The genetics of non-syndromic hearing impairment in South Africa

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Plagiarism declaration

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I hereby confirm that I have been granted permission, by the University of Cape Town's Doctoral Degree Board, to include the following publications in my thesis. Where necessary, my co-authors have agreed to the inclusion of these publications in my thesis.

1. Hotchkiss, J., Manyisa, N., Adadey, S.M., Oluwole, O.G., Wonkam, E., Mnika, K., Yalcouye, A., Nembaware, V., Haendel, M., Vasilevsky, N., Mulder, N.J., Jupp, S., Wonkam, A. & Mazandu, G.K. 2019. The Hearing Impairment Ontology: A Tool for Unifying Hearing Impairment Knowledge to Enhance Collaborative Research. *Genes (Basel).* 10(12). DOI:10.3390/genes10120960.
2. Manyisa, N., Adadey, S.M., Wonkam-Tingang, E., Yalcouye, A. & Wonkam, A. 2022. Hearing Impairment in South Africa and the Lessons Learned for Planetary Health Genomics: A Systematic Review. *Omics.* 26(1):2-18. DOI:10.1089/omi.2021.0181.
3. Manyisa, N., Schrauwen, I., de Souza Rios, L.A., Mowla, S., Tekendo-Ngongang, C., Popel, K., Esoh, K., Bharadwaj, T., Nouel-Saied, L.M. & Acharya, A. 2021. A Monoallelic Variant in REST Is Associated with Non-Syndromic Autosomal Dominant Hearing Impairment in a South African Family. *Genes.* 12(11):1765.

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List of articles the candidate has contributed to that were excluded from the thesis.

1. Adadey, S.M., Manyisa, N., Mnika, K., de Kock, C., Nembaware, V., Quaye, O., Amedofu, G.K., Awandare, G.A. & Wonkam, A. 2019. GJB2 and GJB6 Mutations in Non-Syndromic Childhood Hearing Impairment in Ghana. *Frontiers in Genetics.* 10:841. DOI:10.3389/fgene.2019.00841.
2. Lebeko, K., Manyisa, N., Chimusa, E.R., Mulder, N., Dandara, C. & Wonkam, A. 2017. A Genomic and Protein-Protein Interaction Analyses of Nonsyndromic Hearing Impairment in Cameroon Using Targeted Genomic Enrichment and Massively Parallel Sequencing. *Omics: A Journal of Integrative Biology*. 21(2):90-99. DOI:10.1089/omi.2016.0171.
3. Oluwole, O.G., Esoh, K.K., Wonkam-Tingang, E., Manyisa, N., Noubiap, J.J., Chimusa, E.R. & Wonkam, A. 2021. Whole exome sequencing identifies rare coding variants in novel human-mouse ortholog genes in African individuals diagnosed with non-syndromic hearing impairment. *Experimental Biolgy and Medicine.* 246(2):197-206. DOI:10.1177/1535370220960388.
4. Wonkam, A., Manyisa, N., Bope, C.D., Dandara, C. & Chimusa, E.R. 2020. Whole Exome Sequencing Reveals Pathogenic Variants in MYO3A, MYO15A and COL9A3, and Differential Frequencies in Ancestral Alleles in Hearing Impairment Genes Among Individuals from Cameroon. *Human Molecular Genetics.*
5. Wonkam, A., Adadey, S.M., Schrauwen, I., Aboagye, E.T., Wonkam-Tingang, E., Esoh, K., Popel, K., Manyisa, N., Jonas, M., deKock, C., Nembaware, V., Cornejo Sanchez, D.M., Bharadwaj, T., Nasir, A., Everard, J.L., Kadlubowska, M.K., Nouel-Saied, L.M., Acharya, A., Quaye, O., Amedofu, G.K., Awandare, G.A. & Leal, S.M. 2022. Exome sequencing of families from Ghana reveals known and candidate hearing impairment genes. *Communications Biology.* 5(1):369. DOI:10.1038/s42003-022-03326-8.

Preface

Hearing impairment (HI) is a sensory condition resulting in the partial or complete loss of hearing. It is a heterogenous condition that may arise due to genetic, environmental, or unknown factors. Hearing impairment is estimated to affect 1 in 1000 live births, and there is an estimated that 1.57 billion people live with HI worldwide.

Genetics accounts for approximately 50% of HI in developing countries. Environmental factors account for 25% of HI and unknown factors account for 25% of HI in developing countries. There is however a greater contribution of environmental factors in HI on the African continent (52%).

The gene *GJB2* is a significant contributor to HI in individuals of European, North American and Asian descent. This gene, however, has been shown to have minimal significance in patients of African descent. There are currently no known genes significantly associated with HI in patients of African descent.

Understanding the genetic causes of HI within a population will allow for better management of HI. This is due to a strong correlation between the genotype and phenotype. As such, it is necessary to study HI within the African population, including the Black South African population. This may also allow the innovation of a panel that will allow rapid identification of causative variants in the patients.

Research concept

The study concept and all the methodology were conceived by the candidate in conjunction with the principal investigator Professor Ambroise Wonkam. The research proposal including methods for samples and data collection, experiments, and analysis was designed by the candidate with the advice and guidance of the supervisors. Besides, drafting the full manuscripts and incorporating revisions from co-authors and journals reviewers were performed by the candidate.

Data collection, experimentation, and data analysis

The data were obtained from a cross-sectional study from 2018 to 2022 from the South African population. Patients were recruited from schools for the deaf in eight provinces in South Africa and two hospitals in Cape Town, Western Cape.

A proposal was submitted to the University of Cape Town, Faculty of Health Sciences Human Research Ethics Committee, Cape Town, South Africa by the candidate for approval.

Epidemiological data management, cleaning, and analysis was performed by the candidate. Where samples preparation or laboratory experiments was necessary, this was performed by the candidate with involvement of collaborators was requested by the principal investigator. Where bioinformatics support was necessary, the involvement of collaborators was requested by the principal investigator, and with the involvement of the candidate. The work and contribution of these collaborators to the study is indicated in each publication where applicable.

Publications

Synthesis of all the results and drafting of manuscripts of the publications included in this thesis were executed by the candidate, after which revisions from all co-authors were similarly incorporated before submission to the journal by the principal investigator. After review, all reviewer comments were addressed by the candidate in conjunction with the principal investigator. The detailed role of the candidate in the publications included is stated in each chapter.

The inclusion of publications in this thesis, is to 1) demonstrate the candidate's competency and proficiency in the relevant field. This will also aid in reinforcing the academic career of the candidate; 2) enhance the candidacy for continued financial support from the organisations that supported this work: Hearing Impairment Genetic Studies in Africa (HI-Genes Africa), Genetic Medicine of African Populations (GeneMAP), and the University of Cape Town; 3) enable participation in relevant conferences and training workshops; 4) the publications provide essential parts of the consistent body of research that fits the Faculty of Sciences policies. The policies promote the publication of thesis work, as much as possible, to allow the dissemination of knowledge generates and improve the profile of the institution and candidate.

The thesis contains three publications. These publications are presented in chapters 3, 4 and 6. Chapter 5 of the thesis includes unpublished, original work.

The candidate performed the recruitment drive and molecular work performed in chapter 5. Bioinformatics analysis was performed by collaborators at the University of Columbia.

Analysis of the results was performed by the candidate. The chapter was synthesized by the candidate, under the guidance of the supervisor, Professor Wonkam.

The candidate has met all requirements and requests approval of UCT's Doctoral Degrees Board, under Rules GP6.7 as follows:

a. The candidate's proposal to include publications in the current thesis was approved by the UCT Faculty of Health Sciences Doctoral Degree Board.
b. The thesis contains an adequate summary, introduction, a chapter on the aims and objectives, a comprehensive academic discussion of the results, forming the basis of the conclusions and perspectives drawn from this research.
c. Each results chapter with publications included is preceded by a synopsis of how the publications directly tie to the aims and objectives of the project, as well as to the thesis.
d. All included publications were written and published during the candidate's tenure as a PhD student since 2019.

_____

Noluthando Manyisa

Abstract

Background

Hearing impairment (HI) is a sensory disorder resulting in the partial or complete disability to perceive sound in the better-hearing ear. It is defined as the inability to hear better than 25dB in the better-hearing ear. Subsequently, it is considered disabling when a child cannot perceive sound better the 30dB, in the better hearing ear, and an adult cannot perceive sound better than 40dB, in the better hearing ear. Hearing impairment may result from genetic, environmental, or unknown factors. The connexin gene, *GJB2*, is the prevalent gene resulting in congenital HI in most children with European, North American, and East Asian ancestry. Apart from the founder mutations present in *GJB2* in Morocco, Ghana and Senegal, the prevalent causative genes resulting in congenital HI in African populations are yet to be fully elucidated.

Congenital Hearing impairment in South Africa (RSA), has been estimated to have an incidence rate of 5.5 per 1000 live births, which is 5 times higher than the birth incidence in high-income countries (approximately 1 to 2 per 1000 live births). Patients are generally diagnosed late with HI, at approximately 3 years old, and the most prevalent environmental factors associated with HI in RSA are middle ear infections, with several reports implicating ototoxicity as a cause of HI. Variants in connexin genes i.e. *GJB2* associated with HI have been shown to be irrelevant in the Black South African populations, and the limited genetic studies have identified private mutations in selected families. However, the full extent of prevalent genes associated with HI in the South African populations is still to be investigated.

Methods and results

Through a systematic review, we investigated the state of HI research in South Africa was established. Though studies have been performed since the 1960s, the results showed that genetics of HI in South Africa was not well explored. Universal new-born hearing screening is ideal in detecting congenital HI, but it is currently not standard practice in the country. However, with the advent of modern technology, HI screening may be more accessible to patients through community health workers. Patients who fail the repeated screenings may then be referred for further audiometry testing. This may positively impact the identification of patients with HI and assist with the necessary interventions.

We also collaboratively worked to establish the first disease ontology for HI to further allow standardised and harmonised language surrounding HI. This provides the scalability and interoperability of research going forward. It will allow for all stakeholders in HI research to use the same terminology when discussing HI.

In order to address the dearth of genetics research regarding non-syndromic HI, patients presenting with putative genetic HI were recruited from schools of the deaf across South Africa and two hospitals in Cape Town. The patients were recruited along with their family members, both with and without HI, and their DNA was extracted from whole blood. Twenty-seven families segregating non-syndromic HI, consisting of 65 affected and 35 unaffected individuals were subjected to whole exome sequencing (WES).

The HI was resolved in 20 families (74%), and pathogenic variants were identified in the genes: *WFS1* (c.A2141), *MITF* (cT918A), *ADGRV1*(c.G564T, c.A17450G, c.A11298C), *PDSS1*(c.C641T, c.G754C), *TBC1D24*(c.G1514A), *TMPRSS3*(c.205+6t>A), *NEU1*(c.C1069T, c.G727A), *MYO15A*(c.C1378T, c.9303+5G>A, c.G6634A), *USH2A*(c.T9437A, c.G2990T, c.G101A), *STRC*(c.G225A, c.C4057T, c.G4655C, c.C4351T, c.G4403A), *P2RX2*(c.G1064A, c.C1187G), *OTOG*(c.C2525A, c.G3143A, c.G916A), *LHFPL5*(c.621delC), *SLC26A4*(c.T94C, c.T716A), *GJB2*(c.35delG), *TRIOBP*(c.C3133T, c.C4298T), *REST*(c.G1244C), *CRYM*(c.*6_*2delACAAA), *CDH23*(c.T1585C, c.G8230A), *FGFR2*(c.1297+10G>C), *MYO7A*(c.6255delC). The pathogenic variants presented 8 autosomal dominant alleles and 12 autosomal recessive alleles

Five families presented with pathogenic or likely pathogenic variations associated with Usher Syndrome and the remaining 14 families presented with pathogenic variations associated with non-syndromic HI. One family presented with putative pathogenic variations in *NEU1*, which is a gene associated with Sialidosis.

We specifically investigated, in greater detail, a dominant novel variation in *REST*, present in one family, which encodes a transcription factor, that was identified using whole exome sequencing. This gene was previously suspected to be associated with hearing impairment only once, in an American family. The variation was absent in the unaffected South African family members, unrelated patients, and unaffected controls. *In vitro* cell-based studies indicated that the variation results in the loss of nuclear exclusivity of REST. Luciferase assays indicated that the mutant was unable to repress the expression of one of its target genes, whereas the wild-type effectively inhibited the expression of the target.

Conclusions

This thesis successfully performed the following investigations: 1) development of the first Hearing Impairment Ontology worldwide, 2) review the genetic profile of HI in South Africa, 3) used WES to find known and novel variants in established HI genes, and 4) confirmed *REST* as a novel HI gene. Future work will focus on sequencing all the remaining samples and identifying their putative causative mutations. Further work includes feedbacking the results of the genetic testing to the patients and their families. The data will contribute to improving the HI-genes pairs' curation in Africa, and globally.

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List of abbreviations

Abbreviation	Meaning
ACMG	American College of Medical Genetics'
AD	Autosomal Dominant
ADNSHI	Autosomal Dominant Non-Syndromic Hearing Impairment
ANNOVAR	Annotate Variation
AR	Autosomal Recessive
ARNSHI	Autosomal Recessive Non-Syndromic Hearing Impairment
Comp Het	Compound Heterozygote
CSOM	chronic suppurative otitis media
CT	computerized tomography SCAN
DANN	deleterious annotation of genetic variants using neural networks
dB	Decibel
dbSNP	Single Nucleotide Polymorphism Database
DO	Human Disease Ontology
DVD	Deafness Variation Database
ENT	Ear, Nose and Throat
GATK	genome analysis toolkit
gEAR	Gene Expression Analysis Resource
GHLD	Global Hearing Loss Database
GME	Greater Middle East variome project databases
gnomAD	genome aggregation database
gVCF	genomic variant call files
GWAS	Genome-wide association studies
HEK293	Human Embryonic Kidney cells
Het	Heterozygote
HHL	Hereditary Hearing Loss Homepage
HI	Hearing Impairment
HI-GENES Africa	Hearing Impairment Genetics Studies in Africa
HIO	Hearing Impairment Ontology
HIV	human immunodeficiency virus
HL	Hearing Loss

Hom	Homozygous
HPO	Human Phenotype Ontology
LOVD	Leiden Open Variation Database
MDR-TB	multi-drug resistant tuberculosis
MT	Mutant
NCBO	National Center for Biomedical Ontology
NIDCD	National Institute on Deafness and Other Communication Disorders
NSHI	Non-Syndromic Hearing Impairment
OBO	Open Biomedical Ontology
OME	Otitis media with effusion
OMIM	Online Mendelian Inheritance in Man
OWL	Ontology Web Language
PLP	pathogenic and likely pathogenic
PTA	Pure Tone Audiology
RP	retinitis pigmentosa
SCDO	Sickle Cell Disease Ontology
SHIELD	Shared Harvard Inner Ear Laboratory Database
SNV	single nucleotide variations
TB	Tuberculosis
WES	Whole Exome Sequencing
WT	Wild-type

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Chapter 1: Introduction

Hearing impairment (HI), or hearing loss (HL), is a common sensory disorder, colloquially referred to as the silent epidemic. It is defined as the inability to detect sounds better than 20dB in the better-hearing ear (WHO, 2023a), and it affects approximately 1.57 billion people worldwide (Haile et al., 2021). Disabling hearing loss is defined as the inability to detect sounds better than 35dB in the better-hearing ear, and affects approximately 434 million adults and 34 million children worldwide .(WHO, 2023b)

Normal hearing

The process of normal hearing is essential in understanding HI. The ear is divided into the outer, middle, and inner ear. Sound waves travel from the environment through the auditory/ear canal where they vibrate against the tympanic membrane (Holme and Steel, 1999). These vibrations are amplified by the ossicles in the middle ear, and this results in the generation of waves in the perilymph in the inner ear (Holme and Steel, 1999, Tekin et al., 2001). The waves result in movement of the tectorial membrane, which results in a shearing motion which bends the hair cells in the cochlea (Tekin et al., 2001). This causes the subsequent opening and closing of ion channels in the hair cells, which allows an influx of potassium ions into the hair cells, initiating an action potential across the cells (Tekin et al., 2001, Holme and Steel, 1999). Calcium channels are then activated, which results in the release of neurotransmitters and the subsequent activation of the acoustic nerve (Tekin et al., 2001).

Classification of HI

Considering the process of hearing, HI may be classified according to the mechanism and/or degree of HI. Hearing impairment may be conductive (where conduction of sound from the outside environment is impaired), sensorineural (where there is impairment in either the conversion of sound to an action potential or with the activation of the acoustic nerve), or mixed (where an individual has both sensorineural and conductive HI). The degree of HI is indicated in Table 1.1, where an individual's ability to hear is indicated as performance.

Table 1.1: WHO degree of Hearing Impairment classification

Degree of Hearing Impairment	Corresponding Audiometric ISO Value	Performance
No Impairment	25dB of better	Able to hear whispers
Slight/Mild Impairment	26 – 40dB	Able to hear words spoken in normal voice at 1m
Moderate Impairment	41 – 60dB	Able to hear words using raised voice at 1m
Severe Impairment	61 – 80	Able to hear some words when shouted into better hearing ear
Profound Impairment, including Deafness	81dB or greater	Unable to hear and understand shouted voice

Adapted from Olusanya et al. (2019).

The classification of HI may also be done according to the age of onset of HI, and/or the aetiology of HI. The age of onset may be congenital/prelingual, perilingual, or postlingual; depending on when the HI developed. The aetiology of HI refers to whether the HI results from genetic, environmental or unknown factors.

Genetics of HI

Genetic factors account for approximately 50% of HI cases in developed populations, of which 70% is categorised as non-syndromic HI (NSHI)(Schrijver, 2004). Autosomal recessive inheritance accounts for 70% to 80% (ARNSHI), and autosomal dominant inheritance counts for 10% to 20%. X-linked inheritance accounts for 1 to 5% of NSHI, and mitochondrial inheritance will have variable frequencies, dependent on the population (Schrijver, 2004, Tekin et al., 2001, Smith et al., 2005). Environmental factors are, however, the prevalent cause of HI in developing countries (Wonkam et al., 2013b).

Hearing impairment is associated with over 1000 mutations (Shearer et al., 2011) in over 124 genes (Van Camp G and Smith, 2021). The connexin genes are the prevalent genes associated with HI in European, North American and Asian populations (Chan and Chang, 2014, Hutchin et al., 2005, Liu et al., 2002). These genes have, however, been shown to be irrelevant in Sub-Saharan African populations, including South Africa. (Bosch et al.,

2014a, Bosch et al., 2014b, Wonkam et al., 2015, Gasmelseed et al., 2004, Kabahuma et al., 2011), with the exception of founder mutations in the Ghanaian and Moroccan populations (Gazzaz et al., 2005, Hamelmann et al., 2001a, Adadey et al., 2020, Adadey et al., 2019, Aboagye et al., 2022).

Previous studies have focussed on determining the prevalence of the connexin genes in the South African population (Bosch et al., 2014b, Lebeko et al., 2017, Kabahuma et al., 2011) and recent work by Kabahuma et al. (2021) focussed on identifying the spectrum of pathogenic variations in *MYO7A*. This study aims to address the gap in understanding the genetics of HI in South Africa by recruiting HI patients and using next-generation sequencing to determine the genes associated with HI in the population.

Chapter 2: Aims and objectives

This study aimed to identify prevalent genes associated with NSHI in the Black, Indian, and Coloured/Mixed Ancestry South African population. The aim was achieved using the following objectives:

1. Systemic review of HI in the South African population
2. Establish an ontology for uniform terminology related to HI
3. Recruit affected patients and their unaffected family members into the study, using a detailed questionnaire and obtaining DNA extracted from whole blood
4. Determine the clinical presentation of HI in the South African population
5. Use whole exome sequencing (WES) to resolve NSHI in the South African population
    a. Generate WES for all participants in the study
    b. Determine the causative mutations associated with NSHI in familial cases of NSHI in the South African population

Chapter 3: Systematic Review

Synopsis: This chapter presents current scientific knowledge regarding HI in South Africa in the form of a peer reviewed systematic review. The review includes the incidence of HI, aetiology and various technologies that may be used to detect HI in the South African population.

Manyisa, N., Adadey, S.M., Wonkam-Tingang, E., Yalcouye, A. & Wonkam, A. 2022. Hearing Impairment in South Africa and the Lessons Learned for Planetary Health Genomics: A Systematic Review. Omics. 26(1):2-18. DOI:10.1089/omi.2021.0181.

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Co-Author's contribution: S.M.A, E.T.W., and A.Y. double checked the article selection and performed the quality control analysis. A.W. conceived and supervised the entire project.

Hearing Impairment in South Africa and the Lessons Learned for Planetary Health Genomics: A Systematic Review

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Abstract

Hearing impairment (HI) is a silent planetary health crisis that requires attention worldwide. The prevalence of HI in South Africa is estimated as 5.5 in 100 live births, which is about five times higher than the prevalence in high-income countries. This also offers opportunity to drive progressive science, technology and innovation policy, and health systems. We present here a systematic analyses and review on the prevalence, aetiologies, clinical patterns, and genetics/genomics of HI in South Africa. We searched PubMed, Scopus, African Journals Online, AFROLIB, and African Index Medicus to identify the pertinent studies on HI in South Africa published from inception to 30 April 2021, and the data were summarized narratively. We screened 944 records, of which 27 studies were included in the review. The age at diagnosis is approximately 3 years old and the most common factor associated with acquired HI was middle ear infections. There were numerous reports on medication toxicity, with kanamycin-induced ototoxicity requiring specific attention when considering the high burden of tuberculosis in South Africa. The Waardenburg Syndrome is the most common reported syndromic HI. The Usher Syndrome is the only syndrome with genetic investigations, whereby a founder mutation was identified amongst black South Africans (*MYO7A*- c.6377delC). *GJB2* and *GJB6* genes are not major contributors to non-syndromic HI among Black South Africans. Furthermore, emerging data using targeted panel sequencing have shown a low-resolution rate in Black South Africans in known HI genes. Importantly, mutations in known non-syndromic HI genes are infrequent in South Africa. Therefore, whole-exome sequencing appears as the most effective way forward to identify variants associated with HI in South Africa. Taken together, this paper contributes to the emerging field of planetary health genomics with a focus on HI and offers new insights and lessons learned for future roadmaps on genomics/multi-omics and clinical studies of HI around the world.

Key Words: Hearing impairment; South Africa; systematic review; next generation sequencing; epidemiology; genetics; science and innovation policy; planetary health genomics

Introduction

Hearing impairment (HI) is a sensory condition, whereby an individual cannot hear sounds softer than 25dB for children and 30dB for adults in their better hearing ear (the ear they

hear best with)(WHO, 2021a). Hearing impairment affect approximately 1.57 billion people (Haile et al., 2021) and disabling HI affects approximately 433 million people worldwide (WHO, 2021a). Disabling HI affects approximately 49.66 million people in Sub-Saharan Africa (WHO, 2018d) and has a prevalence of 5.5 per 1000 live births in South Africa(Swanepoel et al., 2009). The South African prevalence was reported about 12 years ago and needs an update.

Hearing impairment may be classified according to the age of onset of the HI: the HI is either congenital (present at birth, prelingual (developed before the child acquired language), or post-lingual (developed after the child acquires language) (Tekin et al., 2001, Schrijver, 2004). It is also classified according to the number of ears affected, either one ear (unilateral HI) or both ears (bilateral HI) (Tekin et al., 2001, Schrijver, 2004). It may also be classified according to the type of hearing impairment (sensorineural, conductive, or mixed HI) or the aetiology of the hearing impairment (either genetic, environmental or unknown aetiology) (Tekin et al., 2001, Schrijver, 2004). Furthermore, HI may be classified according to whether it is associated with other clinical manifestations, as in syndromic HI, or not, as in non-syndromic HI (Schrijver, 2004, Tekin et al., 2001).

Genetic factors account for approximately 50 % of congenital HI in high-income countries; among which 70% of HI is non-syndromic (Schrijver, 2004); and approximately 70 to 80% of the non-syndromic HI is due to autosomal recessive inheritance (Chung et al., 1959, Morton, 1991). Environmental factors account for a greater proportion of HI in developing countries, as was indicated in Cameroon (Wonkam et al., 2013a). Environmental factors that result in HI may include, but are not limited to, infectious disease, otitis media (collection of fluid in the ear), injury to the head or ear, excessive noise, ageing, and/or exposure to ototoxic drugs (WHO, 2021a, Schrijver, 2004).

The connexin genes, *GJB2* and *GJB6,* are the most prevalent genes associated with HI in European, Asian, and North American populations (Chan and Chang, 2014, Hutchin et al., 2005, Liu et al., 2002, Najmabadi and Kahrizi, 2014, Pandya et al., 2003). The variations in the connexin genes have however been shown to be insignificant in the majority of studied sub-Saharan African populations (Bosch et al., 2014a, Bosch et al., 2014b, Wonkam et al., 2015, Tingang Wonkam et al., 2019), including South Africa (Kabahuma et al., 2011). The exception is that of the Ghanaian and Moroccan populations, whereby

Ghana has a founder mutation in *GJB2* and Morocco has the 35delG mutation in *GJB2* present in the populations (Hamelmann et al., 2001a, Gazzaz et al., 2005, Adadey et al., 2019).

South Africa is a multicultural, multi-racial (including 80.8% of Black African, 8.8% Coloured/Mixed Ancestry, 2.6% Indian/Asian, and 7.8% White (Stats-SA, 2020)), developing country on the southern tip of Africa. It has a population of 55.7 million people (Stats-SA), with a high burden of infectious disease with; where an estimated 7.1 to 8.3 million are affected with HIV (WHO, 2019) and 215 000 to 400 000 individuals infected with tuberculosis (TB) (WHO, 2019) in 2018. 60% of tested TB patients are HIV positive in South Africa in 2017 (WHO, 2018b). However, there is an increasing burden of non-communicable disease over the past decades (Mayosi et al., 2009), some of which are of genetic origin such as congenital hearing impairment. Despite the relatively high incidence of HI in South Africa, (Swanepoel et al., 2009) a review of this condition has not been investigated.

This systematic review aims to provide a landscape of HI in South Africa, (1) by examining the diagnostic approaches, prevalence, and aetiology, in particular the genetics of HI in the country, and (2) with an eye to inform HI scholarship elsewhere in a context of the emerging field of planetary health genomics.

Materials and Methods

This review is reported in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement (Moher et al., 2009).

Ethics approval

This analysis and the research that informed the present review were approved by and granted ethics clearance from the Human Research Ethics Committee of the University of Cape Town (HREC: 104/2018). Approval to recruit patients from schools was obtained from the relevant provincial education departments and permission for the recruitment was obtained from the schools from which patients were recruited. Written and informed consent was obtained from individuals 18 years old and older or from the guardian/parent, with verbal and/or written assent obtained from minor children, including approval to published identifiable photographs.

Selection Criteria

We included observational studies published from inception to 30 April 2021, that report on various aspects of HI in South Africa. This includes data on the prevalence, the aetiologies, the clinical profiles, and genetics of HI. For duplicate studies, the most comprehensive and/or initial article with the largest sample size was considered. We excluded qualitative studies, letters to the editor, reviews, and commentaries. Studies with either unavailable full text or missing key data, that could not be accessed after a reasonable request was excluded.

Search Strategy

We searched PubMed, Scopus, and African-specific databases (African Journals Online, AFROLIB, and African Index Medicus) for relevant articles. The keywords used, to search for publications were: “hearing impairment” OR “hearing loss” OR “hearing disorder” OR “hearing disorders” OR “deaf” OR “deafness” AND “South Africa”. In addition, specific researchers active in the field of hearing loss in South Africa were contacted to identify additional sources of information, and additional specific articles were thus added based on their relevance to this review.

Selection of Studies

Titles and abstracts obtained from searches were imported into the software EndNote, version X9.3.3, for the removal of duplicates. Regarding our inclusion and exclusion criteria, the initial search and review were performed by one author (NM). They screened unduplicated titles and abstracts before reviewing the full text of all selected studies for final inclusion. A second author, a medical geneticist, (AW) verified that the study screening and selection process was performed correctly. Any disagreement between the two authors was solved through discussion and consensus.

Data Extraction Process

One researcher (NM) used a predesigned data extraction sheet, to summarize data from relevant studies. Extracted data included: the last name of the first author, the year of publication, the province(s) where the study was conducted, the settings (hospital, schools, community), the study design, data collection (prospective versus retrospective), study population including demographic information (gender, age, self-declared “racial

background" according to the South African official classification), sample size and the number of cases of HI (for prevalence studies), methods used to diagnose HI, various classifications of HI: according to the types (sensorineural, conductive, mixed), levels of HI (mild, moderate, severe, profound), inheritance patterns, clinical profiles (syndromic versus non-syndromic), data on genetic testing. For some studies, relevant proportions were calculated from their raw data. Data were summarized narratively. A second researcher (SMA) checked the accuracy of the data extraction process; any discrepancy was resolved through discussion and consensus.

Assessment of Methodological Quality

The quality of included studies included was assessed by two investigators (NM and AW), with the quality of genetic studies using a risk of bias tool (Q-Genie) developed by Sohani et al. in 2015 (Sohani et al., 2015); and for prevalence and other studies, the risk of bias assessment tool developed by Hoy et al. in 2012 (Hoy et al., 2012). Discrepancies were solved by discussion and consensus.

Results

The review processes

Initially, 939 records and five targeted articles were identified through the literature search. Removing 191 duplicates retained 753 records. Two-hundred and seventy-seven records were excluded based on their titles and 476 articles were scrutinised, and 449 articles were further excluded. This rendered 27 articles eligible for inclusion in this study (**Figure 1**). The characteristics of the studies included in this review are summarized in Table 1.

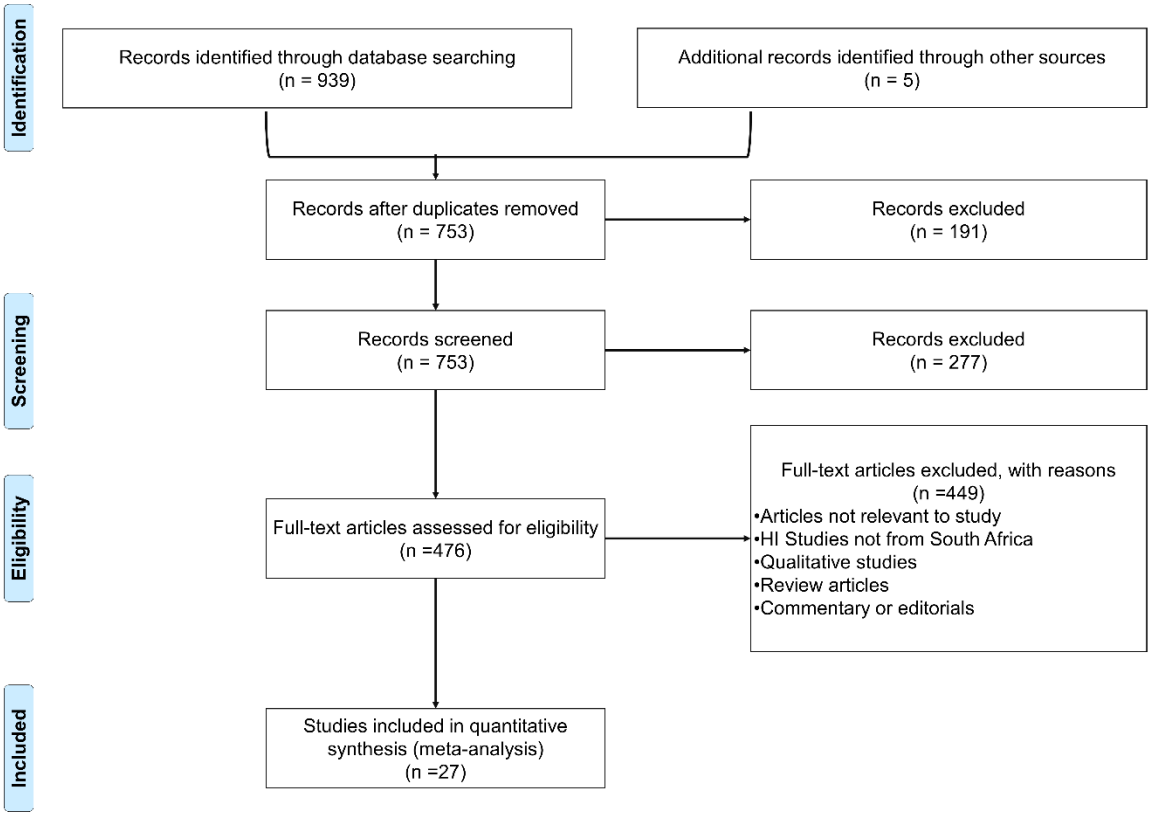

Figure 3.1: Schematic diagram of the review process.

Table 3.1 Summary of Articles reviewed for inclusion

First author's name, Publication year	Province	Study Setting	Study Design	Data collection	Study populations	Female (%)	Mean age (years)	Age range (years)	Sample size	Diagnostic tool
Appana, 2016	KwaZulu-Natal	Hospital	Longitudinal	Prospective	MDR-TB patients	48	34	18-56	52	PTA
Beighton, 1979	NR	NR	Case report	Retrospective	Family presenting with a rare syndrome	NR	NR	NR	41	
Beighton, 1993	NR	Research	Case report	Retrospective	Patients identified from an Usher syndrome survey	NR	NR	NR	3	NR
Bosch, 2014	Eastern Cape	School	Cross-sectional	Retrospective	Students from a school of the deaf	NR	NR	NR	25	NR
Butler, 2013	Free State	Hospital	Cross-sectional	Retrospective	Children referred for HI screening	NR	3.4[#]	1 months -5.9 years	260	PTA

Chaya, 2018	Eastern Cape	Hospital	Case report	Observational	A child presenting with an undiagnosed progressive condition	0	NA	11 months	1	ABR
Clarke, 2006	NR	Hospital	Case Report	Retrospective	Family presenting with BOR	NR	NR	NR	3	NR
Eksteen, 2019	Western Cape	Preschool	Cross-sectional	Retrospective	Children attending preschool	50.5	NR	4-7	8023	HearScreen, PTA
Els, 2018	Gauteng	Hospital	Cross-sectional	Observational	OME patients	49.7	$25.54; 6.75	2-12	109	PTA & POT
Ghafari, 2020	Western Cape	Hospital	Cohort study	Retrospective	MDR-TB patients	43	34.9#	NR	102	PTA
Kabahuma, 2011	Limpopo	School	Cross-sectional	Retrospective	Students two schools for the deaf	65.9	NRr	5-21	182	PTA
Kabahuma, 2021	Limpopo	NR	Cross-sectional	Retrpspective	Congenital HI individuals from Limpopo province	NR	NR	NR	94	PTA
Levin, 1966	NR	Hospital	Case Report	Observational	Two brothers presenting with Pendred Syndrome	0	9	7,100	2	Audiometry
Louw, 2018	Gauteng	Clinic	Cross-sectional		Patients at clinic	74	41.2	16-97	1084	PTA

Potgieter, 2014	Gauteng	Hospital	Cross-sectional	Prospective	Sclerosteosis patients	60	32	10-72	10	PTA, DPOAEs and ABR
Rappoport, 1970	Gauteng	Hospital	Case Report	Retrospective	Patient identified in hospital	23	NR	NR	NR	Tuning fork
Roberts, 2015	NR	NR	Cross-sectional	Retrospective	Retinal degenerative disease patients presenting with Usher syndrome	23	NR	NR	NR	NR
Roberts 2020	NR	NR	Cross-sectional	Retrospective	Retinal degenerative disease patients presenting with rod-cone dystrophy, HI and renal dysfunction	NR	NR	NR	4	NR
Sandstrom, 2020	Gauteng	Clinic	Cross-sectional	Prospective	Patients at community clinics	71	52	20-88	63	HearScreen, PTA
Spracklen, 2017	Western Cape	Hospital	Cross-sectional	Retrospective	Cancer patients treated with cisplatin	28.8	48[#]	14-75	222	PTA
Swanepoel, 2007	Gauteng	Hospital	Cross-sectional	Retrospective	New-born babies	NR	0	0	6241	TEOAE

Tekendo-Ngongang, 2019	Western Cape	Hospital	Cross-sectional	Retrospective	Patients with Noonan Syndrome	NR	[#]4.5	1 month-51 years	26	NR
Tiedt, 2013	Free State	Hospital	Cross-sectional	Prospective	Children with CSOM	47.7	4.6[#]	1-12	86	PTA
Tshifularo,2013	Gauteng	Hospital	Cross-sectional	Prospective	HIV patients	58.8	31.5	1-76	153	NR
Viljoen, 1983	Western Cape	Hospital	Case Report	Retrospective	The family that was seen at the genetic clinic	62.5	21	11-49	8	PTA
Winship, 1992	Western Cape	School and Genetic Clinic	Cross-sectional	Retrospective	Children diagnosed with Waardenburg syndrome in a study at school	NR	NR	NR	101	NR
Yaniv, 1987	Eastern Cape	Hospital	Case report	Observational	Patients with tuberculous otitis media	38.7	18	9 months – 67 years	31	NR

Mean age given of male participants and female participants separately, with male participants mean age indicated first; #Median age given instead of mean age; NR= not reported; NA = not applicable PTA = pure-tone audiometry; POT = pneumatic otoscopy and tympanometry; DPOAEs = distortion product otoacoustic emission; ABR = auditory brain response; CSOM = chronic suppurative otitis media; MDR-TB =

multi-drug resistant tuberculosis; TEOAE = transient evoked otoacoustic emissions; HearScreen is a hearing screen mobile application developed by the hearX group. BOR= Beanchio-oto-renal syndrome.

Prevalence and newborn screening

The prevalence of HI in developing countries is estimated to be 6 in 1000 live births (Olusanya and Newton, 2007), but this is a general estimate, and it is not specific to South Africa. Swanepoel et. al., (2007, (Swanepoel et al., 2007)), partially resolved the lack of prevalence data by reporting, over four years, on the universal new-born hearing screening (UNHS) programme that ran at a private hospital in Gauteng. Of the 13 799 births at the hospital, only 6 241 new-borns were screened for HI (Swanepoel et al., 2007). The first 22 months of the project, the screening was subsidized by the hospital, whereas in the succeeding 26 months, the parents were responsible for paying for the screening. Within the first 22 months of UNHS, there was a 75% uptake in the UNHS programme, and this was followed by a drop to 20% in the succeeding 26 months(Swanepoel et al., 2007).

The UNHS starts with an initial hearing screening for the babies and those who failed were rescreened (Swanepoel et al., 2007). Two hundred and nineteen infants of 694 that failed the initial screening, were rescreened in the hospital and 19 presented with HI that required diagnostic testing (Swanepoel et al., 2007). The authors estimated a 3 in 1000 prevalence of HI at birth, in the private sector, after taking into consideration the new-borns who did not return to the hospital for the rescreen (Swanepoel et al., 2007). Swanepoel et. al, (2009) furthered the work to determine an estimated prevalence of 5.5 in 1000 births, after taking into consideration the public health care system (Swanepoel et al., 2009).

Age of diagnosis

The median age of diagnosis for children presenting with HI, at a tertiary public hospital in Bloemfontein, was shown to be 3.7 years old by Butler et. al. (2013, (Butler et al., 2013)). The median age of the first appointment at the hospital was 3.4 years and there was a median delay of 49 days between the first appointment at the hospital and diagnosis (Butler et al., 2013).

The median age of diagnosis for children presenting with HI in a private audiology practice in Bloemfontein was shown to be 2.24 years old by Butler et. al. (2015, (Butler et al., 2015)). The median age of diagnosis was significantly different between patients seen in the private practice and patients seen in the tertiary public hospital (Butler et al., 2015). The patients who were screened at birth had a median age of diagnosis of 1.25 years old whereas patients who weren't screened at birth had a median age of diagnosis of 3.01 years (Butler et al., 2015). The differences in the median age of diagnosis based on new-born screening are, however, not significantly different (Butler et al., 2015).

Technology and techniques used to detect-HI

Community health workers (CHWs) used the hearScreen mobile application, from the hearX group, to screen 8023 children in preschool, for HI, in Khayelitsha and Mitchells Plain in the Western Cape, as reported by Eksteen et. al. in 2019 (Eksteen et al., 2019). Of the 8023 children, 2313 failed the initial test and 435 failed their retest (Eksteen et al., 2019). Three-hundred and eighty-nine children of the 435 who failed the retest, were screened by the project audiologist and 124 children were referred for diagnostic evaluation (Eksteen et al., 2019). Only 94 of the 124 children attended their diagnostic evaluations, of which 54 were diagnosed with HI (Eksteen et al., 2019).

The hearTest application, from the hearX group, and PTA was used to screen 58 individuals for HI after five individuals were excluded from the study (Sandstrom et al., 2020). The application was shown to have a 90.6% sensitivity to detect HI above 40dB (Sandstrom et al., 2020). The HI threshold varied between 0.9 and -5.4dB between the application and PTA in the patients, with the average time for the HI test on the application being 512 seconds (Sandstrom et al., 2020).

Aetiology: acquired hearing impairment

The most frequent cause of acquired HI was due to post-natal complications such a meningoencephalitis, streptomycin induced HI, and middle-ear diseases (Beighton et al., 1991). Meningitis accounted for 230 cases, maternal rubella accounted for 143 cases, severe infections accounted for 118, and jaundice accounted for 66 cases of environmental HI in the 765 children with acquired HI (Beighton et al., 1991).

Otitis media with effusion, chronic suppurative otitis media, and tuberculous otitis media

Otitis media with effusion (OME) was studied, in children admitted for either an adenoidectomy or adenotonsillectomy in the otorhinolaryngology department of an academic hospital in Pretoria, by Els et. al. (2018, (Els and Olwoch, 2018)). The prevalence of OME was 11.9% and 22.9% bilaterally and unilaterally respectively in a group of 102 children (Els and Olwoch, 2018), whereas another study of 136 children had an OME prevalence of 16.5% (Biagio et al., 2014). The OME reported by Els et. al. (2018) resulted in a mean hearing loss of 19.8dB (Els and Olwoch, 2018) whereas Biagio et. al. (2014 (Biagio et al., 2014)) had not reported any audiometry for study participants.

Thirty-one patients presented with tuberculous otitis media, a rare form of chronic suppurative otitis media (CSOM), at the Ear, Nose and Throat department of a hospital in the Eastern Cape, what was then the Ciskei, between 1984 and 1985 (Yaniv, 1987). Twenty-six patients were found to have conductive HI of varying degrees, with five patients also presenting with sensorineural HI (Yaniv, 1987). Six patients could not be tested but one patient was noted to have bone sequestra (i.e. a piece of necrotic bone detached from the healthy tissue) and stapes lying loose in the middle ear (Yaniv, 1987). The study found that, of the 31 patients, only 14 patients (47%) had a healthy second ear (Yaniv, 1987).

Tiedt et. al. (2013) examined CSOM in 86 children from the Free State (Tiedt et al., 2013). In contrast to Yaniv (1987), 68.6% of patients presented with unilateral CSOM and 31.4% was bilateral (Tiedt et al., 2013). Audiometry was performed in 46 patients, a total of 66 ears, with HI present in 44 ears (66.7% of ears tested), with the median for the average HI being 38.3dB (Tiedt et al., 2013).

A study to determine the trends of head, neck, and ENT manifestation of HIV-infected patients looked at 153 patients, at an academic hospital in Pretoria, was undertaken by Tshifularo et. al. (2013, (Tshifularo et al., 2013)). The study determined that 14.29% of the patients presented with sensorineural HI (Tshifularo et al., 2013). The study also indicated that the common otological manifestation was CSOM, which affected 27% of patients, and 15.58% of their patients were affected with OME (Tshifularo et al., 2013).

Medication ototoxicity

Cisplatin-induced ototoxicity

In 222 cancer patients treated with cisplatin, ototoxicity was observed at rates between 39.2 and 66.7% depending on the ototoxicity grading scale used, according to Spracklen et. al., (2017, (Spracklen et al., 2017)). An earlier study had indicated a 55.1% incidence of cisplatin-induced ototoxicity in the South African population, of which 62.7% was bilateral HI (Whitehorn et al., 2014). Spracklen et. al. (2017, (Spracklen et al., 2017)) used the grading scaled indicated by Konrad-Martinet. Al. (2005, (Konrad-Martin et al., 2005)), Chang et. al. (2010, (Chang and Chinosornvatana, 2010)), and the U.S. Department of Health and Human Services (2017, (Services, 2017)).

Ototoxicity was shown to be associated with increased cisplatin dosage, alone, and with cisplatin dosage and rs6721961in *NFE2L2*, on all three grading scales following correction

for multiple testing (Spracklen et al., 2017). SNP rs316019 in *SLC22A2* was significant when using the Chang grading scale (Spracklen et al., 2017, Chang and Chinosornvatana, 2010).

Multi-drug resistant tuberculosis (MDR-TB) and kanamycin induced ototoxicity

Fifty-two patients, from KwaZulu Natal, were included in a study to determine the effects of aminoglycoside treatment, for multi-drug resistant tuberculosis (MDR-TB), over 5 intervals during a 6-month period (Appana et al., 2016). Appana et. al., (2016) observed normal hearing in 44% in the right ear and 40% in the left ear of the patients before treatment (Appana et al., 2016). Sensorineural HI was present in 50% of patients in the right ear and 54% of patients in the left ear before treatment, with all patients receiving kanamycin (Appana et al., 2016). The hearing profile of all patients changed to show bilaterally clinically significant HI for all patients by the fifth post-treatment session (Appana et al., 2016). Following their fifth post-treatment session, sensorineural HI was present in 94% of the patients on their right ear and 96% of patients on their left ear (Appana et al., 2016).

The authors saw a gradual deterioration in the average hearing thresholds following the commencement of treatment when considering the low (125Hz, 250Hz, 500Hz), mid (1000Hz and 2000Hz), high (4000Hz and 8000Hz), and ultra-high (10 000Hz and 12 500Hz) frequencies(Appana et al., 2016). The deterioration in average hearing thresholds in the low frequencies remained within the normal limits throughout the course of the treatment (Appana et al., 2016).

Similarly, Ghafari et. al., (2020) analysed audiometry data in 102 patients receiving kanamycin for treatment of MDR-TB in Cape Town (Ghafari et al., 2020). The patients were tested for HI before treatment and after 4, 8, and 12 weeks after starting treatment (Ghafari et al., 2020). Eighty-four patients (82.4%) developed HI during the course of treatment with Kanamycin and the HI was significantly associated with the exposure of kanamycin (Ghafari et al., 2020).

Streptomycin induced ototoxicity

A report by Viljoen et. al. (1983) investigated a large, nonconsanguineous family with streptomycin-induced ototoxicity (Viljoen et al., 1983). The HI affected 8 family members, of both sexes, and was bilaterally and ranged from moderately severe to very severe sensorineural HI (Viljoen et al., 1983). The ototoxicity susceptibility in the family was determined to be autosomal dominantly inherited (Viljoen et al., 1983) and genetic analysis identified a 1555A to G variation in the mitochondrial DNA as a causative mutation associated with the streptomycin-induced HI (Gardner et al., 1997).

Genetics of Hearing Impairment

Diagnostic surveys of HI in 4452 scholars were undertaken by Beighton et. al. (1991) since 1975 (Beighton et al., 1991). The authors were able to ascertain that genetic syndromes accounted for 8% of HI, non-syndromic HI accounted for 12%, acquired HI accounted for 25% of HI of unknown causes accounted for 55% in the population (Beighton et al., 1991). Waardenburg syndrome was the most common cause of syndromic HI due to genetics, followed by Treacher-Collins, Sclerosteosis, and Usher Syndrome among others (Beighton et al., 1991). A handful of genetic syndromes and non-syndromic genetic cases will be reported below.

Syndromic hearing Impairment

Hearing impairment in Sclerosteosis

The audiological profile of patients with Sclerosteosis was studied by Potgieter et. al. (2014 (Potgieter et al., 2014)). The study consisted of 10 individuals, of the total 36 living individuals diagnosed with Sclerosteosis; of which 18 lived in the Gauteng region (Potgieter et al., 2014). All 10 individuals present with HI, which ranged from moderated, in one individual, to profound in eight individuals (Potgieter et al., 2014). The HI was mixed in all individuals and symmetrical in six participants(Potgieter et al., 2014).

Beighton et. al. (1973) had previously reported on the clinical features of Sclerosteosis, in a study that comprised of 21 individuals (Beighton et al., 1976). Conductive HI was indicated to be apparent in patients with Sclerosteosis once the patient started schooling (Beighton et al., 1976). The conductive HI was present bilaterally in all adults and three children in the study (Beighton et al., 1976). The authors indicated that the sensorineural HI may develop in adulthood due to compression of the vestibulocochlear nerve or due to involvement of the oval and round windows in the cochlear (Beighton et al., 1976).

The involvement of the oval and round windows was observed in CT scans take in nine (18 ears) of the ten patients in the Potgieter et. al. (2014) study (Potgieter et al., 2014). Abnormalities were noted in 16 ears (89%) in the oval window and in 17 ears (94%) in the round window (Potgieter et al., 2014). The abnormalities observed included windows containing bony overgrowths and/or the windows been closed amongst other observations (Potgieter et al., 2014). The authors, furthermore, noted the narrowing of the internal auditory canal or the closure of the canal in 16 ears (Potgieter et al., 2014).

Brown-Vialetto-van Laere Syndrome

The first reported case of Brown-Vialetto-van Laere Syndrome in South Africa was of an 11-month-old baby from the Eastern Cape (Chaya et al., 2018). The case, as reported by Chaya et. al. (2018), indicates that the child had normal Apgar scores at birth but developed progressive dysphagia at 10 weeks and intermittent stridor (Chaya et al., 2018). Symptom progression resulted in constant stridor, persistent dysphagia, regression of motor milestones, ptosis, paradoxical abdominal movements when breathing that were consistent with diaphragmatic paralysis, and bilateral vocal cord palsy at 11 months and auditory brain stem response indicated that the child had sensorineural HI (Chaya et al., 2018). Genetic analysis indicated a heterozygous pathogenic variant and a variant of unknown significance in *SLC5A3* that supported the diagnosis of Brown-Vialetto-van Laere Syndrome (Chaya et al., 2018).

Cranio-metaphyseal dysplasia

Beighton et. al. (1976) investigated a rare syndromic disorder in 41 individuals from a family, of which 15 individuals were determined to be affected (Beighton et al., 1979). The proband presented with bilateral HI, a prominent forehead, right facial paly, and left facial weakness (Beighton et al., 1979). Radiological findings indicated cranial thickening with sclerosis and metaphyseal flaring at the knees (Beighton et al., 1979). The symptoms presented in the proband were observed, to varying degrees, in several relatives with a variable presence of HI in the family (Beighton et al., 1979).

Usher syndrome and Rod-Cone Dystrophy

Beighton et. al. (1993) identified a rare and potentially novel syndrome, which manifests as sensorineural HI, rod-cone dystrophy, and kidney dysfunction (Beighton et al., 1993); with the Fanconi lesions resulting in skeletal changes and kidney failure that may be fatal (Roberts et al., 2020). The patients were identified during a large-scale survey of individuals with Usher syndrome and all patients were of Afrikaans descent (Beighton et al., 1993). The patients studied included two sisters and an unrelated teenage boy; the eldest of the girls and the teenage boy presented with profound HI sensorineural HI and the younger girl presenting with mild sensorineural HI (Beighton et al., 1993). The three children had similar fundal findings (including choroidal depigmentation, a macular scar, and vascular attenuation) and Fanconi-type renal lesions (Beighton et al., 1993). The skeletal changes observed in this condition are indicated in Figure 2a.

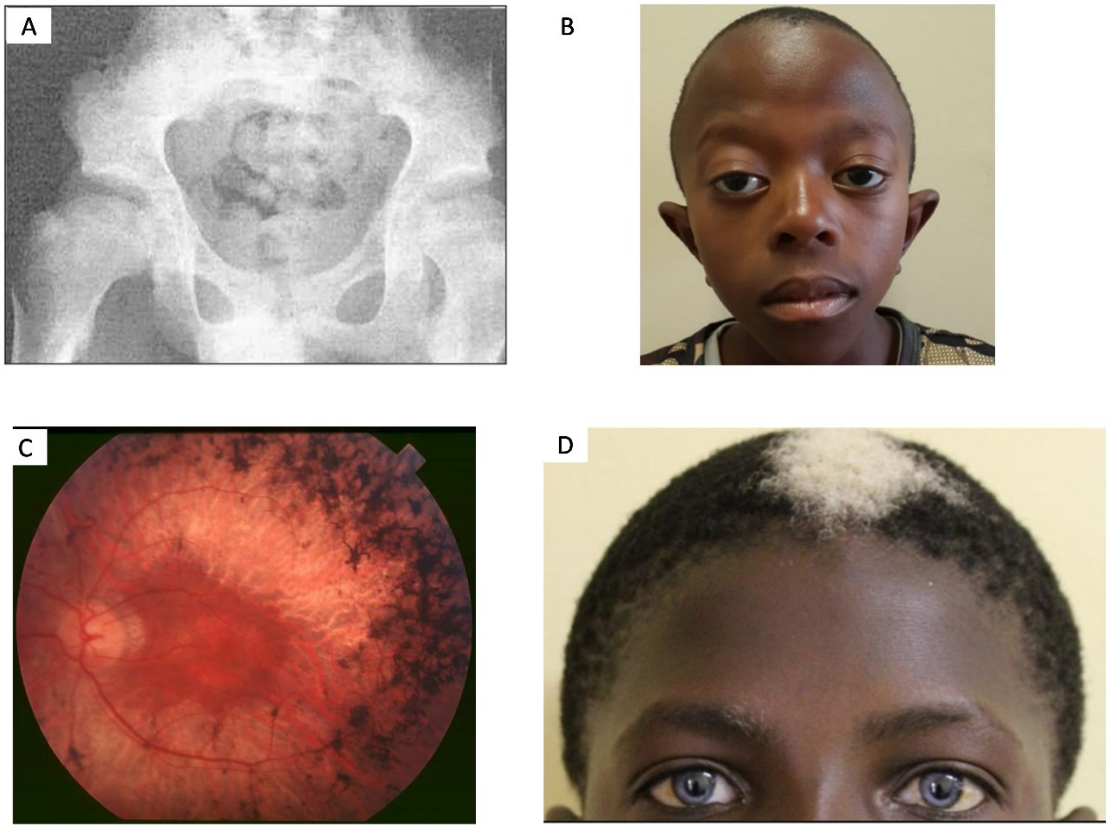

Figure 3.2: Images of the characteristics of various syndromic conditions. (A) Indicates the skeletal changes observed in renal dysfunction, rod-cone, and sensorineural HI. The kidney dysfunction results in rickets-like skeletal changes in the condition. Adapted from Beighton et. al. (1993) © John Wiley and Sons. **(B)** Fundus photographs of a patient with pathologic variation in *USH2*, showing reduction of the retinal arteries and bone-spicule pigment deposits. Adapted from Hamel (2006), with copyright permission. **(C)** Craniofacial features of a child presenting with Noonan syndrome. Adapted from Tekendo-Ngongang et. al. (2019) with copyright permission. **(D)** South African Adolescent child presenting with heterochromia iridium, no dystopia canthorum and a white forelock representative of Type II Waardenburg syndrome, recently observed in our clinic (Written parental consent and verbal assent from minor obtained granting permission to reproduce photograph).

Recent work by Roberts et. al. in 2020, identified the novel homozygous *RRM2B*-c.786G>T variant as a plausible disease-causing mutation in two sisters, an unrelated male and an unrelated female affected with renal dysfunction, rod-cone dystrophy, and sensorineural HI (Roberts et al., 2020). The variant was discovered using whole-exome sequencing on the affected siblings and their carrier parents (Roberts et al., 2020). The two sisters were recruited and reported by Beighton et. al. (1993).

Usher syndrome is an inherited HI syndrome characterised by HI and visual impairment due to retinitis pigmentosa (Kimberling et al., 1992). The condition was first reported by Von Graefe in 1858 (von Graefe, 1858) and its inherited nature was emphasized by C.H. Usher in 1914 (Usher, 1914). Usher Syndrome has 3 subtypes of which Usher I has the most severe phenotype. Usher I is characterised by congenital severe to profound sensorineural HI and vestibular dysfunction and the onset of retinitis pigmentosa in the first decade of life (Kimberling et al., 1992, Van Camp G and Smith, 2021). Usher II is characterised by congenital moderate to severe sensorineural HI, absence of vestibular dysfunction, and the onset of retinitis pigmentosa in the first or second decade of life (Van Camp G and Smith, 2021, Weston et al., 1996, Kimberling et al., 1992). Usher III presents as progressive sensorineural HI with variable vestibular dysfunction and variable onset/presence of retinitis pigmentosa(Weston et al., 1996, Van Camp G and Smith, 2021). The characteristics of retinitis pigmentosa are indicated in Figure 2b.

A study of inherited retinal degenerative disease patients by Roberts et al (2015) identified a founder mutation that is responsible for a significant proportion of Usher Syndrome amongst Black South Africans (Roberts et al., 2015). The mutation in *MYO7A* was first identified in two unrelated individuals and, following screening in another 12 Black Usher patients, it was seen in a further six Black South Africans (Roberts et al., 2015, Roberts et al., 2016). The study had included six Black suspected Usher patients and three Usher patients of mixed ancestry, but the founder mutation was absent within these groups (Roberts et al., 2015). Molecular analysis in a cohort of Usher patients by Roberts et. al. (2015) indicated a homozygous deletion inf *MYO7A* that affected six of 12 (42.86%) Black African individuals (Roberts et al., 2015). The variation *MYO7A* c.6377delC was shown to be a founder mutation, whereby the affected patients had the same haplotype (Roberts et al., 2015).

Hearing impairment in Noonan Syndrome

Noonan syndrome is an autosomal dominantly inherited multisystem disorder characterised by congenital heart disease, small stature, ocular hypertelorism (which is abnormally increased distance between the eyes) and skeletal malformations (Roberts et al., 2013). Patients present with distinct craniofacial dysmorphism and the syndrome is clinically heterogeneous(Tekendo-Ngongang et al., 2019). Approximately 10% of patients with Noonan syndrome will present with HI due to sensorineural hearing impairment(Roberts et al., 2013, Ahituv et al., 2000). Figure 2c illustrates the craniofacial dysmorphism that may be observed in a child with Noonan syndrome.

A study of 26 patients presenting with Noonan syndrome, by Tekendo-Ngongang et. al. (2019), observed that 15% (n=4) of patients presented with HI (available in the supplementary material) (Tekendo-Ngongang et al., 2019). Twenty of the patients were unrelated and 65% of the patient population had at least on cardiovascular abnormality, of which pulmonary stenosis was the most prevalent (Tekendo-Ngongang et al., 2019). The study was able to ascertain causative variations in *CBL, PTPN11* and *MAP2K1* with the variants segregating with the condition in the respective families (Tekendo-Ngongang et al., 2019).

Pendred Syndrome

Pendred syndrome, characterised by HI and goitre with the absence of iodine deficiency, was first described by Vaughan Pendred, in two sisters, in 1896 (Pendred, 1896, Reardon et al., 1997). The autosomal recessive inheritance of Pendred syndrome was ascertained by W.R. Brain in 1927 (Brain, 1927) and the first gene, *SLC26A4*, associated with Pendred syndrome was first described in 1997 by Everett et. al. (Everett et al., 1997).

Levin and Klugman described the case of two brothers with Pendred syndrome in 1966 (Levin and Klugman, 1966). The 7year old, younger brother, was admitted into the hospital due to swelling in the neck (goitre) (Levin and Klugman, 1966). The child had congenital sensorineural HI and subsequently diagnosed with Pendred Syndrome. Familial history indicated an elder brother who also had HI and he was, likewise, diagnosed with Pendred syndrome (Levin and Klugman, 1966). The two brothers were treated with 1-thyroxine sodium, of which the younger brother showed greater improvement than his elder brother(Levin and Klugman, 1966).

Branchio-oto-renal syndrome

Branchio-oto-renal syndrome (BOR) was first described in 1976 by Melnick et. al. following the examination of a father and his 3 affected children (Melnick et al., 1976). The variability of the phenotypic expression of the condition was noted by Heimler et. al. in 1986 following the study of a large family presenting with BOR (Heimler and Lieber, 1986) and the causative gene, *EYA1*, was identified in 1997 by Abdelhak et al. (Abdelhak et al., 1997). The condition is characterised by abnormalities of the ear (inner, middle and outer), HI, branchial cleft sinuses and fistulae, lacrimal duct stenosis and renal abnormalities (Heimler and Lieber, 1986).

Clark et. al. (2006) reported on a pre-natal proband presenting with agenesis of the kidneys in a South African Afrikaans family (Clarke et al., 2006). A previous pregnancy, where the

child passed on after birth, had also presented with BOR syndrome, and clinical manifestations were observed in the father of the children(Clarke et al., 2006). The father had a unilateral pre-auricular pit and HI of unknown aetiology(Clarke et al., 2006). A de novo, novel, heterozygous *EYA1* 727G>T was identified as the causative mutation that originated in the father (Clarke et al., 2006).

Waardenburg Syndrome

Waardenburg syndrome was first described by P.J. Waardenburg in 1951(Waardenburg, 1951). The syndrome is characterised by sensorineural HI associated with pigmentary abnormalities of the hair, eyes and skin (white forelock, characteristic blue irises and depigmentation of the skin) and dystopia canthorum (Waardenburg, 1951, Van Camp G and Smith, 2021). The syndrome has four subtypes; whereby Type I and Type II are differentiated by the presence of dystopia canthorum in Type I (Van Camp G and Smith, 2021). Type III is a combination of Type I Waardenburg with upper limb abnormalities and Type IV is the combination of Type II with Hirschsprung Disease (Van Camp G and Smith, 2021). Waardenburg is inherited, predominantly, in an autosomal dominant pattern (Waardenburg, 1951) and the *MITF* was the first gene associated with Waardenburg (Tassabehji et al., 1994). Figure 2d illustrates the facial characteristics that may be observed in Waardenburg syndrome.

Rappoport et. al., in 1970, reported on a 23-year-old woman who was diagnosed with Waardenburg syndrome following a hospital visit (Rappoport, 1970). The young woman presented with a white forelock and dystopia canthorum (Rappoport, 1970). The Waardenburg syndrome originated from her grandmother; with the white forelock present in all affected members (Rappoport, 1970). Neither HI nor heterochromia iridium was present in the family members, but some members presented with dystopia canthorum and/or hyperplasia and depigmentation of the medial portion of the eyebrows (Rappoport, 1970).

A retrospective study by Seller et. al. (1983) identified 90 children presenting with Waardenburg Syndrome from 3006 HI children in 19 schools (Sellars and Beighton, 1983). The authors noted the presence of dystopia canthorum in 54% of the children and that a proportion of the children presented with heterochromia iridium in the absence of other clinical manifestations (Sellars and Beighton, 1983). Furthermore, the variability of Waardenburg was noted even amongst siblings (Sellars and Beighton, 1983)

The possibility of a founder mutation been responsible for Waardenburg Syndrome amongst the Afrikaans population was examined by de Saxe et. al. in 1984 (de Saxe et al., 1984). The author analysed genealogy of three Afrikaans families and noted that all three families

were related with the Waardenburg phenotype originating from a grandfather who passed it down to his descendants (de Saxe et al., 1984).

Variation in the expression of Waardenburg syndrome was investigated in 68 children attending Schools for the Deaf and 33 individuals, from seven families, who had attended a genetic clinic staffed by Winship et. al. (1992, (Winship and Beighton, 1992)). The children, at the schools, all presented with profound sensorineural HI and 89% of the children had variable pigmentation in the eyes (Winship and Beighton, 1992). Bilateral sapphire blue eyes were present in 28 children, 24 children had heterochromia iridium and nine children had segmental heterochromia either unilaterally or bilaterally and none of the children had Hirschsprung disease (Winship and Beighton, 1992).

The variations observed in the children were also observed in the familial cases. Six of the seven families identified presented with type 1 Waardenburg and the seventh family presented with type 1 Waardenburg. Hearing impairment was present in six individuals, of the 33 individuals in the type 1 families, and in the four siblings of type 2 (Winship and Beighton, 1992).

A cross-sectional study of a 13-member South African family identified a splice site mutation in *PAX3* that segregated with Waardenburg syndrome within the family (Butt et al., 1994). In contrast, a case report detailed the comorbid presence of Waardenburg and tetraphocomelia in a premature neonate that passed away 10 minutes after birth (Wu et al., 2009). The mother had detailed an uneventful pregnancy except for swelling and discomfort in the 29th week of pregnancy (Wu et al., 2009). The mother subsequently went into labour four days after her discomfort complaint and the neonate was born with gross malformations of the limbs, heterochromia iridium and a white forelock (Wu et al., 2009). Family history indicated that the mother, grandmother and uncle of the baby all had white forelocks but genetic testing was inconclusive (Wu et al., 2009).

Genetic aetiology: Non-syndromic hearing impairment

The connexin genes have been studied in 25 Black African students from one school of the deaf in the Eastern Cape (Bosch et al., 2014b, Bosch et al., 2014a) and 183 Black African students from two School of the in Limpopo province (Kabahuma et al., 2011), by Bosch et. al. (2014) and Kabahuma et. al. (2011), respectively. Participants for the study by Kabahuma et. al. (2011) underwent audiometric assessments (including tympanometry, transient otoacoustic emissions and PTA) before inclusion into the study and all participants were determined to have stable sensorineural HI (Kabahuma et al., 2011). The study by

Bosch et. al. (2014) of the 25 patients from Eastern Cape had no audiological data (Bosch et al., 2014b), in comparison to the study performed in Limpopo.

Molecular analysis of potentially causative variants in both the Eastern Cape and Limpopo studies yield no causative mutations in either *GJB2* or *GJB6* (Bosch et al., 2014a, Bosch et al., 2014b, Kabahuma et al., 2011). Lebeko et. al. (2017) furthered the research for putative causative mutations in the 25 patients from the Eastern Cape. The patients were screened for and were negative for variants in *MYO7A, CDH23, LOXHD1, OTOF* and *SLC26A4* (Lebeko et al., 2017), which had been previously discovered in a targeted sequencing approach (Lebeko et al., 2016).

Kabahuma *et. al.* published a follow-up study, in 2021, providing molecular analysis for causative mutations in 94 patients, presenting with non-syndromic HI, from Limpopo (Kabahuma et al., 2021). The study identified eight *MYO7A* variants, of which four variants were novel(Kabahuma et al., 2021). The novel variation p.Thy1780Ser was the most common variation identified, and it was present in four of the eight families segregating variations in *MYO7A*(Kabahuma et al., 2021).

Discussion

The global prevalence of HI is approximately one in five people presenting with HI; which is an estimated 1.57 billion people affected with HI (Haile et al., 2021). Hearing impairment has been classed as the 4th leading cause of disability worldwide (Wilson et al., 2017), but data on the prevalence in low-income countries are sparse (Haile et al., 2021).

This present study is the most comprehensive review of HI in South African to date. The research showed the prevalence of HI has received little attention in South Africa over the last decade, with no study reporting the national prevalence of the condition. The reported prevalence of 5.5 in 1000 live births is much higher than the estimated prevalence of 1.33 in 1000 and 1.86 in 1000 live births in developed countries such as the USA and England respectively (Morton and Nance, 2006). Researchers, such as Swanepoel (2009), indicated that a large-scale study may be necessary to determine the prevalence of HI in South Africa. This may be aided by the use of smartphone screening applications, similar to the ones used by Eksteen et al. (2019); when taking into consideration the validation done by Sandstrom et al. (2020). The smartphone application could putatively be used to screen patients for HI at their community clinics and refer patients who fail the screening for diagnostic testing. This screening method may allow for a more accurate determination of the prevalence of HI in South Africa and early detection of HI in both children and adults.

This screening method may potentially be cost-effective approach in screening for HI in under-resourced settings and may allow for the early detection of HI in patients.

Early diagnosis of HI is essential in the process of giving appropriate and effective interventions to HI patients (Ching et al., 2017). Cochlear implantation, before 6 months after birth, results in better language development in children with congenital/prelingual HI compared to those who received implants after 24 months (Ching et al., 2017). The implementation of universal new-born hearing screening (UNHS), has helped many countries to achieve a reduction in age at diagnoses of HI; an average of 20 months age of HI diagnoses was observed in Italy (Canale et al., 2006) and 2-5 months in the USA (American Academy of Pediatrics, 2007, D'Aguillo et al., 2019), Despite the UNSH achievements, some countries still have a high average age of HI diagnosis; 4.1 years in New Zealand (Gruber et al., 2019), 6-11 years in Ghana (Adadey et al., 2019), and 7.4 years in South Korea (Lim et al., 2018). Even though South Africa has a relatively lower age of HI diagnosis (between 2-4 years) (Butler et al., 2015, Butler et al., 2013) compared to other African countries, there is a need to reduce the age of diagnosis to few months after birth.

Though South Africa is an emerging country, it has one of the highest rates of wealth inequality (Vorster, 2021). Wealth inequality may play a role in the uptake of new-born hearing screening within the country, as seen by Swanepoel et al. (2007), though early diagnosis is shown to be cost-effective intervention in addressing the cost of unaddressed HI (WHO, 2017b). Universal, subsidised hearing screening for both new-borns and other members of the population may mitigate the financial barriers that patients and their families face in seeking a diagnosis.

Determining the barriers to diagnosis of HI and the causes of HI in the South Africa population may allow for better understanding of HI in South Africa. Merugumala et al. (2017) studied the barriers to early diagnosis of HI in an Indian city. One parent noticed that their child was not hearing when the child was eight-to-nine months old, but did not consult with a doctor because their child was not sick; whereas another parent thought the child would start speaking when they were older (Merugumala et al., 2017). These perceptions may not be limited to this Indian city, as in a study pertaining to the perceptions of HI by parents of children with HI in South Africa, seven of the eleven parents could not identify the cause of HI or attributed the HI to environmental aetiology (Gardiner et al., 2019). This calls for thorough engagement with the community, to make the early symptoms of HI and the causes of HI well known, including genetic aetiologies. Dispelling incorrect perceptions within the wider population could be a key objective that could aid in lowering the age of diagnosis in South Africa and in other developing countries.

In Africa, the acquired causes of HI require further attention since they account for a large proportion of cases (Wonkam et al., 2013a, Adadey et al., 2019). In Ghana and Cameroon cerebrospinal meningitis, severe malaria and otitis media were identified among the acquired causes of HI (Wonkam et al., 2013a, Adadey et al., 2019). Available data South African indicate otitis media with effusion as the main cause of acquired HI among children (Yaniv, 1987, Biagio et al., 2014, Els and Olwoch, 2018). Otitis media with effusion is a chronic inflammatory condition, characterised by effusion behind an intact membrane with the absence of the signs and symptoms of acute inflammation (Qureishi et al., 2014). Otitis media with effusion is less severe than CSOM, but it is shown by Els et. al. (2018) to result in a mean HI of 19.8dB (Els and Olwoch, 2018). The prevalence of HI in the OME patient group was not indicated, though caregivers reported HI in 6.6% of 136 patients in the study by Biagio et. al. (2014, (Biagio et al., 2014)). CSOM, conversely, resulted in a mean HI of 38.3dB in the subset of patients who had PTA done (Tiedt et al., 2013). This means the HI threshold included children where there was no complaint of HI, with children with a complaint of HI (72.7% of tested ears) having a mean HI threshold of 41.7dB (Tiedt et al., 2013). The work on OME and CSOM is invaluable in understanding the causes of HI in the South African population, but further studies are necessary to identify all underlying causes of HI in the population. Identifying the underlying causes of HI will indicate the proportion of HI that is due to genetic factors and the proportion due to environmental factors; which may allow for better surveillance and management of HI in the population.

Additionally, ototoxicity was reported by researchers as possible causes of HI in South Africa. Cisplatin-induced HI was observed in some cancer patients. The HI observed with cisplatin usage has been associated with variants in *NFE2L2* and *SLC22A2* genes (Spracklen et al., 2017, Chang and Chinosornvatana, 2010). Genetic testing for the variants in *NFE2L2* and *SLC22A2* may be offered to patients prior to cisplatin based therapy. This may assist in determining the risk of developing HI following chemotherapy for patients. Furthermore, recent clinical trials, in the USA and Canada, have shown sodium thiosulphate as a promising agent to protect against the HI induced by the essential component of cancer chemotherapy, cisplatin (Freyer et al., 2017) and lovastatin and atorvastatin were shown to protect against/reduce cisplatin-induced ototoxicity in mice and human adults, respectively (Fernandez et al., 2021, Fernandez et al., 2020). Putatively, a trial of atorvastatin and sodium thiosulphate may be included in the treatment plans of South African patients undergoing cisplatin-based chemotherapy to mitigate HI due to ototoxicity.

The studies considered for the review also reported HI in patients with MDR-TB (Ghafari et al., 2020, Appana et al., 2016). MDR-TB treatment requires the use of strong drugs, such as an aminoglycoside, which are ototoxic (Ruhl et al., 2019, Jiang et al., 2017) and resulted in HI in 82.4% and 100% of patients in the two reviewed studies (Appana et al., 2016, Ghafari et al., 2020). Aminoglycoside antibiotics induce HI by disrupting the intercellular physiological pathways in sensory cells that take up the drug due to the high rate of aminoglycoside trafficking across the endothelial and epithelial barrier layers in humans (Jiang et al., 2017). The use of aminoglycosides is necessary for the fight against MDR-TB, however meta-analysis of 87 studies, comprising of 12 030 patients with MDR-TB analysed the outcomes associated with different drugs (Collaborative Group for the Meta-Analysis of Individual Patient Data in et al., 2018). The analysis found that kanamycin and capreomycin were associated with worse outcomes in the treatment of MDR-TB(Collaborative Group for the Meta-Analysis of Individual Patient Data in et al., 2018) and both medications are no longer recommended for treatment of MDR-TB by the WHO (WHO, 2018c). It may thus be necessary to update treatment plans to eliminate kanamycin to treat MDR-TB.

This review takes into regard the genetics of HI, where there was a relatively high amount of literature on syndromic HI gathered. In particular, consanguinity was observed in several syndromic studies reported in Africa (Abdi et al., 2016, Ben-Rebeh et al., 2016, Riahi et al., 2015, Hmani-Aifa et al., 2009, Boulouiz et al., 2007, Bousfiha et al., 2017, Ben Said et al., 2012, Ben-Rebeh et al., 2010) and can be considered to have played an active role in the segregation of recessive conditions within the affected family members. Atipo-Tsiba (2016)discussed the impact of consanguinity in his case report on a 40-year-old man and concluded that “Consanguineous marriages are sometimes the source of rare and often serious genetic disease. Doctors, political and religious leaders should join forces to ban such unions between members of the same family”. Unions between related individuals are considered null and void, within the South African legal context (Wille et al., 2007). It is, however, feasible that individuals were unaware of their degree of relatedness; when taking into consideration the possibility of founder mutations in the South African population, which are associated with population bottleneck (Roberts et al., 2015). An example would be the three Waardenburg families investigated by de Saxe et al. (1984), where genealogical tracing determined that they were from a common ancestor, though no consanguinity occurred.

Waardenburg syndrome, in the studies observed, is the most common genetic syndrome associated with HI in the South African population, with a similar observation being reported in Cameroon (Wonkam Tingang et al., 2020). This may be due to the syndrome being

associated with the clinical features that are easily observable, i.e. white forelock, and heterochromia.(Beighton et al., 1991, Winship and Beighton, 1992). Waardenburg is however phenotypically variable and Winship and Beighton (1992) has indicated that careful consideration should take place in order to differentiate the various forms of Waardenburg . A shortcoming in these studies on Waardenburg syndrome, similar to the study on rod-cone-dystrophy (Beighton et al., 1993) and cranio-metaphyseal dysplasia(Beighton et al., 1979), is the absence of the underlying causative variations. This is in contrast to the indication of the causative variations for Brown-Vialetto-van Laere Syndrome (Chaya et al., 2018) and Noonan Syndrome (Tekendo-Ngongang et al., 2019). Fortunately, the use of next generations sequencing is rapidly helping to narrow the gap in South Africa, as evidenced by the 2020 work of Roberts *et. al.* (Roberts et al., 2020).

Unlike South Africa, there are molecular data on syndromic HI in other African settings, mostly from North Africa. Genetic studies in Algeria, Tunisia and Morocco identified causative mutations in Usher 2 and Usher 1 patients (Abdi et al., 2016, Hmani-Aifa et al., 2009, Ben-Rebeh et al., 2016, Riahi et al., 2015, Boulouiz et al., 2007, Bousfiha et al., 2017). Eighteen unrelated Algerian patients, of which 16 had Usher 1 and 2 had Usher 2, were recruited from a larger cohort of HI patients by Abdi et al. (2016). Seventeen putatively pathogenic mutations were identified within the cohort, whereby sixteen patients were homozygous for deleterious mutations and two patients were compound heterozygous for deleterious mutations (Abdi et al., 2016). Eight of the variants were novel and putatively deleterious in the patients. These novel variants were in *MYO7A, CDH23, PCDH15, USH1C, USH1G,* and *USH2A* (Abdi et al., 2016). Similarly, a large consanguineous Tunisian family segregated a novel *GPR98* mutation that segregated with the USH2C phenotype in the family(Hmani-Aifa et al., 2009). The family, additionally, had members that presented with autosomal recessive retinitis pigmentosa; whereby *PDE6B* was identified as the gene responsible for the visual impairment (Hmani-Aifa et al., 2009). Variations in *MYO7A, USH1C* and *PCDh15* were, likewise, identified as possibly causative variations in four consanguineous Tunisian families, with three of the four identified variations being novel(Ben-Rebeh et al., 2016). And private mutations in *MYO7A* and *USH1C*, in four unrelated Tunisian families, were determined to be the causative mutations for the Usher 1 phenotype observed (Riahi et al., 2015). Usher 1B and Usher 2C were identified in two separate studies of two consanguineous Moroccan families (Boulouiz et al., 2007, Bousfiha et al., 2017). In the first family, 2 homozygous variations in *MYO7A*, were identified of which c.1687G>A was determined to be the disease-causing variation and p.Y1719C was a polymorphism within the population (Boulouiz et al., 2007). In the second family, compound

heterozygous mutations, one nonsense and one missense, in GRP68 were identified as the Usher 2C causative mutations in the family (Bousfiha et al., 2017).

El Bouchikhi et al. (2015) undertook mutation screening for putative causative variations in *PTPN11* for Noonan Syndrome, in a North African. setting The study identified 19 participants, of which 16 were reached and had genetic testing performed (El Bouchikhi et al., 2015). Congenital heart disease was present in 17 of the 19 patients and one patient presented with sensorineural HI (El Bouchikhi et al., 2015). Known heterozygous causative variations were identified in four patients; of which 2 patients had the same variation and the other two patients had differing variations (El Bouchikhi et al., 2015).

A novel homozygous frameshift mutation was identified as the causative mutation in a consanguineous Tunisian family by Ben Said et al. (2012), in Pendred Syndrome. The family consisted of six affected family members, whereby diagnosis of Pendred syndrome was based on the HI, enlarged vestibular aqueduct and goitre (Ben Said et al., 2012). Goitre was present in 2 of the six affected family members, with five patients presenting with profound HI and one patient presenting with severe HI (Ben Said et al., 2012). Enlarged vestibular aqueduct was present in 5 patients and the five patients were determined to be homozygous for a c.451delG frameshift mutation in *SLC26A4* (Ben Said et al., 2012). The sixth family member was homozygous for p.E47X in *GJB2* and had no goitre or enlarged vestibular aqueduct (Ben Said et al., 2012). Charfeddine et al. (2010) and Ben-Rebeh et al. (2010) identified homozygous p.L445W in three and eight consanguineous Tunisian families respectively, presenting with Pendred Syndrome. There was variable presence of goitre within the eight consanguineous families, of which only three individuals, of the total 41 individuals, presented with goitre (Ben-Rebeh et al., 2010).

Generally, comparative data on syndromic HI has been rather scarce from other African countries. Visual abnormalities have been studied within the Nigerian population, whereby two separate studies screen school going individuals for visual impairments (Abah et al., 2011, Onakpoya and Omotoye, 2010). Retinitis pigmentosa, and derivatively Usher Syndrome, was identified in 4/620 (Abah et al., 2011) and 1/156 (Onakpoya and Omotoye, 2010). The studies, however, did not include genetic testing. In Nigeria, BOR was diagnosed in a 4-year-old child who presented with bilateral HI at an ear, nose and throat speciality hospital (Nasir et al., 2018). The child had bilateral branchial fistulae and pre-auricular sinuses (Nasir et al., 2018). Her hearing impairment was determined to be mixed HI and an ultrasound indicated agenesis of the right kidney (Nasir et al., 2018). The causative variation was determined to be *de novo* in the child; as both her parents and siblings had no

phenotypic manifestations, but genetic testing was unattainable due to the absence of the facility at the hospital (Nasir et al., 2018).

Although there are over 120 HI genes globally identified (Van Camp G and Smith, 2021), the prevalent genes associated with non-syndromic HI in South Africa remain unknown. The South African population is genetically diverse, and it is necessary to investigate the contribution of *GJB2* and *GJB6* at different locations in the country (Bosch et al., 2014a, Bosch et al., 2014b, Kabahuma et al., 2011). There was, however, no significant contribution of *GJB2* or *GJB6* to HI in South Africa (Kabahuma et al., 2011, Bosch et al., 2014a, Bosch et al., 2014b). The above observations provide further supporting evidence that the contribution of *GJB2* to HI in Africa is negligible; with exceptions from Morocco (Gazzaz et al., 2005, Ratbi et al., 2007), Ghana (Brobby et al., 1998, Hamelmann et al., 2001b, Adadey et al., 2019), Sudan and Kenya (Gasmelseed et al., 2004). Furthermore, other HI genes including *REST, CDH23, LOXHD1, OTOF,* and *SLC26A4* were investigated and found negative within the population (Lebeko et al., 2017, Manyisa et al., 2021). Kabahuma et al. (2021)has pointed to the potential of *MYO7A* being a prevalent gene within the population. The variations identified within the study, however, need to be ascertained in a larger cohort of unrelated patients presenting with non-syndromic HI, as was done by Lebeko et al. (2017). The findings, of the abovementioned genetic studies, support the need for large studies in South Africa in order to understand the genetics of HI within the population and identify prevalent mutations that result in HI in South Africa.

Genetic HI studies within the country may also benefit from modelling Retinal Degenerative Disease project which follows patients on a long-term scale (Roberts et al., 2016). The causative mutations that segregate within the family may not necessarily be stagnant and instances where family members carry different mutations may arise, as observed in the family studied by Ben Said et al. (2012) and the presence of both Usher Syndrome and ARRP in the family studied by Hmani-Aifa et al. (2009). Long term follow-up may allow for the larger pedigrees and, coincidently, assist in the study of the genetics of HI within the South African population, likely with the use of whole-exome sequencing.

Conclusions

The study revealed a lack of appropriate prevalence study on HI in South Africa, with the best estimate being 5.5 per 1000 birth. The age at diagnosis is relatively late, around 3 years of age and the most common environmental factor associated with acquired HI was infection of the middle ear. There were numerous reports on medication toxicity; including kanamycin

(treatment used in multi-drug resistant tuberculosis (MDR-TB)) induced ototoxicity, that should deserve specific attention considering the high burden of TB in South Africa.

The Waardenburg Syndrome is the most common reported syndromic hearing impairment, but it has not been investigated at the molecular level. Usher Syndrome is the only syndrome that has benefited for intensive genetic study, with identification of a specific founder mutation *(MYO7A*- c.6377delC), among Black South Africans, and there are emerging data using next-generation sequencing on Noonan syndrome as well as Renal dysfunction, rod-cone dystrophy, and sensorineural hearing loss syndrome. *GJB2* and *GJB6* are not major contributors of non-syndromic HI among Black South Africans, and the few available data using targeted panel sequencing on limited sample size, have shown a very low pick-up rate in known HI genes. The use of mobile screening approaches in primary health care settings, along with extensive research into the causes of HI in the South African population are necessary to understand the prevalence and factors that result in HI. Through the study of the causes of HI, the preventable cases of HI may be identified, and further work may be done in order to ease the burden of HI within the population. The use of Whole-exome sequencing in multiplex families will be necessary for the better understanding of genetic HI, particularly the non-syndromic form within South Africa.

Local and global impact statement

Hearing impairment is a silent planetary health crisis that requires attention within South Africa and internationally. The prevalence of HI in South Africa is estimated to be 5.5 in 1000 live births in South Africa, which is at least five times higher than the prevalence of HI in developed countries. The prevalence of HI within the population requires policy changes to implement systems that will allow for diagnosis and management of HI within the population. There is however a dearth of structured information regarding HI within the population and this systematic review remedies this gap by collating key information regarding HI into a single source.

Taken together, this systematic review contributes to the emerging field of planetary health genomics with a focus on HI and offers new insights and lessons learned for future roadmaps on genomics/multi-omics and clinical studies of HI around the world.

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Chapter 4: Development of a Hearing Impairment Ontology (HIO)

Synopsis: This chapter present the hearing impairment ontology. The ontology presents hierarchical, logical and standardised terms and definitions used by researchers, clinicians and individuals when referring to hearing impairment.

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The Hearing Impairment Ontology: A Tool for Unifying Hearing Impairment Knowledge to Enhance Collaborative Research

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Abstract

Hearing impairment (HI) is a common sensory disorder that is defined as the partial or complete inability to detect sound in one or both ears. This diverse pathology is associated with a myriad of phenotypic expressions and can be non-syndromic or syndromic. HI can be caused by various genetic, environmental, and/or unknown factors. Some ontologies capture some HI forms, phenotypes, and syndromes, but there is no comprehensive knowledge portal which includes aspects specific to the HI disease state. This hampers inter-study comparability, integration, and interoperability within and across disciplines. This work describes the HI Ontology (HIO) that was developed based on the Sickle Cell Disease Ontology (SCDO) model. This is a collaboratively developed resource built around the 'Hearing Impairment' concept by a group of experts in different aspects of HI and ontologies. HIO is the first comprehensive, standardized, hierarchical, and logical representation of existing HI knowledge. HIO allows researchers and clinicians alike to readily access standardized HI-related knowledge in a single location and promotes collaborations and HI information sharing, including epidemiological, socio-environmental, biomedical, genetic, and phenotypic information. Furthermore, this ontology illustrates the adaptability of the SCDO framework for use in developing a disease-specific ontology.

Keywords: hearing impairment; hearing loss; ontology; data harmonization; meta-analysis

Introduction

Hearing impairment (HI), the partial or total inability to hear, is a communication barrier and language development impediment. It can thus have a huge effect on one's quality of life (Chadha et al., 2018). HI has the highest rate for age-standardized disability of life in the world (Murray et al., 2015, Vos et al., 2015). According to the most recent World Health Organization (WHO) estimates (WHO, 2018a), over six percent of the world's population, representing approximately 460 million individuals, are currently living with a disabling HI, of which 93% are adults and mostly males (242 million males vs 190 million females). The financial burden associated with HI, which includes costs for healthcare, education, social support, and loss of productivity (Graydon et al., 2019), is estimated to be 750 billion US dollars annually (WHO, 2017a). Even though 60% of HI cases can be prevented (Graydon et al., 2019), the number of cases is expected to significantly increase to over 900 million in 2050 (WHO, 2018a) with huge negative economic implications, unless action is taken. As a matter of urgency, there is a need to strengthen collaborative HI research efforts aimed at curbing the projected increased burden of HI globally. The Global Hearing Loss project (https://thespindle.org/project/global-hearing-loss-database/) has highlighted that HI research data is commonly unstructured, stored in natural language format, and hardly shared. The general lack of standardization of research data on rare or neglected diseases across studies (Adekile et al., 2019) hampers presentation, sharing, integration, and interoperability of important information, such as prevalence, socio-environmental, biomedical, and phenotypic information. The need for harmonized HI datasets motivated the World-Wide Hearing group to develop a standard platform, the Global Hearing Loss Database (GHLD). The GHLD is based on WHO protocols with a web portal and a smartphone application to ease HI data collection and sharing processes. However, given the complexity of HI etiologies and phenotypes, analyses of these datasets and inter-study comparability would require a standard knowledge representation of the HI knowledge domain (Löhler et al., 2019). A standard knowledge representation of the HI concepts or terms would include concise descriptions to ensure a common understanding of the domain and to enable automated reasoning and inferencing. Moreso, with the constant evolution of biomedical knowledge (Adekile et al., 2019), a human- and machine-readable upgradeable system is needed for standardized and well-defined HI knowledge representation to enhance collaborative research in the field.

Recent advances in artificial intelligence have fostered the use of ontology models to represent knowledge and information-based systems (Martinez-Cruz et al., 2012) in a

human- and machine-readable format to help process, reuse, and re-apply knowledge (Gruber, 1995, Mazandu and Mulder, 2012). An ontology is useful in establishing a common and controlled vocabulary system, describing key concepts, properties, and hierarchical relationships between concepts (Mazandu et al., 2017), with precise definitions for clear and unambiguous communication. In the biomedical research context, several human disease-related ontologies have been introduced, including the Human Disease Ontology (DO), which consistently defines various concepts encountered in disease domains (Kibbe et al., 2015), the Mondo Disease Ontology, which provides a merged and comprehensive cross-species disease ontology (https://monarch-initiative.github.io/mondo/), and the Human Phenotype Ontology (HPO), providing controlled vocabularies of abnormal phenotypes encountered in human disease (Kohler et al., 2017, Kohler et al., 2019). However, as previously argued in support of developing the Sickle Cell Disease Ontology (SCDO) (Graydon et al., 2019), none of the existing ontologies comprehensively captures related concepts specific to HI due to the complex nature of HI etiologies and phenotypes.

We present the Hearing Impairment Ontology (HIO), built upon the SCDO framework. The HIO was compiled by a working group, which includes HI and ontology experts, who defined, in detail, essential aspects of the HI knowledge domain (e.g., phenotypes, genetics, therapeutics, diagnostics, etc.) and how these aspects are related. Similar to how the SCDO was built around the central concept, 'Hemoglobinopathy' (Adekile et al., 2019), the HIO is built around the 'Hearing Impairment' concept. However, because HI can be associated with a myriad of phenotypes and/or syndromes, caused by various genetic, environmental, and/or unknown factors, the SCDO framework was adapted to account for the additional complexities of HI. To date, this developing HIO represents the most comprehensive standardized HI domain knowledge portal, which will allow for the application of ontology-driven mining approaches for the identification of pertinent research questions.

Materials and Methods

For this first version of the HIO, the working group consisted of experts from an existing Hearing Impairment Genetics Studies in Africa (HI-GENES Africa) project at the University of Cape Town, Division of Human Genetics in the Department of Pathology, and expert ontology developers to provide technical guidance. The experts from the HI-GENES Africa project included PhD students and Postdocs, with a varied range of expertise which included clinicians, biomedical scientists, geneticists, and bioinformatics experts. SCDO curators and developers led the design, following the published ontology development reporting guidelines (Matentzoglu et al., 2018) and best practice.

SCDO Model-Based HIO Development

After attempting to "fit" different forms of HI and their associated causes into the same upper classes used in the SCDO (except replacing 'Hemoglobinopathy' with 'Hearing Impairment'), it was apparent that the HIO model needed to be carefully adjusted to account for the marked differences in the causes and pathophysiology between these diseases. A schema for the HIO was drawn up to formalize how the HIO would be modelled (what main classes would be needed and what relationships would be described between these classes). It is worth noting that these two diseases have complex phenotypic expressions, influenced by several genetic and environmental factors. Since SCDO was built around the central concept 'Hemoglobinopathy' to include more factors influencing its phenotypes (Adekile et al., 2019), likewise, HIO is built around the central concept 'Hearing Impairment' to ensure that all aspects influencing the disease outcome and phenotypic manifestations are captured. This is achieved by relating different HI concepts specified in the ontology to various factors, including genetic and environmental factors, that contribute to the disease outcome. The overview of different steps in the modeling, from populating the ontology, checking different concepts and relations to the release of the HIO by curators, domain and ontology experts via internal and external reviews, is described in Figure 1.

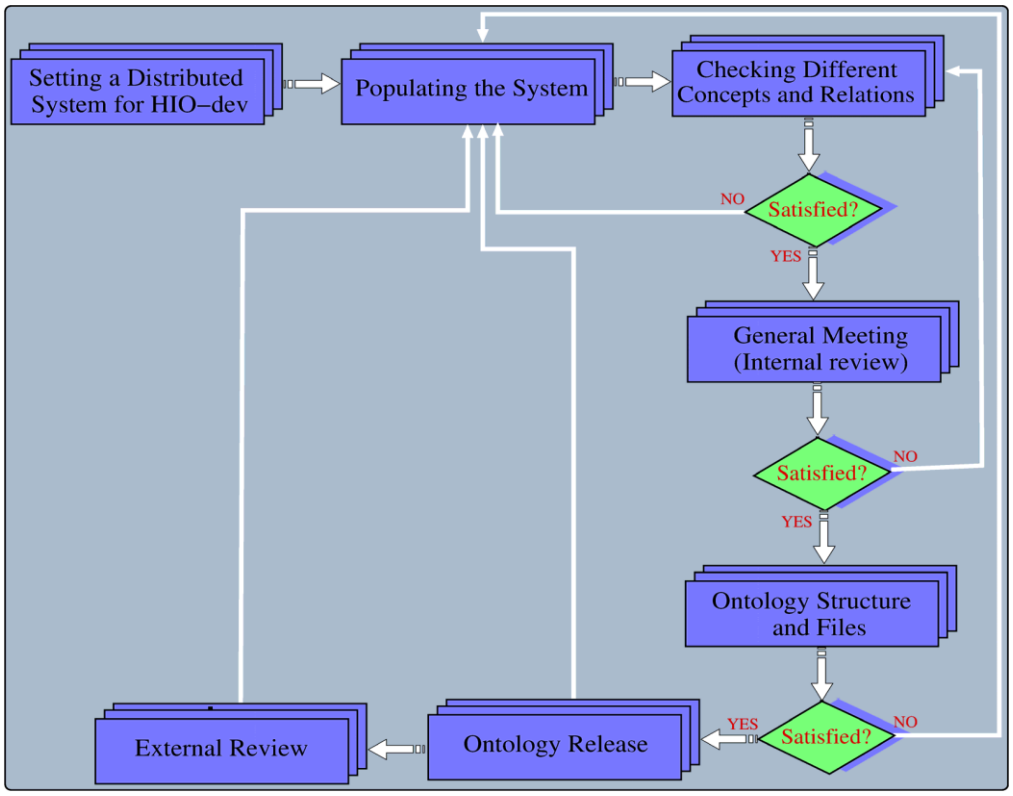

Figure 4.1. Flow chart of the dynamic and iterative ontology development process. It starts by setting up an online collaborative ontology development tool, WebProtege, which provides a highly distributed ontology content management system, enabling domain experts, ontology curators, and developers to share and update information, and easily visualise the ontology classes and structure. A general discussion meeting (or internal review) is called to share a common understanding of existing Hearing Impairment (HI) knowledge currently included in the ontology and resolve any disagreement about a given concept.

Building Different HIO Objects

Annotation properties (both required and optional) were re-used from the SCDO. The additional annotation property, 'deprecated synonym', was included by the working group in order to indicate when a term has a synonym that is no longer acceptable. Terms to be included in the ontology were added and annotated in a shared online spreadsheet by the working group. The relationships between classes were also captured in shared online spreadsheets (each sheet dedicated to the relationships made by a certain object property). To keep track of terms reused from existing ontologies, the 'existence in other ontologies' annotation property was used to assign an 'existence status' to each term. The frequency of existence statuses was subsequently used also to evaluate ontology terms unique to the HIO and its contribution to updating HI terms in other ontologies.

Distributed Model-Based HIO Design

Coordinating an ontology development with groups of contributors from heterogeneous specialized backgrounds to derive a unified domain conceptualization is challenging. To ease the process, the online collaborative ontology development tool, WebProtege (Horridge et al., 2019), was used to draft the skeleton structure (labels of terms only) of the HIO in Ontology Web Language (OWL) format. This tool provides a highly distributed ontology content management system, enabling domain experts, ontology curators, and developers to share and update information, and easily visualise the ontology classes and structure.

HIO File Refinement and Evolution

For quality control assurance of different concepts in the ontology and considering the dynamic evolution of the ontology structure, an iterative and collaborative process was used for refining different definitions and properties, as well as the topological structure. These concepts were validated by experts before being included into the hio-edit.owl file in WebProtege. Each term had to be checked by at least two members of the working group, including at least one HI expert. Once terms were validated during the general online discussion meeting (or internal review), curators added their annotations from the spreadsheet into the WebProtege project (recording in the spreadsheet which terms had been added). Thereafter, ROBOT, an open-source tool for automating ontology development workflows and tasks (Jackson et al., 2019), was used to compile the complete

ontology release files, which are Ontology Web Language (OWL) and Open Biomedical Ontology (OBO) formats.

Results

HIO General Description

Other than the 'HIO' and 'deprecated terms' classes, the HIO currently consists of 10 upper classes (see Figure 2). It contains 495 terms that are topologically connected by 543 links (is_a relationships) and expected to be associated with each other by 45 object properties (excluding the owl:topObjectProperty). Figure 2 shows how the SCDO upper classes were adapted for the HIO. Figure 3 shows the number of concepts in the HIO's upper-level classes (Graph A) and the number of relationships currently asserted between concepts via object properties (Graph B) in this first release version of the ontology.

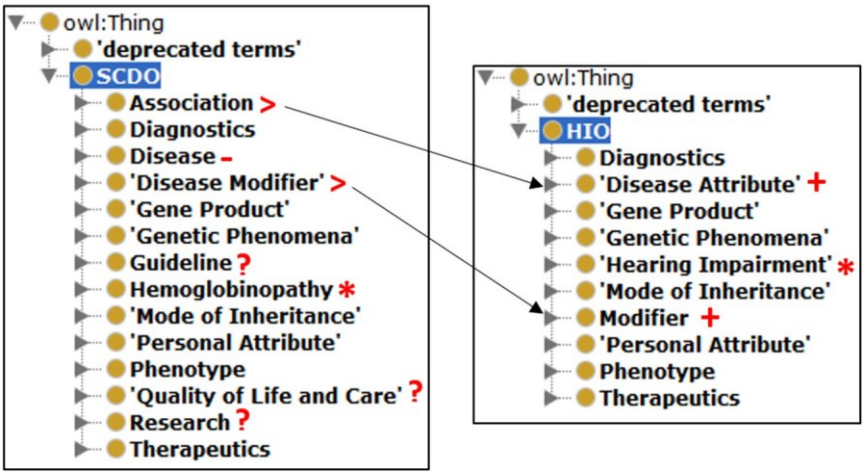

Figure 4.2. The upper classes of the Sickle Cell Disease Ontology (SCDO) and HI Ontology (HIO). (*) indicates the ontologies' central classes. (+) indicates classes in HIO but not in the SCDO. (-) indicates a class in SCDO but not in HIO. (>) indicates classes in SCDO that were incorporated in other HIO classes. (?) indicates SCDO classes that still need to be reviewed and adapted as necessary for inclusion into the HIO.

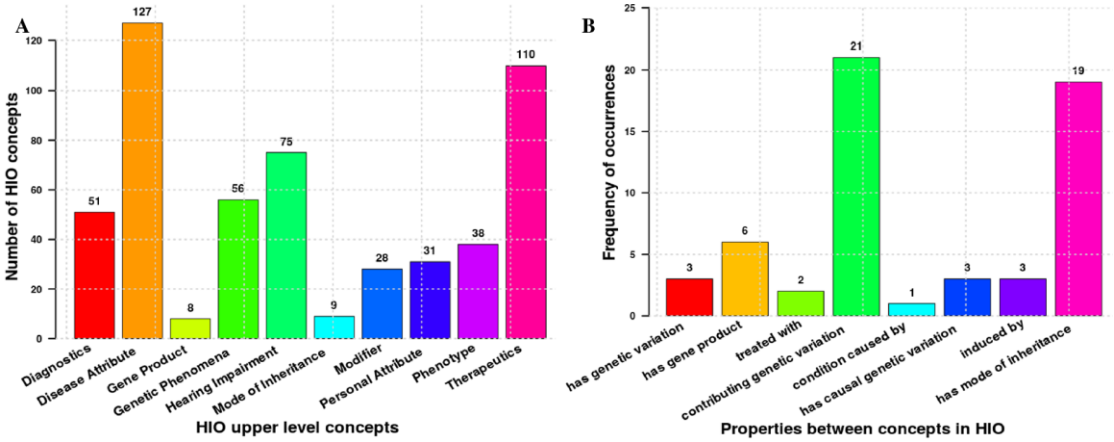

Figure 4.3. Summary statistics of current concepts and properties in the current HIO. Numbers at the top of bars represent the number of different HIO sub-classes topologically linked to upper-level classes (**A**) and the occurrence frequency of a given property or association in the ontology (**B**). Note that 'contributing genetic variation' is used as the short hand label for the 'gene carrying contributing genetic variation' property and 'has causal genetic variation' for the 'condition has causal or contributing genetic variation' property.

The following upper classes from the SCDO are included in this first draft of the HIO: Diagnostics, Gene Product, Genetic Phenomena, Mode of Inheritance, Personal Attribute, Phenotype and Therapeutics. A new HIO identifier is assigned to each reused concept from other ontologies and attached to cross references to the source ontology. The SCDO's

central ‘Hemoglobinopathy’ class has been replaced by a new central ‘Hearing Impairment’ class, which contains four main subclasses: ‘Hearing Impairment by Cause’, ‘Hearing Impairment by Ear Affected’, ‘Hearing Impairment by Onset’, and ‘Hearing Impairment by Physiopathology Mechanism’ (see Figure 4), which are comprehensively populated with the current HI domain knowledge, capturing various aspects associated with HI.

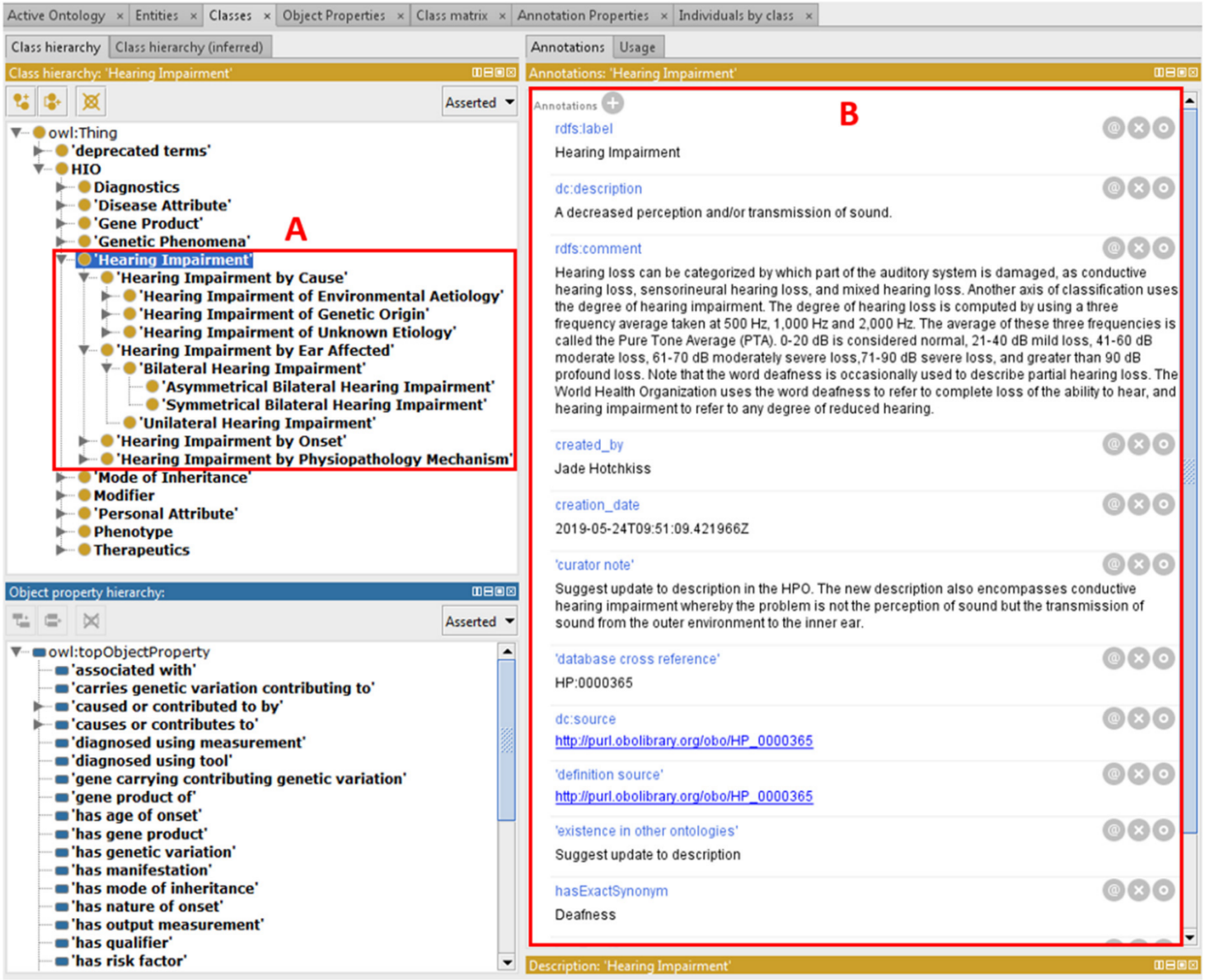

Figure 4.4. The 'Hearing Impairment' class within the HIO. (**A**) General categorization of hearing impairments in the 'Hearing Impairment' upper class and (**B**) annotations of the 'Hearing Impairment' class.

Two new (compared to the SCDO) upper classes have been included, namely: 'Disease Attribute' and 'Modifier' (see Figure 2). The 'Disease Attribute' class (see Figure 5) incorporates content similar to the SCDO's 'Association' class but notably also includes a 'Disease Cause' sub-class, which was found necessary due to the varied and often complex causes of hearing impairments (Nance, 2003, Adadey et al., 2017, Fook and Morgan, 2000, Szyfter et al., 2013). The 'Disease Cause' class contains the term 'Unknown Etiology' and the two subclasses 'Environmental Disease Cause' and 'Intrinsic Disease Cause' (Cunningham and Tucci, 2017, Rudman et al., 2017, Angeli et al., 2012), which are populated comprehensively with factors that cause or contribute in some way to HI.

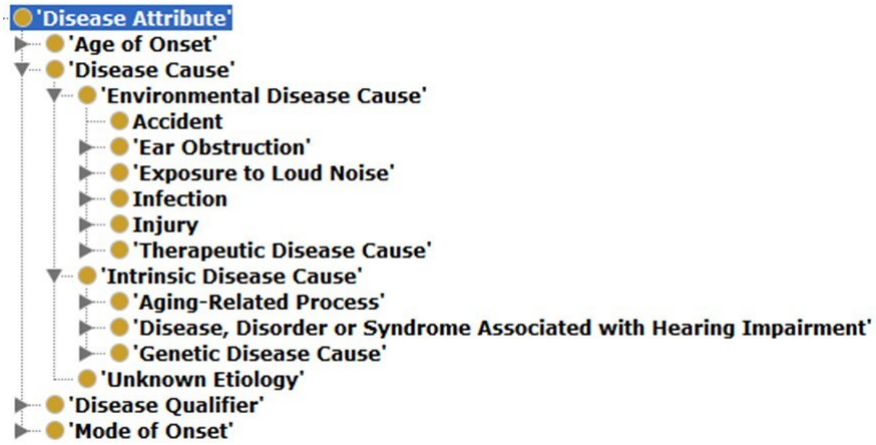

Figure 4.5. The 'Disease Attribute' class structure within the HIO. This hierarchy is intended to contain all possible features specific to or leading to HI.

The 'Modifier' class includes the SCDO's 'Disease Modifier' upper class as a sub-class, along with a new 'Disease Cause Modifier' class. This additional type of modifier was included because causes of hearing impairment sometimes have modifying factors that determine whether or not the disease

is in fact caused/present, e.g., ototoxicity induced by drugs (one cause of hearing impairment) has numerous modifying factors (e.g., dose, duration of therapy, concurrent renal failure, infusion rate, lifetime dose, coadministration with other drugs having ototoxic potential, genetic susceptibility) (Lustig, 2018).

The HIO's central 'Hearing Impairment' class links, either directly or indirectly, to sub-classes of all other upper classes through numerous associations, as can be seen in Figure 6. For simplicity, associations made with terms in the 'Modifier' upper class are not shown (see these in Figure 7) and only associations with the 'Disease Cause' sub-class of the

‘Disease Attribute’ class are displayed. As shown by the large yellow c-shape in Figure 6, the ‘Intrinsic Disease Cause’ class encompasses portions of most of the other upper classes, namely ‘Genetic Phenomena’, ‘Phenotype’, ‘Gene Product’, ‘Personal Attribute’ and ‘Therapeutics’.

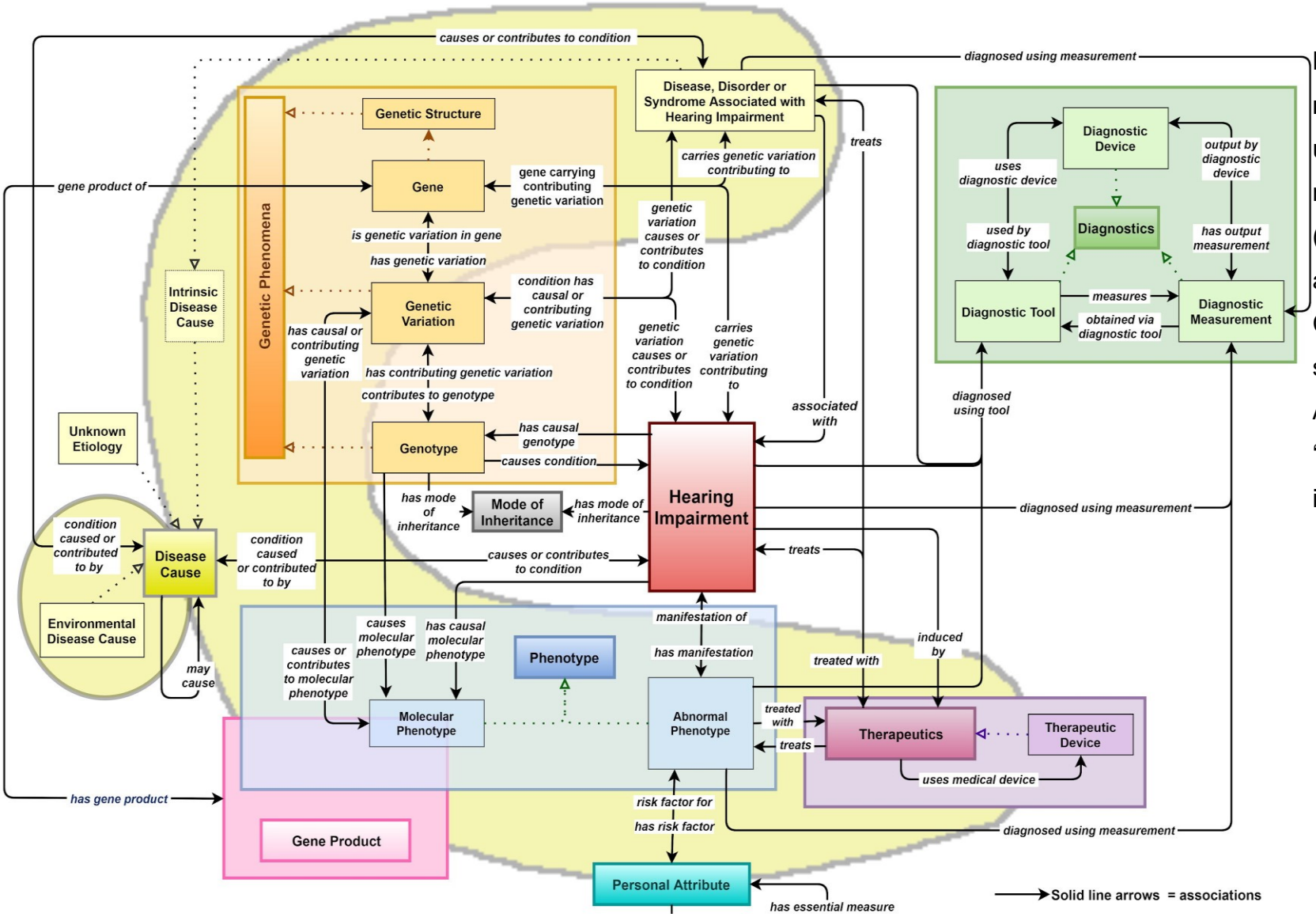

Figure 4.6. Associations made in the HIO between upper level (close to the root of the ontology) classes (excluding 'Modifier' class and only including 'Disease Cause' sub-class (yellow shapes) of the 'Disease Attribute' class). The 'Hearing Impairment' class is the central class.

The remaining associations made in the ontology, i.e., with the other disease attributes not in the 'Disease Cause' class (namely: 'Age of Onset', 'Mode of Onset', and 'Disease Qualifier' (which includes qualifiers such as 'Rare and 'Acquired')) and with the 'Modifier' class, are shown in Figure 7.

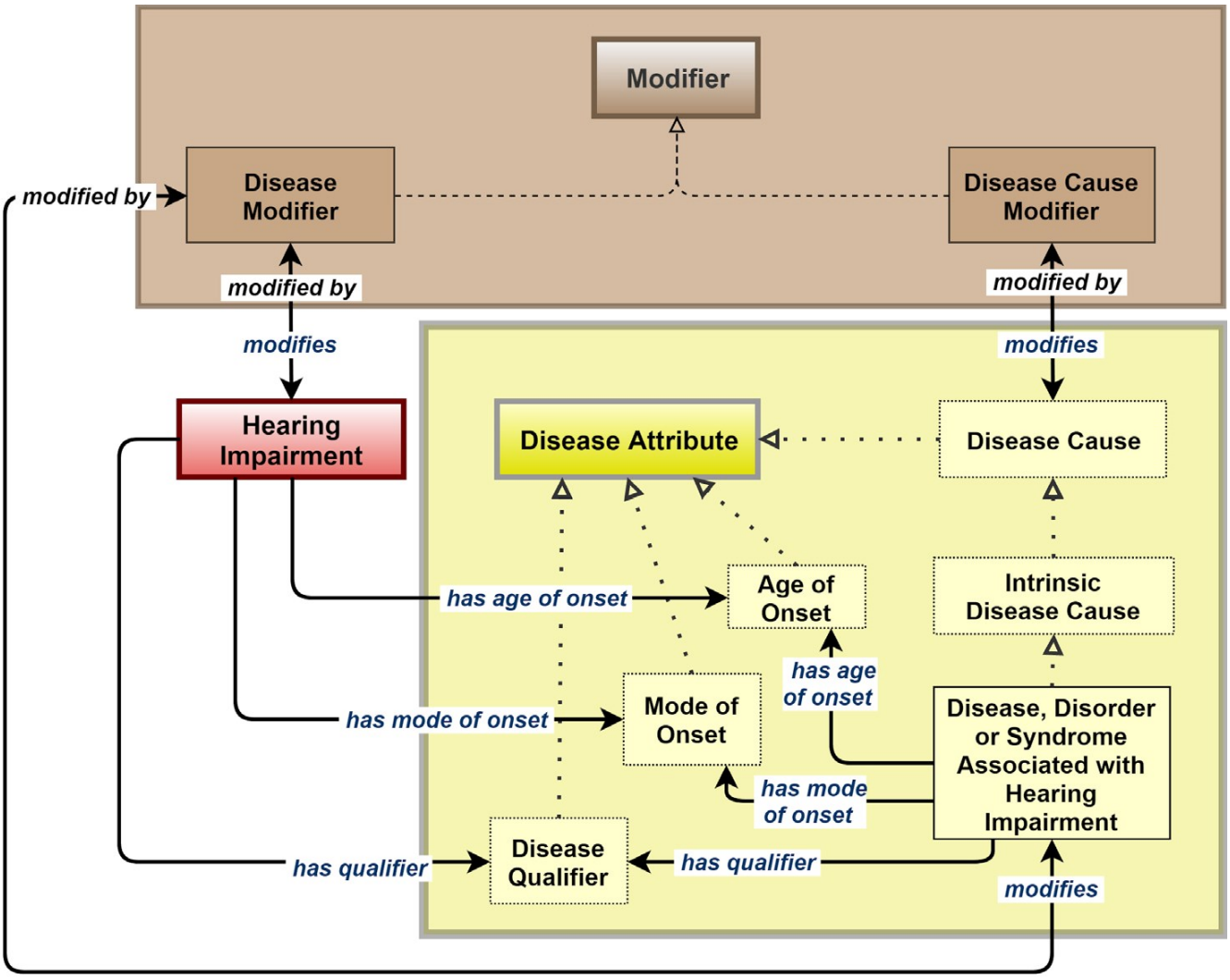

Figure 7. Associations made in the HIO to and from the 'Modifier' and 'Disease Attribute' classes, excluding those already shown in Figure 5 for the 'Disease Cause' class.

Assessing the Relevance of HIO

Of the 493 terms in the ontology (excluding 'deprecated terms' and upper 'owl:Thing'), at the time of writing this, 399 terms have descriptions and are considered as having all minimum required annotations (i.e., *rdfs:label*; *dc:description*; *definition source*, if relevant; *database cross reference*, if applicable; *dc:creator*, if the term was defined by an HIO curator using a source other than available ontologies; *existence in other ontologies*, used to record

the existence status of the term prior to inclusion in the HIO; and *hasExactSynonyms,* to indicate synonyms, where relevant). Analysis of the existence statuses ascribed to these terms using the 'existence in other ontologies' annotation property (see Table 1) shows that the HIO, even in its first draft, is making contributions towards including and standardizing HI terms that were previously not included in other ontologies. Some of these terms are provided in Table 2 for illlustration. Where applicable, terms unique to the HIO will be recommended for inclusion into other related ontologies, such as the HPO, Orphanet, DO, MESH, and NCIT.

HIO Release and License

The HIO is released every two months with possible special releases when there are significant incidental changes. It is freely available under the Creative Commons Attribution 4.0 Unported License (CC:https://creativecommons.org/licenses/by/4.0/legalcode) and further copyrighted to maintain the quality and integrity of the term vocabulary, meaning that any modification to the HIO can only be done by HIO developers and curators.

Different HIO Access Platforms

In order to foster the dissemination of and easy access to this novel ontology the latest OWL file produced has been uploaded to the NCBO BioPortal at https://bioportal.bioontology.org/ontologies/HIO. This also facilitates the searching and viewing of different HIO concepts. In addition, the OWL and OBO files can be accessible via the GitHub repository at https://github.com/hiodev/hi-ontology.

Table 4.1. Summary of terms' existence statuses prior to inclusion in the HIO

Existence Status	Explanation of Status	No. Terms	% Terms
Sufficient	Exists in other ontology and has appropriate description	284	71.2
Suggest update to description	Used term from existing ontology but will suggest they update their description to ours	27	6.8
Suggest update to label	Used term from existing ontology but will suggest they update their label to ours	0	0
Suggest update to label and description	Used term from existing ontology but will suggest they update their label and description to ours	0	0
Few but definitions not available	Term exists in a few ontologies but has not been given a description in any	3	0.8
Few but definitions not freely available	Term exists in a few ontologies but the description is not freely available	8	2.0
Few but definitions not specific enough	Term exists in a few ontologies but the definitions are not specific enough for the HIO's needs	9	2.3
Not relevant to context of hearing impairment	Term exists in other ontologies but the definitions are not relevant to the HI field	3	0.8
Negligible	No description or outdated ontology	2	0.5
None	Not in any existing ontology	63	15.8

Table 4.2. Some of terms that are unique to HIO.

Term Label	Term ID	Term Description
Symmetrical Bilateral Hearing Impairment	HIO:0000365	When the severity and configuration of hearing impairment is approximately the same in both ears.
Asymmetrical Bilateral Hearing.	HIO:0000366	When each ear has a di_erent severity and configuration of hearing impairment
Postlingual Hearing Impairment	HIO:0000475	Hearing impairment which develops after the acquisition of speech and language, usually after the age of six.
Prelingual Hearing Impairment	HIO:0000476	Hearing impairment which is either congenital or develops before the acquisition of speech and language, usually before the age of 6.
Temporal Bone Fracture with Otic Capsule Involvement	HIO:0000287 Traumatic injury to the temporal bone in which the continuity of the bone is broken and violation of the otic	Traumatic injury to the temporal bone in which the continuity of the bone is broken and violation of the otic capsule is involved
Temporal Bone Fracture without Otic Capsule Involvement	HIO:0000288	Traumatic injury to the temporal bone in which the continuity of the bone is broken and violation of the otic capsule is not involved.

Pseudo-Dominant Inheritance	HIO:0000228	When the inheritance of a recessive trait mimics a dominant pattern of inheritance.
Cisplatin-Induced Hearing Impairment	HIO:0000215	Hearing loss caused by cisplatin (a chemotherapeutic agent) ototoxicity.
Neomycin-Induced Hearing Impairment	HIO:0000285	Partial or complete loss of hearing following ingestion of neomycin.
Maternal Medical History	HIO:0000362	A record of a patient's biological mother's background regarding health and the occurrence of disease events of the mother
Hearing Impairment based on Immaturity	HIO:0000514	Hearing impairment that occurs due to premature birth (birth at or before 37 weeks of gestational age

Discussion

Making use of ontological reasoning approaches may play a significant role in solving scalability and interoperability issues associated with current large-scale biological high-throughput datasets. This implies that building and maintaining biomedical ontologies is essential, especially in this current data rich era with an extensive consideration of big data analytics. With the contribution of HI domain experts, we have designed the HIO, which enables knowledge acquisition and harmonization, verification and validation of data available in different databases. This ontology is set to be the most comprehensive standardized HI domain knowledge portal, which will allow for the application of ontology-driven mining approaches for the identification of pertinent research questions. The HIO will foster clear and unambiguous communication and also facilitate sharing of information within the field.

HIO Structure, Other Disease Ontologies and HI Online Datasets

As pointed out previously, the HIO reuses concepts from other ontologies, including HPO, DO, and especially SCDO (see Table 1: summarizing the number of HI specific concepts vs reused concepts). These concepts were adjusted, where applicable, to incorporate new concepts specific to HI and relevant in various areas, such as HI subtypes, phenotypic expressions, genetic phenomena and different modes of inheritance. It is worth noting that, although we did not foresee all the adaptations that would be required, the use of the SCDO as a template for the HIO has been a very useful exercise. Whereas the general structure of the SCDO is more readily transferable to other monogenic diseases, we believe the HIO can be used as a template for designing disease-specific ontologies for diseases with a broader range of causes. Finally, note that there exist several online resources storing HI datasets and containing or enabling the retrieval of HI information. Table 3 lists some of these resources.

Table 4.3. Some existing online hearing impairment resources.

Scheme	Description	Types	URL	Reference
HHL	Hereditary Hearing Loss Homepage	An up-to-date overview of the genetics of hereditary hearing impairment for researchers and clinicians working in the field.	https://hereditaryhearingloss.org/	-
SHIELD The	Shared Harvard Inner Ear Laboratory Database	An integrative gene expression database for inner ear research	https://shield.hms.harvard.edu	[(Shen et al., 2015)
DVD	Deafness Variation Database	A comprehensive resource integrating available genetic, genomic, and clinical data together with expert curation to generate a single classification for each variant in 152 genes implicated in syndromic and non-syndromic deafness.	http:// deafnessvariationdatabase.org/	(Azaiez et al., 2018)

LOVD	Leiden Open Variation Database	Retinal and hearing impairment genetic variant database	https://databases.lovd.nl/shared/genes/OTOF	(Fokkema et al., 2011)
NIDCD	National Institute on Deafness and Other Communication Disorders	A resource providing knowledge about Hearing, Ear Infections, and Deafness Diseases and Conditions. It also provides NIDCD Temporal Bone Registry at https://www.tbregistry.org/, a resource for learning about the pathology and pathophysiology of otologic disorders, which serves as a resource for scientists to analyze data from a collection of more than 12,000 temporal bone specimens.	https://www.nidcd.nih.gov/health/hearing-ear-infectionsdeafness	

gEAR	Gene Expression Analysis Resource	Visualization and analysis of multiomic data both in public and private domains.	https://umgear.org/ -	
OMIM	Online Mendelian Inheritance in Man	An Online Catalog of Human Genes and Genetic Disorders	https://www.omim.org	(Amberger et al., 2019)

HIO Potential Future Applications

Even though we have shown the relevance of this new ontology by looking at how many of the classes are HI specific by querying against NCBO BioPortal, it should be noted that an ontology should be applied in order to appropriately assess its impact and suitability. We plan to use this ontology in data representation, which includes data harmonization, interoperability, and integration. For this, the HIO will be an essential resource in designing an ontology-based case report forms, providing essential data elements and controlled terminology. Different datasets can then be mapped to these data elements, making these datasets interoperable, thus easing the data integration process and meta-analysis. In the context of HI research, this will orient data analysis and enable the use of machine learning approaches with sufficient statistical power (K. Mazandu et al., 2019) for predicting disease clinical outcomes (Zhao et al., 2019) and optimal therapeutic interventions, based on the disease pathophysiology mechanisms and other clinical parameters in patient records. It is expected that this HIO will contribute to fostering the subsequent HI research translation into healthcare, inferring knowledge based on patient clinical records, and the development of ontology-powered artificial intelligence medical tools helping in therapeutic interventions, prognosis, and diagnosis, as well as predictive models for an improved understanding of disease processes.

Challenges and Future Direction

Although the HIO has been designed in a manner that takes into account the various complexities of HI, there is admittedly still much content to be included, both with regards to terms and associations. New discoveries are also regularly being made in this field towards technological advances for diagnostics and therapeutics. This suggests that the ontology should be dynamic, in continuous evolution, keeping the HI knowledge up to date as new knowledge is accessible. There will thus be a need for ongoing input and maintenance of the HIO. For this, there is already a dedicated curation team aiming at assuring the quality and accuracy of the information contained in this ontology and also keeping it updated as HI knowledge evolves.

Going forward, these remaining upper classes of the SCDO will also be evaluated, and where necessary, adapted, for inclusion into the HIO: ‘Research’, ‘Guidelines’, and ‘Quality of Life and Care’. We also plan to use competency questions defined by HI experts to evaluate the scope and domain coverage of the HIO. Beyond the use of competency

questions, this ontology will also be assessed on its HI concept inclusion power, i.e., in terms of percentage of HI clinical terminologies from a given database, such as the GHLD database, or HI associated clinical reports or selected literature found in the HIO. This is particularly useful as it will provide an indication on the HIO ability in HI text mining tasks. Finally, the next critical challenge is to introduce this HIO into the dynamic clinical setting. This necessitates the development of testable and actionable health informatics applications to ensure clinical system-wide adherence. As indicated previously, HIO addresses the issue of unifying research clinical data from diverse sources. This ontology already paves the way towards the integration of clinical data into electronic medical records, which should facilitate the development of effective health informatics tools to potentially assist in the public and clinical management of hearing impairment conditions.

Conclusions

We have developed the HIO, a common controlled HI vocabulary, which is expected to enhance collaborative research. This ontology is currently the most comprehensive and standardized human- and machine-readable resource that unambiguously defines HI concepts and terminology for researchers, patients, and clinicians in order to help process, reuse, and re-apply existing HI knowledge in biomedical research and health-care systems. In the context of big data analytics, this ontology may facilitate retrospective data harmonization and contribute to mapping HI datasets to functional knowledge to enable the subsequent HI research translation into clinical applications and policy guidelines. The HIO will allow researchers, clinicians, and patients to readily access standardized HI-related knowledge in a single location and promote HI data integration, interoperability, and sharing, including epidemiological, socio-environmental, biomedical, genetic, and phenotypic datasets.

Author Contributions:

G.K.M., V.N., and A.W. are leading the H.I.O. implementation. J.H., N.M., E.W., V.N., N.V., G.K.M., and A.W. curated the current H.I.O. content. N.M., J.H., E.W., S.M.A., O.G.O., K.M., A.Y., and A.W. provided the H.I.O. content. G.K.M., N.J.M., M.H., N.V., S.J. contributed to the ontology structure development and implementation. G.K.M., J.H., N.M., and V.N. wrote the manuscript. All authors revised and approved the manuscript.

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Conflicts of Interest:

The authors declare no conflict of interest.

Chapter 5: Clinical and Genetic Profile of Hearing Loss In a group of Families from South Africa

Introduction

The impaired ability to perceive sound is a common sensory condition that is estimated to affect 1 in 1000 people worldwide (WHO, 2021a). Hearing impairment (HI), also known as hearing loss or deafness, is the inability to detect sounds better than 20dB in the better hearing ear (WHO, 2023a). It is considered disabling when an individual cannot detect better than 35dB in their better hearing ear (WHO, 2023a).

Hearing impairment is a heterogeneous condition that may arise from several different factors. Broadly, the aetiology of HI may be as a result of genetic, environmental, or unknown factors. Genetic hearing impairment accounts for 50% of congenital HI in developed nations (Schrijver, 2004), this is contrasted by environmental HI being the leading cause of HI in developing nations (Wonkam et al., 2013b). Non-syndromic HI (NSHI) occurs in 70% of congenital genetic HI; of which recessive inheritance accounts for ~70% to 80% of NSHI (Morton, 1991, Chung et al., 1959). Dominant inheritance accounts for 20% to 30% of NSHI and mitochondrial and sex-linked inheritance accounts for 1% to 2%.

Studies of the genetic causes of HI in African populations have been limited. Most studies have focussed on the connexin genes *GJB2*, which are the leading cause of genetic NSHI in European, Asian, and North American populations(Chan and Chang, 2014, Hutchin et al., 2005, Liu et al., 2002). These genes have however been shown to be insignificant in African populations (Gasmelseed et al., 2004, Kabahuma et al., 2011, Bosch et al., 2014a, Bosch et al., 2014b, Trotta et al., 2011, Wonkam et al., 2015), with only the Ghanaian and Moroccan populations having founder pathogenic variants in *GJB2* (Adadey et al., 2019, Ratbi et al., 2007, Aboagye et al., 2022, Adadey et al., 2020).

This study aims to recruit patients presenting with likely genetic HI in order to perform whole exome sequencing and bioinformatics analysis to identify putative prevalent genes associated with HI in South Africa.

Methods

Ethics declaration

This study was granted ethical clearance from the University of Cape Town Human Research Ethics Committee (HREC: 104/2018). Prior to recruitment, approval was obtained from the relevant provincial education departments and the school principals. Written consent was obtained from all participants over the age of 18. Written consent was obtained from the parents or guardians of individuals under the age of 18, and verbal assent was obtained from the minor children.

Recruitment of participants

Patients were recruited, primarily, from Schools of the Deaf in eight of the nine provinces in South Africa. The patients were recruited based on the procedures described by Bosch et al. (2014a), (2014b).

Family and medical history were obtained from the patients, their parents and/or guardians and pedigrees were drawn for each family. A general and otological assessment was performed by a medical geneticist, followed by pure tone audiometry where possible. Patients of Black, Coloured (Mixed-Ancestry), or Indian ancestry presenting with genetic HI were recruited into the study along with their parents where possible. In familial cases of HI, additional family members were recruited. Additionally, photographs, where possible, were taken of patients presenting with putative syndromic HI.

The control population is composed of ethnically matched individuals who do not have HI or a family history of HI. Peripheral blood samples were collected for all patients and controls for isolation of DNA.

Determining the putative inheritance pattern

The putative inheritance pattern was determined by a process of elimination, where possible. The process in summarised in the appendix as a decision tree.

Firstly, Y-linked inheritance was ruled out if at least one son of an affected father was unaffected. Recessive inheritance was suspected in the affected children who had unaffected parents, or the condition was absent in some of the generations. X-linked recessive was suspected if all the sons of an affected mother were also affected. Autosomal recessive inheritance was suspected if the condition was passed to both male and female children.

Dominant inheritance was suspected if the affected children had at least one affected parent and/or the affected child was the direct descendent of affected individuals across several generations, with no generational gaps. The process is graphically represented in the appendix (A2).

DNA extraction and whole exome sequencing

DNA was extracted from whole blood using the chemagen 360 Instrument (PerkinElmer, Massachusetts, USA) per the manufacturer's instructions. DNA concentration and quality were assessed using the Promega QuantiFluor dsDNA System on a Quantus Fluorometer (Madison, WI, USA). The DNA samples underwent WES at OmegaBioservices (Norcross, GA, USA) according to the procedure shown in Manyisa et al. (2021) using the Illumina Rapid Capture Exome Kit (San Diego, CA, USA).

Bioinformatics analysis

Alignment and Quality Checking

High-quality reads were aligned to the human GRCh37/hg19 human reference genome using the DRAGEN software (version 05.021.408.3.4.12). After sorting and marking duplicates, variants were called, and individual genomic variant call files (gVCF) were created. Joint variant calling for single nucleotide variations (SNV) and Insertion/Deletions(Indels) was performed using the genome analysis toolkit (GATK) software (version 4.0.6.0) (McKenna et al., 2010). Using plink (version 1.9), the sex for individuals that underwent exome sequencing, was verified (Chang et al., 2015, Purcell et al., 2007). Additionally, identity-by-descent sharing in plink (version1.9) and Kinship-based Inference for GWAS (KING) algorithms were used to verify their familial relationships (Chang et al., 2015, Purcell et al., 2007, Manichaikul et al., 2010).

Variant Annotation and Filtering

Variant annotation and filtering was done using ANNOVAR and custom scripts (Wang et al., 2010). Prioritisation was initially done using AR and AD modes of inheritance. Rare variants for all populations in the genome aggregation database (gnomAD) (Chakchouk et al., 2019), with minor allele frequencies < 0.0005, and known likely pathogenic and pathogenic variants in ClinVar were retained (Landrum et al., 2016).

The deleterious effect of missense variations was predicted by annotating the variants using dbNSFP (version4.0) (Liu et al., 2011, Liu et al., 2020). dbNSFP includes Sorting Intolerant from Tolerant (SIFT), polymorphism phenotyping v2 (PolyPhen-2), MutationAssessor, the likelihood ratio test (LRT), Mendelian clinically applicable pathogenicity (M-CAP) score, Rare Exome Variant Ensemble Learner (REVEL), MutPred, PROtein Variation Effect Analyzer (PROVEAN), MetaSVM, and MetaLR [27,28]. Whereas the tools MutationTaster, Eigen, Eigen-PC, functional analysis through Hidden Markov models (FATHMM-MKL), combined annotation dependent depletion (CADD) score, and deleterious annotation of genetic variants using neural networks (DANN) were used to evaluate both coding and non-coding variants(Liu et al., 2011, Liu et al., 2020).

Adaptive boosting and random forest scores were analysed using dbscSNV (Jian et al., 2014). The investigation of the detrimental effect of variations within conserved splicing areas was made possible by dbscSNV(Jian et al., 2014). Furthermore, the conservation of nucleotides and amino acids was estimated, at which the variations occur, using phyloP, Genomic Evolutionary Rate Profiling (GERP), SiPhy, and phastCons scores (Liu et al., 2011, Liu et al., 2020, Cooper et al., 2005, Pollard et al., 2010).

The online Mendelian inheritance in man (OMIM), ClinVar, and gnomAD databases were analysed to see if there were any known connections between identified genes and/or variations and HI.(McKusick, 1998). Variants were regarded as putatively causal if they were in known HI genes or genes expressed in the inner ear, if the variant had a predicted influence on protein function or mRNA, and if the variation segregated with HI within the family.

Results

Patients' demographics

Five hundred and eleven patients (511), and their families, were enrolled in this study from schools of the deaf in eight of the nine provinces of South Africa. Table 5.1 indicates the breakdown of the patient population based on their demographic information.

Table 5.1: Demographic breakdown of the patients' population

Demographic		Summary Statistics(n)	Percentage (%)
Gender	Male	257	50.3
	Female	251	49,1
	Undetermined	3	0,6
Ethnicity	Black	415	81,2
	Coloured/Mixed Ancestry	84	16,4
	Indian	4	0,8
	Unspecified	9	1,8
Syndromic or Non-syndromic	Syndromic	58	11,4
	Non-syndromic	351	68,7
	Unknown	102	20,0
Bilateral or Unilateral	Bilateral	412	80,6
	Unilateral	13	2,5
	Undetermined	86	16,8
Symmetrical or Asymmetrical	Symmetric	234	45,8
	Asymmetric	77	15,1
	Undetermined	200	39,1
Unilateral HI	Left Ear	5	1,0
	Right Ear	6	1,2
	Undetermined	2	0,4
Degree of HI Left	Mild	5	1,0
	Moderate	11	2,2
	Severe	31	6,1

	Profound	270	52,8
	Undetermined	190	37,2
Degree of HI Right	Mild	3	0,6
	Moderate	10	2,0
	Severe	28	5,5
	Profound	275	53,8
	Undetermined	190	37,2
Transmission	Familial	111	21,7
	Sporadic	280	54,8
	Undetermined	120	23,5
Mechanism	Sensorineural	231	45,2
	Conductive	6	1,2
	Mixed	37	7,2
	undetermined	237	46,4
Consanguinity	Yes	10	2,0
	No	412	80,6
	Unknown	7	1,4
	Undetermined	82	16,0
Schooling	Primary	266	52,1
	Secondary	136	26,6
	Tertiary	9	1,8
	No school/Pre-school	41	8,0
	Special school	19	3,7
	Other	1	0,2
	Undetermined	39	7,6

The patients had an average age at recruitment, of 14.08 (±8.63) years and a median age of 12 years. The hearing impairment was noticed at approximately at 1.93 (±1.51) years and the hearing impairment was diagnosed at approximately at 2.69 (±2.26) years.

Eighty-three percent (*n* = 424) of the patient population was deemed to have putative genetic hearing impairment. Fourteen percent (*n* = 73) of the population had HI due to

environmental factors and three percent (*n* = 14) of the population had HI whose aetiology was unknown.

Patients presented with several environmental factors that could have, individually, resulted in HI. Most cases of environmental HI were attributed to prematurity (*n* = 69; 94.5%) and low birth weight (*n* = 65; 89%). The breakdown of the proportion of environmental factors is indicate in Table 5.2. Each putative causative environmental factor is calculated as a percentage against the total number of patients presenting with putative environmental HI (*n* = 73).

Table 5.2: Proportion of environmental factors

Environmental cause	Count (n)	%
Prematurity	69	94.5
Low birth weight	65	89.0
Neonatal asphyxia	54	74.0
Head trauma	53	72.6
Recurrent/Chromic otitis	50	68.5
Ototoxic drugs during pregnancy	44	60.1
Nuclear jaundice	41	56.2
Congenital Infection	41	56.2
Ototoxic drug	37	50.7
Meningitis	36	49.3
Radiation during pregnancy	34	46.6
Mumps	26	35.6
Noise pollution	25	34.2
Measles	24	32.9

The inheritance pattern for the cohort is indicated in Table 5.3. Inheritance patterns are indicated for the entire cohort, the familial cases in the cohort, and the isolated cases in the cohort. The putative inheritance pattern is determined based on the family history and pedigrees provided by the participants and/or their families.

Table 5.3: Inheritance pattern breakdown for the cohort

Cohort subsection	Inheritance patterns	Count (n)	Percentage (%)
All Participants	Autosomal recessive	238	46,6
	Autosomal dominant	51	10,0
	X-Linked	0	0,0
	Mitochondrial	0	0,0
	Unknown	222	43,4
Familial cases	Autosomal recessive	22	7,9
	Autosomal dominant	41	14,6
	X-Linked	0	0,0
	Mitochondrial	0	0,0
	Unknown	48	17,1
Isolated cases	Autosomal recessive	202	72,1
	Autosomal dominant	3	1,1
	X-Linked	0	0,0
	Mitochondrial	0	0,0
	Unknown	75	26,8

Familial and isolated cases

Most patients (54.8%, n = 280) presented with isolated HI, whereby the patient was the only person in the family with HI. The transmission was undetermined in 120(23.5%) patients.

Familial cases of HI, whereby there was two or more affected individuals in the family, accounted for 111 (21.7%) cases in the cohort.

Isolated cases

One hundred and thirty-four individuals (47.9%) were male and 146 (52.1%) individuals were female, in the cohort of 280 isolated cases. The cohort consisted of 234 individuals (83.6%) of Black descent, 42individuals (15.0%) of Coloured descent, 1 individual (0.4%) who was Indian, and 4 individuals (1.4%) chose to not disclose their ethnicity.

The hearing impairment was non-syndromic in 243 individuals (86.8%) and syndromic in 25 individuals (8.9%), with the type of HI in 12 individuals (4.3%) being undetermined.

Sensorineural HI accounted for 152 cases (54.3%), conductive HI accounted for 5 cases (1.8%) and 23 patients (8.2%) had mixed HI. The mechanism of HI was unknown in 100 patients (35.7%).

The HI was bilateral in 264(94.3%) patients, unilateral in seven (2.5%) patients and undetermined in nine (3.2%) patients. The symmetry of the HI, in bilateral HI, is indicated in Figure 5.1.1, which shows that most patients had symmetric HI. Seven patients had unilateral HI, of which one patient had the left ear affected, four patients had the right ear affected, and the affected ear could not be determined in two patients (their audiograms were unavailable).

The degree of hearing impairment is indicated in figures 5.1.2 and 5.1.3 for the left and the right ear respectively.

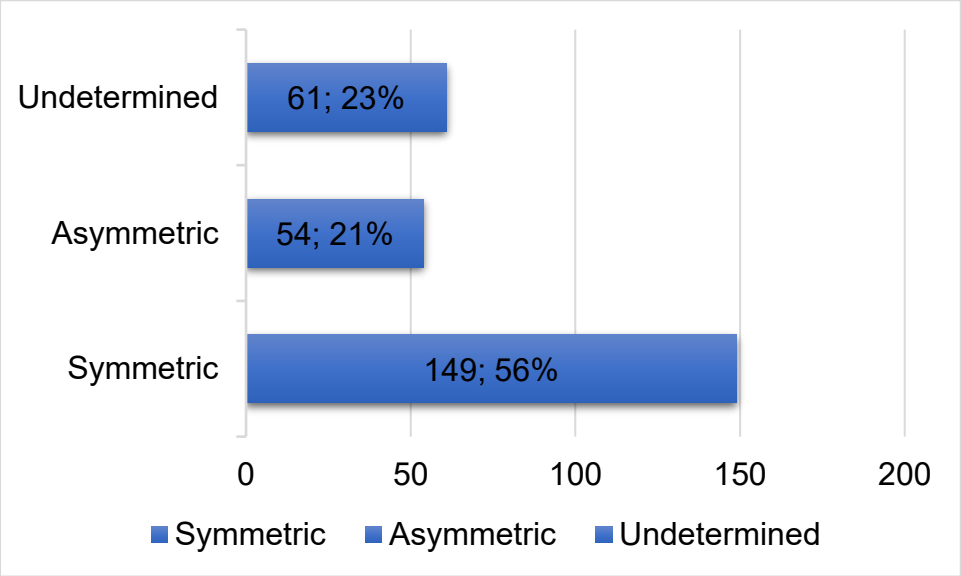


Figure 5.1.1: Symmetry of HI in Isolated cases. The bar chart indicates the number of patients presenting with symmetrical, asymmetrical, and undetermined symmetry in the patients with bilateral HI.

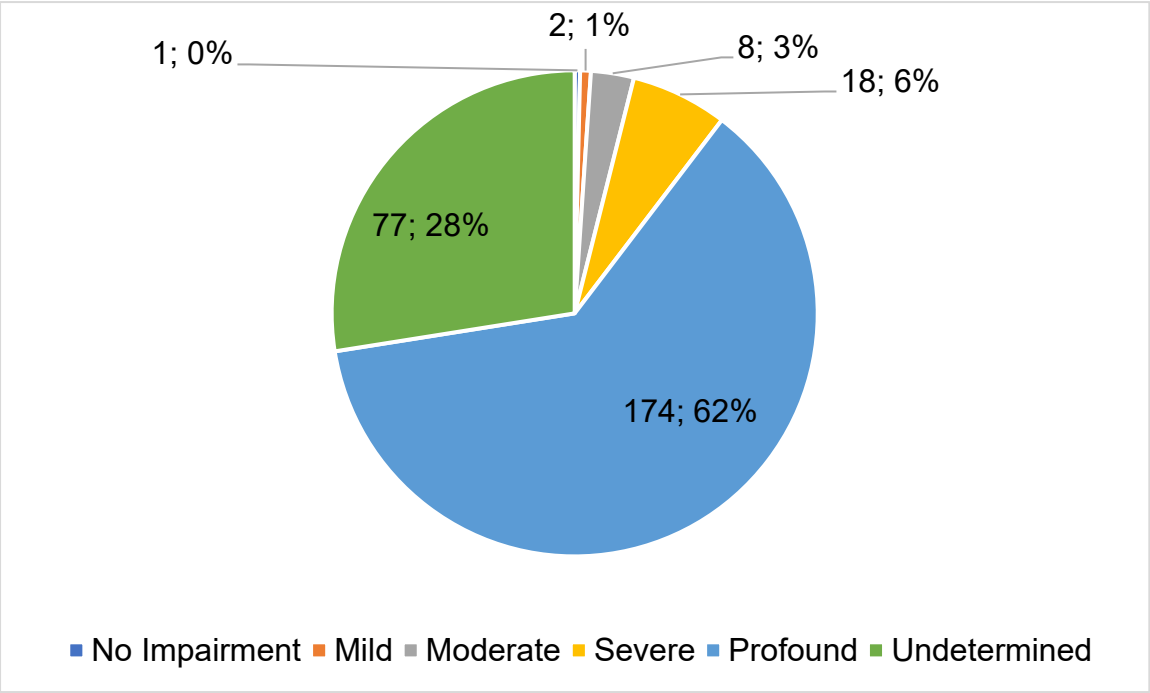


Figure 5.1.2: The degree of HI in the left ear for isolated cases. The pie chart indicates the number of patients that have no impairment, mild HI, severe HI, and undetermined degree of HI in the left ear.

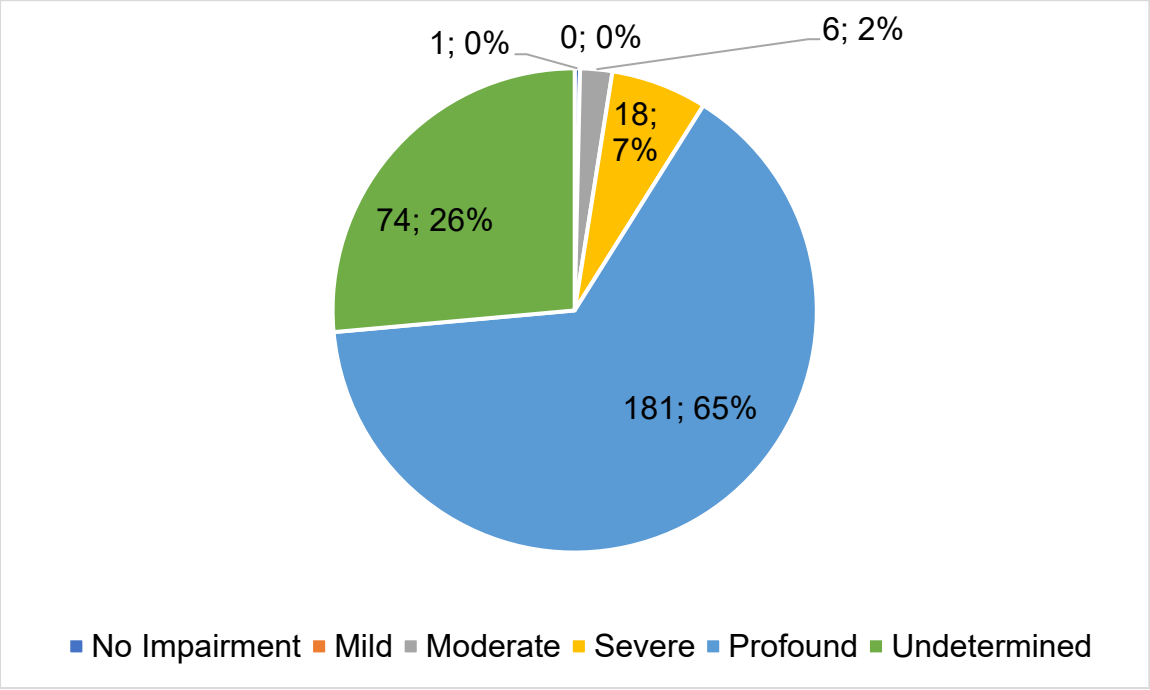


Figure 5.1.3: The degree of HI in the right ear for isolated cases. The pie chart indicates the number of the patients presenting with no impairment, mild HI, severe HI, profound HI, and undetermined degree of HI in the right ear.

Familial cases

The familial cases consisted of 63 males and 47 female individuals as index patients, with one individual preferring not to disclose their gender. Eighty-four patients were Black, 24 patients were Coloured/Mixed Ancestry. 2 were Indian, and one individual preferred not to disclose their ethnic identity. The predominant form of the HI was syndromic HI (n=77), with 26 non-syndromic cases and eight undetermined cases. Sensorineural HI accounted for 62 cases, conductive HI accounted for 1 case, mixed HI accounted for 10 cases, and 10 individuals had an undetermined mechanism of HI.

One hundred and five patients had bilateral HI, two had unilateral HI and one patient had unidentified localisation. Of the two patients with unilateral HI, one had the left ear affected and the other had the right ear affected. Figures 5.1.4 ,5.1.5 and 5.1.6 indicates the symmetry of the HI in individuals with both ears affected, the degree of HI in the left ear, and the degree of HI in the right ear respectively.

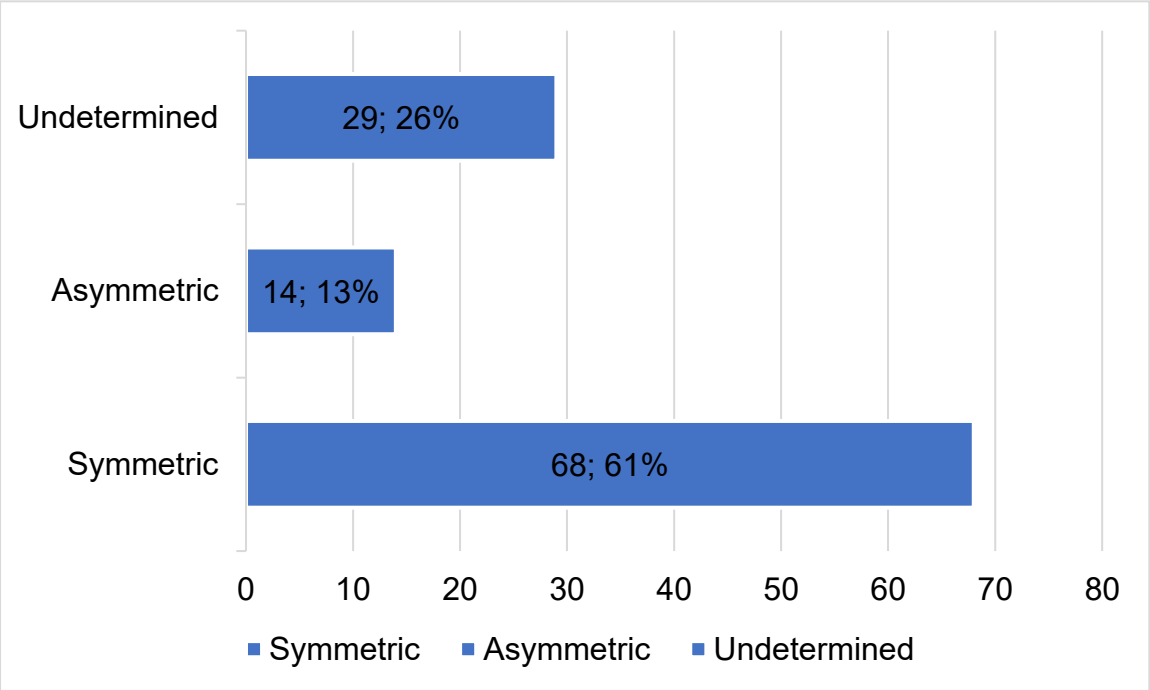


Figure 5.1.4: Symmetry of HI in familial cases. The bar chart indicates the proportion of patients with symmetric, asymmetric and undetermined symmetry in the familial cases with bilateral HI.

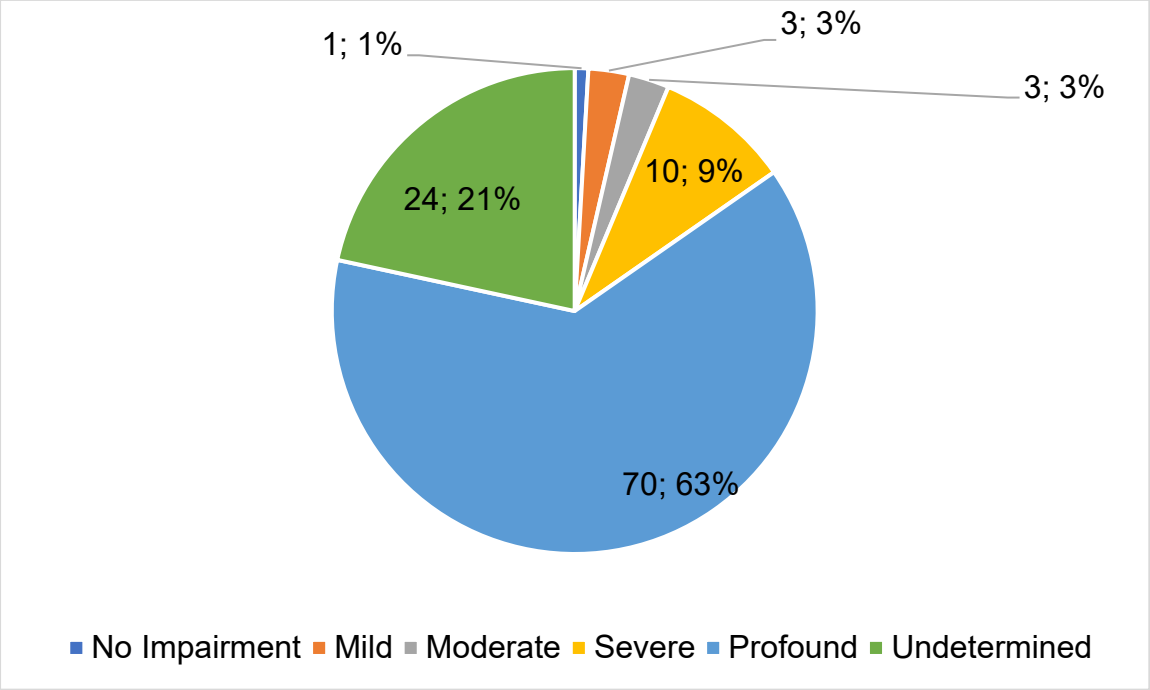


Figure 5.1.5: The degree of hearing impartment in the left ear for familial cases. The pie chart indicates the number of index cases with no impairment, mild impairment, moderate impairment, severe impairment and profound impairment in the left ear probands with familial HI

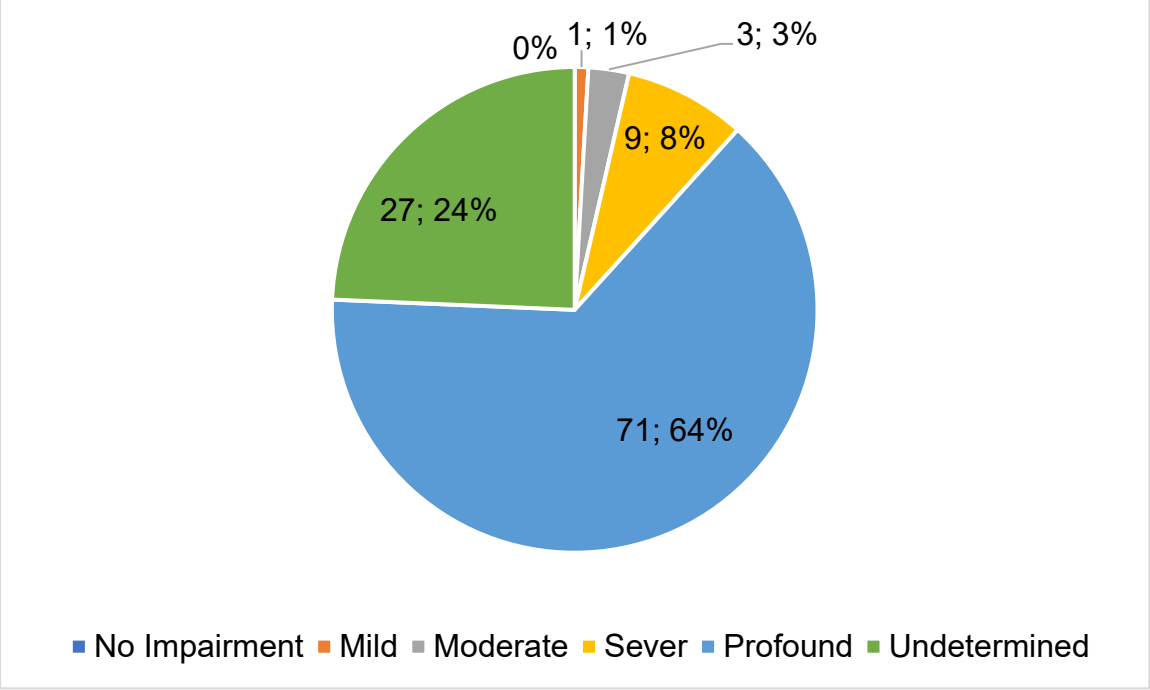


Figure 5.1.6: Degree of HI in the left ear for familial cases. The pie chart indicates the number of index cases with no impairment, mild impairment, moderate impairment, severe impairment, and profound impairment in the right ear of the probands with familial HI

The inheritance pattern was unknown in the majority of families (48; 43.2%). Dominant inheritance accounted for 48 families (36.9%) and autosomal recessive inheritance accounted for 22 families (19.8%). There were no cases of X-linked or mitochondrial inheritance of HI. representative pedigrees for autosomal dominant and autosomal recessive inheritance are indicated in Figure5.1.7.

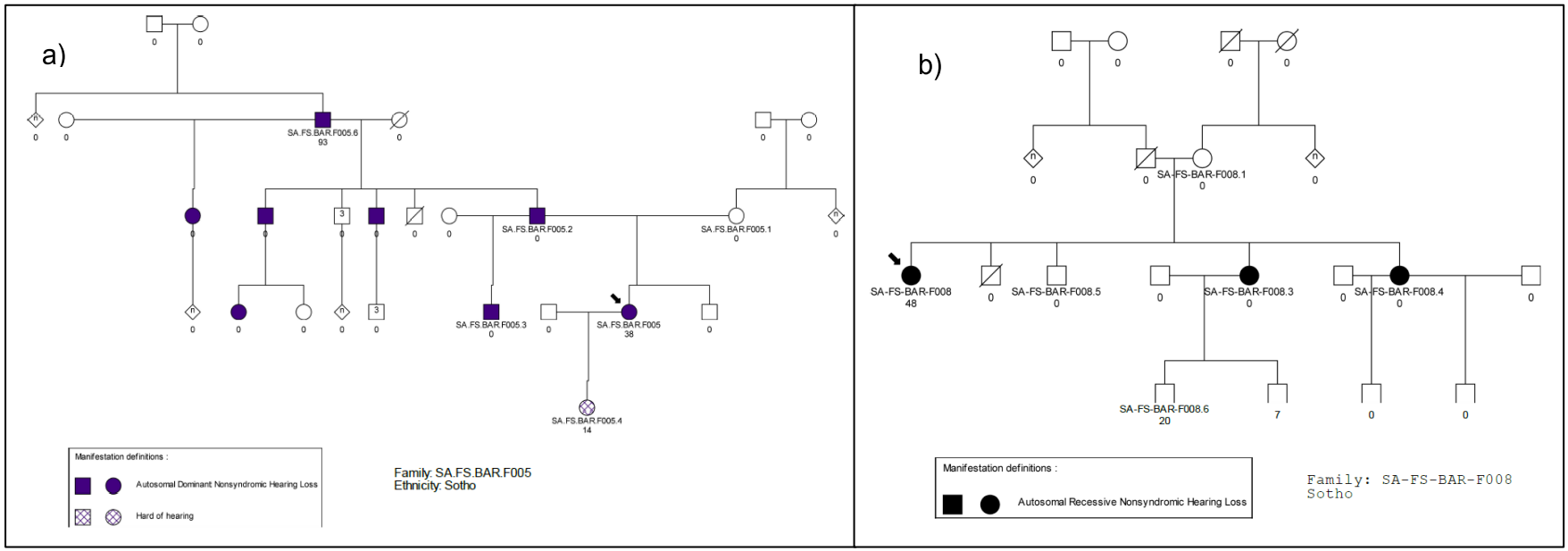


Figure 5.1.7: Representative pedigrees for autosomal dominant and autosomal recessive familial cases of hearing impairment. a) Multigenerational pedigree of a family presenting with autosomal dominant hearing impairment. b) Multigenerational pedigree of a family presenting with autosomal recessive hearing impairment.

Non-syndromic and syndromic cases of HI

The majority of cases presented as non-syndromic HI (*n* = 351) (NSHI). Syndromic cases of HI accounted for 69 cases and the type of HI was undetermined in 102 cases.

Non-syndromic cases

One hundred and sixty-three males and 243 females were enrolled as patients presenting with non-syndromic HI. The familial cases accounted for 77 probands, the isolated cases accounted for 243 probands, and familial history was undetermined in 31 cases. Black patients accounted for 280 probands, Mixed Ancestry/Coloured accounted for 63 probands, four individuals were of Indian descent, and five individuals did not disclose their ethnic background.

The majority of patients presented with bilateral hearing impairment (n=335) HI and 12 patients were undetermined, four patients had unilateral HI, of which two had the right ear affected and the affected ear was undetermined in 2 patients. Sensorineural HI accounted for 186, two patients had conductive HI, 28 patients had mixed HI, and the mechanism was undetermined in 135 patients. Figures 5.1.8, 5.1.9, and 5.1.10 indicate the symmetry in bilateral cases, the degree of HI in the left ear, and the degree of HI in the right ear respectively. Illustrative audiograms indicating sensorineural HI, mixed HI, and conductive HI are presented in Figure 5.1.11.

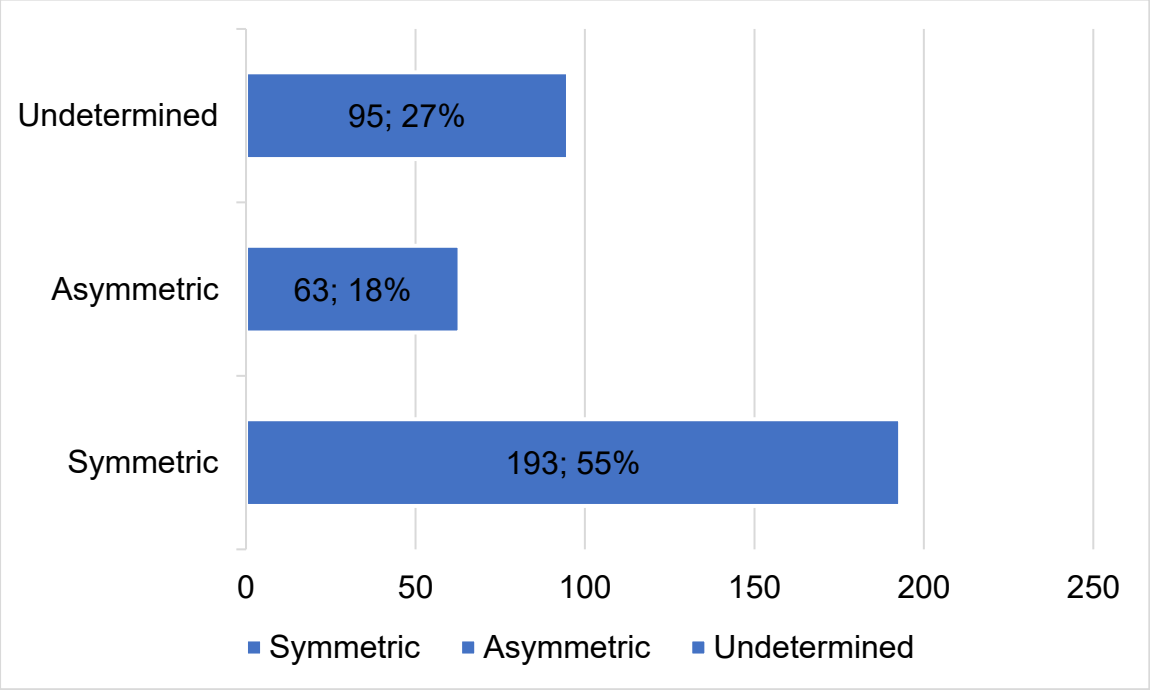


Figure 5.1.8: Symmetry in bilateral cases of NSHI. The bar graph depicts the number of symmetric, asymmetric and undetermined cases in the cohort presenting with NSHI

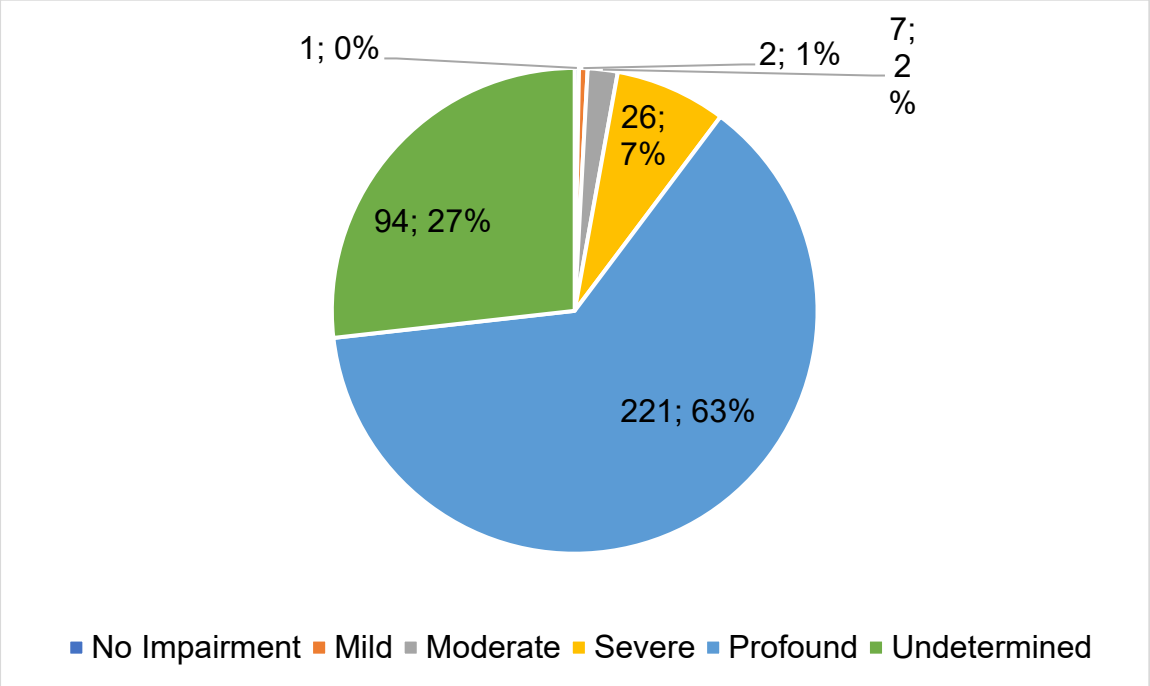


Figure 5.1.9: The degree of HI in the left ear in NSHI cohort. The pie chart indicates the number of NSHI patients presenting with no impairment, mild, moderate, severe, and profound HI. It also indicates the proportion of HI where the degree is undetermined.

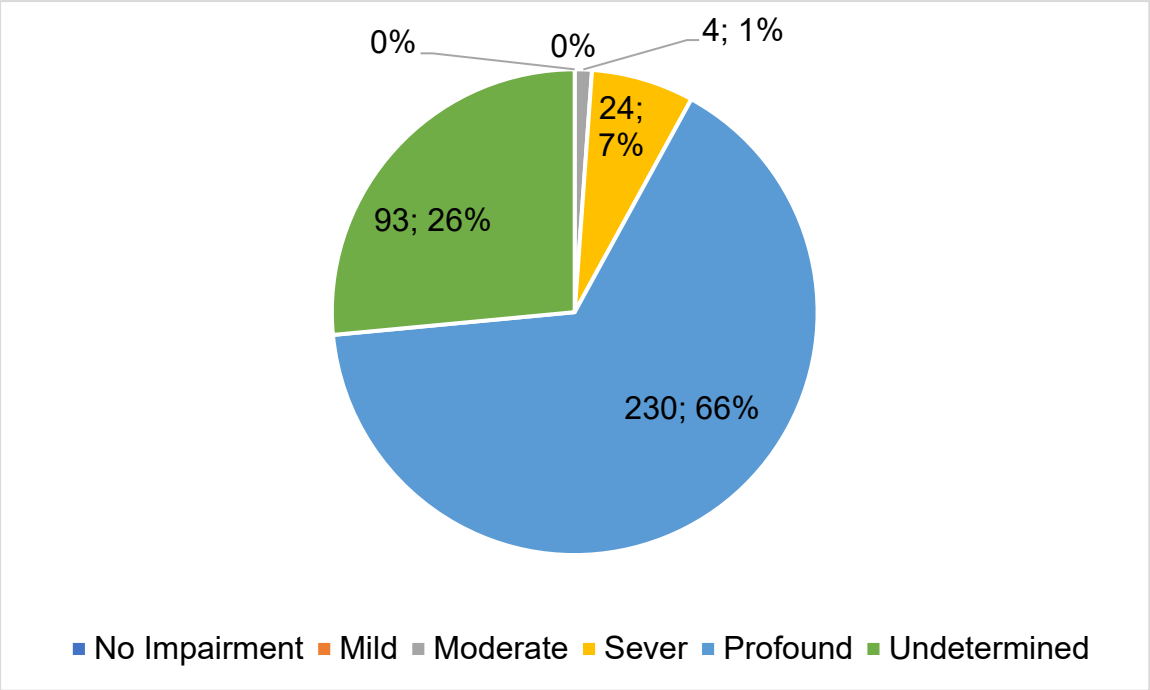


Figure 5.1.10: The degree of HI in the right ear in NSHI cohort. The pie chart indicates the number of NSHI patients presenting with no impairment, mild, moderate, severe, and profound HI. It also indicates the proportion of HI where the degree is undetermined.

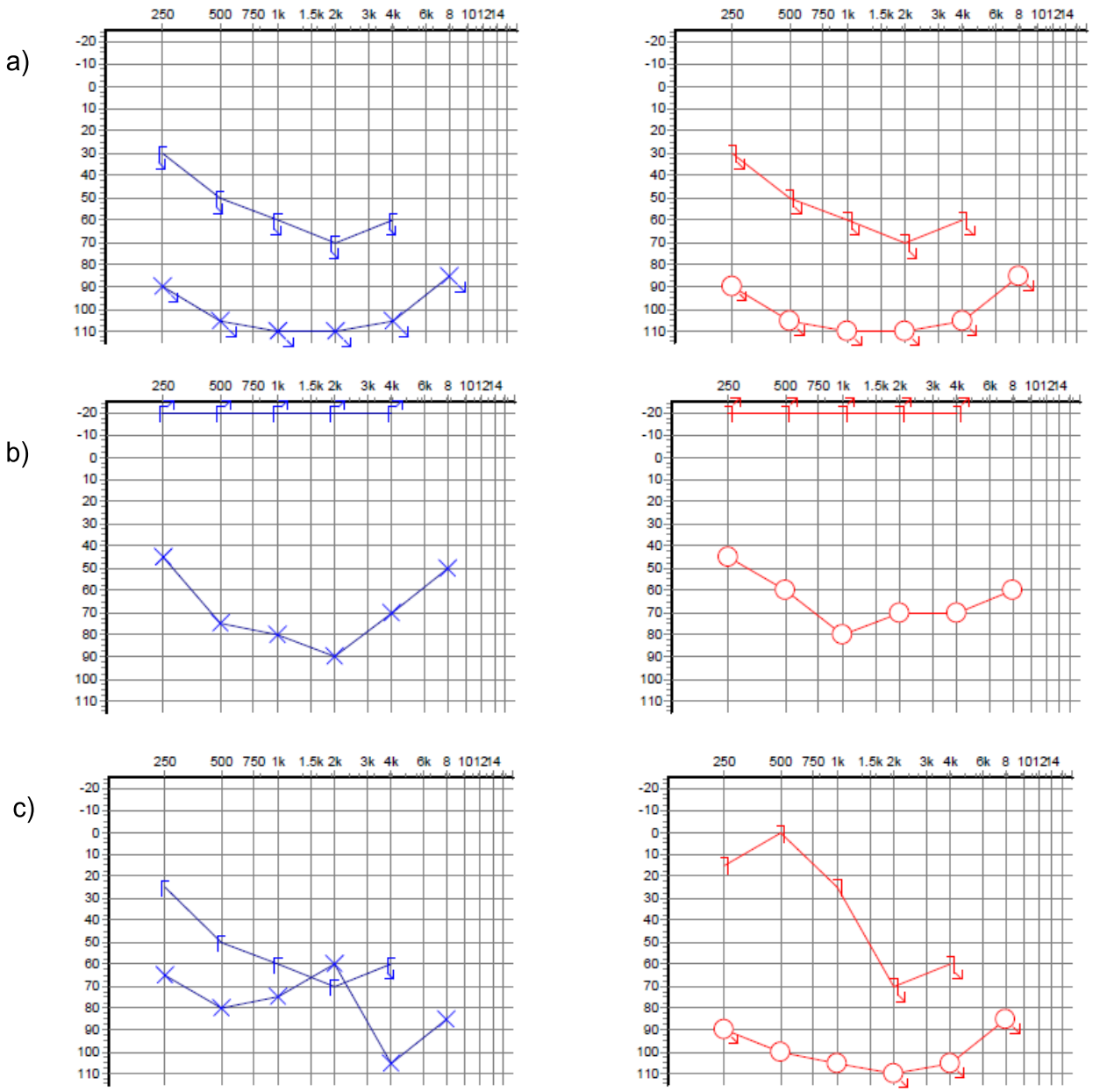


Figure 5.1.11: Representative audiograms of the different mechanisms of HI in the patient population. The figure indicates sensorineural (a), conductive (b), and mixed(c) HI.

Syndromic cases

The cohort consisted of 58 cases of syndromic HI (SHI). Males accounted for 39 probands and females accounted for 19 probands. There were 26 familial cases of SHI, 25 cases of isolated SHI and 7 cases where the family history was undetermined. The ethnic backgrounds of the cohort consisted of 13 probands of Coloured/Mixed Ancestry.

Bilateral HI was present in 49 probands, 6 probands had unilateral HI, and three probands were undetermined. Three patients' left ears were affected, and three patients' right ears were affected.

The majority of the patients had sensorineural HI (31), conductive HI was present in four cases, mixed HI was present in 5 patients, and the mechanism was undetermined in 18 patients. The type of syndrome, symmetry of the HI, degree of HI in the left and the right ear are indicated in figures 5.1.12, 5.1.13, 5.1.14 and 5.1.15. Figure 5.1.16 presents illustrative images of patients presenting with Waardenburg, Branchio-oto-renal, and Treacher-Collins syndrome in the patient population. Illustrative pedigrees for patients presenting with Alport, Usher, Stickler, Branchi-oto-renal, Keratitis-ichthyosis-deafness and Waardenburg Syndromes are indicated individually, after Figure 5.1.16, to allow for readability.

Three cases of Usher syndrome were identified during recruitment, following examination by a qualified medical geneticist, and they are indicated in Figure 5.1.12. There were an additional six cases of Usher Syndrome identified from the patient cohort, following whole exome sequencing analysis, which had previously putatively identified as non-syndromic HI.

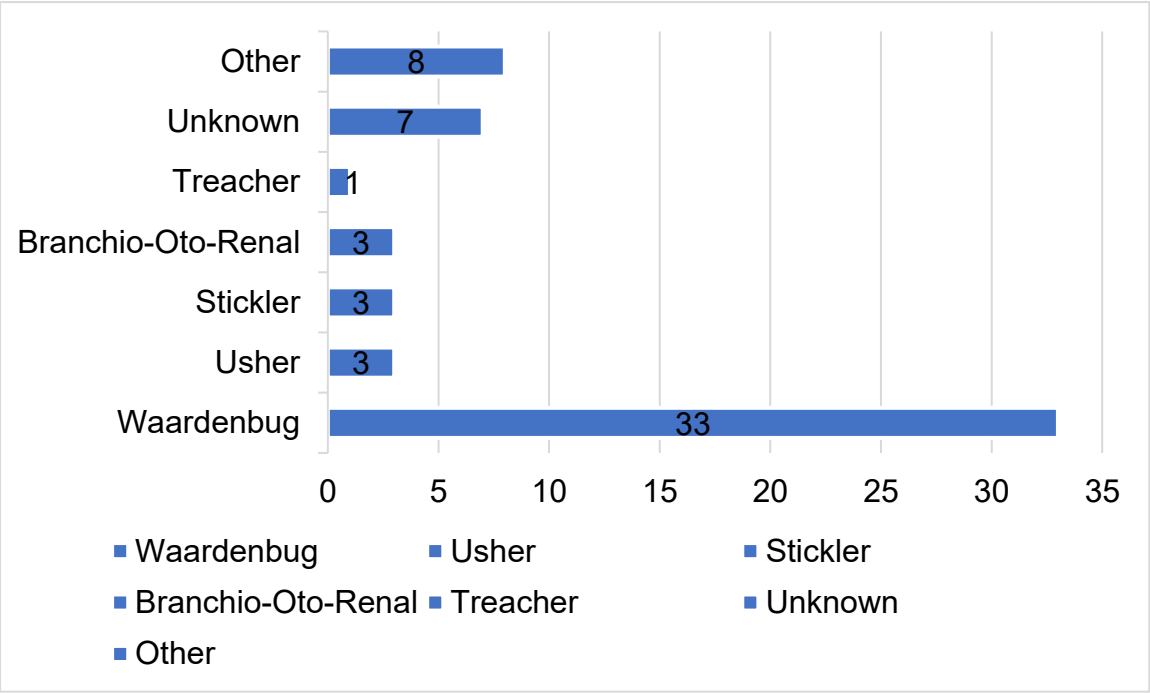


Figure 5.1.12: Types of syndromes in the SHI cohort. The graph indicates the distribution of syndromes for the SHI cohort. Syndromes grouped as "other" are KID syndrome (Keratitis-ichthyosis-deafness), Haemifacial Microsomia, Goldenhar, Chondroplasia punctata, OAV(Oculo-auriculo-vertebral spectrum), Alport syndrome, and congenital rubella syndrome.

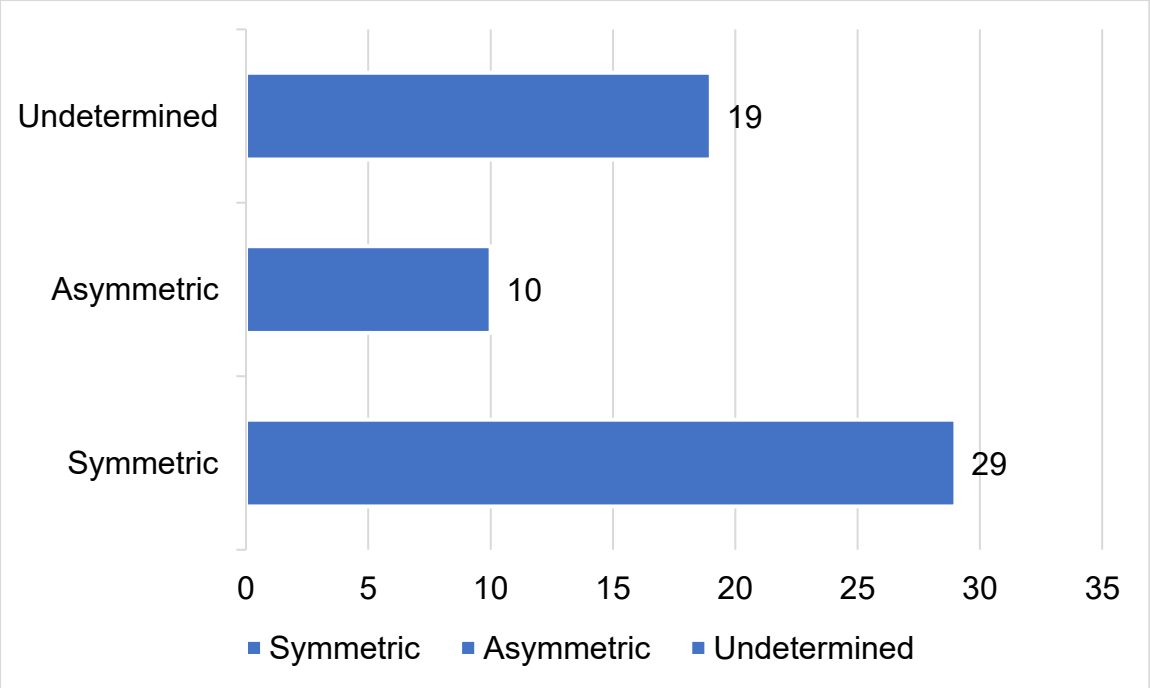


Figure 5.1.13: Symmetry of bilateral HI in SHI cohort. The bar chart indicates the number of patients in the of the cohort presenting with symmetric, asymmetric and undefined symmetry in the patients with bilateral SHI.

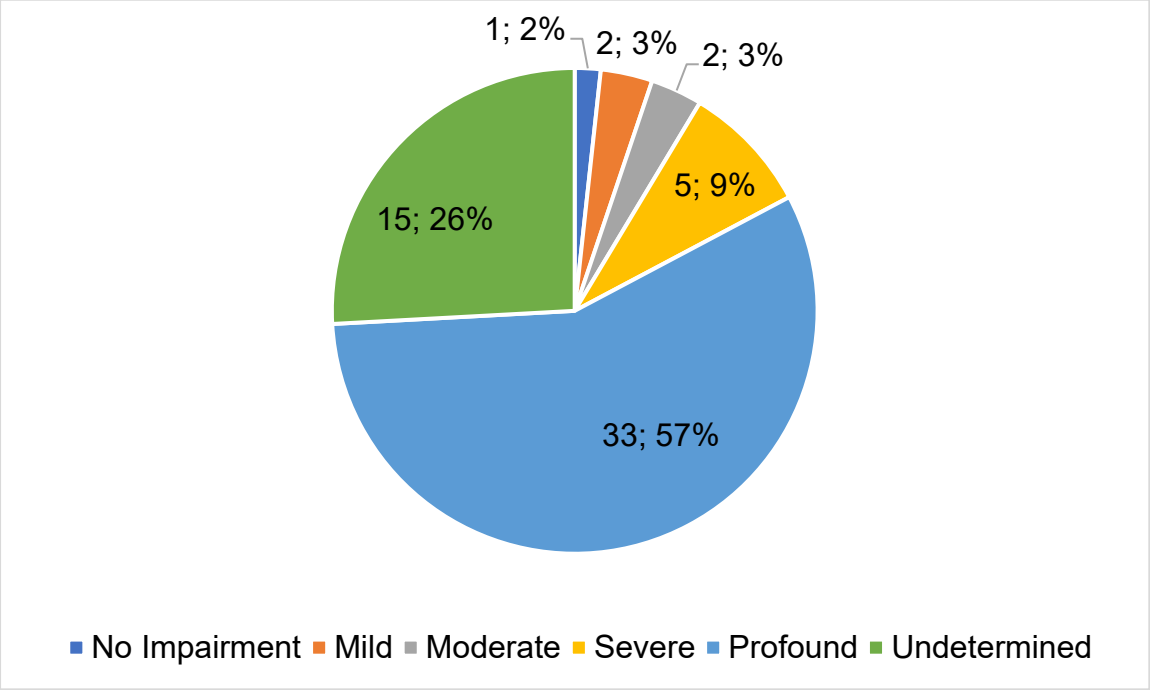


Figure 5.1.14: Degree of HI in the left ear for SHI cohort. The pie chart indicates the number of patience presenting with no impairment, mild, moderate, severe, profound and undetermined degree of HI in the left ear.

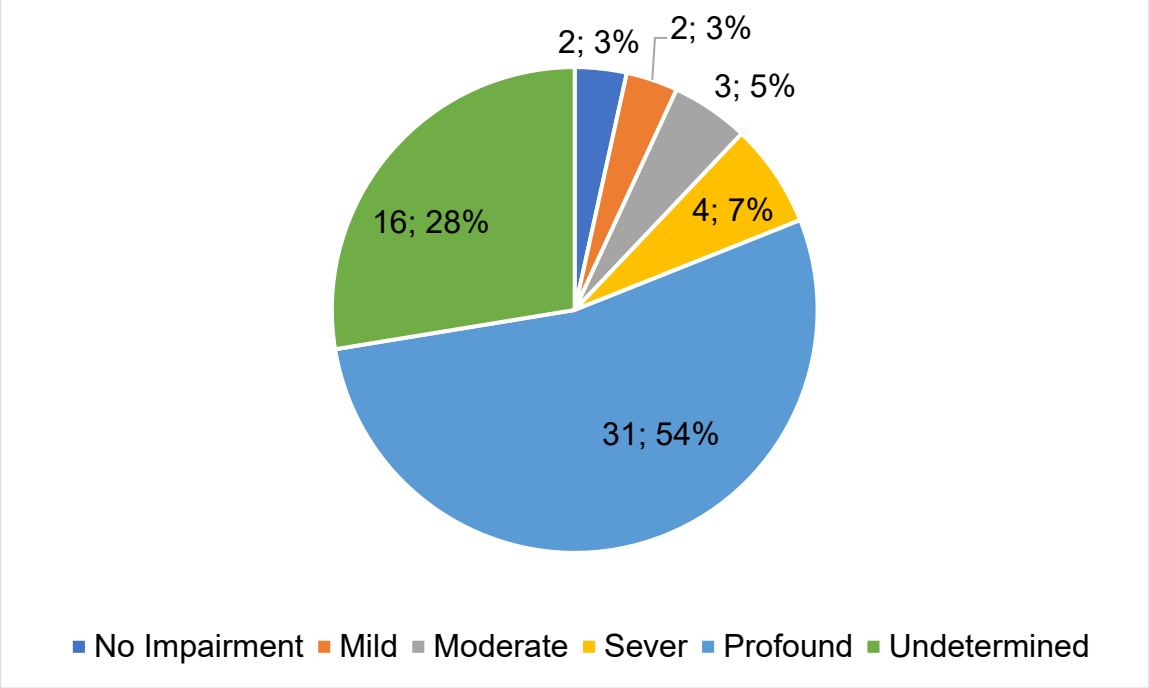
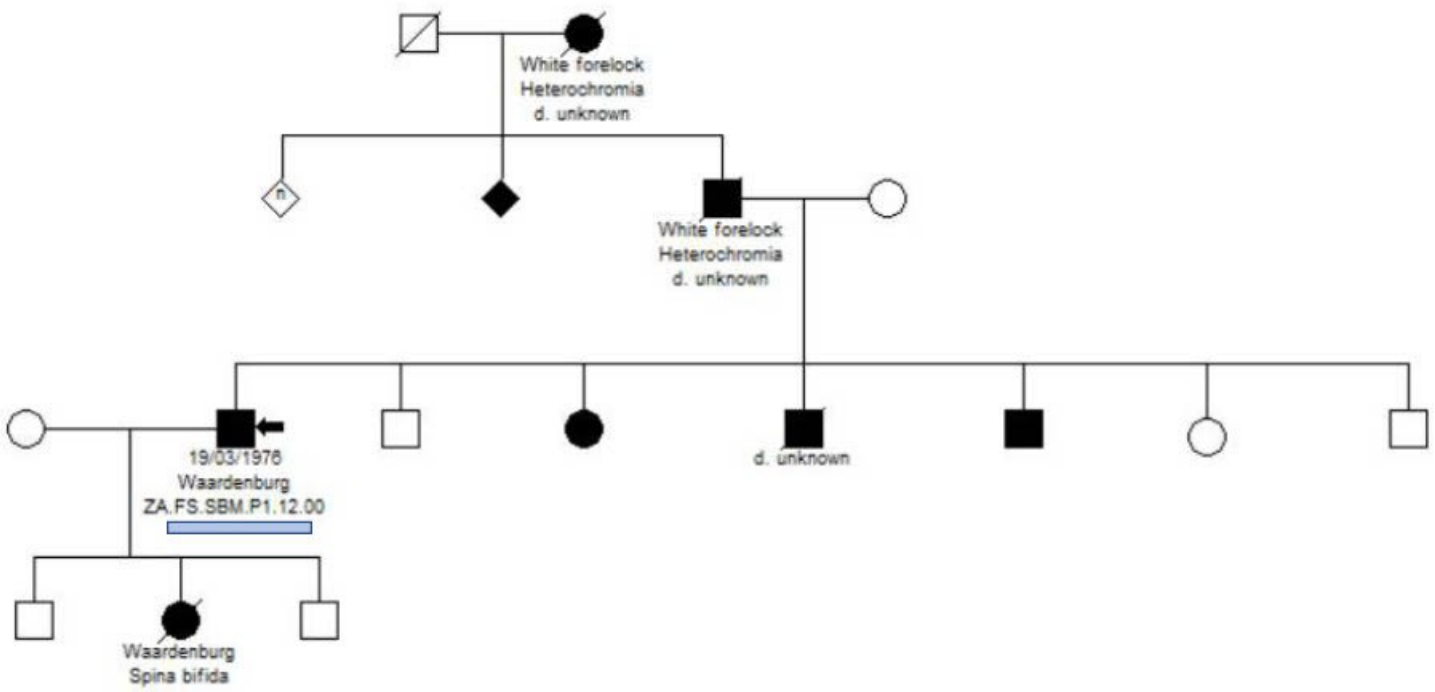


Figure 5.1.15: Degree of HI in the right ear for SHI cohort. The pie chart indicates the number of patients presenting with no impairment, mild, moderate, severe, profound and undetermined degree of HI in the left ear.

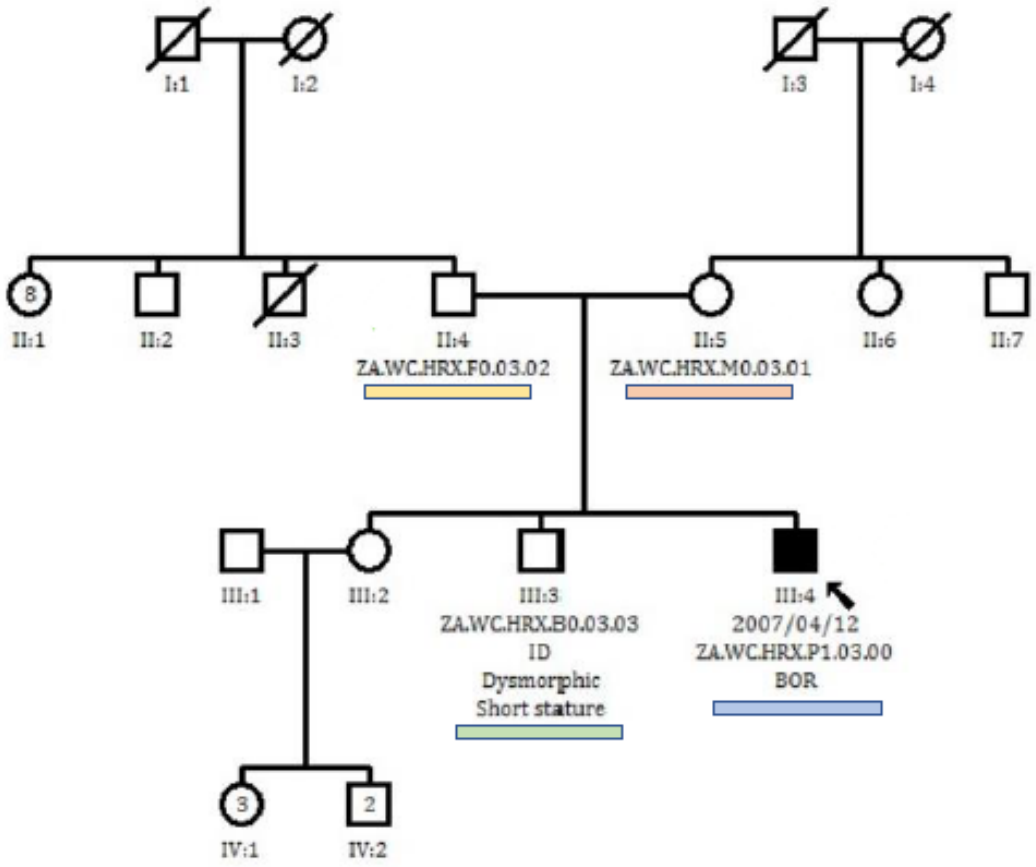
Figure 5.1.16: Illustrative images presenting three syndromes seen in the patient population. Panel a) Presents 3 patients presenting with Waardenburg syndrome, with hearing impairment. Patient a1) presents with bilateral heterochromia, white forelock, and skin depigmentation. Patient a2) presents with unilateral heterochromia and white forelock. Patient a3) presented with unilateral heterochromia. **Panel b)** Indicates a patient presenting with Treacher-Collins Syndrome. Patient presents with cranial dysmorphism, auricle malformation, and microtia. **Panel c)** Presents two patients presenting with Branchio-oto-renal syndrome. Both patients presented with unilateral skin tags.

Illustrative pedigrees for eight syndromes.

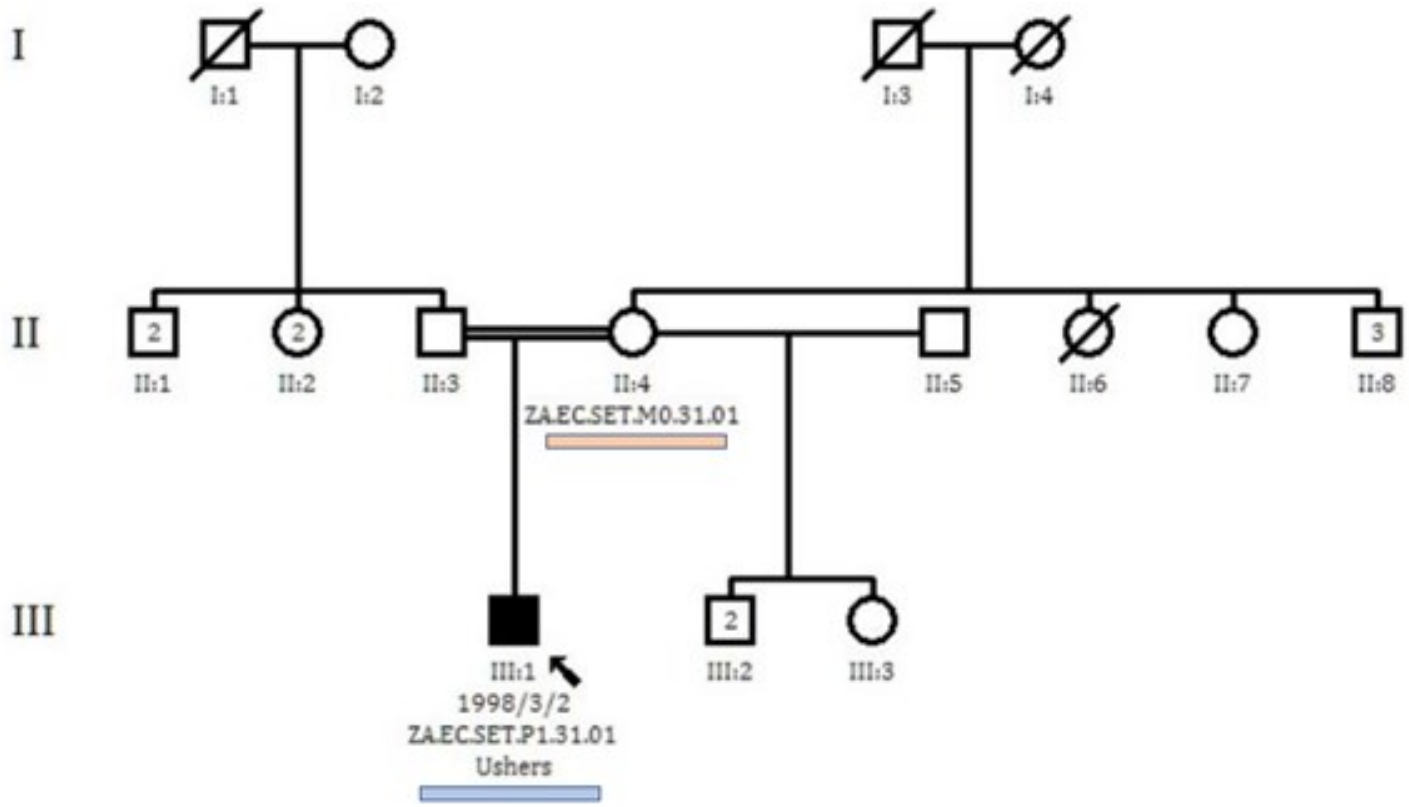
Family 362 – South Africa



Family 362	Member	Gender	Status	Type
ZA.FS.SBM.P1.12.00	Proband	Male	Affected	Waardenburg

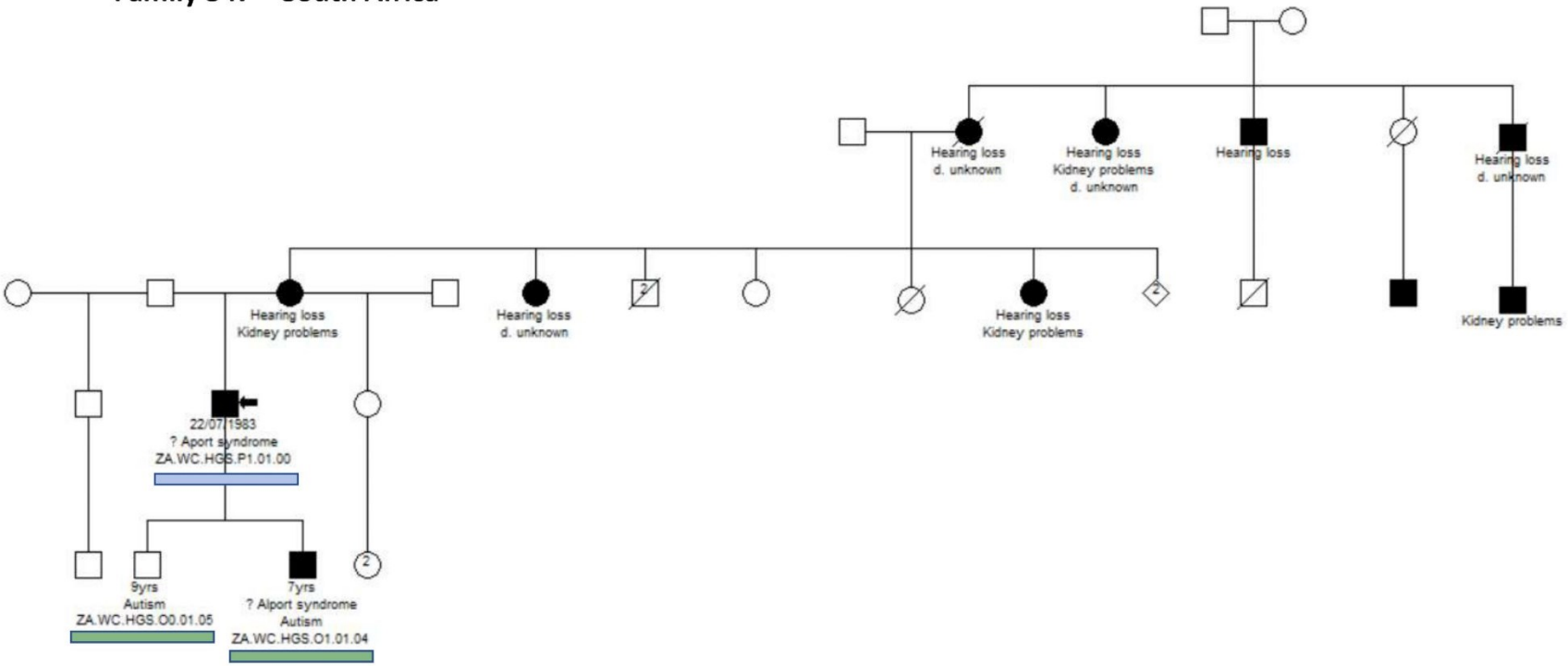


Family 20	Status	Type
ZA.WC.HRX.P1.03.00	Affected	BOR
ZA.WC.HRX.M0.03.01	Unaffected	
ZA.WC.HRX.F0.03.02	Unaffected	
ZA.WC.HRX.B0.03.03	Unaffected	

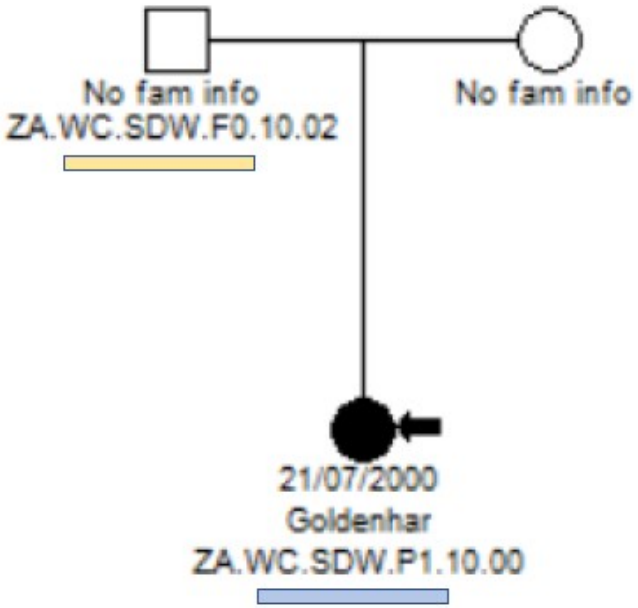


Family 16	Status	Type
ZA.EC.SET.P1.31.00	Affected	Ushers
ZA.EC.SET.M0.31.01	Unaffected	

Family 347 – South Africa

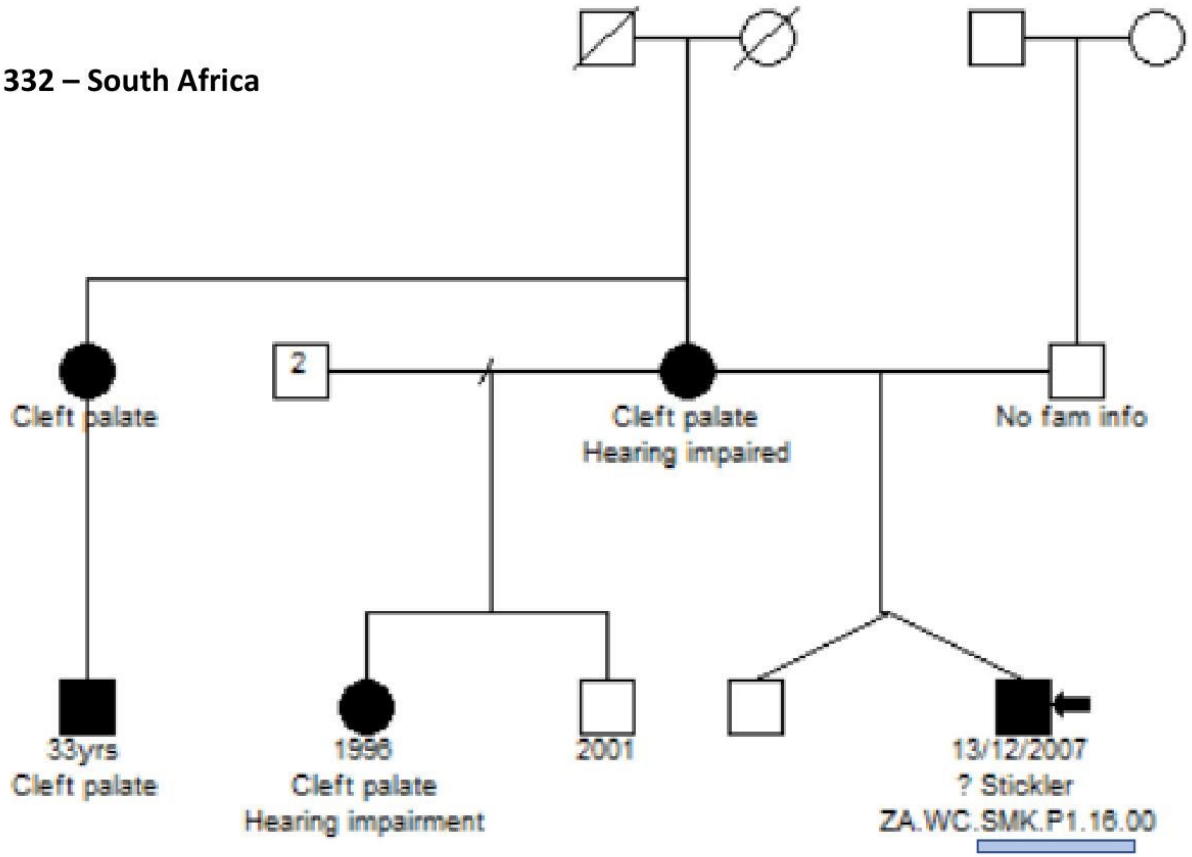


Family 347	Member	Gender	Status	Type
ZA.WC.HGS.P1.01.00	Proband	Male	Affected	? Alport syndrome
ZA.WC.HGS.O1.01.04	Son	Male	Affected	
ZA.WC.HGS.O0.01.05	Son	Male	Unaffected	

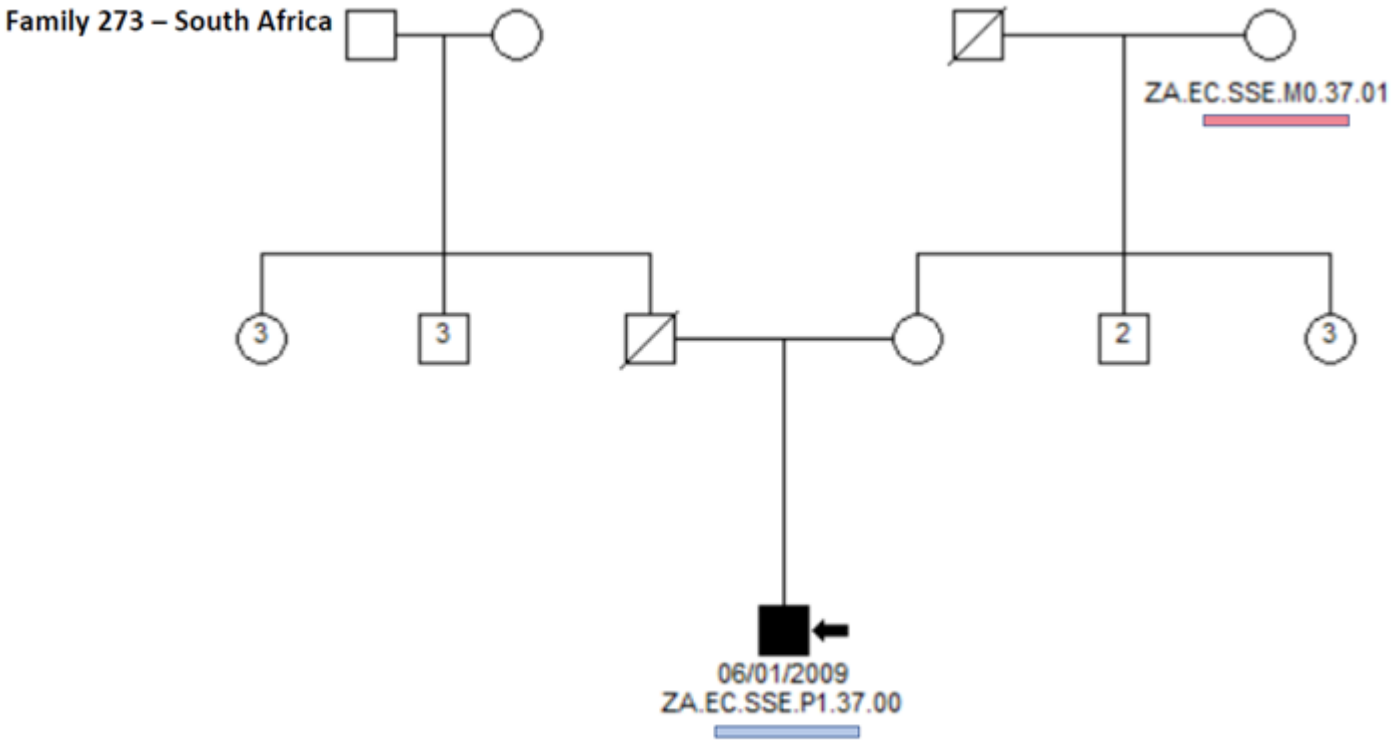


Family 290	Member	Gender	Status	Type
ZA.WC.SDW.P1.10.00	Proband	Female	Affected	Goldenhar
ZA.WC.SDW.F0.10.02	Father	Male	Unaffected	

Family 332 – South Africa



Family 332	Member	Gender	Status	Type
ZA.WC.SMK.P1.16.00	Proband	Male	Affected	Stickler syndrome ?



Family 273	Member	Gender	Status	Type
ZA.EC.SSE.P1.37.00	Proband	Male	Affected	? KID Syndrome
ZA.EC.SSE.G0.37.03	Grandmother	Female	Unaffected	

Whole Exome Sequencing Results

Whole exome sequencing identified putatively pathogenic variants in the following genes in the 27 multiplex families, consisting of a total of 100 individuals. Causative variations were identified in 20 families and are indicated below in Table 5.4, as well as the putative consequences of the variations according to the standards set by the American College of Medical Genetics and Genomics (Richards et al., 2015) using InterVar (Li and Wang, 2017), Varsome,(Kopanos et al., 2019), and Genetic Variant Interpretation Tool (Kleinberger et al., 2016). Pedigrees for the 27 families are indicated in the appendix.

Table 5.4: Whole exome sequencing for non-syndromic HI in the cohort.

Family name	Family	Inheritance pattern	Genotype	Gene	Region	Ascension number	cDNA change	Protein Change	Clinical Consequence	Other HI Genes that do not segregate in the family
SA.FS.BAR.F005	1	AD	Het	*WFS1*	4p16.1	NM_006005.3	c.A2141G	p.Y137Cfs	LP	*ECHS1:* c.410_411del; p.Y137Cfs*7 (carrier)
SA.FS.BAR.F016	2	AD	Het	*MITF*	3p13	NM_198177.2	cT918A	p.N306K	LP	
SA.FS.THI.F003.	5	AR	Comp Het	*ADGRV1*[1]	5q14.3	NM_032119.3	c.G564T	p,E188D	LB	
							c.A17450G	p.N5817S	VUS	
							c.A11298C	p.T3766T	LB	
SA.FS.THI.F016	6	AR	Comp Het	*PDSS1*	10p12.1	NM_014317.4	c.C641T	p.A214V	LP	*TRIOBP:* c.A202G; p.T68A; c.A5048G; p.Q1683R (Comp Het)
							c.G754C	p.E252Q	LP	

SA.LIM.SED.F002	7	AD	Het	*TBC1D24*	16p13.3	NM_001199107.1	c.G1514A	p.C505Y	LP	*COL4A3;* c.1029+3A>G (Het)
SA.LIM.SED.F028	8	AR	Hom	*TMPRSS3*	21q22.3	NM_032405.1	c.205+6t>A		LP	
SA.LIM.TSH.F010	11	AR	Comp Het	*NEU1*	6p21.33	NM_000434.3	c.C1069T	p.R357W	VUS	
							c.G727A	p.G243R	P	
SA.NC.RET.F005	12	AR	Comp Het	*MYO15A*[2]	17p11.2	NM_016239	c.C1378T	p.Q460X	P	
							c.9303+5G>A		LP	
							c.G6634A	p.E2212K	P	
SA.NC.RET.F003	14	AR	Comp Het	*USH2A*[3]	1q41	NM_206933.2	c.T9437A	p.L3146Q	VUS	
							c.G2990T	p.G997V	VUS	
							c.G101A	p.R34Q	LB	
SA.NC.RET.F011	15	AR	Comp Het	*STRC*[4]	15q15.3	NM_153700.2	c.G225A	p.M75I	LP	
							c.C4057T	p.Q1353X	P	
							c.G4655C	p.G1552A	VUS	
SA.NC.RET.F012	16	AD	Het	*P2RX2*[5]	12q24.33	NM_174873.2	c.G1064A	p.G355D	LP	
							c.C1187G	p.T396R	LP	
SA.NC.RET.F023	17	AR	Comp Het	*STRC*[6]	15q15.3	NM_153700.2	c.C4351T	p.R1451X	P	*ADGRV1*: c.A2459G; p.N820S; c.C5809A; p.P1937T (Comp Het)
							c.G4403A	p.R1468Q	LP	
		AR	Comp Het	*OTOG*[6]	11p15.1	NM_001292063.1	c.C2525A	p.P842H	VUS	
							c.G3143A	p.S1048N	VUS	
							c.G916A	p.A306T	VUS	
SA.WC.GRI.F001	19	AR	Hom	*LHFPL5*	6p21.31	NM_182548.3	c.621delC	p.P208Lfs*36	VUS	
SA.WC.GRI.F002	20	AR	Comp Het	*SLC26A4*	7q22.3	NM_000441.1	c.T94C	p.F32L	LP	
							c.T716A	p.V239D	P	

SA.WC.GRI.F004	21	AR	Hom	*GJB2*	13q12.11	NM_004004.5	c.35delG	p.G12Vfs*2	P	
SA.WC.GRI.F016	22	AR	Comp Het	*TRIOBP*	22q13.1	NM_001039141.2	c.C3133T	p.R1045C	VUS	*OTOA:* full gene del; *SLC17A8:* c.G310A; p.V104I (Het)
							c.C4298T	p.P1433L	VUS	
SA.WC.GRI.F017	23	AD	Het	*REST*	4q12	NM_005612.4	c.G1244C:	p.C415S	LP	*CDH23:* c.C5653T; p.R1885C; (Het); *NDUFAF3:* c.17dupA; p.Y6Ter (Het)
SA.WC.WIT.F023	25	AD	Het	*CRYM*[7]	16p12.2	NM_001888.4	c.*6_*2delACAAA		*VUS*	*ADGRV1* ; c.C7150T; p.R2384W; (Hom) MYO7A; c.G19A; p.G7R (Het)
		AR	Comp Het	*CDH23*[6]	10q22.1	NM_022124.5	c.T1585C	p.F529L	VUS	
							c.G8230A	p.G2744S	VUS	
SA.WC.WIT.F027	26b	AD	Het	*FGFR2*	10q26.13	NM_001144919.1	c.1297+10G>C		*LB*	*PLEC:* c.G5504A; p.R1835Q (hom)
SA.WC.WIT.F028	27	AR	Hom	*MYO7A*	11q13.5	NM_001127180.1	c.6255delC	p.P2086Lfs*5	P	

One hundred samples consisting of 65 affected and 35 unaffected individuals. Forty five of the 65 affected individuals (69.2%) had the genetic of the hearing impairment resolved and 20 patients remain unresolved. The study resolved 20 out of the 27 families (74.1%).

Pathogenic non-syndromic variations were identified in 14 families and syndromic pathogenic variations were identified in six families. Homozygous or compound homozygous pathogenic variations in genes associated with Type 2 Usher Syndrome were identified in four families(genes *ADGRV1, PDSS, USH2A,* and *CDH23*). Family 27 presents with a homozygous founder pathogenic variant in *MYO7A* associated with Usher Syndrome Type 1B. Family 11 has variations in the *NEU1* genes, which is associated with sialidosis. The putative consequences are indicated where P= pathogenic, LP= likely pathogenic, LB= likely benign, B= benign, and VUS= variant of unknown significance.

Footnotes: [1]Affected patients share the variant c.A11298C:p.T3766T. [2]Affected patients share the variant c.9303+5G>A. [3]Patient carries unphased variants in *USH2A*. [4]Variant c.C4057T:p.Q1353X and c.G4655C:p.G1552A are on the same haplotype. Variant c.G225A"p.M75I is on the second allele. [5]Affected patient presents with both pathogenic variations on the same allele. [6]Father has compound heterozygote *STRC* pathogenic variations. Child carries *STRC* c.C4351T: p.R1451X. Child has compound heterozygote *OTOG*, with c.G2525A:p.P842H and c. G3143A:p.S1048N are on the same haplotype. Father carriers c.G916A:p.A306T. Mother carries *OTOG* c.G2525A:p.P842H and c. G3143A:p.S1048N haplotype. [7]Affected family members carry dominant *CRYM* c.*6_*2delACAAA variant. One individual has compound heterozygote *CDH23* variants.

Nineteen of the 38 variants identified as putative causative variants are known variants. The variant rs ID and the publication reference are indicated in Table 5.5. Not all known variations had published literature, as some variations were only published to databases such as gnomAD(Karczewski et al., 2020) and/or ClinVar(Landrum et al., 2016).

Table 5.5: Variant classification according to novelty.

Gene	**Inheritance pattern**	**Genotype**	**cDNA change**	**Protein Change**	**Known or novel**	Rs ID	**Publication**
WFS1	AD	Het	c.A2141G	p.Y137Cfs	Novel		
MITF	AD	Het	c.T918A	p.N306K	Novel		

ADGRV1	AR	Comp Het	c.G564T	p,E188D	Known	rs377529304	Richards et al. (2015)
			c.A17450G	p.N5817S	Known	rs1040515250	
			c.A11298C	p.T3766T	Novel		
PDSS1	AR	Comp Het	c.C641T	p.A214V	Novel		
			c.G754C	p.E252Q	Known	rs376818531	
TBC1D24	AD	Het	c.G1514A	p.C505Y	Novel		
TMPRSS3	AR	Hom	c.205+6T>A		Novel		
NEU1	AR	Comp Het	c.C1069T	p.R357W	Known	rs772426069	Uchihara et al. (2010), Naganawa et al. (2000)
			c.G727A	p.G243R	Known	rs104893983	
MYO15A	AR	Comp Het	c.C1378T	p.Q460X	Novel		
			c.9303+5G>A		Novel		
			c.G6634A	p.E2212K	Known	rs371352836	
USH2A[2]	AR	Comp Het	c.T9437A	p.L3146Q	Known	rs1307351188	

			c.G2990T	p.G997V	Novel		
			c.G101A	p.R34Q	Novel		
STRC	AR	Comp Het	c.G225A	p.M75I	Novel		
			c.C4057T	p.Q1353X	Novel		
			c.G4655C	p.G1552A	Novel		
P2RX2	AD	Het	c.G1064A	p.G355D	Novel		
			c.C1187G	p.T396R	Novel		
STRC	AR	Comp Het	c.C4351T	p.R1451X	Known	rs778909195	Richards et al. (2008)
			c.G4403A	p.R1468Q	Novel		
OTOG	AR	Comp Het	c.C2525A	p.P842H	Known	rs560987450	
			c.G3143A	p.S1048N	Known	rs561196208	
			c.G916A	p.A306T	Known	rs553079779	
LHFPL5	AR	Hom	c.621delC	p.P208Lfs*36	Novel		
SLC26A4	AR	Comp Het	c.T94C	p.F32L	Novel		

			c.T716A	p.V239D	Known	rs111033256	Tekin et al. (2003)
GJB2	AR	Hom	c.35delG	p.G12Vfs*2	Known	rs80338939	Carrasquillo et al. (1997)
TRIOBP	AR	Comp Het	c.C3133T	p.R1045C	Known	rs145115226	
			c.C4298T	p.P1433L	Known	rs761830009	
REST	AD	Het	c.G1244C	p.C415S	Novel		Manyisa et al. (2021)
CRYM	AD	Het	c.*6_*2delACAAA		Known	rs749834549	
CDH23[6]	AR	Comp Het	c.T1585C	p.F529L	Novel		
			c.G8230A	p.G2744S	Known	rs376189742	
FGFR2	AD	Het	c.1297+10G>C		Known	rs758605716	
MYO7A	AR	Hom	c.6255delC	p.P2086Lfs*5	Known		Roberts et al. (2015)

Discussion

Hearing impairment has seen a rise in disease burden since 2005 (Kassebaum et al., 2016). It currently affects 1.5 billion people, of which approximately 430 million people present with disabling HI (WHO, 2021b). Hearing impairment was estimated to affect 5.5 live births in South Africa (Swanepoel, 2009). This estimate, while useful, is most certainly out of date, and a new study would be required to determine the current prevalence of HI. In South Africa, research into the genetic cause of HI has shown an increase over time. The initial studies of HI in South Africa were to identify the frequency of pathogenic *GJB2* variations in the genetic HI population (Bosch et al., 2014a, Bosch et al., 2014b, Kabahuma et al., 2011). Work by Yan et al. (2016) investigated putative genetic variation in a multi-population cohort, and reported putative causative variants in *POUF3, SIX1, TRIOBP,* and *MARVELD2*. Kabahuma et al. (2021) identified eight pathogenic or likely pathogenic *MYO7A* variants in ten patients from a cohort of 94 Indigenous South Africans.

Acquired and Syndromic HI

This study was performed to identify the genetic causes of NSHI in the South African population. It, however, inadvertently identified several cases of acquired and syndromic HI. The identified cases of acquired HI are not at a scale to determine the prevalent causes of acquired HI in the population. Similarly, the number of Syndromic cases of HI, in this study, is not at a scale where it can provide a near-accurate glimpse of the types of syndromes present in the South African HI population. This, however, is an area of research that is of public health interest and should be explored further.

Fifty-eight cases of Syndromic HI were identified during recruitment, of which 33 cases were identified as cases of Waardenburg syndrome. Three cases of Usher syndrome were identified when patients were examined during the recruitment process. Whole exome sequencing analysis, further, identified putative pathogenic variations associated with Type 2 Usher syndrome in 5 patients and one family presented with the known p.P2086Lfs*5 founder pathogenic variant in *MYO7A* associated with Usher 1B in the South African population (Roberts et al., 2015). Thus, Usher Syndrome accounted for nine patients in the cohort.

Type I and Type II usher syndrome presents with congenital HI and retinitis pigmentosa (RP) within the first decade, type 1, or between the first or second decade, type 2, of life.

This means that the diagnosis of Usher Syndrome may be missed in several patients in the absence of ophthalmological examination. This is illustrated in the fact that 6 families within the cohort presented with homozygous or compound heterozygous pathogenic variations in genes associated with Type 2 Usher Syndrome and one family was homozygous for the p.P2086Lfs*5 founder pathogenic variant associated with Type 1b Usher Syndrome.

It is necessary to consider an extensive study to identify cases of SHI. This is import for early intervention, management, and surveillance of disease progression in patients presenting with putative SHI.

Whole exome sequencing

Whole exome sequencing identified a diverse spectrum of genes associated with HI in 27 families, which were the familial putative non-syndromic cases recruited within the first year of the recruitment drive. The study putatively resolved the genetic cause of HI in 20 of the 27 families submitted, with a resolution rate of 74.1%.

Interestingly, two families presented pathogenic variations in the *STRC* gene. *STRC* encodes the sterocilin protein which in expressed in the hair cells (Verpy et al., 2001). The presence of two families segregating *STRC* pathogenic variations is not significant, but it is worth noting when performing WES in other South African families. This will provide an estimate of the frequency of *STRC* putative pathogenic variations in the South African population and establish the prevalence of the gene in the population.

It was relatively surprising two families presented where one individual carried pathogenic variations in two genes. The first family, the family presented with compound heterozygous pathogenic or likely pathogenic variations in *STRC*, but the proband had an additional compound heterozygous pathogenic variation in *OTOG*. In the second family, a dominant pathogenic variation in *CRYM* resulted in the HI in the family. However, one individual also had compound heterozygous pathogenic variations in *CDH23*. Further study may indicate whether the presence of variations in two genes has an effect on the overall HI in these two particular individuals and the clinical consequences as they are both variations of unknown significance.

Surprisingly, only three cases of Usher syndrome were identified in the field during the recruitment process. However, an additional five cases of Usher Syndrome were identified following the analysis of the whole exome sequencing results. Four families presented with

Usher Syndrome Type 2 variations and one family presented with Usher Syndrome Type 1b. Usher syndrome presents as congenital HI and vision loss due to retinitis pigmentosa, with or without vestibular dysfunction (Van Camp G and Smith, 2021).

A 6th family presented with compound heterozygous variations in the *NEU1* gene. The variantion rs772426069 has been associated with sialidosis when homozygous (Uchihara et al., 2010, Naganawa et al., 2000). The impact of *NEU1* will need to be investigated separately as the second variant is a variant of unknown significance.

The error in phenotyping the families may be attributed to the delay in the presentation of retinitis pigmentosa, which normally presents in the first or second decade of life (Van Camp G and Smith, 2021).

Finally, the novel *REST* variant identified in this study is reported in Chapter 6. The gene had, prior to this study, only been associated with HI in a North American family.(Nakano et al., 2018).

Conclusion

This study resulted in the recruitment of 511 patients and their families into the study. Twenty-seven families were submitted for WES; of which 15 families were resolved and 11 families were partially resolved. This study identified a novel variant in *REST*, which is the second recorded case of *REST* associated HI. It also identified pathogenic variations in *MYO15A* which may support the idea that *MYO15A* is a notable gene in African populations.

Chapter 6: RE1 Variant in Silencing Transcription Factor (*REST*) explains a dominant hearing impairment in one family

Synopsis: This chapter presents an original article detailing the identification of *REST* as the causative gene resulting in HI in a South African family.

Manyisa, N., Schrauwen, L., de Souza Rios, L.A., Mowla, S., Tekendo-Ngongang, C., Popel, K., Esoh, K., Bharadwaj, T., Nouel-Saied, L.M. & Acharya, A. 2021. A Monoallelic Variant in *REST* Is Associated with Non-Syndromic Autosomal Dominant Hearing Impairment in a South African Family. *Genes.* 12(11):1765.

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Co-Author's contribution: Conception of the project, A.W. and S.M.L.; recruitment, C.T.-N.,K.P.; cellular localization and expression, L.A.d.S.R., S.M.; bioinformatics analysis, I.S., T.B., L.M.N.-S., A.A.; protein modelling, A.N., K.E.; review and editing, I.S, L.A.d.S.R., S.M., C.T.-N., K.P., K.E., T.B., L.M.N.-S., A.A,A.N., E.W.-T., C.d.K., C.D., S.M.L., A.W.; supervision of the project: A.W., S.M.L. and S.M. All authors have read and agreed to the published version of the manuscript.

A Monoallelic variant in *REST* is associated with non-syndromic autosomal dominant hearing impairment in a South African family

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Abstract

Hearing impairment (HI) is a sensory disorder with a prevalence of 0.0055 live births in South Africa. DNA samples from a South African family presenting with progressive, autosomal dominant non-syndromic HI were subjected to whole-exome sequencing, and a novel monoallelic variant in *REST* [c.1244G>C; p.(C415S)], was identified as the putative causative variant. The co- segregation of the variant was confirmed with Sanger Sequencing. The variant is absent from databases, 103 healthy South African controls, and 52 South African probands with isolated HI. In silico analysis indicates that the p.C415S variant in *REST* substitutes a conserved cysteine and results in changes to the surrounding secondary structure and the disulphide bonds, culminating in alteration of the tertiary structure of REST. Localization studies using ectopically expressed GFP- tagged Wild type (WT) and mutant *REST* in HEK-293 cells show that WT REST localizes exclusively to the nucleus; however, the mutant protein localizes throughout the cell. Additionally, mutant REST has an impaired ability to repress its known target *AF1q*. The data demonstrates that the identified mutation compromises the function of REST and support its implication in HI. This study is the second report, worldwide, to implicate *REST* in HI and suggests that it should be included in diagnostic HI panels.

Keywords: *REST*, RE1-silencing transcription factor, Nonsyndromic Hearing Impairment, South Africa, Africa; DFNA27

Introduction

Hearing impairment (HI) is a sensory disorder that affects 466 million people (WHO, 2021a). HI affects approximately 1 in 1000 newborns worldwide, but it has an estimated prevalence of 5.5 in 1000 live births in South Africa (Swanepoel, 2009). HI is defined as the inability to detect sound better than 25 dB in the better hearing ear and it is considered disabling HI when a child cannot hear better than 30 dB and an adult cannot hear better than 40 dB in their better hearing ears (WHO, 2021a).

Hearing impairment has a heterogeneous etiology with the causes of HI being broadly classified as either genetic, environmental, or due to unknown factors. Genetic factors may account for 50% of HI in high income countries (Wonkam et al., 2013b). Approximately 121 genes have been identified as being associated with non-syndromic hearing impairment (NSHI) (Van Camp G and Smith, 2021). Non-syndromic hearing impairment accounts for

about 70% of HI cases of genetic origin and is inherited on an autosomal recessive (AR) mode in approximately 80% of cases, while autosomal dominant (AD) account for 15 to 20% (Smith et al., 2005). Variants in *GJB2* and *GJB6* genes are the major contributors to NSHI in Europeans, Asians, and Arabs (Chan and Chang, 2014, Hutchin et al., 2005, Liu et al., 2002, Najmabadi and Kahrizi, 2014, Pandya et al., 2003), but are infrequent in most populations of African descent, including black South Africans. In addition, using data from the genome aggregation database (gnomAD) database, for ARNSHI the prevalence of identified likely pathogenic and pathogenic (PLP) variants (Karczewski et al., 2020) was estimated at 96.9 per 100,000 individuals for Ashkenazi Jews for ARNSHI based on sequence data compared to only 5.2 per 100,000 individuals among Africans/African Americans (Chakchouk et al., 2019) indicating for African populations many variants remain to be discovered. Therefore, there is an urgent need to investigate HI in African populations using next generation sequencing and multiplex HI families.

RE1-silencing transcription factor (*REST*) is a transcriptional repressor that binds to a 23-bp RE1 consensus sequence in the promoter region of its target genes (Chong et al., 1995, Schoenherr and Anderson, 1995, Kraner et al., 1992). The DFNA27 locus, containing REST, was mapped in 2008 in a North American family (Peters et al., 2008). *REST* was reported as a strong candidate associated with HI in the family in 2018, wherein an intronic variant (NC_000004.12:g.56927594C>G) was identified (Nakano et al., 2018). *REST* contains 4 exons and encodes a 1097aa protein that restricts the expression of neuronal genes in non-neuronal cells (Chong et al., 1995, Schoenherr and Anderson, 1995, Bayram et al., 2017). This variant (g.56927594C>G) results in the prevention of alternative splicing of the *REST* mRNA which is necessary for the regulation of REST in the inner ear (Nakano et al., 2018).

In this study, whole exome sequence (WES) data was generated from DNA samples obtained from a non-consanguineous South African family. The family presented with progressive ADNSHI and a novel monoallelic variant in *REST (*NM_005612.5:c.1244G>C, p.C415S, GRCh37:4:57796268:G:C), in the locus DFNA27, was identified.

Materials and Methods

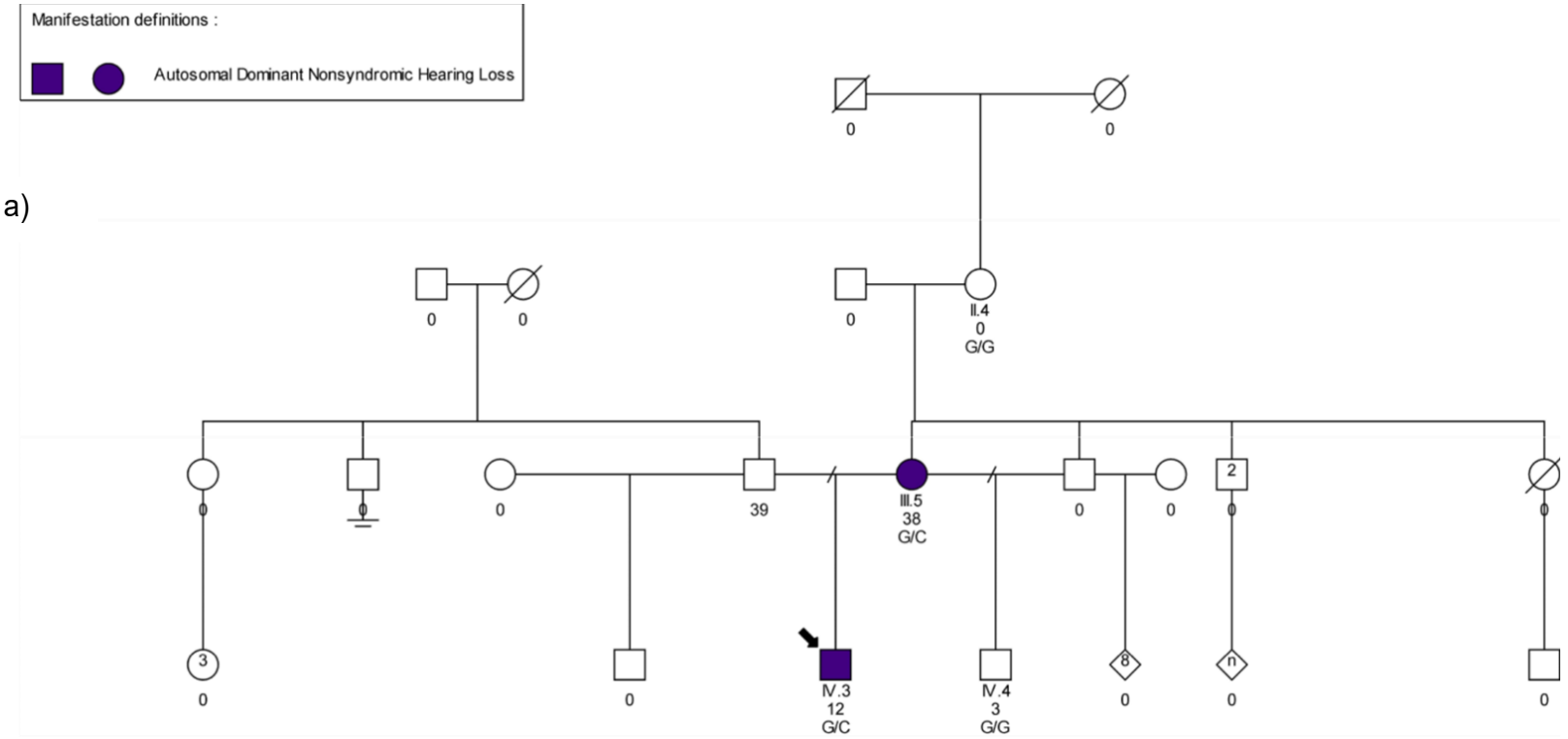
Participants' Recruitment

The participants' selection process followed the protocol reported by reported Bosch et al. (2014a, 2014b) (Bosch et al., 2014b, Bosch et al., 2014a). The hearing-impaired members

of a South African Xhosa family of African ancestry (Family 1, Figure1a) were identified through a national recruitment program for the deaf. The detailed personal history and medical records of the hearing- impaired participants (mother and son) were reviewed by two medical geneticists (C.T.N and A.W.). A general systemic and otological examination was performed, including pure tone audiometry. We followed the recommendation number 02/1 of the Bureau International d'Audiophonologie (BIAP), Belgium.

In addition, a total of 52 unrelated probands with sporadic NSHI of putative genetic origin comprising of both Black South African and South Africans of Mixed Ancestry (Table S2) were recruited, to investigate the frequencies of possible PLP variants identified. Moreover, 103 healthy Black South African controls without personal or familial history of HI were randomly recruited at outpatient clinics at Groote Schuur Hospital, Cape Town, South Africa (Table S3).

a)


Manifestation definitions :
Autosomal Dominant Nonsyndromic Hearing Loss
0
0
0
0
0
II.4
0
G/G
0
0
0
39
III.5
38
G/C
0
0
2
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3
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IV.3
12
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n
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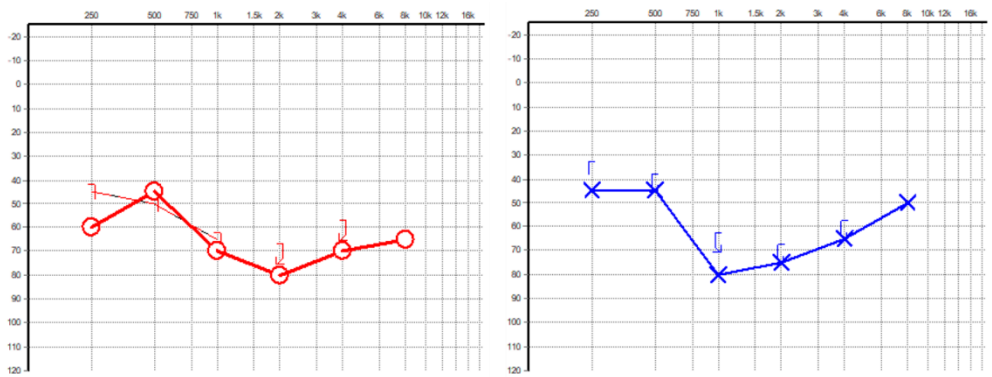
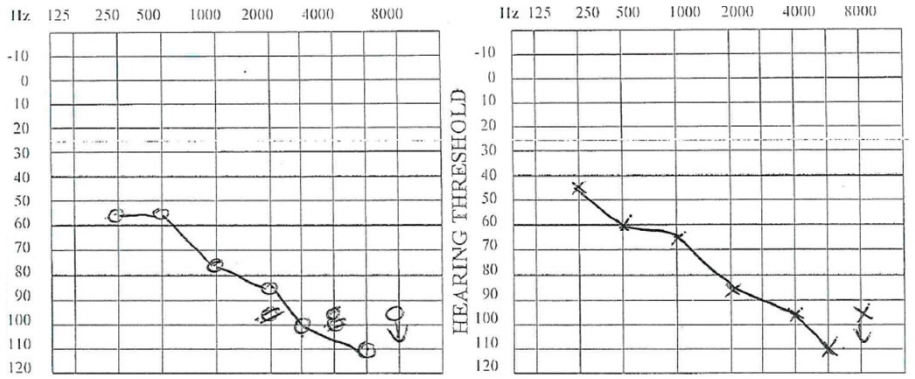
b




Hz 125 250 500 1000 2000 4000 8000
HEARING THRESHOLD

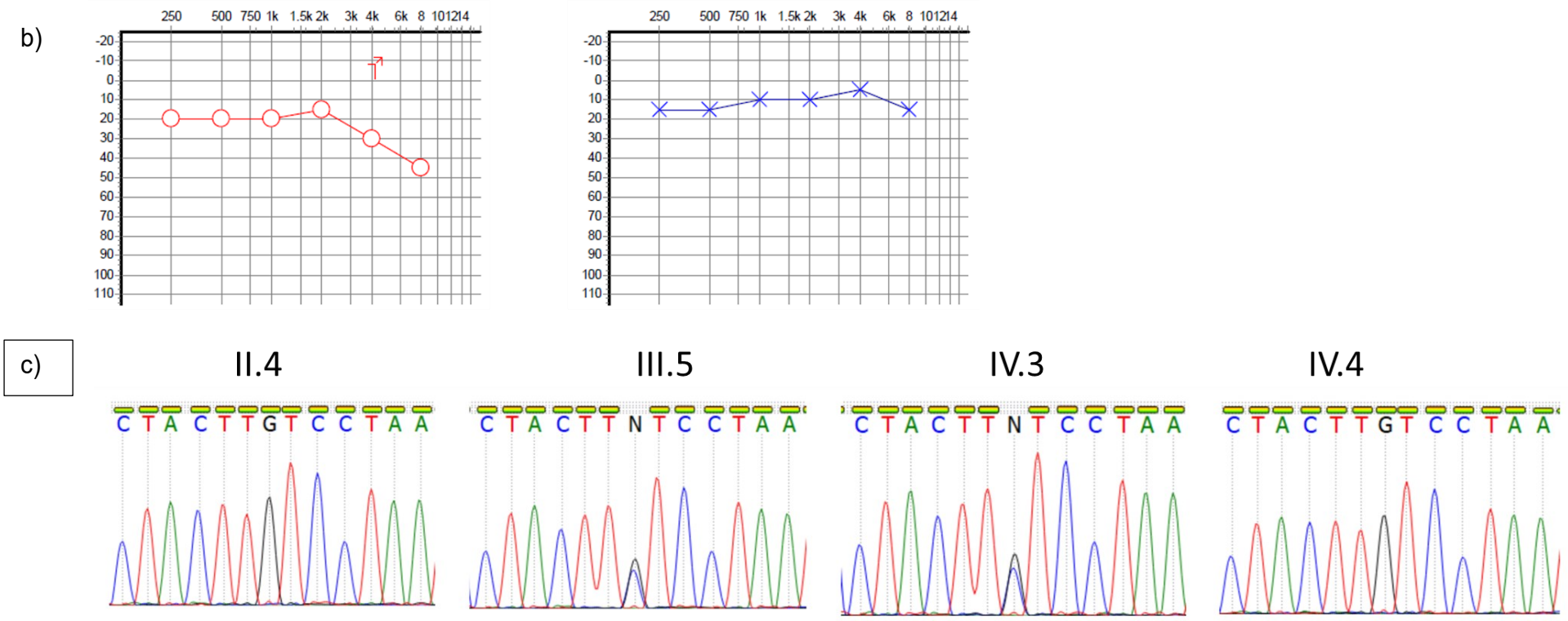


Figure 6.1: The pedigree of the family presenting with non-syndromic hearing impairment. (a) The pedigree suggesting a dominant inheritance model of hearing impairment (HI). The age and genotype are indicated under the ID for the four family members that were available for sequencing. (b) audiograms indicate severe hearing impairment in the affected mother and son and normal hearing in the unaffected child. The left ear is blue and the right ear is red in the audiograms for subject III.5 and IV.4. (c) The missense mutation segregates within the family with the affected mother and child being heterozygous for the c.1244G>C. The unaffected grandmother and half-brother are homozygous wild-type. (Audiology sizes have been increased to facilitate readability).

Molecular Methods

DNA Extraction

Peripheral Blood samples were obtained from the proband (IV.3) and three family members: the proband's affected mother (III.5), unaffected half-brother (IV.4), and maternal grandmother (II.4) (Figure1a). DNA was extracted using the chemagen 360 Instrument (PerkinElmer, Massachusetts, USA) per the manufacturer's instructions.

Whole Exome Sequencing

DNA samples, from the two affected members of Family 1, underwent whole-exome sequencing at OmegaBioservices (Norcross, GA, USA). An Illumina Nextera Rapid Capture Kit® (37Mb; San Diego, CA, USA) was used for library preparation, according to the manufacturer's instructions. The library was then sequenced using the Illumina HiSeq 2500 sequencer using the pair-end 150bp run format. The sequencing data were processed using the Illumina DRAGEN Germline Pipeline v3.2.8.

Bioinformatics Analysis

Alignment and Quality Checking

High-quality reads were aligned to the human GRCh37/hg19 human reference genome using the DRAGEN software (version 05.021.408.3.4.12). Variants were called following sorting and marking of duplicates and individual genomic variant call files (gVCF) were generated. Joint variant calling for single nucleotide variations (SNV) and Insertion/Deletions(Indels) was performed using the genome analysis toolkit (GATK) software (version 4.0.6.0) (McKenna et al., 2010). For those individuals that underwent exome sequencing, their sex of was verified using plink (version 1.9) (Chang et al., 2015, Purcell et al., 2007). Additionally, identity-by-descent sharing in plink (version1.9) and Kinship-based Inference for GWAS (KING) algorithms were used to verify their familial relationships (Chang et al., 2015, Purcell et al., 2007, Manichaikul et al., 2010).

Variant Annotation and Filtering

ANNOVAR and custom scripts were used for variant annotation and filtering (Wang et al., 2010). Variants were initially prioritized using an AD mode of inheritance. Rare variants for all populations in the genome aggregation database (gnomAD) (Karczewski et al., 2020,

Landrum et al., 2016), with minor allele frequencies < 0.0005, and known likely pathogenic and pathogenic variants in ClinVar were retained (Landrum et al., 2016).

Functional prediction of missense variants was performed by annotating dbNSFP (version4.0) (Liu et al., 2011, Liu et al., 2020). dbNSFP includes Sorting Intolerant from Tolerant (SIFT), polymor- phism phenotyping v2 (PolyPhen-2) 2, MutationAssessor, the likelihood ratio test (LRT), Mendelian clinically applicable pathogenicity (M-CAP) score, Rare Exome Variant Ensem- ble Learner (REVEL), MutPred, PROtein Variation Effect Analyzer (PROVEAN), MetaSVM, and MetaLR (Liu et al., 2011, Liu et al., 2020). Whereas the tools MutationTaster, Eigen, Eigen-PC, functional analysis through Hidden Markov models (FATHMM-MKL), combined annotation dependent deple- tion (CADD) score, and deleterious annotation of genetic variants using neural networks (DANN) were used to evaluate both coding and non-coding variants.

Splice site variants were analyzed using dbscSNV which used Adaptive boosting and random forest scores (Jian et al., 2014). This tool allowed for the analysis of the deleterious effect of variants within conserved splicing regions, 3 to +8 at the 5J splice site and 12 to +2 at the 3J splice site (Jian et al., 2014). Furthermore, the conservation of nucleotides and amino acids was estimated, at which the variations occur, using phyloP, Genomic Evolutionary Rate Profiling (GERP), SiPhy, and phastCons scores (Liu et al., 2011, Liu et al., 2020, Cooper et al., 2005, Pollard et al., 2010).

The online Mendelian inheritance in man (OMIM) (McKusick, 1998), ClinVar, and gnomAD were used to determine if there were known associations between identified genes and/or variants and HI. Variants were considered to be putatively causative if they occurred in known HI genes or genes that were expressed in the inner ear, if the variant had a predicted effect on protein function or the mRNA, and if the variation segregated with HI within the family.

Direct Cycle Sequencing

Direct cycle sequencing was performed to validate the segregation of the candidate variation in the family. Cycle sequencing was also performed on 52 probands with sporadic NSHI of putative genetic origin, and 103 presumably healthy, ethnically controls who had no family history of HI. Primers (forward 5'-GTTCTTTAGTAGTGCTTGAGG-3' and reverse 5'-GGTGACTACCAGAACTCG-3') that target the variant of interest in exon 4 were designed based on the genomic sequence of REST (OMIM: 600571), from Ensembl (Yates et al., 2019). The primer specificity was evaluated using primerBlast (Ye et al., 2012) and

In silico PCR. The annealing temperature was determined to be 60 ◦C for 45 s and fragment elongation occurred at 72 ◦C for 1 min. Sequencing of the PCR was performed using BigDye™ Terminator v3.1 Cycle Sequencing Kit and an ABI 3130XL Genetic Analyzer® (Applied Biosystems, Foster City, CA, USA). UniPro UGENE (version 38.1) (Okonechnikov et al., 2012).

Secondary Structure Analysis and Multiple Sequence Alignment

The secondary structure of the wild-type (WT) and mutant (MT) REST was viewed using psipred 4.0 (Buchan and Jones, 2019, Jones, 1999) and this was followed by performing a multiple sequence align- ment (MSA). A PSI-Blast search was performed for REST, using the non-redundant protein data and default search parameters. The PSI-Blast hits were retrieved as FASTA files and the MSA was performed using CLUSTAL Omega (version 1.2.4) (Madeira et al., 2019) and the MSA was viewed using Jalview (version 2.11.1.4) (Waterhouse et al., 2009).

Protein Modelling and Disulphide Bond Formation

The three-dimensional structure of the longest isoform of REST was used to generate protein models for the WT and MT REST. A homology model of the WT and MT REST was constructed using MODELLER (version 9.4) (Webb and Sali, 2016, Sali and Blundell, 1993, Marti-Renom et al., 2000, Fiser and Do, 2000), based on the available crystal structure (6DU2(Burkholder et al., 2018)) as a template. PYMOL Viewer (version 2.4) (Schrödinger, 2015) was used for visualization of the structure and image processing. The disulphide bonds within the tertiary structure of REST were analyzed using DiANNA 1.1 Web Server (Ferre and Clote, 2005, Ferrè and Clote, 2005, Ferrè and Clote, 2006). Finally, the domains of REST were generated using InterPro (Blum et al., 2021).

Localisation and Expression Analysis

Mammalian expression plasmids expressing GFP-tagged REST (NM_001193508), and myc-DDK-tagged REST (RG235166 and RC235166) were purchased from Origene (Rockville, MD, USA), and used as templates for generating mutant versions using custom primers (Forward primer 5’ CTTCAAATCTAAGCATCCTACTTCTCCTAATAAAACAATG GATGTC 3’ and reverse primer 5’ GACATCCATTGTTTTATTAGGAGAAGTAGGATGCTT AGATTTGAAG 3’) acquired from Whitehead Scientific (Stikland, Western Cape, South Africa), based on the protocol adapted from Stratagene QuikChange system. Site-directed

mutagenesis (SDM) was performed using the KAPA HiFi HotStart ReadyMIx (Roche, Basel, Switzerland). The plasmids were sequenced by Inqaba Biotec (Gauteng, South Africa) to determine if the SDM was successful.

Cell Culture, Transfections and Visualization Using Confocal Microscopy

HEK-293 human embryonic kidney(Graham et al., 1977) cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% (v/v) fetal bovine serum (Thermo Fisher Scientific, Waltham, MA, USA) and 1% (v/v) penicillin/streptomycin (Sigma-Aldrich, St. Louis, MO, USA). Cells were cultured in humidified incubator at 37 ◦C with 5% CO2. Mycoplasma contamination was screened using bisBenzimide H 33342 trihydrochloride (14533, Sigma-Aldrich, St. Louis, MO, USA) DNA staining. The HEK-293 cells were plated in 35 mm dishes (density 4 104 cells per mL) 16 h before transfection. Cells were transiently transfected using X-tremeGENE™ HP DNA Transfection Reagent according to the manufacturer's instructions (Roche, Basel, Switzer- land), with 250 ng of plasmid (Empty, GFP-only, GFP-tagged WT REST or GFP-tagged MT REST). Live viewing was performed 24 h after transfection, using a Zeiss LSM8800 with Airyscan confocal microscope (Zeiss, Oberkochen, Germany). Cells were spiked with 1 in 100,000 Hoechst for co-visualization of nuclear material. The detector of the confocal was the photomultiplier tube (PMT) and allowed detection of the green fluorescence signal through the Argon laser at 488 nm. Images were visualized and processed using the ZEN Blue Software (latest version) provided by Zeiss (Zeiss, Oberkochen, Germany).

Quantitative Real-Time PCR

HEK-293 cells were plated (density 8 104 cells per ml) in 12-well plates, 16 h before transfection. Cells were transfected using X-tremeGENE™ HP DNA Transfection Reagent, according to the manufacturer's instructions, with 500 ng of either the WT or MT REST plas- mid containing the myc-DDK tag and non-transfected cells received X-tremeGENE™ HP DNA Transfection Reagent (Roche, Basel, Switzerland) with no plasmid DNA. Total RNA was extracted 24 h post-transfection using the High Pure RNA Isolation Kit (Roche, Basel, Switzerland) per the manufacturer's instructions, quantified with Nanodrop1000 (Thermo Fisher Scientific, Waltham, MA, USA), and verified using agarose gel electrophoresis. cDNA was produced from 500 ng of total RNA using the iScript™ Reverse

Transcription Supermix for RT-qPCR (BioRad, Hercules, CA, USA), according to the manufacturer's instructions, and used in qPCR experiment using AF1q primers (5J GGACCCTGTGAG- TAGCCAGT 3J, for the forward primer, and 5J TTGCCAACGCTGCTGTCTTT 3J, for the reverse primer) and GAPDH as an internal control (5J GAAGGCTGGGGCTCATTT 3J, for the forward primer, and 5J CAGGAGGCATTGCTGATGAT 3J, for the reverse primer). qPCR was performed using KAPA SYBR® FAST (Roche, Basel, Switzerland) on the Rotor- Gene Q (Qiagen, Hilden, Germany) real-time PCR machine. The comparison was made between the negative control, WT and MT using the fold-change (2 ΔΔCT), where the control group was set to 1.

The expression data, from two experiments, was pooled and the mean and standard deviation was determined. The significance of the data was evaluated using the Student's t-test.

Luciferase Assay

HEK-293 cells were plated (density of 8 104 cells per ml) in 12-well plates, 16 h before transfection. Cells were transfected using X-tremeGENE™ HP DNA Transfection Reagent (Roche, Basel, Switzerland), according to the manufacturer's instructions with 400 ng of either WT or MT REST plasmid containing the myc-DDK tag or empty pCMV plasmid and 200 ng of pGL2-basic containing AF1q. The cells were harvested and 36 h and lysed using the Passive Lysis Buffer (Promega, Madison, WI, USA). The lysate was frozen at 80 ◦C overnight before thawing and centrifugation at 12,000 rpm at 4 ◦C. The luciferase assay was performed according to the manufacturer's instructions and luminometer readings were recorded from the GloMax®-Multi Detection System (Promega, Madison, WI, USA). The expression data, from two experiments, was pooled and the mean and standard deviation was determined. The significance of the data was evaluated using the Student's t-test.

Results

Phenotypic Description

Despite the small size of the family, the most likely mode of inheritance was ADNSHI. From anamnesis, we did no identified any environmental factors as a possible cause of HI, and no HI participant had a history of ophthalmological clinical expression (blurred or distorted

vision, photophobia, eye pain, etc.), or any neurological symptoms such as vertigo or dizziness. Additionally, no vestibular, neurologic, or any other systemic abnormalities were detected by physical examination.

A medical history of prelingual progressive HI was described for the two affected family members; however, prior to this study, no formal audiological assessment was performed on the affected mother and the unaffected grandmother and unaffected half- brother. Audiological assessment of the proband and his mother revealed symmetrical bilateral sensorineural HI (Figure1b).

The index patient (IV.3) was 12 years old at the time of the recruitment. He was diagnosed with HI at three years of age. He has progressive HI, air conduction thresholds had decreased in his 2019 audiogram when compared to his 2017 audiogram, according to the report from his audiologist. He has severe (pure tone average was evaluated as 71 dB at 500, 1000 and 2000 Hz), sensorineural HI in both ears. He previously had surgery on his ears, to insert grommets and had undergone speech therapy and was using hearing aids when first contacted. There were no associated anomalies in the patient and the parents were unrelated. The HI was determined to be familial non-syndromic HI and putatively of autosomal dominant inheritance.

The affected mother (III.5); was 37 years old at the time of recruitment. She presented with severe HI, in both ears (pure tone average was evaluated as 65 dB in right ear and 67 dB in left ear, at 500, 1000 and 2000 Hz), that was identified and diagnosed at 27 years of age. Her HI is sensorineural in both ears. She had not undergone any speech therapy but used hearing aids when first contacted. She did not present with any associated anomalies and her parents were unrelated.

Conventional pure tone audiometry indicated a mild high frequency hearing impairment in the half-brother of the proband (IV.4). A lack of focus was, however, noted in the child during the assessment and is the most likely cause of the result, rather than the child having high frequency HI.

WES Identification of Candidate Novel Variant in REST

The average target region coverage was about 225, with 96.30% of the target region being covered to a depth of 10 or more. Through using the filtering criteria described in the methods section, a candidate variant was identified in a known candidate HI gene REST (OMIM: 600571). The variation was present in the proband and mother, and thus segregated with the HI phenotype. The variant was confirmed in the mother and the

proband through direct Sanger sequencing, and absent in the unaffected younger half-brother and the putatively unaffected grandmother. The novel NM_005612.4:c.1244G>C; p.(C415S) variant occurs in the 4th exon and within the lysine-rich protein domain (Figure S1) and was predicted to be damaging by 16 of the 17 bioinformatics tools used (Table S4), including MutationTaster, FATHMM-MKL, Eigen-PC, CADD, and DANN. The variant was predicted to occur in a conserved position of the genome and was absent from gnomAD, UK10K, Greater Middle East (GME) variome project databases, ClinVar as well as the Single Nucleotide Polymorphism Database (dbSNP). Based on the hearing loss specific American College of Medical Genetics and Genomics (ACMG) guidelines for the interpretation of sequence variants, the variant (Oza et al., 2018), was classified as likely pathogenic.

Additional heterozygous variants were identified in the proband in two known syndromic autosomal recessive HI genes. This includes a variant of unknown significance (VUS) in CDH23 (c.5653C>T), previously reported as a VUS in a case with Usher Syndrome(Bahena et al., 2021), and a ClinVar reported pathogenic variant in NDUFAF3 (c.188dupA) (Landrum et al., 2016), a gene associated with Mitochondrial complex I deficiency, nuclear type 18. These two variants are displayed Table S1 and were found in a heterozygous state in the proband and are both absent in the mother. Thus, they do not segregate with HI in the family and the proband is merely a carrier of these variants.

Sanger Sequencing Confirmation of the Variant

Sanger sequencing confirms the monoallelic candidate variants and its co-segregation with the HI phenotype (Figure1a,c). The two affected individuals (III.5, and IV.3) were heterozygous for the variant. The unaffected maternal grandmother (II.4), and an unaffected bother (IV.4) did not have the variant (Figure1a,c).

This variant was not detected in the 103 controls or 52 sporadic NSHI South African probands of Black or Mixed Ancestry (Table S1). The demographic information of the controls is presented in Table S2.

Analysis of the REST p.(C415S) Variant on the Protein

Evolutionary Conservation of Amino Acids

Multiple sequence alignment of REST from human and other species retrieved from the non-redundant database using PSI-BLAST indicates loss of a highly conserved cysteine

residue at position 415 in the protein sequence (Figure2a). This was consistent with the GERP++RS score of 4.96 (scores of 4–6 indicate fewer observed substitutions as compared to what is expected under neutrality) indicating a strong evolutionary and functional constraint on the position across multiple mammalian species. The variation occurs after the DNA binding domain and before the second nuclear localization signal of REST (Figure S1).

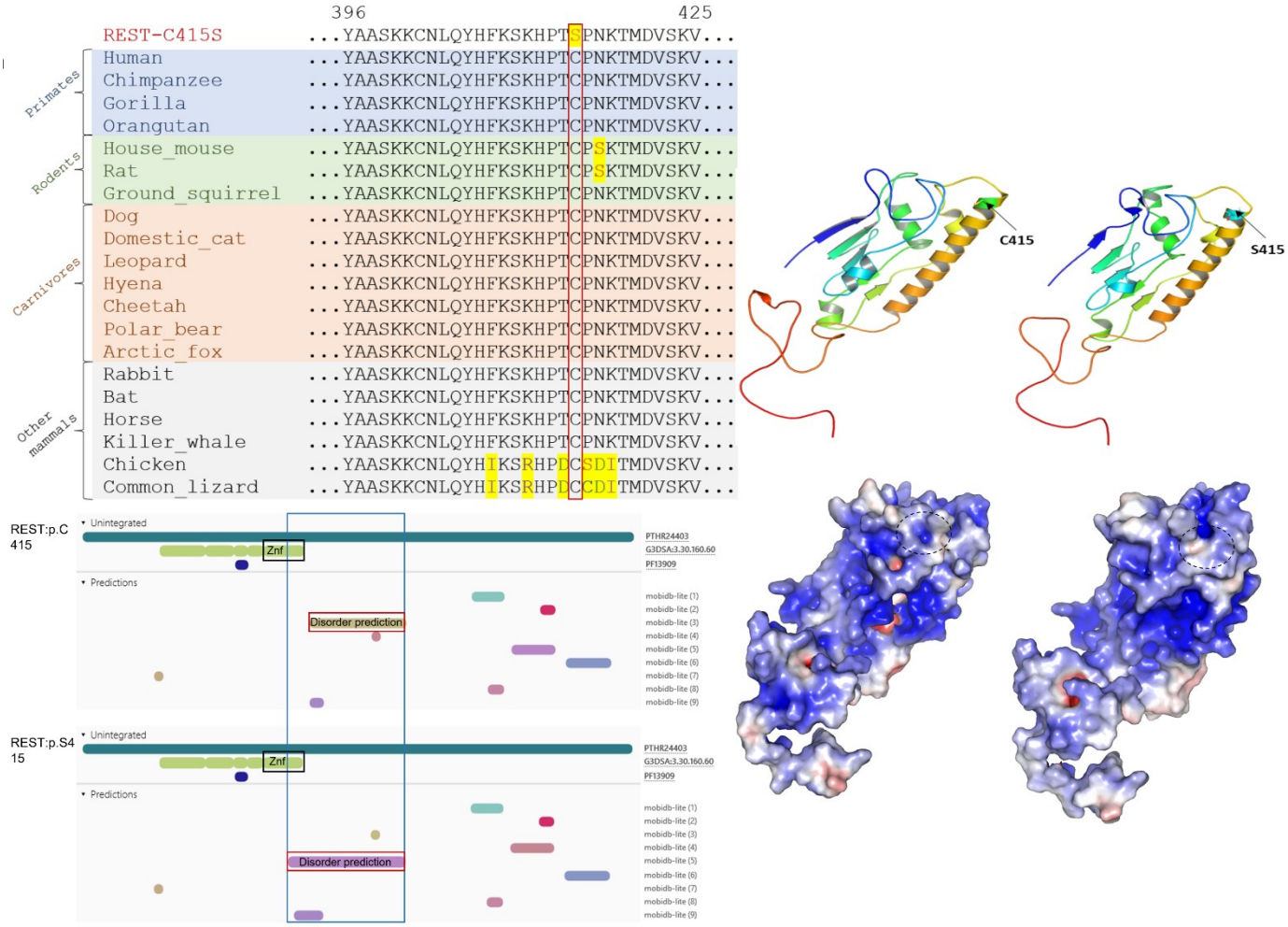


Figure 6.2. (a) The evolutionary conservation of the Cysteine amino acid at position 415 in REST. The position is indicated by the red box. (b) The domain structure of the WT and MT protein are shown. The mutant protein has an extended disordered domain when compared to the WT. (c) Protein modelling of REST comparing the WT (right) to the MT (left) in the ribbon form. The mutation results in changes in the β sheets and α helixes in the protein. This results in either the extension or the retraction of some the structures, as well as the formation and loss of other structures. (d) Protein modelling of REST comparing the WT (right) and the MT (left) in the space filling form indicating the consequences of the

changes illustrated in (2c). The p.C415S variation results in a change in the tertiary structure of the protein such that it is slightly smaller than the WT protein. The MT protein also has several, previously exposed moieties, hidden.

Secondary Structural Changes in REST Due to p.C415S

Secondary structure analysis was performed in PSIPRED 4.0 [37,38]. The variation (Figure S2) caused substantial disruptions in multiple secondary structural features of the protein demonstrated by the black boxes in Figure2B. This included (1) the complete abrogation of β strands at ^{170}EEQ^{172}, ^{264}RKHLR^{268}, ^{434}EAD^{436}, ^{461}KNEKSVK^{467}, and ^{543}KKKK^{546}, (2) the loss of helices at ^{52}VA^{53}, ^{312}SS^{313}, ^{409}KS^{410}, ^{513}FS^{514}, and ^{522}KLEVD^{526}, (3) disruptions in the composition of β strands within ^{60}GSCCDYLVGEERQMAEL^{76} and ^{171}EEQFVHHIRVH^{181} among other regions, and (4) disruptions in helices at ^{105}GLEN^{108}, ^{501}EMDVH^{505}, and ^{493}RKSV^{496} among other regions. These secondary structural changes were predicted to cause disorder within a classic zinc finger (Znf) domain located between residues 360–439 based on a search against the InterPro (Blum et al., 2021) resource (Figure S2). This is expected to affect the overall function of REST.

Conformational Changes in the Tertiary Structure Due to Variant REST

In-Silico modelling of the tertiary structure of the protein indicated that the variation resulted in structural changes in the protein. The variation results in a shift in the formation of disulphide bonds within the protein. It results in the loss of a disulphide bond between the cysteines at positions 415 and 1062 and the addition of a new bond between the cysteines at positions 337 and 363. It results in a change in the positions of the cysteines involved in all disulphide bonds, except for one, with bonds forming between new partners. It results in conformational changes in the protein when compared to the WT, whereby there is a difference in the predicted structure of the WT protein as compared to the MT. The tertiary structure of REST is indicated in Figure2c,d, with the disulphide bond changes in disulphide bond formation indicated in Figure S3.

Furthermore, the mutation results in an extension of a disordered domain in the protein structure. This disordered domain extends in the last zinc finger in the mutant. In the wildtype, the disordered domain is, rather, adjacent to the zinc finger domain. This is indicated in Figure2b.

In Vitro Functional Assay: Mutant REST Loses Exclusive Nuclear Localization and Ability to Repress Target

Using confocal microscopy (Figure3a), the WT and MT REST proteins, tagged with GFP on the c-terminus, could be visualized in HEK-293 cells. As expected, cells transfected with an empty vector (expressing GFP only) showed strong and uniform GFP expression throughout the cells. The WT REST protein is located exclusively within the nucleus, as can be seen via co-localization of GFP-WT-REST with Hoechst, a DNA stain, as is expected of transcription factors. In contrast to this, the GFP-MT-REST displays a localization pattern similar to that of GFP-only, although to a seemingly lower intensity, indicating that the mutant REST protein losses exclusive nuclear shuttling/localization. To compare the transcriptional repressive activity of the mutant REST with that of the WT, the ability of the proteins to repress a known REST target, AF1q, was assessed. WT or MT REST were transiently expressed in HEK-293 cells for 24 h, followed by qualitative PCR to measure levels of AF1q mRNA in these cells. This revealed that WT REST could competently repress AF1q transcription, by an average fold change of 0.51 (±0.07), while this transcriptional repression was lost in cells expressing the mutant REST protein, indicating that the mutation is of functional significance (Figure3b).

Relative luciferase activity was also used to assess the ability of REST to repress AF1q. WT or MT REST were transiently expressed in HEK-293 cells for 36 h followed by luciferase assay. The assay revealed that WT REST could repress AF1Q transcription with a relative luciferase activity of 0.34 (±74.97), whereas transcriptional repression abated in cells expressing mutant REST protein. The difference in luciferase activity was however not significantly different ($p > 0.05$).

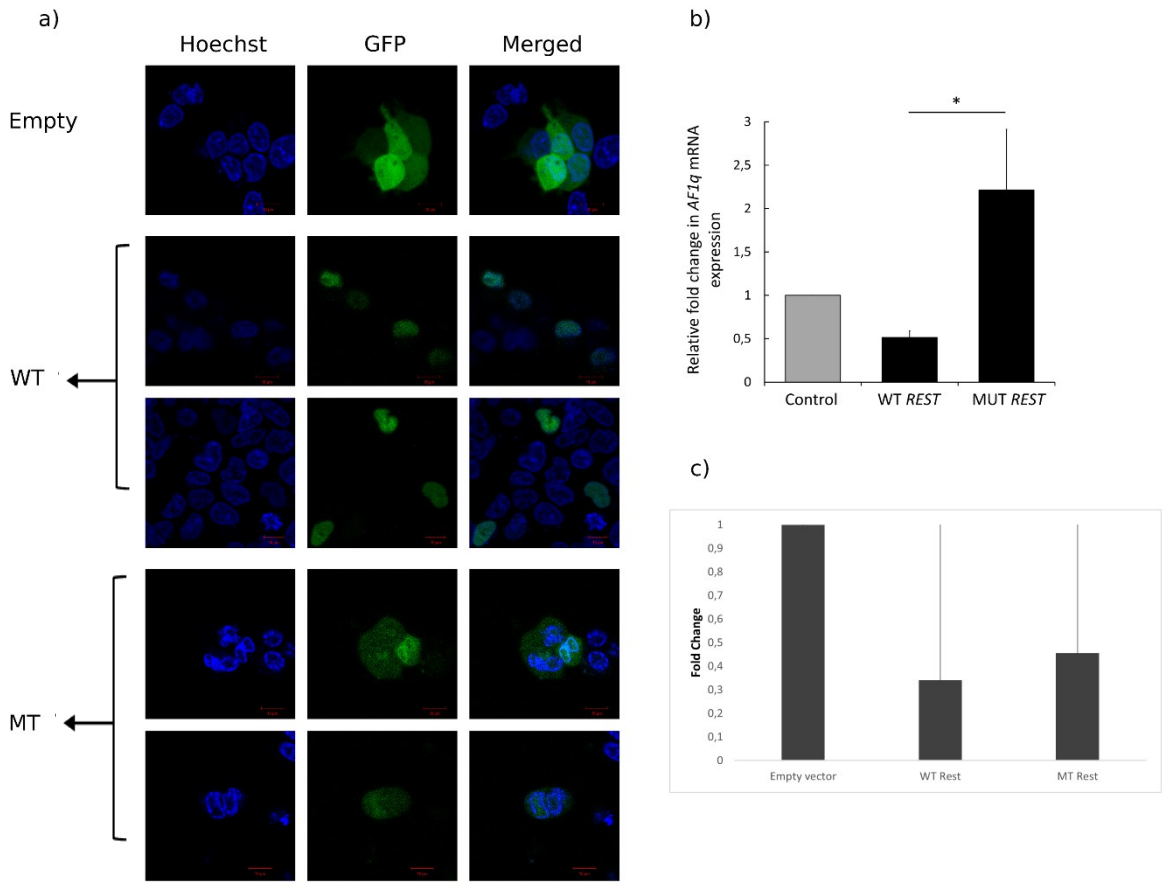


Figure 6.3: WT REST localizes to the nucleus, while MT REST displays localization patterns similar to GFP-only and loses repressive ability of target gene. (a) Micrographs of HEK-293 cells transfected with GFP-only (one representative image), GFP-tagged WT REST and GFP-tagged MT REST (two representative images each). The LHS row indicates Hoechst staining, the middle row indicates GFP, and the RHS row represents merged images. HEK-293 cells were transiently transfected with the respective constructs and visualized live after 24 h (spiked with Hoechst stain) under the confocal microscope (b) HEK-293 cells were transiently transfected with WT or MT REST expression constructs, and 24 h later the expression of AF1q mRNA was measured using qPCR and plotted relative to empty vector control. (c) HEK-293 cells were transiently transfected with WT and MT REST expression constructs and harvested and lysed 36 h later. Luciferase activity was measured with a luminometer and plotted relative to the empty vector control.

Discussion

To our knowledge, this study is the first to investigate the association of HI with *REST* variants in individuals of African ancestry, and the second to demonstrate this gene's association with ADNSHI globally. Thus, the data confirms *REST* as a novel HI gene.

Moreover, the likely pathogenic variant reported is novel, and was not found in 52 unrelated sporadic cases of NSHI cases in a group of Black and Mixed ancestry South Africans. The data reinforce the genetic and locus heterogeneity nature of HI, and the urgent need of investigating diverse populations, particularly the understudied African populations, as the studies will help refining HI disease-gene curation.

REST encodes a transcription factor that represses the transcription of neuronal genes in non-neuronal cells (Chong et al., 1995, Kraner et al., 1992, Schoenherr and Anderson, 1995). The gene is associated with genetic stability and the lack of functional REST results in embryonic lethality during embryonic development (Nechiporuk et al., 2016, Chen et al., 1998). REST is essential for neuronal development and a premature loss of REST results in the progenitor cells prematurely exiting the cell cycle (Nechiporuk et al., 2016). Furthermore, REST is inactivated by alternative splicing, where the REST protein that contains the 4th exon is inactive (Nakano et al., 2018).

In silico analyses show that the putative causative variation in exon 4 leads to changes in the protein structure (both secondary and tertiary), leading to re-arrangement of disulphide bonds, and protein folding. Transcription factors often work with other proteins within a complex to perform specific functions such as nuclear shuttling, recruitment of RNA polymerase and modelling of chromatin, and therefore a change in protein structure may lead to a disruption in any of these functions, which in turn impact target gene regulation (Spitz and Furlong, 2012). *REST* is not only associated with HI, but it is also associated with a predisposition to Wilm's Tumors (Mahamdallie et al., 2015) and has been implicated in colon cancer, small cell lung cancer, and neuroblastomas (Palm et al., 1999, Coulson et al., 2000, Westbrook et al., 2005), and could suggest targeted long term follow up of the affected individuals.

In vitro assays showed the variants found in this family perturbs cellular localization of the protein. The fact that the mutant loses nuclear exclusivity may indicate a disruption in nuclear transportation. Although a nuclear localization signal (NLS) has been defined for REST (Figure S1), experiments by Shimojo (2006) showed that disruption of this domain did not affect nuclear shuttling, but instead disruption of the 5th zinc finger resulted in REST localizing to the cytoplasm (Shimojo, 2006). The mutation may thus be responsible for disruptions to the nuclear localization machinery as modelling has indicated that the secondary structure and tertiary structure the DNA binding domain of REST (amino acids 159 to 412, see Figure S1) in the mutant is aberrated.

This change in REST structure may also be the cause of the loss of repression of the *AF1q* gene, a target of REST, when comparing the MT to the WT. This may be due to REST being unable to access its binding element, the RE1-like sequence in the *AF1q* promoter, which could be a result of the altered ability of the mutant REST to form com- plexes with partner proteins. REST has been defined as a master transcriptional regulator of neuron-specific genes via epigenetic remodeling, with the ability to recruit several partner proteins (Hwang and Zukin, 2018). It is possible that the latter ability is impaired in the mutant REST protein. Future work should focus on elucidating the protein-protein and protein-DNA interactions of REST when considering the variation. The use of an HI in vivo model would be useful in further elucidating the pathogenic role of REST mutants in this disease. Causative variations in *REST* are rare, as only one other family has been reported (Peters et al., 2008), but Nakano et al. (2020) have shown that the HI phenotype in mice may be rescued if REST inactivation is prevented (Nakano et al., 2020).

Although the variant identified in the present study is predicted to be deleterious (Table S4) and affect the structure and function of the protein (Figure2). The functional predication is further supported by an in vitro functional assay (Figure3), more studies in other populations will likely inform and strengthen the HI disease gene-pair curation, globally.

Conclusions

We identified a monoallelic novel likely pathogenic variant in REST (OMIM: 600571). The variant NM_005612.4:c.1244G>C co-segregated with non-syndromic autosomal domi- nant hearing impairment in an affected mother and son from South Africa. This study is the second report, worldwide, to describe the *REST*–HI gene-disease pair in humans, and thus confirms *REST* as a novel ADNSHI that should be included in targeted diagnostic gene panels. Our study emphasizes the urgent need of using WES to investigate hearing impairment in understudied African populations, to reveal the relevant valid gene vari- ants to be investigated in clinical practice, and to enhance our understanding of hearing pathobiology, globally.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/genes12111765/s1, Table S1: Variations identified using whole-exome sequencing in Family1. Table S2: Demographic and phenotypic of individuals of sporadic HI of putative genetic origin. The mean age was 18 years old (±21)

and the median age was 15 years old. Table S3: Demographic information of the control population. The mean age was 55.7 years old (±15.1) and the median age was 58. Table S4: Functional prediction for the candidate variant identified in REST. Figure S1: Schematic Diagram of REST, isoform 1, with some of its transcriptional co-repressors interacting with the repressor domains. The protein consists of 2 repressor domains (RD1 and RD2); one at the amino terminal and one at the carboxyl-terminal of the protein. It has 8 zinc fingers in the DNA binding domain (DBD) and two nuclear localization domains (shown in red; of which one is a zinc finger). REST has a ninth zinc finger close to RD2 and has a lysine-rich domain (from position 400 to 603) and a proline-rich domain (from position 595 to position 815). Adapted from the Atlas of Genetics and Cytogenetics in Oncology and Haematology. Figure S2: The secondary structure of the wild type and MT proteins and the key. The WT protein is represented in (A) and the MT protein is represented in (B), with the WT and MT variation, highlighted by a red square in both (A) and (B). The variation disrupts the secondary structure of amino acids upstream and downstream of it, resulting in the extension or contraction of some of the structure or the addition or loss of other structures. Figure S3: The shift in disulphide bonds is indicated with the WT represented in (A) and the MT represented in (B). the mutation results in far-reaching changes in the disulphide bond formation with only the bond between cysteines 574 and 1065 being preserved between the WT and MT proteins.

Author Contributions: Conception of the project, A.W. and S.M.L.; recruitment, N.M., C.T.-N., K.P., molecular experiments, N.M.; cellular localization and expression, N.M., L.A.d.S.R., S.M.; bioinformatics analysis, I.S., T.B., L.M.N.-S., A.A.; protein modelling, A.N., K.E.; issue of the first draft, N.M.; review and editing, N.M, I.S, L.A.d.S.R., S.M., C.T.-N., K.P., K.E., T.B., L.M.N.-S., A.A, A.N., E.W.-T., C.d.K., C.D., S.M.L., A.W.; supervision of the project: A.W., S.M.L. and S.M. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Human Research Ethics Committee of the University of Cape Town (HREC: 104/2018) and the Institution Review Board (IRB) at Columbia University (IRB- AAAS2343). Clearance to recruit patients from schools was obtained from the relevant provincial education departments and permission was obtained from the schools from which patients were recruited. Written and informed consent was obtained from individuals 18 years and older or from the guardian/parent, with verbal and/or written assent obtained from minor children.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare no conflict of interest.

Supplementary material

Table S1: Variations Identified using whole-exome sequencing in Family 1

Gene	Refseq ID	Variation	Protein consequence	Inheritance model	Homozygous/ Heterozygous	Known/Novel variant	ACMG Classification	Verdict
CDH23	NM_022124.5	c.5653C>T	p.(R1885C)	Autosomal recessive	Heterozygous	Known	PM1_M, PM2_M, PP2_P, PP3_P	LP
NDUFAF3	NM_199069.2	c.188dupA	p.(Y63*)	Autosomal recessive	Heterozygous	Known	PVS1_VS, PM2_M, PP3_P	P
REST	NM_005612.4	c.1244G>C	p.(C415S)	Autosomal Dominant	Heterozygous	Novel	PM1_M, PM2_M, PP1_P	VUS

xxx_VS = Very Strong; xxx_M = Moderate; xxx_P = Supportive; P = Pathogenic; LP = Likely Pathogenic; VUS = Variant of uncertain significance

Table S2: Demographic and phenotypic of individuals of sporadic HI of putative genetic origin

Statistic		Frequency	Percentage
Sex	Male	22	42,3
	Female	30	57,7
Age	Median	15	
	Mean	18	
Ethnicity	Black	43	82,7
	Mixed Ancestry	9	17,3
Mechanism of HI	Sensorineural	45	86,5
	Mixed HI	7	13,5
Localisation of HI	Unilateral	0	0,0
	Bilateral	52	100,0
	Unspecified	0	0,0
Degree of HI Right	No Impairment	0	0,0
	Mild	0	0,0
	Moderate	0	0,0
	Severe	4	7,7
	Profound	45	86,5
	Unspecified	3	5,8
Degree of HI Left	No Impairment	0	0,0
	Mild	1	1,9
	Moderate	0	0,0

	Severe	7	13,5
	Profound	40	76,9
	Unspecified	4	7,7
Onset of HI	Congenital	31	59,6
	Perilingual	11	21,2
	Postlingual	7	13,5
	Unspecified	3	5,8

Table S3: Demographic information of the control population

Statistic		Frequency	Percentage
Gender	Female	78	75.7
	Male	25	24.3
Age	Mean	55.7	
	Median	58	
Ethnicity	Black	103	100.0
	Mixed Ancestry	0	0.0

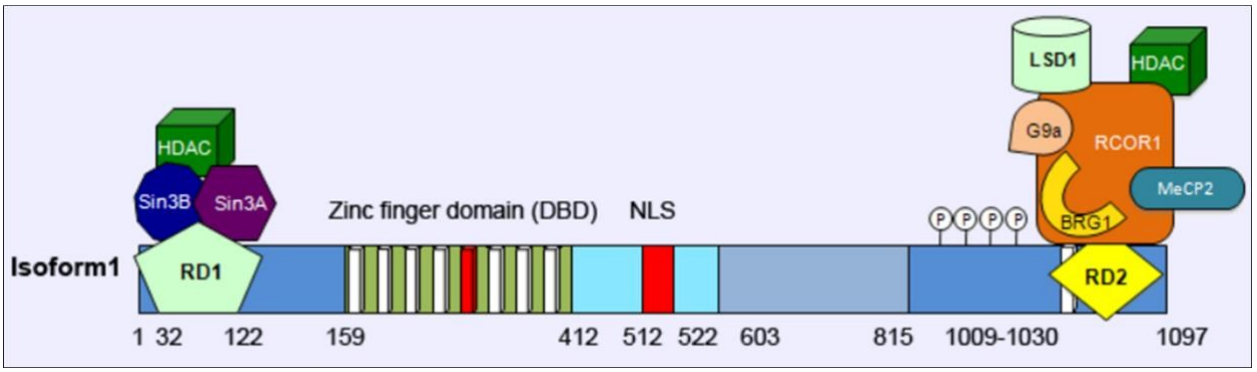
Table S4: Functional prediction for the candidate variant identified in *REST*

Prediction Tool	c.1244G>C
CADD	11.26
DANN	0.892
dbSNP rs number	Absent
Eigen	0.374
Eigen-PC	0.31
FATHMM	Tolerated

FATHMM-MKL	Damaging
Frequency in gnomAD	0
GERP++	4.96
LRT	Deleterious
M-CAP	Tolerated
MetaLR	Tolerated
MetaSVM	Tolerated
MutationAssessor	Low
MutationTaster	Disease Causing
MutPred	0.308
PhastCons	1
PhyloP	9.504
Polyphen2 HDIV prediction	Probably Damaging
Polyphen2 HVAR prediction	Probably Damaging
Predicted effect	Missense
PROVEAN	Damaging
REVEL	0.354
SIFT	Tolerated
SiPhy	14.723

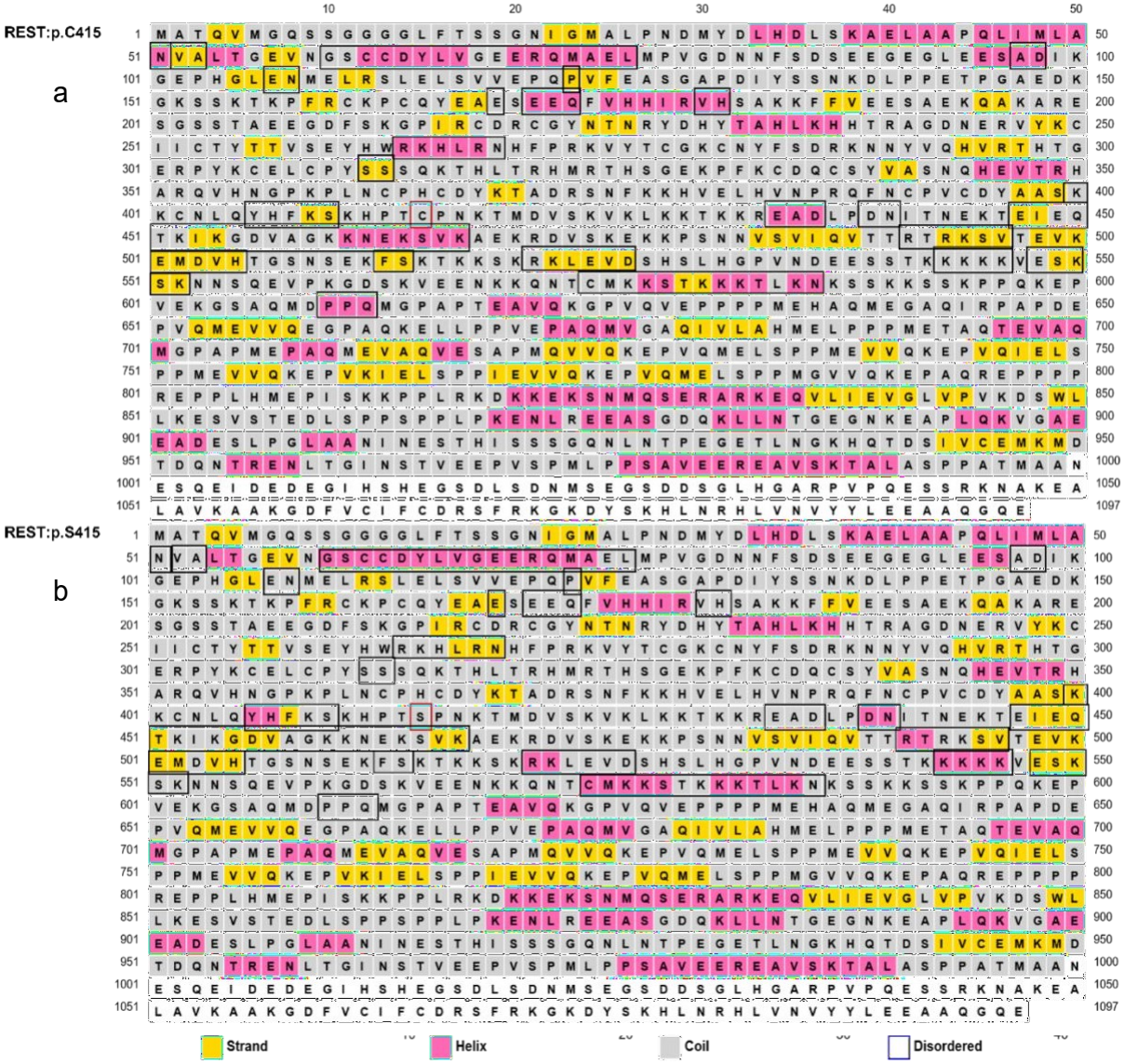
RefSeq used: NM_005612.5

Figure S1: Schematic Diagram of REST, isoform 1, with some of its transcriptional co-repressors interacting with the repressor domains



The protein consists of 2 repressor domains (RD1 and RD2); one at the amino terminal and one at the carboxyl-terminal of the protein. It has 8 zinc fingers in the DNA binding domain (DBD) and two nuclear localization domains (shown in red; of which one is a zinc finger). REST has a ninth zinc finger close to RD2 and has a lysine-rich domain (from position 400 to 603) and a proline-rich domain (from position 595 to position 815). Adapted from the Atlas of Genetics and Cytogenetics in Oncology and Haematology

Figure S2: REST secondary structure prediction by the PSIPRED 4.0 workbench.



Yellow regions represent beta strands, pink regions represent alpha helices, grey regions represent coils, while plain regions (no colours) represent areas of disorder. Black and red boxes indicate positions of difference between the wild type (REST:p.C415) and mutant (REST:p.S415) proteins. The red box shows the mutant site (C415S), while the black boxes show loss of, and attenuation of α helices and β strands in the mutant protein as compared to the wild type protein.

Figure S3: Disulphide bond order in WT REST and C415S REST

a

Predicted bonds	
62 - 63	EVNGSCCDYLV - VNGSCCDYLVG
161 - 278	TKPFRCKPCQY - RKVYTCGKCNY
164 - 306	FRCKPCQYEAE - ERPYKCELCPY
218 - 250	KGPIRCDRCGY - ERVYKCIICTY
221 - 334	IRCDRCGYNTN - EKPFKCDQCSY
253 - 337	YKCIICTYTTV - FKCDQCSYVAS
281 - 391	YTCGKCNYFSD - PRQFNCPVCDY
309 - 945	YKCELCPYSSS - TDSIVCEMKMD
363 - 402	PKPLNCPHCDY - AASKKCNLQYH
366 - 394	LNCPHCDYKTA - FNCPVCDYAAS
415 - 1062	SKHPTCPNKTM - KGDFVCIFCDR
574 - 1065	KKQNTCMKKST - FVCIFCDRSFR

b

Predicted bonds	
62 - 402	EVNGSCCDYLV - AASKKCNLQYH
63 - 278	VNGSCCDYLVG - RKVYTCGKCNY
161 - 250	TKPFRCKPCQY - ERVYKCIICTY
164 - 218	FRCKPCQYEAE - KGPIRCDRCGY
221 - 306	IRCDRCGYNTN - ERPYKCELCPY
253 - 394	YKCIICTYTTV - FNCPVCDYAAS
281 - 334	YTCGKCNYFSD - EKPFKCDQCSY
309 - 391	YKCELCPYSSS - PRQFNCPVCDY
337 - 363	FKCDQCSYVAS - PKPLNCPHCDY
366 - 945	LNCPHCDYKTA - TDSIVCEMKMD
574 - 1065	KKQNTCMKKST - FVCIFCDRSFR

The shift in disulphide bonds is indicated with the WT represented in A) and the MT represented in B). the mutation results in far-reaching changes in the disulphide bond formation with only the bond between cysteines 574 and 1065 being preserved between the WT and MT proteins.

Chapter 7: General Discussion and Conclusion

Discussion

The burden of hearing impairment is increasing worldwide with projected estimates of 700 million people affected by disabling HI by 2050 (WHO, 2021b). Providing a universal language through the HI Ontology allows researchers, clinicians and other health care professionals, and patients, their families, and community members access to the same language when discussing HI. It is necessary for the harmonization of terms and concepts, and to provide scalability to existing projects addressing HI, and is the first hearing impairment ontology.

Though HI may arise from environmental or unknown factors, genetics is a large contributor to congenital HI in developed countries. The prevalent genes associated with HI within, mainly *GJB2*, developed countries are also well known (Hilgert et al., 2009, Snoeckx et al., 2005, Dai et al., 2007, Mutai et al., 2013, Hutchin et al., 2005). This is however not the case for patients from Sub-Saharan Africa, where *GJB2* implication is to date only present in Senegal and as a founder mutation in the Ghanaian population (Dia et al., 2022, Adadey et al., 2019). Additionally, many mutations identified, outside of the noted *GJB2* mutations, are private mutations specific to a family (Lebeko et al., 2016, Wonkam et al., 2021, Wonkam-Tingang et al., 2020, Manyisa et al., 2021). Through studying HI in patients of African descent, the dearth of knowledge regarding genetics in patients of African descent is being addressed.

The recruitment of patients within eight provinces in South Africa, provided a large and diverse cohort to better understanding the genetics of HI in the South African population. Previous studies have sought to determine the prevalence of *GJB2* pathogenic variants, the aetiology of HI and/or associated syndromes indicated in Manyisa et al. (2022). Work from Kabahuma et al. (2021) and Roberts et al. (2015) have contributed to ascertaining the presence of *MYO7A* pathogenic variants in the South African context and the whole exome sequencing undertaken in this study corroborates their work.

Additional work and interest surrounds *MYO15A* pathogenic variants which are, interestingly, showing up in several Sub-Saharan populations (Wonkam et al., 2022,

Wonkam et al., 2020). Additional studies of genetic HI in Sub-Saharan Africa will prove vital for determining the extent of *MYO15A* involvement in Sub-Saharan African HI.

This study saw resolution of the causative genes resulting in HI in 20 of the 27 families (74.1%) that underwent WES. Only two families present with variations in the same gene, *STRC,* which is something to note when analysing the exomes of the remaining families. The genetic heterogeneity within the cohort is expected, due to the diversity of African populations; and may explain the difficulty in determining the prevalent genes associated with genetic HI in the South African population.

A significant finding of this study was the discovery of the novel dominant mutation in *REST*. *REST* had only been implicated as a gene associated with HI in one other case report (Peters et al., 2008). The novelty of the finding is similar to the identification of the *GRXCR2* mutation identified by Wonkam et al. (2021) and the mutation in *CLIC5* identified by Wonkam-Tingang et al. (2020). It indicates that, through WES, researchers are steadily deciphering the genes associated with HI in African populations and that this should be an area of greater focus.

Recommendations

- Further analysis is necessary to identify putative prevalent pathogenic variants in the South African population. This will require the sequencing all existing DNA samples.
- Further analysis of the existing WES data may allow for the identification of possible modifiers that are associated with HI in the South African population.
- Phenotyping errors were observed in patients presenting with Usher Syndrome. This may be addressed by meeting with patients through their second and thirds decades of life.
- The causative pathogenic variants associated with HI will need to be returned to patients and their families.

Conclusion

This thesis successfully performed the following investigations: 1) development of the first Hearing Impairment Ontology worldwide, 2) review the genetic profile of HI in South Africa, 3) used WES to find known In 20 families that were resolved (74%), pathogenic variants identified in established HI genes: *WFS1* (c.A2141), *MITF* (cT918A), *ADGRV1*(c.G564T,

c.A17450G, c.A11298C), *PDSS1*(c.C641T, c.G754C), *TBC1D24*(c.G1514A), *TMPRSS3*(c.205+6t>A), *NEU1*(c.C1069T, c.G727A), *MYO15A*(c.C1378T, c.9303+5G>A, c.G6634A), *USH2A*(c.T9437A, c.G2990T, c.G101A), *STRC*(c.G225A, c.C4057T, c.G4655C, c.C4351T, c.G4403A), *P2RX2*(c.G1064A, c.C1187G), *OTOG*(c.C2525A, c.G3143A, c.G916A), *LHFPL5*(c.621delC), *SLC26A4*(c.T94C, c.T716A), *GJB2*(c.35delG), *TRIOBP*(c.C3133T, c.C4298T), *REST*(c.G1244C), *CRYM*(c.*6_*2delACAAA), *CDH23*(c.T1585C, c.G8230A), *FGFR2*(c.1297+10G>C), *MYO7A*(c.6255delC); and 4) confirmed *REST* as a novel HI gene. Future work will focus on sequencing all the remaining samples and identifying their putative causative mutations. This may include revisiting the putative phenotype of the patients, where the patients presented with a mutation in a syndromic gene, and/or the possibility that the HI is either environmental or due to variations not captured by WES. Further work includes feedbacking the results of the genetic testing to the patients and their families. The data will contribute to improving the HI-genes pairs' curation in Africa, and globally.

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Appendix

A1: Recruitment instrument

UNIVERSITY OF CAPE TOWN
DIVISION OF HUMAN GENETICS

INFORMATION SHEET FOR PARTICIPANTS OF RESEARCH STUDY

Study Title: HEARING IMPAIRMENT GENETICS STUDIES IN AFRICA "HI-GENES AFRICA"
General Information about Research

Good day,

You have been approached to participate in this study because you or your family member has been diagnosed with hearing impairment.

People with hearing impairment have difficulties in hearing or inability to hear in one ear or both ears. Hearing impairment can be caused by a change in one of our genes (a piece of DNA), which are the instructions or building plans of our body. Hearing impairment can also be caused by some conditions such as infections of a child before or after birth and head or ear injuries. People with hearing impairment caused by a change in their genes represents half (~50%) of all people born with hearing impairment. When the cause happens to be a change in the genes, many people including boys and girls are usually affected with hearing impairment in the same family. However, the person diagnosed with hearing impairment might be the first and only person that is affected in his/her family. In most of the cases, both parents do not have hearing problem themselves, but each of them carries a gene with a change that may be passed on to their children. Each child who inherits these two genes with changes from both parents (one from each parent) will have hearing impairment at birth or few months or years after birth. In some cases, however, one or both parents carrying changes in their genes have hearing impairment themselves and can pass it on to children.

Why are we doing this study?
The reason for doing this study is to find the genes with changes that cause hearing impairment in people from Africa, and to better understand how these genes affect hearing in African populations. Knowing these genes can help doctors to improve their ability to detect hearing impairment or other related factors early, to offer appropriate treatment to affected people, and to give useful advice to affected people and their families.
This study is part of the Human Heredity and Health Consortium (H3Africa). The study will be performed by investigators at University of Cape Town (South Africa); we are also working with investigator at the University of Yaoundé 1 (Cameroon), University of Bamako (Mali) and University of Ghana (Ghana).

What would you ask me to do if I agree to participate in this study?
If you agree to be a part of this study, the person with the hearing impairment will be assessed by one of the doctors performing this research, and hearing may also be tested if needed. In addition, blood (about 2 to 4 tea spoons) and saliva will be collected from the child with hearing problems. Blood and saliva will also be collected from both parents and brothers and sisters, or other family members with or without hearing impairment.

What will happen to my samples and information?
Your and your family member's blood and saliva will be analysed to look for changes in the DNA (genes) that would explain why you or your child has hearing impairment. Part of the DNA will be stored for future possible testing. DNA will be shared with researchers in other countries involved in our study. The researchers may also decide to share genetic data gathered from your samples to help further research into hearing loss. We do this by putting information into scientific databases. If you agree to take part in the study, some of your genetic and hearing information might be placed into a scientific database called dbGaP. Any researcher who would want to use dbGaP would need to apply to the database. If they are granted access then they would be able to see your information, but they will not know that it belongs to you because all personal identifiers will be removed if your data is shared and/or if the data is used to produce scientific publications

Confidentiality
All information given is confidential. All the information gathered during this study will be safely stored in locked offices or in password protected computers. Your DNA will be coded and the persons working on the sample will not know who it belongs to. The researcher will not discuss your participation in the research with any other patients or persons not involved with

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your medical care. You may be asked to give permission/consent to publish photos if these are taken during the clinical examination, but this will be your decision.

What are the risks of being involved in the study?
The risk of harm or discomfort expected during this study is not greater than those you encounter in daily life or during routine medical examinations. The prick of the needle during blood taking will be the same as having bloods taken for any other reason. There may be some bruising, but this is not usually a serious problem. Also, the amount of blood and saliva collected is not harmful to your health.

Are there any benefits to me?
Medical examinations, hearing tests and laboratory testing will be offered free of charge to everyone participating in this study. This study may identify the gene(s) with changes that cause hearing impairment in you or your family. Knowledge of these genes can help doctors to improve prevention, early detection and treatment of hearing impairment in you, your family and other affected people. Should something be identified, all effort will be made to contact you and explain the result to you in person. This will provide you with information which can be helpful for you/your child and your family. Please do inform us if your contact details change during the study period so that you may be contacted if needed.

Do I have to participate?
No you do not; involvement in this research study is entirely voluntary. If at any time during the study you wish to withdraw your consent you may do so without any harm to your medical care and without any penalty and compensation to the researchers involved. Please feel free to ask your family members or close friends' opinion's if you need advice before agreeing to participate to the study.

Could my participation be stopped by the researchers?
Yes, your participation in this study may be terminated if your condition is later found to be caused by other conditions such as infections, and if your blood sample collected is later found not to be suitable for testing in the lab.

What happen if another known condition is detected by chance on me during testing of my DNA?
Your DNA won't be tested for any known condition, except for hearing impairment. Therefore, the chance for another condition to be detected is very low. Only results of testing for hearing impairment will be returned to you or your family.

Can I expect any compensation by participating to this study?
No, you will not receive any compensation, being it monetary or any type given to you for participating in this study. However, your transport costs to get to the recruitment venue and to travel home again will be covered, and a light meal provided by the researchers to all participants the day of your medical assessment.

Please feel free to contact the following person if you have any problems or questions about the research

Prof. Ambroise Wonkam (Project Supervisor)
Division of Human Genetics, University of Cape Town
Anzio Road, Observatory 7925, Cape Town, South Africa
Tel: +27(0)21 406 6307
E-mail : Ambroise.Wonkam@uct.ac.za

This research has been reviewed and approved by the Human Research Ethics Committee (HREC) of the University of Cape Town. If you have any questions regarding your rights as participants in this research project please contact Prof. M. Blockman, Chair of the Research Ethics Committee of the University of Cape Town on +27(0)21 406 6496.

UNIVERSITY OF CAPE TOWN
DIVISION OF HUMAN GENETICS

INFORMED CONSENT FOR RESEARCH PROJECT:

Hearing Impairment Genetics Studies in Africa **(HI-GENES Africa)**

I,……., hereby voluntarily consent to my child's …….. / My (parent/guardian/adult participant) genetic material being investigated for disease causing genes involved in hearing impairment.

I am aware that all participants to this study will have:

- ☐ Require medical and family history where possible
- ☐ A medical assessment done by one of the medical doctors involved in the study
- ☐ Hearing test performed if needed
- ☐ A blood and saliva samples taken to extract DNA for genetic studies

The benefits, risks and procedures for this research have been read and explained to me and I have been given an opportunity to have any questions about the research answered to my satisfaction.

By consenting to this research study:

- ☐ I agree to participate as a volunteer
- ☐ I agree that my DNA and data can be stored, shared, and for the present hearing loss studies and, scientific publications after removal of my personal identifiers.
- ☐ I agree that my DNA and data can be stored, shared, and used for additional studies and scientific publications after removal of my personal identifiers.
- ☐ I agree that my data can be added to the dbGAP database.
- ☐ I agree that my photographs taken can be used in scientific publications after removal of my personal identifiers

The return of results has been discussed with me and I have been given an opportunity to have any questions about the putative results answered to my satisfaction.

By consenting to this research study:

- ☐ I agree to have the results of the genetic tests regarding the hearing loss **and** other putative pathogenic variations returned to me
- ☐ I agree to have the results of the genetic tests regarding the hearing loss **only** returned to me
- ☐ I do not wish to have any results of the genetic tests returned to me

Participation in this study is voluntary and may be withdrawn at any stage, without any harm to future care.

_____ _____
Date Name and signature

_____ _____
Father's signature Mother's signature

_____
Guardian's signature (if applicable)

UNIVERSITY OF CAPE TOWN
DIVISION OF HUMAN GENETICS

ASSENT FOR RESEARCH PROJECT:

Hearing Impairment Genetics Studies in Africa (HI-GENES Africa)

This to confirm the following information has been given and explained to me to my satisfaction:

- ☐ This study is being done by the doctors at the University of Cape Town to try and learn more about genetic causes of a condition called hearing impairment.
- ☐ You and some members of your family have been asked to participate in the study because hearing impairment may run in your family.
- ☐ The doctors are trying to see if they can learn more about how hearing impairment happens in some families. This is so they can better help you and other people's families in the future.
- ☐ If you decide to be a part of the study one of the doctors doing this study will examine you, will test your ears and collect some blood and saliva from you.
- ☐ If you give doctor's permission, pictures of you may be taken and used in scientific publications after removal of your personal identifiers.
- ☐ Nothing that will be done will be harmful to you.
- ☐ Everything that is done during this study will be confidential, meaning that no one who is not a part of the study will know that you participated.
- ☐ You can decide for yourself whether you want to be a part of this study. If you decide to not be a part of it then no one will be angry with you, and you will still get all the help and care that you need.

I am here assenting to participate to the study:

_____	_____
Date	Name and signature

HREC Insurance Clause: UCT carries a No-Fault Insurance Policy to cover non-commercially sponsored interventional clinical research. No-Fault compensation implies that participants incurring a research-related injury are not required to prove wrong-doing to be compensated.

HI-GENE Africa

QUESTIONNAIRE

Date: ……../……../……. Study participant's Code number……

1 SOCIO-DEMOGRAPHICS

1.1 Name: ……

1.2 Date of birth: ……/…../……

1.3 Sex: *(Please encircle code)* **1.** Male **2.** Female

1.4 Ethnic origin (Black, Caucasian, Coloured, Indian, Asian, other/mixed):

……

1.4.1 Cultural origin if known (Xhosa, Zulu, Tswana, Sotho):

……...

1.5 Level of education *(Please encircle code)*

1. Primary	**2.** Secondary	**3.** Tertiary	**4.** Special (not main stream school)	**5.** None/pre-school	**6**. Other (no formal education)

1.6 Phone number (Preferably three telephone numbers):

……

2 EXCLUSION OF AN ACQUIRED/ENVIRONMENTAL ETIOLOGY

2.1 Congenital infection: ☐Yes (1) ☐No (2) ☐ Unknown (3) *(if YES please tick relevant subsections)*

☐Toxoplasmosis ☐Rubella ☐CMV ☐Syphilis

2.2 Usage of ototoxic drugs during pregnancy (streptomycin, gentamicin):

☐Yes (1) ☐No (2) ☐ Unknown (3)

Please check if applicable

2.3 Radiation therapy during the 1st trimester of pregnancy ☐Yes (1) ☐No (2) ☐Unknown

2.4 Prematurity ☐Yes (1) ☐No (2) ☐ Unknown (3)

2.5 Neonatal asphyxia ☐Yes (1) ☐No (2) ☐ Unknown (3)

2.6 Meningitis ☐Yes (1) ☐No (2) ☐ Unknown (3)

2.7 Measles ☐Yes (1) ☐No (2) ☐ Unknown (3)

2.8	Ototoxity	☐Yes (1)	☐No (2)	☐ Unknown (3)
2.9	Noise pollution	☐Yes (1)	☐No (2)	☐ Unknown (3)
2.10	Small for age	☐Yes (1)	☐No (2)	☐ Unknown (3)
2.11	Nuclear jaundice	☐Yes (1)	☐No (2)	☐ Unknown (3)
2.12	Mumps	☐Yes (1)	☐No (2)	☐ Unknown (3)
2.13	Recurrent/chronic otitis	☐Yes (1)	☐No (2)	☐ Unknown (3)
2.14	Trauma (eardrum, head)	☐Yes (1)	☐No (2)	☐ Unknown (3)

Comments:

Researcher: The Condition is likely to be

1. Environmental **2.** Genetic **3.** To be confirmed by Doctor *(Please encircle code)*

Note: IF ENVIRONMENTAL, EXCLUDE THE PATIENT FROM THE STUDY
Comments:

3. PHENOTYPE OF THE HEARING IMPAIRMENT

3.1 Onset of hearing problem *(Please encircle)*

1. From birth **2**. Suddenly (over three days)

3. Quickly (weeks and months) **4**. Gradually (years)

3.2 Age - Hearing loss was noticed:

……

3.3 Age - Medical diagnosis:

……

3.4 Technique used for diagnosis: *(Please encircle code)*

1. Audiometry (hearing test/over ears)

2. ABR (auditory brainstem response, put to sleep/brain images)

3.5 Degree of hearing loss: (Only complete if audiogram is available)

Grade of Impairment	Corresponding audiometric ISO value	Performance	Right ear	Left ear
0- No impairment	25 dB or lower (better ear)	No or very slight hearing problems. Able to hear whispers.		
1- Slight impairment	26 –40 dB (better ear)	Able to hear and repeat words spoken in normal voice at 1m		
2- Moderate impairment	41-60 dB (better ear)	Able to hear and repeat words spoken in raised voice at 1m.		
3- Severe impairment	61-80 dB (better ear)	Able to hear some words when shouted into better ear.		
4- Profound impairment, including deafness	81 dB or greater (better ear)	Unable to hear and understand even a shouted voice.		

3.6 Localisation of the hearing loss *(Please refer to the table above and encircle code/s)*

1. One side

If on one side: **1.** Right **2.** Left

OR

2. Both sides

If on both sides: **1.** Symmetrical *(the same)* **2.** Asymmetrical *(not the same)*

3.7 Mechanism of hearing problem *(Please encircle code)*

1. Sensorineural **2.** Conductive **3.** Mixed

3.8 Management of the hearing loss

3.8.1 Hearing aid ☐Yes (1) ☐No (2) ☐ Unknown (3)

3.8.2 Cochlear implant ☐Yes (1) ☐No (2) ☐ Unknown (3)

3.8.3 Surgery ☐Yes (1) ☐No (2) ☐ Unknown (3)

3.8.4 Speech therapy aid ☐Yes (1) ☐No (2) ☐ Unknown (3)

3.8.5 Alternative communication (sign language, lip reading) ☐Yes ☐No ☐ Unknown

Comments:

4. CLINICAL HISTORY

4.1.1 Intellectual Disability/Learning difficulties ☐Yes (1) ☐No (2) ☐ Unknown (3)

4.1.2 Seizures ☐Yes (1) ☐No (2) ☐ Unknown (3)

4.1.3 Goitre ☐Yes (1) ☐No (2) ☐ Unknown (3)

4.1.4 Diabetes mellitus ☐Yes (1) ☐No (2) ☐ Unknown (3)

4.1.5 Diabetes insipidus ☐Yes (1) ☐No (2) ☐ Unknown (3)

4.1.6 Syncope ☐Yes (1) ☐No (2) ☐ Unknown (3)

4.1.7 Hirschprung's disease ☐Yes (1) ☐No (2) ☐ Unknown (3)

4.1.8 Kidney disease ☐Yes (1) ☐No (2) ☐ Unknown (3)

Specify …….

4.1.9 Haematuria ☐Yes (1) ☐No (2) ☐ Unknown (3)

4.1.10 Visual impairment ☐Yes (1) ☐No (2) ☐ Unknown (3)

Specify……

4.1.11 Other ☐Yes (1) ☐No (2) ☐ Unknown (3)

Specify…….

5. FAMILY HISTORY/ PEDIGREE (AT LEAST 3 GENERATIONS)

5.1 Consanguinity ☐Yes (1) ☐No (2) ☐ Unknown (3)

Comments:

5.2 Likely mode of Inheritance *(Please encircle code)*

1. AR **2**. AD **3**. X-linked **4**. Mitochondrial **5.** Unknown

6 ASSOCIATED ANOMALIES

6.1 Anthropometrics

6.1.1 Weight: ……kg 6.1.2 Height: ……..cm

6.1.3 BP: ……./…….mmHg 6.1.4 COH: …….cm/……..Centile

DOCTOR: The Condition is likely to be

1. Environmental **2.** Genetic *(Please encircle code)*

Note: IF ENVIRONMENTAL, EXCLUDE THE PATIENT FROM THE STUDY
Comments:

_____

_____

_____

6.1.2 Craniofacial dysmorphism:

☐ Yes (1) *(if YES please tick relevant subsections)*
☐ No (2) *(subsections not applicable)*
☐ Unknown (3)

6.1.2.1 Auricle malformation ☐

6.1.2.2 Atresia of the EAM ☐

6.1.2.3 Preauricular sinus ☐

6.1.2.4 Microtia ☐

6.1.2.5 Branchial fistula ☐

6.1.2.6 Cleft palate ☐

6.1.2.7 Branchial cyst ☐

6.1.2.8 Microphtalmia ☐

6.1.2.9 Microcephaly ☐

6.1.2.10 Macrocephaly ☐

6.1.2.11 Goitre ☐

6.1.2.12 Dystopia Canthorum ☐

6.1.2.13 Heterochromia ☐

6.1.2.14 White hair/forelock ☐

6.1.3 Skin defect ☐ Yes (1) ☐ No (2) ☐ Unknown (3) *(if YES please tick relevant subsections)*

6.1.3.1 Ichthyosis ☐

6.1.3.2 Neurofibromas ☐

6.1.3.3 Atrichosis/Hypotrichosis ☐

6.1.3.4 Skin depigmentation ☐

6.1.3.5 Keratoderma ☐

6.1.4 Limbs defect ☐Yes (1) ☐No (2) ☐ Unknown (3)

Specify: ……..

6.1.5 Other abnormality (specify)……

6.1.6 Otoscopy …….

6.1.7 Photographs taken **1.** Yes **2.** No *(Please encircle code)*

7 INVESTIGATIONS (if available)

Investigation	Results
Kidney Ultrasound	
Electrocardiogram	
Eye test	
Fasting blood sugar	
Urine Dipstick	

8 TYPE OF HEARING IMPAIRMENT

8.1 Syndromic ☐Yes (1) ☐No (2) ☐ Unknown (3)

Specify condition ……

8.2 Non-syndromic ☐Yes (1) ☐No (2) ☐ Unknown (3)

8.3 Other condition ……..

9 GENETIC INVESTIGATIONS

9.2 Previous DNA Extraction in the index case ☐Yes (1) ☐No (2) ☐Unknown (3)

9.2.1 If yes, please indicate:

GJB2 mutation excluded ☐

GJB6 mutation excluded ☐

Whole exome sequencing ☐

Whole Genome sequencing ☐

9.3 Previous DNA extraction in family member(s) ☐Yes(1) ☐No (2) ☐Unknown (3)

9.3.1 If yes, please indicate:

GJB2 mutation excluded ☐

GJB6 mutation excluded ☐

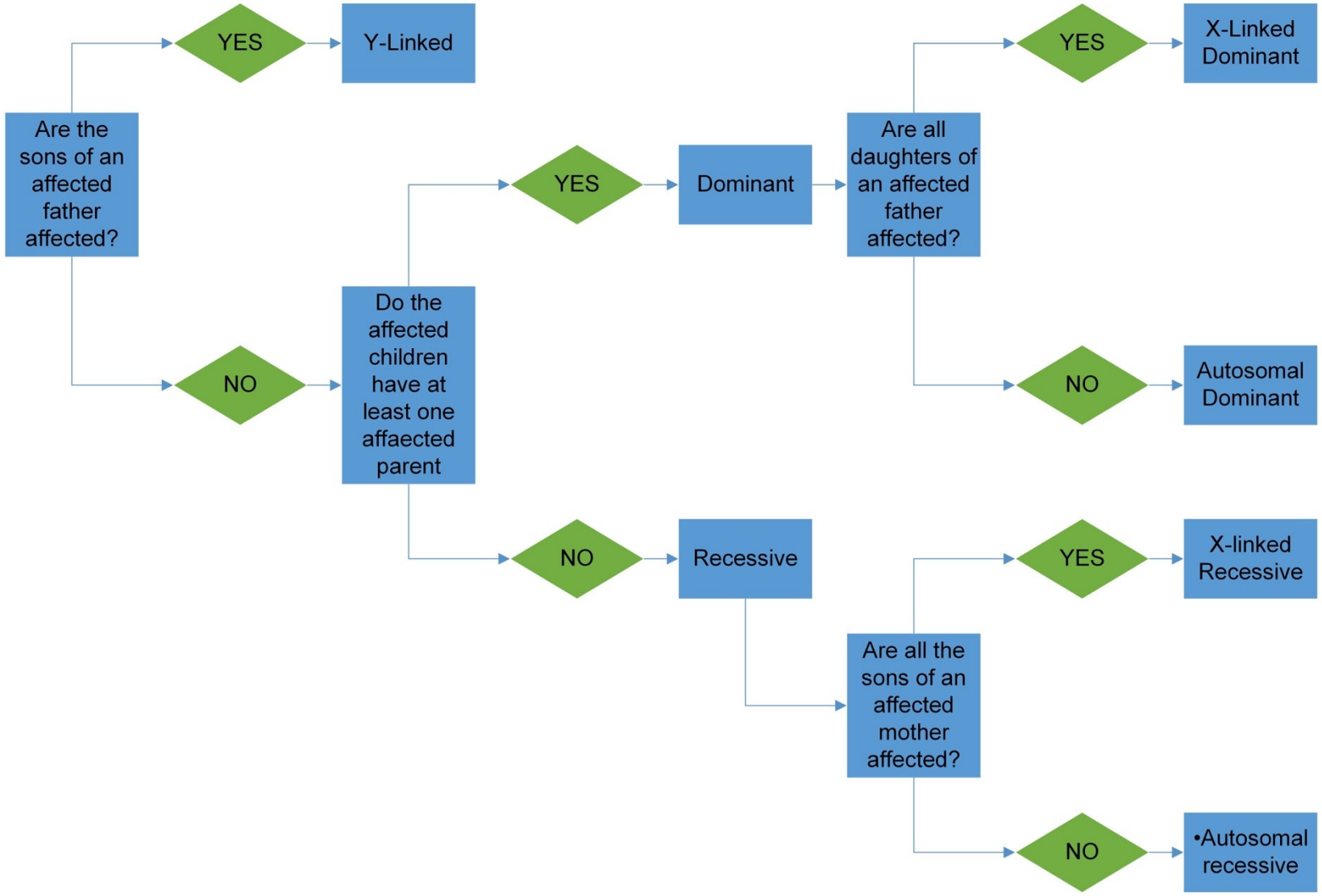
Whole exome sequencing ☐

Whole Genome sequencing ☐

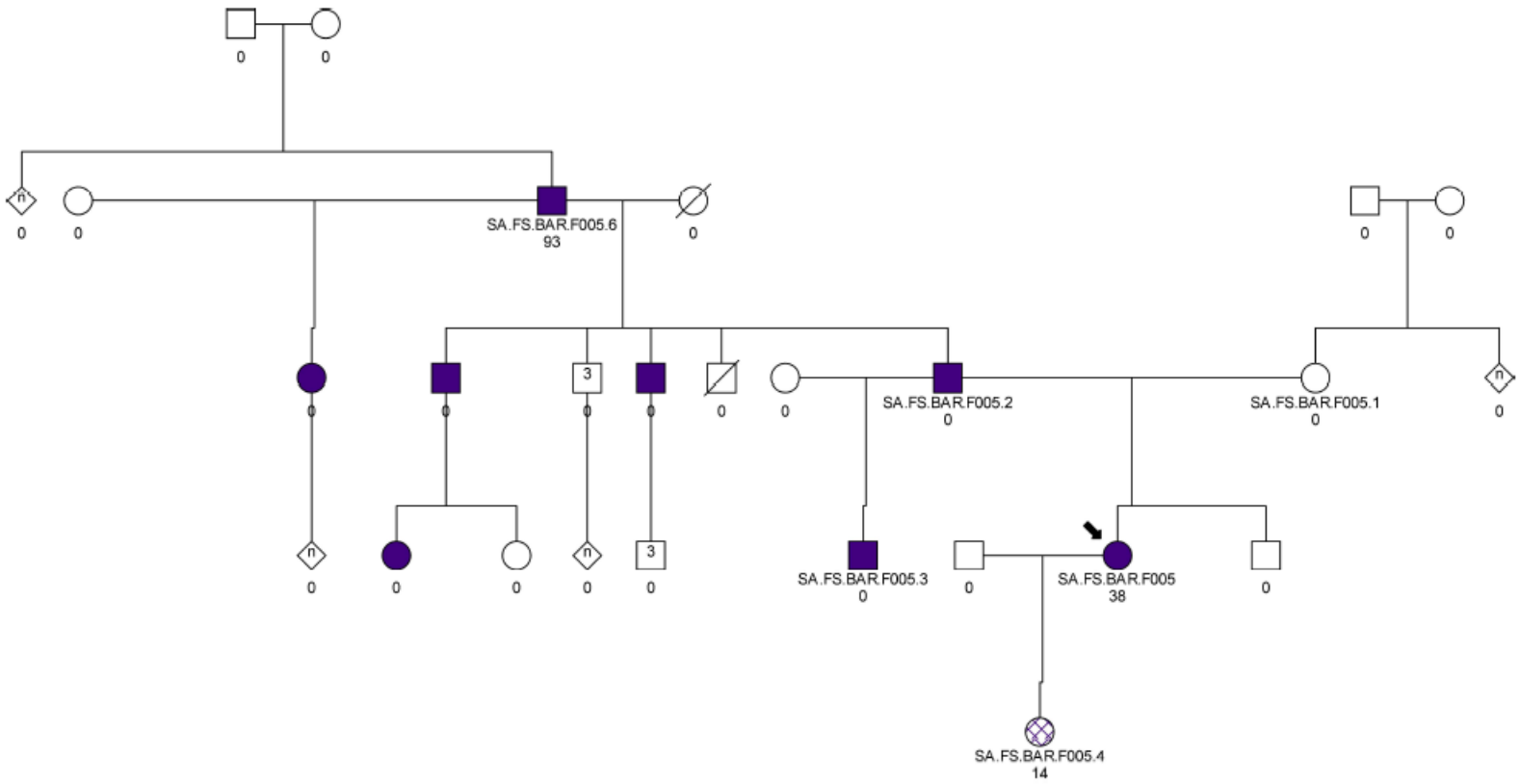
9.3. Any positive genetic testing on the index case ☐Yes (1) ☐No (2) ☐Unknown (3)

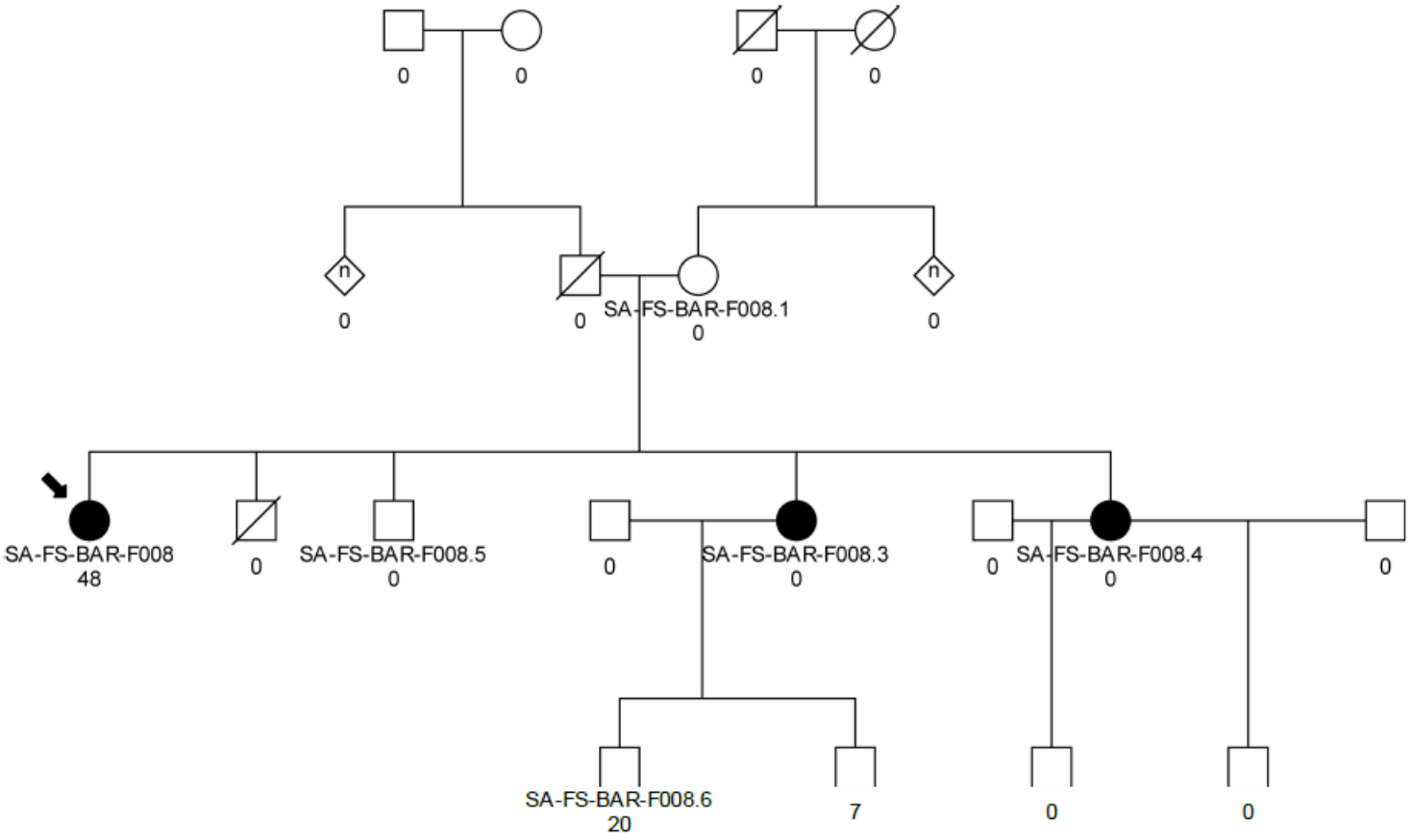
9.3.1 If yes, please indicate: ……..

A2: Decision tree for inferring inheritance pattern



A3: Pedigrees

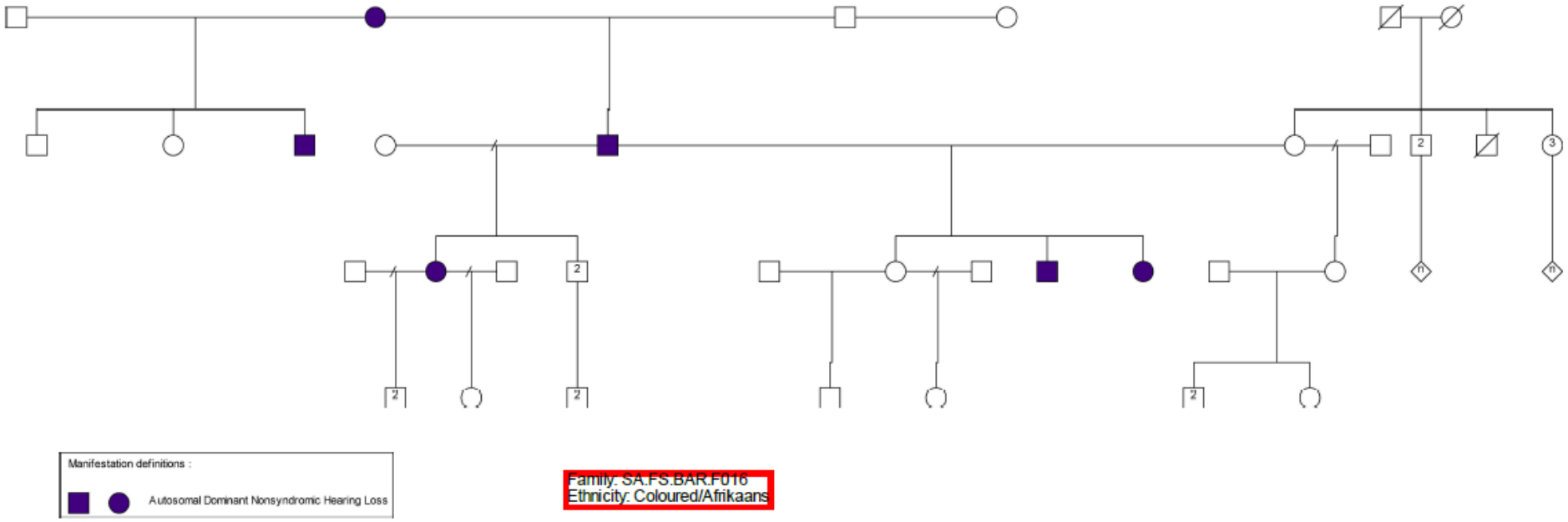


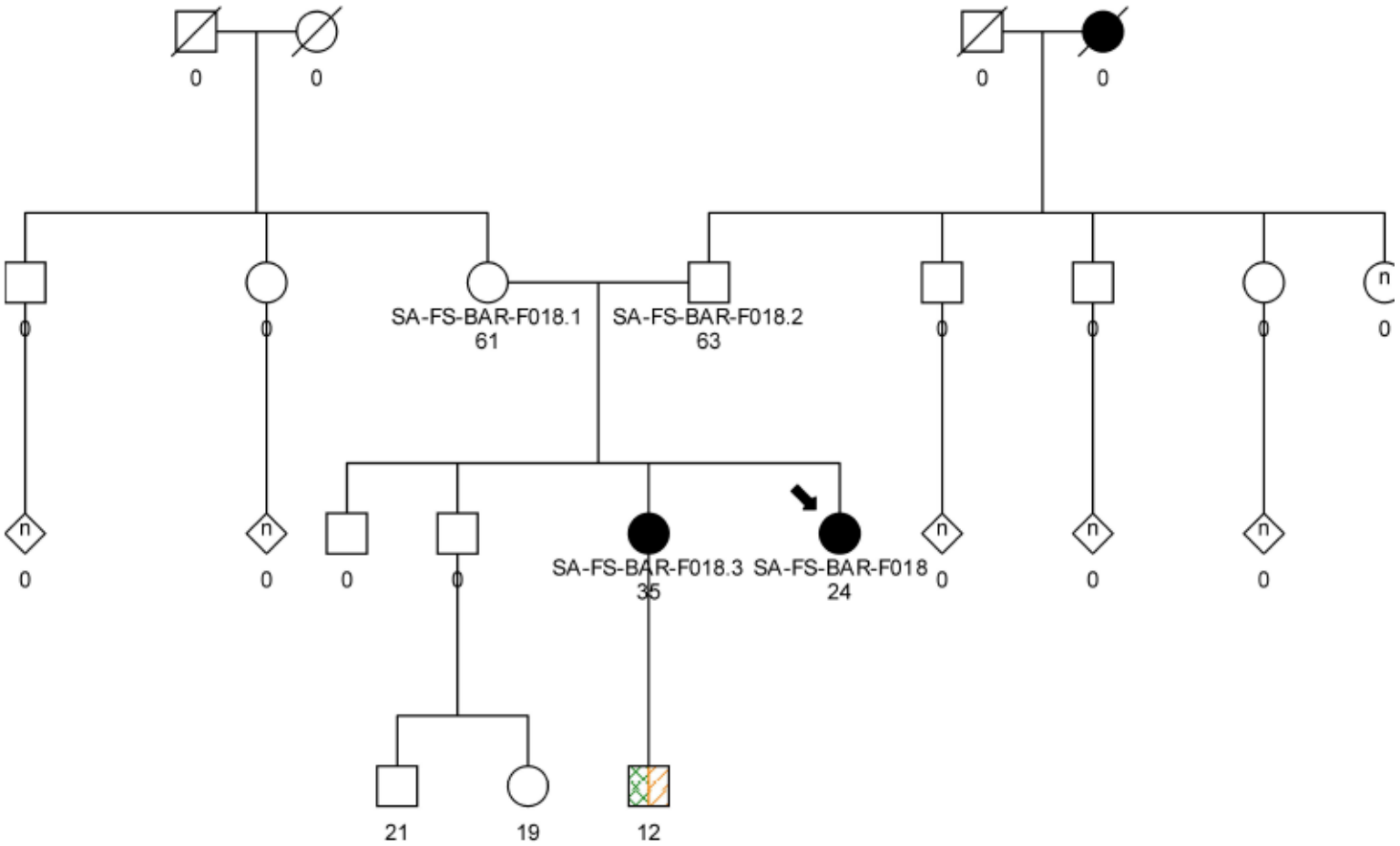


Manifestation definitions :

Autosomal Recessive Nonsyndromic Hearing Loss

Family: SA-FS-BAR-F008
Sotho


Manifestation definitions :
Autosomal Dominant Nonsyndromic Hearing Loss
Family: SA.FS.BAR.F016
Ethnicity: Coloured/Afrikaans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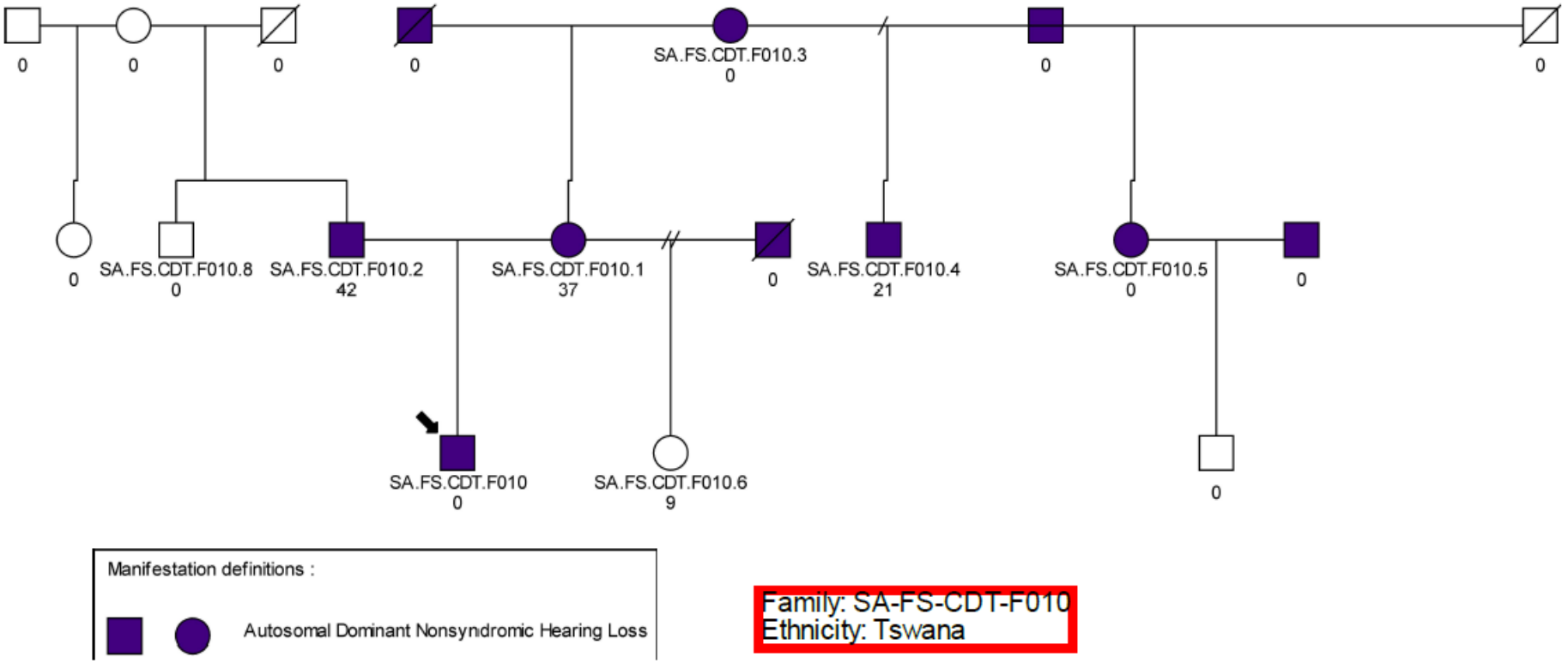
Manifestation definitions :
Autosomal Recessive Nonsyndromic Hearing Loss
Autism
Epilepsy
Family: SA-FS-BAR-F018
Ethnicity: Not stated/Unknown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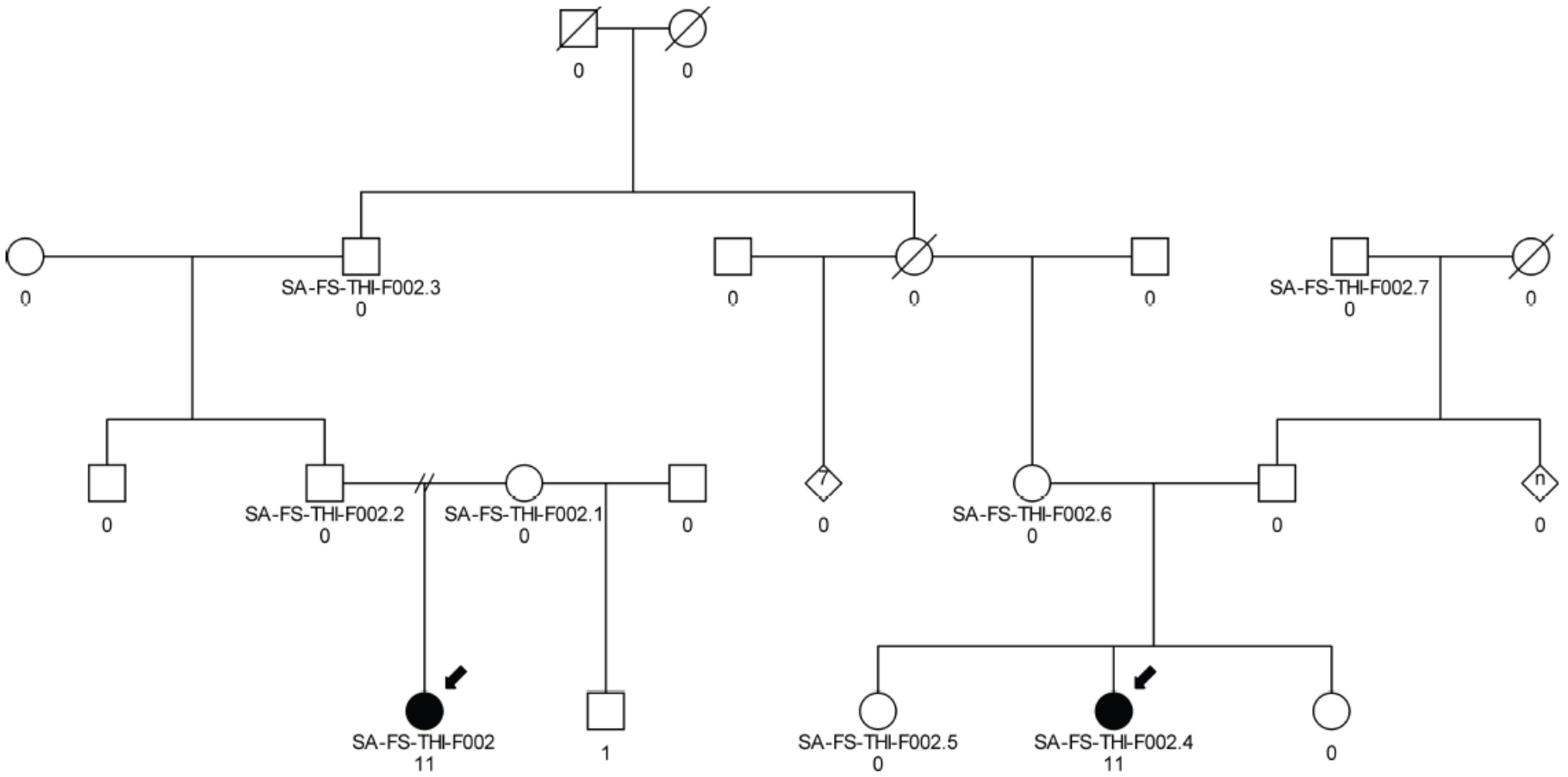
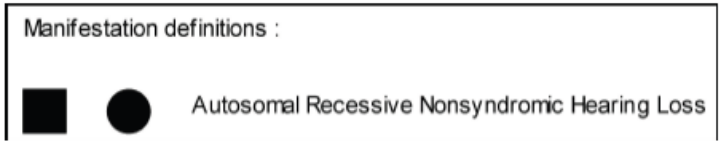
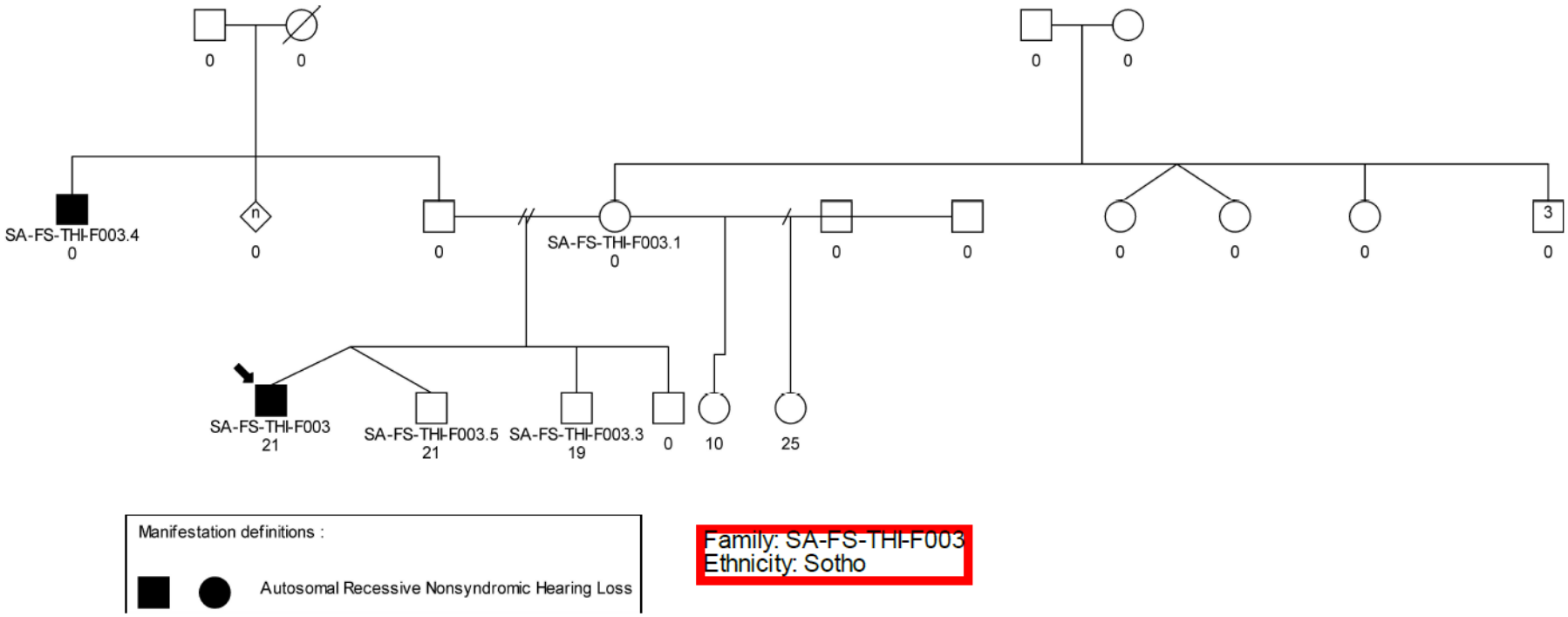
Manifestation definitions :
Autosomal Dominant Nonsyndromic Hearing Loss
Family: SA-FS-CDT-F010
Ethnicity: Tswana





Family: SA-FS-THI-F002
Ethnicity: Sotho/Zulu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Manifestation definitions :
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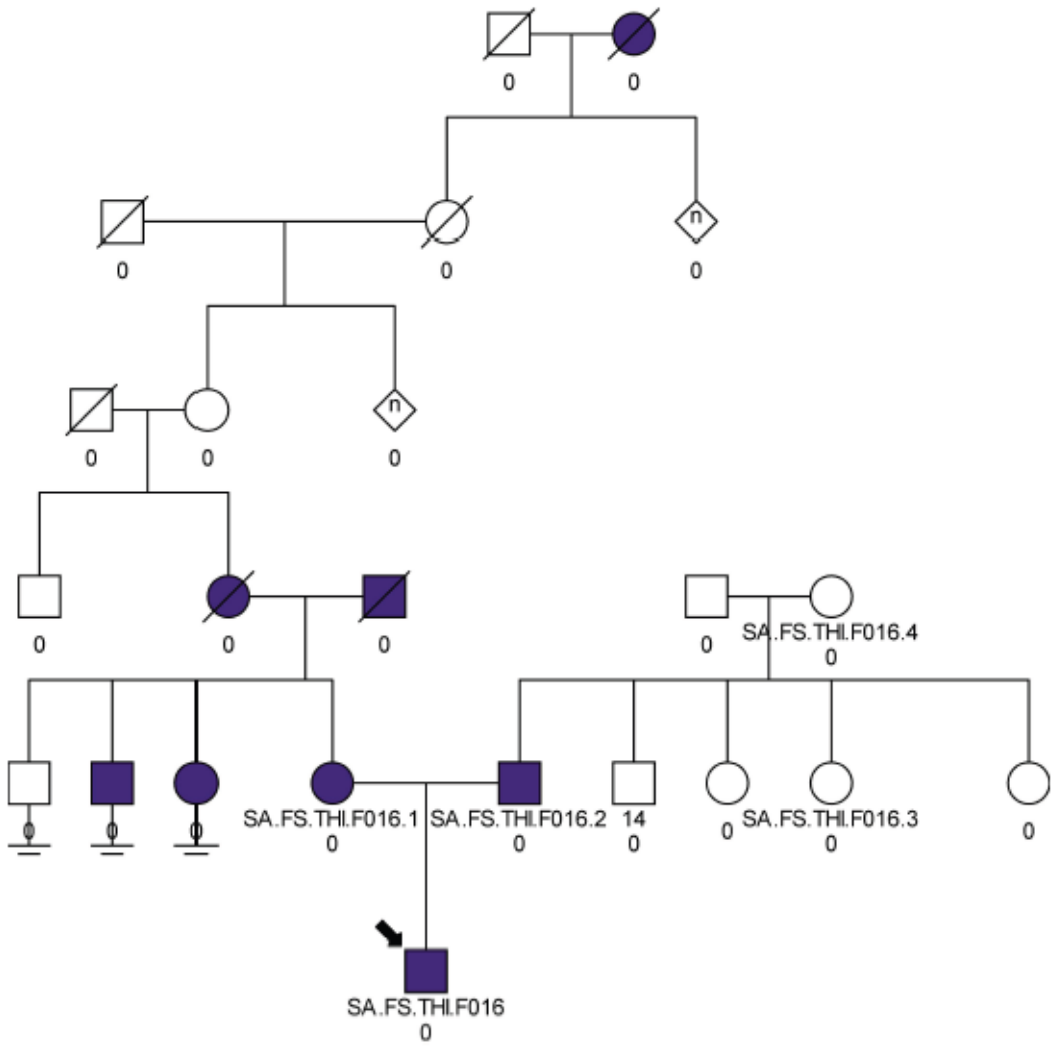
Family: SA-FS-THI-F003
Ethnicity: Sotho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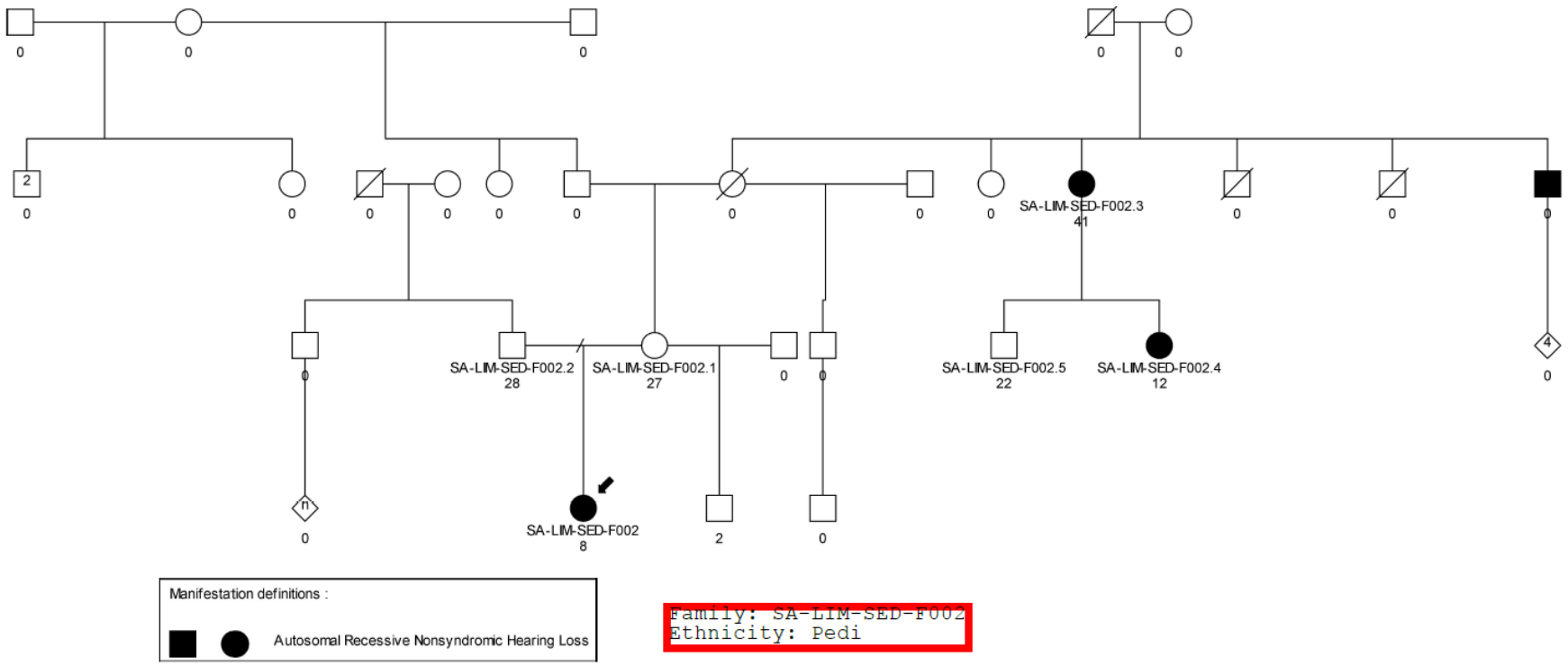
Manifestation definitions :
Autosomal Dominant Nonsyndromic Hearing Loss
Family: SA.FS.THI.F016
Ethnicity: Xhosa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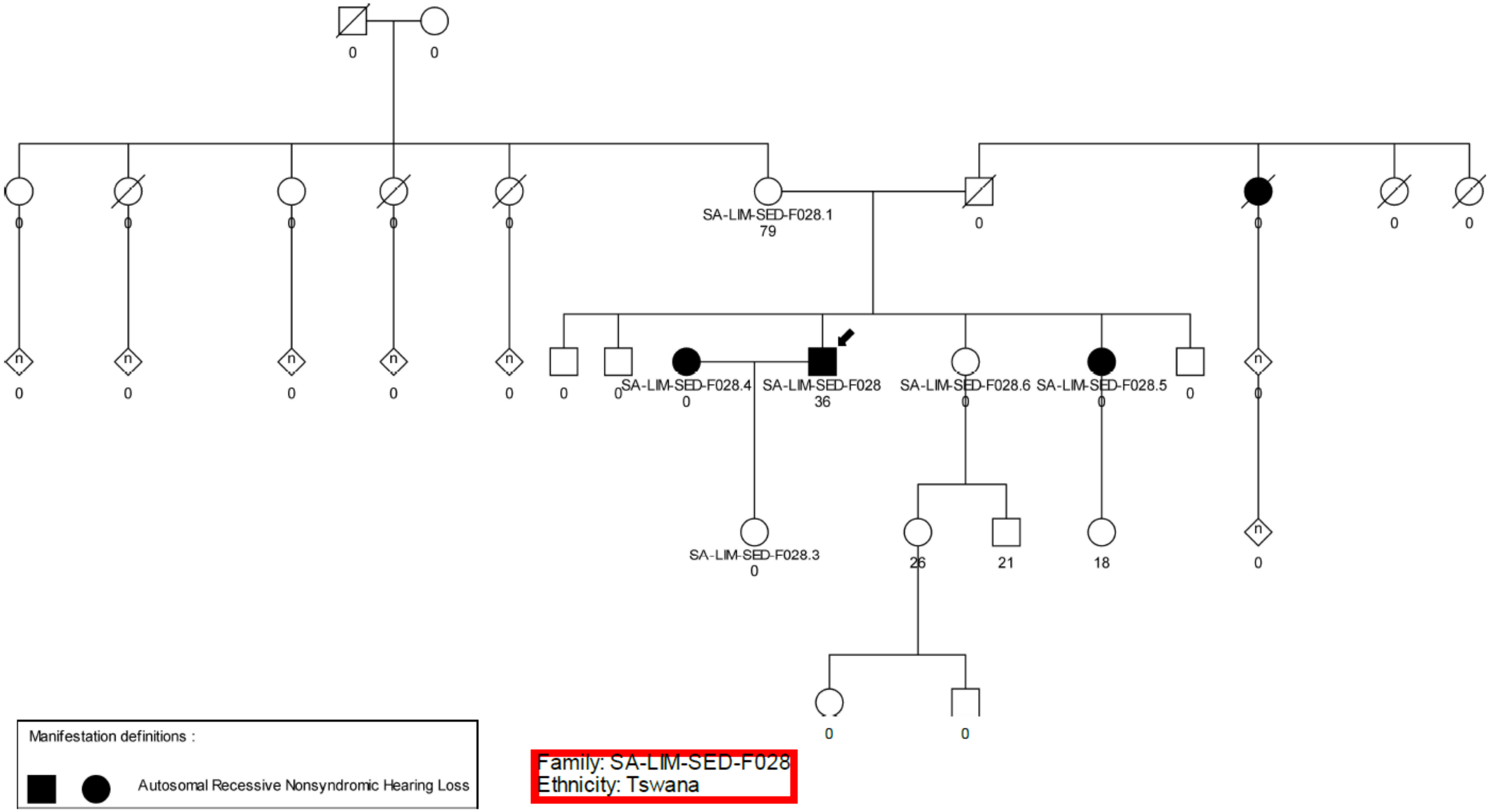
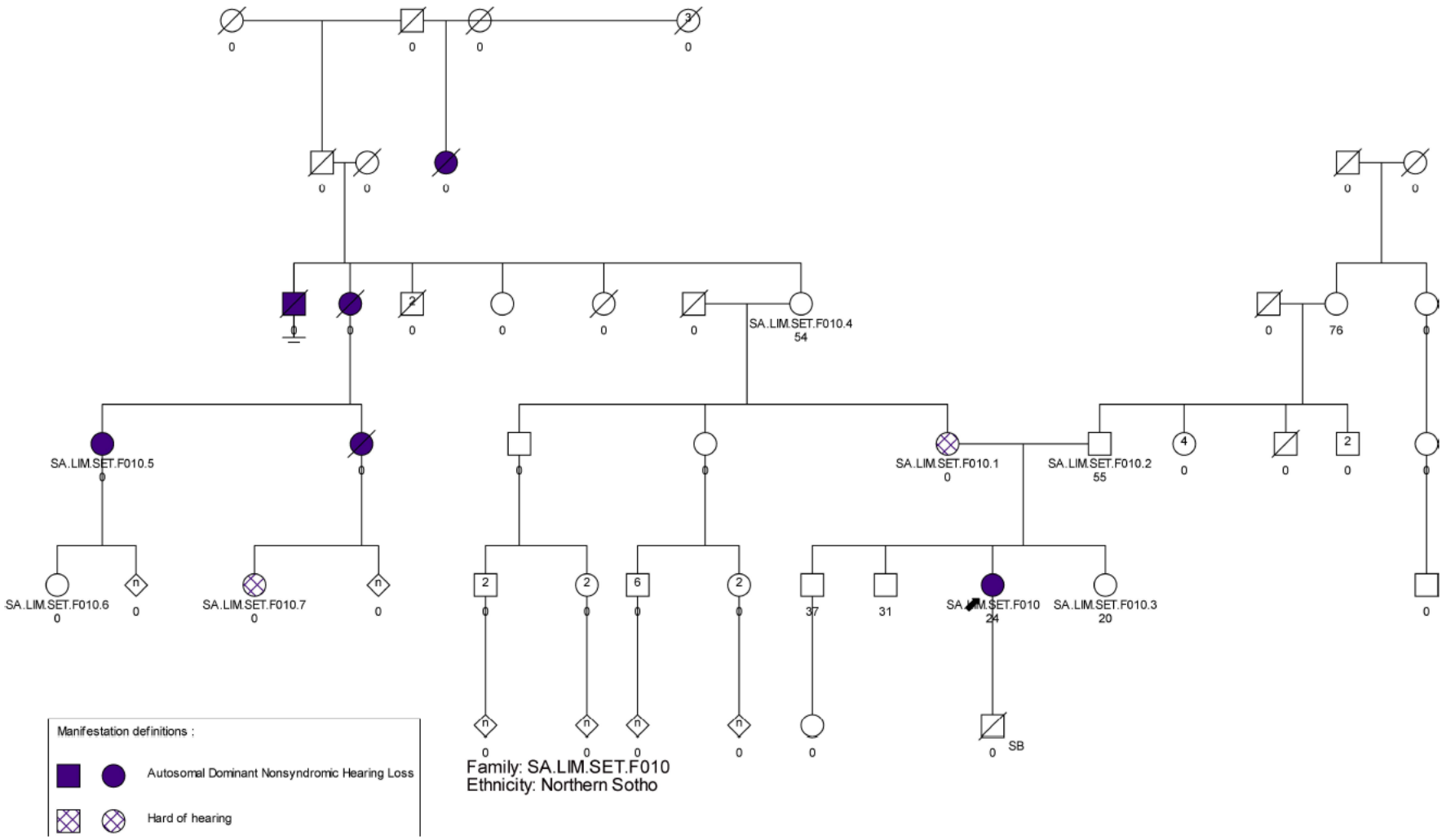
Manifestation definitions :
Autosomal Recessive Nonsyndromic Hearing Loss
Family: SA-LIM-SED-F002
Ethnicity: Pedi



Family: SA-LIM-SED-F028
Ethnicity: Tswana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SA.LIM.SET.F010.2
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SA.LIM.SET.F010
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SA.LIM.SET.F010.3
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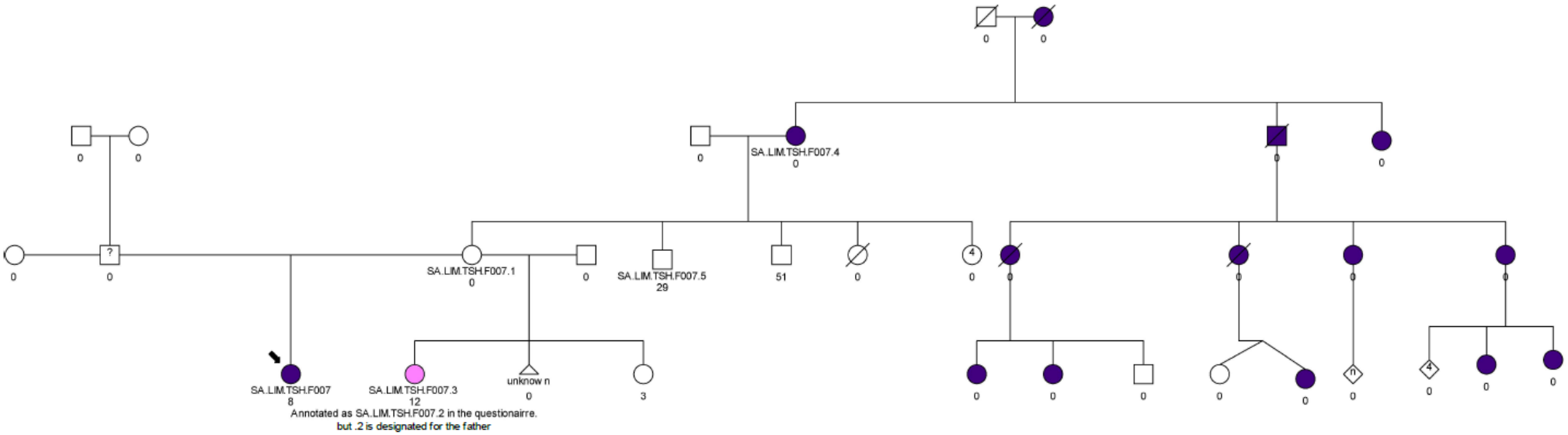
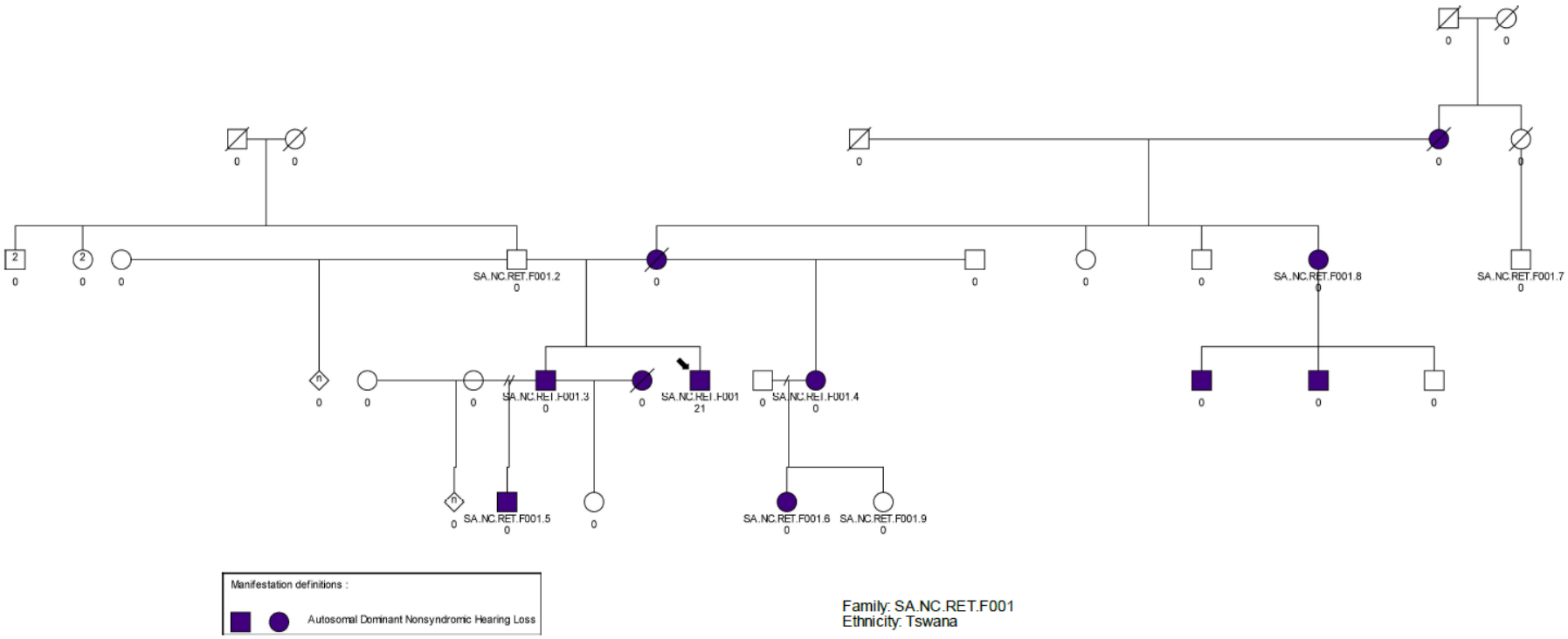
Manifestation definitions :
Autosomal Dominant Nonsyndromic Hearing Loss
Hard of hearing
Family: SA.LIM.SET.F010
Ethnicity: Northern Sotho



Family: SA.LIM.TSH.F007
Ethnicity: Sotho-Vhenda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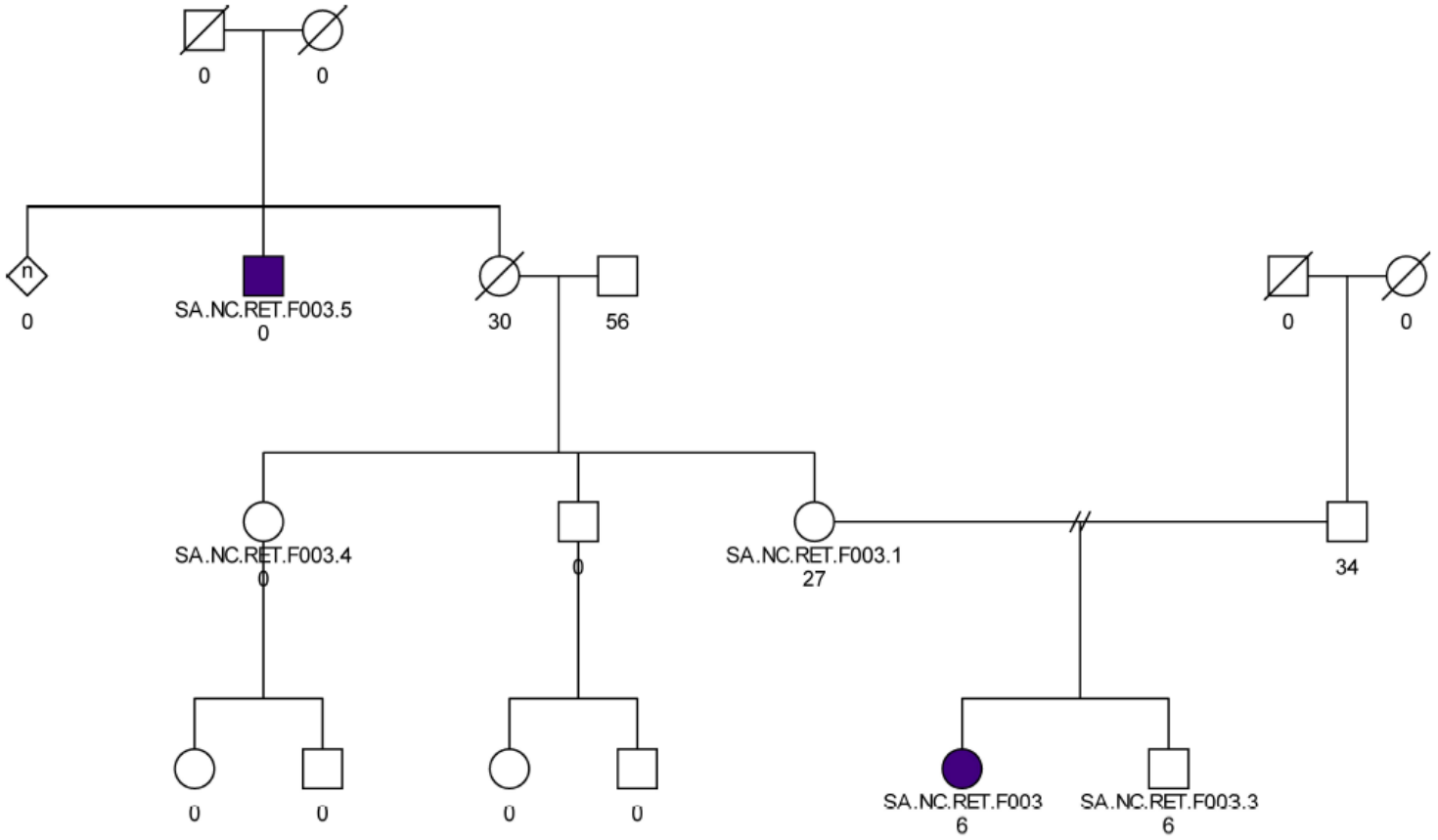
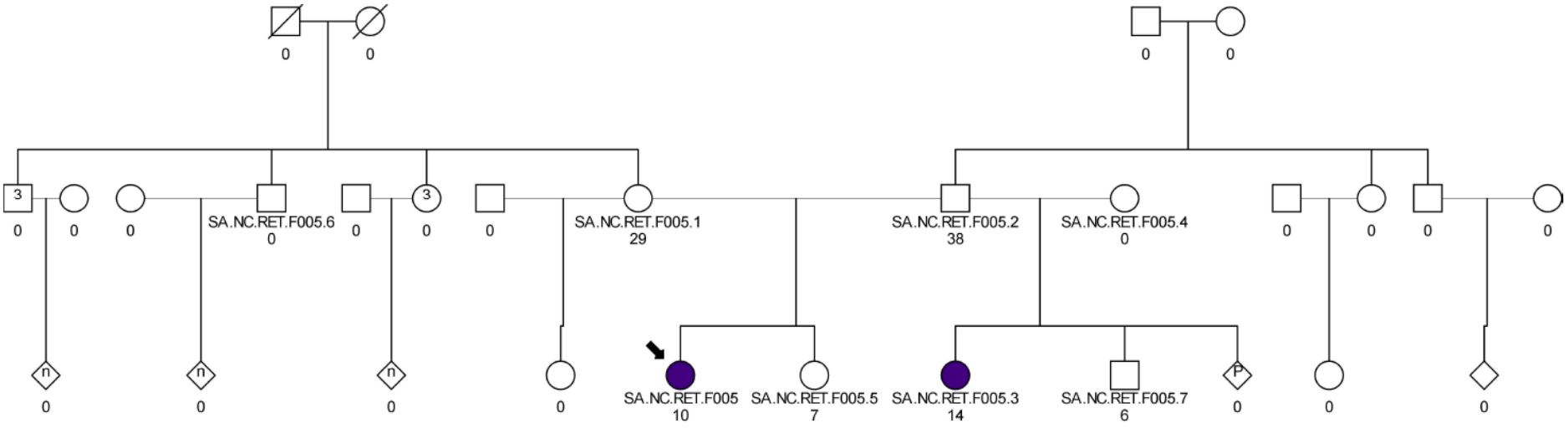
Manifestation definitions :
Autosomal Dominant Nonsyndromic Hearing Loss
Family: SA.NC.RET.F001
Ethnicity: Tswana



Manifestation definitions :

Autosomal Dominant Nonsyndromic Hearing Loss

Family: SA.NC.RET.F003
Ethnicity: Tswana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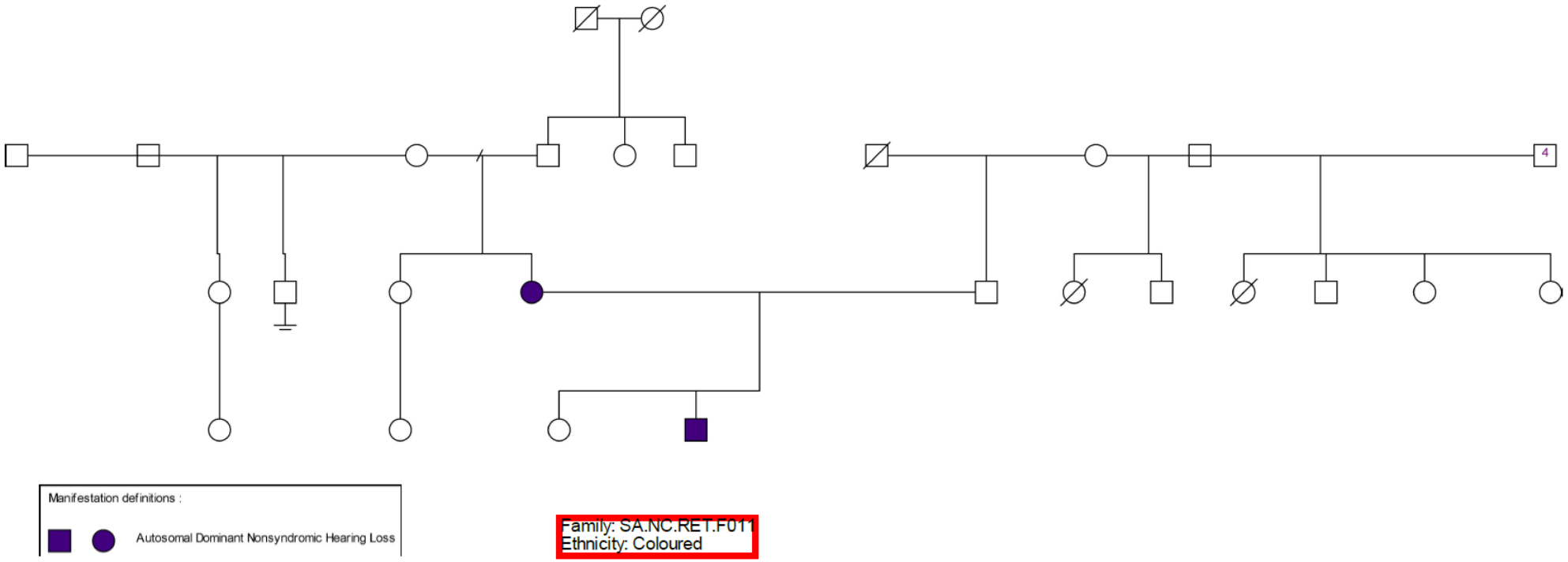
Autosomal Dominant Nonsyndromic Hearing Loss
Family: SA.NC.RET.F005
Ethniciy: Tswana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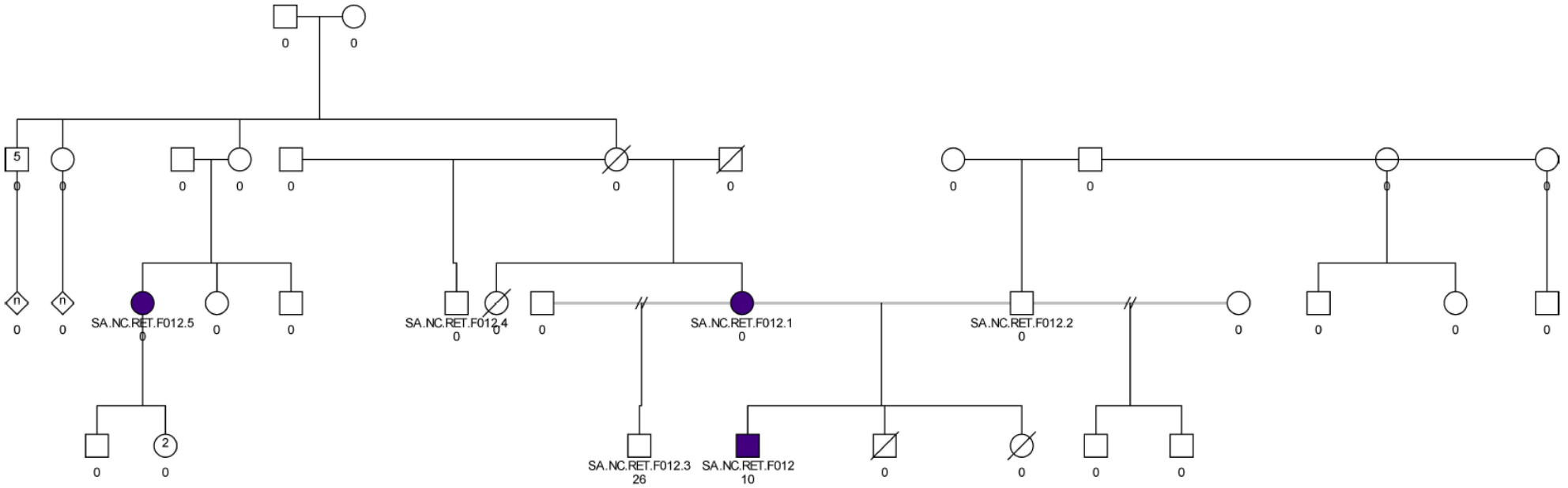
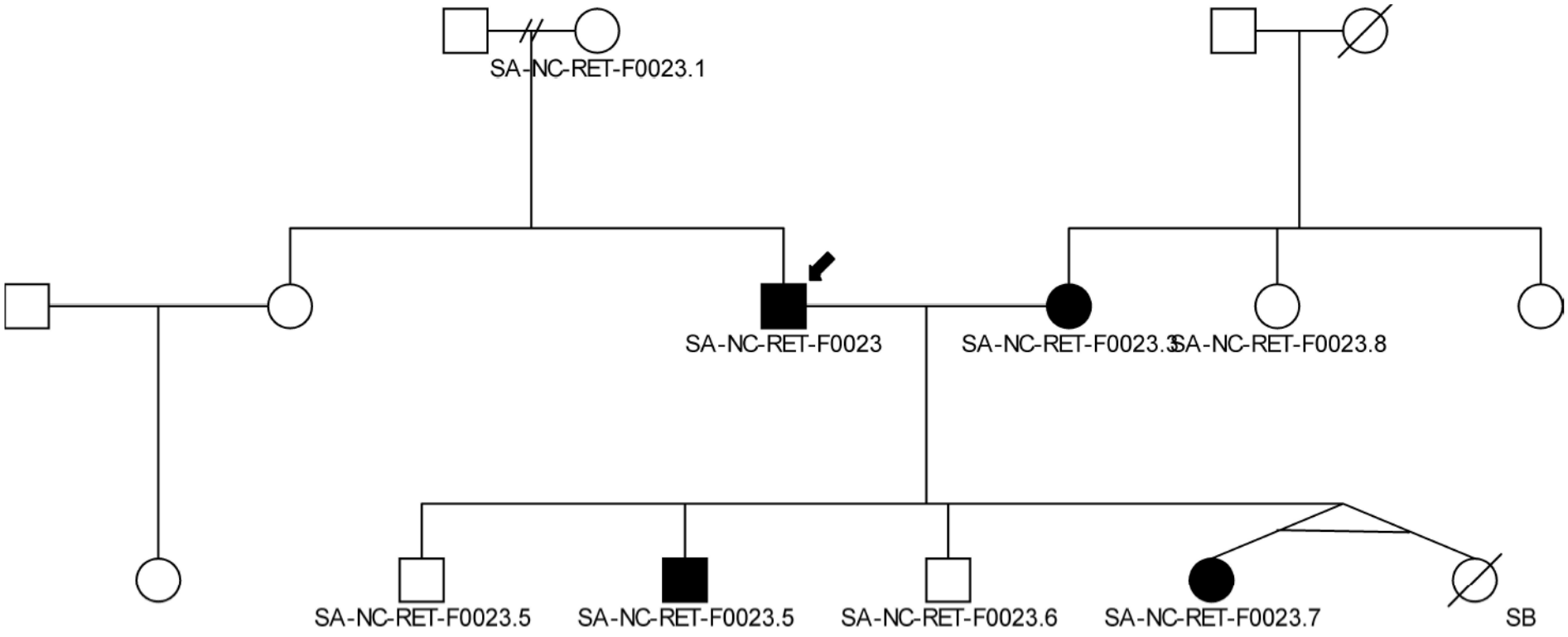
Manifestation definitions :
Autosomal Dominant Nonsyndromic Hearing Loss
Family: SA.NC.RET.F011
Ethnicity: Coloured



Family: SA.NC.RET.F012
Ethnicity: Tswana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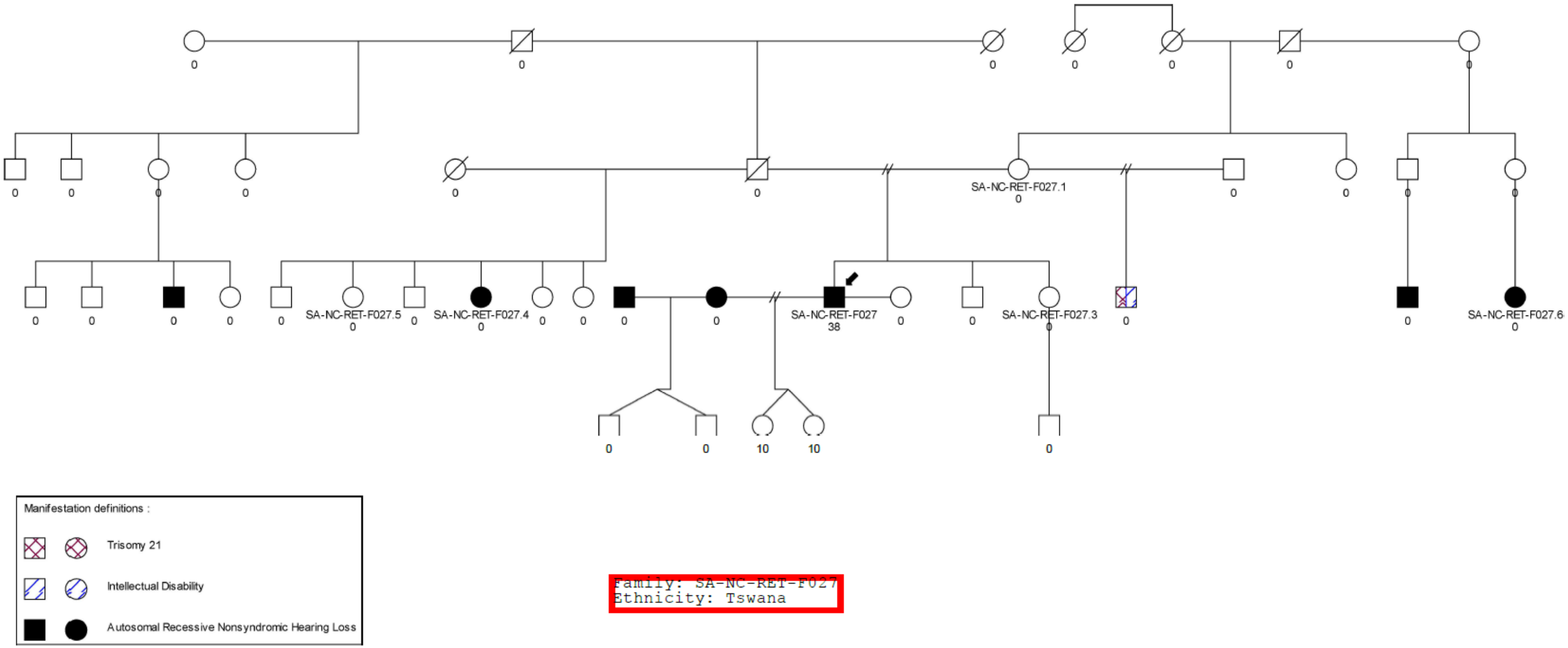
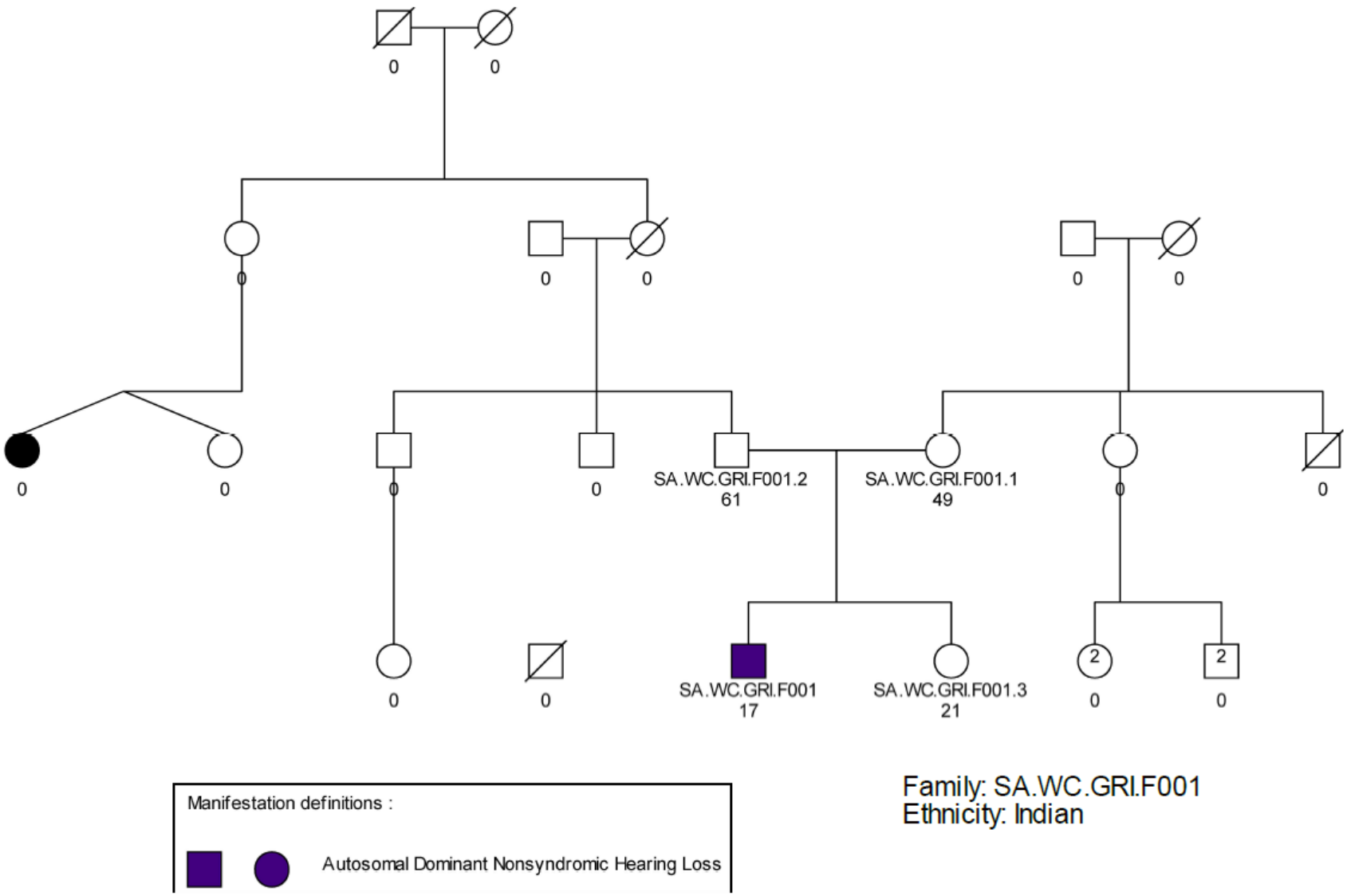
Manifestation definitions :
Autosomal Recessive Nonsyndromic Hearing Loss
Family: SA-NC-RET-F023
Ethnicity: Coloured



Family: SA-NC-RET-F027
Ethnicity: Tswana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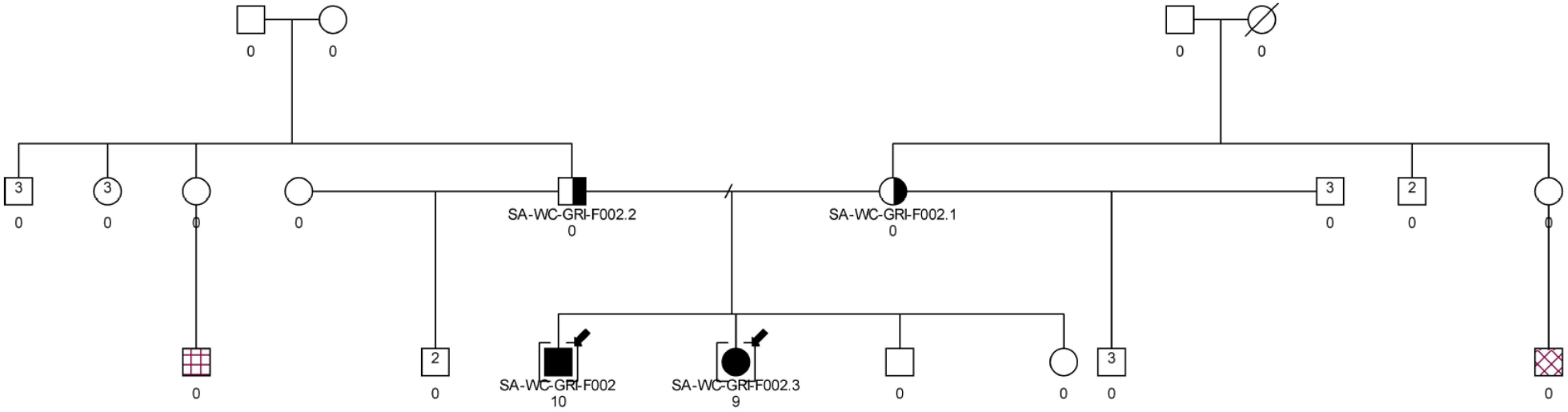
Manifestation definitions :
Autosomal Dominant Nonsyndromic Hearing Loss
Family: SA.WC.GRI.F001
Ethnicity: Indian



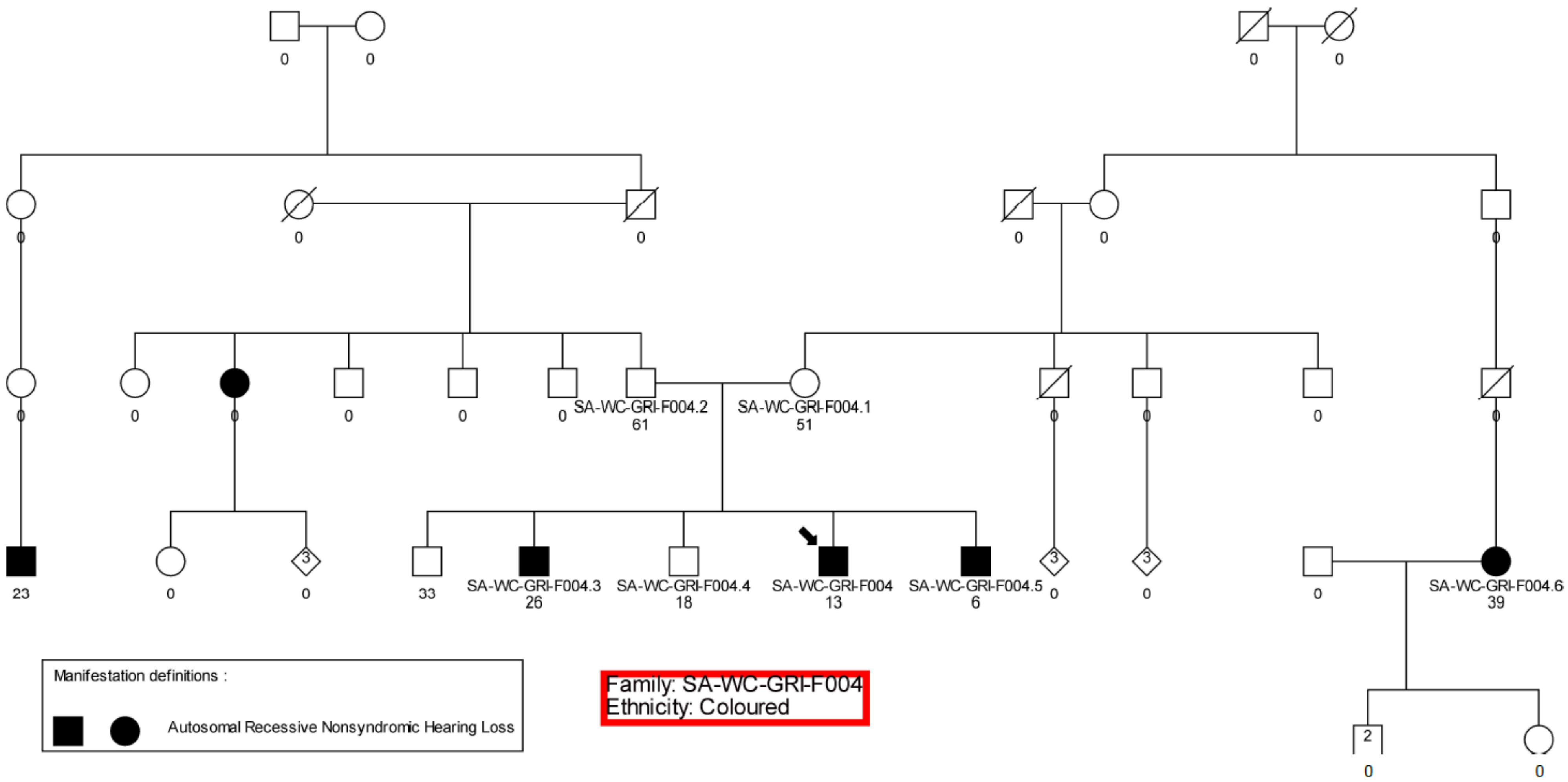
Manifestation definitions :

Trisomy 21

Schizophrenia

Autosomal Recessive Nonsyndromic Hearing Loss

Family: SA-WC-GRI-F002
Ethnicity: Coloured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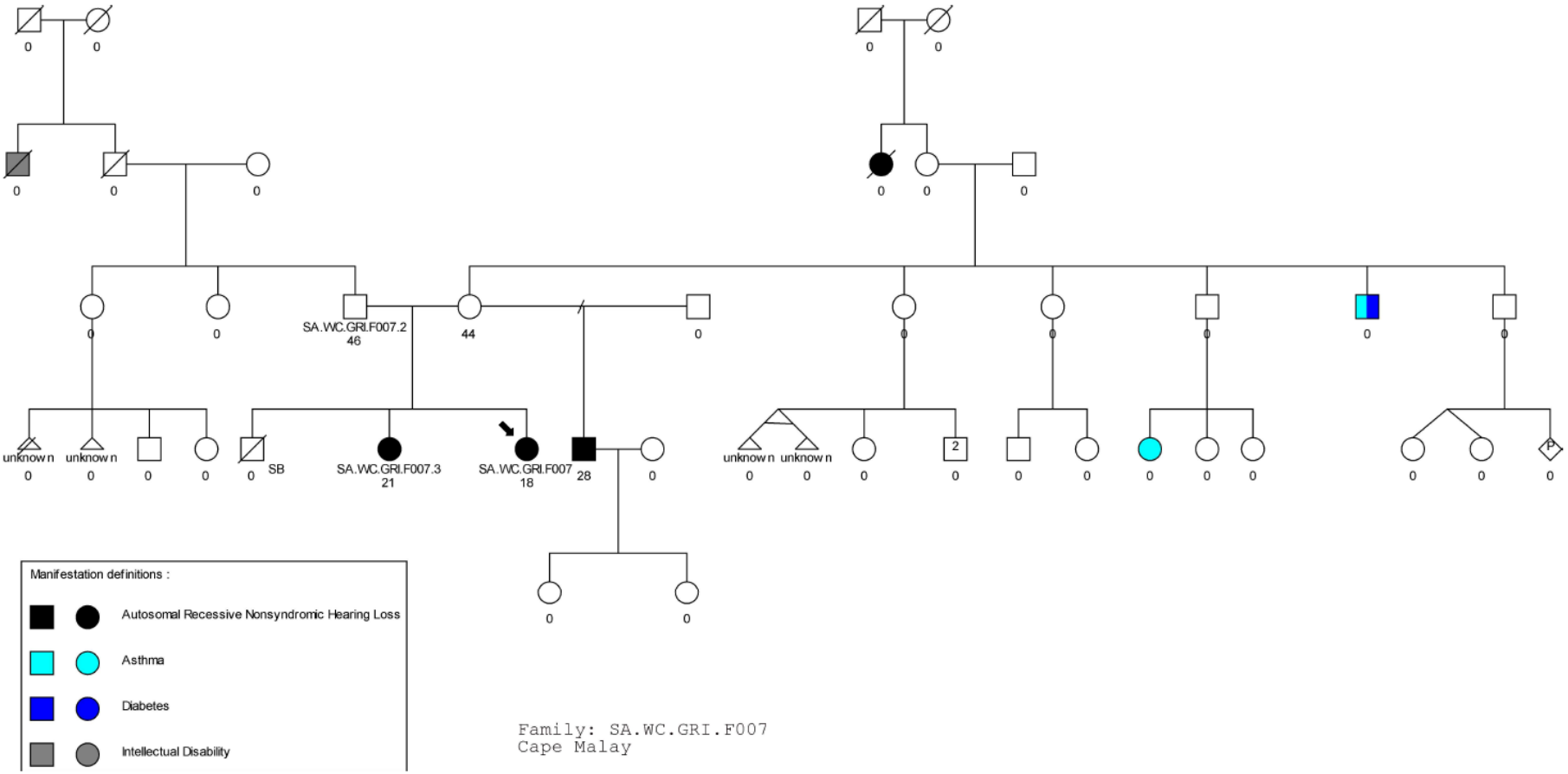
Autosomal Recessive Nonsyndromic Hearing Loss
Family: SA-WC-GRI-F004
Ethnicity: Coloured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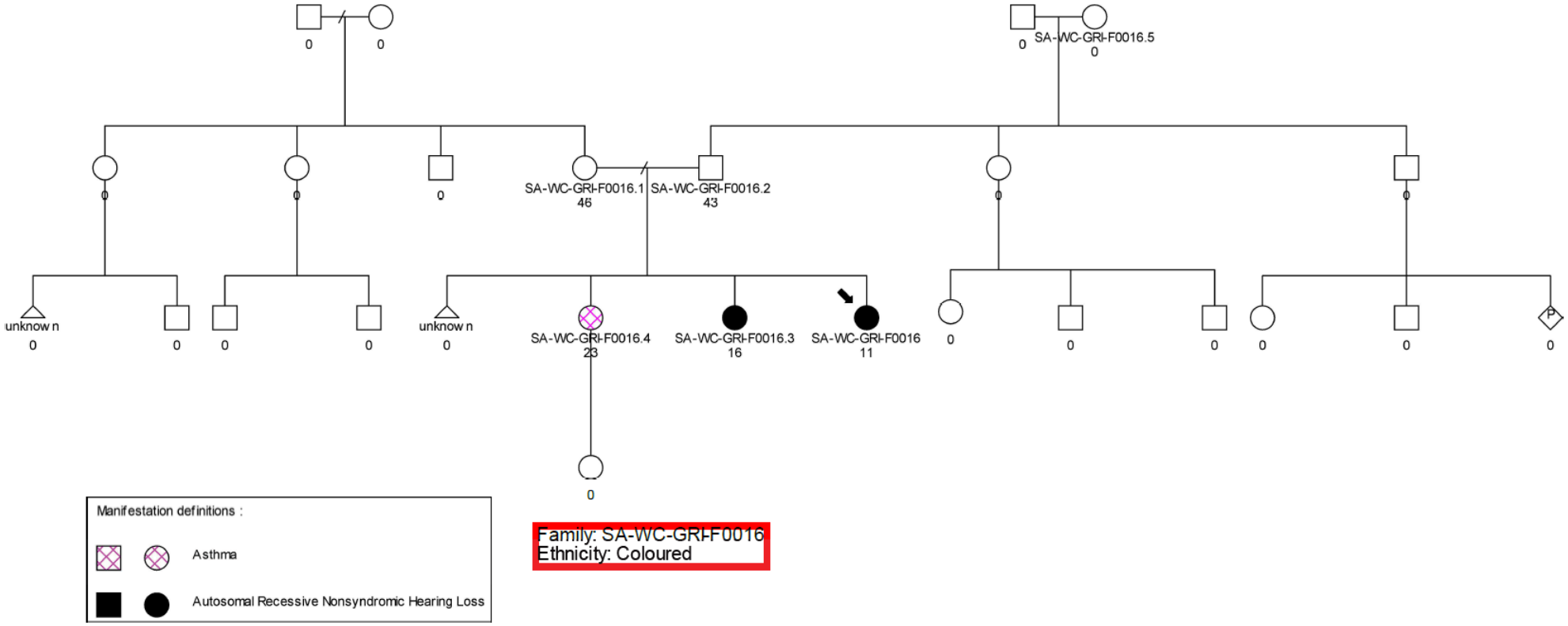
Manifestation definitions :
Autosomal Recessive Nonsyndromic Hearing Loss
Asthma
Diabetes
Intellectual Disability
Family: SA.WC.GRI.F007
Cape Malay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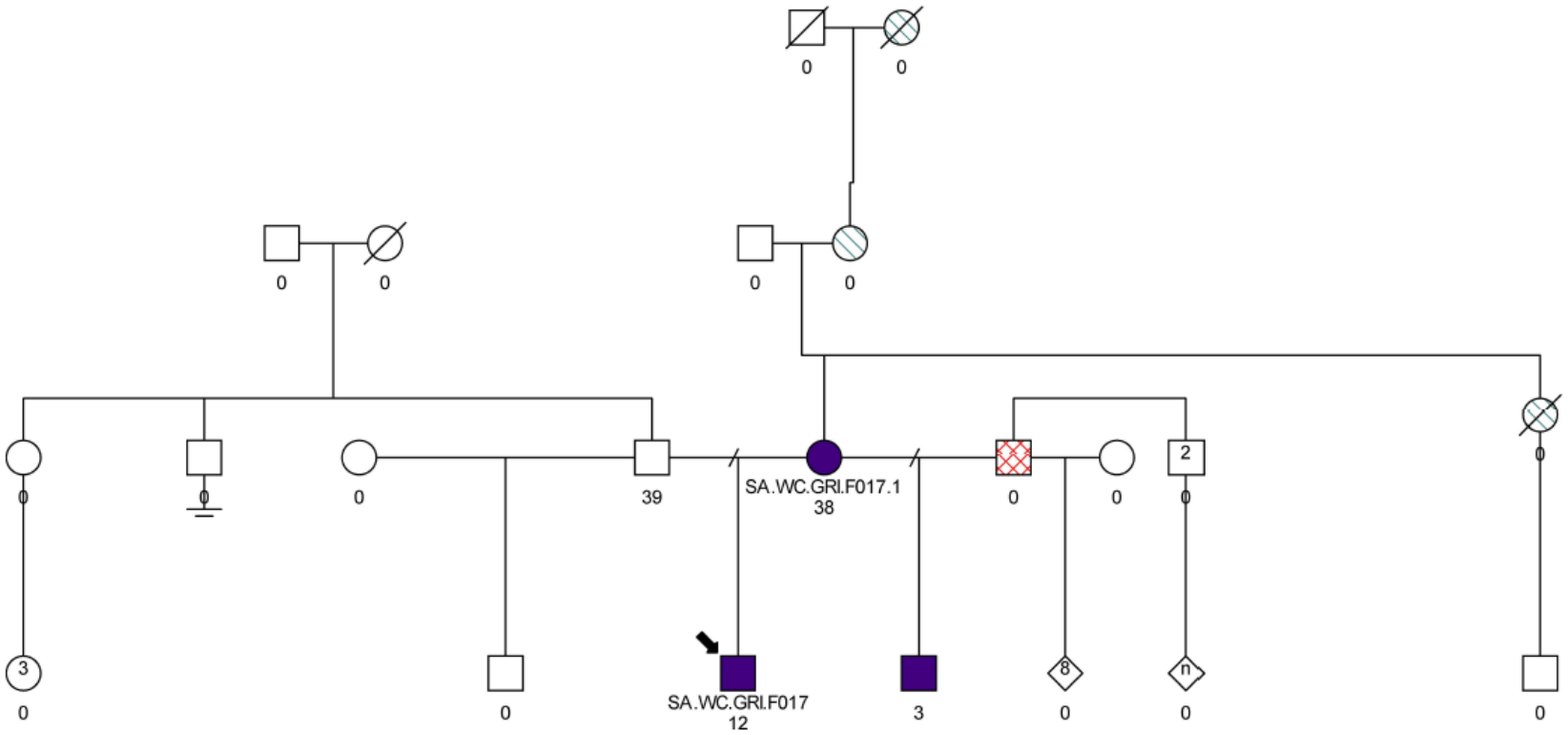
Manifestation definitions :
Asthma
Autosomal Recessive Nonsyndromic Hearing Loss
Family: SA-WC-GRI-F0016
Ethnicity: Coloured



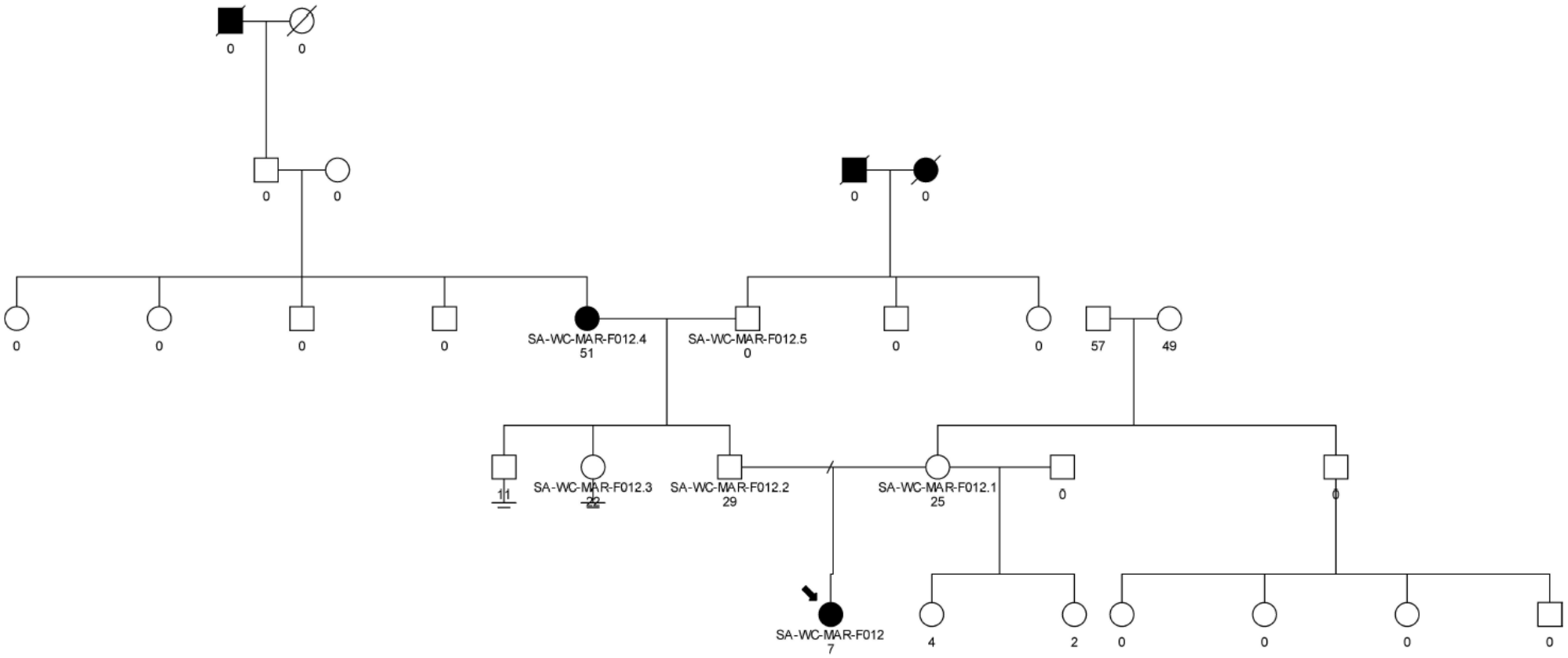
Manifestation definitions :

Autosomal Dominant Nonsyndromic Hearing Loss

Osophogial Cancer

Mental Instability

Family: SA.WC.GRI.F017
Ethnicity: Xhosa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0
57
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1
SA-WC-MAR-F012.3
2
2
SA-WC-MAR-F012.2
29
SA-WC-MAR-F012.1
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SA-WC-MAR-F012
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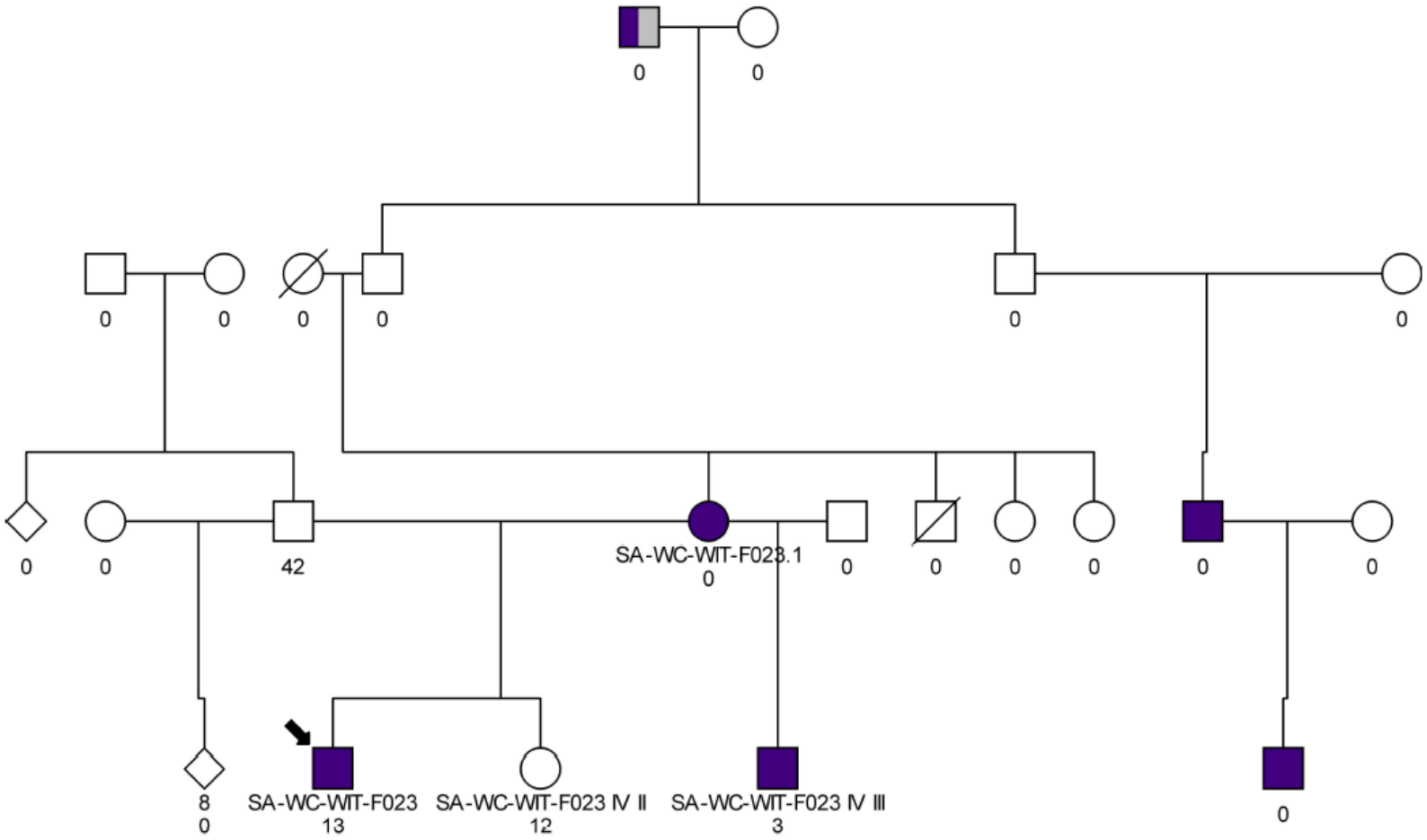
Manifestation definitions :
Autosomal Recessive Nonsyndromic Hearing Loss
Family: SA-WC-MAR-F012
Ethnicity: Coloured



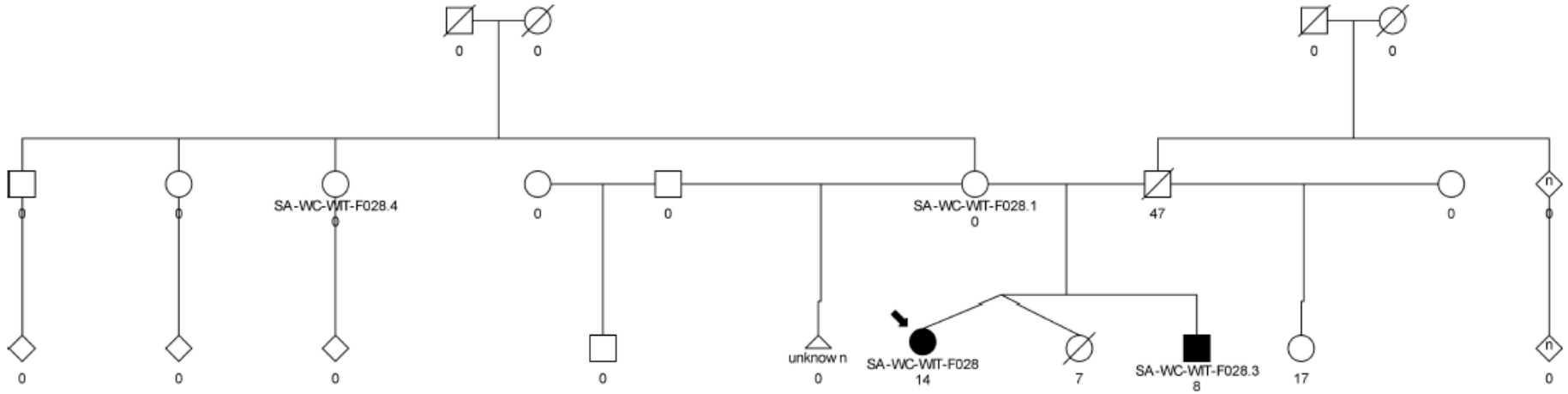
Manifestation definitions :

Autosomal Dominant Nonsyndromic Hearing Loss

Blindness

Family: SA-WC-WIT-F023

Ethnicity: Not Stated



Manifestation definitions :

Autosomal Recessive Nonsyndromic Hearing Loss

Family: SA-WC-WIT-F028
Ethnicity: Xhosa

A4: Variant classification of novelty