Genetic Aspects of Hearing Loss in the

Limpopo Province of South Africa

Rosemary I Kabahuma

MBCHB (Makerere University), MMED ENT Surgery (University of Nairobi),

MSC Audiological Medicine (University of London)

A thesis submitted in fulfillment of the requirements for the degree of

Doctor of Philosophy

Faculty of Humanities, Department of Speech Pathology and Audiology,

University of the Witwatersrand, Johannesburg, South Africa

January 2010

DEDICATION

To Almighty God who has by grace made all this possible.

To my daughters Constance and Theodora, who experienced the cost of a PhD at an early age and yet remained encouraging, supportive, always believing for their mother.

To Ezra whose support gave me the space to fly.

'But he knows the way that I take; when he has tested me, I will come forth as gold.'

Job 23:10

DECLARATION

I, Rosemary Ida Kabahuma, do hereby declare that this dissertation submitted in fulfillment of the requirements for the degree of **Doctor of Philosophy** in the Faculty of Humanities, Department of Speech Pathology and Audiology, University of Witwatersrand, is my own original work. All assistance I have received has been stated in the acknowledgements. This work has not been submitted before for any degree or examination at this or any other university. I declare that the protocol was cleared by the Committee for Research on Human subjects, Ethics committee clearance certificate protocol number **M991005**.

Rosemary Ida Kabahuma

_____ day of ______ 2010

ACKNOWLEDGEMENTS

I would like to thank all the many individuals and organizations who, through their support, ensured the successful completion of this project. It is not possible to mention each one by name but the following are singled out:

- My main supervisor, Prof. Claire Penn, Department of Speech Pathology and Audiology, University of Witwatersrand, Johannesburg, South Africa
- My co-supervisor, Prof. Michele Ramsay, Division of Human Genetics, National Health Laboratory Service and School of Pathology, University of Witwatersrand, Johannesburg, South Africa
- Prof. Jackie L Clarke, Faculty of Audiology, Callier Center for Communication Disorders, University of Texas at Dallas, for the constructive criticism and advice
- The eaf students at the Tshilidzini and Bosele Schools for the Deaf who took part in this study
- The parents and guardians of all the subjects for their co-operation
- The school principals and staff at the Tshilidzini and Bosele Schools for the Deaf, who received us warmly and went out of their way to ensure the successful completion of this project
- All the translators at the two Schools for the Deaf who assisted in the completion of the questionnaires
- The nursing staff at the two Schools for the Deaf, for their assistance with phlebotomy and urine testing
- Prof. RF Mueller, Karl Bromelow and Tim Hutchin, formerly of the Deafness Research Team of the Molecular Medicine Unit, St James' University Hospital,

University of Leeds, UK, for the financial support and training in mutation detection techniques during the attachment at Leeds

- Prof. Andrew Read and James O'Sullivan, Department of Medical Genetics, St Mary's Hospital, University of Manchester, UK, for the invaluable work in the detection of the Waardenburg Syndrome mutations in this study
- Dr Xue Zhong Liu and Xiao Mei of the Research Unit of the Department of Otorhinolaryngology at the University of Miami, USA, for their invaluable training in mutation detection
- Dr Daniels and Mrs Daniels for all their support
- The staff and students of the Department of Speech Pathology and Audiology, University of the Witwatersrand, Johannesburg, South Africa for the assistance with the audiological testing of the subjects
- Ronel Kilian and Philemon Ratshilumela for the audiological testing of the subjects
- The staff and students at the molecular laboratory of the NHLS, University of the Witwatersrand, Johannesburg, South Africa, for the assistance with DNA extraction and processing of samples. A special thanks to Silke Arndt, Fahmida Essop, Robyn Kerr, Tony Lane and Angela Turner
- Jerry Sigudla, a dedicated research assistant who painstakingly worked to track and compile data
- Sam Ntuli for the assistance with the statistical analysis
- The Mellon Foundation for partially funding this PhD
- The Medical Research Council, South Africa for partially funding this project; and
- The Department of Health and Social Welfare, Limpopo Province, for facilitating my study leave and offering financial support for the attachment to the University of Miami.

TABLE OF CONTENTS

DEDICATION	II
DECLARATION	III
ACKNOWLEDGEMENTS	IV
TABLE OF CONTENTS	VI
LIST OF ABBREVIATIONS	XIV
LIST OF TABLES	XVI
LIST OF FIGURES	XXI
ABSTRACT	1
CHAPTER 1: INTRODUCTION	6
1.1 General Introduction	6
1.2 Genes and Populations	11
1.3 The Limpopo Province	14
1.3.1 The Land.	14
1.3.2 Population characteristics	20
1.3.3 Employment	21
1.3.4 Health profile of the people in the Limpopo Province	21
1.3.5 Access to health care in the Limpopo Province	23
1.4 The People, their Cultures and Practices	28
1.4.1 The Venda people and politics	28
1.4.1.1 Betrothal and marriage amongst the Bavhenda	30
1.4.1.2 Attitudes towards disability amongst the Venda	31
1.4.1.3 Ear disease and traditional healing amongst the Venda	31
1.4.2 The Shangaan (Tsonga) people and politics	31

1.4.2.1 Betrothal and marriage among the Shangaan	32
1.4.3 The Pedi people and politics	32
1.4.3.1 Betrothal and marriage among the Pedi	34
1.5 The Rationale for this Study	36
CHAPTER 2: LITERATURE REVIEW AND BACKGROUND INFORMATION	١٧
2.1 Overview of Genetics of Hearing Loss	39
2.1.1 Disease Inheritance	39
2.1.2 Modes of Inheritance	40
2.1.3 Research into Genes for Hearing Loss	43
2.2 Epidemiological Perspectives of Hearing Loss	45
2.2.1 General Considerations in the Aetiology of Hearing Loss	45
2.2.2 Epidemiological Models of Hearing Research.	47
2.2.3 Epidemiological models for SNHL	53
2.2.4 Epidemiology of Hearing Loss in Africa	55
2.2.5 Epidemiological Studies on Genetic Hearing Loss	60
2.3 The Ear in Genetic Hearing Deafness	63
2.3.1 Development of The Ear	63
2.3.2 Overview of the Anatomy of the Mature Inner Ear	66
2.3.3 Gap Junctional Systems of the Human Ear	68
2.3.4 Major Ear Defects in Hereditary Hearing Loss	69
2.3.5 Overview of the Physiology of Hearing	70
CHAPTER 3: LITERATURE REVIEW AND BACKGROUND INFORMATION	N II
3.1 History of Research in Genetic Deafness	73
3.1.1 History of the Genetics of Hearing Loss	73
3.1.2 Clinical Phenotypes of Genetic Deafness	74

3.1.3 Histopathologic Phenotypes of Genetic Deafness	76
3.1.4 Molecular Phenotypes in Syndromic Genetic Disease	79
3.1.5 The Human Genome Project	80
3.1.6 Research using the mouse as a model for human deafness	82
3.2 Gene Localization and Auditory Research	86
3.2.1 Genes implicated in Hearing Loss	86
3.2.2 General functional classification of deafness genes	87
3.2.2.1 Genes controlling hair cell structure	88
3.2.2.2 Extracellular matrix genes	88
3.2.2.3 Genes controlling ion homeostasis	89
3.2.2.4 Genes controlling transcription factors	89
3.2.2.5 Miscellaneous genes	90
3.2.3 Overview of Connexins (Cx) and the Gap Junctional Systems	
of the Ear	91
3.2.4 Gap junction Gene Variants and Hearing Loss	94
3.2.5 GJB2 Mutations and Hearing Loss: Phenotype-Genotype	
Relationship	94
3.2.6 GJB2 Mutations and Type of Hearing Loss	95
3.2.7 Waardenburg syndrome	96
3.2.7.1 Clinical features of Waardenburg Syndrome	97
3.2.7.2 The Clinical Classification of Waardenburg Syndrome	100
3.2.7.3 Variable penetrance of Waardenburg Syndrome	103
3.2.8 Mitochondrial genes	106
3.2.9 Audiological findings in non-syndromic genetic hearing loss	108
3.2.10 The Future Application of Proteomics and Genomics	109

3.3 Clinical Perspectives	114
3.3.1 Detection of Childhood Hearing Loss	117
3.3.1.1 Targeted Screening	119
3.3.1.2 Universal Neonatal Hearing Screening	120
3.3.2 Principles of Assessment	126
3.3.3. Audiological assessment	129
3.3.3.1 Immitance testing	129
3.3.3.2 Evoked otoacoustic emissions	129
3.3.3.3 Auditory brainstem response testing	129
3.3.3.4 Auditory steady-state response testing	130
3.3.3.5 Audiometry	130
3.3.3.6 Audioprofiles	131
3.3.3.7 Description of hearing loss	132
3.3.4 Assessment and Investigations	
3.3.4.1 History	135
3.3.4.2 Clinical examination	137
3.3.4.3 Ophthalmology	138
3.3.4.4 Serology	138
3.3.4.5 Haematology and Biochemistry	139
3.3.4.6 Thyroid tests	139
3.3.4.7 Immunology	139
3.3.4.8 Metabolic screen	139
3.3.4.9 Urinalysis	140
3.3.4.10 Electrocardiography	140
3.3.4.11 Radiology	140

3.3.4.12 Audiology	141
3.3.4.13 Vestibular investigations	141
3.3.4.14 Clinical photographs	142
3.3.4.15 Genetic testing	142
3.3.4.16 Referral to geneticist	145
3.3.5 Aetiological Diagnosis	148
3.3.6 Intervention for the hearing impaired Child	149
CHAPTER 4: METHODOLOGY	
4.1 Problem Statement, Research Question and Purpose of the Study	152
4.2 Aim and Objectives	153
4.3 Study Design	154
4.3.1 Reference Population	157
4.3.2 Setting (Schools for the Deaf)	158
4.3.3 Study Population	159
4.3.4. Inclusion Criteria	160
4.3.5 Exclusion Criteria	160
4.3.6 Limitations of the Study	161
4.3.6.1 Language	161
4.3.6.2 Sample Size	161
4.3.6.3 The Use of Questionnaires	162
4.3.6.4 Attrition	163
4.3.6.5 Pedigrees and family testing	163
4.3.6.6 Unavailability of Investigative Facilities	163
4.3.7 Ethical Considerations	163
4.3.8 Ethics Approval	164

4.4 Methods and Procedures	164
4.4.1 Equipment	164
4.4.2 Audiological Evaluation	164
4.4.3 Procedures	166
4.4.3.1 Phase1	166
4.4.3.2 Phase 2a	168
4.4.3.3 Phase 2b	170
4.5 Methods used for Mutation Detection	174
4.5.1 Specimen Collection	174
4.5.2 DNA Extraction	174
4.5.3 Mutation Detection	175
4.6 Data analysis	180
4.6.1 Mapping techniques used for epidemiological analysis	180
4.6.2 Statistical Analysis	181
4.6.3 The Null Hypothesis (H ₀)	182
CHAPTER 5: RESULTS	
5.1 Demographic Information of Subjects	183
5.1.1 Phase I	183
5.1.2 Phase 2	188
5.2 Geographical Distribution of Hearing Loss	191
5.2.1 Phase I	191
5.2.2 Phase 2	192
5.3 Type and Degree of Hearing Impairment	199
5.3.1 Tympanometry and Transient otoacoustic emissions	199
5.3.2 Audiometry	200

5.4 Aetiological Investigation of Hearing Disorders	
5.4.1 Family History of Hearing Loss Among the Subjects	204
5.4.2 Consanguinity Among Parents	205
5.4.3 Urinalysis Results	211
5.4.4 Reported Pregnancy and Perinatal history	211
5.4.5 Reported Medical Conditions Among the Subjects	211
5.5 Mutation Detection	215
5.5.1 GJB2	215
5.5.2 Waardenburg Syndrome	218
5.5.3 Mitochondrial Mutations	219
5.6 Clinical signs in Hearing Loss	
5.6.1 Eye Findings Among the Subjects	223
5.6.2 Skeletal Findings Among the Subjects	223
5.6.3 Ear, Nose and Throat Findings Among the Subjects	224
5.6.4 Other Systemic Findings Among the Subjects	225
5.7 Tests of association and Binary logistic regression analysis	
5.7.1 Calculation of crude odds ratio	233
5.7.2 Interpretation of the crude odds ratio	233
5.7.3 Assessment of the fitted logistic regression model	233
5.7.4 Interpretation of the odds ratio for family history	233
5.7.5 The Hosmer-Lemeshow goodness-of-fit test	234
5.7.6 Magnitude of area under ROC curve	235
5.7.7 Plot of sensitivity/specificity vs probability cut-off point	236

6.1 Dis	cussion	238
	6.1.1 Geographical Distribution of Hearing Loss in Limpopo	238
	6.1.2 Accounting for Bias in this Study	243
	6.1.2.1 Bias due to migratory labour practice	243
	6.1.2.2 Bias due to non-random admission into schools	244
	6.1.2.3 Bias due to proximity to the schools	244
	6.1.2.4 Bias due to varying population density within the province	244
	6.1.3 Type and Degree of Hearing Loss in Limpopo	245
	6.1.4 Aetiology of Learing Loss in Limpopo	248
	6.1.5 Influence of Consanguinity on Genetic Hearing Loss in Limpopo	255
	6.1.6 Mode of Inheritance of Hearing Loss in the Study Population	258
	6.1.7 Significance of the Candidate Genes for Deafness in the Limpopo	259
	6.1.7.1 GJB2 (Connexin26)	260
	6.1.7.2 Common Mitochondrial Mutations	263
	6.1.7.3 Waardenburg syndrome	264
	6.1.8 Nosological Entities of Hearing Loss in Limpopo	264
6.2 Cor	nclusions	268
	6.2.1 High risk areas for hearing loss in the Limpopo province	268
	6.2.2 Clinical Perspectives	269
	6.2.3 Genetic Perspectives	269
	6.2.4 Policy Issues	271
6.3 Rec	commendations	277
7.1 Ref	Terences	281
72 Aj	ppendices	304

LIST OF ABBREVIATIONS

HL	Hearing Level
StatsSA	Statistics South Africa
DNA	Deoxyribose nucleic acid
EcoG	Electrochocleography
GP	general practioner
OME	Otitis media with effusion
РТА	Pure tone average
ECG	Electrocardiogram
MRI	Magnetic resonance imaging
TORCH	Toxoplasmosis, Rubella, Cytomegalovirus, Herpes
CSF	Cerebral spinal fluid
СТ	Computerised tomography
CME	Continued medical education
SEN	Special education needs
ENT	Ear Nose and Throat
SNHL	Sensorineural hearing loss
TEOAEs	Transient evoked otoacoustic emissions
ART	Acoustic reflex threshold
РМНС	Pietersburg Mankweng Hospital Complex
ENG	Electronystagmography
NHLS	National Health Laboratory Services
PCR	Polymerized chain reaction
WS	Waardenburg syndrome

ARNSHL	Autosomal recessive nonsyndromic hearing loss
NSSNHL	Nonsyndromic sensorineural hearing loss
NSAHL	Nonsyndromic autosomal hearing loss
Cx26	Connexin 26
DOH	Department of Health
WHO	World Health Organisation
BP	Base Pair(s)
EDHI	early detection of hearing impairment

LIST OF TABLES

Table number	Page no.
CHAPTER 1	
Table 1.1: Population of Limpopo Province by home language and district	20
Table 1.2: Limpopo Province population in five – year age groups according	
to race	20
Table 1.3: Disabled population by district in the Limpopo Province	21
Table 1.4: Public sector human resource data, Limpopo Province	27
Table 1.5: Home area of students at Tshilidzini School, August 1997	36
CHAPTER 2	
Table 2.1: Features of some epidemiological methods in use	49
Table 2.2: The domains and measures of auditory dysfunction (adapted from	
Davis et al 1983)	52
Table 2.3: Prevalence of hearing loss in childhood (after Davidson et al 1989)	56
Table 2.4: Depicting time of appearance of ear features	64
CHAPTER 3	
Table 3.1: Gene expression in the human ear	93-94
Table 3.2: Classes and genes identified for Waardenburg syndrome	101
Table 3.3: Phenotypic penetrance of selected Waardenburg syndrome traits	
Table 3.4: Penetrance of pigmentary abnormalities WS patients with and	
without hearing lossin relation to syndrome type	104
Table 3.5: The degree of hearing loss and the frequency of pigmentary abnormalities	es
in relation to syndrome type	105
Table 3.6a: Audiological manifestation of the autosomal dominant nonsyndromic	
hearing impairment genes	109
Table 3.6b: Audiological manifestation of the autosomal recessive nonsyndromic	
hearing impairment genes	110

Table 3.6c: Audiological manifestation of the X-linked nonsyndromic hearingimpairment genes111Table 3.6d: Audiological manifestation of the mitochondrial nonsyndromic111hearing impairment genes111Table 3.7: Evaluation strategy of hearing loss135

CHAPTER 5

Table 5.1: Demographic information of subjects, Phase 1	183
Table 5.2: Age of detection, by parents, of hearing loss among subjects, Phase 1	188
Table 5.3: Demographic information of subjects, Phase 2	189
Table 5.4: Age of detection, by parents, of hearing loss among subjects, Phase 1	189
Table 5.5: Geographical distribution of hearing loss according to district,	
Limpopo Province, both schools Phase 1	191
Table 5.6: Geographical distribution of hearing loss according to district,	
Limpopo Province, both schools Phase 2	192
Table 5.7: Comparison of municipal wards considering high risk areas for hearing loss	
Table 5.8: Municipalities with highest geographical distribution of hearing loss,	
both schools Phase I	193
Table 5.9: Municipalities showing the highest geographical distribution	
of hearing loss according to school, Phase I	194
Table 5.10: The geographical distribution of hearing loss according to Municipalities	
municipalities normalized to African population, both schools: Phase 2	194
Table 5.11: Tympanometric results	199
Table 5.12: Cross tabulation of Tympanometric results between ears	199
Table 5.13: Abnormalities for ear with abnormal tympanogram	

Table 5.14: Severity of hearing impairment, best ear average 0.5-4kHz,

Tshilidzini, Phase 2 201

Table 5.15: Audiogram configuration among subjects, Tshilidzini, Phase 2	201
Table 5.16: Asymmetry of hearing impairment among subjects,	
Tshilidzini, Phase 2	201
Table 5.17: Severity of hearing impairment, best ear average 0.5-4kHz,	
Bosele, Phase 2	201
Table 5.18: Audiogram configuration among subjects, Bosele, Phase 2	201
Table 5.19: Asymmetry of hearing impairment among subjects, Bosele, Phase 2	202
Table 5.20: Severity of hearing impairment, best ear average 0.5-4kHz, both	
schools, Phase 2	202
Table 5.21: Audiogram configuration among subjects, both schools, Phase 2	202
Table 5.22: Asymmetry of Hearing Impairment among subjects, both schools, Phase 2	202
Table 5.23: Family History of hearing loss among subjects, Phase 2	203
Table 5.24: Distribution of family history of hearing loss according to municipality,	
Limpopo Province, both schools, normalized to African Population, Phase2	203
Table 5.25: Cross tabulation of consanguinity of parents by municipality,	
Bosele School, Phase 2	207
Table 5.26: Cross tabulation of consanguinity of parents by municipality,	
Tshilidzini School, Phase 2	210
Table 5.27: Cross tabulation of consanguinity of parents by municipality,	
Bosele School, Phase 2	212
Table 5.28: Cross tabulation of consanguinity of parents by municipality,	
Tshilidzini School, Phase 2	212
Table 5.29: History of consanguinity of parents by school, Phase 2	213
Table 5.30: Cross tabulation of consanguinity of parents by family history	
of hearing loss, Phase 2	213
Table 5.31: Cross tabulation of language group by consanguinity of parent	213
Table 5.32: Results of urinalysis among participants	213
Table 5.33: Cross tabulation of consanguinity of parents by relative with hearing loss	214

Table 5.34: Cross tabulation of consanguinity of parents by relative with hearing loss	214
Table 5.35: History of maternal problems during pregnancy and labour	214
Table 5.36: History of other medical conditions among participants	214
Table 5.37: GJB2 variations observed in a deaf population from the Limpopo	
Province of South Africa.	215
Table 5.38: Cross tabulation of GJB2 variations and language group in a South	
African control population (n=74).	219
Table 5.39: GJB2 (Cx26) variations: genotype versus allele frequency as observed	
in a South African population.	219
Table 5.40: GJB2 (Cx26) variations tested for Hardy-Weinberg equilibrium:	
Position g.3318-34	220
Table 5.41: GJB2 (Cx26) variations tested for Hardy-Weinberg equilibrium:	
Position g.3318-15	221
Table 5.42: Cross tabulation of consanguinity of parents by base variation:	
Position g.3318-34	222
Table 5.43: Cross tabulation of family history of hearing loss by base variation:	
Position g.3318-34	222
Table 5.44: Cross tabulation of ethnic group by base variation: Position g.3318-34	222
Table 5.45 : Levels of significance of results following cross tabulation of	
participants' age at detection with other variables	227
Table 5.46 : Levels of significance of results following cross tabulation of risk	
factors for hearing loss with other variables.	228
Table 5.47 : Levels of significance of results following cross tabulation of	
consanguinity of parents with other variables.	228
Table 5.48: Levels of significance of results following cross tabulation of family	
history of hearing loss with other variables.	229
Table 5.49: Levels of significance of results following cross tabulation of degree	
of first affected relative with other variables.	229

Table 5.50: Levels of significance of results following cross tabulation of degree	
of second affected relative with other variables.	230
Table 5.51 : Levels of significance of results following cross tabulation of	
language group with other variables.	230
Table 5.52: Levels of significance of results following cross tabulation of	
GJB2 variation C>T at position -34 with other variables.	231
Table 5.53 : Levels of significance of results following cross tabulation of	
Table 5.55. Levels of significance of results following cross tabulation of	
GJB2 variation C>T at position -15 with other variables.	231
Table 5.54 : Levels of significance of results following cross tabulation of	
participants' home address with other variables.	232
Table 5.55 Results of binary logistic regression analysis	232
Table 5.56 Logistic model for consanguinity of parents	234
Table 5.57 Hosmer-Lemeshow goodness-of-fit test	235

LIST OF FIGURES

Figure number

Page no.

CHAPTER 1

Fig. 1.1: Aetiological classification of genetic hearing loss	7
Fig. 1.2: Location map of the study areas within the map of South Africa	16
Fig. 1.3: Map of the Limpopo Province showing the districts and municipal boundaries	17
Fig. 1.4: The Baobab tree, the Icon of Limpopo Province	18
Fig. 1.5: The Land of the legends – Lake Fundudzi, Venda	18
Fig. 1.6: The arid landscape of No-Body and Moria regions, the headquarters of the ZC	С
church whose star logo seen in the background is etched in the mountainside.	18
Fig. 1.7: A group following a climbing trail in the mountains in Agatha	18
Fig. 1.8: The Tzaneen Dam with the Drakensberg mountain range in the background	19
Fig. 1.9: Polishing the homestead floor with fresh cow dung in a Giyani village	19
Fig. 1.10: Sharing a meal, the typical homestead arrangement seen in the background	19
Fig. 1.11: A Shangaan (Tsonga) girl greeting visitors to the homestead in a Giyani villag	e 19
Fig. 1.12: One of the reception areas inside the Pietersburg Provincial Hospital	25
Fig. 1.13: Ear, nose and throat outpatient clinic at the Pietersburg Provincial Hospital	26
Fig. 1.14: A Venda woman in full traditional attire	35
Fig. 1.15: Shangaan women dance group	35
Fig. 1.16: Pedi women's dance group from Mashashane in traditional wear	35
CHAPTER 2	
Fig. 2.1: Pedigree showing autosomal dominant inheritance	41
Fig. 2.2: Pedigree showing autosomal recessive inheritance	41
Fig. 2.3: Pedigree showing dominant X-linked inheritance	42
Fig. 2.4: Pedigree showing recessive X-linked inheritance	42
Fig. 2.5: Pedigree showing mitochondrial inheritance	43

Fig. 2.6: The relationship between genetic and environmental factors in causation	
of hearing loss as a function of age (adapted from Davis et al 1983a)	51
Fig. 2.7: Epidemiological model of hearing function	54
Fig. 2.8: Schematic drawing of inner ear development in mammals	
(after Varela-Nieto et al (2004).	66
Fig. 2.9: Diagram showing the structure and gene expression of the human ear	67
CHAPTER 3	
Fig. 3.1a: The progress of deafness gene discovery from 1994-2001	85
Fig. 3.1b: Total number of deafness gene identified annually 1986-2007	86
Fig. 3.2: Aetiological surveys among 3,064 children in Southern Africa	106
Fig. 3.3 Realistic relationship of need, demand and supply in the health services	116
Fig. 3.4: The ideal relationship of need, demand and supply in the ideal health service	117
Fig. 3.5: Medical assessment of the hearing impaired child	128
Fig. 3.6: The areas addressed in a hearing impaired child's management protocol	149
Fig. 3.7: Components of a paediatric audiological medicine service	151
CHAPTER 4	
Fig. 4.1: Parents and Teachers at Prayer in the hall – Bosele School	172
Fig. 4.2: Translator (Nurse) explaining the questionnaire to the parents – Bosele School	172
Fig. 4.3: Parents waiting for assistance in completing the questionnaire- Bosele School	172
Fig. 4.4: Subjects waiting their turn at Bosele School	172
Fig. 4.5: Doctor completing a subject's medical examination form - Bosele School	173
Fig. 4.6: Doctor examines subject's ear at Bosele School	173
Fig. 4.7: TEOAE (transient otoacoustic emission) station - Bosele School	173
Fig. 4.8: Sound-proofed testing room, Tshilidzini School	173
CHAPTER 5	
Fig. 5.1a: Box and Whisker plot showing the ages (in years) of the participants, Phase 1,	,

both schools 184

Fig. 5.1b: Box and Whisker plot showing the ages (in years) of the participants, Phase 1,	
Tshilidzini School	184
Fig. 5.1c: Box and Whisker plot showing the ages (in years) of the participants, Phase 1,	
Bosele School	185
Fig. 5.2a: Box and Whisker plot showing the distance of the participants homes	
from school in kms, Phase 1, both schools	186
Fig. 5.2b: Box and Whisker plot showing the distance of the participants homes	
from school in kms, Phase 1, Bosele School	186
Fig. 5.2c: Box and Whisker plot showing the distance of the participants homes	
from school in kms, Phase 1, Tshilidzini School	187
Fig. 5.3: Box and Whisker plot showing the ages (in years) of the participants,	
Phase 2, both schools	190
Fig. 5.4: Geographical distribution of hearing loss according to municipality,	
Limpopo Province, both schools Phase 1	195
Fig. 5.5: Geographical distribution of hearing loss according to municipality,	
Limpopo Province, both schools Phase 2	195
Fig. 5.6: Spatial distribution of Hearing Loss according to municipality, Limpopo	
Province, Phase 1	196
Fig. 5.7 Spatial distribution of Hearing Loss according to municipality, Limpopo	
Province, normalized to African Population, Phase 2	196
Fig. 5.8: Spatial distribution of hearing loss in the Limpopo Province according to	
language group, Phase 1	197
Fig. 5.9: Spatial distribution of hearing loss in the Limpopo Province according to	
language group, Phase 1	198
Fig. 5.10: A Spatial distribution of subjects according to family history of	
Hearing loss per local municipality, Limpopo province, Phase 1	206
Fig. 5.11: Spatial distribution of subjects without a family history of hearing loss	
per local municipality, Limpopo Province, Phase 2	207

Fig. 5.12: Spatial distribution of subjects with a family history of hearing loss	
per local municipality, Limpopo Province, Phase 2	208
Fig. 5.13: Spatial distribution of subjects with a family history of hearing loss	
per local municipality, Limpopo Province, Normalized to African	
population, Phase 2	208
Fig. 5.14: Spatial distribution of subjects with a history of consanguinity among	
parents, per local municipality, Limpopo Province, Phase 2	209
Fig. 5.15: Spatial distribution of subjects without a history of consanguinity among	
parents, per local municipality, Limpopo Province, Phase 2	209
Fig. 5.16: Spatial distribution of subjects with unknown history of consanguinity	
per local municipality, Limpopo Province, Phase 2	210
Fig. 5.17: Gel electrophoresis showing size of PCR fragment (GJB2)	216
Fig. 5.18: Gel electrophoresis (GJB2) following Fermentas SsiI enzyme digest	
(cutting at position g.3318-15)	216
Fig. 5.19: Gel electrophoresis (GJB2) following Bsml enzyme digest	
(cutting at position g.3318-34)	216
Fig. 5.20:Electropherograms showing GJB2 variation T A at position -6 C T variation	
at position -15	217
Fig. 5.21: Electropherogram showing GJB2 variant at GJB2 position -34	217
Figure 5.22a: Clinical features Waardenburg syndrome Type I.	226
Figure 5.22b: Patchy depigmentation of the skin in participant with WS type I	226
Figure 5.23: The ROC (receiver operating characteristic) curve	236
Figure 5.24: Plot of sensitivity/Specificity versus probability cut-off point	237