A Study of Kindreds with Hereditary Deafness from Sindh Province of Pakistan

By

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بسم الله الرحمن الرحيم

CERTIFICATE

The Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad, accepts this thesis submitted by **Samreen** in its present form, as satisfying the thesis requirements for the degree of Master of Philosophy in Biochemistry/Molecular Biology.

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IN THE NAME OF ALLAH
THE COMPATIONATE, THE MERCIFULL,
ALL PRAISE BE TO ALLAH,
LORD OF ALL THE WORLDS,
KING OF THE DAY OF JUDGEMENT,
YOU ALONE WE WORSHIP, AND TO YOU
ALONE WE TURN FOR HELP.

O'ALLAH GUIDE US TO THE PATH, THAT IS RIGHT,
THE PATH OF THOSE YOU HAVE BLESSED,
NOT TO THOSE WHO HAVE EARNED YOUR ANGER,
NOT THOSE WHO HAVE GONE ASTRAY.

(AL- QURAN)

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This humble effort dedicated to my loving **Papajan** and **Ammi** for their endless love and kind prayers.

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How I made it through them, I can't say.
All your love has helped me reach this day.
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Knowing of the love that's there for me,
Yearning without loss for what is mine.
Only in that love can I define,
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Samreen

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List of Abbreviations

A	Adenine
aa	Amino Acid
ATP	Adenosine TriPhosphate
ARNSHI	Autosomal Recessive Non-Syndromic Hearing Loss
BMP	Bone Morphogenic Protein
bp	Base Pairs
CDH23	Cadherin23
cDNA	Complementry DNA
cM	Centimorgan
COCH	Cochlin
COL	Collagen
dB	Decibles
DFN	Deafness X-Linked
DFNA	Deafness Non-Syndromic Autosomal Dominant
DFNB	Deafness Non-Syndromic Autosomal Recessive
DNA	Deoxyribonucleic Acid
DNR	Dinucleotide Repeats
dNTPs	Deoxy Nucleotide Triphosphates
EDTA	Ethylene Diamine Tetraacetate
ESTs	Expressed Sequence Tags
G	Guanine
GJB	Gap Junction Beta
GJB2	Gap Junction Protein, Beta 2, 26 kDa
GJB3	Gap Junction Protein Beta 3
GJB6	Gap Junction Protein, Beta 6
HBD	Homozygous By Descent
HL	Hearing Level
HHI	Hereditary Hearing Impairment
IHCs	Inner Hair Cell
Hz	Hertz
kDa	Kilo Dalton
mM	Millimolar
mRNA	Messenger RNA
MSR	Microsatellite Repeat
mtDNA	Mitochondrial DNA
MYO7A	Myosin VIIA
MYO15	Myosin XVA
MYO6	Myosin VI
ng	Nanogram
NSHI	Non-Syndromic Hearing Impairement
OTOF	Otoferlin
P	Short Arm of Chromosome
PCR	Polymerase Chain Reaction
PDS	Pendrin
RNA	Ribonucleic Acid

Q	Long Arm of Chromosome
SDS	Sodium Dodesyl Sulphate
TBE	Tris-Borate EDTA
TNR	Tetranucleotide Repeat
TECTA	Tectorin
TEMED	N' N' N' N'-Tetra Methyl Ethylene Diamine
TetraNR	Tetranucleotide Repeat
TMC1	Transmembrane Channel-Like 1
TMIE	Transmembrane Inner Ear Protein
TMPRSS3	Transmembrane Protease, Serine 3
TSR	Template Suppression Reagent
ul	Microlitre
USH	Usher Syndrome
UV	Ultraviolet

Abstract

Deafness is one of the most complex birth defects in human population, which affect as many as three of every 1,000 babies born. Given the unique biological requirements of sound transduction and the selective advantage conferred upon a species capable of sensitive sound detection, it is not surprising that up to 1% of the approximately 30,000 or more human genes are necessary for hearing. The autosomal recessive forms of deafness are generally the most severe and are most exclusively caused by cochlear defects (sensorineural deafness). Out of 68 known DFNB loci, most of the recessively inherited forms of hearing impairment cause a phenotypically identical severe to profound, prelingual hearing loss except DFNB2-*MYO7A*, DFNB8/10-*TMPRSS3* and DFNB16-*STRC*, which cause a delayed, childhood onset hearing impairment. Until now, about 37 genes have been identified causing autosomal recessive deafness.

The study at hand incorporates three Sindhi families (A, B, C) showing symptoms of recessive non-syndromic deafness. Affected individuals from all the three families have a history of prelingual profound hearing impairment and use sign language for communication.

Linkage in the three families was searched by using polymorphic microsatellite markers corresponding to candidate genes involved in related autosomal recessive non-syndromic deafness phenotypes. Linkage to several known deafness loci was conclusively excluded, thus indicating the involvement of novel loci responsible for deafness in these three families.

INTRODUCTION

INTRODUCTION

Deafness

Deafness means loss of the sense of hearing, which may be partial or complete. Partial loss of hearing is often called hearing loss rather than deafness.

A person who cannot detect sound at amplitude of decibels in a frequency range of from 800 to 1,800 vibrations per seconds is said to be hard of hearing. The ear normally perceives sounds in the range of 20 to 20,000 vibrations per second. Hearing impairment is the most prevalent sensorial deficit in the general population and its prevalence increases with the age (Rabionet *et al.*, 2000). The overall impact of hearing impairment is greatly influenced by the severity of hearing defect and by the age of onset. The mutation show in conformational changes of protein and influence the phenotype of the affected individual. In developed countries, genetic factors are the major cause of hearing loss accounting for about 60% of cases (Morton 1991; Cohen and Gorlin, 1995). About 75 percent of individuals with genetically determined deafness have no other clinical features; the other 25% have identifiable syndromes (Morton, 1991; Reardon, 1992; Marazita *et al.*, 1993).

Prevalence

Studies of the epidemiology of hearing impairment have suggested that approximately 1 in 1000 to 1 in 2000 show a profound hearing loss at birth or in early childhood (Parving, 1983; Newton, 1985; Fortnum and Davis, 1997). More then 4000 infants are born deaf each year. It is estimated that 50 to 70% of childhood deafness is due to hereditary causes. Almost every day 2 babies are born with significant hearing loss. Adult onset hearing loss is also a significant health problem with 5% of people under 45 years of age have a handicapping loss of hearing. Fourteen percent of individuals between the ages of 45 and 64 and 30% of those older then 65 years are having hearing problems (Bronya *et al.*, 1999).

Types of Hearing loss

On the Basis of Damage to Inner Ear

► Conductive Hearing Loss

Conductive hearing loss disrupt the conduction of sound through the outer and middle ear affecting hearing before the sound reaches the cochlea and the nerve receptors of the inner ear. Conductive deafness is temporary or curable. Most such cases are caused by otitus media, an infection that spreads to the middle ear from the upper respiratory tract. In adults, cause of conductive deafness is otosclerosis, a chronic hereditary condition in which spongy bone formation results in fixation of the stapes (the bone that connects the middle ear to the inner ear) and restricts its vibration. Deafness can also be caused by perforation or rupture of the eardrum by a sudden loud noise, by physical puncture, or as a result of an infectious disease.

►Sensorineural Hearing Loss

Sensorineural hearing loss results from damage to the neural receptors of the inner ear (the hair cells, organ of corti), the nerve pathways to the brain (notably the auditory nerve), or the area of the brain that receives sound information. Deafness of this type is usually permanent. Low frequency sensorineural hearing loss is an unusual type of hearing loss in which frequencies of 2000 Hz and below are predominantly affected. It can be congenital or caused by tumours, injury, toxic substances (mercury), loud noise or by the side effect of medicine.

►Mixed Hearing Loss

It is simply the combination of above two types. It can occur when a person has permanently sensorineural hearing loss and then also develops a temporary conductive hearing loss.

Quantification of Hearing Loss

The severity of hearing loss is measured by the degree of loudness, as measured in decibels. Hearing loss may be ranked as mild, moderate, severe or profound. The following list shows the ranking and their corresponding decibels ranges.

1. Normal range 10-15 dB
2. Mild
   - ▶ For adults 25-40 dB loss
   - ▶ For children 15-49 dB loss
3. Moderate 41-55 dB loss
4. Moderate severe 56-70 dB loss
5. Severe 71-90 dB loss
6. Profound > 90 dB loss

On the Basis of Inheritance

▶ Non-syndromic Hearing Loss

This is the type of deafness in which there is no other recognizable abnormal phenotype with deafness. It is more common cause of hearing loss than syndromic deafness. It is classifieds by its mode of inheritance into three categories, namely, autosomal dominant DFNA, autosomal recessive DFNB, X-chromosome linked DFN.

▶ Autosomal Recessive

The most common pattern of transmission in hereditary hearing loss in autosomal recessive. A child must have both copies of mutated gene to exhibit deafness. The parents will most likely have normal or near normal hearing even though they posses the recessive gene. Typically, there is a 25% chance that the offspring will be affected and manifest hearing impairment or deafness.

► Autosomal Dominant

If a genetic change (mutation) acts in a "dominant" way it means that even though the gene in the other autosome of the pair is normal, the dominant gene will override it. So a person with an autosomal dominant gene for hearing loss has 1 in 2 (50%) chance of passing that gene to their child.

► X-Linked Inheritance

X-linked inheritance involves particular genes located on the x chromosomes. Males are more affected because they only have a single x chromosome there will be no gene for normal hearing on his y- chromosome. Female can carry the mutation on one of the x chromosome without phenotypic expression. Her sons have a 50% chance of inheriting the mutation and express phenotypically. The daughters have a 50% chance of inheriting the mutation and become a carrier of the mutation.

► Mitochondrial Inheritance

Inherited mitochondrial mutations also have been found to be a cause of non-syndromic hearing loss, and predispose to aminoglycoside induced hearing loss. (Fischel, 2003). In 22 autosomes, each cell also contains a tiny additional piece of DNA, in mitochondria known as mitochondrial DNA and it is coding for 37 genes. Only mothers can pass on the mutation because only the eggs carry mitochondrion DNA.

► Syndromic Hearing Loss

It is the hearing loss associated with other defects in the body. Over 400 genetic syndromes that include hearing loss have been described (Gorlin, 1995). Syndromic deafness can be either dominant (Wardenburg syndrome, Branchial-oto-renal syndrome, Stickler syndrome) recessive (Usher syndrome, Penderd syndrome), X-linked (Alport syndrome, Nance syndrome, Hunter syndrome) or mitochondrial.

Loci and Genes for Non–Syndromic Deafness

The identification of deafness genes is an early and essential step in understanding the molecular mechanism of hearing and hearing loss. Clinical features and genetic heterogeneity suggest a complex process in NSHL. So far, 37 non-syndromic hearing impairment (NSHI) genes have been identified, of which 21 are associated, with autosomal recessive. NSHI (Hereditary Hearing Loss Homepage http://www.uia.ac.be/dnalab/hhh). The human genome project, cDNA libraries constructed from human fetal cochlear and auditory tissues of other organisms, mapped EST from human fetal cochlea, are all new resources which should help pinpoint good candidates and facilitate the identification of the remaining deafness genes. By identifying genes responsible for monogenic hearing impairment, more insight may be gained into the molecular process of hearing and the pathology of hearing loss.

Table 1.1: Loci and Genes for Autosomal Dominant Non-Syndromic Deafness

Locus	Localization	Gene	Gene Product	Biological Role	Mouse Model (Gene Symbol)
DFNA1	5q31	*DIAPH1*	Diaphanous 1	Cytoskeleton	
DFNA2	1p34	*GJB3* *KCNQ4*	Connexin 31 KCNQ4	Gap junction Ion channel, Transporter	*Gjb3*$^{-/-}$
DFNA3	13q12	*GJB2* *GJB6*	Connexin 26 Connexin 30	Gap junction Gap junction	*Gjb2*$^{-/-}$ *Gjb6*$^{-/-}$
DFNA4	19q13	*MYH14*			
DFNA5	7p15	*DFNA5*	DFNA5	*Unknown*	
DFNA6	4p16.3	*WFS1*	Wolframin	*Unknown*	
DFNA7	1q21-q23	*Unknown*			
DFNA8	11q22-q24	*TECTA*	α-Tectorin	Extracellula Matrix	*Tecta*$^{-/-}$
DFNA9	14q12-q13	*COCH*	Cochlin	Extracellular Matrix	*Col11a2*$^{-/-}$
DFNA10	6q22-q23	*TECTA*	α-Tectorin	Extracellular	*Tecta*$^{-/-}$
DFNA11	11q12.3-q21	*MYO7A*	Myosin VIIA	Motor	*Shaker 1 (sh l)*
DFNA12	11q22-q24	*TECTA*	α-Tectorin	Extracellular Matrix	*Tecta*$^{-/-}$
DFNA13	7q22.1	*COL11A2*	α2(XI) Collagen	Extracellular Matrix	*Col11a2*$^{-/-}$
DFNA14	4p16	*WFS1*	Wolframin	*Unknown*	
DFNA15	5q31	*POU4F3*	POU4F3	Transcription Regulator	*Brn3c*$^{-/-}$ *Dreidel (ddl)*
DFNA16	2q24	*Unknown*			
DFNA17	22q13	*MYH9*	Myosin IIA	Motor	
DFNA18	3q22	*Unknown*			
DFNA19	10	*Unknown*			
DFNA20	17q25	*ACTG1*			

Locus	Localization	Gene	Gene Product	Biological Role	Mouse Model (Gene Symbol)
DFNA21	6p21	*Unknown*			
DFNA22	6q13	*MYO6*	Myosin VI	Motor	*Snell's waltzer (sv)*
DFNA23	14q21-q22	*Unknown*			
DFNA24	4q	*Unknown*			
DFNA25	12q21-24	*Unknown*			
DFNA26	17q25	*ACTG1*			
DFNA27	4q12	*Unknown*			
DFNA28	8q22	*TFCP2L3*	TFCP2L3	Transcription regulator	
DFNA29		*Unknown*			
DFNA30	15q25-26	*Unknown*			
DFNA31	6p21.3	*Unknown*			
DFNA32	11p15	*Unknown*			
DFNA33		*Unknown*			
DFNA34	1q44	*Unknown*			
DFNA35		*Unknown*			
DFNA36	9q13-q21	*TMC1*	TMC1	Integral membrane protein	*Deafness(dn)*
DFNA37	1p21	*Unknown*			
DFNA38	4p16	*WFS1*	Wolframin	*Unknown*	
DFNA39	4q21.3	*DSPP*			
DFNA40	16p12	*Unknown*			
DFNA41	12q24-qter	*Unknown*			
DFNA42	4q28	*Unknown*			
DFNA43	3q28-29	*Unknown*			
DFNA44	3q28-29				
DFNA45		*Unknown*			
DFNA46		*Unknown*			
DFNA47	9p21-22				
DFNA48	12q13-q14	*MYO1A*			
DFNA49	1q21-q23	*Unknown*			
DFNA50	7q32	*Unknown*			
DFNA51	19q21	*Unknown*			
DFNA52		*Unknown*			
DFNA53	14q11-q12	*Unknown*			
DFNA54		*Unknown*			

Table 1.2: Loci and Genes for Autosomal Recessive Non-Syndromic Deafness

Locus	Localization	Gene	Gene Product	Biological Role	Mouse Model (Gene Symbol)
DFNB1	13q12	*GJB2* *GJB6*	Connexin 26 Connexin 30	Gap junction Gap junction	*Gjb2*$^{-/-}$ *Gjb6*$^{-/-}$
DFNB2	11q12.3	*MYO7A*	Myosin VIIA	Motor	*shaker 2 (sh 1)*
DFNB3	17p11.2	*MYO15*	Myosin XVIA	Motor	*shaker 2 (sh 2)*
DFNB4	7q31	*SLC26A4*	Pendrin	Ion channel, Transporter	*Pds*$^{-/-}$
DFNB5	14q12	*Unknown*			
DFNB6	3p13-p21	*TMIE*	TMIE	Integral membrane Protein (predicted)	*spinner (sr)*
DFNB7	9q13-q21	*TMC1*	TMC1	Integral membrane Protein (predicted)	*Beethoven(Bt h)*
DFNB8	21q22	*TMPRSS3*	TMPRSS3	Enzyme	
DFNB9	2p22-p23	*OTOF*	Otoferlin	Neuron/synapse	
DFNB10	21q22.3	*TMPRSS3*	TMPRSS3	Enzyme	
DFNB11	9q13-q21	*TMC1*	TMC1	Integral membrane Protein	*Beethoven(Bt h)*
DFNB12	10q21-q22	*CDH23*	Cadherin 23	Adhesion	*Waltzer (v)*
DFNB13	7q34-q36	*Unknown*			
DFNB14	7q31	*Unknown*			
DFNB15	3q21-q25, 19p13	*Unknown*			
DFNB16	15q21-q22	*STRC*	Stereocilin	*Unknown*	
DFNB17	7q31	*Unknown*			
DFNB18	11p14-p15.1	*USH1C*	Harmonin	Macromolecular Organizer	
DFNB19	18p11	*Unknown*			
DFNB20	11q25	*Unknown*			

Locus	Localization	Gene	Gene Product	Biological Role	Mouse Model (Gene Symbol)
DFNB21	11q	*TECTA*	A-Tectorin	Extracellular Matrix	*Tecta* $^{-/-}$
DFNB22	16p12.2	*OTOA*	Otoancorin	Extracellular Matrix	
DFNB23	10p11.2-q21	*PCDH15*			
DFNB24	11q23	*Unknown*			
DFNB25	4p15.3-q12	*Unknown*			
DFNB26	4q31	*Unknown*			
DFNB27	2q23-q31	*Unknown*			
DFNB28	22q13	*Unknown*			
DFNB29	21q22	*CLDN14*	Claudin 14	Tight junction	
DFNB30	10p	*MYO3A*	Myosiniiia	Motor	
DFNB31	9q32-q34	*WHRN*			
DFNB32	1p13.3-22.1	*Unknown*			
DFNB33	9q34.3	*Unknown*			
DFNB34		*Unknown*			
DFNB35	14q	*Unknown*			
DFNB36	1p36	*ESPN*	Espin	Cytoskeleton	
DFNB37	6q13	*MYO6*	Myosin VI	Motor	*Snell's waltzer (sv)*
DFNB38	6q26-q27	*Unknown*			
DFNB39	7q11.22-q21.12	*Unknown*			
DFNB40	22q11.21-12.1	*Unknown*			
DFNB41		*Unknown*			
DFNB42	3q13.31-q22.3	*Unknown*			
DFNB43		*Unknown*			
DFNB44	7p14.1-q11.22	*Unknown*			
DFNB45	1q42.3-q44				
DFNB46	18p11.32-p11.31	*Unknown*			

Locus	Localization	Gene	Gene Product	Biological Role	Mouse Model
DFNB47		*Unknown*			
DFNB48		*Unknown*			
DFNB49	5q12.3q14.1.	*Unknown*			
DFNB50	12q23	*Unknown*			
DFNB51		*Unknown*			
DFNB52		*Unknown*			
DFNB53		*Unknown*			
DFNB54		*Unknown*			
DFNB55		*Unknown*			
DFNB56		*Unknown*			
DFNB57		*Unknown*			
DFNB58		*Unknown*			
DFNB59		*Unknown*			

Gene Discovery in the Auditory System

Traditional methods for mapping disease genes, such as genetic linkage analysis have a less than totally optimal use in gene discovery efforts for hearing disorders. Successful use has been restricted largely to consanguineous kindreds in which there has been a limited immigration (Morton, 2002). A complementary method to genetic linkage analysis for gene identification is one that utilizes tissue or organ specific cDNA libraries to provide candidate genes (Hedrick *et al.,* 1984; Jones and Reed, 1989; Gurish *et al.,* 1992). Cochlear cDNA libraries constructed from human (Robertson *et al.,* 1994; Jacob *et al.,* 1997) and mouse (Cohen-Salmon *et al.*, 2002) have provided precious biological tools for gene discovery in the mammalian cochlea, in particular, DFNA9 (Robertson *et al.,* 1998).

►Cadherine Related 23

It is located at 10q21-q22. *CDH23* gene produces a protein called cadherin (related) 23, a type of protein that causes cells to stick together. Cadherins (calcium dependent cell adhesion molecule) are membrane bound glycoprotein receptors that function in cell to cell contact at adherens junctions (Nagafuchi, 2001). *CDH23* encodes 23, a putative calcium-dependent adhesion molecule required for proper morphogenesis of mechanosensitive hair bundles of the inner-ear neurosensory cells (Frolenkov *et al.,* 2004). The shaping of the hair bundle relies on a functional unit composed of *MYO7A*, harmonin b, and *CDH23* and that the interaction of these proteins ensures the cohesion of the stereocilia, further identify a close spatio-temporal relationship between *CDH23* and harmonin b in developing hair cell (Beoda *et al.*, 2002). Cadherins are grouped on the basis of tandem repeats of the extracellular cadherin specific motif (EC domain), and they usually have a single predicted membrane-spanning domain with a cytoplasmic domain involved in linkage to the cytoskeleton (Kemler, 1993; Gumbiner, 1996). The cadherin 23-harmonin-myosin VIIA complex may organize hair cell bundles by cross-linking stereocilia. It may also promote the transmission of sound waves. Allelic mutations of *CDH23* encoding cadherin 23 cause both non-syndromic deafness DFNB12 and *USH1D* (Bolz *et al.*, 2001; Bork *et al.*, 2001). All reported *CDH23* alleles identified in non-

syndromic deafness patients are missense mutations (Bork *et al.*, 2001; Astuto *et al.*, 2002).

►Trans membrane Protease, Serine 23 (*TMPRSS3*)

TMPRSS3 gene is located at 21q22.3. This gene encodes a protein that belongs to the serine protease family. The protein appears to be bound to the endoplasmic reticulum and has a conserved serine protease signature sequence at the carboxyl-terminus. *TMPRSS3* is expressed in supporting cells of organ of corti, in the stria vascularis and in the spiral ganglion cells of the cochlea (Guipponi *et al.*, 2002). Serine proteases are enzymes that use the amino acid serine to cleave, or cut apart, other proteins. *TMPRSS3* mutations account for hearing loss in 1.8% (8 of 449) of Pakistani families segregating congenital deafness as an autosomal recessive trait (Ahmed *et al.*, 2004).

►Collagen XI (alpha2 chain)

Collagen XI protein, codified by gene *COL11A2* located in chromosome 6, is one of the component of tectorial membrane (De Leenher *et al.*, 2002). It is an acellular membrane comprising many different types of collagen (II, V, IX, XI), non collagen protein and it is involved in deflection of ciliary's bundle of cochlear outer hair cells, immediately after sound stimulus (Libby and Steel, 2000). Mutation in the collagen gene causes some allelic connective tissue disorders such as Marshall Syndrome (Griffith *et al.*, 2000). There are no mouse models for these dominant disorders, but mice that are homozygous for a recessive knockout allele of *Col11a2* have severe sensorineural hearing loss (McGuirt *et al.*, 1999).

►Tectorin (*TECTA)*

Gene *TECTA* is located in chromosome 11q22-q24. It codifies α-tectorin protein a major non-collagenous glycoprotein component of the tectorial membrane (Denoyelle *et al.*, 2002). The tectorial membrane overlying hair cells plays a crucial role in the mechanosensory transduction process. Many different types of cells synthesize alpha-tectorine protein during development of inner ear. Tectorin mutations probably inhibit the

function of the tectorial membrane as a resonator. Mutations in gene cause two forms of autosomal dominant hearing loss (DFNA8 and DFNA12) both pre-lingual and they may be progressive or non progressive and an autosomal recessive form (DFNB21) prelingual, severe to profound (Denoyelle *et al.*, 2002). Homozygosity for functional null alleles of *TECTA* at the DFNB21 locus causes recessive, prelingual, and severe to profound stable hearing loss with a flat or shallow U-shaped audiometric configuration (Naz *et al.*, 2003).

►Cochlin

Protein cochlin is codified by *COCH,* located in chromosome 14q11–13. It is ubiquitously present in the bovine inner ear (Ikezono *et al.*, 2001). It is expressed in the cochlea as well as vestibular organs. The function of the *COCH* gene is not known. Mutations are responsible for DFNA9, which starts between the ages of 20 and 30 years, approximately. Initially, it is profound in high frequencies with variable progression at the age of 40-50 years. Mutations of gene *COCH* may be one of the genetics factors that contribute to the symptoms of Meniere disease and this hypothesis should be considered in patients with symptoms of the disease (Usami *et al.*, 2003).

►Protein Connexin

Protein connexin is the structural component of intercellular gap junctions. These gap junction channels permit the rapid exchange of ions and are thought to play an important role in maintaining hearing function by circulation of potassium ions between the fluids of the inner ear (Goodenough *et al.*, 1996; Bruzzone *et al.*, 1996). Six connexin subunits assemble into a half-channel that dock with its counterpart from an adjacent cell to form an intercellular channel (Bruzzone *et al.*, 1996; Kumar *and Gil NB,* 1996). Blockage of k+ circulation causes hearing impairment. Gap junctions also important for homeostasis in the inner ear. Mutations in the connexins are responsible for a diversity of diseases, including deafness. At least four connexins are known to be expressed in the ear.

►Gap Junction Protein, Beta 2, (connexin 26)

In 1997, gene connexin 26, which is located at chromosome 13q11-q12, was discovered, whose mutation caused DFNA3 and DFNB1 (Wang *et al.*, 2003). *GJB2* and other

members of the connexin gene family have simple genomic structure composed of two exons and are responsible for both forms of hearing loss. *GJB2* mutation disturbs the homeostasis of the cortilymph, an extracellular space surrounding the sensory hair cells, due to impaired K+ transport by supporting cells, resulting in a degradation of the organ of corti rather than affecting the endolymph homeostasis, in mice and probably in human (Kudo *et al.*, 2003). Some recessive mutations delete or insert base pairs, the building material of DNA. 80 recessive and 6 dominant mutations have been found in *GJB2*. The most common mutation, 35delG (one guanosine residue deletion from nucleotide position 35), is very frequent in Caucasian population. This mutation results in shifting of the reading frame and protein truncation. Because of the high prevalence of this mutation in western countries and the small size of *GJB2* gene, diagnostic testing is available (Chang *et al.*, 2003).

► Gap Junction Protein Beta 3 -*GJB3* (Cx31)

GJB3 is located at 1p34. The *GJB3* gene provides instructions to make a protein called gap junction protein, beta 3, 31k Da, which is a member of the gap junction or connexin family of proteins. It has not been determined yet if protein connexin 31 is present in all gap junctions of the inner ear. The site of Cx31 is the same for gene *KCNQ4*, expressed in both hair cells, and if mutant, it causes DFNA2 (Mhatre *et al.*, 2003). Few *GJB3* mutations in individuals with non-syndromic deafness inherited in an autosomal recessive manner. *GJB3* is expressed in the cochlea and has been evaluated as a candidate gene for various forms of hereditary deafness in the absence of statistically significant linkage data (Friedman and Griffith, 2003).

► Gap Junction Protein, Beta 6 *GJB6* (Connexin 30)

GJB6 is located in chromosome 13 (13q12). The *GJB6* gene provides instructions to make a protein called gap junction beta 6, which is a member of the gap junction or connexin family of proteins. The distribution and expression of connexin 30 were of to be nearly same as that of connexin 26 (Xia *et al.*, 2001). A 342-kb deletion in the *GJB6* gene, which encodes protein connexin 30, that is reported to be expressed with connexin

26 (Del Castello *et al.*, 2002). Therefore, pathophysiological hypotheses concerning hearing loss associated with connexin 26 and connexin 30 are similar.

► ***KCNQ4***

KCNQ4 mapped in chromosome 1 (1p34). It is a component of potassium channel and is involved in the potassium recycling pathway as well. In the cochlea, channels *KCNQ4* are expressed not only in outer hair cells, but also in the inner hair cells, whose main function is to promote the outflow of potassium from the cells to supporting cells (De Leenher *et al.*, 2002). The potassium ions then recirculate through connexin channels between the supporting cells to the stria vascularis, where they are secreted into the endolymph through potassium channels formed by the *KCNQ1* and *KCNE1* gene products. It is the first ion channel shown to be specifically expressed in a sensory pathway (Kharkovets *et al.*, 2000). Mutations of this gene were identified in families affected by progressive hearing loss and started at the age of 20 years with high frequencies.

► **Trans membrane channel-like 1 *(TMCI)***

TMC1 gene is located at chromosome 9q21.12. It provides instructions to make a protein called Trans membrane channel-like 1. It is an ion channel, which is mainly localized in the inner hair cells (IHC), then it might be involved in the most basic auditory process of hair-cell transduction but its function remains unknown. *TMC1* protein assists in the transport of charged atoms (ions). The proper level of particular ions, such as sodium and potassium, is required for the conversion of sound to nerve impulses. The deafness mouse is the homologous model for recessive *TMC1* mutations while the Beethoven (Bth) mouse is identified with dominant *TMC1* mutations that cause postlingual hearing impairment (Kurima et al., 2002; Vreugde et al., 2002). In the mouse *TMC1*, expression has been found to localize to cochlear hair cells (Kurima *et al.*, 2002).

► **Trans membrane Inner Ear Protein *(TMIE)***

The *TMIE* gene is located at 3p21. It makes a protein called Trans membrane inner ear protein. The function of this protein is unknown, but it appears to be important for normal

hearing. It has no similarity with other proteins (Mitchem *et al.*, 2002).). At least five mutations in the *TMIE* gene have been identified in families with non-syndromic deafness. Several of the mutations substitute one amino acid for another in the protein made by *TMIE*. The other mutations insert or delete a small amount of DNA in the gene. *TMIE* mutations co-segregating with DFNB6 deafness in 5 consanguineous families was identified (Naz *et al.*, 2002).

► Myosine Genes

Myosine genes are members of large super family of genes that encodes proteins that exert mechanical force. Myosines are molecular motor proteins that bind to actin and that hydrolyze ATP to generate the force to move across actin filaments. They are important for the structural integrity of the stereocilia. In the cochlea, they have been implied in the formation and movements of expansions of cytoplasmatic membrane and transduction signals of outer and inner hair cells (Libby and Steel, 2000).

► Myosine V11A (*MYO7A*)

Gene *MYO7A*, located in chromosome 11 (11q13.5). In the cochlea, the protein is present along the sterocilia, close to the junction between hair cells and supporting cells and presented in the synaptic region (Tamagawa *et al.*, 2002). Mutations in the gene cause structural defects of the protein and consequent affections in auditory function. DFNB2 comprising different grades of vestibular dysfunction and variable age of onset. *MYO7A* that causes DFNB2, DFNB11 and Usher 1B were the first one to show that one single gene could determine both forms of hearing loss, syndromic and non-syndromic (Ahmed *et al.*, 2003).

► Myosine XVA (*MYO15*)

The *MYO15A* gene is located on chromosome 17 (17p11.2). It makes a protein called myosin XVA, which is part of a group of proteins called unconventional myosins. In the inner ear, the expression of this gene seems to be restricted to hair cells, on the cuticular plaque. Mutations of this gene determine DFNB3 (Belyantseva *et al.*, 2003).

►Myosine VIA *(MYO6)*

The *MYO6A* gene is located on chromosome 6 (6q13), codifies non-conventional protein myosine6 concentrated of cuticular plaque of hair cells. (Bitner-Glinddzicz, 2002). *MYO6* is thought to function as both a transporter and an anchor (Altman *et al.*, 2004). Mutation in *MYO6* was reported in a Pakistani family in which 6 individuals had bilateral, profound, congenital sensorineural hearing loss segregating as an autosomal recessive disorder DFNB37 (Ahmed *et al.*, 2003).

►Myosine IIIA (*MYO3*)

Non-conventional protein codified by gene *MYO3A*, located on chromosome 10p11.1 (Walsh *et al.*, 2002). Mutations determine DFNB30, characterized by bilateral progressive hearing loss that affects primarily high frequencies.

►Otoferlin Gene

Otoferlin gene is located at 2p23. The *OTOF* gene provides instructions to make a protein called otoferlin, which is present in the inner ear and brain and involved in the fusion, triggered by the calcium, of synaptic vesicles with the plasma membrane (Denoyelle and Petit, 2002). Otoferlin may play a role in releasing chemical signals (neurotransmitters) from nerve cells that are involved in hearing. Mutation determines DFNB9, characterized by pre-lingual profound hearing loss involving all frequencies.

►Pendrin Gene

Pendrin is codified by gene *PDS* located at chromosome 7q. Mutations in this gene are responsible both by Penderd Syndrome and by DFNB4 gene *SLC26A4* (Wilcox *et al.*, 2000). *PDS,* now designated as solute carrier (SCL) family, *SLC26A4,* is mutated in Pandered syndrome and in some types of non-syndromic deafness (Li *et al.*, 1998; Everett *et al.*, 1999). The pendrin gene product act as an iodide/chloride transporter (Everett *et al.*, 1999). DFNB4 is characterized by progressive hearing loss and widening of vestibular aqueduct, without thyroid affection. In the mature cochlea, protein pendrin is expressed in prominent spiral cells and cells adjacent to external spiril (Wilcox *et al.*,

2000). To date 42 different *PDS* mutations have been identified in people with classic pendred syndrome (Everett *et al.*, 1997; Bogazzi *et al.*, 2000).

►Transcription Regulators

Many transcription factors are necessary for developing the auditory system (Cantos *et al.*, 2000; Anagnostopoulous, 2002). Four transcription factor genes have been identified: *POU3F4, POU4F3, EYA4* and *TFCP2L3. POU3F4* is responsible for X-linked and mixed hearing loss. The conductive hearing loss is from stapes fixation. These patients suffer from an increased per lymphatic pressure causing the typical "gusher" that appears during stapes footplate surgery. Mutation of some of these genes (*POUF4, POU4F3, EYA4* and *TFCP2L3)* expressed in specialized cell types of the inner ear are associated with non-syndromic, post lingual progressive hearing loss.

►*POU* Transcription-Factor Genes

The *POU3F4* gene is expressed in the mesenchyma of the inner and middle ear, in which it is involved in the maturation of bone; mutant mice with targeted inactivation of *POU3F4* have abnormal development of the bony labyrinth. Mutations in or around the gene for *POU3F4* on chromosome Xq21 are responsible for DFN3 (De Kok *et al.*, 1995), an X-linked form of non-syndromic, progressive hearing loss with fixation of the footplate of the stapes. A deletion of only 8 base pairs was the mutation found in gene *POU4F3,* located in chromosome 5 (5q31), starting between 18 and 30 years, progressive and reaches moderate to severe level at the age of 50, approximately (Wiess *et al.*, 2003). Gene *POU4F3* codifies transcription factor belonging to the family of proteins of domain *POU.* The *POU4F3* gene is expressed only in hair cells, in which it is responsible for the regulation of transcription of target genes that are important for the survival of cells in the organ of corti. Targeted deletions of both alleles of *POU4F3* in mice have been shown to cause deafness and balance impairments.

In the present study, three families showing non-syndromic hearing loss were ascertained from District Hyderabad, province of Sindh. Linkage to known deafness loci was tested by PCR-based microsatellite genotyping.

MATERIALS & METHODS

Materials and Methods

Families Studies

Three families referred here with as family 'A' 'B' and 'C' with autosomal recessive non-syndromic hearing loss (NSHL) were studied from Hyderabad, Sindh province of Pakistan.

Pedigree Analysis

The families were visited at their places of residence. The elders and relatives of the families were interviewed to obtain information about the diseases and other relevant matters. The case history, number of affected individuals, number of generations involved, the associated defects if any and onset of the disorder was carefully recorded. All the information was checked and rechecked by interviewing different members of the families. After a detailed and thorough discussion with elders of three families, genetic pedigrees were drawn for each family, by the standard methods described by Bennett *et al.* (1995). The males were symbolized by squares and females by circles. Filled circles or squares were indicative of affected individuals. Each generation has been numbered consecutively. Individuals within a generation have appeared as Arabic numerals. A number enclosed within a symbol indicates the number of sibs. A double line between the partners showed cousin marriages. The mode of inheritance of hearing impairment was deduced by observing the segregation of hearing loss within the family.

Blood Sampling

Blood samples from both normal and affected individuals including their parents were collected through 10 ml syringes (0.8 X 38 mm 21_G x $1^1/_2$) in standard potassium EDTA tubes. The samples were kept at 4°C till the DNA was extracted.

Extraction and Purification of Genomic DNA from Blood

Two methods were used for the extraction and purification of the genomic DNA from blood samples:

► Organic preparation using 1.5 ml microcentrifuge tubes.

► Commercially available Kit.

Organic preparation using 1.5 ml microcentrifuge tubes

In organic (Phenol-Chloroform) method, 0.75 ml of blood was taken in a 1.5 ml microcentrifuge tube and mixed it with equal volume of solution A and was kept at room temperature for 10-15 minutes. The tube was then centrifuged at 13,000 rpm for 1 minute in a Microfuge® 18 centrifuge (Beckman Coulter™). Supernatant was discarded and pellet was resuspended in 400 µl of solution A. Centrifugation was repeated, and after discarding the supernatant the nuclear pellet was resuspended in 400 µl of solution B, 12 µl of 20% SDS and 20 µl of proteinase K (100 µl/ml final concentration) and incubated at 37°C overnight. On the following day, 0.5 ml of fresh mixture of equal volume of solution C and solution D was added in sample, mixed and centrifuged for 10 minutes at 13,000 rpm. The aqueous phase (upper layer) was transferred into a new microcentrifuge tube and equal volume of solution D was added. Centrifugation was then carried out again at 13,000 rpm for 10 minutes. The aqueous phase was placed in a new tube, and after adding 55 µl of 3 M sodium acetate (pH 6) and equal volume of isopropanol, the tubes were inverted several times to precipitate DNA. The DNA pellet was washed with 70% ethanol and dried in the incubator at 37°C. After evaporation of residual ethanol, DNA was dissolved in appropriate amount of DNA dissolving buffer (DDB).

Composition of solutions

Solution A	**Solution B**
0.32 M sucrose	10 mM Tris (pH 7.5)
10 mM Tris (pH 7.5)	400 mM NaCl
5 mM $MgCl_2$	2 mM EDTA (pH 8.0)
1% (v/v) Triton X-100	

Solution C

Phenol

10 mM Tris

Solution D

Chloroform/Isoamylalcohol 24:1 (v/v)

DNA dissolving buffer (DDB)

10 mM Tris (pH 8.0)

0.1 mM EDTA

DNA Extraction by Commercially Available Kit

DNA extraction was also carried out by using Genomic Isolation Kit (Sigma Chemical Co. USA).

One hundred and fifty microlitres of blood was taken in a 1.5 ml microcentrifuge tube along with 250 µl of lysis solution A, mixed by inversion, incubated at 65°C for 6 minutes. 450 µl of chloroform was added and vortexed vigorously for 2-3 minutes. Clear aqueous phase was transferred to a new 1.5 ml microcentrifuge tube after adding 100 µl of precipitation solution B and centrifugation at 14,000 rpm for 5-10 minutes. DNA was then precipitated by adding 500 µl of 100% ethanol. Ethanol was removed after centrifugation at maximum speed for 2 minutes, and then washed with 70% ethanol. After evaporation of residual ethanol, DNA was dissolved in appropriate amount of Tris-EDTA (TE) buffer by incubation at 65°C for 5 minutes.

DNA Dilution and Micropipetting

The stock DNA was diluted to 40-50 ng/µl for PCR amplification. Micropipetting was carried out using adjustable micropipettors with disposable tips ranging from 10 – 1000 µl of upper volume limit.

Polymerase Chain Reaction (PCR)

Polymerase chain reaction was performed in 0.2 ml tubes (Axygen, USA) containing 25 µl total reaction mixture. The reaction mixture was prepared by adding 1 µl sample DNA (40 ng), 2.5 µl 10 X PCR buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 25 mM $MgCl_2$), 0.3 µl Taq DNA polymerase (one unit) in 20.1 µl PCR water. The reaction mixture was centrifuged for few seconds for thorough mixing. The reaction mixture was taken through thermocycling conditions consisting: 5 minutes of 95°C for template DNA denaturation followed by 40 cycles of amplification, each consisting of 3 steps: one minute at 95°C for DNA denaturation into single strands; 1 minute at 57°C for primers to hybridize or "anneal" to their complementary sequences on either side of the target sequence; and one minute at 72°C for extension of complementary DNA strands from each primer, final 10 minutes at 72°C for Taq DNA polymerase to synthesize any unextended strands left.

PCR was performed using Gene Amp PCR system 2400 and 9600 thermocycler (Perkin Elmer volume limit).

Horizontal Gel Electrophoresis

Amplified PCR products were analyzed on 2% agarose gel, prepared by melting 1 g of agarose in 50 ml 1 X TBE (0.89 M Tris-Borate, 0.032 M EDTA, pH 8.3) in a microwave oven for two minutes. 5 µl of ethidium bromide (0.5 µg/ml final concentration) was added to stain DNA.

PCR samples were mixed with loading dye (0.25% bromophenol blue, 40% sucrose) and loaded into the wells. Electrophoresis was performed at 100 volts (80 mA) for half an hour in 1 X TBE buffer. Amplified products were visualized by placing the gel on UV transilluminator (Biometra, Germany).

Vertical Gel Electrophoresis

The amplified products were resolved on 8% non-denaturing polyacrylamide gel. Gel solution was made in a 250 ml conical flask, and was poured in the space between the two glass plates separated at a distance of 1.5 mm. After placing the comb, it was allowed

to polymerize for 45-60 minutes at room temperature. Samples were mixed with loading dye and loaded into the wells. Electrophoresis was performed in a vertical gel tank of Whatman Biometra (Life Technologies, USA) at 100 volts (30 mA) electric current for 90 minutes depending upon the size of amplified length. The gel was stained with ethidium bromide solution (10 mg/ml) and visualized on UV transilluminator for photography by digital camera DC120 (Kodak, USA).

Composition of 8% Polyacrymalide Gel (50 ml)

13.5 ml 30% acrylamide solution (29 g polyacrylamide, 1 g N, N methylene-bis-acrylamide)

5 ml 10 X TBE

350 µl 10% Ammonium pesulphate

17.5 µl TEMED (NNN'N'-tetra methyl ethylene diamine)

31.13 ml distilled water

Genotyping and Primer Database Analysis

Analysis of microsatellite markers was performed by PCR; the amplified products were resolved in 8% standard non-denaturing polyacrylamide gel as described above. Microsatellite markers were visualized by placing the ethidium bromide stained gel on UV transilluminator and genotypes were assigned by visual inspection of the gel photograph taken. Microsatellite markers mapped by Cooperative Human Linkage Centre (CHLC), were obtained from Research Genetics, Inc. (USA). Information about the cytogenetic location of these markers as well as the length of the amplified products was obtained from genome database homepage (www.gdb.org) and Marshfield Medical Centre (www.marshmed.org.gov/genetics/). The numbers of tri, tetra nucleotide repeat sequence polymorphic markers used in current study were approximately 94%. Average heterozygosity of each marker was above 70%, implying that these markers are highly informative for allelotyping pedigree members. The autosomal recessive non-syndromic

hearing impairment loci initially investigated and the microsatellite markers with their cytogenetic locations are shown in Table 2.2

Linkage Studies

a) Linkage to DFNB1 locus (Connexin 26)

To exclude the DFNB1 locus from linkage two approaches were followed:

- Linkage of the families to DFNB1 locus was investigated by typing the microsatellite markers (D13S292, D13S787 and D13S1275) mapped in the linkage interval of the locus.

- Genomic DNA of an affected individual was screened for mutation in the *GJB2* gene. For this purpose, exon 2 of *GJB2* gene, containing the entire open reading frame of 681bp encoding 226 amino acids, was amplified by primers set (Table 2.1) of exon 2, sequenced through Automated Genetic Analyzer, ABI Prism 310® (Applied Biosystem, USA).

b) Mutation screening in *GJB2* gene

To search for a mutation in the *GJB2* gene, exon 2 and intron-exon boundaries were amplified by two sets of overlapping primers (Table 2.1).

C) Linkage to known DFNB loci

To elucidate the gene defect in the families presented here, an initial search for linkage was carried out by using polymorphic markers mapped within several autosomal recessive non-syndromic deafness loci listed on the Hereditary Hearing Loss Homepage (http://dnalab-ww.uia.ac.be/dnalab/hhh).

Table 2.2 summarizes microsatellite markers located in the region of known deafness loci, which were used as first pass analysis for genetic linkage in families with non-syndromic recessive deafness. Selected markers had an average heterozygosity of >70 %. Genotyping of these markers was performed as described above.

DNA Sequencing

Thermo-cycling conditions were described above. The concentration of genomic DNA was 100 ng and the concentration of primers used was 2.5 µl (20 ng/µl) in 25 µl of reaction mixture. PCR products were analyzed on 2% agarose gel along with 100 bp DNA Ladder (O' Range Ruler™, MBI Fermentas, UK).

The amplified products were purified using Rapid PCR Purification Kit (Marligen, USA). Three hundred microlitres of binding solution (H1) (concentrated Guanidine HCl, EDTA, Tris-HCl, and Isopropanol) was added to the amplification reaction and mixture was applied to a spin cartridge containing silica-based membranes where the double stranded DNA was selectively adsorbed. Adsorption to the membrane is influenced by buffer composition and temperature. DNA polymerases, buffer, unreacted primers and dNTPs were removed with 500 µl of alcohol-containing wash buffer (H2) (NaCl, EDTA, Tris-HCl). DNA was eluted in Tris-EDTA buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA) at 70°C.

Purified products were subjected to cycle sequencing using Big Dye terminator V 3.1 ready reaction mix and sequencing buffer (PE Applied Biosystems, Foster city, CA, USA). The reaction mixture was taken through thermocycling conditions consisting: 1 minute of 95°C for template DNA denaturation followed by 30 cycles of amplification each consisting of 3 steps: 30 seconds at 95°C for DNA denaturation into single strands; 30 seconds at 63°C for primer to hybridize or "anneal" to their complementary sequences on either side of the target sequence; and 4 minutes at 72°C for extension of complementary DNA strands from primer, final 10 minutes at 72°C for Taq polymerase to synthesize any unextended strands left.

The sequencing products were purified by ethanol precipitation protocol (POP6 Protocol). Sequencing product was transferred to 1.5 ml microcentrifuge tube, containing 16 µl of distilled water and 64 µl 100% ethanol. Tubes were kept at room temperature for 15 minutes, and centrifuged at 14, 000 rpm for 20 minutes. Supernatant was removed and 250 µl of 70% ethanol was added into the tubes. Tubes were centrifuged at 14,000 rpm for 10 minutes after thorough mixing. Supernatant was discarded, and 20 µl of T.S.R.

(Template Suppression Reagent) was added into the tube. Mixture thus obtained was placed in 0.5 ml septa tubes. After applying denaturation temperature of 95°C for 2 minutes, the sample was sequenced through Automated Genetic Analyzer, ABI Prism 310® (Applied Biosystem, USA).

The chromatograms obtained of the affected individuals were compared with the corresponding control gene sequences from NCBI (National Centre for Biotechnology Information) database to identify the aberrant nucleotide base pair change (http://www.ncbi.nlm.nih.gov/).

Table 2.1: Primers for PCR amplification of *GJB2* gene exon 2.

	Primers (5`→ 3`)		Amp. Length (bp)	Annealing Temp. (°C)
Exon 2 (*GJB2* gene)	**Forward**	**Reverse**		
	GTAAGAGTTGGTGTTTGCTC	GATGACCCGGAAGAAGATGC	**576**	**63**
	CAGCTGATCTTCGTGTCCAC	GAGTTTCACCTGAGGCCTAC	**600**	**63**

Table 2.2: A list of microsatellite markers used to test linkage to known DFNB loci.

S.NO	LOCUS	MARKERS	LOCATION (cM)
1	DFNB1	D13S292 D13S787 D13S1275	9.00 8.87 6.99
2	DFNB6	D3S1767 D3S3647	69.09 68.47
3	DFNB8/10	D21S1260	46.71
4	DFNB7/11	D9S1876	67.93
5	DFNB31	D9S1881 D9S1776 D9S302	135.85 123.33
6	DFNB35	D14S53 D14S77 D14S258	86.29 80.82 76.28
7	DFNB36	D1S214 D1S2870	14.04 14.04
8	DFNB37	D6S1031 D6S1659 D6S1619	88.63 88.63 87.29

9	DFNB38	D6S1277 D6S1599	173.31 169.95
10	DFNB39	D7S2410 D7S2212	100.81 95.43
11	DFNB42	D3S4523 D3S1575 D3S2460	220.00 131.87
12	DFNB43	D15S652 D15S655 D15S643	90.02 82.84 52.33
13	DFNB44	D7S670 D7S1818 D7S817 D7S460	69.56 69.56
14	DFNB45	D1S1609 D1S404 D1S1594	274.53 273.46 265.49
15	DFNB46	D18S976 D18S52 D18S54 D18S63 D18S481 D18S1376 D18S471 D18S452	 12.08 09.26 08.03 08.03 06.94
16	DFNB47	D2S1400 D2S423	27.6 23.0

17	DFNB49	D5S647 D5S2500 D5S1501	74.07 69.23
18	DFNB54	D1S193 D1S3721	73.21 59.00
19	DFNB55	D4S428 D4S1645	187.0 72.52

cM: centiMorgan

RESULTS

Results

Pedigree Analysis

Family A

The family A resides in Hyderabad district in the province of Sindh. The family history presented in the pedigree (Figure 3.1) indicates four-generations consisted of 23 individuals including one affected female (1V-3) and two affected males (1V-5, 1V-6). Parents (III-3 and III-4) of the affected family members (1V-3, 1V-5, 1V-6), although phenotypically normal, carry the recessive disease allele in heterozygous condition and the trait appears independent of the sex suggest that the trait is transmitted in autosomal recessive manner. After a general examination and interviews regarding the complete medical history of the individuals and family relationships it was concluded that there was no possibility of the environmental factors and infections to be the cause of deafness. The affected persons are deaf and mute since birth.

Blood samples were collected from all the participating members of the family including three normal (III-3, III-4, and IV-7) and three affected individuals (IV-3, IV-5, and IV-6) and then processed for DNA extraction.

Family B

These kindred reside in Hyderabad district in the Sindh province. The family members traditionally marry within the family due to strict social customs. The family pedigree (Figure 3.2) consists of four generations and three affected individuals (IV-3, IV-4 and IV-5). Parents (III-2 and III-3) of the affected family members, although phenotypically normal, carry the disease allele in heterozygous condition. After a general examination and interviews regarding the complete medical history of the individuals and family relationships it was concluded that there was no possibility of deafness due to environmental factors and infections. The affected persons are deaf and mute since birth and all affected individuals had profound prelingual hearing impairments. Blood samples were collected from six members including three affected (IV-3, IV-4, and IV-5) and three normal individuals (III-2, III-3 and 1V-1).

Family C

This family was located in Tando Adam in district Sanghar in province of Sindh. Consanguineous marriages are common within the community where the kindred reside. The family pedigree (Figure 3.3) consists of four generations and two affected individuals (IV-1, IV-2). Normal parents produce affected individuals after skipping the generations, therefore, suggests that the trait is transmitted in the autosomal recessive mode. After a general examination and interviews regarding the complete medical history of the individuals and family relationships it was concluded that there was no possibility of the environmental factors and infections to be the cause of deafness. The affected persons are deaf and mute since birth.

Blood samples were collected from all the participants including two normal (III-3, IV-4) and two affected (IV-1, IV-2) individuals.

Genetic Mapping

On the basis of genetic linkage studies in other forms of hereditary hearing impairment, it is clear that at least some candidate intervals should be tested for linkage or exclusion prior to embarking on genome-wide search. Sixty eight gene loci (DFNB) for autosomal recessive non-syndromic deafness have been identified so far. In the present study, all the three families were tested for the linkage to several known DFNB loci. Table 2.2 summarizes the microsatellite markers in the region of known loci, which were used in the present study for candidate gene mapping. Average heterozygosity for the selected markers is greater than 80%. Analysis of microsatellite markers was carried out using a standard PCR reaction and electrophoresis in 8% non-denaturing polyacrylamide gel. Microsatellite markers were visualized by staining the gel with ethidium bromide and genotypes were assigned by visual inspection.

Linkage to known loci

Majority of the non-syndromic hearing loss show autosomal recessive inheritance. Mutation in the *GJB2* gene (connexin) at DFNB1 locus accounts for more than 50% of the recessive non-syndromic deafness.

In family A (Figure 3.1), initially three microsatellite markers D13S292, D13S787, D13S1275 from DFNB1 genetic interval, were used to test the linkage. From the results obtained (Figure 3.4-3.6), it was clear that affected individuals were heterozygous for different combinations of parental alleles, thus linkage to DFNB1 locus was excluded. In order to verify the results obtained with DFNB1 linked markers in family A, *GJB2* gene (Kelsell *et al.*, 1997), implicated earlier in causing deafness at this locus (Guilford *et al.*, 1994), was sequenced in one affected individual. Sequence Analysis of the coding exon of *GJB2* gene in the affected individual of family A showed wild type sequence (Figure 3.7-3.8), thus further excluding the family from linkage to DFNB1 locus.

In family A, several other loci including DFNB6 (Figure 3.9), DFNB7/11 (Figure 3.10), DFNB31 (Figure 3.11), DFNB35 (Figure 3.12-3.13), DFNB36 (Figure 3.14-3.15), DFNB37 (Figure 3.16), DFNB38 (Figure 3.17-3.18), DFNB39 (Figure 3.19), DFNB42 (Figure 3.20-3.21), DFNB43 (Figure 3.22), DFNB44 (Figure 3.23), DFNB45 (Figure 3.24), DFNB46 (Figure 3.25-3.28), DFNB47 (Figure 3.29), DFNB49 (Figure 3.30), DFNB54 (Figure 3.31) and DFNB55 (Figure 3.32-3.33) were tested for linkage. Most of these loci were mapped in previously Pakistani families showing non-syndromic hearing impairment. Analysis of the results indicates that family A was not linked to any of these loci.

In family B (Figure 3.2), initially three microsatellite markers D13S292, D13S787, D13S1275 from DFNB1 genetic interval, were used to test the linkage. From the results obtained (Figure 3.34-3.36), it was clear that affected individuals were heterozygous for different combination of parental allele thus linkage to DFNB1 locus was excluded. Exclusion to DFNB1 locus was further confirmed by sequencing the coding exon of *GJB2* gene in affected individuals. Sequence analysis showed the wild type sequence (Figure 3.37-3.38). Several other loci were tested for linkage in this family, segregating autosomal recessive hearing loss, two to three polymorphic microsatellite markers tightly linked to the known loci were genotyped. Table 2.2 summarizes microsatellite markers in the region of known deafness loci, which were used for genetic linkage in this family. Results obtained with DFNB6 (Figure 3.39-3.40), DFNB8/10 (Figure 3.41), DFNB7/11 (Figure 3.42), DFNB31 (Figure 3.43-3.44), DFNB35 (Figure 3.45), DFNB36 (Figure 3.46), DFNB37 (Figure 3.47),

DFNB38 (Figure 3.48), DFNB39 (Figure 3.49), DFNB42 (Figure 3.50), DFNB43 (Figure 3.51- 3.52), DFNB44 (Figure 3.53-3.54), DFNB45 (Figure 3.55-3.56), DFNB46 (Figure 3.57-3.62), DFNB47 (Figure 3.63-3.64), DFNB49 (Figure 3.65), DFNB54 (Figure 3.66) and DFNB55 (Figure 3.67), were conclusively excluded the family B from the linkage by the detection of heterozygote for the microsatellite markers in the affected individuals of the family.

In family C (Figure 3.3), initially two microsatellite markers D13S292 and D13S787, from DFNB1 genetic interval, were used to test the linkage. From the results obtained (Figure 3.68-3.69), it was clear that affected individuals were heterozygous for different combinations of parental alleles, thus linkage to DFNB1 locus was excluded.

In family C several other loci including DFNB6 (Figure 3.70), DFNB8/10 (Figure 3.71), DFNB7/11 (Figure 3.72-3.73), DFNB31 (Figure 3.74-3.75), DFNB35 (Figure 3.76), DFNB36 (Figure 3.77), DFNB37 (Figure 3.78-3.79), DFNB38 (Figure 3.80), DFNB39 (Figure 3.81), DFNB42 (Figure 3.82), DFNB43 (Figure 3.83), DFNB44 (Figure 3.84), DFNB45 (Figure 3.85-3.86), DFNB46 (Figure 3.87), DFNB47 (Figure 3.88), DFNB49 (Figure 3.89-3.90) and DFNB55 (Figure 3.91), were tested for linkage in family C. Analysis of the results indicate that family C was not linked to any of these loci.

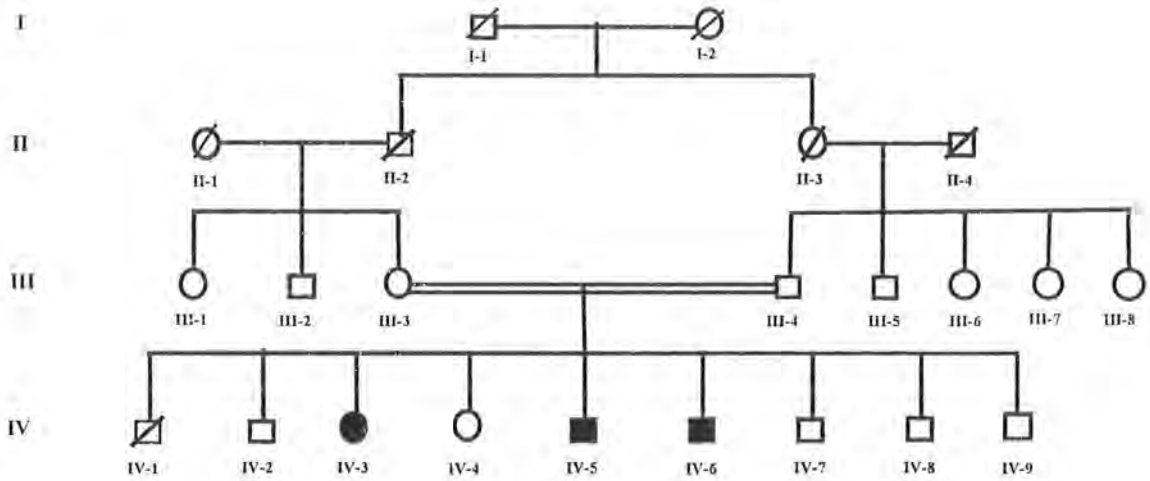


Figure 3.1: Pedigree of family **A** with non-syndromic hearing loss. Squares represent males while circles represent females. Affected individuals are represented by filled squares and circles. Double lines indicate family inter-marriages.

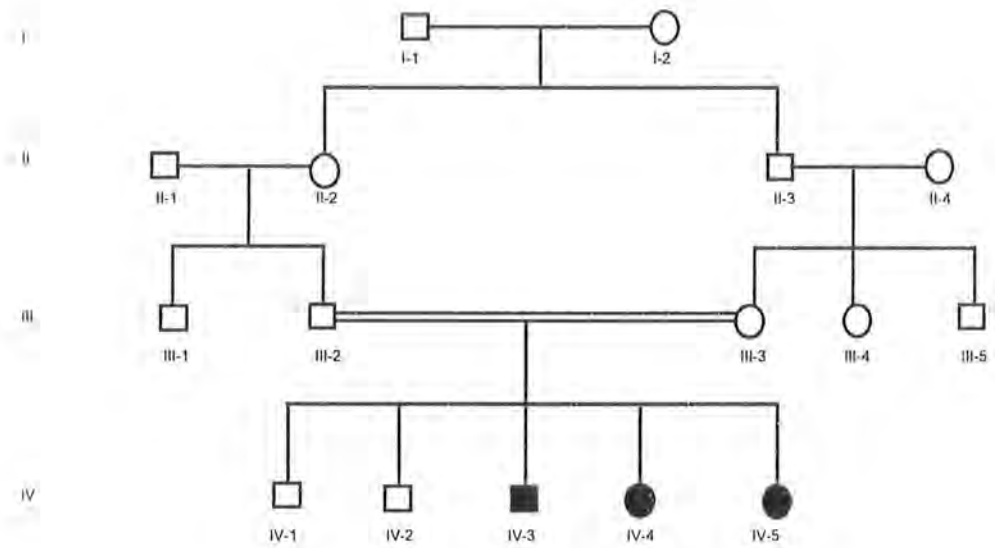


Figure 3.2: Pedigree of family **B** with non-syndromic hearing loss. Squares represent males while circles represent females. Affected individuals are represented by filled squares and circles. Double lines indicate family inter-marriages.

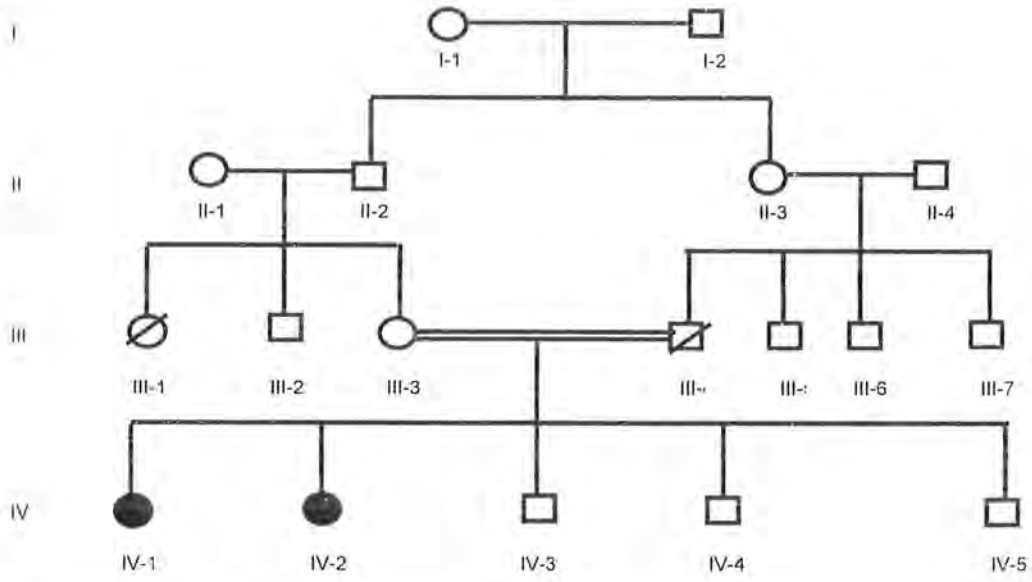
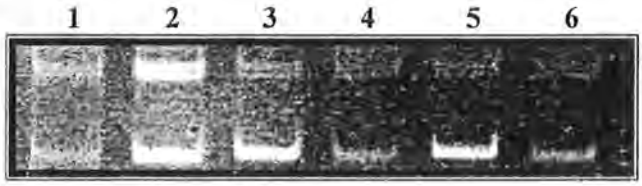


Figure 3.3: Pedigree of family **C** with non-syndromic hearing loss. Squares represent males while circles represent females. Affected individuals are represented by filled squares and circles. Double lines indicate family inter-marriages.

Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

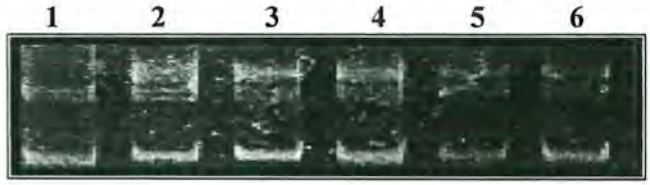
Figure 3.4: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D13S292 at DFNB1, on chromosome 13. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

Figure 3.5: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D13S787 at DFNB1, on chromosome 13. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

Figure 3.6: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D13S1275 at 6.99 cM, linked to DFNB1, on chromosome 13. The Roman with Arabic numerals refers to the individuals in the pedigree.

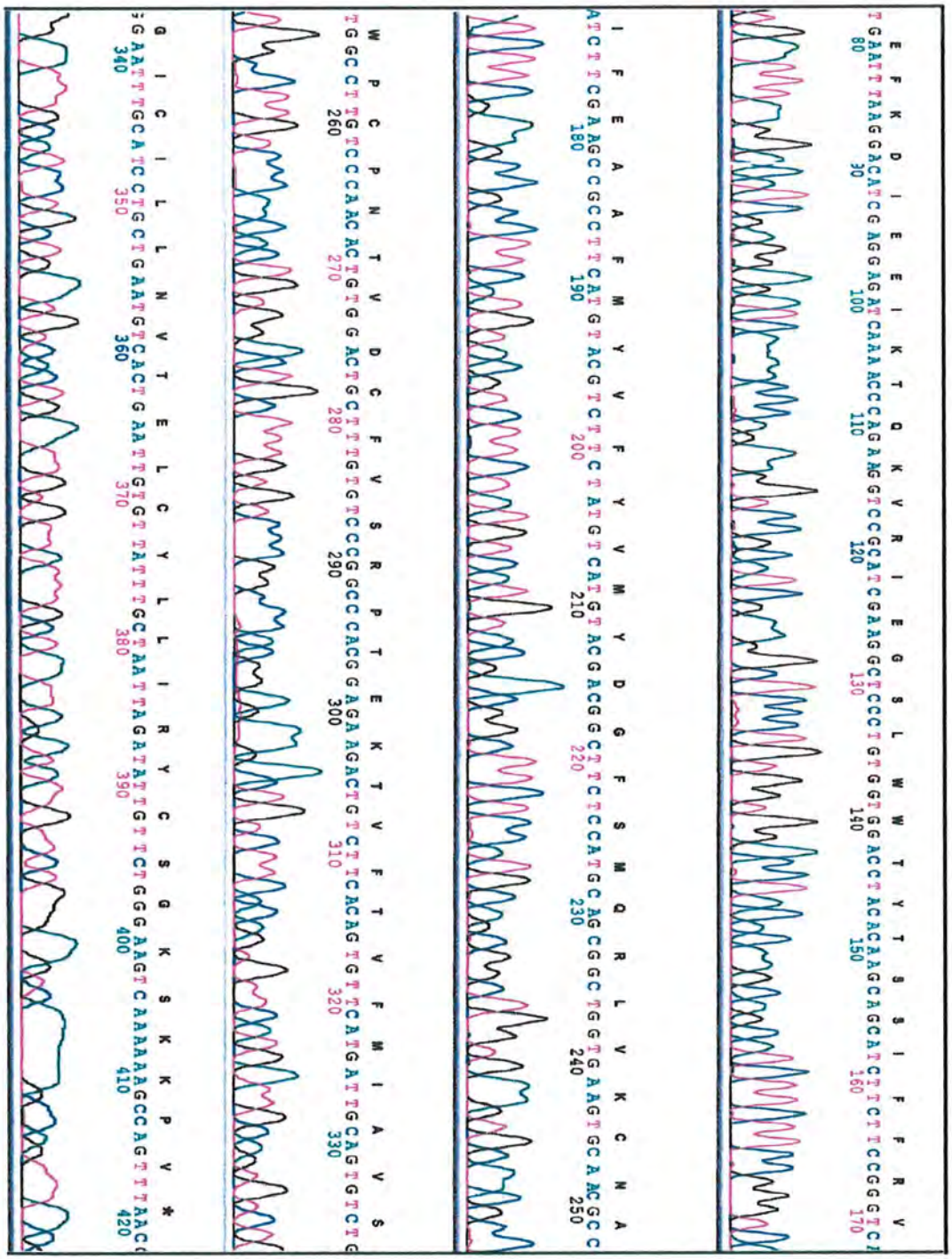
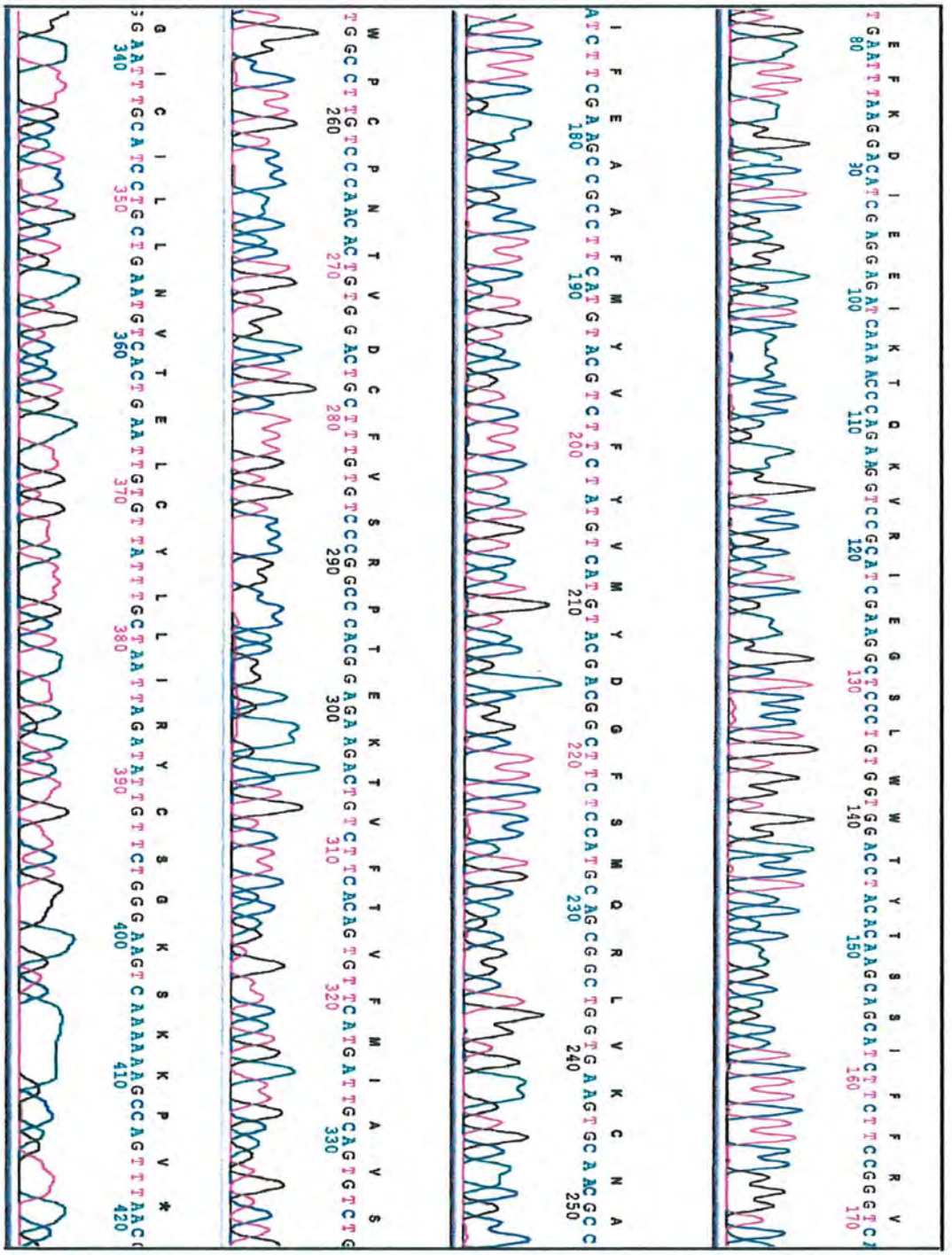
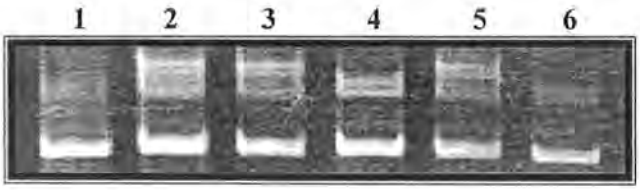


Figure 3.7 Representative electropherogram generated by Big Dye terminater, sequencing of translated exon 2 of *GJB2* gene from an affected individual (IV: 5) of family **A**

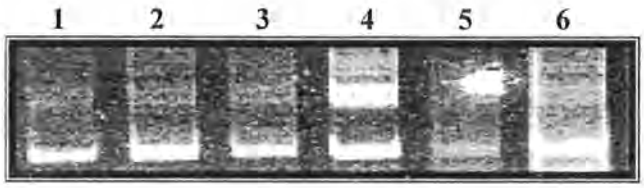




Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

Figure 3.9: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D3S1767 at 70.61 cM, linked to DFNB6, on chromosome 3. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

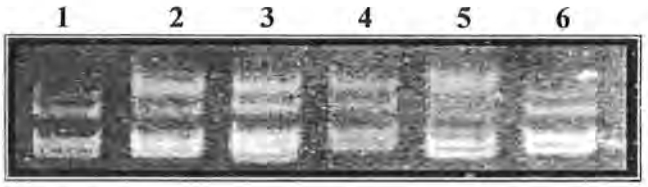
1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

Figure 3.10: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S1876 at 67.93 cM, linked to DFNB11, on chromosome 9. The Roman with Arabic numerals refers to the individuals in the pedigree.

Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

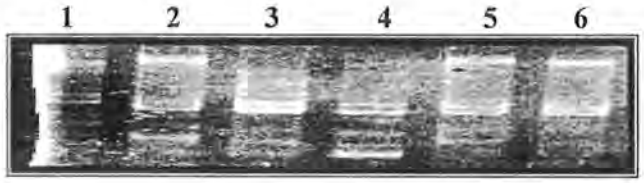
Figure 3:11 Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S302 at 120 cM, linked to DFNB31, on chromosome 9. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

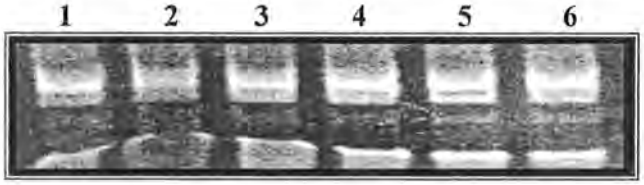
Figure 3.12: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D14S53 at 86.29 cM, linked to DFNB35, on chromosome 14. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

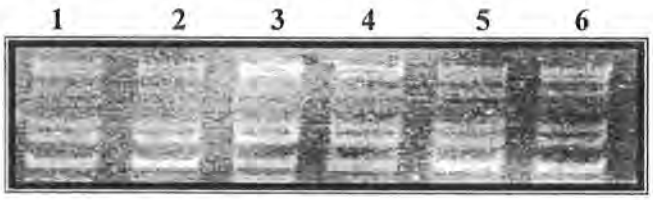
Figure 3.13: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D14S77 at 80.82 cM, linked to DFNB35, on chromosome 14. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

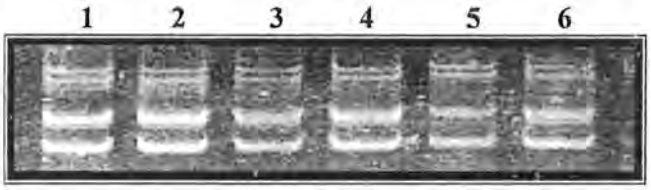
Figure 3.14: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D1S214 at 14.04 cM, linked to DFNB36, on chromosome 1. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

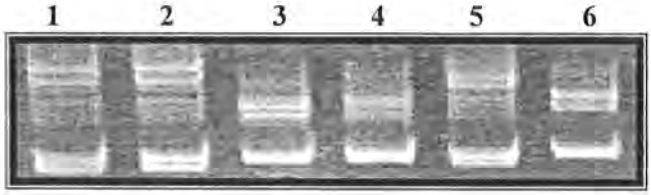
Figure 3.15: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D1S2870 at 14.04 cM, linked to DFNB36, on chromosome 1. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

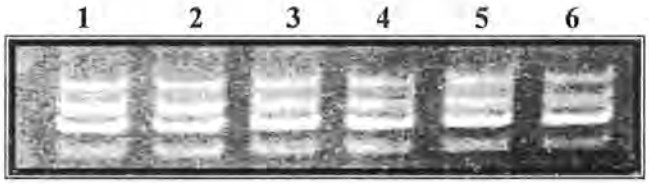
Figure 3.16: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D6S1031 at 88.63 cM, linked to DFNB37, on chromosome 6. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

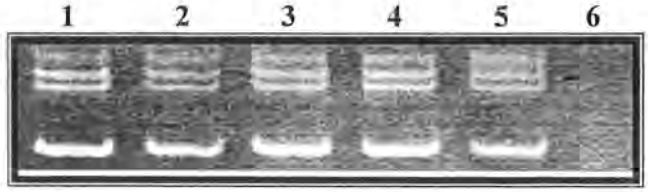
Figure 3.17: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D6S1277 at 173.31 cM, linked to DFNB38, on chromosome 6. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

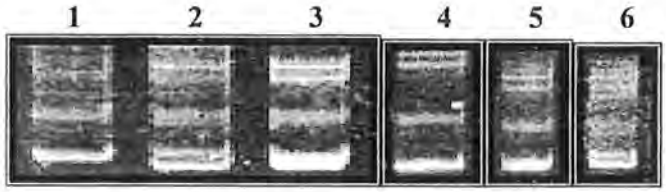
Figure 3.18: Electrophergram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D6S1599 at 169.95 cM, linked to DFNB38, on chromosome 6. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

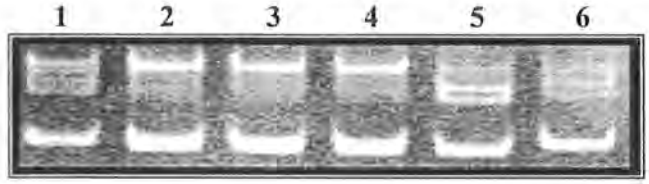
Figure 3.19: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D7S2212 at 95.43 cM, linked to DFNB39, on chromosome 7. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

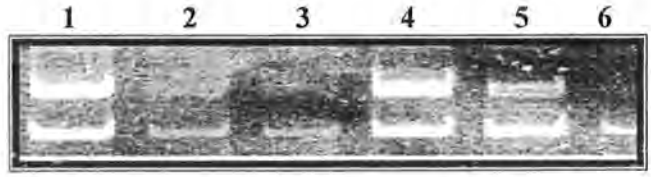
Figure 3.20: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D3S4523 at 220 cM, linked to DFNB42, on chromosome 3. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

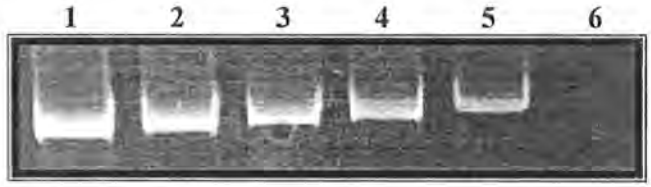
Figure 3.21: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D3S2460 at 134.60 cM, linked to DFNB42, on chromosome 3. The Roman with Arabic numerals refer to the individuals in the pedigree



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

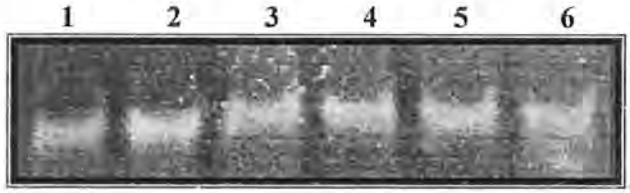
Figure 3.22: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D15S652 at 90.02 cM, linked to DFNB43, on chromosome 15. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

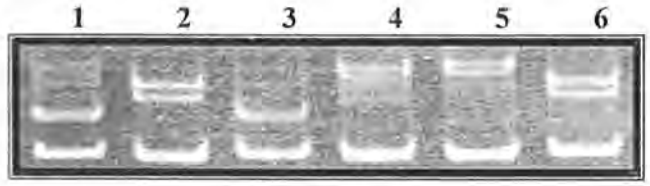
Figure 3.23: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D7S670 at 69.56 cM, linked to DFNB44, on chromosome7. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

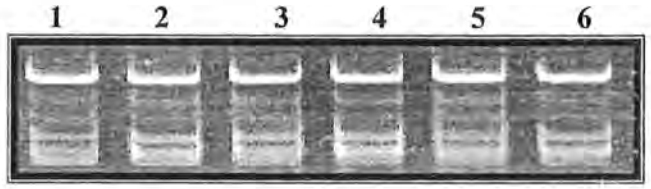
Figure 3.24: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D1S1609 at 274.53 cM, linked to DFNB45, on chromosome 1. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

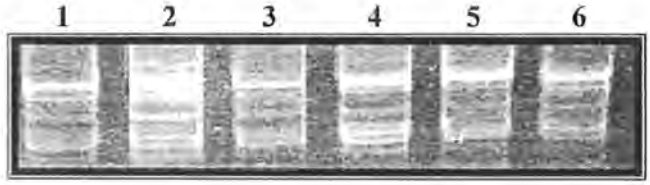
Figure 3.25: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D18S976 at 12.81 cM, linked to DFNB46, on chromosome 18. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

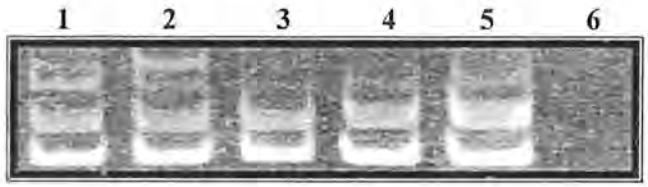
Figure 3.26: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D18S54 at 8.3 cM, linked to DFNB46, on chromosome 18. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

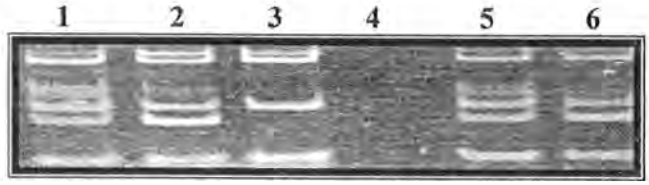
Figure 3.27: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D18S481 at 6.94 cM, linked to DFNB46, on chromosome 18. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

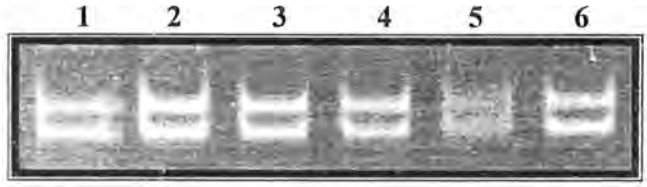
Figure 3.28: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D18S1376, linked to DFNB46, on chromosome 18. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

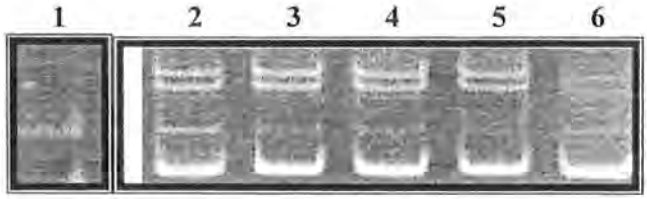
Figure 3.29: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D2S423 at 23.0cM, linked to DFNB47, on chromosome 2. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

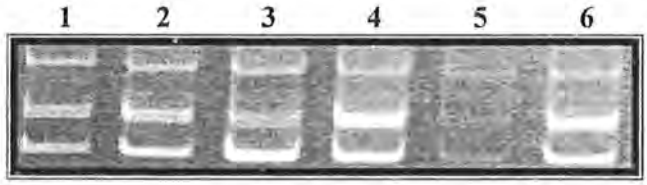
Figure 3.30: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D5S647 at 74.0cM, linked to DFNB49, on chromosome 5. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

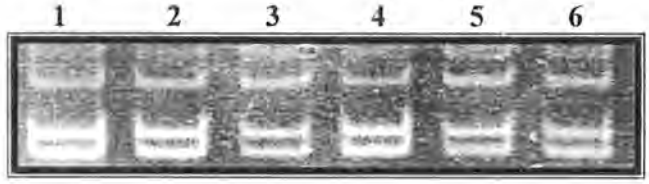
Figure 3.31: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D1S3721 at 72.59 cM, linked to DFNB54, on chromosome 1. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

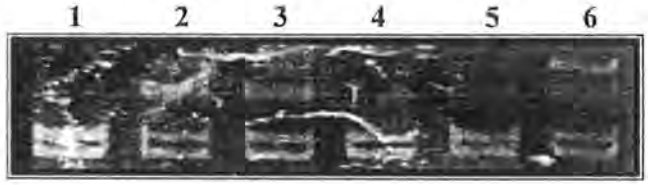
Figure 3.32: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D4S1645 at 72.52 cM, linked to DFNB55, on chromosome 4. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family A

1	IV-5	Affected
2	IV-6	Affected
3	IV-3	Affected
4	IV-7	Normal
5	III-3	Normal
6	III-4	Normal

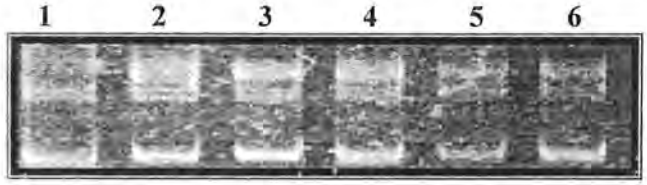
Figure 3.33: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D4S428 at 64.24 cM, linked to DFNB55, on chromosome 4. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

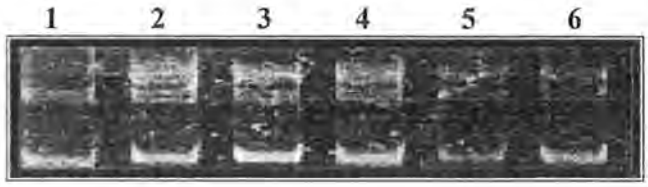
Figure 3.34: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D13S292 at 9.0 cM, linked to DFNB1, on chromosome 13. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

Figure 3.35: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D13S787 at 8.87 cM, linked to DFNB1, on chromosome 13. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

Figure 3.36: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D13S1275 at 6.99 cM, linked to DFNB1, on chromosome 13. The Roman with Arabic numerals refers to the individuals in the pedigree.

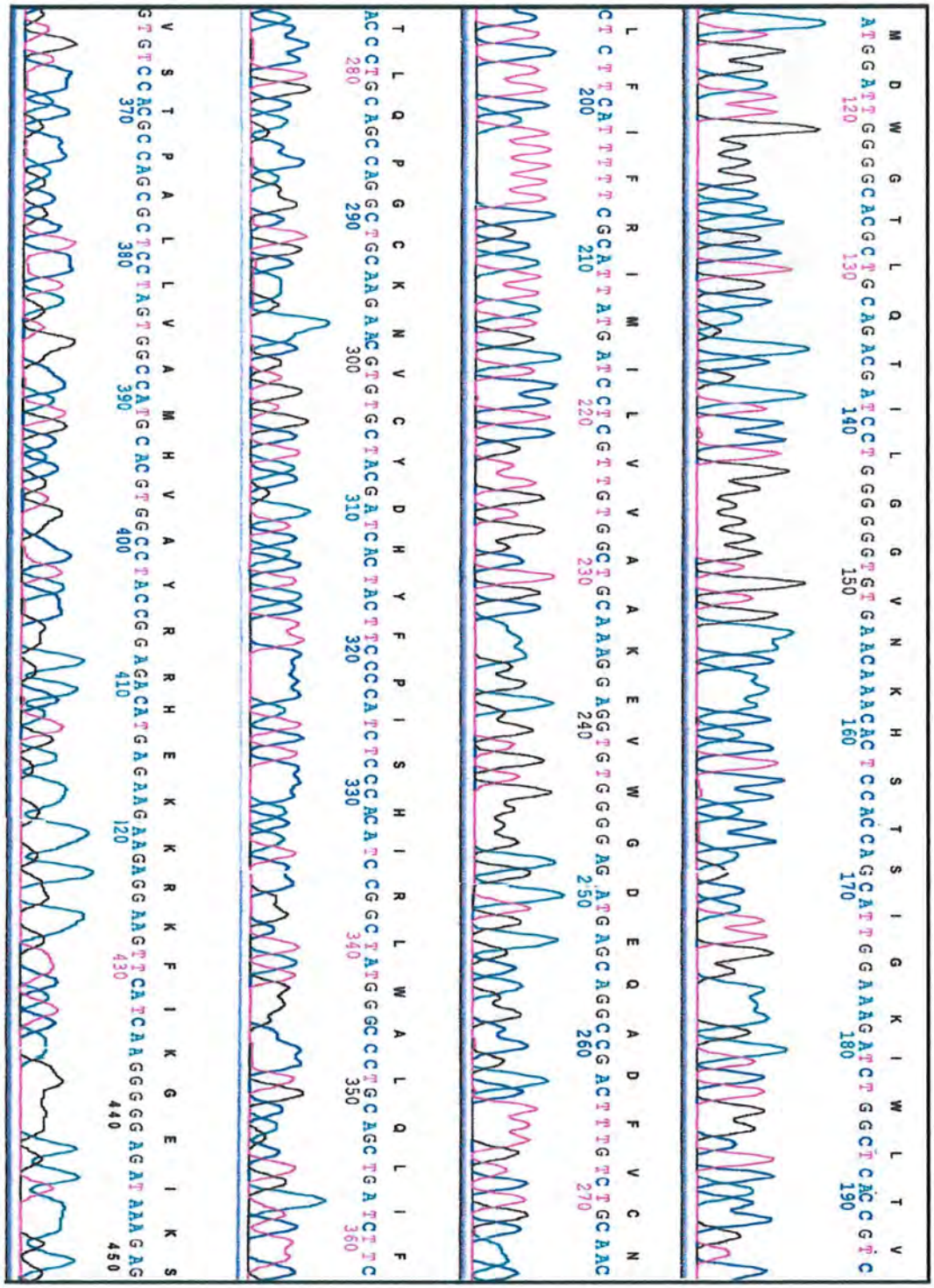
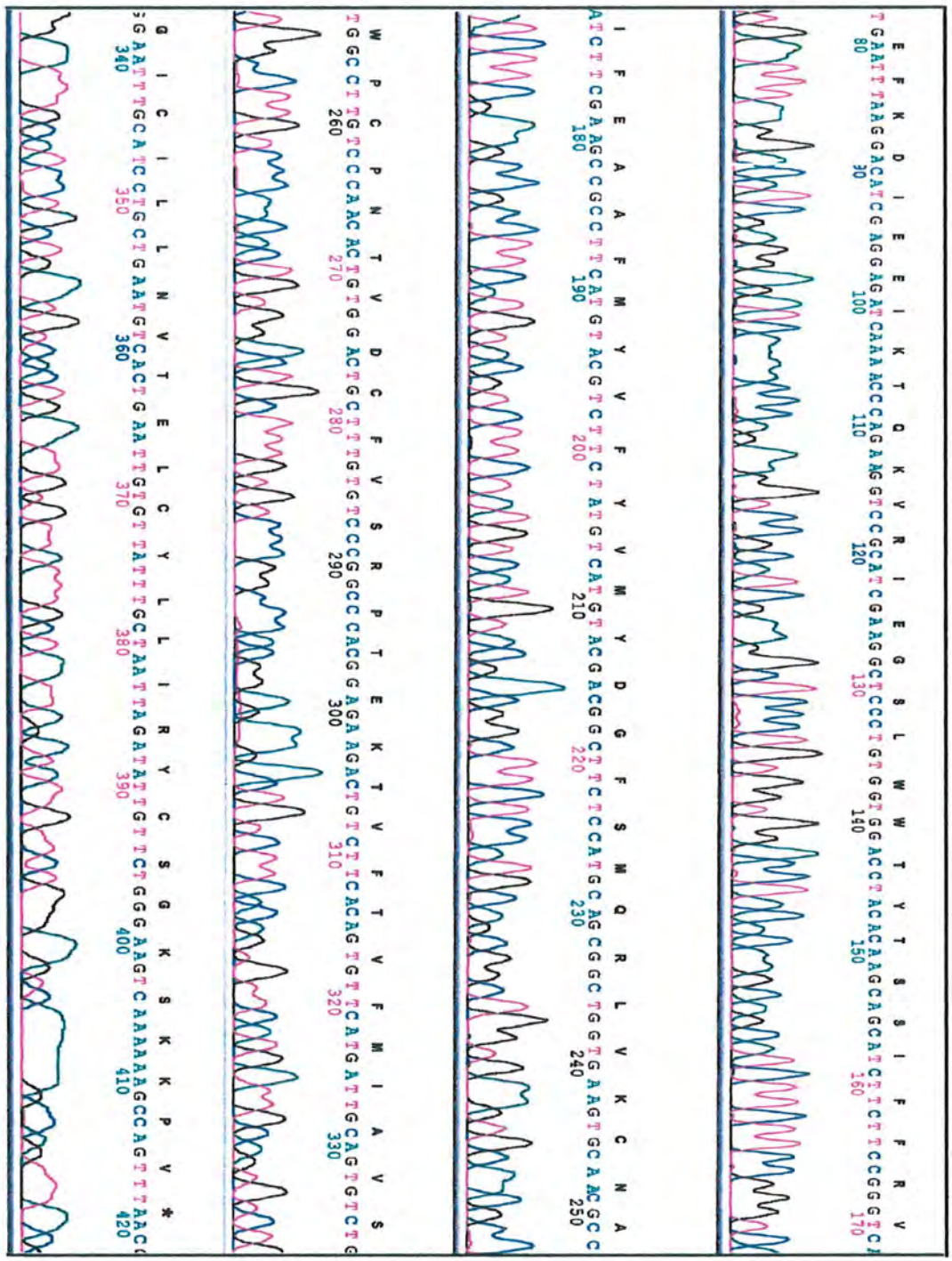
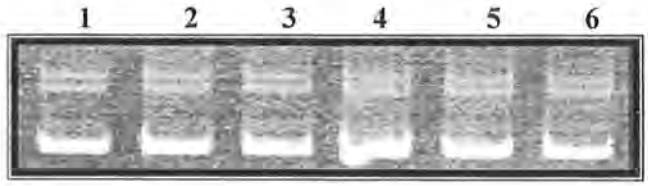


Figure 3.36 Representative electropherogram generated by Big Dye terminater, sequencing of translated exon 2 of *GJB2* gene from an affected individual (IV: 3) of family **B**

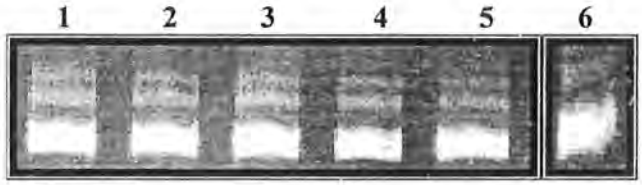




Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

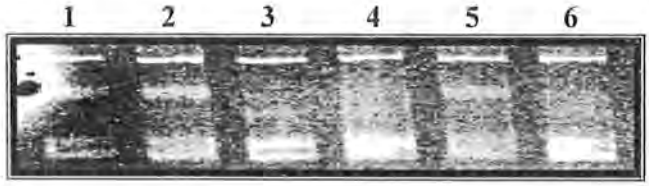
Figure 3.39: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D3S1767 at 70.67 cM, linked to DFNB6, on chromosome 3. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

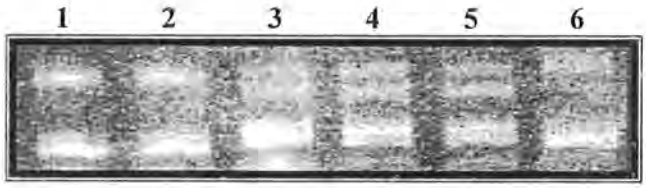
Figure 3.40: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D3S3647 at 68.47 cM, linked to DFNB6, on chromosome 3. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

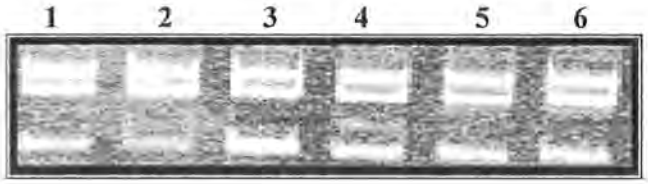
Figure 3.41: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D21S1260 at 64.71 cM, linked to DFNB8-10, on chromosome 21. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

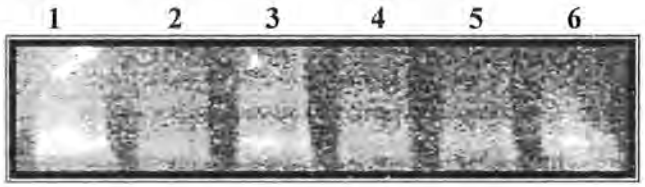
Figure 3.42: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S1876 at 67.93 cM, linked to DFNB11, on chromosome 9. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

Figure 3.43: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S1881 at 135.85 cM, linked to DFNB31, on chromosome 9. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

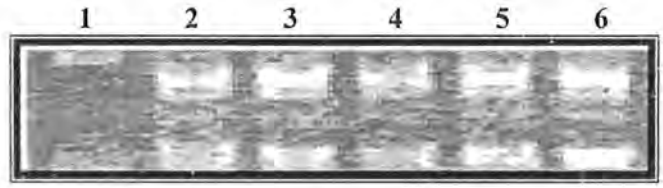
1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

Figure 3.44: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S1776 at 123.33 cM, linked to DFNB31, on chromosome 9. The Roman with Arabic numerals refers to the individuals in the pedigree.

Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

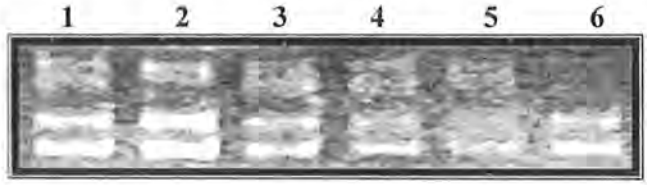
Figure 3.45: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D14S258 at 76.28 cM, linked to DFNB35, on chromosome 14. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

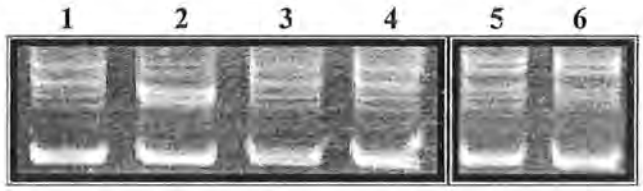
Figure 3.46: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D1S214 at 14.04 cM, linked to DFNB36, on chromosome 1. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

Figure 3.47: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D6S1031 at 88.63 cM, linked to DFNB37, on chromosome 6. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

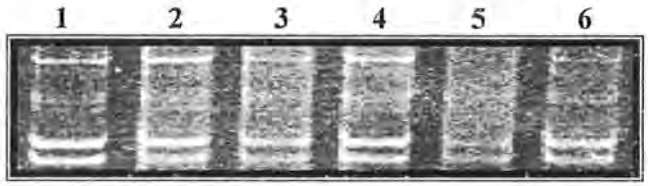
1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

Figure 3.48: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D6S1277 at 173.31 cM, linked to DFNB38, on chromosome 6. The Roman with Arabic numerals refers to the individuals in the pedigree.

Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

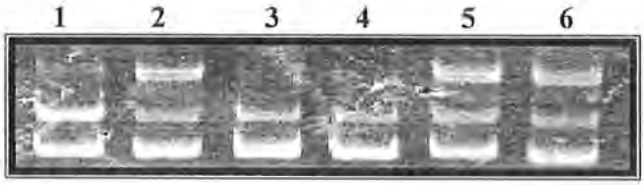
Figure 3.49: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D7S2410 at 100.81 cM, linked to DFNB39, on chromosome 7. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

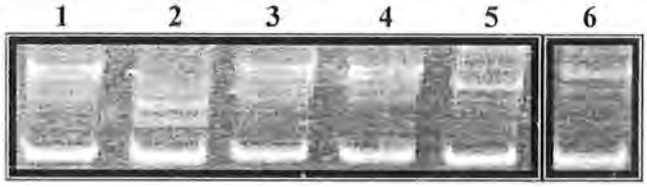
Figure 3.50: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D3S1575 at 131.83 cM, linked to DFNB42, on chromosome3. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

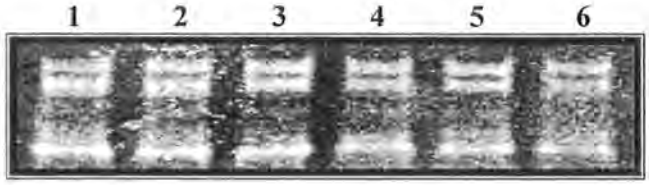
Figure 3.51: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D15S655 at 82.84 cM, linked to DFNB43, on chromosome15. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

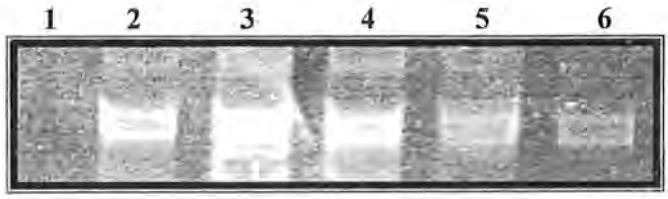
Figure 3.52: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D15S643 at 52.33 cM, linked to DFNB43, on chromosome15. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

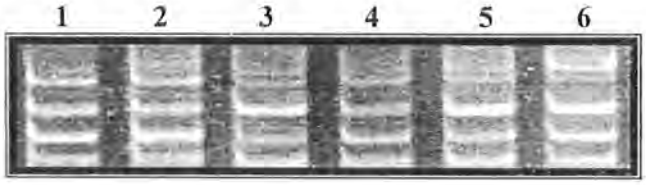
Figure 3.53: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D7S670 at 69.56 cM, linked to DFNB44, on chromosome 7. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

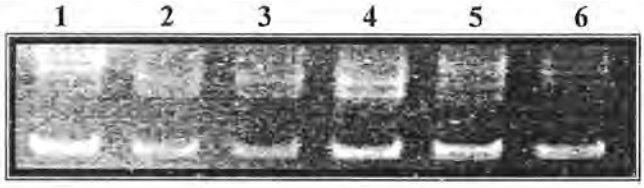
Figure 3.54: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D7S460, linked to DFNB44, on chromosome 7. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

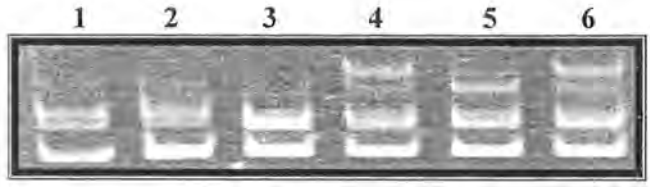
Figure 3.55: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D1S404 at 273.46 cM, linked to DFNB45, on chromosome 1. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

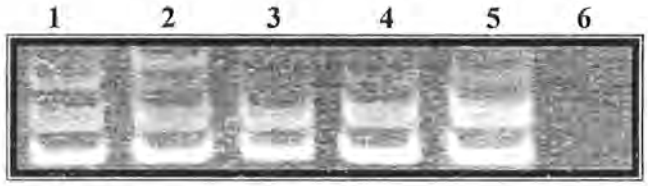
Figure 3.56: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D1S1594 at 265.49cM, linked to DFNB45, on chromosome 1. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

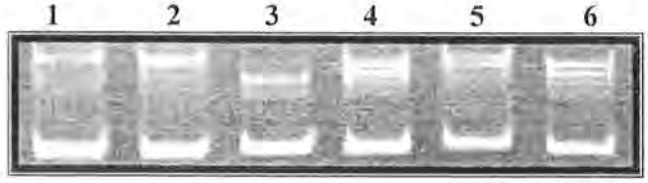
Figure 3.57: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D18S62 at 18.70 cM, linked to DFNB46, on chromosome 18. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

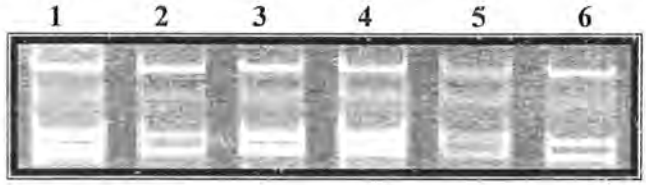
Figure 3.58: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D18S1376 at 16.54 cM, linked to DFNB46, on chromosome 18. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

Figure 3.59: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D18S976 at 12.81 cM, linked to DFNB46, on chromosome 18. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

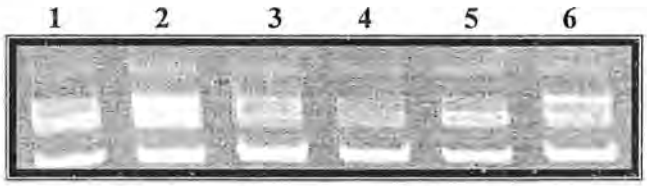
1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

Figure 3.60: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D18S54 at 8.3 cM, linked to DFNB46, on chromosome 18. The Roman with Arabic numerals refers to the individuals in the pedigree.

Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

Figure 3.61: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D18S63 at 8.3 cM, linked to DFNB46, on chromosome 18. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

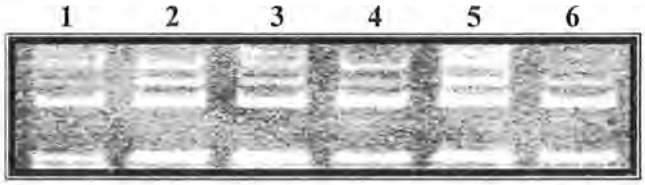
1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

Figure 3.62: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D18S452, linked to DFNB46, on chromosome 18. The Roman with Arabic numerals refers to the individuals in the pedigree.

Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

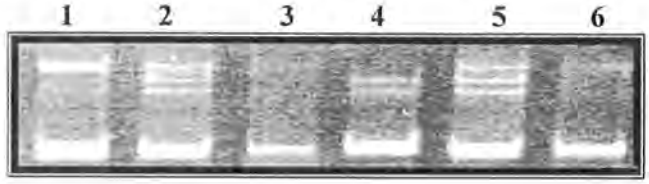
Figure 3.63: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D2S1400 at 27.6 cM, linked to DFNB47, on chromosome 2. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

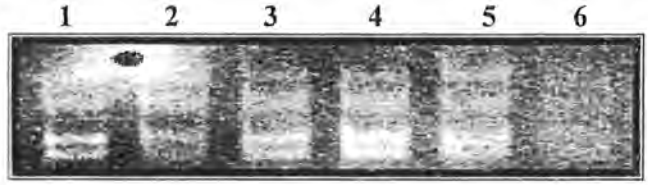
Figure 3.64: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D2S423 at 23.0 cM, linked to DFNB47, on chromosome 2. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

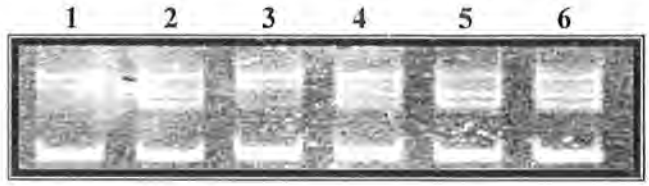
Figure 3.65: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D5S2500 at 69.23 cM, linked to DFNB49, on chromosome 5. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

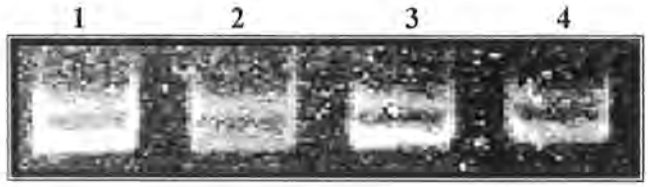
Figure 3.66: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D1S193 at 73.21cM, linked to DFNB54, on chromosome 1. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family B

1	IV-3	Affected
2	IV-4	Affected
3	IV-5	Affected
4	IV-1	Normal
5	III-2	Normal
6	III-3	Normal

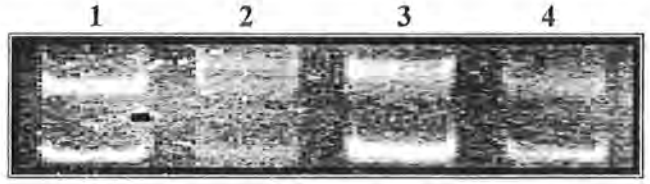
Figure 3.67: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D4S1645 at 72.52 cM, linked to DFNB55, on chromosome 4. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family C

1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

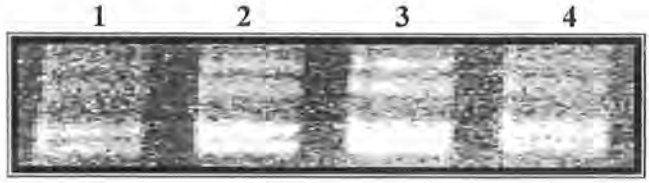
Figure 3.68: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D13S292 at 9.0 cM, linked to DFNB1, on chromosome 13. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family C

1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

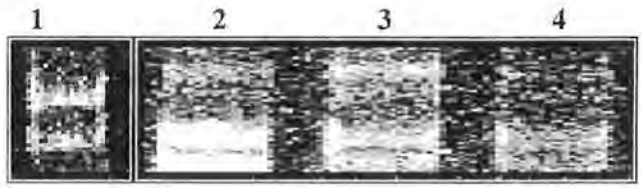
Figure 3.69: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D13S787 at 8.87 cM, linked to DFNB1, on chromosome 13. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family C

1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

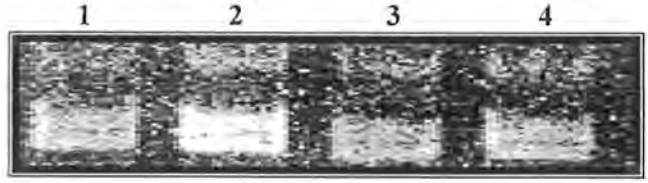
Figure 3.70: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D3S647 at 68.47 cM, linked to DFNB6, on chromosome 3. The Roman with Arabic numerals refers to the individuals in the pedigree.



]Family C

1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

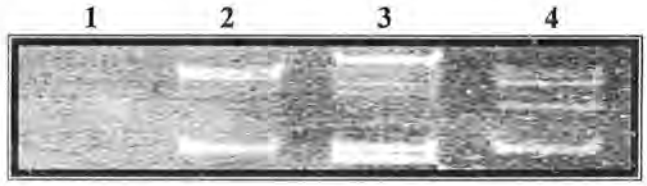
Figure 3.71: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D2S1260 at 46.71cM, linked to DFNB8-10, on chromosome 2. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family C

1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

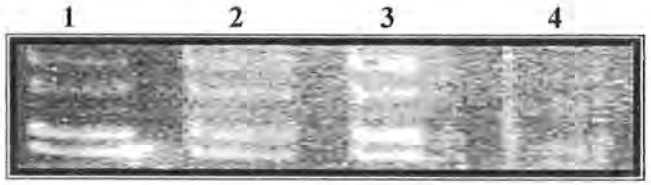
Figure 3.72: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S1876 at 67.93 cM, linked to DFNB11, on chromosome 9. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family C

1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

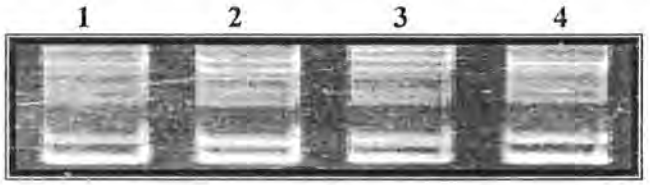
Figure 3.73: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S301 at 66.32 cM, linked to DFNB11, on chromosome 9. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family C

1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

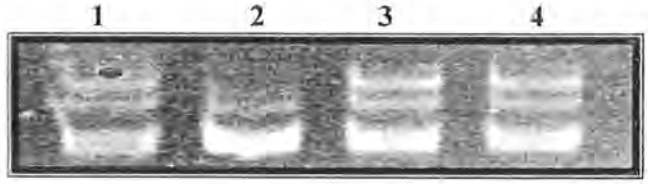
Figure 3.74: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S1881 at 135.85 cM, linked to DFNB31, on chromosome 9. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family C

1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

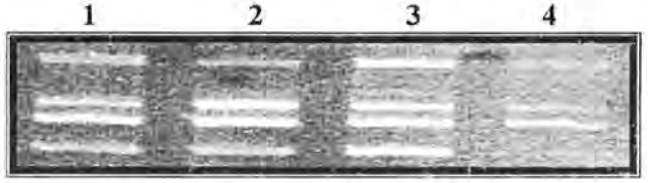
Figure 3.75: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S302 at 120 cM, linked to DFNB31, on chromosome 9. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family C

1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

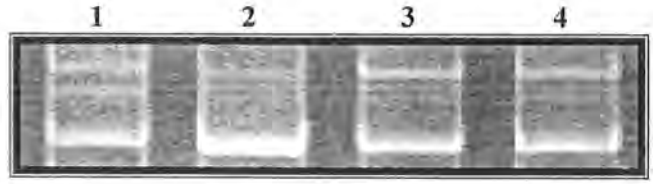
Figure 3.76: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D14S53 at 86.29 cM, linked to DFNB35, on chromosome 14. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family C

1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

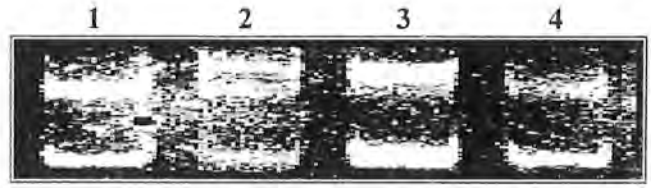
Figure 3.77: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D1S2870 at 14.04 cM, linked to DFNB36, on chromosome 1. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family C

1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

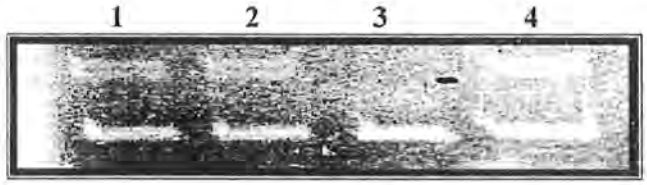
Figure 3.78: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D6S16590 at 88.63 cM, linked to DFNB37, on chromosome6. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family C

1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

Figure 3.79: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D6S1031 at 88.63 cM, linked to DFNB37, on chromosome6. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family C

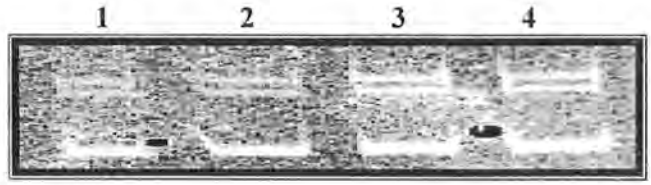
1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

Figure 3.80: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D6S1599 at 173.31 cM, linked to DFNB38, on chromosome 6. The Roman with Arabic numerals refers to the individuals in the pedigree.

Family C

1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

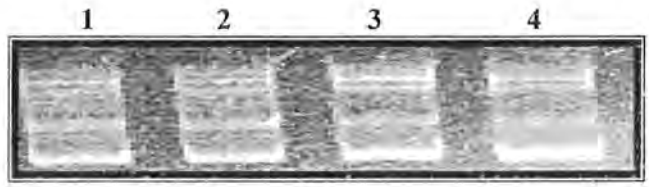
Figure 3.81: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D7S2212 at 95.43 cM, linked to DFNB39, on chromosome 7. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family C

1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

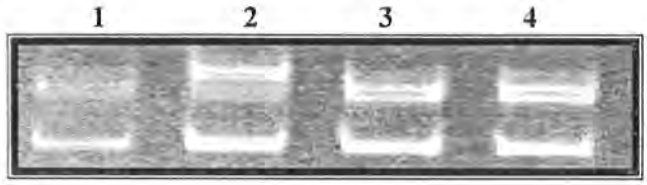
Figure 3.82: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D3S4523 at 135.85 cM, linked to DFNB42, on chromosome 3. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family C

1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

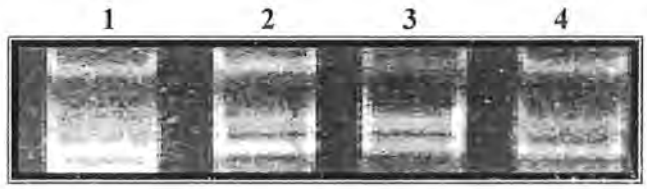
Figure 3.83: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D15S652 at 90.0 cM, linked to DFNB43, on chromosome 15. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family C

1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

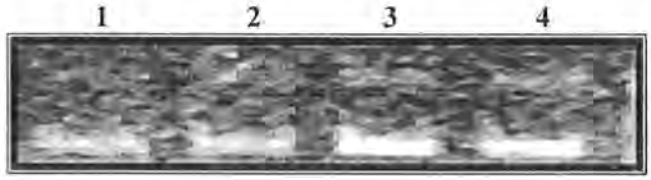
Figure 3.84: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D7S1818 at 69.56 cM, linked to DFNB44, on chromosome 7. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family C

1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

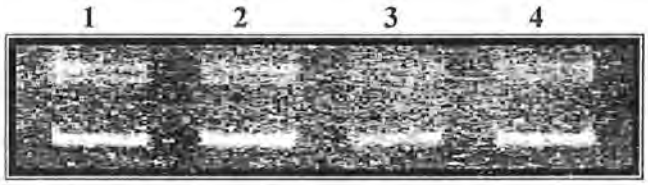
Figure 3.85: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D1S404 at 273.86 cM, linked to DFNB45, on chromosome 1. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family C

1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

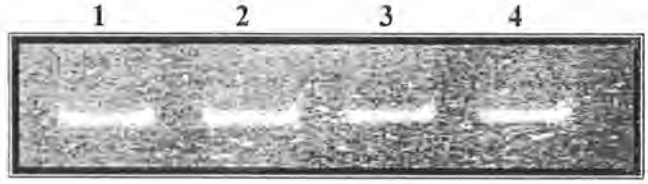
Figure 3.86: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D1S1594 at 265.49 cM, linked to DFNB45, on chromosome 1. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family C

1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

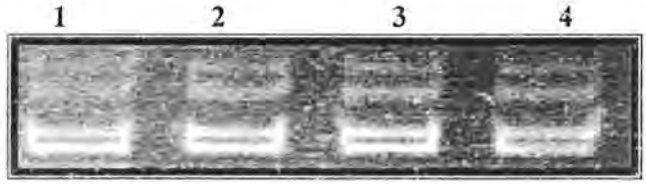
Figure 3.87: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D18S1376 at 16.54 cM, linked to DFNB46, on chromosome 18. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family C

1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

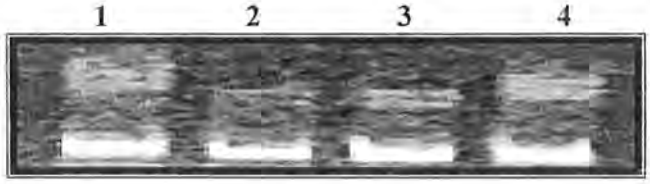
Figure 3.88: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D2S1400 at 27.6 cM, linked to DFNB47, on chromosome 2. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family C

1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

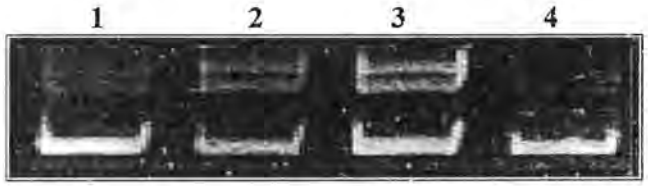
Figure 3.89: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D5S647 at 74.07 cM, linked to DFNB49, on chromosome 5. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family C

1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

Figure 3.90: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D5S2500 at 69.23 cM, linked to DFNB49, on chromosome 5. The Roman with Arabic numerals refers to the individuals in the pedigree.



Family C

1	IV-1	Affected
2	IV-2	Affected
3	IV-4	Normal
4	III-3	Normal

Figure 3.91: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D4S1645 at 72.52 cM, linked to DFNB55, on chromosome 4. The Roman with Arabic numerals refers to the

Individuals in the pedigree.

DISCUSSION

DISCUSSION

The successful mapping of the human genome has created an explosion of clinically relevant knowledge that continues to expand as the function of our 35,000 or more genes are being identified. Geneticists can play a major role in the management of infants with newly detected hearing loss by facilitating the establishment of an etiologic diagnosis.

In general, deafness differs from many other "complex" genetic diseases in that the hearing loss usually results from abnormalities in single gene or gene pairs. However, some forms of deafness are beginning to be recognized in which the hearing loss may result from the combined effects of genes at two or more loci. The fact that the same mutation leads to different clinical presentations may be the indication that the knowledge of molecular genetics has not reached the details of auditory dynamics yet, as well as the myriad of neurological abnormalities involved. However, new mutations are described, new genes are cloned and mapped. To date 68 autosomal recessive deafness loci (DFNB1-DFNB68) have been mapped and 37 of the corresponding genes have been identified. The phenotype in autosomal recessive non-syndromic hearing loss is remarkably similar, profound sensorineural hearing loss across all frequencies. Severe to profound hearing impairment with onset before 12 months of age (prelingual) is a characteristic of the affected individuals of most of the families for which gene causing recessive hearing impairment have been localized. Exception of DFNB2 (*MYO7A*), DFNB8/10 (*TMPRSS3*), DFNB4 (*STRC)* in which the age of onset may occur in later phases of childhood.

In the present study, three highly consanguineous families (A, B, C), demonstrating autosomal recessive form of non-syndromic deafness, were ascertained from Hyderabad district in the province of Sindh. The affected individuals in the families had prelingual severe to profound hearing loss with no associated features of syndromic or acquired form of deafness. The affected individuals from various age groups showed the same level of severe hearing loss implying that deafness was not progressive in any of the families studied.

To search for loci that harbored the candidate genes, responsible for autosomal recessive non-syndromic hearing loss in these families, linkage studies were performed by a method known as homozygosity mapping. The hereditary nature of non-syndromic

deafness was first reported in the sixteenth century by Johannes Schenck (Stephens, 1985). In 1621, the papal physicians Zacchias recommended deaf people not to marry because of the risk of having deaf children (Cranefield and Federn, 1970). Nearly a century ago Sir Archilbad Garrod (1908) noted that a large proportion of patients with an autosomal recessive inborn error of metabolism termed alkaptonuria were the offspring of consanguineous unions. Smith (1953) observed that offspring of consanguineous matings would be homozygous for genetic markers near the disease gene. A recessive trait could be mapped using offspring of the consanguineous matings by homozygosity mapping. A fraction of genome of offspring of consanguineous matings would be expected to be homozygous because of identity by descent (Lander and Botstein, 1987). In multiplex families, in particular parental consanguinity provides a high degree of assurance that affected children will be homozygous by descent (HBD) over a genomic region that include the autosomal recessive non-syndromic hearing loss (ARNSHL) gene. On average, 1/16th of the genome of offspring of first cousin matings would be expected to be homozygous. The region of homozygosity would be expected to be random between different offspring of these matings, except a common disease locus shared by affected offspring. Thus, the use of offspring from several first cousin matings can be used to identify markers linked to a recessive disorder. To establish a linkage, only four affected sibs in first cousin marriage or three affected sibs in the second cousin marriage are required. Miano *et al.* (2000) have indicated potential problems encountered during linkage studies performed by homozygosity mapping:

1. Unexpected allelic heterogeneity, causing region containing the disease locus to be missed.

2. Identification of homozygosity identical by descent (IBD) region unrelated to disease locus.

3. Potential for inflation of LOD scores as a result of underestimation of the extent of inbreeding increasing the chance of false positive linkage.

An obvious factor in the use of homozygosity mapping is the problem created by genetic heterogeneity. Various investigators have, in practice, overcome this complication by the

use of isolated inbred populations to identify large inbred marker density of screening set and their heterozygosity. For markers with 70% heterozygosity, a homozygous segment as short as 9 cM may be detected when the markers are 1 cM apart.

In the present study, family A, was tested for mapping to several known autosomal recessive non-syndromic loci by using polymorphic microsatellite markers from their candidate linkage intervals. Two to three markers per locus were used to genotype three affected individuals of family 'A'. Linkage to DFNB1 locus was also checked by sequencing the codon region of exon 2 of *GJB2* gene. Electropherogram obtained by genotyping the markers revealed that the affected individuals were heterozygous for different combinations of parental alleles, thus indicating exclusion of family 'A' from linkage to known autosomal recessive non-syndromic hearing loss loci.

In family B, DFNB1 locus was excluded from linkage by genotyping markers mapped within DFNB1 locus and sequences analysis of coding exon of *GJB2* gene. This indicated presence of a causative gene other than *GJB2,* responsible for deafness, in this family. Therefore, family 'B' was tested for linkage to several other known deafness loci. However, no evidence for linkage to these loci was found. Electropherogram obtained by genotyping the markers revealed that the affected individuals were heterozygous for different combinations of parental alleles, thus indicating exclusion of family 'B' from linkage to several known autosomal recessive non-syndromic hearing loss loci.

Family C, was too tested for linkage to DFNB1 and 18 other known deafness loci. However, no evidence for linkage to these loci was found. Electropherogram obtained by genotyping the markers revealed that the affected individuals were heterozygous for different combinations of parental alleles, thus indicating exclusion of family 'C' from linkage to 18 of the known autosomal recessive non-syndromic hearing loss loci.

Since all the three families, presented here, failed to show linkage to known deafness loci, therefore, future studies involving these families should include testing of linkage to other known loci and, if required, scanning of the total genome to identify the disease causing deafness loci.

Despite the recent success in discovering hearing loss genes, the vast majority of NSHL genes remain to be identified. This is particularly due to the fact that many families in which NSHL segregates are small, with an insufficient number of informative recombination events to allow narrowing of the genetic interval where NSHL gene maps. Thus in many cases, several megabases of genomic DNA must be analyzed to identify candidate genes for each of the NSHL loci. The rate of discovery of deafness genes by positional cloning in human will be accelerated by the freely available human genome sequence and by a catalogue of Expressed Sequence Tags (ESTs) within genetic intervals known to contain locus for human hereditary hearing loss. To assist in the identification of deafness genes cDNA library has been synthesized, partially sequenced and many ESTs assigned map position (Skvorak *et al.*, 1999).

Although significant advances have been made, it is clear that more genes and mutations await discovery. Information about these genes and their protein products is revolutionizing our knowledge of the molecular processes involved in hearing and enhancing our understanding of how alteration of these processes can lead to hearing loss. This knowledge may lead mutation-specific therapies that can delay or prevent certain forms of genetic deafness such as the avoidance of aminoglycoside therapy in those with specific mitochondrial mutations.

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Electronic Database Information

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- Hereditary hearing loss homepage http://www.uia.ac.be/dnalab/hhh
- Marshfield Medical Center www.marshmed.org.gov/genetics/
- National center for biotechnology information http://www.ncbi.nlm.nih.gov/