Genetic Mapping of Candidates of Non-Syndromic Deafness Genes

By

Syed Irfan Raza

Department of Biological Sciences
Quaid-i-Azam University
Islamabad

2006

Genetic Mapping of Candidates of Non-Syndromic Deafness Genes

A thesis submitted in the partial fulfilment of
the requirement for the degree of
Master of Philosophy

in

(Biochemistry/Molecular Biology)

By

Syed Irfan Raza

Department of Biological Sciences
Quaid-i-Azam University
Islamabad

CERTIFICATE

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

Supervisor: _____
Dr. Wasim Ahmad

External Examiner: _____

Chairperson: _____

Date: _____, **2006**

IN THE NAME OF

ALLAH,

THE MOST BENEVOLENT, EVER MERCIFUL.

He (Allah the Almighty) taught (man) the use of the Pen (the basic implement in the field of knowledge) and taught man which he knew not (a divine invitation for research and advancement of knowledge so as to lead the life in conformity and obedience to Divine Pleasure)."

(Al –Quran)

DEDICATION

Dedicated this effort to my beloved Brother in-law (Late) and Baji for their endless love

CONTENTS

- **Acknowledgements** I
- **List of Abbreviations** II
- **List of Figures** III-VII
- **List of Tables** VIII
- **Abstract** IX

INTRODUCTION

- Types of Hearing Loss 1
- Mode of Inheritance and Genetical Etiology 2
- Prevalence 2
- Clinical Diagnosis of Sensorineural Hearing Loss 3
- Audiometry 3
- Gene-Localization Studies 4
- Nuclear Gene 4
- Cadherins related 23 (*CDH23*) 9
- Transmembrane Channel like Gene 1 (*TMC1*) 10
- Transmembrane protease, serine 3 (*TMPRSS3*) 11
- *ESPIN* 11
- Gap Junctions 11
- Gap Junction Protein Beta 2, 26kDa (*connexin 26*) 12
- Gap Junction Protein Beta 3 –GJB3 (*Cx31*) 13
- Gap Junction Protein, Beta 6 GJB6 (*connexin 30*) 13
- Otoanchorin (*OTOA*) 13
- Otoferlin (*OTOF*) 14
- Solute Carrier Family 26, Member 4 (*SLC26A4*) 14
- Myosin VII (*MYO7A*) 14
- Diaphanous 15

- Myosin XVA (*MYO15*) 15
- Cochlin (*COCH*) 15
- Transmembrane Inner Ear Protein (*TMIE*) 16
- KCNQ4 16
- POU Domain, Class 3, Transcription Factor 4 (*POU3F4*) 17

MATERIALS AND METHODS

- Families Studied 18
- Blood Sampling 18
- Extraction and Purification of Genomic DNA from Blood 18
  - Organic Preparation Using 1.5 ml Microcentrifuge Tubes 18
  - DNA Extraction by Commercially available Kit 19
- DNA Dilution and Micropipetting 20
- Polymerase Chain Reaction (PCR) 20
- Horizontal Gel Electrophoresis 20
- Vertical Gel Electrophoresis 21
- Genotyping and Primer Database Analysis 22

  Linkage Studies

  - Linkage to DFNB1 locus (*Connexin 26*) 22
  - Linkage to Known DFNB loci 22
  - Mutation Screening in *GJB2* gene 23
- Mutation Screening in *MYO15* gene 23
- DNA Sequencing 24

RESULTS

Families Studied

- Family A 30
- Family B 30
- Family C 31
- Family D 31
- Genetic Mapping of Candidate of Hearing Impairment Genes 31

DISCUSSION 59-61

REFERENCES 62-73

ACKNOWLEDGEMENTS

Praise be to Thee! We do not know anything except what thou hast made known to us: indeed Thou art the best Knower, the Wisest (Al-Quran).

At last the efforts have borne fruit and my work gets completed. It is a matter of pleasure for me. But the cause of honor for me is my supervisor, Dr. Wasim Ahmad Associate Professor, Department of Biological Sciences, Quaid-i-Azam University, Islamabad. He is the one who has brought life into this work with his passion and utmost devotion. He has been with me with his heart and soul at each moment of entire duration of the work. Whenever I needed him, he was already there for me with a tangible sense of patronage. I may not be able to pay my gratitude to him for the rest of my life because that seems finite and the things I had for him mean an eternity to me.

The credit goes to Professor Dr. Samina Jalali, Chairperson, Department of Biological Sciences, Quaid-i-Azam University Islamabad. She is the one who played the part of back bone, not exclusively in my work but all kind of ongoing productive activities in the department. Without her support this work would have taken a lot of time and still be incomplete. I am thankful to her.

My sincere regard and thanks are overdue to Sir Saeed-ul-Haque for his constructive suggestions and technical guidance.

I am thankful to all my worthy colleagues Mr. Jawad Hassan, Mr. Peter John, Mr. Muhammad Salman Chishti, Mr. Ghazanfar Ali, Mr. M. Naeem, Miss Saba Irshad, Miss Attya Bhatti, Miss Shaheen Skindar, Mr. Musharraf Jeelani, Mr. Sabir Hussain, Mr. Zahid Azeem, Mr. Mohsin Shah, Miss Nazish Anjum, Miss Iram Fatima, Miss Iram Afshan, Miss Aroob Sultana and Miss Samreen, who bestowed me with their fruitful and valuable suggestions.

My humble and heart felt gratitude is reserved for my beloved parents. Without their prayers, support and encouragement, the completion of this study task would have been a dream.

Syed Irfan Raza

LIST OF ABBREVIATIONS

AHL	Age Related Hearing Loss
ARNSHL	Autosomal Recessive Non-Syndromic Hearing Loss
BOA	Behavioral Observation Audiometry
Bth	Beethoven
CDH23	Cadherins23
CHLC	Cooperative Human Linkage Centre
cM	Centimorgan
COCH	Cochlin
dB	Decibels
DDB	DNA Dissolving Buffer
DFN	Deafness
DFNA	Autosomal Dominant Non-Syndromic Deafness
DFNB	Autosomal Recessive Non-Syndromic Deafness
dn	Deafness
EDTA	Ethylenediaminetetraacetic acid
GJB	Gap Junction Beta
HIH	Hereditary Impaired Hearing
HL	Hearing Loss
Mdfw	Modifier of Deafwaddler
MYO	Myosine
NSRD	Non Syndromic Autosomal Recessive Deafness
OTOA	Otoanchorin
OTOF	Otoferlin
SLC	Solute Carrier Family
SNHL	Sensori Neural Hearing Loss
STR	Short Tandem Repeat
TBE	Tris-Borate EDTA
TMC1	Transmembrane Channel-like 1
TMIE	Transmembrane Inner Ear
TMPRSS3	Transmembrane Protease, Serine-3
TSR	Template Suppression Reagent
VRA	Visual Reinforcement Audiometry

LIST OF FIGURES

Figure No.	Title	Page No.
3.1	Pedigree of the family A with autosomal recessive non-syndromic hearing loss. Circles represent females, squares represent males. Filled circles and squares represent affected individuals.	34
3.2	Pedigree of the family B with autosomal recessive non-syndromic hearing loss. Circles represent females, squares represent males. Filled circles and squares represent affected individuals.	35
3.3	Pedigree of the family C with autosomal recessive non-syndromic hearing loss. Circles represent females, squares represent males. Filled circles and squares represent affected individuals.	36
3.4	Pedigree of the family D with autosomal recessive non-syndromic hearing loss. Circles represent females, squares represent males. Filled circles and squares represent affected individuals.	37
3.5	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. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their position within a generation.	38
3.6	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. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.	38
3.7	Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D13S292 at 8.87 cM linked to DFNB1. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.	39
3.8	A representative chromatogram generated by big dye terminator sequencing of translated exon 2 of *GJB2* gene from control individual (**a**), a homozygous affected male IV-5 (**b**), and a heterozygous carrier (**c**). Arrow indicates the substitution of G with A, at nucleotide position 71, leading to non-sense mutation (W24X).	40
3.9	Representative chromatogram generated by big Dye Terminator; sequencing of the translated exon 2 of *GJB2* gene from an affected male individual (IV-2) of family B, showing wild type sequence.	41-42
3.10	Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S302 at 123.33 cM linked to DFNB31. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.	43

3.11 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. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation. 43

3.12 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. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation. 44

3.13 Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D5S2042 at 78.31 cM linked to DFNB49. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation. 44

3.14 Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D5S1501 at 85.25 cM linked to DFNB49. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation. 45

3.15 Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D17S1294 at 50.70 cM linked to DFNB3. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation. 45

3.16 Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D17S1293 at 56.58 cM linked to DFNB3. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation. 46

3.17 A representative chromatogram generated by big dye terminator sequencing of exon 3 of *MYO15A* gene from an affected female individual (IV-4) of family C showing wild type sequence. Arrow indicates a nucleotide C at position 3685, which has been substituted with T, resulting in a non-sense mutation (Wang *et al.*, 1998). 47

3.18 A representative chromatograms generated by big dye terminator, sequencing of exon 29 of *MYO15A* gene from an affected female individual (IV-4) of family C showing wild type sequence. Arrow indicates a nucleotide A at position 6331, which has been substituted with T, resulting in a missense mutation (Wang *et al.* 1998). 47

3.19 A representative chromatograms generated by big dye terminator, sequencing of exon 29 of *MYO15A* gene from an affected female individual (IV-4) of family C showing wild type sequence. Arrow indicates a nucleotide A at position 6337, which has been substituted with C, resulting in a missense mutation (Wang *et al.* 1998). 48

3.20 A representative chromatograms generated by big dye terminator, sequencing of exon 30 of *MYO15A* gene from an affected female individual (IV-4) of family C showing wild type sequence. Arrow indicates a nucleotide C at position 6613, which has been substituted with T, resulting in a missense mutation (Liburd *et al.* 2001). 48

3.21 A representative chromatograms generated by big dye terminator, sequencing of exon 40 of *MYO15A* gene from an affected female individual (IV-4) of family C showing wild type sequence. Arrow indicates a nucleotide A at position 7801, which has been substituted with C, resulting in a non-sense mutation (Wang *et al.* 1998). 49

3.22 A representative chromatograms generated by big dye terminator, sequencing of exon 43 of *MYO15A* gene from an affected female individual (IV-4) of family C showing wild type sequence. Arrow indicates a nucleotide G at position 8147, which has been substituted with T, resulting in a missense mutation (Liburd *et al.* 2001). 49

3.23 Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S169 at 49.20 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation. 51

3.24 Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S104 at 51.81 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation. 51

3.25 Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S1118 at 58.2 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation. 52

3.26 Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S50 at 60.59 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation. 52

3.27 Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S166 at 66.32 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation. 53

3.28 Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S301 at 66.32 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation. 53

3.29 Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S175 at 70.33 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation. 54

3.30 Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S1122 at 76.0 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation. 54

3.31 Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S922 at 80.31 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation. 55

3.32 Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D10S1653 at 40.36 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation. 55

3.33 Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D10S1661 at 42.50 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation. 56

3.34 Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D10S1423 at 43.26 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation. 56

3.35 Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D10S1775 at 52 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation. 57

3.36 Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D10S2481 at 52.1 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation. 57

3.37 Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D10S1160 at 54.23 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation. 58

LIST OF TABLES

Table No	Title	Page No
1.1	Loci and genes for autosomal dominant non-syndromic deafness	5 – 6
1.2	Loci and genes for autosomal recessive non-syndromic deafness	7 – 8
2.1	Primers for PCR amplification of *GJB2* gene exon 2	26
2.2	Primers for PCR amplification of *MYO-15* gene	26
2.3	A list of microsatellite markers used for linkage to known DFNB Loci	27-28
2.4	List of microsatellite markers used for linkage analysis of Chromosome number 9 and chromosome number 10	29
3.1	List of allelic variants in *MYO15A* reported to date	50

Abstract

Hearing loss represents an important social and medical phenomenon affecting a significant proportion of the population. Congenital or childhood-onset deafness affects approximately one in thousand children with the majority of cases having no associated syndromal features (non-syndromic deafness). A genetic cause is responsible for 60% of cases, most of which display an autosomal recessive mode of inheritance. Non-syndromic sensorineural deafness is genetically heterogenous. To date approximately 68 autosomal recessive (DFNB) and 53 autosomal dominant (DFNA) hearing impairment loci have been identified. 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, DFNB8/10, and DFNB16.

In the present study, four Pakistani families (A, B, C, D) with autosomal recessive non-syndromic form of deafness, were ascertained from Azad Jammu and Kashmir, Peshawar and Punjab. Family A has three, B and C have six each, and family D has four affected individuals. All the affected individuals were deaf since birth showing prelingual deafness and using sign language for communication.

Linkage in the four families was studied by genotyping microsatellite markers corresponding to candidate genes, involved in autosomal recessive non-syndromic deafness phenotypes. In family A, linkage was established with DFNB1 locus on chromosome 13q12. DNA sequence analysis of exon 2 of *GJB2* gene in affected individual (VI-5) detected a non-sense mutation (W24X).

In family B, analysis of genotyped markers showed linkage to two loci, DFNB31 on chromosome 9q32-q34 and DFNB49 on chromosome 5q12.3-q14.1, identifying one (or possibly both) as the site of a novel autosomal recessive non-syndromic sensorineural hearing loss gene.

In family C linkage was detected with DFNB3 on chromosome 17p11.2. *MYO15A* gene was screened for the reported mutations in exons 3, 29, 30, 40, and 43 in affected individual of the family. However, no disease causing mutation was detected, suggesting the presence of a novel mutation in the family.

In family D, analysis of the results of the genotyped microsatellite markers located on chromosome 9 and 10, showed that the affected individuals were heterozygous with different combinations of parental alleles thus, excluding family D from linkage to chromosme 9 and 10.

INTRODUCTION

INTRODUCTION

The human ear is the most complex organ in the body, capable of turning the tiniest disturbances in air into a form the brain can understand and doing so instantaneously, over an enormous range of pitch and loudness. Hearing loss or deafness is the total inability to hear sound in one or both ears. Hearing impairment (HI) is the most common sensory disorder. Approximately 1 in 1000 children is born deaf or hearing impaired or develops HI during childhood (Morton, 1991).

Types of Hearing Loss

Hereditary impaired hearing is classified as syndromic or non-syndromic, or according to its transmission via an autosomal dominant, autosomal recessive, X-chromosome recessive or maternal trait. Another useful classification of hereditary impaired hearing (HIH) according to its onset before (prelingual) or after acquisition of speech (posts lingual). As a rule of thumb, most prelingual cases with HIH follow a recessive segregation, whereas most cases of post-lingual HIH follow an autosomal dominant trait (Bitner-Glindzicz *et al.*, 2002; Petersen *et al.*, 2002; Smith *et al.*, 2002). Among hereditary non-syndromic deafness, autosomal-recessive inheritance predominates (about 80% of the cases), autosomal dominant inheritance is observed in about 20% of the cases, and X-linked (≈ 1%) and mitochondrial (<1%) inheritance is more rarely encountered (Morton, 1991). Hearing loss can be classified as:

- Conductive hearing loss results from abnormalities of the external ear and /or the ossicles of the middle ear.
- Sensorineural hearing loss results from malfunction of inner ear structures (i.e., cochlea).
- Mixed hearing loss is a combination of conductive and sensorineural hearing loss.
- Central auditory dysfunction results from damage or dysfunction at the level of the VIIIth cranial nerve, auditory brain stem, or cerebral cortex.

Mode of Inheritance and Genetical Etiology

Congenital or childhood-onset deafness affects approximately 1/1000 children with the majority of cases (approx. 70%) having no associated syndromal features. Causes of hearing impairment are numerous and, in a particular population, the relative contribution of genetic and environmental causes may be determined by social factors such as population structure and consanguinity, infection control and immunization, and provision of neonatal and postnatal medical care (Petit *et al.*, 1996; Morton *et al.*, 2002). In most of cases the hearing loss is a multifactor disorders caused by both genetic and environmental factors. However, single gene mutation can lead to hearing loss. In this case, hearing loss is monogenic disorder with following mode of inheritance:

- Autosomal dominant
- Autosomal recessive
- X-linked
- Mitochondrial

These monogenic forms of hearing loss can be: Syndromic (characterize by hearing loss in combination with other abnormalities or Non syndromic (Willems, 2000). Autosomal recessive non-syndromic hearing loss (ARNSHL) is the most common form of severe inherited childhood deafness. This genetically heterogenous disorder can be caused by mutations in a number of different genes. To date, 68 ARNSHL loci have been reported and mutations in 22 genes have been known so far (http://www.uia.ac.be/dnalab/hhh).

Prevalence

Between 1/2000 (0.05%) and 1/1000 (0.1%) children are born with profound hearing loss (Marazita *et al.*, 1993). Among heredity non-syndromic deafness, autosomal-recessive inheritance predominates (accounting for about 80% of the cases), autosomal-dominant inheritance is observed in about 20% of the cases, and X-linked ($\approx$ 1%) and mitochondrial (<1%) inheritance is more rarely encountered (Morton, 1991). The autosomal-recessive forms of deafness are generally the most severe and are almost exclusively caused by cochlear defects sensorineural deafness), in contrast to the syndromic forms of deafness (Petit, 1996).

Clinical Diagnosis of Sensorineural Hearing Loss

Genetic forms of hearing loss must be carefully distinguished from acquired (non-genetic) cause of hearing loss. The genetic forms of hearing loss are diagnosed by otologic, audiologic, and physical examination, family history, ancillary testing (such as CT examination of the temporal bone), and DNA based testing.

Hearing is measured in decibels (dB). The threshold or 0 dB marks for each frequency refers to the lever at which normal young adults perceive a tone burst 50% of the time. Hearing is considered normal if an individual's thresholds are with in 15 dB of normal thresholds. Severity of hearing loss is graded as:

- Mild (26-40 dB)
- Moderate (41-55 dB)
- Moderately sever (56-70 dB)
- Severe (71-90 dB)
- Profound (90 dB)

Audiometry

Audiometry subjectively determines how the individual processes auditory information, i.e., hears. Audiometry consists of behavioral testing and pure tone audiometry. Behavioral testing includes behavioral observation audiometry (BOA) and visual reinforcement audiometry (VRA). Behavioral observation audiometry is used in infants from birth to six months of age. Visual reinforcement audiometry is used in children from six to 2.5 years.

Pure–tone audiometry involves determination of the lowest intensity at which an individual "hears" a pure tone, as a function of frequency (or pitch). Octave frequencies from 250 to 8000 Hz are tested using earphones. Intensity or loudness is measured in decibels (dB), defined as the ratio between two sound pressures. Conventional audiometry is used to test individuals age five years and older; the individual indicates when the sound is heard (Smith *et al.*, 2005).

Gene-Localization Studies

Initially only genes implicated in syndromic hearing loss were localized and until 1994 only three gene loci involved in non-syndromic hearing loss had been mapped on the human genome. Recently, linkage analysis has mapped many genes for non-syndromic hearing loss, to different chromosomes. The different gene loci for the numerous non-syndromic forms of deafness have been called DFN (for deafness) and are numbered in chronologic order of discovery. Autosomal dominant loci referred to as DFNA, autosomal recessive loci as DFNB, and X-linked loci as DFN. Till 2005, 120 loci had been identified: 54 DFNA loci, 68 DFNB loci, and 6 DFN loci (http://www.uia.ac.be/dnalab/hhh).

Nuclear Genes

Of the 30,000-50,000 human genes, only 1%, i.e. 300-500 genes are estimated to be necessary for hearing (Friedmann *et al.*, 2003). Today, approximately 120 independent genes for HIH, approximately 80 for syndromic and 41 for non-syndromic HIH, have been identified, which is about one third of the total (Nance *et al.*, 2003). Of the 41 mutated genes, which cause non-syndromic HIH, 15 causes autosomal dominant HIH, 15 cause autosomal recessive HIH, 6 both autosomal dominant and recessive HIH, 2 X-linked HIH and 3 maternally inherited HIH.

The loci and genes reported to date for non-syndromic deafness are presented in Table 1.1. These have been numbered in chronologic order. DFN is the root of the locus symbol for deafness. An A or B suffix indicates that mutant allele is segregating in an autosomal dominant or recessive pattern, respectively. Sex linked non-syndromic hearing loss is designated with a DFN symbol and a numerical suffix http://www.uia.ac.be/dnalab/hhh.

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

Locus	Localization	Genes	Gene products	Biological Role	Mousemodel (Gene Symbol)
DFNA1	5q31	*DIAPH1*	Diaphanous 1	Cyotoskelen	
DFNA2	1p34	*GJB3* *KCNQ4*	Connexin31 KCNQ4	Gap Junction Ion channel, transporter	Gjb3 -/-
DFNA3	13q12	*GJB2* *GJB6*	Connexin26 Connexin30	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	Extracellular matrix	
DFNA9	14q12-q13	*COCH*	Cochlin	Extracellular matrix	Coll 1a2-/-
DFNA10	6q22-q23	*EYA4*	α-tectorin EYA4	Extracellular matrix transcription regulator	
DFNA11	11q12.3q21	*MYO7A*	myosinVIIA	Motor	Shaker 1 (sh1)
DFNA12	11q22-q24	*TECTA*	α-tectorin	Extracellular matrix	
DFNA13	6q21	*COL11A2*	α2(XI) collagen	Extracellular matrix	
DFNA14	4p16	*WFS1*	wolframin	Unknown	
DFNA15	5q31	*POU4F3*	POU4F3	Transcription regulator	Brn3c-/- Driedel(ddl)
DFNA16	2q24	Uknown			
DFNA17	22q	*MYH9*	Myosin IIA	Myosin heavy-chain-9	
DFNA18	3q22	Unknown			
DFNA19	10 pericentre	Unknown			
DFNA20	17q25	*ACTG1*	Gamma actine-1		
DFNA21	6p21	Unknown			
DFNA22	6p13	*MYO6*	Myosin VI	Motor	Snell's walter (sv)
DFNA23	14q21-q22	Unknown			
DFNA24	4q	Unknown			
DFNA25	12q21-24	Unknown			

DFNA26	17q25	*ACTG1*			
DFNA27	4q12	Unknown			
DFNA28	8q22	*TFCP2L3*	Transcription factor		
DFNA30	15q25-26	Unknown			
DFNA31	6p21.3	Unknown			
DFNA32	11p15	Unknown			
DFNA34	1q44	Unknown			
DFNA36	9q13-q21	*TMC1*	TMC1	Integral membrane Protein (TECG1)	Deafness (dn)
DFNA37	1p21	Unknown			
DFNA38	4p16	*WFS1*	wolframin	Unknown	
DFNA39	4q21.3	*DSPP*	DSPP		
DFNA40	16p12	Unknown			
DFNA41	12q24-qter	Unknown			
DFNA42	4q28	Unknown			
DFNA43	2p12	Unknown			
DFNA44	3q28-29	Unknown			
DFNA47	9p21-22	Unknown			
DFNA48	12q13-q14	*MYO1A*	Myosin-1A		
DFNA49	1q21-q23	Unknown			
DFNA50	7q32	Unknown			
DFNA53	14q11-q12	Unknown			
DFNA54	5q31	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 -/- Gjb2 -/-
DFNB2	11q12.3	*MYO7A*	Myosin VIIA	Motor	Shak/er 1 (sh 1)
DFNB3	17q11.2	*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*	TCMI	Integral membrane Protein (Predicted)	Beethoven (Bth)
DFNB8	21q22	*TMPRSS3*	TMPRSS3	Enzyme	
DFNB9	2p22-p23	*OTOF*	Otoferlin	Neuron/synapse	
DFNB10	21q22.3	*TMPRSS3*	TMPRSS3	Enzyme	
DFNB11	9q13-q21	*TMC1*	TMC1	Internal membrane Protein (Predicted)	Beethoven (Bth)
DFNB12	10q21-q22	*CDH23*	Cadherin23	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	*USHIC*	Harmonin	Macromolecuar Organizer	
DFNB19	18p11	Unknown			
DFNB20	11q25	Unknown			
DFNB21	11q	*TECTA*	A-tectorin	Extracellular matrix	
DFNB22	16p12.2	*OTOA*	Otoancorin	Extracellular matrix	
DFNB23	10p11-q21	*PCDH15*	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*	Myosin IIIA	Motor	
DFNB31	9q32-q34	*WHRN*			
DFNB32	1p13.3-22.1	Unknown			
DFNB33	9q34.3	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			
DFNB42	3q13.31-q22.3	Unknown			
DFNB44	7p14.1-q11.22	Unknown			
DFNB46	18p11.32-p11.31	Unknown			
DFNB48	15q23-q25.1	Unknown			
DFNB49	5q12.3-q14.1	Unknown			
DFNB50	12q23	Unknown			
DFNB53	6p21.3	Unknown			
DFNB55	4q12-q13.2	Unknown			
DFNB60	5q22-q31	Unknown			

Cadherins related 23 (*CDH23*)

Cadherins (calcium dependent cell adhesion molecule) are membrane bound glycoprotein receptors that function in cell-to-cell contact at adhesion junctions (Nagafuchi, 2001) and possibly form extracellular connections between adjacent hair cell stereocilia. Cadherins are grouped on the basis of the tandem repeats of the extracellular cadherins-specific motif (EC domain), (Suzuki, 1996; Nolle *et al.*, 2000). The largest *CDH23* isoform consist of 69 exons encoding a deduced 3354 amino acid protein. *CDH23* exon 68 is expressed preferentially in the inner ear and not in the brain or retina (Boeda *et al.*, 2002; Siemens *et al.*, 2002). The 10.5-kb *CDH23* cDNA encodes a large, single-pass transmembrane protein that (Palma *et al.*, 2001) referred to as otocadherin. *CDH23* has an extracellular domain containing 27 repeats that show significant homology to the cadherin ectodomain. They identified loss-of-function mutations in the *CDH23* gene in each of 3 different waltzer alleles. Palma *et al.* (2001) demonstrated that Cdh23 is expressed in the neurosensory epithelium, and during early hair-cell differentiation, stereocilia organization was disrupted in homozygotes for one of the mutant alleles. Allelic mutations of *CDH23* encoding cadherin 23 cause both non-syndromic deafness DFNB12 Bork *et al.* (2001) and USH1D (Bolz *et al.*, 2001; Bork *et al.*, 2001).

Only missense mutations of *CDH23* have been observed in families with non-syndromic deafness, whereas nonsense, frame shift, splice-site, and missense mutations have been identified in families with Usher syndrome. Astuto *et al.* (2002) screened a panel of 69 probands with Usher syndrome and 38 probands with recessive non-syndromic deafness for the presence of mutations in the entire coding region of *CDH23*, by heteroduplex, SSCP, and direct sequence analyses.

Siemens *et al.* (2002) showed that *CDH23* and harmonin, another protein that is the site of mutations causing Usher syndrome, form a protein complex. Two PDZ domains in harmonin interact with 2 complementary binding surfaces in the *CDH23* cytoplasmic domain. One of the binding surfaces is disrupted by sequences encoded by an alternatively spliced *CDH23* exon that is expressed in the ear, but not in the retina. In the ear, *CDH23* and harmonin are expressed in the stereocilia of hair cells, and in the retina within the photoreceptor cell layer. Age-related hearing loss (AHL) in common inbred mouse strains is a genetically complex quantitative trait. Noben-Trauth *et al.* (2003) found a single-nucleotide polymorphism (SNP) in exon 7 of the Cdh23 gene that showed significant association with AHL and the deafness modifier

mdfw (modifier of deafwaddler). The hypomorphic Cdh23 (753A) allele caused in-frame skipping of exon 7. Altered adhesion or reduced stability of *CDH23* may confer susceptibility to AHL. Homozygosity at Cdh23 (753A) or in combination with heterogeneous secondary factors was found to be a primary determinant of AHL in mice.

Transmembrane channel like gene 1 (*TMC1*)

The DFNA36 locus was defined in a single-family co-segregating dominant, progressive sensorineural hearing loss with short tandem repeat (STR) markers on chromosome 9q13-q21 (Kurima *et al.*, 2002). The DFNA36 interval contained the previously mapped DFNB7 and DFNB11 intervals (Jain *et al.*, 1995; Scott *et al.*, 1996; Kurima *et al.*, 2002). In this region a new gene, *TMC1* was detected. *TMC1* is predicted to encode a multipass transmembrane protein with a cytoplasmic orientation of the *COOH*-terminus (Kurima *et al.*, 2002). The Tmc1 mouse ortholog was cloned and specific Tmc1 mRNA expression was detected in cochlear and vestibular hair cells (Kurima *et al.*, 2002).

By alignment of the *TMC1* cDNA with genomic sequences Kurima *et al.* (2002), showed that 24 exons probably encode full-length mRNA, including 4 exons encoding sequence upstream of a methionine codon in exon 5. There are many in-frame stop codons directly upstream of this methionine codon, which was predicted to be an adequate Kozak translation initiation codon. Kurima *et al.* (2002) identified eight different mutations in a novel gene, transmembrane channel-like gene 1 (*TMC1*). Mutations in this gene cause DFNB7/11 in 11 families and DFNA36 in a single family.

Tmc1 mutations were also identified in the recessive deafness (*dn*) and in dominant Beethoven (*Bth*) mouse mutant strain segregating hearing loss and postnatal hair cell degeneration, indicating that Tmc1 is required for postnatal hair cell development or maintenance (Kurima *et al.*, 2002; Vreugde *et al.*, 2002). Mouse Tmc2 is a candidate gene for the dominant Tailchaser mutation, which causes progressive auditory and vestibular dysfunction associated with hair cell stereocilia abnormalities (Kiernan *et al.*, 1999).

Transmembrane protease, serine 3 (*TMPRSS3*)

Scott *et al.* (2001) identified a novel gene within the DFNB8 /DFNB10 critical region on 21q, which they designated *TMPRSS3* (transmembrane protease, serine-3). This gene has been mapped to chromosome 21q22.3 and encodes for the transmembrane serine protease *TMPRSS3* or *ECHOS1*, which contains LDRA and SRCR domains (Gullponi *et al.*, 2002; Wattenhofer *et al.*, 2002). The gene contains 13 exons spanning 24 kb. Scott *et al.* (2001) detected 4 alternative transcripts encoding putative polypeptides of 454, 327, 327, and 344 amino acids. Mutations in this gene are made responsible for DFNB8/10. Mutations found so far were a 1 bp deletion, resulting in a frameshift and an amino acid substitution in the LDLRA domain of the protein (Wattenhofer *et al.*, 2002), an 8 bp deletion an insertion of 18 monomeric beta-satellite repeat units, and a 4 bp mRNA insertion (Scot *et al.*, 2001). Gullponi *et al.* (2002) cloned the mouse ortholog of *TMPRSS3*, which is structurally similar to the human gene and encodes a polypeptide with 88% identity to the human protein. In corresponding mouse model, tmprss3 mutations clinically manifest as >70dB congenital hearing loss (Masmoudi *et al.*, 2001).

(*ESPIN*)

This gene has been recently mapped to chromosome 1p36.3 and encodes for a calcium-insensitive actin binding protein called *ESPIN* (Naz *et al.*, 2004). These authors cloned human *ESPN* and determined that the human *ESPN* gene contains 13 exons. The deduced 854-amino acid protein has 8 ankyrin-like repeats at the N terminus, 2 proline-rich regions, and a consensus site for ATP or GTP binding (P loop). Naz *et al.* (2004) identified a homozygous 4 bp deletion (2469 del GTCA) in exon 13 of the ESPN gene, leading to a stop codon at nucleotide 2533. The resultant protein is predicted to lack one of the C-terminal actin-binding sites necessary for espin activity.

Gap Junctions

Connexins are membrane proteins that are involved in the formation of channels through the plasma membrane of many cells. Six connexons subunits assemble into a half channel that dock with its counterpart from an adjacent cell to form an intercellular channel. A cluster of these channels form a gap junction, which allows intercellular exchange of small molecules and has an important role in intercellular

communication (Bruzzone *et al.*, 1996; Kumar *et al.*, 1996). Gap junctions are clusters of intercellular channels (connexins) that couple the cytoplasmic compartments of adjacent cells. A connexin is a hemichannel compartment of a hexamer of proteins (Connexon) subunits in the plasma membrane, and two connexons on opposing cells form a functional gap junction channel by docking with each other in the intercellular space (Bruzzone *et al.*, 1996; Goodenough *et al.*, 1996). Ultra structure studies preceding the genetic dissection of deafness indicates that gap junctions were present and important for intercellular communication and homeostasis in the inner ear, where they were postulated to permit cell-to-cell signaling and the passage of water and small molecules such as ions between adjacent cells (Kikuchi *et al.*, 1995).

Connexins mutations implicated in hearing loss include mutations in the *connexins26* and *Connexin31* genes in families with non-syndromic hearing loss and in the *connexins32* gene in hearing loss associated with X-linked Charcot- Marie-Tooth syndrome. The *Connexin26* gene, also referred to as the first gene to be implicated in non-syndromic hearing loss, and mutations in this gene are associated with two forms of non-syndromic hearing. One is DFNB1, the most common form of non-syndromic autosomal recessive hearing loss, which accounts for approximately 20 percent of cases of prelingual deafness, which is usually congenital and stable, with little progression, and its severity varies from moderate to profound (Zelante *et al.*, 1997; Scott *et al.*, 1998).

Gap Junction Protein Beta 2, 26kDa (*connexin 26*)

DFNB1 was mapped to chromosome 13q12 in 1994 (Guilford *et al.*, 1994). The DFNB1 gene was identified as gap-junction beta-2 (GJB2) (Kelsell *et al.*, 1997; Lopponen *et al.*, 2003). *GJB2* has a single coding exon encoding for the gap-junction protein connexin-26, a member of the Connexin family. Connexin are integral membrane proteins with four transmembrane domains, forming aqueous gap-junction channel, which allow the diffusional transmission and exchange of potassium and calcium and small signaling molecules, thus coordinating metabolic activities in multi-cellular tissues (Hand *et al.*, 2002; Jan *et al.*, 2004). Six connexin-26 molecules aggregate in a group around a central pore of 2-3nm to form a torus-like structure called connexin (Chang *et al.*, 1999; Smith *et al.*, 2002). Mutations in the *GJB2* gene are the major cause of non-syndromic autosomal recessive and sporadic deafness

(Gurtler *et al.*, 2002). The most frequent mutation is the deletion 35delG, which require homozygosity for manifestation. The second frequent mutation is the M34T substitution, which also requires homozygosity for phenotypic expression. The frequency of *GJB2* mutation is ethnic specific. The 35delG mutation is the most frequent among Europeans, The 167delT the most frequent among Ashkenazi Jews (Morell *et al.*, 1998) and the 235delC the most frequent among Asians (Abe *et al.*, 2000). GJB2 mutations also cause dominant non-syndromic SNHL (DFNA3). True dominant *GJB2*-related deafness is rare but has been identified in families with the *GJB2* mutations R184G, W44S, and C20F (Denoyelle *et al.*, 1998; Martin *et al.*, 1999).

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

This gene was mapped to chromosome 1p34 and encodes for connexin-31, another member of the connexon family (Liu *et al.*, 1997). Mutations in this gene cause DFNB2. In two Chinese families; however, compound heterozygous mutations in the *GJB3* gene were detected, demonstrating autosomal recessive transmission (Liu *et al.*, 2000). A 3 bp deletion in the *GJB3* gene in a Spanish family segregated with autosomal dominant HIH and peripheral neuropathy (Lopez *et al.*, 2001).

Gap Junction Protein, Beta 6 GJB6 (*connexin 30*)

This gene was mapped to chromosome 13q12 encodes for connexin-30, member of the connexon family. Connexin-30 shows 77% identity in amino acid sequence with connexin26 (Lautermall *et al.*, 1998; Kelley *et al.*, 2000). Mutations in this gene cause DFNA3, DFNB1, together with *GJB2* mutations, Kid Syndrome. In DFNB, SNHL is usually severe or profound, and stable in the majority of the cases. Onset is always prelingual but not necessarily congenital (Tekin *et al.*, 2001).

Otoanchorin (*OTOA*)

This gene has been mapped to chromosome 16p12.2 (DFNB22) and encodes for the glycocyclophosphatidyl-inositol anchored protein otoaancorin (Jovine *et al.*, 2002). By sequence analysis using a gene-prediction program (Zwaenepoel *et al.*, 2002) determined that the *OTOA* gene has 28 exons spanning approximately 82 kb. Otoancorin is made up of 1088 amino acids and is located at the interface between the apical surface of the inner-ear sensory epithelia and their overlying a-cellular gels

(Jovine *et al.*, 2002). In a Palestine family, the splice site mutation IVS12 + 2T >C in the OTOA gene was detected as the cause of DFNB22 (Zwaenepoel *et al.*, 2002)

Otoferlin (*OTOF*)

The *OTOF* gene has been mapped to chromosome 2p23 and consists of 48 exons (Mirghomizadeh *et al.*, 2002). The gene encodes for otoferlin, which is a C-terminus anchored cytosolic protein (Yasunaga *et al.*, 2000). The gene is mutated in patients with DFNB9. Mutations described so far were the substitutions Pro490Glu and Ile515Thr located in the Ca-binding site of the C2C domain (Mirghomizadeh *et al.*, 2002; Rodriguez-Ballesteros *et al.*, 2003).

Solute Carrier Family 26, Member 4 (*SLC26A4*)

Pandered syndrome (autosomal recessive deafness with goiter) and non-syndromic deafness DFNB4 are allelic disorders caused by mutations of the *SLC26A4* (PDZ) gene (Everett *et al.*, 1997; Li *et al.*, 1998). This gene was mapped to a 9 cM region on 7q31 (Shffield *et al.*, 1996). The gene encode for a trans-membrane Chloride iodide transporter, expressed in the kidney, thyroid and inner ear (Shffield *et al.*, 1996). Mutations in this gene cause DFNB4 and Pandered syndrome (Campbell *et al.*, 2001). To date >60 mutations have been reported in this gene. Four of these mutations (L236P, IVS8, + IG>A, T416P, H723R) account for approximately 60% of the total Pendred syndrome mutation load (Campbell *et al.*, 2001). Usually, mutations occur as homozygous missense mutations or compound heterozygous mutations (Borck *et al.*, 2003). Pendred syndrome is allelic to DFNB4. In patients with DFNB4 the aqueduct is enlarged but there is no goiter (Tekin *et al.*, 2001).

Myosin VIIA (*MYO7A*)

This gene was mapped to chromosome 11q12.3-21, has 49 coding exons, and spans approximately 87 Kb of genomic DNA (Ahmad *et al.*, 2003). *MYO7A* encodes for the non-muscle myosin heavy-chain-7A, which is made up of 2215 amino acids. *MYO7A* belongs to the unconventional myosin, implicated in intracellular movement of non-muscle cells (Petersen *et al.*, 2002). The gene is expressed in cochlear and vestibular epithelia. Mutation in this gene accounts for DFNA11 and DFNB2. *MYO7A* mutations were first detected in a large, consanguineous Tunisian family with autosomal recessive sensorineural hearing loss (Weil *et al.*, 1997). At least one mutant

allele of *MYO7A* is associated with dominant non-syndromic hearing loss. Eight affected members of a single Japanese family co-segregate progressive hearing loss with a 9 bp in-frame deletion in exon 22 of *MYO7A* (Tamagawa *et al.*, 1996). Four other mutations of *MYO7A* are reportedly associated with non-syndromic recessive deafness DFNB2 (Liu *et al.*, 1997; Weil *et al.*, 1997).

Diaphanus

It belongs to family of proteins related to formins, involved in cell polarization and cytokinesis. Gene DIAPH1 or HDIA1 (26 exons), located at chromosome 5 (5q31), codifies protein diaphanous-1 (1252 amino acids), (Leon and Lalwani, 2002) homologous to protein diaphanous of Drosophila.

Myosin XVA (*MYO15*)

A genome-wide homozygosity mapping strategy identified a locus (DFNB3) on chromosome 17p11.2 for NSRD segregation in a Balinese village (Friedman *et al.*, 1995; Liang *et al.*, 1998). The gene is build up of 66 exons and encodes for myosin-15A. In half of the DFNB3 families, the mutation occurs in the homozygous form. Sequence analysis of the *MYO15* exons in the Pakistani family revealed two missense mutations and one non-sense mutation, which co-segregated with DFNB3 (Wang *et al.*, 1998).

Cochlin (*COCH*)

Mutations in the *COCH* (coagulation factor C homology) gene have been shown to correlate with DFNA9, a late-onset autosomal dominant non-syndromic hearing disorder (Manolis *et al.*, 1996; Robertson *et al.*, 1998; Fransen *et al.*, 1999; Kok *et al.*, 1999; Verhagen *et al.*, 2000; Kamarinos *et al.*, 2001; Verstreken *et al.*, 2001). *COCH* maps to the long arm of human chromosome 14 in bands q11.2–13 (Robertson *et al.*, 1997). The genomic structure of *COCH* has been determined (Robertson *et al.*, 1998; Kok *et al.*, 1999). Cochlin has 12 exons with the initiator ATG start codon being located in exon 2 and the reading frame ending in exon 12. Hearing loss begins in the 4th or 5th decade of life and initially involves the high frequencies (Versreken *et al.*, 2001). Deafness is progressive and is usually complete by the 6th decade. Five different *COCH* mutations are currently known (Roberstson *et al.*, 1998; Fransen *et al.*, 1999; Kok *et al.*, 1999; Kamarinos *et al.*, 2001) all of which are predicted to cause

missense substitutions at highly conserved amino acid residues in the LCCL domain (Robertson *et al.*, 1998; Liepinsh *et al.*, 2001). One of these mutations, resulting in the missense substitution P51S is a founder mutation that appears to be a prevalent cause of autosomal dominant non-syndromic hearing loss with vestibular dysfunction in Belgium and the Netherlands (Frensen *et al.*, 1999). The LCCL domain is encoded by exons 4 and 5 (Robertson *et al.*, 1998). Although the *COCH's* specific function in the auditory system is unknown, there are several indications that it plays an important role in the extracellular matrix of the cochlea (Khetarpal *et al.*, 1991). The majority of the *COCH* mutations lead to misfolding of the FCH/LCCL domain, associated with eosinophilic deposits in the inner-ear structure. Mutated cochlin is not retained intercellularly, is secreted normally by the Golgi/endoplasmatic reticulum pathway, and undergoes proteolytic cleavage and glycosilation (Robertson *et al.*, 2003), suggesting that DFNA9 mutations manifest in the extracellular matrix. Mutant cochlin varies in amount and pattern in the extracellular matrix. Some mutations exhibit almost normal deposition pattern and some show complete of deposition (Grabski *et al.*, 2003).

Transmembrane Inner Ear Protein (*TMIE*)

This gene was mapped to chromosome 3p13 and encodes for a protein made up of 156 amino acids, which exhibits no significant similarity to any other gene (Naz *et al.*, 2002), and contains four exons. The identification of loss-of-function mutations causing deafness in mice and humans indicates that the gene has a conserved, critical role in the auditory system (Naz *et al.*, 2002). The inner ear pathology in affected spinner mice suggests that Tmie is required for normal postnatal maturation of sensory hair cells in the cochlea, including correct development of stereocilia bundles (Mitchem *et al.*, 2002). One insertion, one deletion, and three missense mutations in the TMIE gene have been described causative for DFNB6 thus for (Naz *et al.*, 2002).

KCNQ4

Gene KCNQ4, with 14 exons, mapped in chromosome 1 (1p34), codifies a protein subunit of family KCNQ of potassium channels, protein KCNQ4 (695 amino acids). In the cochlea, channels KCNQ4 are expressed not only in outer hair cells, but also in inner hair cells, whose main function is to promote the outflow of potassium from the cells to supporting cells (Leenheer *et al.*, 2002).

POU Domain, Class 3, Transcription Factor 4 (*POU3F4*)

This gene has been localized to a 500 Kb segment of the xq21.1 band. The gene encodes for a transcription factor with a POU domain (*POU3F4*) (Kok *et al.*, 1995). Mutations in this gene cause DFN3 by truncation of the predicted proteins or non-conservative amino acid substitutions. DFN3 is allelic to stapes gusher syndrome, characterized by fixation of the POU domain (Kok *et al.*, 1995; Oh *et al.*, 2003).

Most of the mutations affect the POU domain (Kok *et al.*, 1997). Deletions account for half of the known *POUF3* mutations (Kok *et al.*, 1997). A *POU3F4* mutation in homozygotic twins with different phenotype, however, suggests that exogenous factors influence the phenotype expression of these mutations (Oh *et al.*, 2003).

The identification of deafness genes has lead to tremendous progress in the discovery of key components that are essential for auditory transduction. There are still numerous unidentified HI genes, and growing evidence exists that modifier genes also play an important role in HI.

In the present study four consanguineous families from different isolated areas of Pakistan with non-syndromic hearing loss have been genotyped with microsatellite markers linked to known and other potential hearing loss loci.

MATERIALS AND METHODS

MATERIALS AND METHODS

Families Studied

Four Families referred hereafter as A, B, C and D with autosomal recessive non-syndromic hearing loss were located in different isolated regions of Pakistan.

After a detailed and thorough discussion with elders of these families, genetic pedigrees were drawn. 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. Cousin marriages were shown by double line between the partners. The mode of inheritance of hearing impairment was deduced by observing segregation of hearing loss within family.

Blood Sampling

Blood samples from both normal and affected individuals including their parents were collected by 05 ml and 10 ml syringes and from children below 2 years of age by butterflies. Blood was saved in Potassium EDTA tubes. The samples were kept at 4°C till 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 is then centrifuged at 13,000 rpm for 1 minute in a Microfuge® 18 centrifuge (Bechman Coulter™).

Supernatant was discarded and pellet was reususpended in 400 µl of solution A. Centrifuge was repeated, and after discarding the supernatant, nuclear pellet was

resuspended in 400 µl of solution B, 12 µl of 20% SDS and 20 µl 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 samples, 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. Centrifuge 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 3M 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 residue ethanol, DNA was dissolved in appropriate amount of DNA dissolving buffer (DDB).

Composition of solutions

Solution A

0.32 M sucrose

10 mM Tris (pH 7.5)

5 mM $MgCl_2$

1% (v/v) Triton X-100

Solution B

10 mM Tris (pH 7.5)

400 mM NaCl

2 mM EDTA (pH 8.0)

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 using Genomic isolation Kit (Sigma Chemical Co. USA).

One hundred and fifty microlitre 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. 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 37°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 $MgCl_2$, 1.5 mM 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 polymerase to synthesize any unextended strands left. PCR was performed using Gene Amp PCR system 2400 and Gene Amp PCR system 9600 thermocyclers (Perkin Elmer, USA).

Horizontal Gel Electrophoresis

Amplified PCR products were analyzed on 2% agarose gel prepared by melting 1g 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 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 (Life Technology, USA).

Vertical Gel Electrophoresis

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 glasses 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% Polyacrylamide 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 per sulphate

17.5 µl TEMED

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 photography taken. Microsatellite markers mapped by Cooperative Human Linkage Centre (CHLC), were obtained from Research Genetics, Inc. (USA). Information about the cytogentic 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 Center (www.marshmed.org/gentics/). The numbers of tri, tetra nucleotide repeat sequence polymorphic markers used in current study were approximately 94%. Average hetrozygosity 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 cytogentic location are shown in the 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 (D13S143, D13S175, D13S787 and D13S292) mapped in the linkage interval of the locus.
- Genomic DNA of an affected individual was screened for mutation in the GJB2 gene, containing the entire open reading frame of 681 bp encoding 226 amino acid, was sequenced through Automated Genetic Analyzer, ABI Prism 3100® (Applied Biosystem, USA).

b) 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 61 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 the genetic linkage in the families with non-syndromic recessive deafness. Selected markers had an average heterozygosity of >70%. Genotyping of these markers was performed as described above.

c) Mutation Screening in *GJB2* gene

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

Mutation Screening in *MYO15A* gene

To search for the known allelic variants of *Myo15A* gene, exon 3, 4, 28, 29, 30, 40, 43 and 44 of the gene were PCR amplified from DNA of affected individual of family 'C' linked to DFNB3. The amplified products were directly sequenced in automated DNA sequencer ABI 310. The primer sets used for amplification of these exons are given in Table 2.2.

DNA Sequencing

Thermo-cycling conditions were described above. The condition 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 analysed on 2% agarose gel along with 100 bp DNA Ladder (O Range Ruler^{TM}, MBI Fermentas, UK).

The amplified products were purified using Rapid PCR purification Kit (Marligen, USA). Three hundred microliters 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 polymerase, 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 mixed 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 primers to hybridize or "anneal" to their complementary sequences.
- 4 minutes at 72°C for extension of complementary DNA strands from primers.
- 10 minutes at 72°C for Taq polymerase to synthesize any unextended stands left.

The sequencing products were purified by ethanol precipitation protocol (POP6 Protocol). Sequencing products were 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 centrifuge 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 tube.

After applying denaturation temperature of 95°C for 2 minutes, the samples were sequenced through Automated Genetic Analyzer, ABI Prism 3100 ® (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.nih.gov/).

Table 2.1: Primers for PCR Amplification of *GJB2* Gene Exon 2

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

Table 2.2: Primers for PCR Amplification of *MYO15A* Gene

Exon No	5′′ → 3′		Amp. Length (bp)	Annealing Temp (°C)
	Forward	Reverse		
3	TCTGTAATGAGGAGACCTGC	ACACTTCATGACTGAGGCTC	319	55
4	ATCTGATCAAGGCACTGCAC	AGTTCTAGGCTCTGGCATTC	252	57
28	CATACACTGAGGACCAAAGC	AGAGAGGAAGCCAGATTTCC	357	57
29	GAGGGACATGAGAGGGATGC	GATCTGACTCCTGGCCTGAC	380	55
30	GCCTTTCTCAGACTAGCCTC	AACATCCAGTGGCTTCTGCC	335	57
39	ATCAGGCTTCTCCAGAAACC	TCCTACAGCACAACCTAGAC	391	55
40	GGAGGAAGTTTCCTACTGTC	GTAGCTGGTTTCTGAGGATC	227	57
43	AAAGAGGTGCCGAGCACAGC	CACACAGCCCTGCTGAGTTC	311	57
44	CAGCTTACAGATGACCCCAC	AGAGCAAGGAGGCACATGTC	294	55

Table 2.3: A List of Microsatellite Markers Used for Linkage to Known DFNB Loci

Locus	Markers	Location (cM)		Locus	Markers	Location (cM)
DFNB1	D13S1275 D13S787 D13S292 D13S1294	6.99 8.87 8.87 12.91		DFNB36	D1S214	14.04
DFNB3	D17S1294 D17S1293 D17S1768	50.70 56.58 61.52		DFNB37	D6S1053 D6S1681 D6S1031	80.45 84.15 88.63
SDFNB6	D3S3647 D3S2409	68.47 70.61		DFNB38	D6S1277	173.31
DFNB7/11	D9S301 D9S1876	66.32 67.93		DFNB39	D7S2204 D7S2212 D7S630 D7S2410 D7S657	90.95 95.43 99.44 100 104.86
DFNB8/10	D9S1260	46.71		DFNB40	D22S686	13.6
DFNB9	D2S171 D2S158	45.30 45.83		DFNB42	D3S4523	135.85
DFNB22	D16S403	43.89		DFNB43	D15S643 D15S652	52.33 90.02
DFNB25	D4S405	56.95		DFNB44	D7S1818	69.56
DFNB29	D21S168 D21S1260	40.45 46.71		DFNB45	D1S1609	274.53
DFNB31	D9S302 D9S1776	123.33 123.33		DFNB46	D18S1376 D18S542	17.0 41.0
DFNB35	D14S588 D14S53	69.82 86.29		DFNB47	D2S2278 D2S1400 D2S262	26.52 27.60 27.60

Locus	Markers	Location (cM)		Locus	Markers	Location (cM)
DFNB49	D5S2500 D5S647 D5S629 D5S2042 D5S1501	69.29 74.07 75.89 78.31 85.25		DFNB55	D4S2389	79.69
				DFNB54	D1S3721 D1S193	72.59 73.21

Table 2.4: List of Microsatellite Markers Used for linkage Analysis of DFNB7 and DFNB31

Locus	Markers	Location (cM)		Locus	Markers	Location (cM)
DFNB7	D9S169	49.20		DFNB30	D10S1653	40.36
	D9S104	51.81			D10S1661	42.50
	D9S772	54.12			D10S1423	43.26
	D9S43	55.25			D10S1749	48.90
	D9S1118	58.26			D10S1775	52.00
	D9S1817	59.34			D10S2481	52.10
	D9S1794	59.87			D10S1160	54.23
	D9S50	60.59				
	D9S773	61.76				
	D9S758	63.65				
	D9S1862	64.72				
	D9S301	66.32				
	D9S166	66.32				
	D9S1876	67.93				
	D9S175	70.33				
	D9S1122	75.88				
	D9S922	80.31				
	D9S1835	117.37				

RESULTS

RESULTS

Families Studied

Family A

Family A was located in a small village Kotli, Azad Jammu Kashmir. The family history presented in the pedigree (Figure 3.1) indicates four generations consisting of 22 members including three affected (IV-4, IV-5 and IV-6). According to pedigree analysis disease appeared in fourth generation through parents, which appeared phenotypically normal, suggests that the trait is transmitted in autosomal recessive manner. Through detailed discussion relating to medical history of the individuals and family relationships it was concluded that there was no possibility of the environmental factors and infections as a cause of deafness. The affected persons are deaf and mute by birth showing prelingual deafness.

Blood samples were collected from all the participating members of the family including III-4, III-5, IV-4, IV-5, IV-6 and IV-7.

Family B

Family 'B' was located in Peshawar. After a careful investigation about the history of the family, a genetic pedigree was drawn (Figure 3.2). The four generations pedigree consists of 23 individuals, including five affected females (IV-2, IV-3, IV-4, IV-7 and IV-9) and one affected male (IV-6). All affected individuals were deaf since birth (prelingual hearing loss). The deafness was not associated with any other disorder, reflecting the non-syndromic hearing loss. The pedigree analysis revealed that the disease was fully penetrant with autosomal recessive mode of inheritance. Moreover, although the parents III-1 and III-2, and III-3 and III-4 of the affected individuals were phenotypically normal, yet they had carried a recessive disease allele in heterozygous condition.

Blood samples were collected from the three affected (IV-6, IV-7 and IV-9) and four normal individuals (III-3, III-4, IV-5 and IV-8) of the family and processed for DNA extraction.

Family C

Family "C" was resident of a small village of Dera Ghazi Khan. According to the detailed information collected from elders of the family, a genetic pedigree was constructed. Six generations pedigree (Figure 3.3) consists of 40 individuals including six affected (V-5, V-8, VI-4, VI-5, VI-6 and VI-7). Disease was found to be fully penetrant in the affected individuals. The parents IV-1 and IV-2 of the affected individuals (V-5 and V-8) were phenotypically normal while one of the parents of affected individuals of the sixth generation was affected i.e., V-8.

Blood samples were collected from four affected (VI-4, VI-5, VI-6 and VI-7) and two normal family members (V-9 and VI-8).

Family D

This family resides in a small village Chishtian in the Punjab province. After a thorough investigation about the family, genetic pedigree was constructed (Figure 3.4). The five generation pedigree consists of 34 individuals, including 5 affected individuals (IV-2, IV-3, IV-4, IV-5, and V-2). With careful pedigree analysis, disease was found to be fully penetrant. Although, the parents III-3 and III-4, and IV-1 and IV-6 were phenotypically normal, yet they had carried a recessive disease allele in heterozygous condition. Because of the consanguineous marriages, affected alleles remained in the family in heterozygous form up to 3rd generation. The disease appeared in 4th and 5th generations of the pedigree.

Blood samples were collected from 5 affected (IV-2, IV-3, IV-4, IV-5, V-2) and 5 normal individuals (III-4, IV-1, IV-6, IV-8 and V-4) of the family.

Genetic Mapping of Candidates of Hearing Impairment Genes

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 candidate gene loci (DFNB1-DFNB68) for autosomal recessive non-syndromic deafness have been identified so far. In the present study, all the four families were tested for the linkage to several known DFNB loci. Information about genetic location of recessive deafness was obtained from hereditary hearing loss home page (www.uis.ac.be/dnalab/hhh). Table 2.3 and 2.4 summarizes the microsatellite markers used for genetic mapping. All the markers used in present study have an average

heterozygosity of greater than 80%. Analysis of microsatellite markers was carried out using a standard PCR reaction and electrophoresis in 8% non-denaturing polyacrylamide gel. The amplified PCR products were visualized by staining the gel with ethidium bromide and genotypes were assigned by visual inspection.

Mutations in *GJB2* gene (connexin 26) at DFNB1 locus on chromosome 13q account for more than 50% of the recessive non-syndromic deafness. To test linkage to DFNB1 locus in family A and B genotyping was performed by using microsatellite markers located in its genetic interval.

In family A (Figure 3.1), three affected (IV-4, IV-5 and IV-6) and three normal (III-4, III-5 and IV-7) individuals were genotyped with DFNB1 linked markers (D13S1275, D13S787 and D13S292). All the affected individuals were homozygous while the normal individuals were heterozygous with the typed markers thus establishing linkage of family A to DFNB1 (Figure 3.5-3.7). Sequence analysis of exon 2 of *GJB2* gene, located at DFNB1 locus, in the affected individual (IV-5) revealed substitution of G with A at nucleotide position 71, resulting in conversion of an amino acid tryptophane to premature stop codon (W24X) (Figure 3.8).

In family B (Figure 3.2), four normal (III-3, III-4, IV-5 and IV-8) and three affected (IV-6, IV-7 and IV-9) individuals were tested for co-segregation of disease with several known DFNB loci. To test linkage of family B with DFNB1 locus, exon 2 of the *GJB2* gene was sequenced in an affected individual (VI-2) of the family. However, sequence analysis failed to detect any sequence variant (Figure 3.9), thus excluding family B from linkage to DFNB1 locus. Genotyping of markers, linked to other Known loci, showed linkage of family B to DFNB31 on chromosome 9q32 (Figures 3.10-3.11) and DFNB49 on chromosome 5q12.3 (Figure 3.12-3.14).

In family C (Figure 3.3), two normal (V-9 and VI-8) and four affected (VI-4, VI-5, VI-6 and VI-7) individuals were selected for genotyping with microsatellite markers linked to the candidate genetic loci. It is evident from the analysis of the results obtained (Figures 3.15-3.16) that the affected individuals in the kindred showed homozygosity with markers (D17S1293 and D17S1294), thus establishing linkage to DFNB3 locus. The candidate gene *MYO15A* at DFNB3 locus was identified earlier by Wang *et al.* (1998). So far six disease causing mutations in exons 3, 29, 30, 40 and 43 of *MYO15A* gene have been reported. In the present study, sequence analysis of exon 3 (Figure 3.17), exon 29 (Figure 3.18), exon 29 (Figure 3.19), exon 30 (Figure 3.20),

exon 40 (Figure 3.21), exon 43 (Figure 3.22) of *MYO15A* in an affected individual (IV-4) of family C failed to detect any sequence variant, thus predicting the presence of a novel mutation in the family.

In Family 'D' (Figure 3.4) five normal (III-4, IV-6, IV-8, IV-1 and V-4) and five affected (IV-2, IV-3, IV-4, IV5 and V-2) individuals were selected for genotyping with the markers located on chromosome 9 and 10. Analysis of the results (Figure 3.23-3.37) showed that the affected individuals were heterozygous with different combinations of parental alleles thus, conclusively excluding the linkage of family 'D' to these genetic intervals.

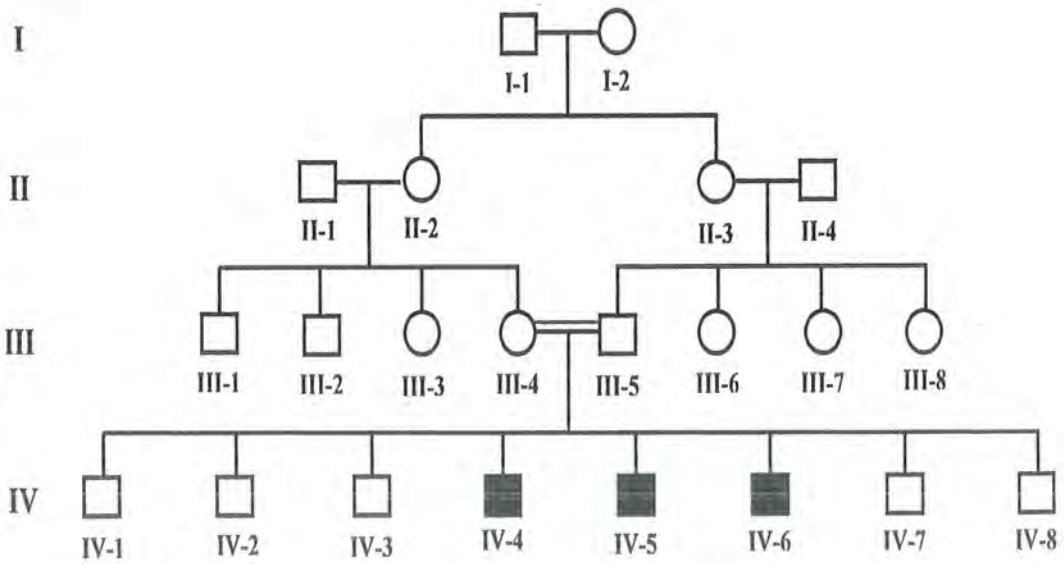


Figure 3.1: Pedigree of the family A with autosomal recessive non-syndromic hearing loss. Circles represent females, squares represent males. Filled circles and squares represent affected individuals.

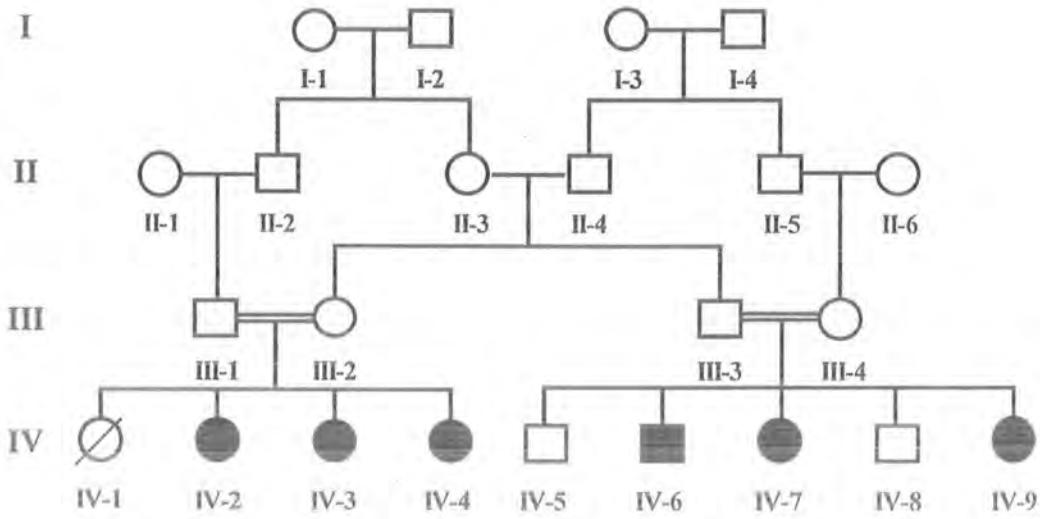


Figure 3.2: Pedigree of the family B with autosomal recessive non-syndromic hearing loss. Circles represent females, squares represent males. Filled circles and squares represent affected individuals.

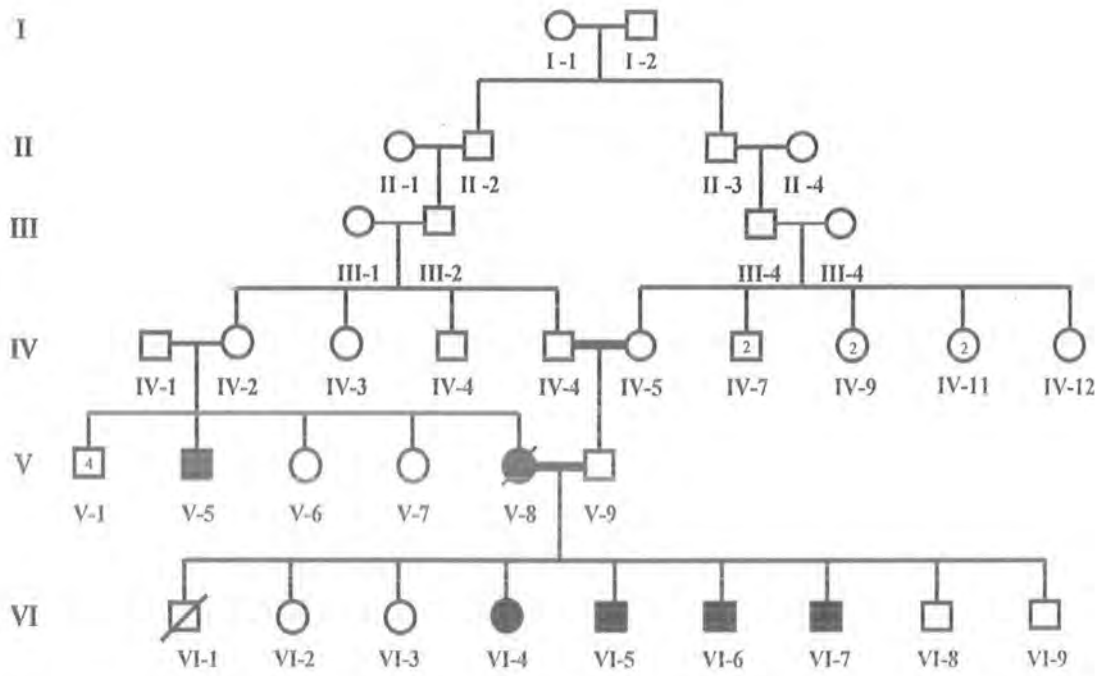


Figure 3.3: Pedigree of the family C with autosomal recessive non-syndromic hearing loss. Circles represent, squares represent males. Filled circles and squares represent affected individuals.

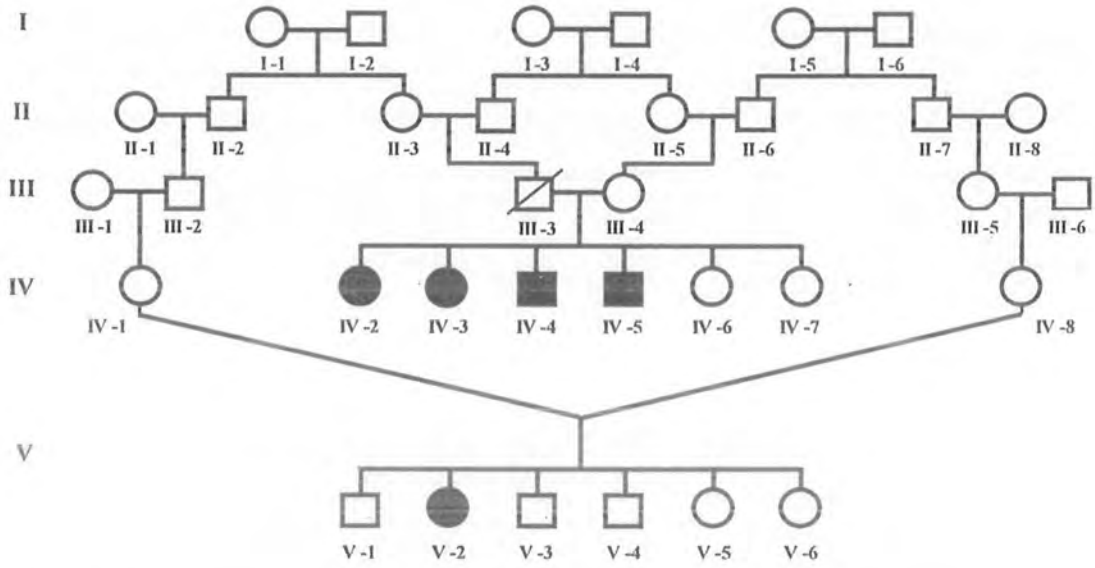
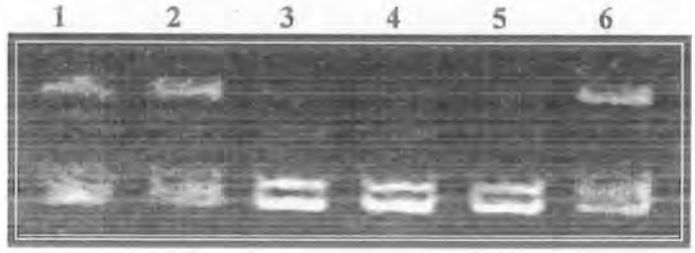


Figure 3.4: Pedigree of the family D with autosomal recessive non-syndromic hearing loss. Circles represent females, squares represent males. Filled circles and squares represent affected individuals.

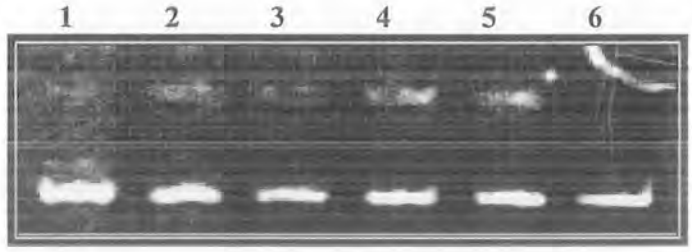


Family A

D13S1275

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

Figure 3.5: 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**. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.

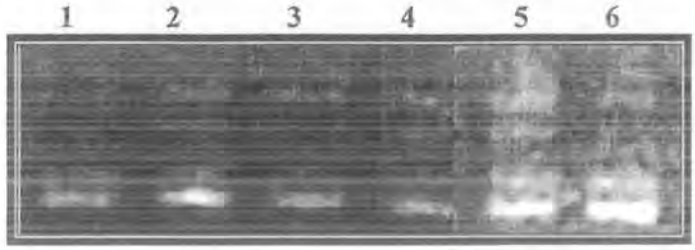


Family A

D13S787

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

Figure 3.6: 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**. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.



Family A

D13S292

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

Figure: 3.7: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D13S292 at 8.87 cM linked to **DFNB1**. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.

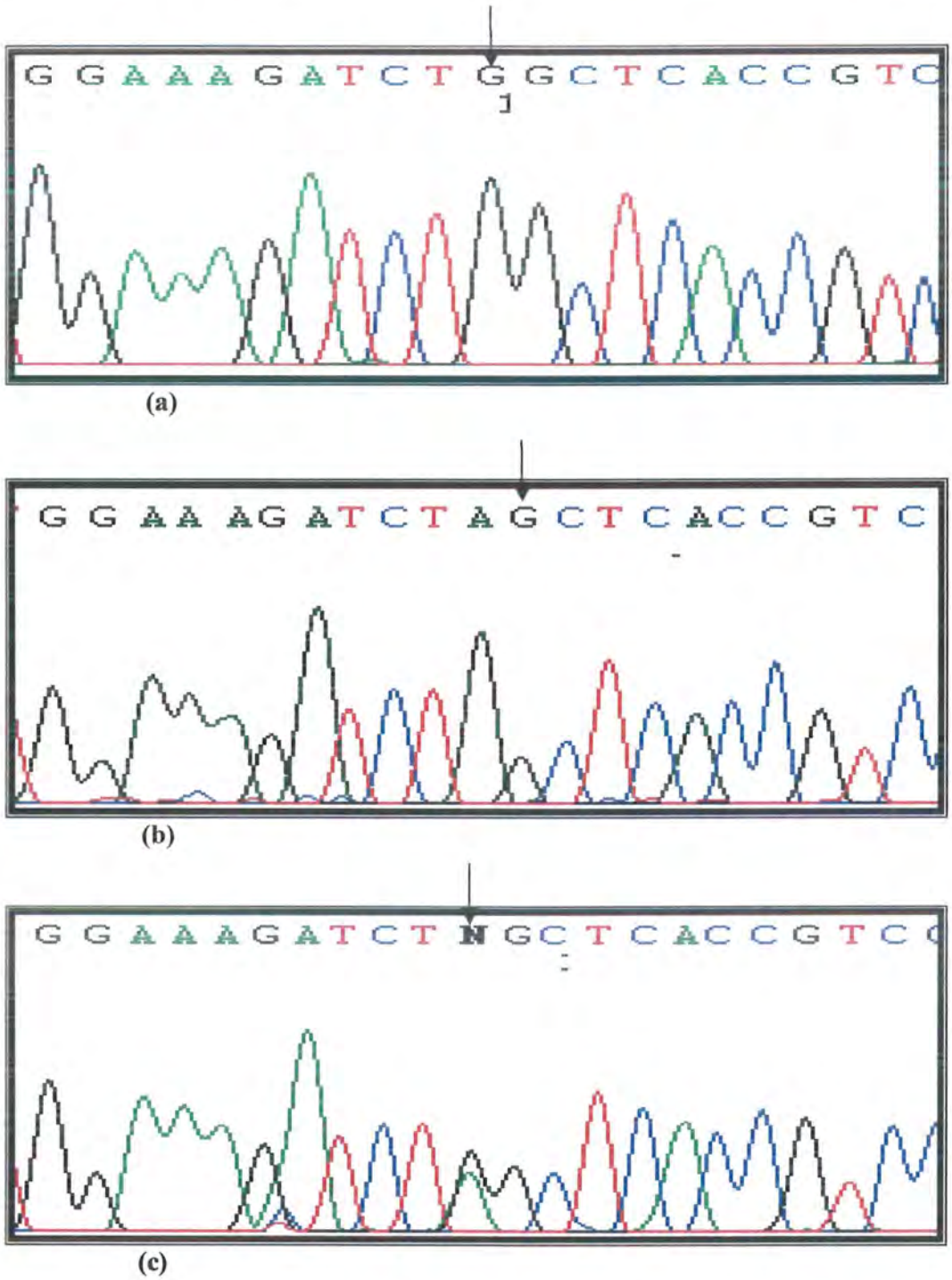
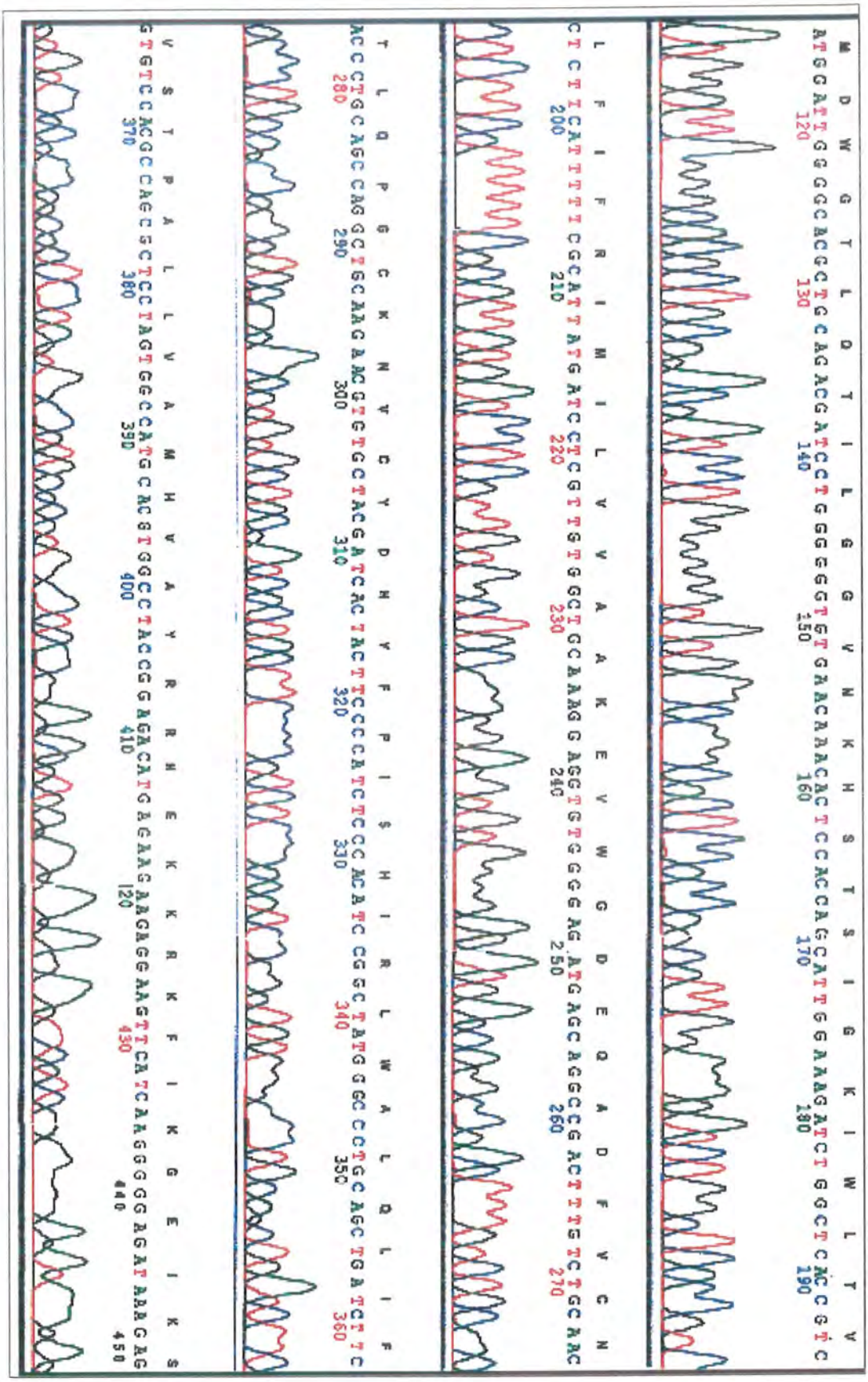


Figure 3.8: A representative chromatogram generated by big dye terminator sequencing of translated exon 2 of *GJB2* gene from control individual (**a**), a homozygous affected male IV-5 (**b**), and a heterozygous carrier (**c**). Arrow indicates the substitution of G with A, at nucleotide position 71, leading to non-sense mutation (W24X).



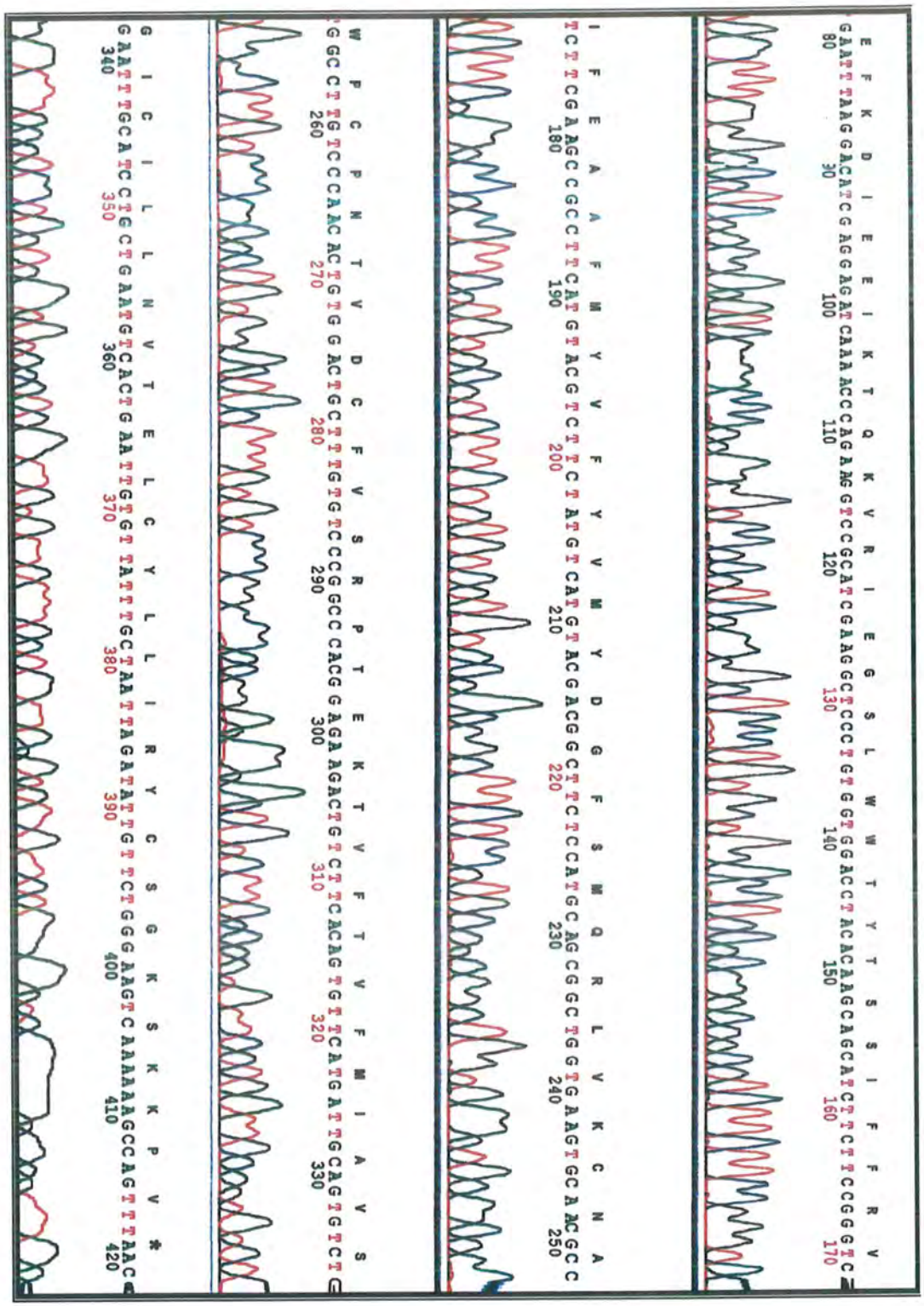
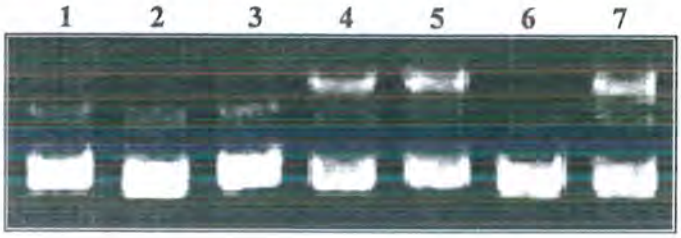


Figure 3.9: Representative chromatogram generated by big Dye Terminator; sequencing of the translated exon 2 of *GJB2* gene from an affected male individual (IV-2) of family B, showing wild type sequence.

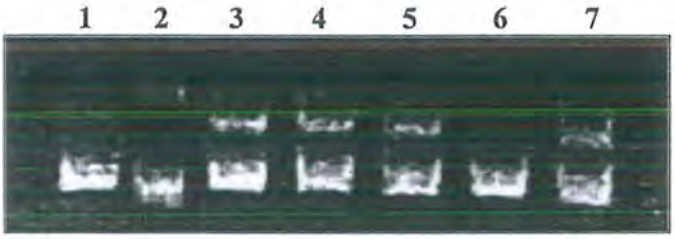


Family B

D9S302

1	IV-6	Affected	**5**	III-3	Normal
2	IV-9	Affected	**6**	IV-7	Affected
3	IV-5	Normal	7	III-4	Normal
4	IV-8	Normal			

Figure 3.10: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S302 at 123.33 cM linked to **DFNB31.** The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.

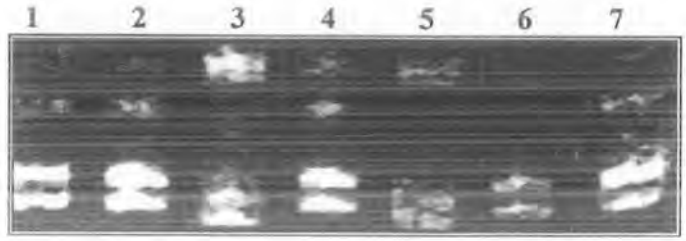


Family B

D9S1776

1	IV-6	Affected	**5**	III-3	Normal
2	IV-9	Affected	**6**	IV-7	Affected
3	IV-5	Normal	7	III-4	Normal
4	IV-8	Normal			

Figure 3.11: 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.** The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.

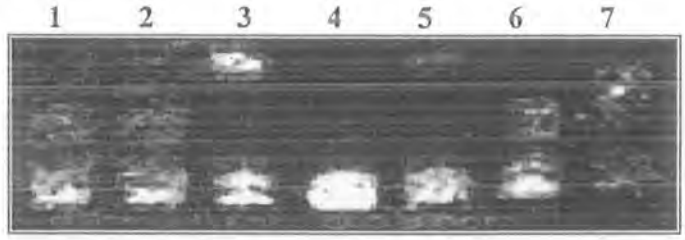


Family B

D5S647

1	IV-6	Affected	5	III-3	Normal
2	IV-9	Affected	6	IV-7	Affected
3	IV-5	Normal	7	III-4	Normal
4	IV-8	Normal			

Figure 3.12: 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.** The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.

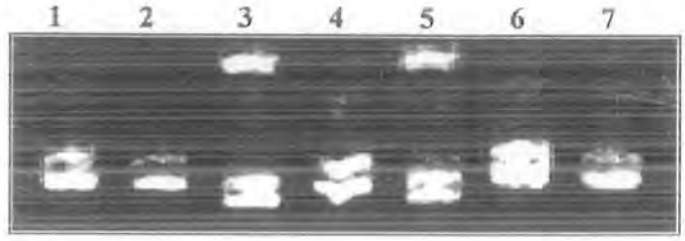


Family B

D5S2042

1	IV-6	Affected	5	III-3	Normal
2	IV-9	Affected	6	IV-7	Affected
3	IV-5	Normal	7	III-4	Normal
4	IV-8	Normal			

Figure 3.13: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D5S2042 at 78.31 cM linked to **DFNB49.** The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.

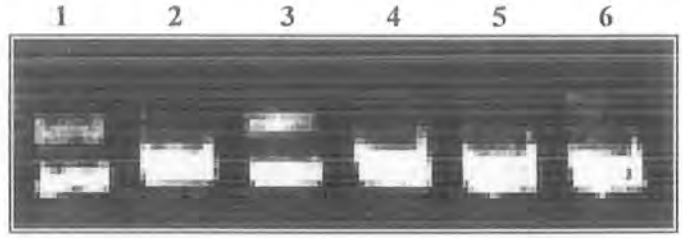


Family B

D5S1501

1	IV-6	Affected	5	III-3	Normal
2	IV-9	Affected	6	IV-7	Affected
3	IV-5	Normal	7	III-4	Normal
4	IV-8	Normal			

Figure 3.14: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D5S1501 at 85.25 cM linked to **DFNB49.** The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.

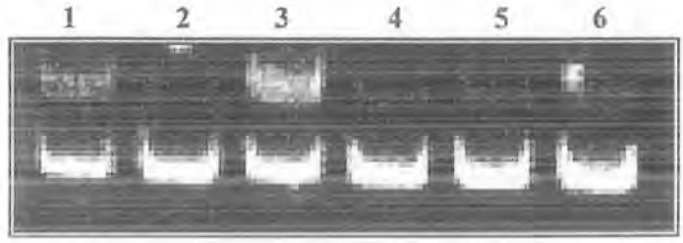


Family C

D12S1294

1	V-9	Normal	5	VI-7	Affected
2	VI-5	Affected	6	VI-4	Affected
3	VI-8	Normal			
4	VI-6	Affected			

Figure 3.15: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D17S1294 at 50.70 cM linked to **DFNB3.** The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.



Family C

D17S1293

1	V-9	Normal	**5**	VI-7	Affected
2	VI-5	Affected	**6**	VI-4	Affected
3	VI-8	Normal			
4	VI-6	Affected			

Figure 3.16: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D17S1293 at 56.58 cM linked to **DFNB3.** The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.

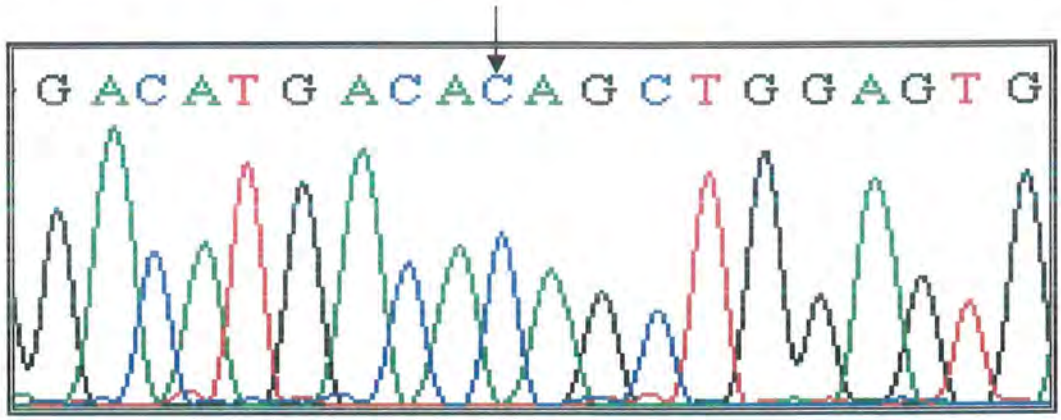


Figure 3.17: A representative chromatogram generated by big dye terminator sequencing of exon 3 of *MYO15A* gene from an affected female individual (IV-4) of family C showing wild type sequence. Arrow indicates a nucleotide C at position 3685, which has been substituted with T, resulting in a non-sense mutation (Wang *et al.*, 1998).

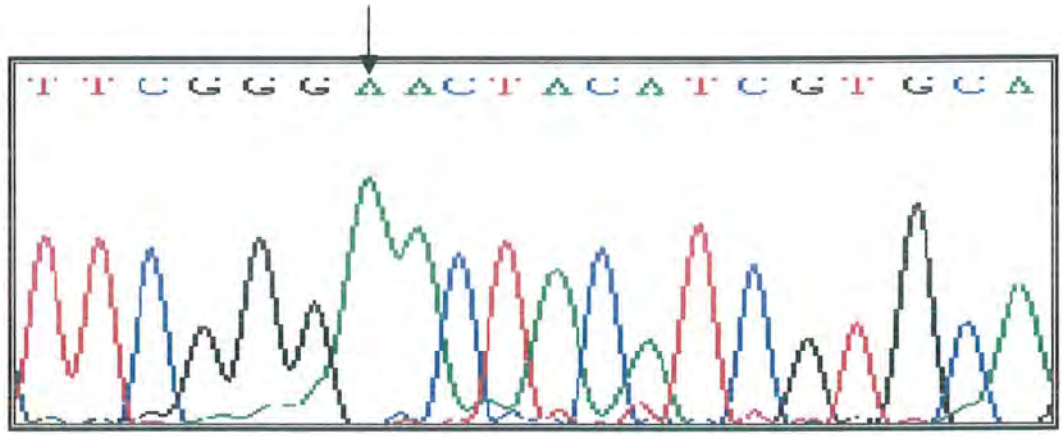


Figure 3.18: A representative chromatograms generated by big dye terminator, sequencing of exon 29 of *MYO15A* gene from an affected female individual (IV-4) of family C showing wild type sequence. Arrow indicates a nucleotide A at position 6331, which has been substituted with T, resulting in a missense mutation (Wang *et al.*, 1998).

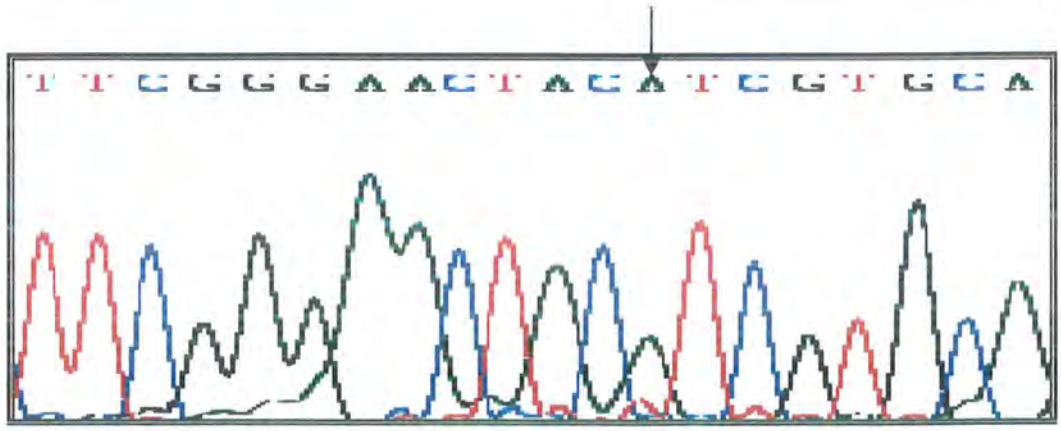


Figure 3.19: A representative chromatograms generated by big dye terminator, sequencing of exon 29 of *MYO15A* gene from an affected female individual (IV-4) of family C showing wild type sequence. Arrow indicates a nucleotide A at position 6337, which has been substituted with C, resulting in a missense mutation (Wang *et al.*, 1998).

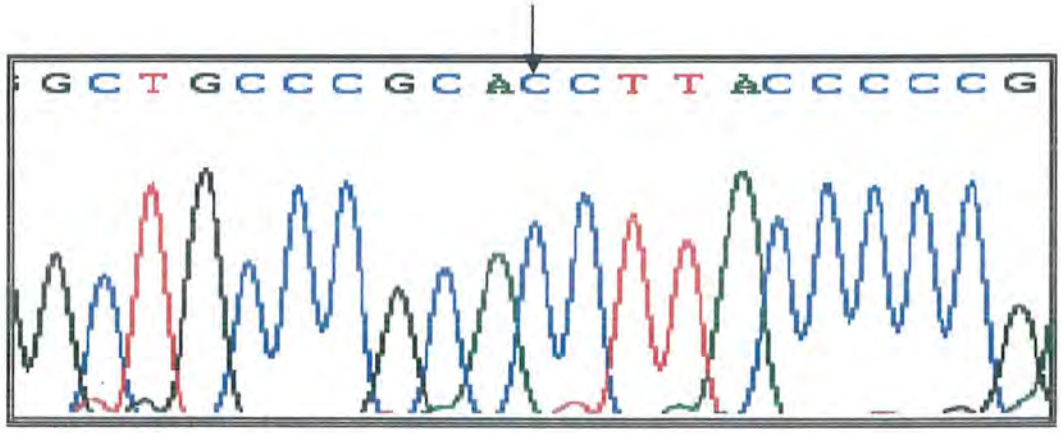


Figure 3.20: A representative chromatograms generated by big dye terminator, sequencing of exon 30 of *MYO15A* gene from an affected female individual (IV-4) of family C showing wild type sequence. Arrow indicates a nucleotide C at position 6613, which has been substituted with T, resulting in a missense mutation (Liburd *et al.*, 2001).

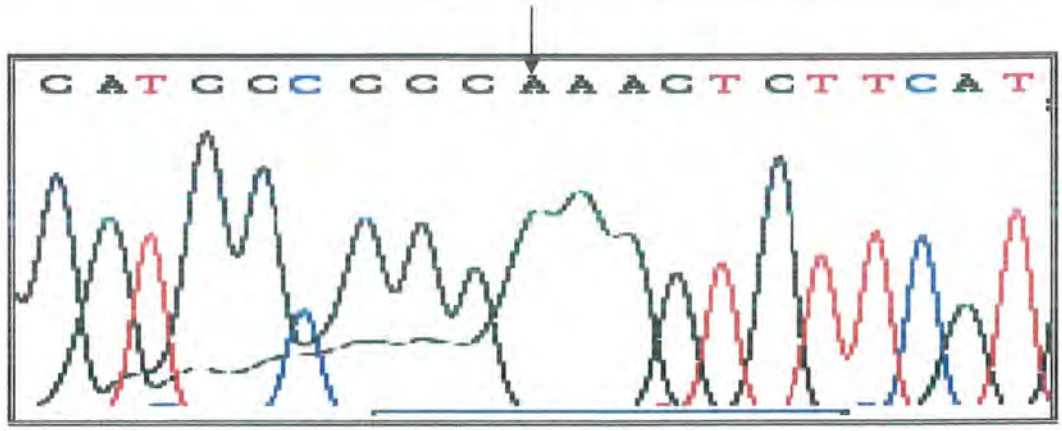


Figure 3.21: A representative chromatograms generated by big dye terminator, sequencing of exon 40 of *MYO15A* gene from an affected female individual (IV-4) of family C showing wild type sequence. Arrow indicates a nucleotide A at position 7801, which has been substituted with C, resulting in a non-sense mutation (Wang *et al.*, 1998).

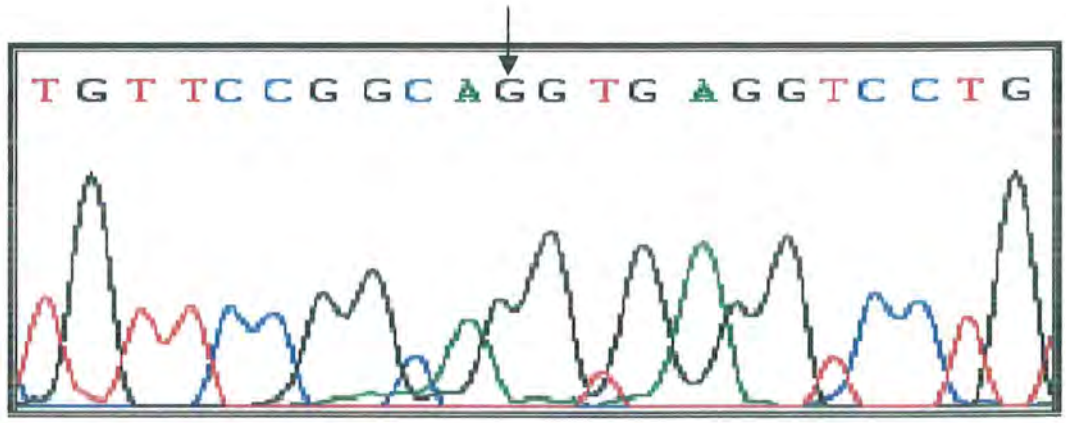
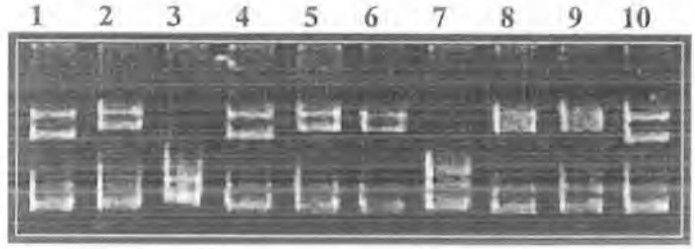


Figure 3.22: A representative chromatograms generated by big dye terminator, sequencing of exon 43 of *MYO15A* gene from an affected female individual (IV-4) of family C showing wild type sequence. Arrow indicates a nucleotide G at position 8147, which has been substituted with T, resulting in a missense mutation (Liburd *et al.*, 2001).

Table 3.1: List of allelic variants in *MYO15A* gene reported to date

Gene	Exon no	Nucleotide Sequence Variant	Amino Acid Change	References
MYO 15A	3	C3685>T	Q1229X	Liburd *et al.* (2001)
	29	A6331>T	N2111Y	Wang *et al.* (1998)
	29	A6337>C	I2113F	Wang *et al.* (1998)
	30	C6613>T	T2205I	Liburd *et al.* (2001)
	40	A7801>C	K2601X	Wang *et al.* (1998)
	43	G8147>T	Q2716H	Liburd *et al.* (2001)

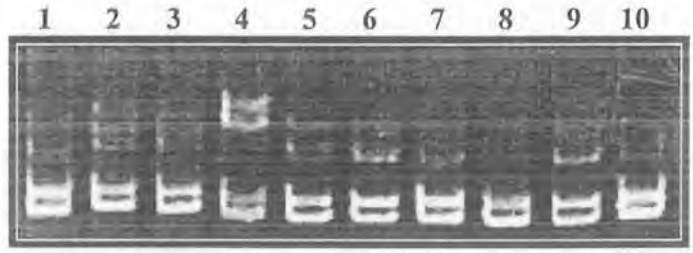


Family D

D9S169

1	IV-2	Affected	**6**	IV-8	Normal
2	IV-3	Affected	**7**	IV-1	Normal
3	III-4	Normal	**8**	V-2	Affected
4	IV-5	Affected	**9**	V-4	Normal
5	IV-6	Normal	**10**	IV-4	Affected

Figure 3.23: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S169 at 49.20 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.

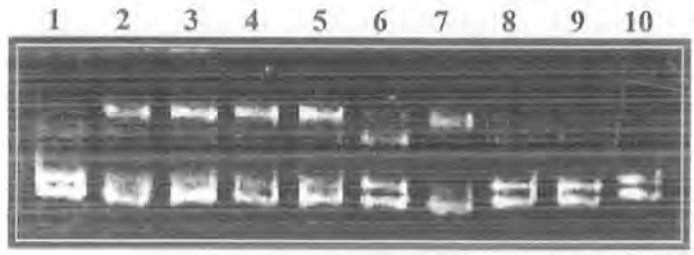


Family D

D9S104

1	IV-2	Affected	**6**	IV-8	Normal
2	IV-3	Affected	**7**	IV-1	Normal
3	III-4	Normal	**8**	V-2	Affected
4	IV-5	Affected	**9**	V-4	Normal
5	IV-6	Normal	**10**	IV-4	Affected

Figure 3.24: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S104 at 51.81 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.

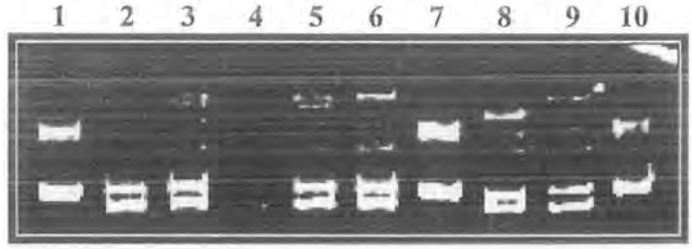


Family D

D9S1118

1	IV-2	Affected	**6**	IV-8	Normal
2	IV-3	Affected	**7**	IV-1	Normal
3	III-4	Normal	**8**	V-2	Affected
4	IV-5	Affected	**9**	V-4	Normal
5	IV-6	Normal	**10**	IV-4	Affected

Figure 3.25: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S1118 at 58.2 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.

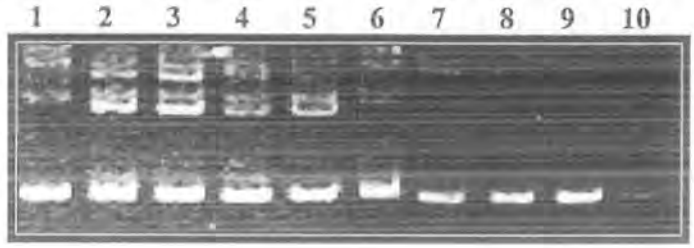


Family D

D9S50

1	IV-2	Affected	**6**	IV-8	Normal
2	IV-3	Affected	**7**	IV-1	Normal
3	III-4	Normal	**8**	V-2	Affected
4	IV-5	Affected	**9**	V-4	Normal
5	IV-6	Normal	**10**	IV-4	Affected

Figure 3.26: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S50 at 60.59 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.

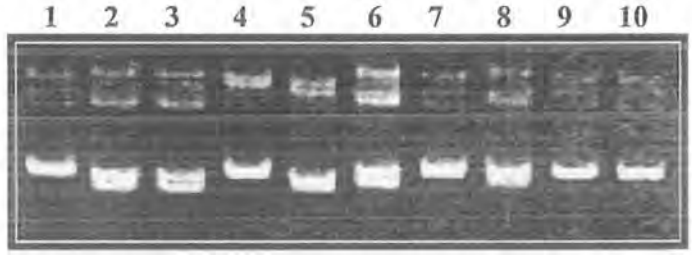


Family D

D9S166

1	IV-2	Affected	**6**	IV-8	Normal
2	IV-3	Affected	**7**	IV-1	Normal
3	III-4	Normal	**8**	V-2	Affected
4	IV-5	Affected	**9**	V-4	Normal
5	IV-6	Normal	**10**	IV-4	Affected

Figure 3.27: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S166 at 66.32 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.

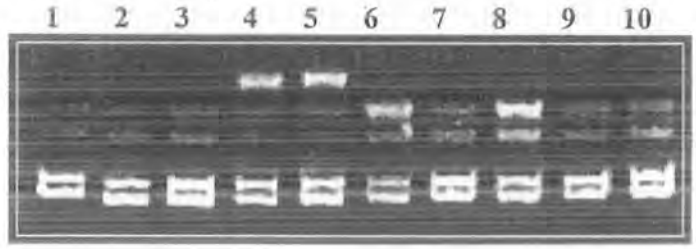


Family D

D9S301

1	IV-2	Affected	**6**	IV-8	Normal
2	IV-3	Affected	**7**	IV-1	Normal
3	III-4	Normal	**8**	V-2	Affected
4	IV-5	Affected	**9**	V-4	Normal
5	IV-6	Normal	**10**	IV-4	Affected

Figure 3.28: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S301 at 66.32 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation

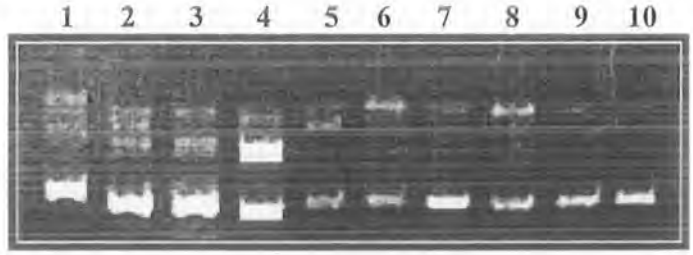


Family D

D9S175

1	IV-2	Affected	6	IV-8	Normal
2	IV-3	Affected	7	IV-1	Normal
3	III-4	Normal	8	V-2	Affected
4	IV-5	Affected	9	V-4	Normal
5	IV-6	Normal	10	IV-4	Affected

Figure 3.29: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S175 at 70.33 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.

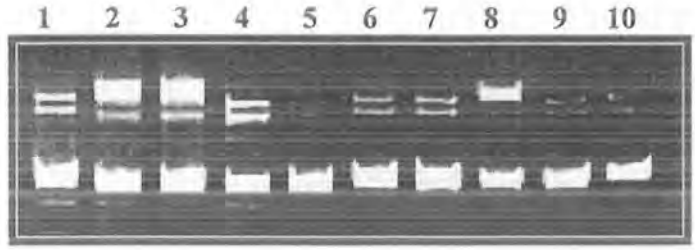


Family D

D9S1122

1	IV-2	Affected	6	IV-8	Normal
2	IV-3	Affected	7	IV-1	Normal
3	III-4	Normal	8	V-2	Affected
4	IV-5	Affected	9	V-4	Normal
5	IV-6	Normal	10	IV-4	Affected

Figure 3.30: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S1122 at 76.0 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.

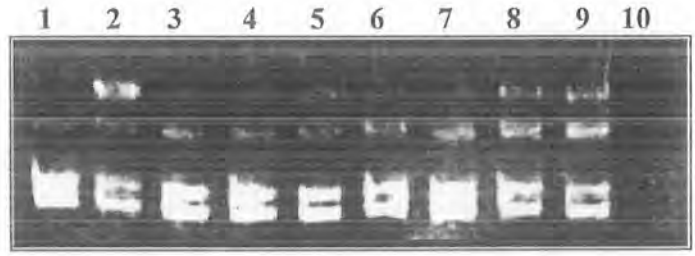


Family D

D9S922

1	IV-2	Affected	**6**	IV-8	Normal
2	IV-3	Affected	**7**	IV-1	Normal
3	III-4	Normal	**8**	V-2	Affected
4	IV-5	Affected	**9**	V-4	Normal
5	IV-6	Normal	**10**	IV-4	Affected

Figure 3.31: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D9S922 at 80.31 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.

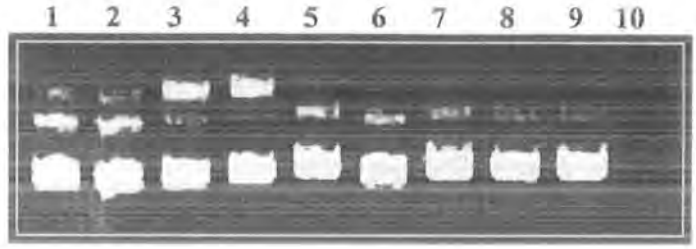


Family D

D10S1653

1	IV-2	Affected	**6**	IV-8	Normal
2	IV-3	Affected	**7**	IV-1	Normal
3	III-4	Normal	**8**	V-2	Affected
4	IV-5	Affected	**9**	V-4	Normal
5	IV-6	Normal	**10**	IV-4	Affected

Figure 3.32: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D10S1653 at 40.36 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.

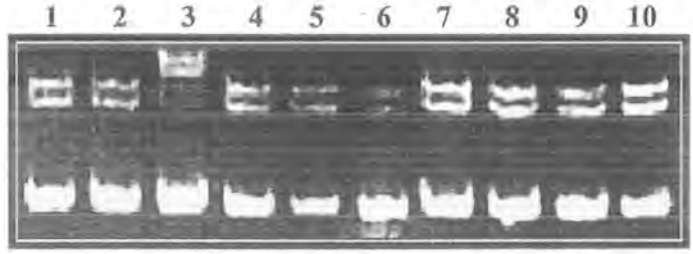


Family D

D10S1661

1	IV-2	Affected	6	IV-8	Normal
2	IV-3	Affected	7	IV-1	Normal
3	III-4	Normal	8	V-2	Affected
4	IV-5	Affected	9	V-4	Normal
5	IV-6	Normal	10	IV-4	Affected

Figure 3.33: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D10S1661 at 42.50 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.



Family D

D10S1423

1	IV-2	Affected	6	IV-8	Normal
2	IV-3	Affected	7	IV-1	Normal
3	III-4	Normal	8	V-2	Affected
4	IV-5	Affected	9	V-4	Normal
5	IV-6	Normal	10	IV-4	Affected

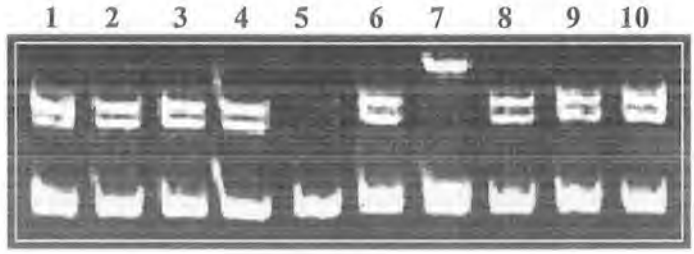
Figure 3.34: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D10S1423 at 43.26 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.

Family D

D10S1775

1	IV-2	Affected	**6**	IV-8	Normal
2	IV-3	Affected	**7**	IV-1	Normal
3	III-4	Normal	**8**	V-2	Affected
4	IV-5	Affected	**9**	V-4	Normal
5	IV-6	Normal	**10**	IV-4	Affected

Figure 3.35: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D10S1775 at 52 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.

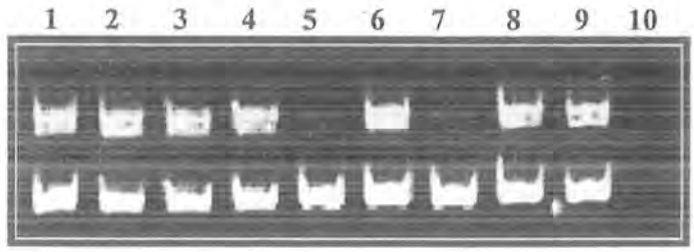


Family D

D10S2481

1	IV-2	Affected	**6**	IV-8	Normal
2	IV-3	Affected	**7**	IV-1	Normal
3	III-4	Normal	**8**	V-2	Affected
4	IV-5	Affected	**9**	V-4	Normal
5	IV-6	Normal	**10**	IV-4	Affected

Figure 3.36: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D10S2481 at 52.1 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.



Family D

D10S1160

1	IV-2	Affected	**6**	IV-8	Normal
2	IV-3	Affected	**7**	IV-1	Normal
3	III-4	Normal	**8**	V-2	Affected
4	IV-5	Affected	**9**	V-4	Normal
5	IV-6	Normal	**10**	IV-4	Affected

Figure 3.37: Electropherogram of the ethidium bromide stained 8% non-denaturing polyacrylamide gel showing allele pattern obtained with marker D10S1160 at 54.23 cM. The Roman numerals indicate the generation number of the individuals within a pedigree while Arabic numerals indicate their positions within a generation.

DISCUSSION

DISCUSSION

Non-syndromic hearing loss in human is the most genetically heterogeneous trait known. To date 68 autosomal recessive deafness loci (DFNB1-DFNB68) have been mapped and 22 of the corresponding genes have been identified so far. 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. An exception to preverbal onset is the DFNB8 locus which mapped to the long arm of chromosome 21, where the affected individuals have normal hearing until 10 years of age but the loss profound within 4-5 years (Veske *et al.*, 1996).

In the present study, four highly consanguineous families A, B, C, and D, demonstrating autosomal recessive form of non-syndromic hearing loss, were ascertained from isolated areas of Azad Jammu Kashmir, NWFP and Punjab. 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.

To search for a locus that harbored the candidate gene responsible for autosomal recessive non-syndromic hearing loss in these families, linkage studies were performed by a method known as homozygosity mapping. Nearly a century ago Sir Archebald 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 the genome of offspring of consanguineous matings would be expected to be homozygous because of identity by descent (Lander and Bostein, 1987). On average, $1/16^{th}$ 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 at a common disease locus shared by the 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 a first cousin marriage or three affected sibs in the second cousin marriage are required.

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 segment as short as 9 cM may be detected when the markers are 1 cM apart.

In the present study family 'A' showed linkage to DFNB1 locus. Sequence analysis of *GJB2* gene in an affected individual (IV-5) (Figure 3.8) revealed a substitution of G with A at nucleotide position 71 resulting in conversion of an amino acid tryptophane to premature stop codon (W24X).

Family B, showed linkage to DFNB31 (Figure 3.10-3.11) on chromosome 9q32 and DFNB49 (3.12-3.14) on chromosome 5q12.3. The candidate gene involved in autosomal recessive deafness DFNB31 locus is *WHRN* (Mburu *et al.*, 2003). *WHRN* consists of 12 exons and spans 103 Kb and encodes for a protein called whirlin. A non-sense mutation has been reported in this gene by Mburu *et al.* (2003). The linkage region for DFNB49 is 11 cM (Ramazan *et al.*, 2004). Candidate gene for this locus has not been identified so far.

In family 'C' linkage was detected to DFNB3 locus on chromosome 17p11.2. The candidate gene involved in autosomal recessive deafness at this locus is *MYO15A* (Wang *et al.*, 1998). The gene comprises of 66 exons. Six disease causing mutations have been reported till date in exon number 3, 29, 30, 40, and 43 of *MYO15A* gene. In the present study, sequence analysis of these exons in an affected individual (IV-4) of family 'C' (Figure 3.17-3.22) failed to detect any sequence variant, thus predicting the presence of a novel mutation in the family.

In family 'D' the selected individuals were tested for the co-segregation of disease with regions on chromosome 9 and 10. Analysis of the results of the genotyped microsatellite markers (Figure 3.23-3.37) showed that the affected individuals were heterozygous with different combinations of parental alleles thus, excluding family D from linkage to these genetic intervals.

Genetics and genomics approaches in human and model organisms have provided a powerful and rapid entry into gene discovery in the auditory system. The recent

identification of several deafness genes by molecular genetic studies has enabled the molecular basis of normal and pathological auditory function. In the coming years, further deafness genes are sure to be identified and mouse models for the human disease will be constructed as start in the long process of understanding the pathological process involved in deafness. 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 (EST's) within genetic intervals known to contain locus for human hereditary hearing loss. Recent progress in the identification of short tandem repeats polymorphic markers and the construction of high density genetic maps utilizing these markers have greatly improved the efficiency of genetic linkage studies. These markers and maps have contributed to efficient linkage mapping by increasing the overall availabilities of these resources to many laboratories:

- by reducing the amount of labor required for genotyping
- by allowing for the development of new genotyping technologies
- by facilitating novel approaches to genome-wide linkage searches

To assist in the identification of deafness genes cDNA library has been synthesized, partially sequences and many ESTs assigned map position (Skvorak *et al.*, 1999). The combination of efficient approaches to phenotype mapping with the development of an accurate transition map of the human genome will greatly expedite the identification of disease-causing and –susceptibility genes.

To this end we ever close to an enhanced understanding of the hearing process, which lead to increased availabilities of diagnostic and presymptomatic genetic testing option, early interventions disease based treatments.

REFERENCES

References

Abe S, Usami S, Shinkawa H, Kelley PM, Kimberling WJ (2000) Prevalent connexin 26 gene (*GJB2*) mutations in Japanese. J Med Genet 37:41-43

Ahmad ZM, Riazudin S, Wilcox ER (2003) The molecular genetics of Usher syndrome. Clin Genet 63:431-444

Astuto LM, Bork JM, Weston MD, Askew JW, Fields RR, Orten DJ, Ohliger SJ, Riazuddin S, Morell RJ, Khan S, Riazuddin S, Kremer H, van Hauwe P, Moller CG, Cremers CW, Ayuso C, Heckenlively JR, Rohrschneider K, Spandau U, Greenberg J, Ramesar R, Reardon W, Bitoun P, Millan J, Legge R, Friedman TB, Kimberling WJ (2002) *CDH23* mutation and phenotype heterogeneity: a profile of 107 diverse families with Usher syndrome and non-syndromic deafness. Am J Hum Genet 2:262-275

Bitner-Glindzicz M (2002) Hereditary deafness and phenotyping in humans. Br Med Bull 63:73-94

Boeda B, El-Amraoui A, Bahloul A, Goodyear R, Daviet L, Blanchard S, Perfettini I, Fath KR, Shorte S, Reiners J, Houdusse A, Legrain P, Wolfrum U, Richardson G, Petit C (2002) Myosin VIIA, harmonin and cadherin 23, three Usher I gene products that cooperate to shape the sensory hair cell bundle. EMBO J 21:6689-6699

Bolz H, von Brederlow B, Ramirez A, Bryda EC, Kutsche K, Nothwang HG, Seeliger M, del C-Salcedo, Cabrera M, Vila MC, Molina OP, Gal A, Kubisch C (2001) Mutation of *CDH23*, encoding a new member of the cadherin gene family, causes Usher syndrome type 1D. Nat Genet 27:108-112

Borck G, Roth C, Martine U, Wildhardt G, Pohlenz J (2003) Mutations in PDZ gene in German families with pendreds syndrome: V138F is a founder mutation. J Clin Endocrinol Metab 88:2916-2921

Bork JM, Peters LM, Riazuddin S, Bernstein SL, Ahmed ZM, Ness SL, Polomeno R, Ramesh A, Schloss M, Srisailpathy CR, Wayne S, Bellman S, Desmukh D, Ahmed Z, Khan SN, Kaloustian VM, Li XC, Lalwani A, Riazuddin S, Bitner-Glindzicz M, Nance WE, Liu XZ, Wistow G, Smith RJ, Griffith AJ, Wilcox ER, Friedman TB, Morell RJ (2001) Usher syndrome 1D and non-syndromic

autosomal recessive deafness DFNB12 are caused by allelic mutations of the novel Cadherin-like gene *CDH23*. Am J Hum Genet 68:26-37

Bruzzone R, White TW, Goodenough DA (1996) The cellular internet On-line with connexins. Bioessay 18:709-718

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

Campbell C, Cucci RA, Prasad S, Green GE, Edeal JB, Galer CE, Karniski LP, Scheffield VC, Smith RJ (2001) Pendred syndrome, DFNB4, and PDZ/*SLC26A4* identification of eight novel mutations and possible genotype-phenotype correlation Hum Mutat 17:403-411

Chang EH, Van Camp G, Smith RJ (2003) The role of connexins in human disease. Ear Hear 24:314-323

Denoyelle F, Lina-Granade G, Plauchu H, Bruzzone R, Chaib H, Levi-acobas F, Weil D, Petit C (1998) Connexin 26 gene linked to a dominant deafness. Nature 393:319-319

Everett La, Glaser B, Beck JC, Idol JR, Buchs A, Everett LA, Glaser B, Beck JC, Idol JR, Buchs A, Heyman M, Adawi F, Hazani E, Nassir E, Baxevanis AD, Sheffield VC, Green ED (1997) Pendred syndrome is caused by mutations in a putative sulphate transporter gene (PDZ). Nat Genet 17:411-422

Fransen E, Verstreken M, Verhagen WIM, Wuyts FL, Huygen PLM, D'Haese P, Robertson NG, Morton CC, McGuirt WT, Smith RJH, Declau F, Heyning PH, Camp G (1999) High prevalence of symptoms of Meniére's disease in three families with a mutation in the *COCH* gene. Hum Mol Genet 8:1425-1429

Friedman TB, Grifith AJ (2003) Human non-syndromic sensorineural deafness. Annu Rev Genom Hum Genet 4:341-402

Friedman TB, Liang Y, Webber JL, Hinnant JT, Barber TD, Winata S, Arhya IN, Asher JH Jr (1995) A gene for congenital, recessive deafness DFNB3 maps to the pericentromeric region of chromosome 17. Nat Genet 9:86-91

Goodenough DA, Goliger JA, Paul DL (1996) Connexins, connexons, and intercellular Communication. Annu Rev Biochem 65:475-502

Grabski R, Szul T, Sasaki T, Timpl R, Mayne R, Hicks B, Sztul E (2003) Mutations in *COCH* that results in non-syndromic autosomal dominant deafness (DFNA9) affect matrix deposition of cochlin. Hum Genet 113:406-416

Guilford P, Ben Arad S, Blanchard S, Levilliers J, Weissenbach J, Belkahia A, Petit C (1994) A non-syndromic form of neurosensory, recessive deafness maps to the pericentromeric region of chromosome 13q. Nat Genet 6:24-28

Gullponi M, Vuagniaux G, Wattenhofer M, Shibuya K, Vazquez M, Dougjerty L, Scamuffa N, Guida E, Okul M, Rossier C, Hancock M, Buchet K, Reymond A, Hummler E, Marzella PL, Kudoh J, Shimizu N, Scott HS, Antonarakis SE, Rossier BC (2002) The transmembrane serine protease (*TMPRSS3)* mutated in deafness DFNB8/10 activates the epithelial sodium channel (ENaC) in vitro. Hum Mol Genet 11:2829-2836

Gurtler N, Lalwani AK (2002) Etiology and non-syndromic sensorineural hearing loss. Otolaryngol Clin North Am 35:891-908

Hand GM, Muller DJ, Nicholson BJ, Engel A, Sosinsky GE (2002) Isolation and characterization of gap junction from tissue culture cells. J Mol Biol 315:587-600

Jain PK, Fukushima K, Deshmukh D, Ramesh A, Thomas E, Lalwani Ak, Kumar S, Plopis B, Skarka H, Srisailapathy CR (1995) A human recessive neurosensory non-syndromic hearing impairment locus is potential homologue of murine deafness (dn) locus. Hum Mol Genet 4:2391-2394

Jan AY, Amin S, Ratajczak P, Richard G, Sybert VP (2004) Genetic heterogeneity of KID syndrome identification of a Cx30 gene (*GJB6*) mutation in a patient with KID syndrome and congenital artichia. J Invest Dermatol 122:1108-1113

Jovine L, Park J, Wassarman PM (2002) Sequence similarity between stereocilin and otoancorin points to a unified mechanism for mechanotransduction in the mammalian inner ear. BMC Cell Biol 3:28-32

Kamarinos M, McGill J, Lynch M, Dahl H (2001) Identification of a novel *COCH* mutation, 1109 N highlights the similar clinical features observed in DFNA9 families. Hum Mut 408:1-6

Kelley PM, Cohn E, Kimberling WJ (2000) Connexin 26: required for normal auditory function. Brain Res Rev 32:184-188

Kelsell DP, Dunlop J, Stevens HP, Lench NJ, Liang JN, Parry G, Muller RF, Leigh IM (1997) Connexin 26 mutation in hereditary non-syndromic sensorineural deafness. Nature 387:80-83

Khetarpal U (1993) Autosomal dominant sensorineuralhearing loss. Further temporal bone findings. Arch Otolaryngol Head Neck Surg 119:106-108

Khetarpal U, Schuknecht HF, Gaecek RR, Holmes LB (1991) Autosomal dominant sensorineural hearing loss. Pedigrees, audiologic findings, and temporal bone findings in two kindreds. Arch Otolaryngol Head Neck Surg 117:1032-1042

Kiernan AE, Zalzman M, Fuchs H, de Angelis MH, Balling R, Balling M, Steel KP, Avraham KB (1999) Tailchaser (Tlc): a new mouse mutation affecting hair bundle differentiation and hair cell survival. J Neurocytol 28:969-985

Kikuchi T, Kimura RS, Paul DL, Adams JC (1995) Gap junctions in the rat cochlea: immunohistochemical and ultrastructural analysis. Anat Embryol 191:101-188

Kok de, Cremers C.W, Ropers H, Cremers F (1997) The molecular basis of X-linked deafness type 3 (DFN3) in two sporadic cases: identification of a somatic mosaicism for a *POU3F* missense mutation. Hum Mut 10:207-211

Kok de, Maarel van der, Bitner- Glindzicz M, Huber I, Monaco A, Malcolm A, Pembrey M, Ropers H, Cremers F (1995) Association between X-linked mixed deafness and mutation in the POU domain gene *POU3f4*. Science 267:685-688

Kok YJM de, Bom SJH, Brunt TM, Kemperman MH, Beusekom E van, Velde-Visser SD, Robertson NG, Morton CC, Huygen PLM, Verhagen WIM, Brunner HG, Cremers CWRJ, Cremers FPM (1999) A Pro51Ser mutation in the *COCH* gene is associated with late onset autosomal dominant progressive sensorineural hearing loss with vestibular defects. Hum Mol Genet 8:361-366

Kumar NM, Gilula NB (1996) The gap junction communication channel. Cell 84:381-388

Kurima K, Peters LM, Yang Y, Riazuddin S, Ahmed ZM, Naz S, Arnaud D, Drury S, Mo J, Makishima T, Ghosh M, Menon P S, Deshmukh D, Oddoux C, Ostrer H, Khan S, Riazuddin S, Deininger PL, Hampton LL, Sullivan SL, Battey JF, Keats BJ, Wilcox ER, Friedman TB, Griffith AJ (2002) Dominant and

recessive deafness caused by mutations of a novel gene, *TMC1*, required for cochlear hair-cell function. Nat Genet 30:277-284

Lander ES, Bostein D (1987) Homozygosity mapping: a way to map human recessive traits with the DNA of inbred Children. Science 236:1567-1570

Lautermall J, ten-Cate WJ, Alltenhoff P, Grummer R, Traub O, Frank H, Jahnke K, Winterhager E (1998) Expression of the gap-junction connexins 26 and 30 in the rate cochlea. Cell Tissue Res 294:415-420

Leenheer EM, Ensink RJ, Kunst HP, Marres HA, Talebizadeh Z, Declau F, Smith SD, Usami S, Van de Heyning PH, Van Camp G, Huygen PL, Cremers CW (2002) DFNA2/KCNQ4 and its manifestations. Adv Otorhinolaryngol 61:41-46

Leon PE, Lalwani AK (2002) Auditory phenotype of DFNA. Adv Otorhinolaryngol 6:34-40

Li XC, Everett LA, Lalwani AK, Desmukh D, Friedman TB, Green ED, Wilcox ER (1998) A mutation in PDS causes non-syndromic recessive deafness. Nat Genet 18:215-217

Liang Y, Wang A, Probst FJ, Arhya IN, Barber TD, Chen KS, Deshmukh D, Dolan DF, Hinnant JT, Carter LE, Jain PK, Lalwani AK, Li XC, Lupski JR, Moeljopawiro S, Morell R, Negrini C, Wilcox ER, Winata S, Camper SA, Friedman TB (1998) Genetic mapping refines DFNB3 to 17p11.2, suggests multiple alleles of DFNB3, and supports homology to the mouse model shaker-2. Am J Hum Genet 62:904-915

Liburd N, Ghosh M, Riazuddin S, Naz S, Khan S, Ahmed Z, Riazuddin S, Liang Y, Menon PS, Smith T, Smith AC, Chen KS, Lupski JR, Wilcox ER, Potocki L, Friedman TB (2001) Novel mutations of *MYO15A* associated with profound deafness in consanguineous families and moderately severe hearing loss in a patient with Smith-Magenis syndrome. Hum Genet 109:535-541

Liepinsh E, Baryshev M, Sharipo A, Ingelman-Sundberg M, Otting G, Mkrt-Chian S (2001) Thioredoxin fold as homodimerization module in the putative chaperon ERp29: NMR structures of the domains and experimental model of the 51 kDa dimmer. Structure 9:457-471

Liu XZ, Walsh J, Mburu P, Kendrick-jones J, Cope MJ, Steel KP, Brown SD (1997) Mutation in the myosin VIIA gene cause non-syndromic recessive deafness. Nat Genet 16:188-190

Liu XZ, Walsh J, Tamagawa Y, Kitamura K, Nishizawa M, Steel KP, Brown SD (1997) Autosomal dominant non-syndromic deafness caused by a mutation in the myosin VIIA gene. Nat Genet 16:191-193

Liu XZ, Xia XJ, Xu LR, Pandya A, Liang CY, Blanton SH, Brown SD, Steel KP, Nance WE (2000) Mutation in connexin 31 underlie recessive as well as dominant non-syndromic hearing loss. Hum Mol Genet 9:63-67

Lopez-Bigas N, Olive M, Rabionet R, Ben-David O, Martinez-Matos JA, Bravo O, Banchs I, Volpini V, Gasparini P, Avraham KB, Ferrer I, Arbones ML, Estivill X (2001) Connexin 31 (*GJB3*) is expressed in the peripheral and auditory nerves and cause neuropathy and hearing impairment. Hum Mol Genet 10:947-952

Lopponen T, Vaisanen ML, Luotonen M, Allinen M, Uusimaa J, Lindholm P, Maki-Torkko E, Vayrynen M, Lopponen H, Leisti J (2003) Connexin 26 muations and non-syndromic hearing impairment in northern Finland. Laryngoscope 113:1758-1763

Manolis EN, Yandavi N, Nadol JB, Eavey RD, Mckenna M, Rosenbaum S, Khetarpal U, Halpin C, Merchant SN, Duyk GM, Macrae C, Seidman CE, Siedman JG (1996) A gene for non-syndromic autosomal dominant progressive postlingual sensorineural hearing loss maps to chromosome 14q12-13. Hum Mol Genet 5:1047-1050

Marazita ML, Ploughman LM, Rawalings B, Remington E, Arnos KS, Nance WE (1993) Genetic epidemiology studies of early-onset deafness in the US School-age population. Am J Med Genet 46:486-491

Martin PE, Coleman SL, Casalotti SO, Forge A, Evans WH (1999) Properties of connexin 26 gap junction protein derived from mutations associated with non-syndromic hereditary deafness. Hum Mol Genet 8:2369-2376

Masmoudi S, Antonarakis SE, Schwede T, Ghorbel AM, Gratri M, PappaSavas MP, Drira M, Elgaied-Boulila A, Wattenhofer M, Rossier C, Scott HS, Ayadi H, Gulpponi M (2001) Novel missense mutations of *TMPRSS3* in two

consanguineous Tunisian families with non-syndromic autosomal recessive deafness. Hum Mut 18:101-108

Mburu P, Mustapha M, Varela A, Weil D, El-Amraoui A, Holme RH, Rump A, Hardisty RE, Blanchard S, Coimbra RS, Perfettini I, Parkinson N, Mallon AM, Glenister P, Rogers MJ, Paige AJ, Moir L, Clay J, Rosenthal A, Liu XZ, Blanco G, Steel KP, Petit C, Brown SD (2003) Defects in whirlin, a PDZ domain molecule involved in stereocilia elongation, cause deafness in the whirler mouse and families with DFNB31. Nat Genet 4:421-428

Miano, Maria G, Samuel G, Jacobson, Andrew Carothers, Isabel Hanson, Peter Teague, Lovell J, Artur V, Cideciyan, Neena Haider, Edwin M Stone, Val C, Sheffield, Alan F Wright (2000) Pitfalls in Homozygosity Mapping. Am J Hum Genet 67:1348-1351

Mirghomizadeh F, Pfister M, Apaydin F, Petit C, Kupka S, Pusch CM, Zenner HP, Blin N (2002) Substitution in the conserved C2C domain of *otoferlin* cause DFNB9, a form of non-syndromic autosomal recessive deafness. Neurobiol Dis 10:157-164

Mitchem K, Hibbard E, Beyer L, Bosom K, Dolan D, Johnson K, Raphael Y, Kohram D (2002) Mutation of the novel gene *TMIE* results in sensory cell defects in the inner ear of spinner, a mouse model of human hearing loss DFNB6. Hum Mol Genet 11:1887-1898

Morell RJ, Kim HJ, Hood Lj, Goforth L, friderici K, Fisher R, Van camp G, Berlin CL, Oddoux C, Ostrer H, keats B, Friedman TB (1998) Mutations in the connexin 26 gene (*GJB2*) among Ashkenazi jews with non-syndromic recessive deafness. N Engl J Med 339:1500-1505

Morton CC (2002) Genetics, genomics and gene discovery in theauditory system. Hum Mol Genet 11:1229-1240

Motron NE (1991) Genetic epidemiology of hearing impairment. Ann NY Acad Sci 630:16-31

Nagafuchi A (2001) Molecular architecture of adherens junctions. Curr Opin Cell Biol 13: 600-603

Nance WE (2003) The genetics of deafness. Ment Retard Dev Disabil Res 9:109-119

Naz S, Giguere C, Kohrman D, Mitchem K, Riazuddin S, Morell R, Ramesh A, Srisailpathy S, Deshmukh D, Riazuddin S, Griffith A, Friedman T, Smith R, Wilcox E (2002) Mutations in a novel gene, *TMIE*, are associated with hearing loss linked to the DFNB6 Locus. Am J Hum Genet 71:632-636

Naz S, Grifith AJ, Riazuddin S, Hampton LL, Battey JF, Khan SN, Riazuddin S, Wilcox ER, Friedman TB (2004) Mutations of *ESPN* cause autosomal recessive deafness and vestibular dysfunction. J Med Genet 41:591-595

Noben-Trauth K, Zheng QY, Johnson KR (2003) Association of cadherin 23 with polygenic inheritance and genetic modification of sensorineural hearing loss. Nat Genet 35:21-23

Nollet F, Kools P, Roy F (2000) Phylogeneti analysis of the cadherin superfamily allows identification of six major subfamilies besides several solitary members. J Mol Biol 299:551-572

Oh N, Kupka S, Mirghomizadeh F, Arold R, Zimmermann N, Blin N, Zenner HP, Pfister M (2003) Clinical and molecular genetics analysis of monozygotic twins displaying stapes gusher syndrome DFN3. HNO 51:629-633

Palma F, Holme RH, Bryda EC, Belyantseva IA, Pellegrino R, Kachar B, Steel KP, Noben-Trauth K (2001) Mutations in Cdh23, encoding a new type of cadherin, cause stereocilia disorganization in waltzer, the mouse model for Usher syndrome type 1D. Nat Genet 27:103-107

Petersen MB (2002) Non-syndromic autosomal-dominant deafness. Clin Genet 62:1 -13

Petit C (1996) Genes responsible for human hereditary deafness: symphony of a thousand. Nat Genet 14:385-391

Ramazan K, Shaikh SR, Jamil A, Shaheen NK, R, Sima R, Zubair MA, Thomas B, Friedman TB, Wilcox ER, Riazuddin S (2004) A new locus for non-syndromic deafness DFNB49 maps to chromosome 5q12.3-q14.1. Hum Genet 116:17-22

Robertson N, Hamaker S, Patriub V, Aseter J, Morton C (2003) Subcellular localization, section, and post-translational processing of normal cochlin, and of mutants causing the sensorineural deafness and vestibular disorder DFNA9. J Med Genet 40:479-486

Robertson NG, Lu L, Heller S, Merchant SN, Eavey RD, Mckenna M, Nadol JB, Miyamoto RT, Linthicum FH, Lubianca Neto JF, Hudspeth AJ, Seidman CE, Morton CC Siedman JG (1998) Mutations in a novel cochlear gene cause DFNA49, a human nonsyndromic deafness deafness with vestibular dysfunction. Nat Genet 20:299-303

Robertson NG, Skvorak AB, Yin Y, Weremowicz S, Johnson KR, Kovatch KA, Battey JF, Bieber FR, Morton CC (1997) Mapping and characterization of a novel cochlear gene in human and in mouse: a positional candidate gene for a deafness disorder. DFNA9. Genomics 46:345–354

Rodriguez-Ballesteros M, Castillo FJ, Martin Y, Moreno-Pelayo MA, Morera C, Prieto F, Marco J, Morant A, Gallo-Teran J, Morales-Angulo C, Navas C, Trinidad G, Tapia MC, Moreno F, Castillo I (2003) Auditory neuropathy in patients carrying muatations in otofelrin gene (*otof*). Hum Mut 22:451-456

Sheffield VC, Kraiem Z, Beck JC, Nishimura D, Stone EM, Salameh M, Sadeh O, Glaser B (1996) Pendered syndrome maps to chromosome 7q21-34 and is caused by an intrinsic defect in thyroid iodine organification. Nat Genet 12:424-426

Scott DA, Carmi R, Elbedour K, Yousefsberg S, Stone EM, Sheffield VC (1996) An autosomal recessive non-syndromic hearing-loss locus identified by DNA pooling using two inbred Bedouin Kindreds. Am J Hum Genet 59:385-391

Scott DA, Kraft ML, Stone EM, Sheffield VC, Smith RJH (1998) Connexin mutations and hearing loss. Nature 391:32-32

Scott HS, Kudoh J, Wattenhofer M, Shibuya K, Berry A, Chrast R, Guipponi M, Wang J, Kawasaki K, Asakawa S, Minoshima S, Younus F (2001) Insertion of beta-satellite repeats identifies a transmembrane protease causing both congenital and childhood onset autosomal recessive deafness. Nat Genet 27: 59-63

Siemens J, Kazmierczak P Reynolds A, Sticker M, Littlewood-Evans A, Muller U (2002) The Usher syndrome proteins cadherin 23 and harmonin form a complex by means of PDZ-domain interactions. Proc Nat Acad Sci 99:14946-14951

Steel KP (2002) Beethoven, a mouse model for dominant, progressive hearing loss DFNA36. Nat Genet 30:257-258

Wang A, Liang Y, Fridell RA, Probst FJ, Wilcox ER, Touchman JW, Morton CC, Morell RJ, Noben-Trauth K, Camper SA, Friedman TB (1998) Association of unconventional myosin *MYO15* mutations with human non-syndromic deafness DFNB3. Science 280:1447-1451

Wattenhofer M, Di Iorio MV, Rabionet R, Dougherty L, Pampnos A, Schede T, Montserrat-Sentis B, Arbones ML, Iliades T, Pasquadibisceglie A, Amelio MD, Alwan S, Rossier C, Dahl HH, Petersen Mb, Estivill X, Gasparini P, Scott HS, Antonarakis SE (2002) Mutations in the *TMPRSS3* gene are a rare cause of childhood non-syndromic deafness in cauasian patients. J Mol Med 80:124-131

Weil D, Kussel P, Blanchard S, Levy G, Levi-Acobas F, Durira M, Ayadi H, Petit C (1997) The autosomal recessive isolated deafness, DFNB2, and the usher 1B syndrome are allelic defects of the myosin-VIIA gene. Nature 16: 191-193

Willems PJ (2000) Genetic causes of hearing loss. N Engl J Med 342:1101-1109

Yasunaga S, Grati M, Petit C (2000) DFNB9 and DFNB12. Adv Otorhinolaryngol. 56:164-167

Yasunaga S, Petit C (2000) Physical map of the region surrounding the *OTOFERLIN* locus on chromosme 2p22-p23. Genomics 66:110-112

Zelante L, Gasparini P, Estivill X, Melchionda S, D'Agruma L, Govea N, Mila M, Monica MD, Lutfi J, Shohat M, Mansfield E, Delgrosso K, Rappaport E, Surrey S, Fortina P (1997) *Connexin26* mutations associated with the most common form of non-syndromic neurosensory autosomal recessive deafness (DFNB1) in Mediterraneans. Hum Mol Genet 6:1605-1609

Zwaenepoel I, Mustapha M, Leibovici M, Verpy E, Goodyear R, Liu XZ, Nouaille S, Nance WE, Kanaan M, Avraham KB, Tekaia F, Loiselet J, Lathrop M, Richardson G, Petit C (2002) Otoancorin, an inner ear protein restricted to the interface between the apical surface of sensory epithelia and their overlying acellular gels, is defective in autosomal recessive deafness DFNB22. Proc Natl Acad Sci USA 99:240-245

Electronic Database Information

- Deafness and hereditary hearing loss overview www.genetests.org
- Genome database homepage www.gdp.org
- Hereditary hearing loss homepage http://www.uia.ac.be/dnalab/hhh
- Marshfield Medical Centre http://www.marshmed.org/gentics/
- National Centre for biotechnology information http://www.ncbi.nih.gov/