# INVESTIGATION OF THE GENETIC CAUSE OF HEARING LOSS IN 28 AUTOSOMAL DOMINANT FAMILIES WITHIN THE NEWFOUNDLAND FOUNDER POPULATION

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# Investigation of the Genetic Cause of Hearing Loss in 28 Autosomal Dominant Families within the Newfoundland Founder Population

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## Abstract

The purpose of this study was to determine the genetic cause of hearing loss in 28 Newfoundland families with Autosomal Dominant hearing loss. AD hearing loss is highly genetically heterogeneous, and is mainly associated with a late onset, progressive phenotype. After a comprehensive literature search, genotype-phenotype evaluations, and a functional candidate gene approach, all 28 probands were sequenced to identify mutations in four genes known to cause autosomal dominant hearing loss, *COCH*, *KCNQ4, TECTA,* and *MYO1A*. First, a known Dutch founder mutation within exon 4 of *COCH*, c.151 C>CT, was found in a Newfoundland proband of Family 2094. All affected family members (n=7) shared this mutation, while unaffected members did not. This is only the second family found to harbor this mutation outside of Europe. This mutation is strongly associated with severe vestibular decline. Affected Family 2094 members carrying the mutation do present vestibular decline in the form of vertigo and balance difficulties. As this mutation is considered to be a Dutch founder mutation, DNA samples from a Dutch p.P51P/S family were genotyped and compared with Family 2094 genotypes. Fragment analysis confirmed haplotype sharing of five markers closely bordering the c.151 C>CT mutation between Newfoundland and Dutch mutation carriers. Second, a novel 3bp deletion in exon 5 of *KCNQ4* was found in 13 affected members of Family 2071. While the mutation was not seen in four other affected family members, audiology test results suggest that these four individuals are phenocopies. Sequencing of the full *KCNQ4* gene was done in all individuals, to rule out another mutation on the same gene. Further investigation, through the construction of an intragenic haplotype, did

not point to any further hearing loss associated variants within *KCNQ4,* and confirmed that all deletion carriers share a common hearing loss haplotype and deletion. Third, a nonsense mutation was found in exon 4 of *MYO1A* in the proband of Newfoundland Family 2102. This is a C→T nucleotide substitution (c.2435 C>CT) that causes a change (p.R93X) in the motor domain of myosin 1A. Of four individuals in Family 2102, three were found to carry the p.R93X mutation, while one unaffected sibling was not. This mutation has been reported once before in a small Italian family. No mutations were discovered in the *TECTA* gene. When each of the causative mutations in *COCH, KCNQ4, and MYO1A* was detected, additional Newfoundland hearing loss probands were screened, to rule out the possibility of a founder mutation. In no case were additional mutation carriers identified. While no founder mutations were discovered in this study, the genetic cause of hearing loss was identified in three families.

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# List of Abbreviations & Symbols

**ABI** Applied Biosystems International

**AD** Autosomal Dominant

**ADNSHL** Autosomal Dominant Non-Syndromic Hearing Loss

**AHC** Auditory Hair Cell

**AR** Autosomal Recessive

**C9orf75** C9 open reading frame 75

**cM** Centimorgan

**COCH** Coagulation Factor C Homolog

**CP** Cytoplasmic

**CT** Computed Tomography

**dB** Decibels

**DFNA** Autosomal dominant deafness gene

**DFNB** Autosomal recessive deafness gene

**DFN** X-Linked deafness gene

**$dH_2O$** De-ionized water

## List of Abbreviations & Symbols (cont)

**DIAPH1** Protein diaphanous homolog 1

**dNTP** Dideoxynucleotide Triphosphate

**DMF** Deionized Formamide

**DMSO** Dimethyl Sulfoxide

**DNA** Deoxyribonucleic acid

**EDTA** Ethylenediaminetetraacetic acid

**EtOH** Ethanol

**EYA4** Eyes absent homolog 4

**FCH** Factor C Homologous Domain

**GS500 (-250) LIZ** GeneScan 500 (-250) LIZ Size Standard

**GJB2** Gap junction protein, beta 2

**GJB6** Gap junction protein, beta 6

**GWS** Genome Wide Scan

**HIC** Human Investigations Committee

**Hz** Hertz

**KCNQ4** Potassium Voltage-Gated Channel 4

## List of Abbreviations & Symbols (cont)

**kD** Kilodalton

**L** Liter

**LD** Linkage Disequilibrium

**Mb** Megabase

**$MgCl_2$** Magnesium Chloride

**mM** Millimolar

**MP3** MPEG-1 Audio Layer 3

**MSH2** MutS homolog 2

**MYO1A** Myosin IA

**MYO7A** Myosin VIIA

**ng** Nanogram

**OMIM** Online Mendelian Inheritance in Man

**PAX3** Paired box gene

**PCR** Polymerase Chain Reaction

**rpm** revolutions per minute

## List of Abbreviations & Symbols (cont)

**SSCP** Single strand conformation polymorphism

**SNP** Single Nucleotide Polymorphism

**Taq** Thermus aquaticus DNA Polymerase

**TBE** Tris/Borate/EDTA Buffer

**TECTA** Tectorin Alpha

**TMPRSS3** Transmembrane protease 3, serine 3

**TORCH** Toxoplasmosis, rubella, cmv, and herpes

**TPRN** Taperin

**U** Units

**$\mu$l** Microlitre

**$\mu$M** Micromolar

**USA** United States of America

**USH** Usher Syndrome

**WFS1** Wolfram syndrome 1 (wolframin)

**WS** Waardenburg Syndrome

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# Chapter 1: Introduction

## Purpose

The aim of this research project is to determine the genetic etiology of autosomal dominant (AD) hearing loss in 28 Newfoundland families.

## Overview

Hearing loss is the most common sensory disorder in humans. For example, one in every 500 newborns has hearing loss (Morton & Nance, 2006). The prevalence of hearing loss increases dramatically with age, and by puberty, the number of affected persons doubles (Morton & Nance, 2006). Hearing loss is even more prevalent in adults, as 60 % of people older than 70 years have a hearing loss of 25 dB or more (Gratton & Vazquez, 2003).

Hearing loss is a multi-factorial disorder caused by both genetic and environmental factors. Genetic factors account for 50 % of all hearing loss cases, while environmental factors cause 25 %. The remaining 25 % are classified as being of unknown etiology (Willems, 2001). Environmental causes of hearing loss include exposure to high sound decibel levels, head trauma, prematurity, neonatal hypoxia, low birth weight, prenatal infections from "TORCH" organisms (i.e., **to**xoplasmosis, **r**ubella, **C**MV, and **h**erpes), and postnatal infections like bacterial meningitis (Willems, 2001; Bitner-Glindzicz, 2002).

Approximately 30 % of genetic cases are syndromic: the phenotype includes other signs and symptoms throughout the body in addition to deafness. Over 400 genetic syndromes include some degree of hearing loss (Gorlin et al. 1995; Nie et al. 2008). Two examples are Usher syndrome (USH): hearing loss accompanied by retinitis pigmentosa, and Pendred syndrome: a hearing loss disorder accompanied by goiter, which is a swelling in the thyroid gland. However, the vast majority, around 70%, of inherited hearing disorders are non-syndromic (Cremers et al. 1991; Van Camp et al. 1997). Worldwide, within non-syndromic cases, 88 % of the hearing loss genes identified cause autosomal recessive (AR) hearing loss, 11 % AD, and the remaining 1% either mitochondrial or X-linked (Smith & Van Camp, 2007).

The five factors used to describe hearing loss are age of onset, sound frequencies affected (low, middle or high), degree of hearing loss (measured in dBs), affected part of the auditory system (conductive, sensorineural or mixed), and configuration (unilateral, or bilateral).

Hearing loss has a high degree of genetic heterogeneity. A large number of mutations within many different genes cause similar hearing loss phenotypes. As of May 2010, the Hereditary Hearing Loss Homepage listed 141 non-syndromic deafness loci that have been mapped and 50 genes that have been identified. Twenty-two of the 50 known genes harbor mutations that cause AD hearing loss (Table 1.1), 33 cause AR hearing loss, and 2 cause X-linked hearing loss (Van Camp G, Smith RJH http://hereditaryhearingloss.org). Loci for non-syndromic hearing loss are denoted 'DFNA' for AD inheritance, 'DFNB' for AR inheritance and 'DFN' for X-linked

inheritance (Griffith & Friedman, 2002). Some genes cause both AD and AR hearing loss. For example, Grifa et al. (1999) found a C→T change in gap junction protein, beta 6 (*GJB6*) that resulted in the substitution of a highly conserved threonine residue for a methionine at amino acid position 5 (p.T5M), resulting in nonsyndromic AD hearing loss. While this *GJB26* mutation causes AD hearing loss, Del Castillo et al. (2002) identified a 342 kb deletion in *GJB6* by studying 422 unrelated subjects from Spain and Cuba with an AR pattern of inheritance.

## Pedigrees

When studying hearing loss, or any hereditary disorder, family members are visualized on a pedigree chart, which in this study shows all known hearing loss phenotypes presented at the time of clinical and audiological testing. This allows easier identification of the inheritance pattern and of the relationships among haplotypes. A haplotype is a combination of alleles that are transmitted together. When a causative mutation is found, alleles of linked markers are assessed in order to develop a haplotype, or pedigree that illustrates shared genetic variants between family members. These haplotypes are then compared between members of the same family or between members of different families that share the same mutation. A common haplotype with the same mutation suggests a common ancestor for that mutation. Furthermore, a haplotype can point to associations between different variants that may be combining to affect the phenotype.

## Audiograms

Audiograms are graphs of the minimal level of sound that a given person can hear at various frequencies (Figure 1.1; Figure 1.2). They are produced using an audiometer, a machine that tests hearing by exposing patients to a range of sounds at different pitches and decibel (dB) levels. During hearing tests, separate audiograms are obtained for each ear. Each line on the audiogram represents one ear. The y-axis measures sound intensity in units of dB, which increases logarithmically. The x-axis of the audiogram measures the frequency, or pitch, of a sound in Hz (Hertz). Low pitch sounds have low frequencies (< 500 Hz), medium pitch sounds have medium frequencies (500 - 2000 Hz), and high pitch sounds have high frequencies (> 2000 Hz). Hearing loss is characterized by intensity, which can be mild, moderate, severe or profound, and by which frequency is affected, such as low, middle or high.

An individual with normal hearing can detect sounds between 0 dB and 20 dB. The minimum level of hearing, 0 dB, is equivalent to a barely audible whisper. Those affected with hearing loss, however, have a higher than normal minimum hearing level. This means that any given sound intensity must be greater than 0 dB for them to hear it. People with a mild degree of hearing loss can only hear sound at intensities between 20 – 40 dB for the frequencies of 500 – 4000 Hz. Individuals with moderate hearing loss can only hear sound from 40-70 dB, and those with severe hearing loss can only hear sound between 70-95 dB in intensity. Lastly, those with profound hearing loss cannot detect sound at all unless it is 95 dB or greater (such as the sound produced by an .MP3 player at maximum volume; Mazzoli et al. 2003).

Figure 1.1 shows a series of simple audiograms: audiogram A shows an individual with normal hearing, B an individual with moderate bilateral (affecting both ears) hearing loss, and C an individual with severe bilateral hearing loss. However, audiograms are often not so simple to read. Figure 1.2 shows two additional audiograms: audiogram A shows an individual with moderate to mild hearing loss in the left ear and normal hearing in the right ear (unilateral), and B shows an individual with bilateral hearing loss sloping to moderate and profound at the higher frequencies. This audioprofile is typical of presbyacusis, or age-related hearing loss. 40 % of the population older than 65 years of age is affected, and 80 % of hearing loss cases occur in elderly people (Gates & Mills, 2005). It is now generally accepted that presbyacusis is most often caused by age-related declines in the auditory system, such as loss or deterioration of sensory cells within the cochlea. Moreover, impaired temporal processing is associated with age-related factors that affect neural synchrony of hearing (Schuknecht et al. 1993; Friedman 2003; Wu et al. 2003; Fitzgibbons et al. 2010). Temporal processing refers to the processing of acoustic stimuli over time. Temporal processing allows us to distinguish speech from background noise, as the decibel levels of the background noise varies over time.

Another common and important characteristic of presbyacusis, and of any sensorineural hearing loss, is the level of speech discrimination a patient demonstrates. Hearing a sound does not always translate into properly distinguishing speech. Tests are also performed to determine a patient's speech discrimination. The measure of speech discrimination is often a percentage, and describes the ability of a patient to correctly

identify words when the sound is loud enough for them to comfortably hear. When a patient has low speech discrimination, a hearing aid will successfully amplify sound in the patient's ear, but will not necessarily improve speech perception. The amplified sound remains gibberish to the patient because he/she is unable to identify the words. (McAlister, 1990; Kodera et al. 1994). A cochlear implant, a surgically implanted electronic device that provides sound to profoundly deaf or severely hard of hearing individuals, has been found in many cases to markedly improve speech discrimination (Leung et al. 2005; Cambron, 2006; Yueh & Shekelle, 2007).

## Autosomal Dominant Hearing Loss

Autosomal dominant non-syndromic hearing loss (ADNSHL) accounts for approximately 15 % of inherited hearing loss (Hildebrand et al. 2008). To date, 59 loci for ADNSHL have been identified, along with 22 causally related genes (Table 1.1; Van Camp G, Smith RJH. Hereditary Hearing Loss Homepage. http://hereditaryhearingloss.org). The majority of AR hearing loss cases are caused by mutations in just a few genes, most notably gap junction protein beta 2 (*GJB2*) and *GJB6*. This contrasts sharply with AD hearing loss, which is significantly more genetically heterogeneous (Griffith & Friedman, 2002), making cost-effective screening for diagnosis in a clinical setting highly problematic. Mutations within the genes wolfram syndrome 1 (*WFS1*), cochlin (*COCH*), potassium voltage-gated channel 4 (*KCNQ4*), and tectorin alpha (*TECTA*) are marginally more frequently reported in comparison to the

other reported causative genes. The audioprofile sometimes provides clues to the underlying causative gene. For example, *WFS1* harbors mutations found in 75 % of families segregating for AD, non-syndromic hearing loss that initially affect only the low frequencies (Young et al. 2001; Bespolova et al. 2001).

ADNSHL is often characterized by a post-lingual, late-onset, progressive phenotype that affects mainly adults. Post-lingual hearing loss is much more frequent than pre-lingual hearing loss, and affects 10 % of the population by the age of 60 (Van Camp et al. 1997). This most often results from damage to auditory hair cells (AHCs) or their innervation (Gates & Mills, 2005). For example, one late-onset progressive hearing loss associated gene is eyes absent homolog 4 (*EYA4*), a member of the vertebrate *Eya* family of transcriptional activators. Mutations in this gene were found in Belgian and USA families, and create premature stop codons leading to post-lingual, progressive, AD hearing loss. *EYA4* was subsequently shown to be critical in the continued function of the mature organ of Corti, an organ in the cochlea that contains the AHCs (Wayne et al. 2001).

## Critical Considerations When Researching Autosomal Dominant Hearing Loss

Of the 28 families researched in this study that are classified as having an AD pattern of inheritance, one or more may have been incorrectly classified due to a lack of sufficient data. Many individuals in these pedigrees (Figure 2.1) are ascertained through

relatives' word of mouth. For this reason, it is important to discuss the role that different factors may be playing in confusing the ascertainment of individuals and thus the search for causative hearing loss mutations.

Digenic inheritance is when a phenotype is expressed only if an interaction between two mutant alleles in two separate genes occurs (Strachan & Read, 2003). Digenic inheritance does not cause AD hearing loss, but digenic inheritance may play a role in the hearing loss of one of the families under investigation in this study. For example, Chen et al. (1997) reported a small consanguineous family with three affected and three unaffected members. Two regions shared by the three affected individuals were identified, one on 3q21.3-q25.2 (LOD = 2.78) and 19p13.3-p13.1 (LOD = 2.78). LOD (Logarithm (base 10) of odds) is a statistical test used to determine the likelihood of obtaining test data if two loci are linked compared to the likelihood of observing the data by chance. Chen et al. (1997) speculated that two non-allelic recessive mutations accounted for the profound congenital deafness in this family. In a Chinese family, Liu et al. (2009) demonstrated through DNA sequencing that mutations in *GJB2* and *GJB3* interact to cause hearing loss in digenic heterozygotes. To support this, they discovered overlapping expression patterns of *GJB2* and *GJB3* in the cochlea, along with co-assembly of the *GJB2* and *GJB3* proteins when co-transfected in human embryonic kidney (HEK) cells (Liu et al. 2009). And a third example was seen recently when mutations within ATP sensitive inward rectifier potassium channel 10 (*KCNJ10*) and solute carrier family 26, member 4 (*SLC26A4*) were found to cause digenic non-syndromic hearing loss associated with enlarged vestibular aqueduct syndrome (EVA)

(Yang et al. 2009). Mutations in *SLC26A4* were previously shown to cause Pendred syndrome (PS), a genetic disorder leading to hearing loss and goiter with occasional hypothyroidism. Many individuals with an EVA/PS phenotype had only one disease-causing variant in *SLC26A4.* Yang et al. (2009) identified double heterozygosity in affected individuals from two separate families. These patients carry single mutations in both *KCNJ10* and *SLC26A4*, and the mutation in *SLC26A4* has been previously associated with the EVA/PS phenotype. The *KCNJ10* mutation reduces potassium conductance activity, which is critical for generating and maintaining proper ion homeostasis in the ear. To add further support to their digenic interaction hypothesis, Yang et al. (2009) demonstrated haploinsufficiency of *Slc26a4* in *Slc26a4+/-* mouse mutants resulted in reduced protein expression of *Kcnj10* in the inner ear.

One important term to keep in mind when researching AD hearing loss is penetrance. Penetrance refers to the proportion of individuals with a mutation who exhibit clinical symptoms. For example, if a mutation in a gene responsible for a type of AD hearing loss is 95 % penetrant, then 95 % of individuals with the mutation will exhibit symptoms, while 5 % will not during their lifetime. Penetrance is often expressed as a frequency at different ages because, for many hereditary diseases, onset of symptoms is age-related (Strachan & Read, 2003). This is particularly important because AD hearing loss is often late-onset and progressive. For this reason, a family's inheritance pattern could appear to be sporadic, when in fact the disorder segregates autosomal dominantly, and the individuals under study simply haven't yet presented the hearing loss phenotype, as the age of onset varies widely and can range well into 50 years of age. A

related but distinct potential problem is expressivity. Expressivity refers to variations of a phenotype for a particular genotype. When a condition has highly variable signs and symptoms, it can be difficult to diagnose.

Mitochondrial inheritance could also be confusing the ascertainment of the families investigated in this study. Mitochondrial inheritance is the inheritance of a trait encoded in the mitochondrial genome, and is always of maternal origin. It is therefore often also called maternal inheritance. When a woman harbors a mitochondrial mutation, and her egg cells are forming an ovary, these egg cells contain a random distribution of both normal and mutated copies of the mitochondrial gene (St. John et al. 2010). Therefore, all children of this mother may inherit some mutated mitochondria, but if the number of mutated mitochondria reaches a critical level, termed the "threshold effect", then an adverse phenotype is seen (St. John et al. 2010; Van Camp G, Smith RJH. Hereditary Hearing Loss Homepage). These mitochondrial hearing loss mutations can be late-onset, or if the carrier is administered certain antibiotics, the phenotype will be "drawn out". This is the case with mutations in MT-RNR1, which are known to cause non-syndromic deafness (Casano et al. 1999; Bates 2003). Mitochondrial mutations are beyond the scope of this study, but have previously been shown to cause late-onset hearing loss that is comparable to the phenotypes of Families 2093, 2112, and 2125 investigated here (Casano et al. 1999). While there are no incidences of male to male transmission in these families, additional clinical ascertainment could potentially reveal a mitochondrial inheritance pattern. This study primarily targets only genes and mutations known to be associated with AD hearing loss, but the possibility of maternally inherited

mutations causing hearing loss in the above mentioned families should not be ruled out, and should be investigated in future studies.

As a result of random genetic drift in the founder population of Newfoundland, there is an elevated incidence of particular rare disorders, such as Bardet-Biedl syndrome (Webb et al. 2009). This makes the founder population of Newfoundland ideal for the study of genetic disorders and increases the chance of detecting novel causative genes and mutations. However, due to the nature of Newfoundland as a genetic isolate, some potential pitfalls arise. One potential pitfall is the uncertainty in inheritance ascertainment. For example, assortative mating could confuse the ascertainment of families and therefore the search for hearing loss mutations. Assortative mating occurs when sexually reproducing organisms choose to mate with individuals that are similar (positive assortative mating) or dissimilar (negative assortative mating) to themselves in some specific way. One family under investigation in this study (Family 2069) is a potential example of positive assortative mating (see p.42, bottom pedigree, $5^{th}$ generation). This is critically important. Positive assortative mating could result in both parents carrying a mutation that causes hearing loss. This may, however, simply be a result of studying a genetic disorder in a highly isolated population, and it is possible that this mating took place not because both individuals were affected by hearing loss, but instead due simply to the low level of mating choice in small out-port community. Either way, our current inheritance classification of Newfoundland Family 2069 could possibly be incorrectly stated as AD, and our candidate gene selection would then be based on unfounded and false assumptions. It is important to keep this possibility under

unfounded and false assumptions. It is important to keep this possibility under consideration, and to investigate and screen for commonly occurring recessive mutations in the proband of Family 2069 as well as dominant mutations. Pseudo-dominance should also be taken into account. This is the situation where the inheritance of an AR trait mimics an AD pattern, and due to the limited extent of clinical ascertainment in these families' histories, it is possible that one of these AD families is in fact affected by an AR mutation segregating in a pseudo-dominant fashion.

Another critical factor to judge when researching genetic hearing loss is the possible presence of phenocopies. A phenocopy is an affected individual who has the same disease, but due to a different cause, as relatives affected with the genetic condition under study. Hearing loss is a very common type of sensory loss in humans. Many types of environmental and genetic factors account for hearing loss so individuals within families affected with hearing loss can be afflicted due to a plethora of different reasons (Griffith & Friedman 2002). For example, a study of heterozygous *WFS1* mutations in two low frequency sensorineural hearing loss families showed that these two families' hearing loss were linked to adjacent but non-overlapping loci on 4p16, DFNA6 and DFNA14 (Van Camp et al. 1999). Upon further study, it was found that an individual in the DFNA6 family who had a recombination event excluding the DFNA14 candidate region was actually a phenocopy. The cause of hearing loss in this phenocopy was reported as unknown, but as a consequence they were able to determine that DFNA6 and DFNA14 are allelic (Bespalova et al. 2001).

Throughout this study genes are investigated through targeted gene sequencing. However, it must be mentioned that it is possible larger genomic abnormalities may account for hearing loss in some of the Newfoundland families under investigation (Lisenka et al. 2003; Shaffer et al. 2006). Large genomic rearrangements, deletions, inversions, etc. can cause and affect the degree and severity of diseases, and these large-scale anomalies are not detected through traditional DNA sequencing methods (Lisenka et al. 2003; Idbaih et al. 2010).

## The Pioneering of Hearing Loss Gene Discovery

The first genes to be implicated in hearing loss were found in the syndromic disorders. Syndromic forms of hearing loss are classified by their associated symptoms. For example, Waardenburg syndrome (WS) is the most common cause of AD syndromic hearing loss. WS is characterized by varying degrees of hearing loss associated with pigmentation anomalies and neural crest defects. It was first described in 1951, but it took several decades to identify the causative genes. Asher and Friedman (1990) studied mice and hamsters with four mutations causing phenotypes similar to those seen in human WS patients. They used the chromosomal locations and syntenic relationships associated with three of these four mutant mouse genes to predict human chromosomal locations for the causative WS gene. Synteny is the situation whereby organisms of relatively recent divergence show similar blocks of genes in the same relative positions in the genome. Asher and Friedman (1990) predicted four possible locations for the causative gene, and

one turned out to be correct. In 1992, mutations causing WS were discovered in the paired box gene (*PAX3*) gene (on chromosome 2q) (Tassabehji et al. 1992). A second common cause of AR syndromic hearing loss is Usher syndrome (Toriello et al. 2004). Usher syndrome was first described in 1858 when Van Graefe reported the case of a deaf and "dumb" male patient presenting with retinal pigment degeneration, who had two similarly affected brothers (Van Graefe, 1858). This was the first syndrome to demonstrate that phenotypes, in this case deafness and blindness, could be inherited in tandem. Usher syndrome is characterized by profound congenital hearing impairment, retinitis pigmentosa, and vestibular dysfunction. It has three clinical types, denoted as I, II, and III, in decreasing order of severity (Saihan et al. 2009). In 1995 one of several causative genes for Usher Syndrome Type 1 was discovered. Weil et al. (1995) chose myosin 7A (*MYOVIIA*) as a functional candidate gene, based on observations that cytoskeletal abnormalities seen in Usher syndrome patients are also seen in mouse mutants with myosin mutations. Two different premature stop codons, a six bp deletion, and two different missense mutations were detected in five unrelated families. In one family, these mutations were identified in both alleles, and resulted in the absence of a functional protein and subsequent Usher syndrome (Weil et al. 1995). Currently, 10 different types of Usher syndrome have been recognized, with more than 100 pathogenic mutations alone for the two most common molecular forms, Usher 1B (USH1B) and Usher 2a (USH2A; Ahmed et al. 2003; Saihan et al. 2009).

The first ADNSHL family investigated was from the small town of Taras, Costa Rica. The hearing loss was described as low frequency AD with a post-lingual age of

onset at 10 years of age (Leon et al. 1981). Leon et al. (1992) performed linkage analysis to determine that the causative gene was linked to markers defining a 7 cM critical region on chromosome 5q31. Genetic markers are DNA sequences with a known location on a chromosome, and are useful in linkage analysis because they are easily identifiable, associated with a specific locus, and highly polymorphic. LOD scores for linkage of deafness to markers in the 7 cM region showed a score of 13.55 at markers D5S2119 and D5S2010 (Leon et al. 1992). This was not only the first AD critical region described, but the first autosomal non-syndromic hearing loss gene to be mapped altogether. It was not until 1997, 26 years after first being reported, that the region was narrowed down further. Positional cloning, sometimes referred to as reverse genetics, is the cloning of an area known to be associated with a disease. It involves the isolation of overlapping DNA segments that progress along the chromosome toward a candidate gene. Lynch et al. (1997) performed fine mapping using positional cloning techniques to narrow the critical region to 1 cM. This revealed protein diaphanous homolog 1 (*DIAPH1*), a previously unidentified human gene. *DIAPH1*, in this case, is a positional candidate gene, a gene identified based upon a determined critical region. This differs from functional candidate genes, which are known to play a role in the disease pathology, or have been previously shown to harbor mutations that cause a disease, like the p.A716T mutation in *WFS1* in Newfoundland (Young et al. 2001; Bespalova et al. 2001). Lynch et al. (1997) sequenced the positional candidate gene *DIAPH1* in all affected family members using Single Strand Conformation Polymorphism (SSCP) analysis. SSCP is the electrophoretic separation of single-stranded nucleic acids based on differences in sequences which bring about a

different secondary structure and thus a measureable difference in mobility through a gel. The causative mutation, a G→T substitution, was now revealed, and *DIAPH1* was also found to be highly expressed in the cochlea (Lynch et al. 1997). Lynch et al. (1997) speculate that the protein this gene encodes, protein diaphanous homolog 1, plays a role in the regulation of actin polymerization in the hair cells of the cochlea.

## Founder Populations & Mutations

No summary of hearing loss associated genes and mutations would be complete without mentioning the importance of founder populations, the founder effect, and founder mutations. A founder population is a small subpopulation that has been isolated due to geography, culture, religion or a combination of these. This subpopulation has a significantly decreased amount of genetic diversity, causing certain genetic traits to either vanish or become very abundant in further generations. When a founder population is isolated individuals in later generations are likely to share many genes, because a mutation in a founder will be passed on to a large proportion of the population in subsequent generations (Nurhousen, 2000). Founder populations, therefore, possess much promise in determining the genes involved in genetic diseases. As there is little genetic heterogeneity, the majority of the individuals with a given disease will carry the same gene mutation. For example, the Ashkenazi Jews were a reproductively isolated population in Europe for roughly a thousand years, with very little out-migration or inter-marriage with other groups (Nebel et al. 2005). As a result of this event, the *GJB2*

mutation c.167delT was found to be highly prevalent in the Ashkanazi Jewish population (Morell et al. 1998). Other examples of founder populations include the Canadian province of Québec, which was established by as few as 2600 individuals, the United States Amish population, and the population of Pingelap, a small island in Micronesia.

A founder mutation is a mutation found as an allele and shared by several individuals from a founder population and derived from a single ancestor. For example, *COCH* p.P51P/S mutation carriers are considered to have originated from a common ancestor (de Kok et al. 1999). A second example was recently seen when Park et al. (2010) investigated the 3-bp deletion in intron 7 (c.991-15_991-13del) of DFNA5, and identified a conserved haplotype between a Korean family and a Chinese family segregating the deletion in DFNA5, suggesting that this deletion represented a founder mutation originating from a common ancestor.

## Colonization of Newfoundland: A Founder Population

The island of Newfoundland makes up a large part of the Canadian province of Newfoundland & Labrador. It is the most easterly landmass on the North American continent. In 1497 the European explorer Giovanni Gabotto (John Cabot) "discovered" Newfoundland, though Vikings had landed earlier. Europeans voyaged across the North Atlantic from England, Scotland, Ireland, France and Portugal to harvest the rich fish stocks. When each fishing season ended they returned to their countries, as permanent

those considering permanent settlement, and with a lack of basic supplies even a one year stay would have been very difficult (Poole & Cuff, 1994). This deterrence lessened throughout the 17th century, however, as small groups of English, Scottish, and Irish settlers set sail from western England in 1610 and throughout the 17th century. These colonists excluded other nations from fishing off of Newfoundland's east coast, but were discouraged in their settlement by the English government, who saw their presence as a threat to the monopoly control that Western England fishing centers had established. Meanwhile, fisherman from France dominated the island's south coast and northern peninsula (Bennett, 2002). Throughout the 1600s, the French began to permanently settle, but in 1713, with the Treaty of Utrecht, the English gained control of the south and north shores of the island. Permanent settlement increased rapidly by the late 18th century, peaking in the early years of the 1800s.

The colonization of Newfoundland began in earnest in the early 19th century mainly from Southwest England and Southeast Ireland. Fisherman brought their families to the island, intending to settle permanently, and these families are the founders of much of today's Newfoundland population. Ninety percent of Newfoundland's population descends from roughly 30 000 founders (Parfrey et al. 2009). Families settled in small inlets along Newfoundland's coast in groups of one or two families. These communities developed in geographical and cultural isolation, and can be characterized by large families, and a strong founder effect (Bear et al. 1987). This isolation also directly led to many generations of interbreeding (Poole & Cuff, 1994; Hancock, 1989).

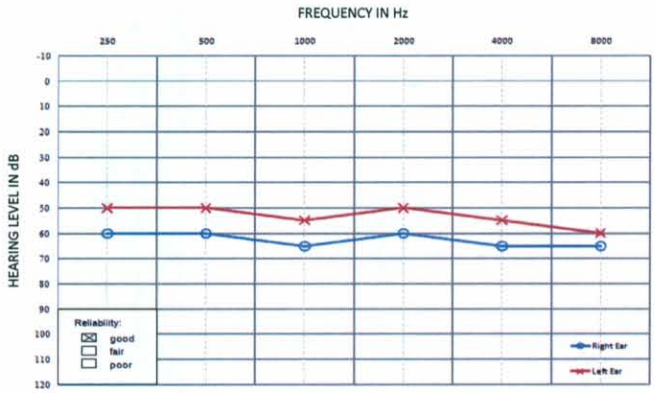
families, and a strong founder effect (Bear et al. 1987). This isolation also directly led to many generations of interbreeding (Poole & Cuff, 1994; Hancock, 1989).

Large extended pedigrees from genetic isolates have been instrumental in the identification of genetic causes of hereditary disorders. Several founder mutations have been identified in Newfoundland. For example, an exon 8 deletion in *MSH2*, a gene causing hereditary non-polyposis colorectal cancer, has been found in five different Newfoundland families (N=74 carriers), and an intron 5 splice site mutation (c.942+3A>T) has been found in 12 different Newfoundland families (N=151 carriers) (Frogatt et al, 1996; Stuckless et al. 2006). A third example is a founder mutation (c.782+3delGAG) found in the deafness gene *TMPRSS3* in two different Newfoundland families (Ahmed et al. 2004; Young et al. unpublished data). These are just a few examples of founder mutations identified in Newfoundland, and serve to highlight the importance of genetically isolated Newfoundland families in the study of hearing loss. At the beginning of this study, only six hearing loss associated genes had been identified in the Newfoundland population (Table 1.2). There is a possibility that a founder mutation exists and could be found in some of these 28 Newfoundland families. When a founder mutation is identified, the prevalence of this mutation in different worldwide populations can be compared, and better estimates of risk for individuals in the founder population can be calculated. The presence of founder mutations in Newfoundland could thus have strong clinical implications in terms of improved diagnosis and the ability to routinely screen individuals if a founder mutation is common enough in the population.

with Bardet-Biedl syndrome (Webb et al. 2009). So while many Newfoundlanders can trace their roots to roughly 30 000 founders, it is critical to keep in mind that these founders came from different towns and different regions. The current population of Newfoundland & Labrador, according to a 2006 census, is 505 469. A map of Newfoundland & Labrador is seen in Figure 1.3.

A)

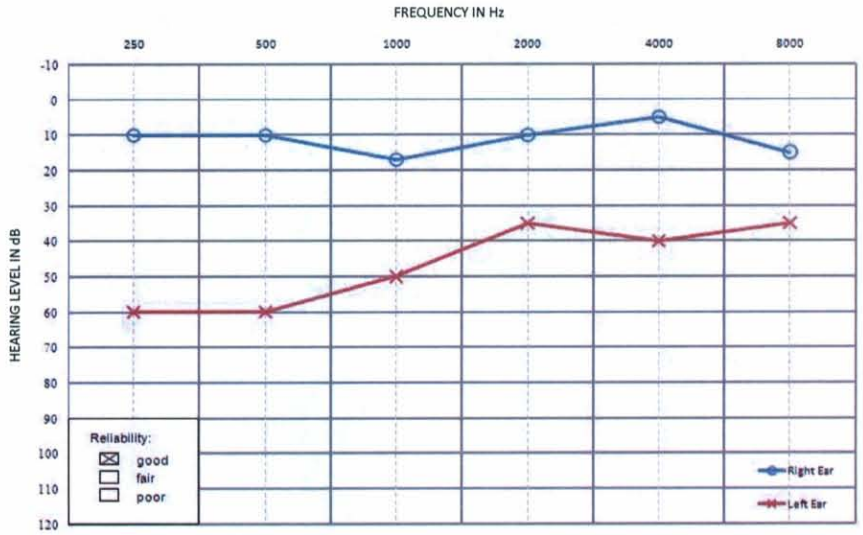

B)




C)


**Figure 1.1 Examples of Audiograms. A) Audiogram of an individual with normal hearing in both ears. B) Audiogram of an individual with moderate hearing loss in both ears. C) Audiogram of an individual with severe hearing loss in both ears.**

A)




B)

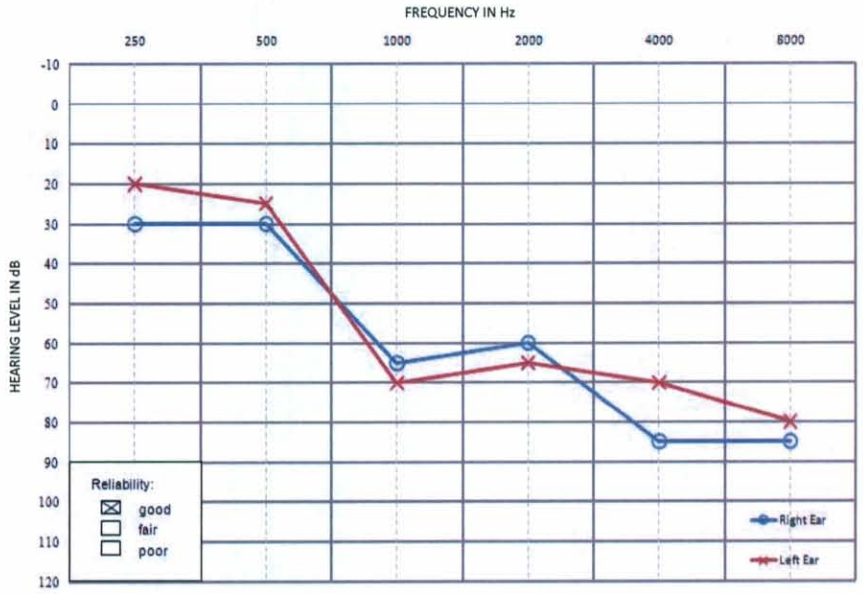



**Figure 1.2 Examples of More Complex Audiograms A) Audiogram of an individual with moderate hearing loss in the low frequencies sloping upwards to mild hearing loss in the mid- to high-frequencies for the left ear only. Hearing in the right ear is normal. B) Audiogram of an individual with progressive hearing loss showing a mild loss at low frequencies which slopes downwards to a severe bilateral loss in the mid- to high-frequencies.**

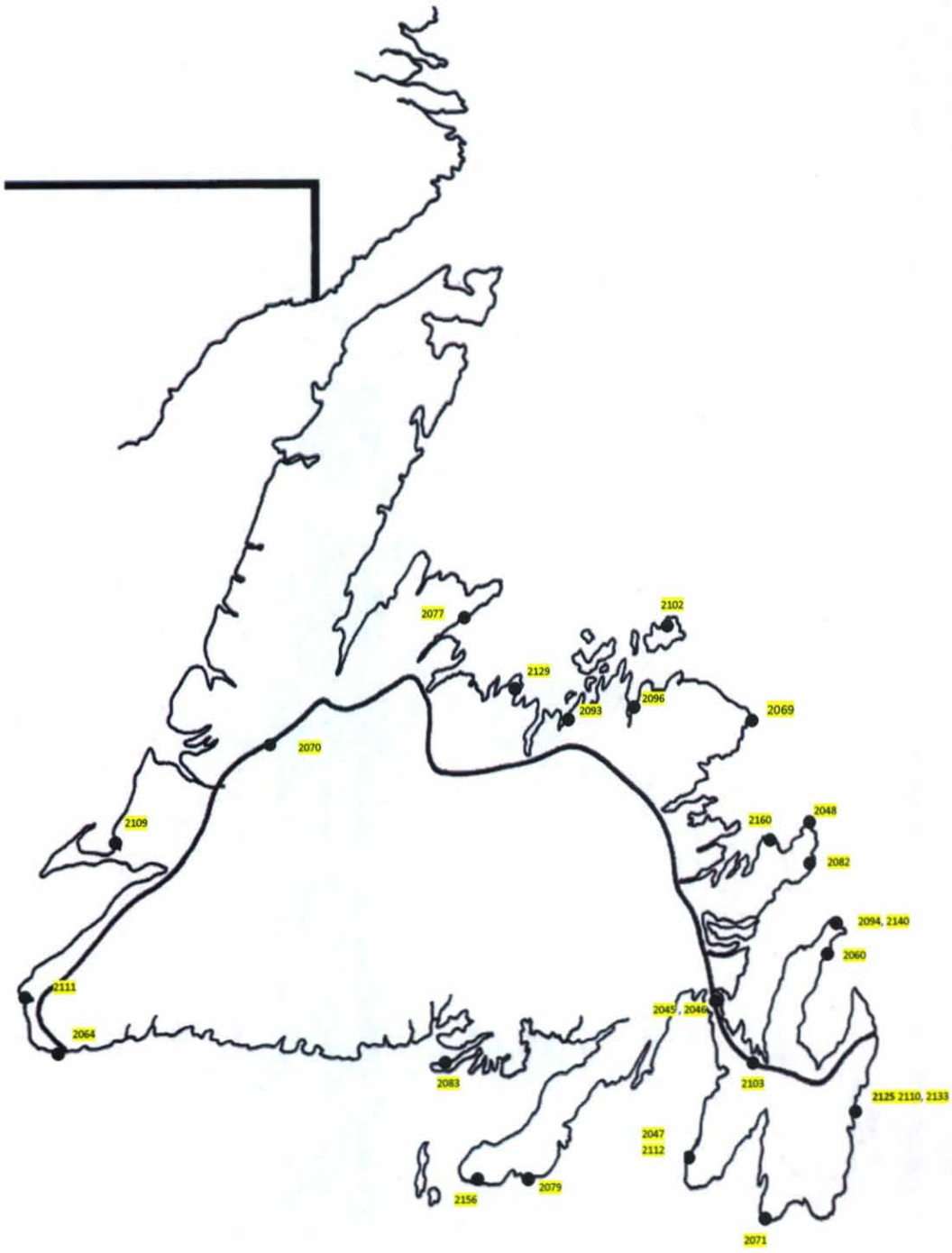



**Figure 1.3 Map of the Island of Newfoundland. AD Families under investigation in this study are indicated with their geographic location.**

**Table 1.1 AD Non-Syndromic Deafness Genes Identified Worldwide to Date (Van Camp G, Smith RJH. Hereditary Hearing Loss Homepage. http://hereditaryhearingloss.org, May 2010)**

| AD Deafness Genes | Protein Encoded | Function |
|---|---|---|
| *CRYM* | Mu-crystallin homolog | Ion Homeostasis |
| *DIAPH1* | Protein diaphanous homolog 1 | Hair Bundle, Cytoskeletal Formation |
| *GJB3* | Gap junction beta-3 protein | Ion Homeostasis |
| *KCNQ4* | Potassium voltage-gated channel subfamily KQT member 4 | Ion Homeostasis |
| *MYH14* | Myosin-14 | Unknown |
| *DFNA5* | Non-syndromic hearing impairment protein 5 | Unknown |
| *WFS1* | Wolframin | Ion Homeostasis |
| *TECTA* | Alpha-tectorin | Extracellular Matrix |
| *COCH* | Cochlin | Extracellular Matrix |
| *EYA4* | Eyes absent homolog 4 | Transcription Factor |
| *COLL11A2* | Collagen, type XI, alpha 2 | Extracellular Matrix |
| *POU4F3* | POU domain, class 4, transcription factor 3 | Transcription Factor |
| *MYH9* | Myosin, heavy chain 9, non muscle | Hair Bundle, Motor Protein |
| *ACTG1* | Actin, gamma 1 | Hair Bundle, Cytoskeletal Formation |
| *MYO6* | Myosin-6 | Hair Bundle, Motor Protein |
| *TFCP2L3* | Grainyhead-like 2 | Transcription Factor |
| *MYO1A* | Myosin-Ia | Unknown |
| *GJB2* | Gap junction beta-2 protein | Ion Homeostasis |
| *GJB6* | Gap junction beta-6 protein | Ion Homeostasis |
| *MYO7A* | Myosin VIIa | Hair Bundle, Motor Protein |
| *TMC1* | Transmembrane channel-like protein 1 | Unknown |
| *CCDC50* | Coiled-coil domain-containing protein 50 | Hair Bundle, Cytoskeletal Formation |

**Table 1.2 Deafness Genes Identified in the Newfoundland Population at Beginning of This Study.**

| Gene | Mutation | # of NL Families | Literary Reference |
|---|---|---|---|
| *GJB2* | c.35delG | 7 | Denoyelle et al. 1997 |
| *GJB6* | D13S1830 | 3 | Del Castillo et al. 2002 |
| *TMPRSS3* | c.207delC | 1 | Ahmed et al. 2004 |
| *TMPRSS3* | c.782+3delGAG | 2 | Ahmed et al. 2004 |
| *PCDH15* | c.1978T>A | 1 | Ahmed et al. 2003 |
| *WFS1* | c.2146G>A | 1 | Young et al. 2001; |

# Chapter 2: Methods & Materials

## Human Subjects & Pedigrees

This study is one part of a larger study to determine the genetic cause of hearing loss in Newfoundland & Labrador. Family members were recruited through the Newfoundland Provincial Genetics Program, and a province-wide ascertainment drive. Informed consent was obtained from all participants, granting researchers permission to access medical records and family history. Blood samples were collected and genomic and mitochondrial DNA was extracted from peripheral leukocytes from participants. Audiological tests were performed to determine the type of hearing loss of each subject and to confirm normal hearing in unaffected subjects. Audiograms are available for all individuals marked with an asterisk in Figure 2.1, and for each of these individuals several audiograms are available at different test ages, with new ones routinely being collected. DNA from several Dutch individuals was provided by Dr. Hannie Kremer of the Radboud University Medical Centre in Nijmegen, Netherlands. This project was approved by The Human Investigations Committee (HIC) (Research Ethics Board of Memorial University, Newfoundland & Labrador) (# 01.186).

So far, 128 probands have been recruited to the study. All probands in the study were routinely screened for mutations previously identified to cause hearing loss in the Newfoundland population. Of these, 28 probands are members of multiplex families with a family history of hearing loss consistent with AD inheritance, and were chosen for this

study (Figure 2.1). Inheritance patterns were determined through an extensive family history questionnaire, and pedigrees were electronically stored using the computer program Progeny. Many individuals' hearing loss was determined by word of mouth from family members. In these cases, the age of onset and the degree and severity of hearing loss are not known. For this reason, the determination of an AD pattern of inheritance is not certain in some cases, but these families were deemed to likely have an AD form of hearing loss, and it was therefore worth testing them for potential AD hearing loss mutations. Due to the extent of genealogy work possible in Newfoundland up to this point, it is important to keep other potential forms of inherited hearing loss, such as mitochondrial inheritance, in mind when searching for causative mutations.

## Experimental Design: Functional Candidate Gene Mutation Screening

Genomic DNA from probands (n=28) was screened using a functional candidate gene approach. A comprehensive literature search was done to collect information on all AD hearing loss genes. One recent review (Hilgert et al. 2009) discussed in depth the genes causing AD hearing loss. It describes how each gene associated with AD hearing loss functions in the ear, and what types of hearing loss they cause. For each of these genes, the mutations found both worldwide and within Caucasian populations are described in detail. Many of these genes may be causative in Newfoundland families (Table 2.1).

This literature search formed the basic foundation from which genotype-phenotype evaluation was performed. Potential candidate genes were investigated for specific case-by-case details on the hearing loss phenotype each mutation caused, and in what population and ethnicity they were reported. These phenotypes were then cross checked with the phenotypes of the 28 families to further narrow down the functional candidate gene list to four: *COCH, KCNQ4, TECTA,* and *MYO1A* (see Figure 2.2). This approach is a form of audioprofiling, a method of categorizing phenotypic data to make genotypic correlations. The audiological data of several members in a family, or in this case several probands from different families, associates with a specific unknown genotype as a function of time (Meyer et al. 2007). From this, we have drawn correlations to the overall phenotype of the group of probands and used this as a foundation for selecting candidate genes previously reported to cause hearing loss with a similar phenotype.

Information on all known hearing loss mutations in these four candidate genes was next collated, including which domain and exon each mutation was reported in (Appendix A). This allowed the identification of the exons most likely to harbor causative mutations in the Newfoundland probands. For example, the majority of mutations reported in *COCH* are reported in the factor c homologous (FCH) domain, spanning exons 4 and 5, and so this area of *COCH* was screened first.

*COCH* has a total of 12 exons, and causes a late-onset, progressive hearing loss most often associated with vestibular dysfunction (Kemperman et al. 2005), which correlates with several AD Newfoundland families. *COCH* is important in maintaining

structural support within the cochlea (Kommareddi et al. 2007). Exons screened were 2-5, and 12. Two deletions within *KCNQ4* have been reported to cause a late-onset, progressive hearing loss (Coucke et al. 1999; Kamada et al. 2006), matching the phenotype of many AD Newfoundland probands. This gene is critical in ion homeostasis with the ear (Kubisch et al. 1999), and is coded by 14 exons, all of which were screened. Missense mutations within *TECTA*, a second gene important in structural support within the auditory system, have been shown to cause late onset hearing loss (Verhoeven et al. 1998). Coded by 23 exons in total, exons 5, 9-14, 17, 18, and 20 were screened. The last candidate gene, *MYO1A* is thought to play a role in sound processing through ion transport (Donaudy et al. 2003), and again, causes a late-onset, progressive hearing loss phenotype. Coded by a total of 28 exons, exons 3, 4, 6, 7, 10-12, 18, and 22 were screened.

While the more "targeted" candidate gene approach outlined above is likely to lead to the identification of mutations, it does restrict the chance of identifying potential "genetic surprises" regarding genotype-phenotype. This method allows for a complete investigation of possible genetic mutations in a given set of promising candidate genes within the two-year time frame of a Master's thesis. However, exons within these candidate genes that have never before been associated with hearing loss mutations are bi-directionally sequenced in this study, and so there is potential for "genetic surprises".

Because Newfoundland is a founder population, it is possible that families would share the same mutation, particularly if those families are from the same geographic area. When a mutation is discovered in an AD Newfoundland proband, all otosclerosis, AD,

and AR Newfoundland probands (n=68) are subsequently screened for that mutation. When appropriate, apparent founder mutations are confirmed by haplotype analysis, to determine the level of sharing for a selection of linked microsatellite markers between families with the same mutation (see Figure 2.2).

## General Strategy for PCR and Sequencing of Candidate Genes

Both forward and reverse strands of specific exon PCR products in each gene were bi-directionally sequenced, along with all intron/exon boundaries to ensure the entire coding region of each exon was covered. PCR primers were designed using Primer 3 software (v. 0.4.0, http://frodo.wi.mit.edu/primer3/). These primer sequences can be found in Appendix B.

## DNA Preparation, PCR Thermocycling, and Electrophoresis

DNA was extracted from whole blood and diluted to10 ng/µl. This blood was stored at 4 °C (performed by research assistant). 1 µL of diluted (stock) DNA was added to 2 µL 10X PCR Buffer (containing $MgCl_2$), 0.4 µL dNTPs (10 mM), 0.08 µL KapaTaq DNA Polymerase (5 U/µL) (Kapa Biosystems, Boston, MA), 12.92 µL of distilled $dH_2O$, 1.0 µL of forward primer (10 µM) and 1.0 µL of reverse primer (10 µM), as per standard PCR protocol. The amount of $dH_2O$ was reduced to add betaine or Dimethyl Sulfoxide (DMSO) when necessary to achieve a successful amplified PCR product. This mix was

centrifuged and addeqd to wells in 20 μL aliquots in a 96-well PCR plate, where it was then sealed, centrifuged, and placed in the GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA). PCR products were electrophoresed on a 1% agarose gel (1.5 g agarose/100 mL TBE) stained with SybrSafe and viewed under UV light on a Kodiak GEL LOGIC 100 Molecular Imaging system (Rochester, NY, Version 4.01, 2005).

## Preparation for ABI Cycle Sequencing

Sephacryl S-300HR was resuspended and 300 μL aliquots were added to wells on a Millipore Multi-screen HTS plate, which was placed over a corresponding 96-well waste plate to catch flow-through. Plates were then balanced and centrifuged at 3000 rpm for 5 minutes. Flow-through was discarded and PCR products were added to wells on the Multi-screen HTS plate. The Multi-screen HTS plate was then positioned and placed over a clean PCR plate, balanced, and centrifuged at 3000 rpm for 5 minutes. The flow-through product collected in the PCR plate contains the purified PCR products.

Successfully amplified PCR products, visualized as bands of the correct size (using a 100 bp marker) when electrophoresed on an agarose gel, were cycle sequenced using the following reaction mix: 0.5 μL of Big Dye Terminator V. 3.1 Sequencing Mix, 2 μL of 5X sequencing buffer, 0.32 μL of Primer (10 μM), 1 μL of purified PCR product DNA template, and 16.18 μL of $dH_2O$, per the Big Dye Terminator V. 3.1 protocol (Applied Biosystems, Foster City, CA). This equals a total reaction volume of 20 μL per

well in a sequencing plate. The resulting plate was centrifuged briefly, loaded onto the thermal cycler, and subjected to a thermal cycling program according to ABI BDT V. 3.1 protocol (Applied Biosystems, Foster City, CA).

Upon completion, 5 µL of 125 mM EDTA followed by 65 µL of 95% Ethanol (EtOH) was added to each reaction well. Plates were briefly centrifuged and then incubated overnight in the dark at ambient temperature. The plate was then centrifuged at 3000 x g for 30 minutes, inverted to decant EtOH, and briefly centrifuged while inverted at 200 rpm for 4 – 5 seconds with folded paper towels placed underneath the sequencing plate to absorb residual ethanol. 150 µL of 70 % EtOH was added to each sample, and the plate was centrifuged at 3000 g for 15 minutes. The plate was again inverted to decant ethanol and spun at 200 rpm for 4 – 5 seconds over a paper towel. Samples were left to air dry in the dark at room temperature for 10 - 15 minutes. 15 µL of Hi-Di Formamide was subsequently added to each well and the plate was vortexed and centrifuged briefly. The final mix was denatured at 95 °C for 2 minutes on a thermal cycler. Once denatured, samples were kept on ice until placed in the ABI 3130 XL DNA Analyzer.

## Automated Sequencing Using the ABI 3130 XL

Automated sequencing was performed using either the ABI 3130 XL DNA Analyzer (Applied Biosystems, Foster City, CA) available in the lab or the ABI 3730 DNA Analyzer in the Genomic & Proteomics Facility, at CREAIT, Memorial University of Newfoundland. The raw sequence data were initially analyzed for quality using

Sequencing Analysis software (Version 5.2, Applied Biosystems, Foster City, CA). High quality sequences were imported into Mutation Surveyor (Version 3.0, Softgenetics, State College, PA). Mutation Surveyor identifies DNA sequence variants in the sample sequence DNA by comparing it to a reference gene sequence.

## Tracing Variants Through Families: Genotype & Haplotype Analysis

Sequencing variants were traced through the pedigrees to see if they co-segregated with hearing loss. Haplotype analysis was performed when necessary. Table 3.4 is a list of microsatellite markers used to characterize the p.P51P/S haplotype shared between a Newfoundland family and a Dutch family. This was done to confirm the founder hypothesis for the *COCH* mutation p.P51P/S (de Kok et al. 1999), identified in this study in a Newfoundland proband. Markers were selected based on location as well as degree of heterozygosity in order to confirm haplotype sharing between the two families, which provided further evidence that p.P51P/S is a Dutch founder mutation.

Initial setup for genotyping required running PCR under standard conditions. Each reaction mix contained 8.5 μL of Hi-Di Formamide, 0.5 μL Genotyping Size Standard *GS500* (*-250*) *LIZ,* and 1 μL of DNA, per manufacturers standard protocol (Applied Biosystems, Foster City, CA). Post-PCR products were electrophoresed on a 1% agarose gel (1 g agarose/100 mL TBE, pH 8.0) containing 4.0 μL of SybrSafe 10-000 X concentrate in DMSO (Invitrogen, Eugene, OR) and viewed under UV light on a

Kodiak GEL LOGIC 100 Molecular Imaging system (Rochester, NY, Version 4.01, 2005). The PCR product was then diluted based on its band intensity to a suitable concentration. Optical plates containing these samples were briefly vortexed and then centrifuged at 1250 rpm for 10 seconds, denatured on a thermal cycler, and immediately loaded onto the ABI 3130 XL DNA Analyzer for genotyping. The PCR products from the fluorescently labeled primers were detected by the ABI Prism 3130 XL DNA Analyzer and genotyped using GeneMapper Software (ABI Prism, Version 4.0).

GeneMapper assisted in making allele calls at each marker for each individual, which were then compared with other individuals and families. Once a pedigree was constructed using the software Progeny (Progeny Software LLC, Delray Beach, FL), markers were integrated for each selected individual. Allele calls, SNPs, and/or common variants were then inputted into each individual's data set to create a pedigree illustrating the segregation of different haplotypes (Progeny Software LLC, Delray Beach, FL; Figure 3.5; Figure 3.10).

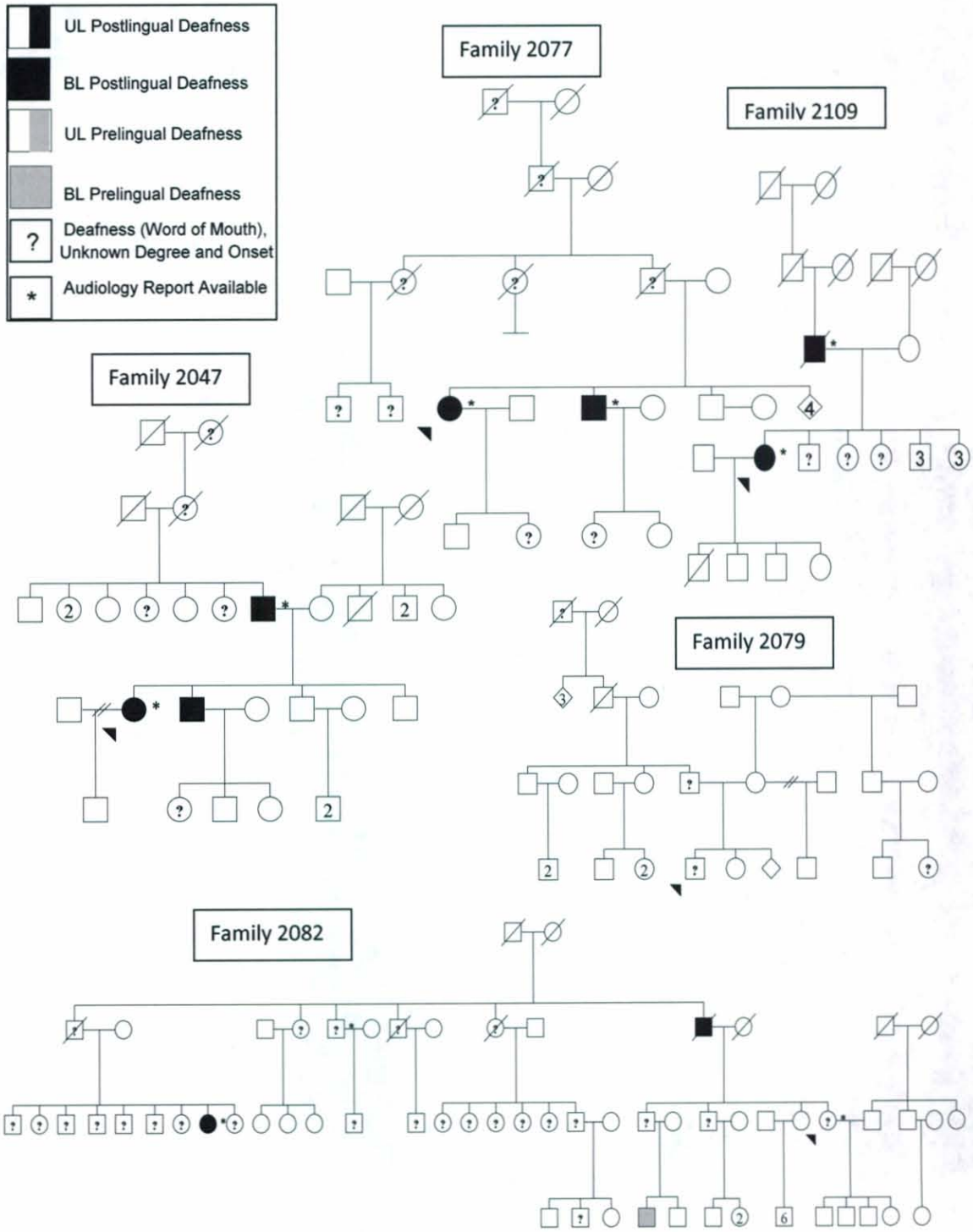



**Figure 2.1 28 Newfoundland Families with AD hearing loss**

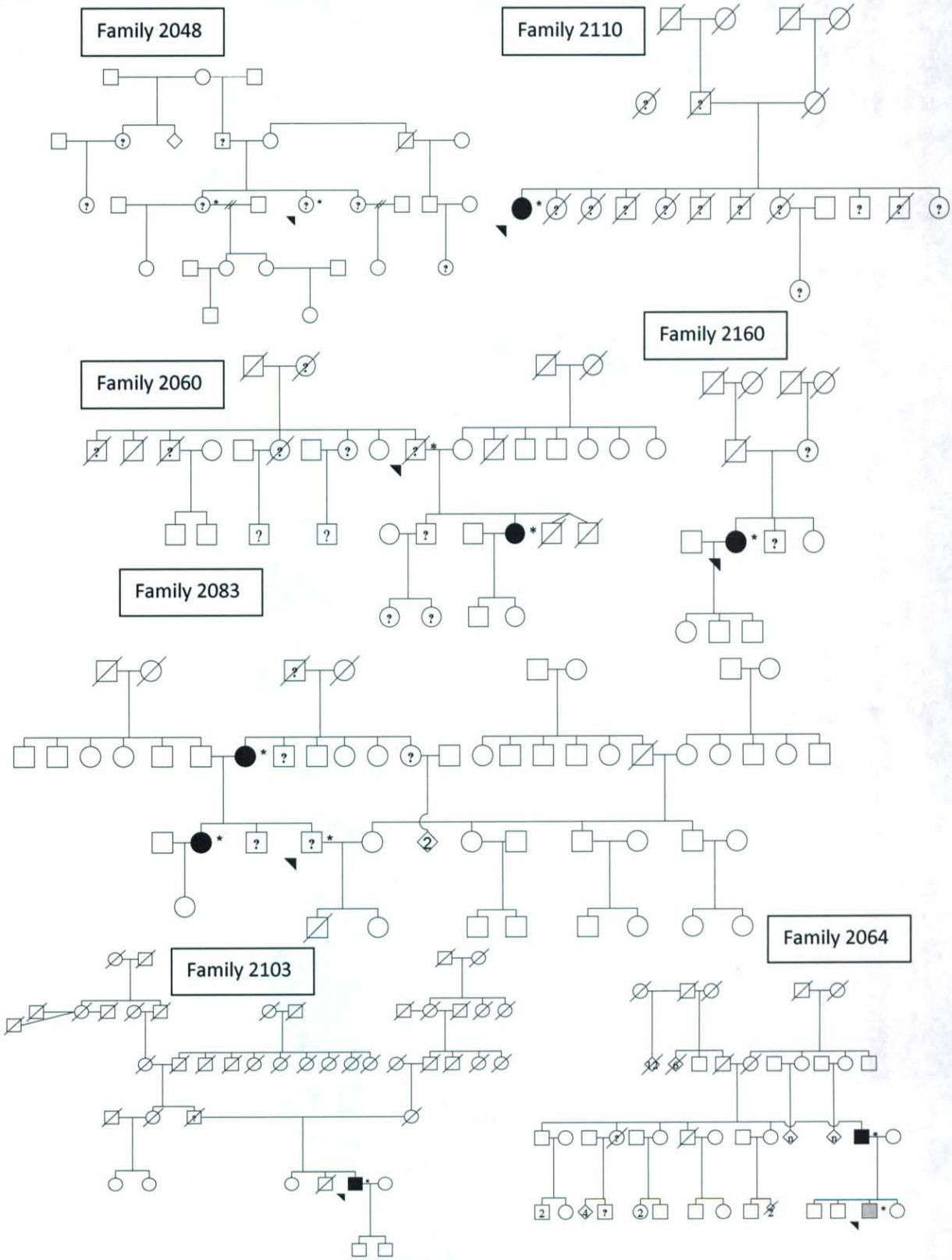



**Figure 2.1 28 Newfoundland Families with AD hearing loss (cont).**

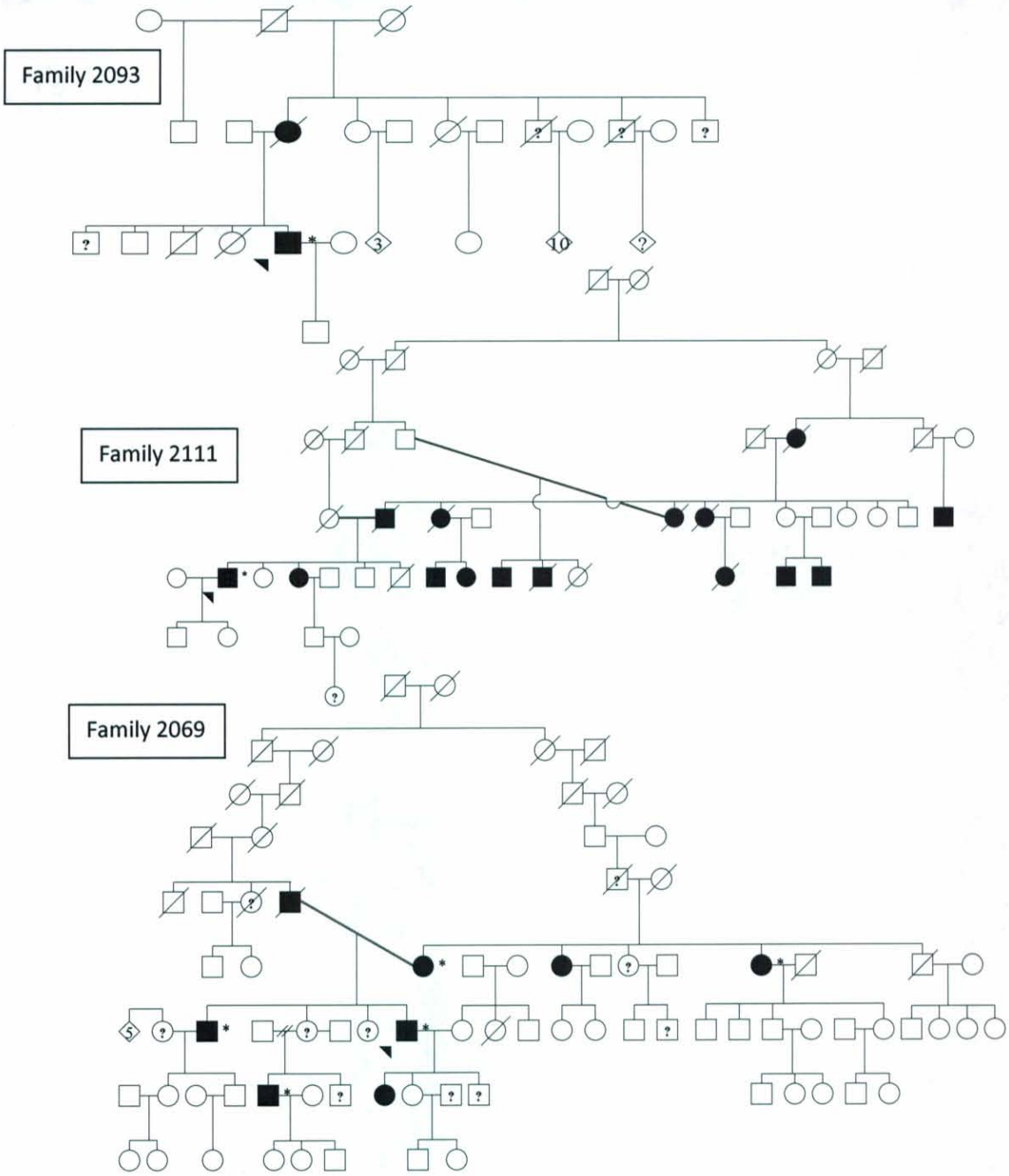



**Figure 2.1 28 Newfoundland Families with AD hearing loss (cont).**

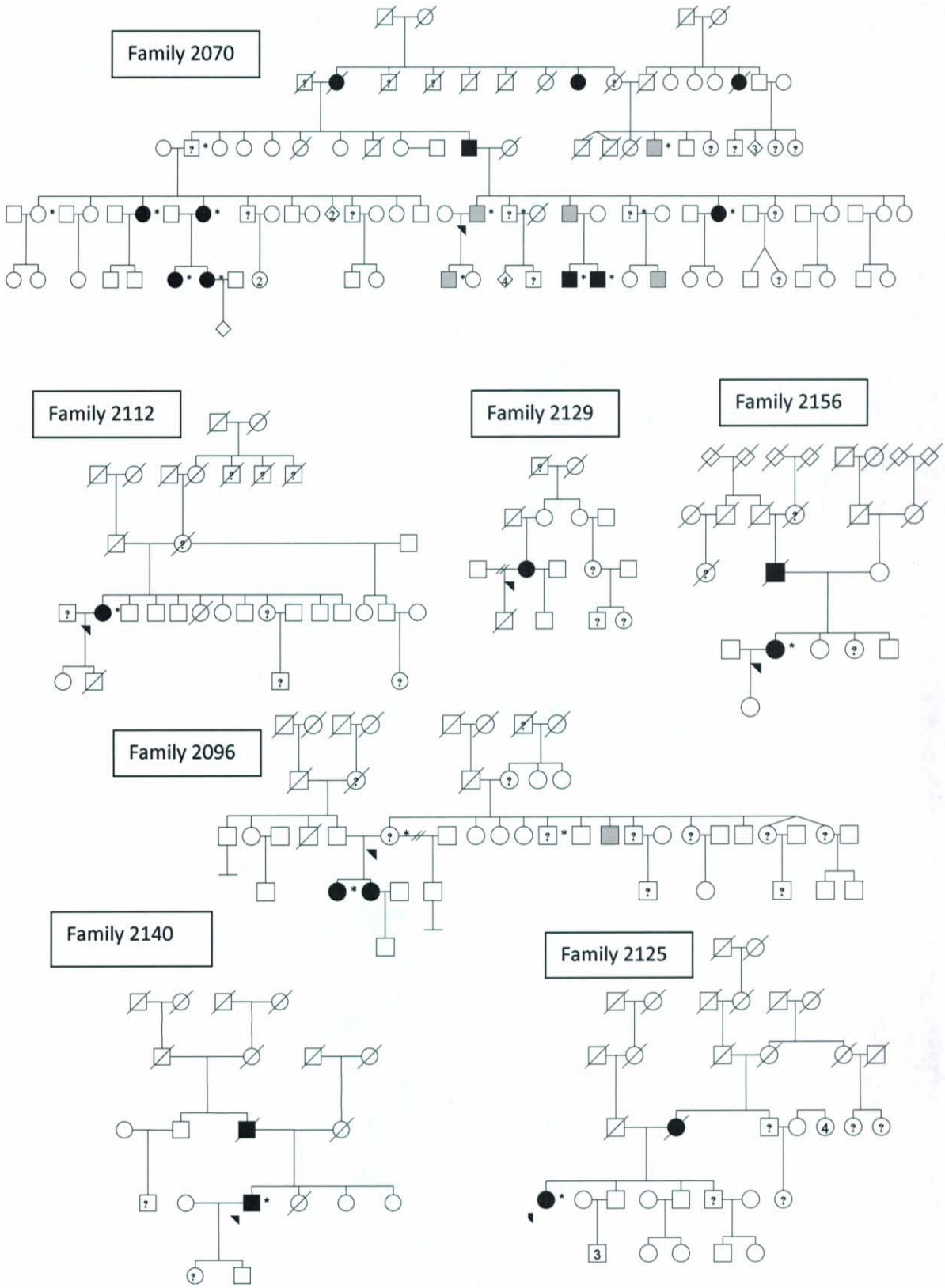



**Figure 2.1 28 Newfoundland Families with AD hearing loss (cont).**

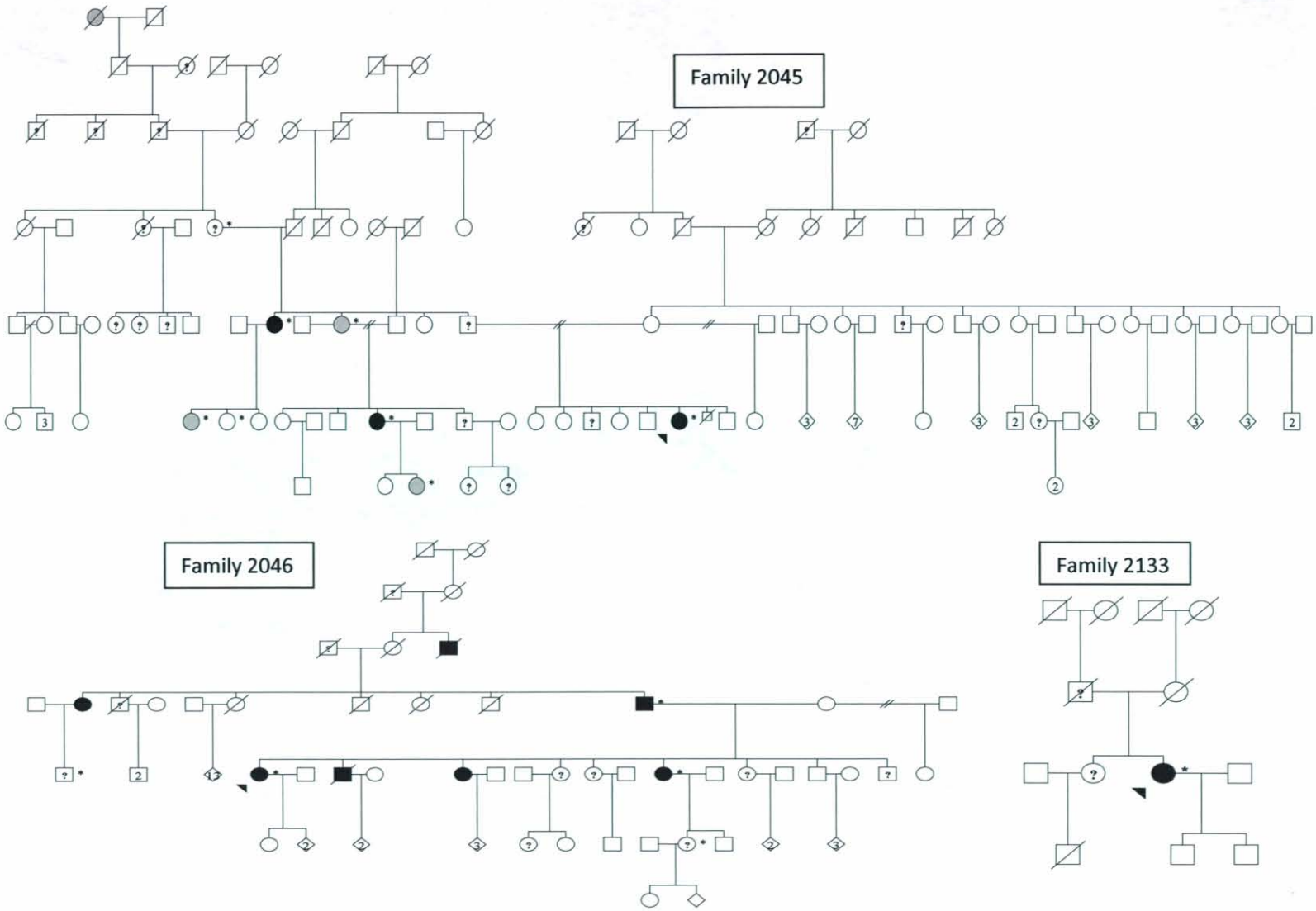



**Figure 2.1 28 Newfoundland Families with AD hearing loss (cont).**

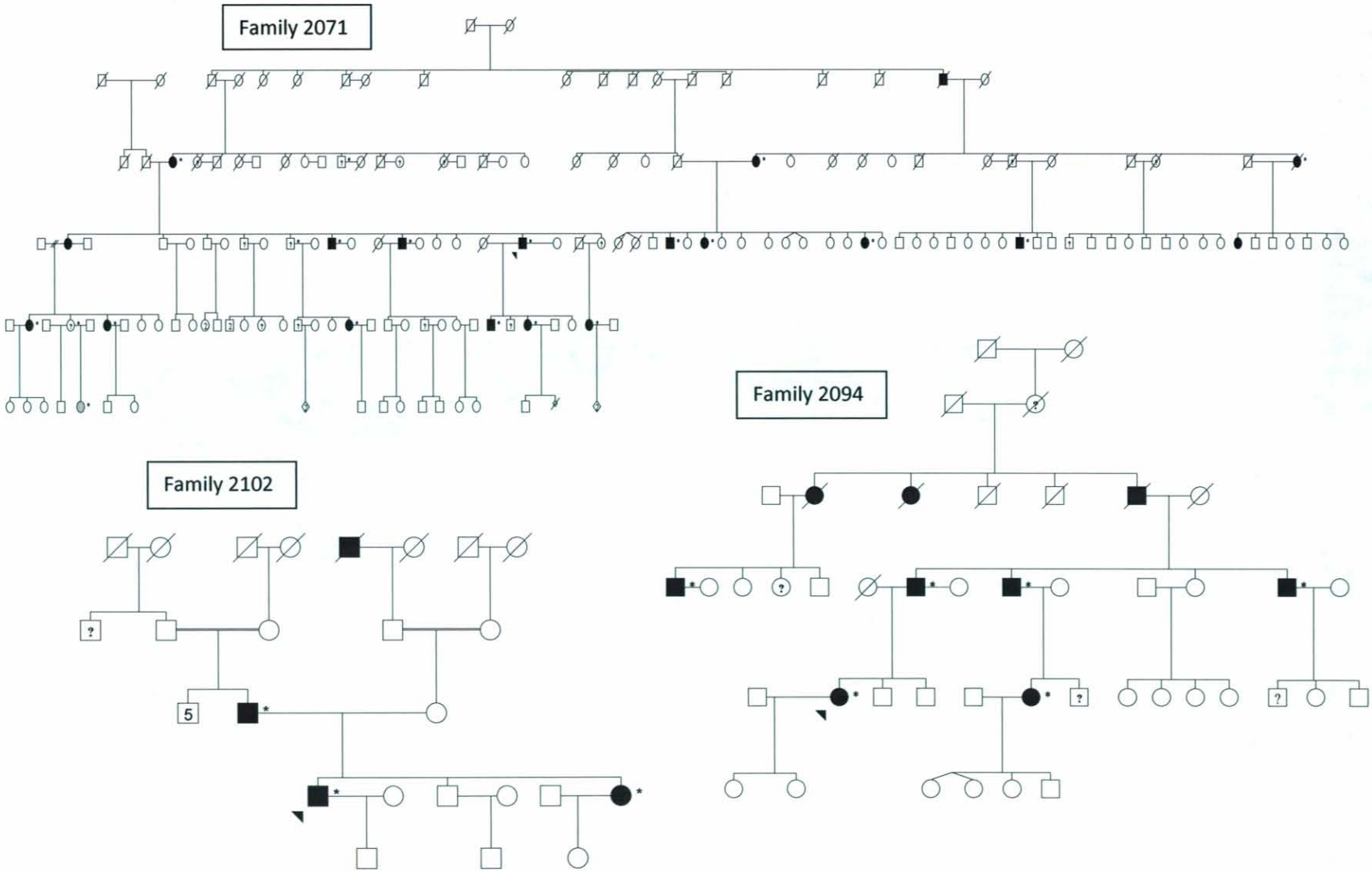



**Figure 2.1 28 Newfoundland Families with AD hearing loss (cont).** Causative hearing loss mutations were identified in these three families.

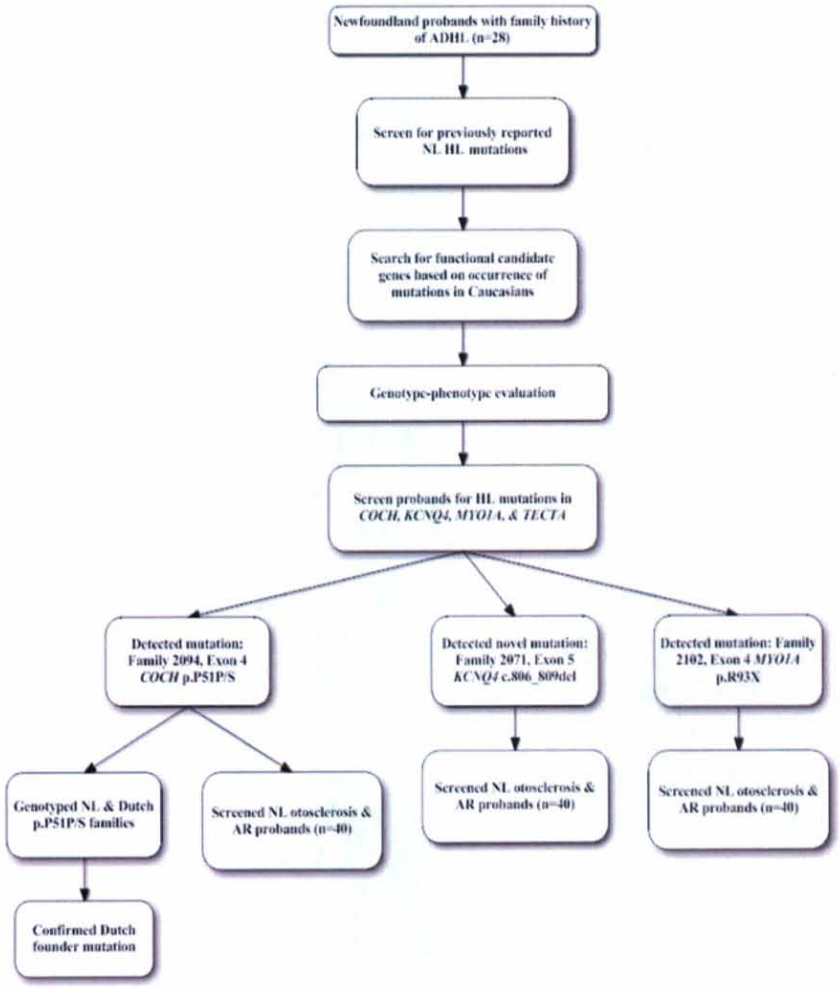



**Figure 2.2 Flow Chart Demonstrating Experimental Design and Progression**

**Table 2.1 Candidate genes for Newfoundland Families Having Late-Onset AD Hearing Loss (Adapted from Hilgert et al. 2009).**

| Candidate Gene | Number of Mutations Found Worldwide | Number of Mutations Found in Caucasians | Function | Original Reference |
|---|---|---|---|---|
| *KCNQ4* | 12 | 8 | Ion Homeostasis | Kubisch et al. 1999 |
| *COCH* | 12 | 8 | Extracellular Matrix | Robertson et al. 1998 |
| *MYO1A* | 8 | 8 | Unknown | Donaudy et al. 2003 |
| *TECTA* | 8 | 7 | Extracellular Matrix | Verhoeven et al. 1998 |
| *ACTG1* | 6 | 6 | Hair Bundle, Cytoskeletal Formation | Zhu et al. 2003; Van Wijk et al. 2003 |
| *EYA4* | 6 | 4 | Transcription Factor | Wayne et al. 2001 |
| *MYH14* | 5 | 5 | Unknown | Donaudy et al. 2004 |
| *MYO6* | 5 | 5 | Hair Bundle, Motor Protein | Melchionda et al. 2001 |
| *MYO7A* | 5 | 4 | Hair Bundle, Motor Protein | Liu et al. 1997 |
| *ESPN* | 4 | 4 | Hair Bundle, Cytoskeletal Formation | Naz et al. 2004 |
| *DFNA5* | 4 | 2 | Unknown | Van Laer et al. 1998 |
| *GJB3* | 3 | 1 | Ion Homeostasis | Xia et al. 1998 |
| *POU4F3* | 3 | 2 | Transcription Factor | Vahava et al. 1998 |
| *TMC1* | 2 | 2 | Unknown | Kurima et al. 2002 |
| *COL11A2* | 2 | 2 | Extracellular Matrix | McGuirt et al. 1999 |
| *CRYM* | 2 | 0 | Ion Homeostasis | Abe et al. 2003 |
| *TFCP2L3* | 1 | 1 | Transcription Factor | Peters et al. 2002 |

**Table 2.1 Candidate Genes for Newfoundland Families Having Late-Onset AD Hearing Loss (Adapted from Hilgert et al. 2009) (cont).**

| | | | | |
|---|---|---|---|---|
| *MYH9* | 1 | 1 | Hair Bundle, Motor Protein | Lalwani et al. 2000 |
| *CCDC50* | 1 | 1 | Hair Bundle, Cytoskeletal Formation | Modamio-Hoybjor et al. 2007 |
| *DIAPH1* | 1 | 0 | Hair Bundle, Cytoskeletal Formation | Lynch et al. 1997 |

# Chapter 3: Results

## Overview

The purpose of this study was to determine the genetic etiology of hearing loss in 28 autosomal dominant hearing loss Newfoundland probands using a functional candidate gene approach. Candidate genes *COCH, TECTA, KCNQ4,* and *MYO1A* were chosen primarily because they have a higher frequency of mutations in autosomal dominant families (Hilgert et al. 2009). The full *GJB2* gene and the del13S1830 mutation in *GJB6,* which underlies the majority of congenital deafness worldwide, were first excluded in all 28 probands. A heterozygous mutation in *WFS1* - a gene now known to cause both syndromic and non-syndromic deafness (Young et al. 2001; Bespalova et al. 2001) - previously found to cause autosomal dominant hearing loss (p.A716T) in a large Newfoundland family (Young et al. 2001) was also excluded in all 28 probands. Figure 2.2 illustrates the research projects experimental progression.

Results of candidate gene screening revealed three distinct mutations causing hearing loss in three separate Newfoundland families. First, a C→T base change in exon 4 of *COCH* in the proband of Family 2094 resulted in the substitution of a conserved proline residue for a serine residue at amino acid position 51 (p.P51P/S). Second, a novel 3bp heterozygous deletion in exon 5 of *KCNQ4* was found in the proband of Family 2071. Third, a nonsense mutation was discovered at amino acid position 93 within exon 4 of *MYO1A*. This nonsense mutation, p.R93X, is due to a C→T nucleotide change, and was found in the proband of Newfoundland Family 2102.

## Family 2094

Within *COCH,* exons 2, 3, 4, 5, and 12 were sequenced in all 28 AD hearing loss families. Of the 28 probands, one proband was identified with a C→T base change in exon 4 of *COCH.* The proband is a member of a family (Family 2094) with four generations of documented hearing loss. The complete pedigree documents 44 individuals and extends back six generations. A partial pedigree is seen in Figure 3.1. Twelve family members have been diagnosed with AD hearing loss. A summary of audiology reports and a phenotype summary are found in Tables 3.2 and 3.3 respectively. The proband (V-2, Figure 3.1), is a 39-year-old female presenting with hearing loss. Reports showed a bilateral hearing loss sloping to moderate loss at high-frequencies (Figure 3.3). Three more audiology tests were conducted over the next three years, showing a gradual worsening of high-frequency hearing loss.

The proband is heterozygous for the C→T base change in exon 4 of *COCH,* which substitutes a highly conserved proline residue for a serine residue at amino acid position 51 (p.P51P/S) (Figure 3.2). Upon identification of p.P51P/S in the proband, DNA from all available individuals was amplified and sequenced for exon 4. Seven out of seven with documented hearing loss harbored the C→T transition; one unaffected (IV-10) did not.

## Search for a Vestibular Phenotype in Family 2094 Mutation Carriers

Previous studies of mutation carriers of p.P51P/S show severe vestibular phenotypes associated with hearing loss (de Kok et al. 1999), so medical and audiological records for all available family members were reviewed (see Figure 3.1). The proband (V-2) has not yet had any episodes of vertigo or associated vestibular problems. She had a Computed Tomography (CT) scan at 40 years of age which did not detect any abnormalities. The probands cousin, V- 6, complained of episodes of dizziness and vertigo at age 32. She has had two CT scans at ages 36 and 38, which did not detect any abnormalities. Her hearing loss was first reported at age 35 as mild hearing loss in the high-frequency range. This cousin also first reported a scratch on her left cornea at age 31. IV-1 has stated he has had balance problems when walking at night since his early 50's. He had a CT scan at age 62, which detected no abnormalities. His hearing loss was diagnosed at age 49 as moderate in the high-frequency range. IV-5 reported balance problems and episodes of dizziness from the age of 50. Audiology testing at age 57 showed a moderate bilateral loss that over the following 15 years progressed to severe hearing loss across all frequencies. IV-7 presented the typical p.P51P/S hearing loss phenotype, and also had occupational noise exposure. IV-10 is an unaffected woman who has not reported any vestibular problems and is not affected with hearing loss. IV-11 reported spinning dizziness and unsteadiness, along with balance problems in his early 30s. His hearing loss was first documented at age 49, and further audiology tests over the following 15 years show bilateral loss beginning in the high-frequencies and then

flattening out later in life to profound hearing loss across all frequencies. III-6 had documented hearing loss from middle age, but no other data was available.

Because Newfoundland is a founder population, and this study is focused on the identification of founder mutations, all otosclerosis, AD, and AR probands in the Newfoundland hearing loss study (n=40) were screened for this mutation, but no additional cases were found.

## Identification of a Dutch Founder Mutation

As p.P51P/S is widely believed to be a Dutch founder mutation (de Kok et al. 1999; Fransen et al. 2001), we genotyped the seven affected Family 2094 members, along with three Dutch p.P51P/S carriers, for seven microsatellite markers closely flanking the *COCH* gene, (Fransen et al. 2001) in order to construct an ancestral haplotype. Affected Family 2094 individuals and the Dutch affected individuals share a contiguous five-microsatellite-marker haplotype at markers D14S257, D14S1071, D14S1040, D14S1034, and D14S1060: 179-281-234-169-201. *COCH* sits ~0.4 Mb downstream of marker D14S257. These markers closely flank the *COCH* gene and constitute a total minimum shared region of ~2.1 Mb on chromosome 14q12 (Figure 3.4; Table 3.5). The full hearing loss haplotype shared among Newfoundland and Dutch families is shown in their respective pedigrees in Figure 3.5. Unaffected individual IV-10 does not share this haplotype. Further genotyping was conducted for additional microsatellite markers

upstream of D14S1060. Markers D14S70 and D14S1014 both displayed allelic disparity between the two families, demonstrating the relatively short length of this putative ancestral haplotype (Table 3.5).

## Family 2071

Of the 28 probands, one proband was identified to have a novel 3 bp deletion, p.Ser269del in exon 5 of *KCNQ4* (DFNA2A). The proband is a member of a family (Family 2071) with four generations of documented hearing loss. The complete pedigree documents 97 individuals, extends back five generations, and reports no cases of consanguinity (Figure 3.7). Twenty-four family members have been diagnosed with AD non-syndromic hearing loss. A summary of audiology reports for participating individuals is found in Table 3.7, with phenotype data shown in Table 3.6.

The proband (III-12, Figure 3.7) is a 62-year-old male presenting with a bilateral hearing loss sloping to moderate loss at mid-frequencies and profound at high-frequencies. The proband harbors a novel heterozygous 3 bp deletion, p.Ser269del, in exon 5 of *KCNQ4* (Figure 3.8). All available family members were sequenced for exon 5. Thirteen members with documented hearing loss shared p.Ser269del. No unaffected family members (n=18) carried the deletion, and of 90 ethnically matched population controls, none carried the deletion. While p.Ser269del is not seen in four members with hearing loss, these four individuals present a distinctly different audioprofile (Figure 3.9;

Table 3.6). Audiology reports of affected relatives with the novel deletion show a high-frequency, late onset hearing loss (Figure 3.9).

This deletion predicts an in-frame removal of a serine residue at amino acid position 269 within the P-loop domain of the KCNQ4 protein (Figure 3.10). We next constructed an intragenic haplotype using commonly occurring SNPs and variants within and surrounding exon 5 of *KCNQ4*. This was done to determine whether or not any interesting and possibly causative variants within *KCNQ4* were shared by affected individuals of Family 2071, and to determine the level of sharing among of intragenic SNPs and variants among deletion carriers. No markers brought any additional interesting information, and no family members without the deletion, or without hearing loss, shared this hearing loss haplotype.

Upon discovering this novel deletion, all otosclerosis, AD, and AR probands in the Newfoundland hearing loss study (n=68) were screened to determine whether or not any additional Newfoundland families shared the mutation, thereby suggesting a possible founder mutation. However, no additional cases outside of Family 2071 were detected. All exons (1-14) were sequenced in the 28 AD probands for this gene.

## Family 2102

A third proband was identified with a C→T base change in exon 4 of *MYO1A* (DFNA48). The proband is a member of a family (Family 2102) with four generations of

documented hearing loss. The complete pedigree documents 22 individuals, extends back five generations, and indicates two consanguineous marriages in early generations (Figure 3.12). Three family members have been diagnosed with AD non-syndromic hearing loss from age 5 with progressive deterioration. A summary of audiology reports is found in Table 3.9. The proband (IV-1) is heterozygous for a C→T nucleotide change, which substitutes a highly conserved arginine residue for a stop signal at amino acid position 98 in exon 4 of *MYO1A* (Figure 3.13). DNA from four available family members was sequenced for exon 4 of *MYO1A.* Three members with documented hearing loss (III-1; IV-1; IV-5) shared the mutation, and one unaffected (III-2) did not. All affected individuals first reported their hearing loss near the age of five, with hearing coming and going but progressively deteriorating (Figure 3.14).

Again, all otosclerosis, AD, and AR probands in the Newfoundland hearing loss study (n=68) were screened to determine if this was a Newfoundland founder mutation. No additional cases were found. Exons 3-4, 6-7, 10-12, 18, and exon 22 were sequenced.

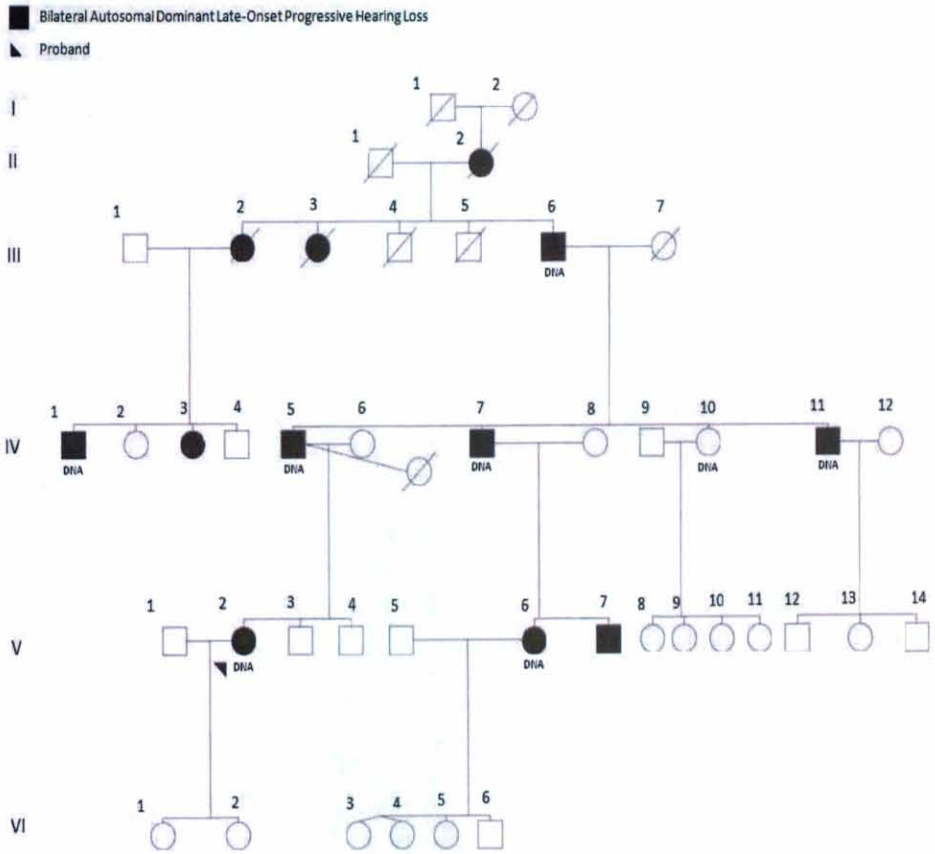



**Figure 3.1 A Six Generation Newfoundland Family (2094) Segregating an Autosomal Dominant Form of Late-Onset Progressive Hearing Loss (partial pedigree). The proband (PID V-2: arrow) was first found to carry the p.P51P/S mutation. DNA from all available affected relatives were screened and also carry the p.P51P/S mutation. Unaffected individual IV-10 does not carry the mutation.**

c.151C>CT:p.P51P/S
PID V-2
Family 2094

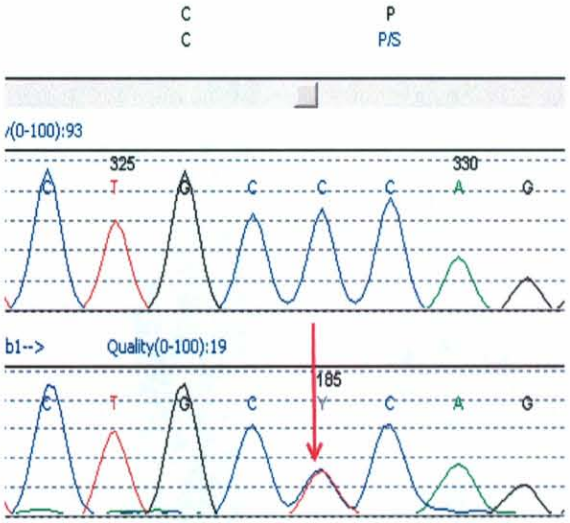



**Figure 3.2 Electropherogram of the Substitution Mutation in *COCH* (c.151C>CT:pP51P/S) Identified in All Affected Family Members. The top trace is from the proband (PID IV-2); the bottom trace is from a reference sequence (obtained from NCBI; NM_004086).**

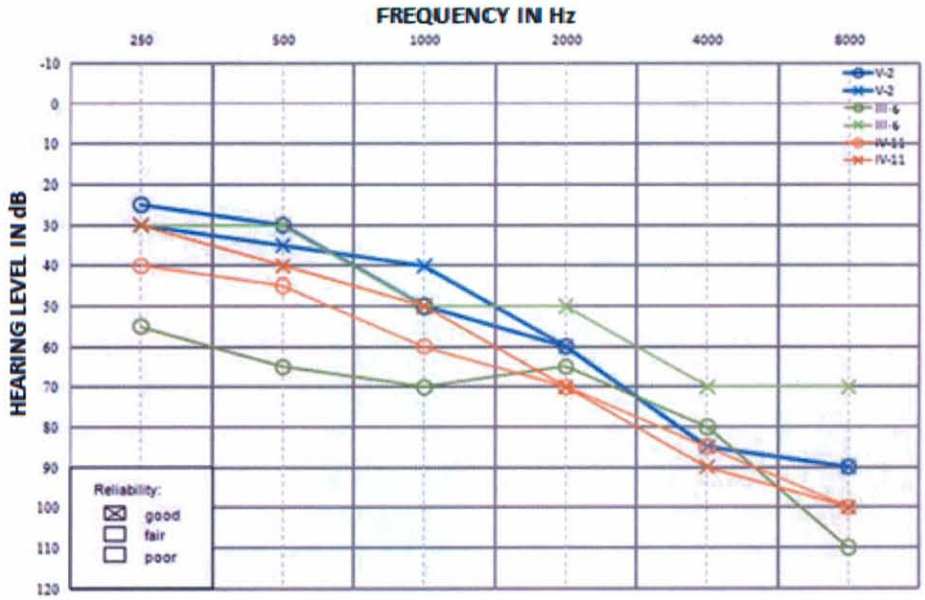



**Figure 3.3 Hearing Loss Phenotype of *COCH* p.P51P/S carriers III-6 , IV-11, and V-2 in Newfoundland Family 2094 (Figure 3.1). Hearing loss is most pronounced in the high frequencies. *Audiology reports were randomly selected, and the same trend is observed for for remaining Family 2094 members, which can be seen in Table 3.2.**

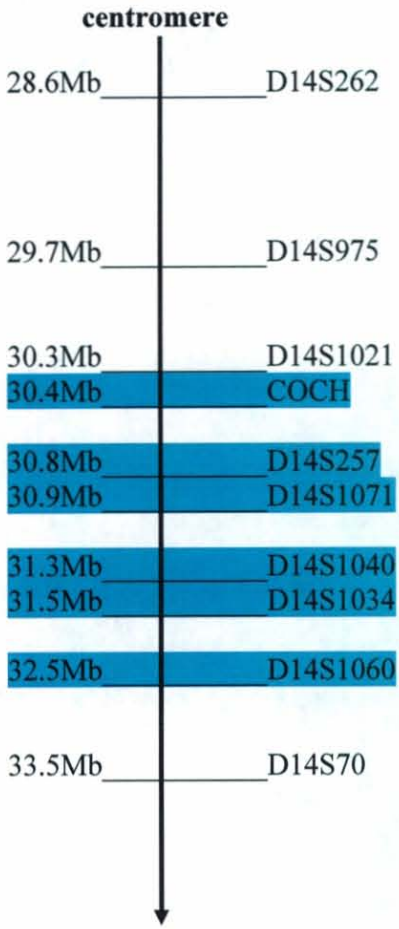



**Figure 3.4 Genetic Map of Markers Used to Construct the p.P51P/S Deafness Haplotype for Newfoundland & Dutch Carriers. Yellow denotes minimum shared region between families. Markers start from centromere. Markers selected from Fransen et al (2001).**

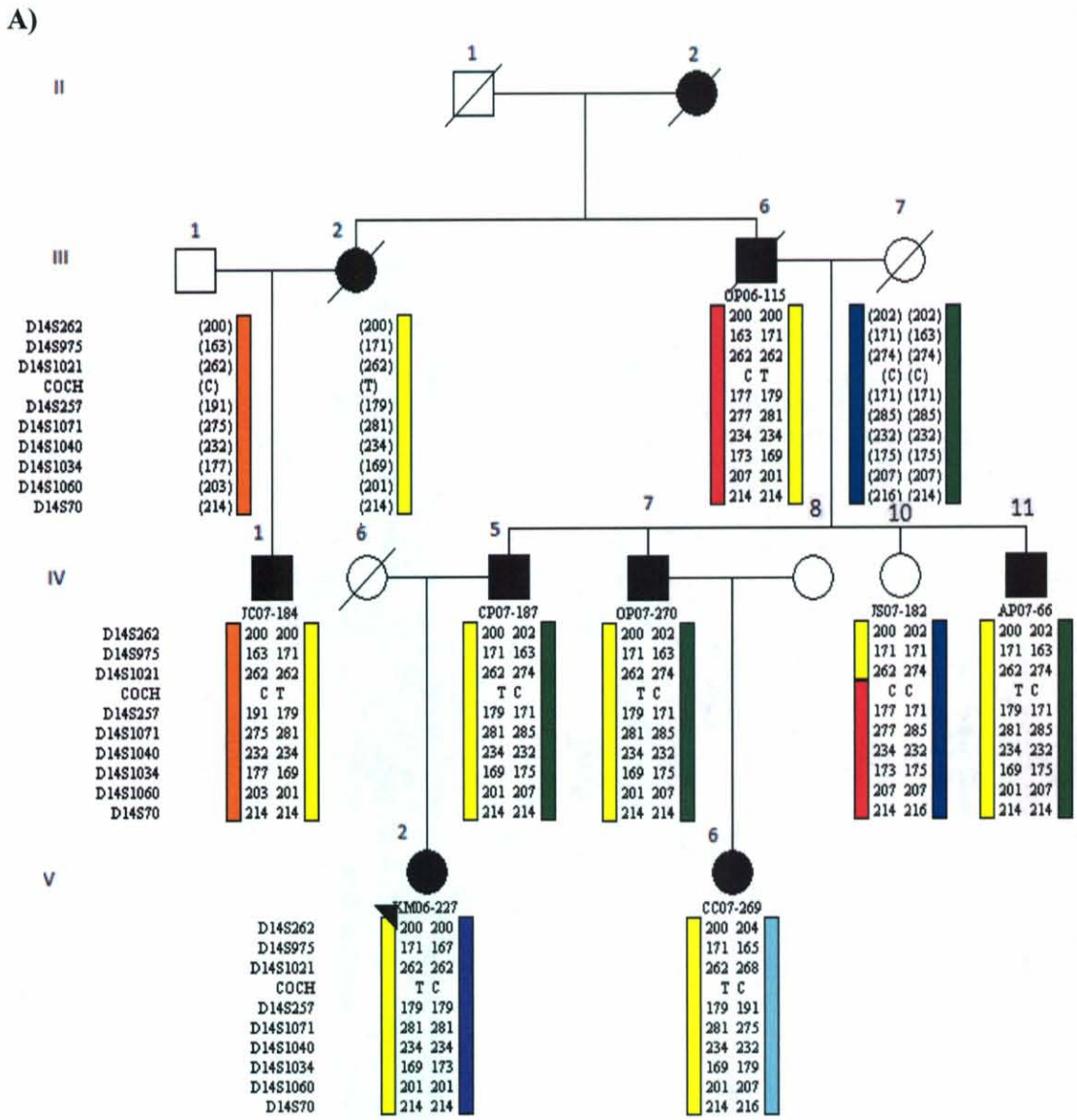



**Figure 3.5 p.P51P/S Family Haplotypes. A) Haplotype for Newfoundland p.P51P/S Family 2094 (partial pedigree). Sharing between families is seen for markers D14S257, D14S1071, D14S1040, D14S1034, and D14S1060, spanning a minimum shared region of ~2.1 Mb.**

B)

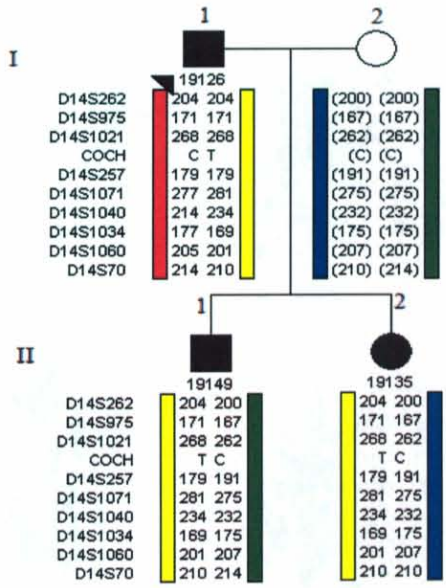
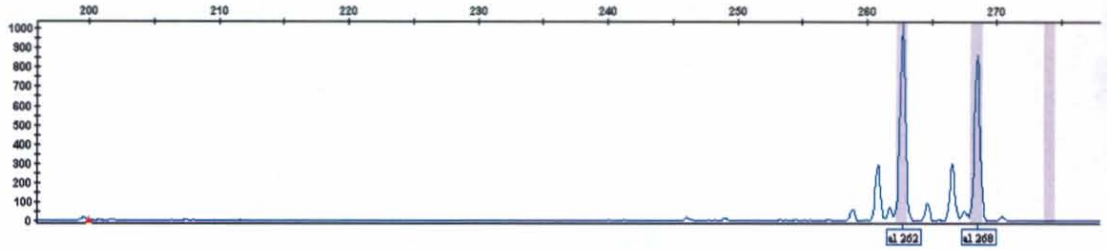
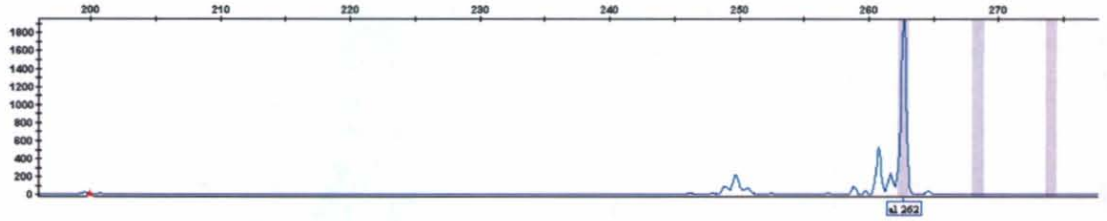



**Figure 3.5 p.P51P/S Family Haplotypes. B) Haplotype for Dutch p.P51P/S family (partial pedigree); associated haplotype is colored yellow. Sharing between families is seen for markers D14S257, D14S1071, D14S1040, D14S1034, and D14S1060, spanning a minimum shared region of ~2.1 Mb.**

A) II-1 (Figure 3.5B)




B) IV-1 (Figure 3.1)




C) IV-10 (Figure 3.1)

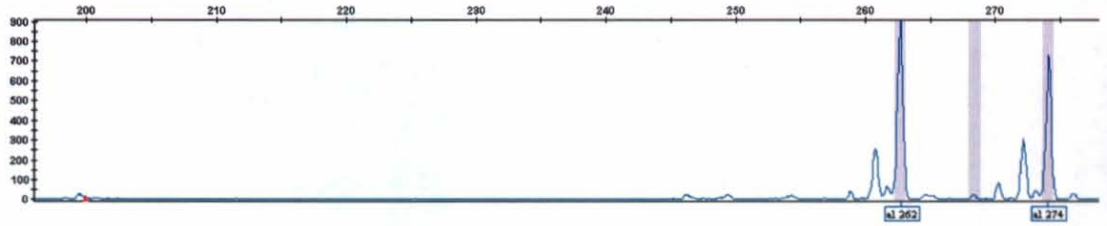



**Figure 3.6 Genotype Examples From Fragment Analysis of Newfoundland and Dutch Families. Homozygosity and Heterozygosity at a marker is described in GeneMapper by the number of times a strong vertical peak is present. If only one strong vertical peak is present, the person is a homozygote; if more than one strong vertical peaks are seen, the person is heterozygous. The numbers in the boxes under these vertical peaks represent the genotypes, or alleles, for that individual. A) II-1 is a Dutch individual (Figure 3.5B) and is a heterozygote (262, 268) for marker D14S1021. B) Family 2094 individual IV-1 (Figure 3.1) is a homozygote (262) for marker D14S1021. C) Family 2094 individual IV-10 (Figure 3.1) is a heterozygote (262, 274) for marker D14S1021.**

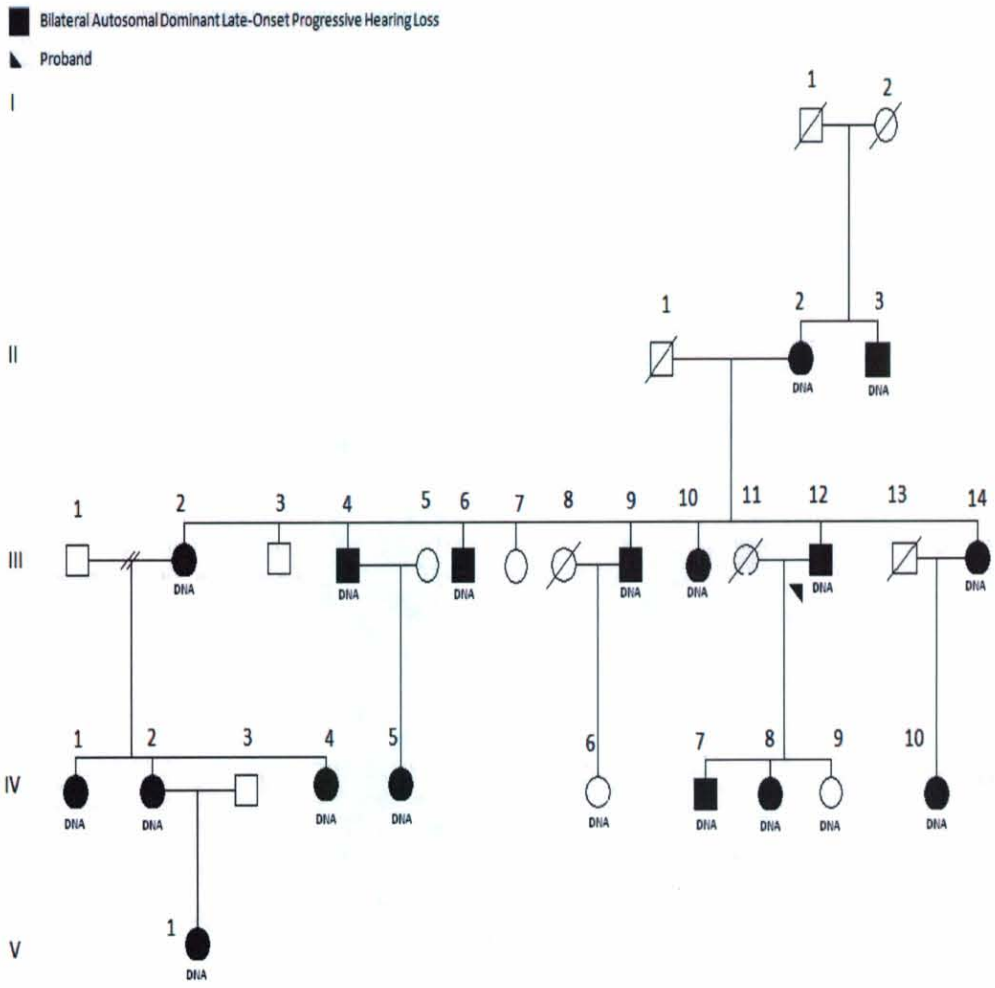



**Figure 3.7 Family 2071. A five generation Newfoundland family affected with autosomal dominant, late-onset, progressive hearing loss (partial pedigree); the 3bp deletion in *KCNQ4* (DFN2A) was first detected in the proband (arrow).**

p.Ser269del
PID III-12
Family 2071

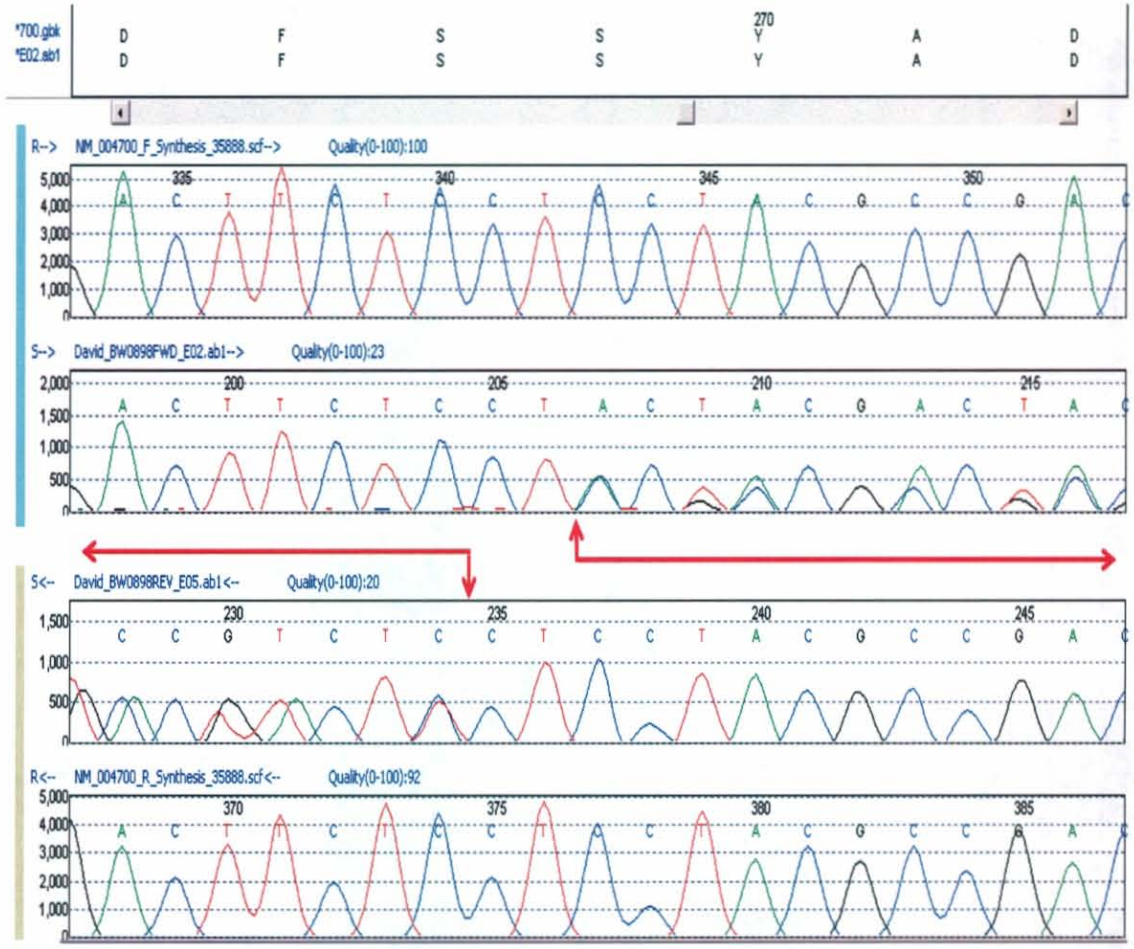
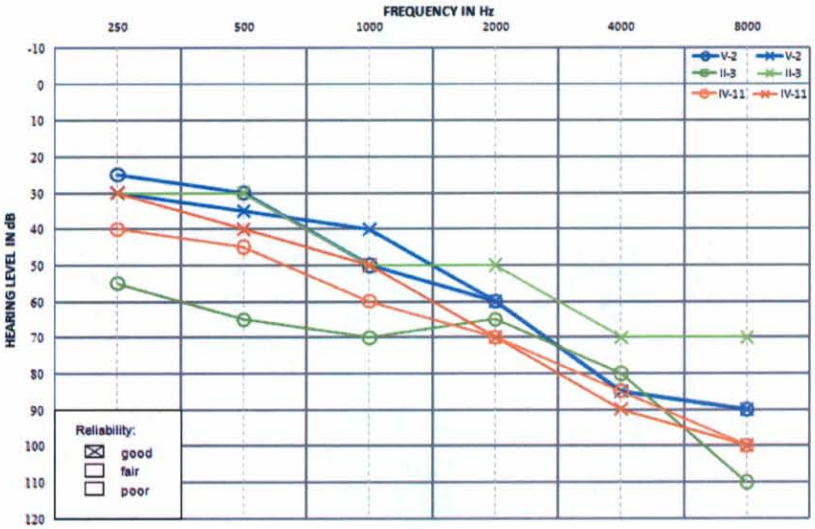



**Figure 3.8 Electropherogram of the 3 bp Deletion in *(*p.Ser269del) Identified in 13 Affected Family 2071 Individuals. The heterozygous deletion causes a 3 bp shift in the Electropherogram of the affected proband's forward and reverse strands, causing the above bidirectional pattern. The top and bottom traces are from a reference sequence, while the middle two traces are the forward and reverse strand traces of the proband III-12 (obtained from NCBI; NM_004700). The bottom trace is from the proband (PID III-12).**

A




B

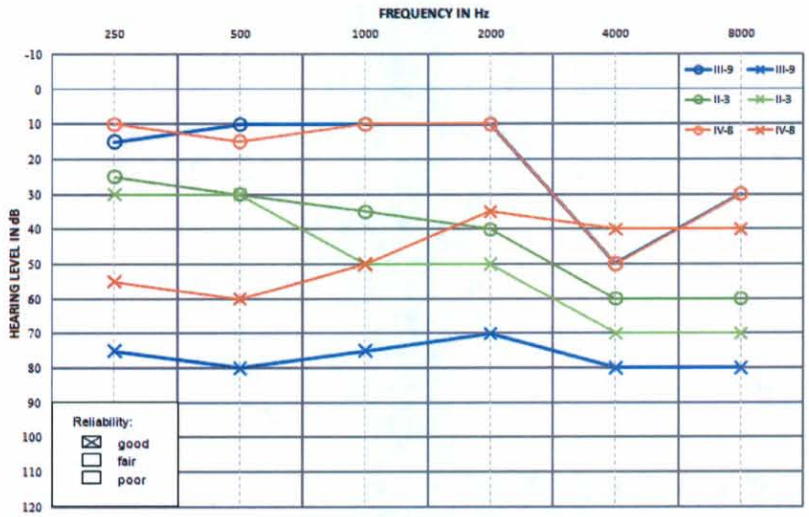



**Figure 3.9 Audiological Summary of Family 2071 Family Members. A: Hearing loss phenotype of *KCNQ4* deletion carriers III-12, V-1, and IV-1 (Figure 3.7) Remaining 2071 deletion carriers all present the same trend observed above, and can be observed in Tables 3.6 & 3.7. Audiology reports were randomly selected. Hearing loss is most pronounced in the high frequencies. B: Hearing loss phenotype of affected family members not carrying the deletion: III-9, II-3, and IV-8.**

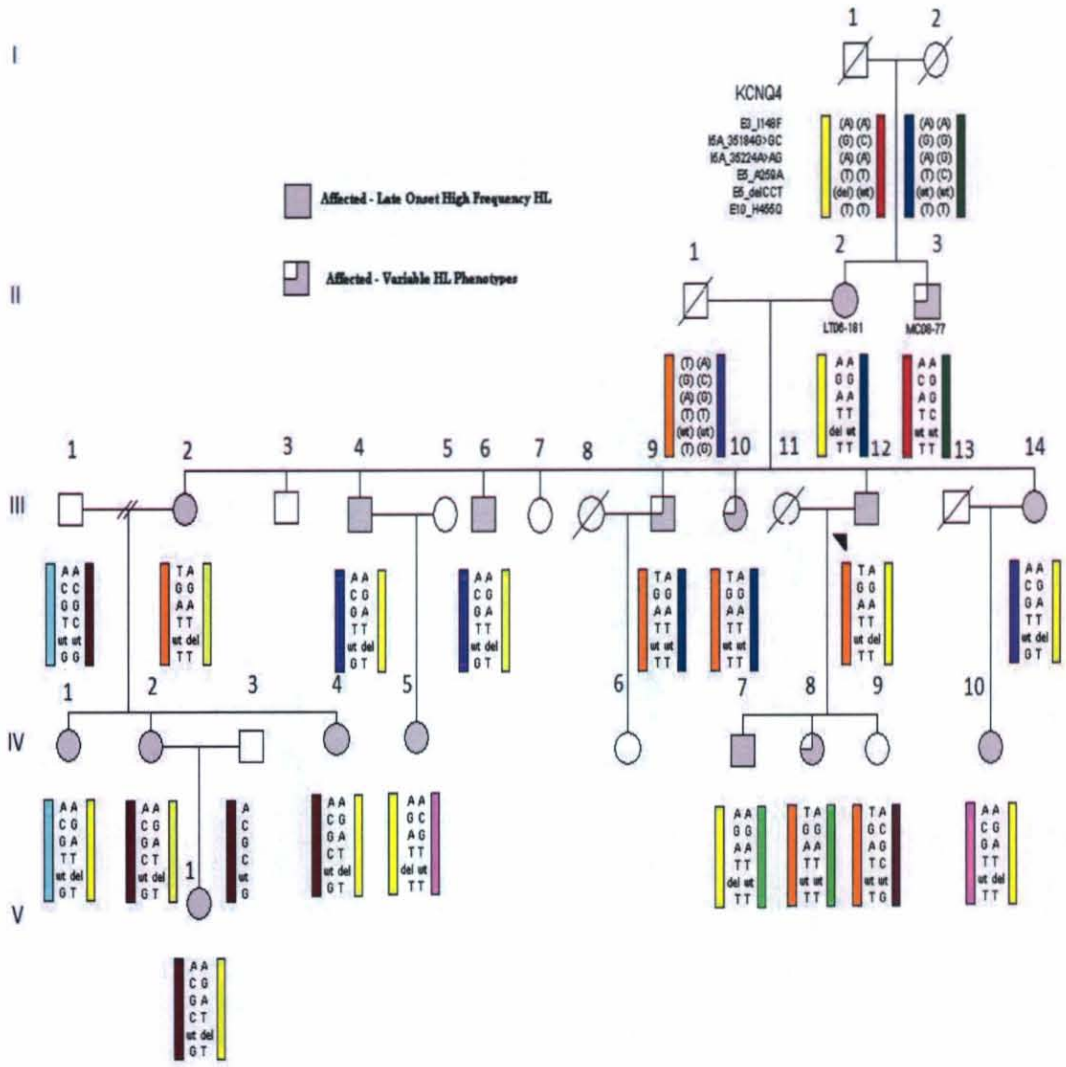



**Figure 3.10 Family 2071 Pedigree With Haplotype. The deafness associated haplotype is shown in yellow. The proband (III-12) and 13 first and second degree relatives harbor the *KCNQ4* deletion. Normal hearing individuals do not carry the deletion.**

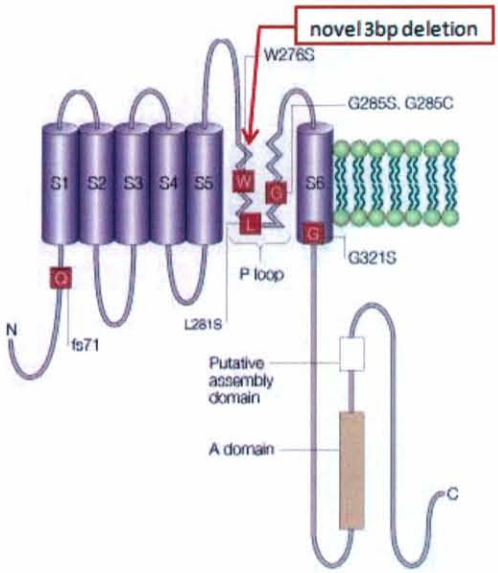



**Figure 3.11 Structure of *KCNQ4*. Arrow denotes novel 3bp deletion discovered in Newfoundland Family 2071. Also shows previously reported missense and deletion mutations found to cause hearing loss. Adapted (Jentsch, 2000).**

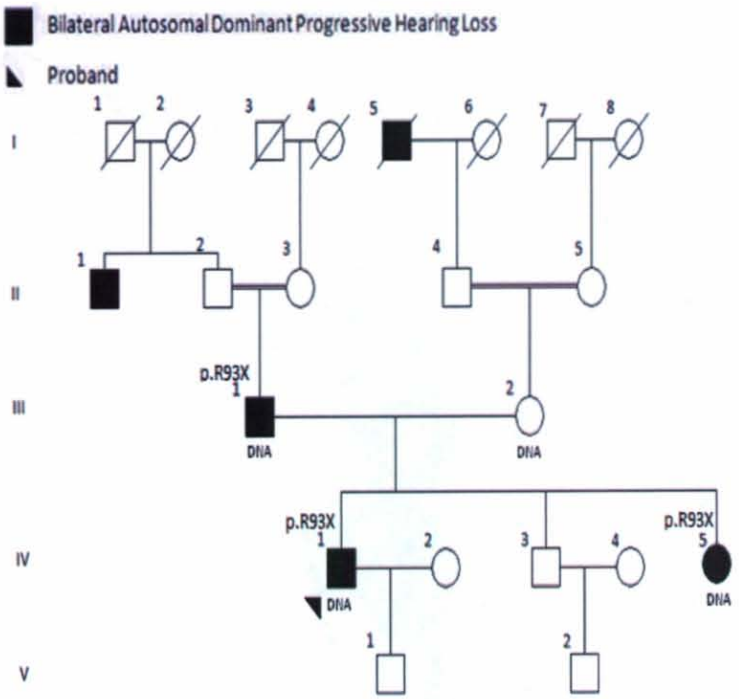



**Figure 3.12 Pedigree of Newfoundland Family 2102. A five generation family originating from Fogo Island, Newfoundland, segregating an apparent AD form of late onset progressive hearing loss. DNA not available for individuals I-5 and II-1.**

p.R93X
PID IV-1
Family 2102

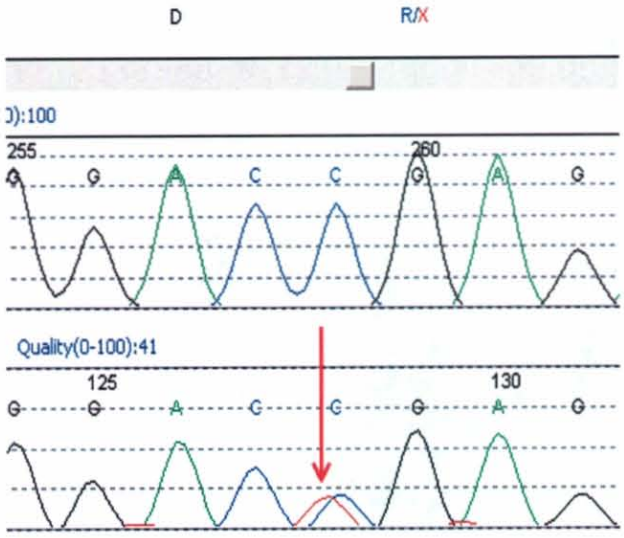



**Figure 3.13 Electropherogram of p.R93X Mutation in *MYO1A*. Individual IV-1 of Family 2102 (Figure 3.12).**

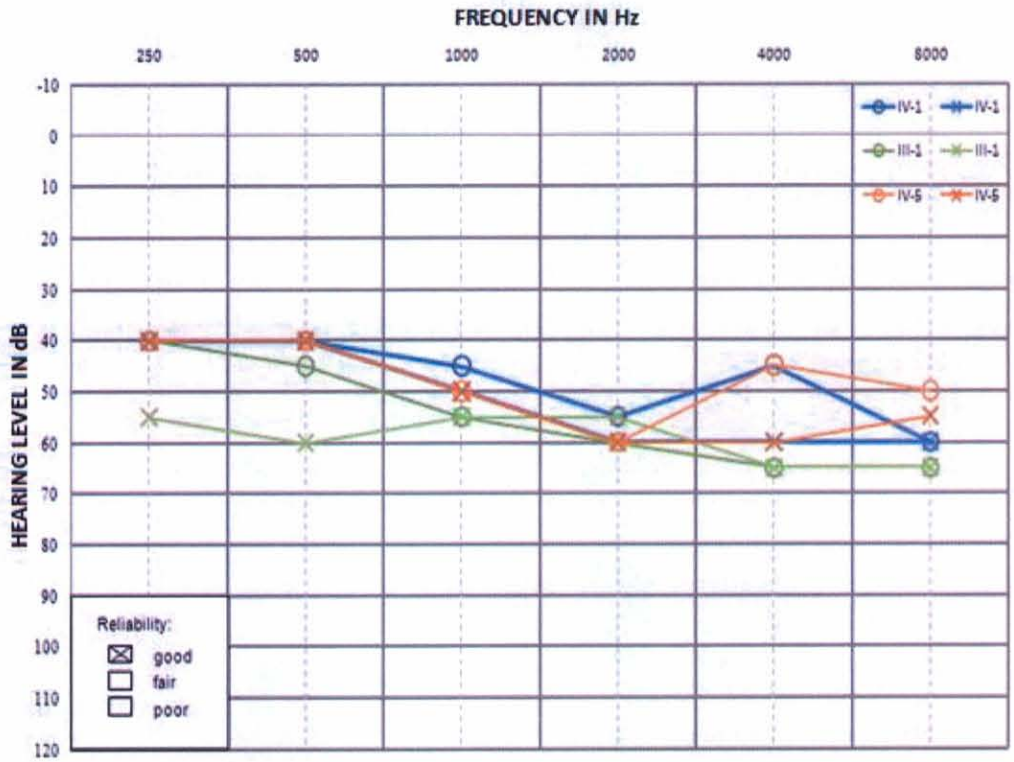



**Figure 3.14 Hearing Loss Phenotype of Newfoundland Family 2102 *MYO1A* Nonsense Mutation Carriers IV-1, III-1, and IV-5 (Figure 3.12). Onset of hearing loss is 5 years of age.**

**Table 3.1 Candidate Genes Screened For Mutations in Newfoundland Families Having Late-Onset Autosomal Dominant Hearing Loss.**

| Candidate Gene | Function | Number of Mutations Found in Caucasian Populations | Number of Mutations Associated with Progressive ADHL |
|---|---|---|---|
| *KCNQ4* | Ion Homeostasis | 8 | 2 |
| *COCH* | Extracellular Matrix | 8 | 6 |
| *MYO1A* | Unknown | 8 | 4 |
| *TECTA* | Extracellular Matrix | 7 | 7 |

**Table 3.2 Audiology Summary For Family 2094 Family Members (pedigree shown in Figure 3.1).**

| Family Member | Age | Onset (yrs) | Sex | Hearing Threshold (dB) | | | | | | | | | | Test Age |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 500 Hz | | 1000 Hz | | 2000 Hz | | 4000 Hz | | 8000 Hz | | |
| | | | | Right | Left | Right | Left | Right | Left | Right | Left | Right | Left | |
| V-2 | 42 | | Female | 30 | 35 | 50 | 40 | 60 | 60 | 85 | 90 | 90 | 90 | 63 |
| III-6 | 98 | | Male | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| IV-1 | 71 | | Male | 65 | 55 | 70 | 65 | 65 | 65 | 80 | 80 | 110 | 110 | 62 |
| IV-5 | 74 | | Male | 50 | 45 | 70 | 70 | 90 | 110 | 100 | 110 | 95 | 100 | 63 |
| IV-7 | 73 | | Female | 45 | 30 | 55 | 40 | 70 | 60 | 90 | 90 | 100 | 100 | 30 |
| IV-11 | 66 | | Female | 45 | 40 | 60 | 50 | 70 | 70 | 85 | 90 | 100 | 100 | 18 |
| V-6 | 37 | | Female | 10 | 10 | 20 | 10 | 30 | 25 | 35 | 30 | 25 | 20 | 35 |

N/A No information was available

**Table 3.3 Phenotype Summary of Family 2094 Individuals (pedigree shown in Figure 3.1).**

| Family Member | Variant | Status | Recorded Vestibular Defects? | Gender | Ear Affected | Noise Exposure | High Frequency | Mid Frequency | Low Frequency | Age | Test-Age |
|---|---|---|---|---|---|---|---|---|---|---|---|
| V-2 | p.P51P/S | Affected | None | Female | Bilateral | None | Profound | Severe | Mild | 42 | 39 |
| III-6 | p.P51P/S | Affected | N/A | Male | Bilateral | None | N/A | N/A | N/A | 98* | 55 |
| IV-1 | p.P51P/S | Affected | Unsteadiness | Male | Bilateral | None | Profound | Severe | Moderate | 71 | 53 |
| IV-5 | p.P51P/S | Affected | Dizziness, Unsteadiness | Male | Bilateral | None | Profound | Profound | Moderate | 74 | 57 |
| IV-7 | p.P51P/S | Affected | Dizziness | Female | Bilateral | Yes | Profound | Severe | Mild | 73 | 55 |
| IV-11 | p.P51P/S | Affected | Unsteadiness | Female | Bilateral | None | Profound | Severe | Mild | 66 | 49 |
| V06 | p.P51P/S | Affected | Vertigo | Female | Bilateral | None | Mild | Mild | Normal | 37 | 35 |
| JS07-182 | No Variant Detected | Not Affected | N/A | Female | N/A | None | N/A | N/A | N/A | N/A | N/A |

N/A Information Not Available
* III-6 died in 2006

**Table 3.4 Physical Location of Markers Used to Create the p.P51P/S Deafness Haplotype. The markers were taken from Fransen et al, 2001.**

| Marker/Gene | Genomic Starting Position on 14q12 (bp) | Type of Nucleotide Repeat | Heterozygosity |
|---|---|---|---|
| D14S262 | 28,630,354 | Dinucleotide | 0.57 |
| D14S975 | 29,749,271 | Dinucleotide | - |
| D14S1021 | 30,341,868 | Dinucleotide | - |
| COCH | 30,413,441 | N/A | N/A |
| D14S257 | 30,799,447 | Dinucleotide | 0.69 |
| D14S1071 | 30,898,090 | Dinucleotide | 0.72 |
| D14S1040 | 31,281,164 | Dinucleotide | 0.73 |
| D14S1034 | 31,537,191 | Dinucleotide | 0.75 |
| D14S1060 | 32,485,191 | Dinucleotide | 0.79 |
| D14S70 | 33,528,945 | Dinucleotide | 0.76 |

**Table 3.5 Haplotype Sharing Across Markers Nearby the *COCH* Gene Between Newfoundland Family 2094 and a Dutch p.P51P/S Family.**

| Location On 14q12 (bp) | Marker | Family 2094 | | | | | | | | Family Dutch | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | JS07-182 | OP06-115 | JC07-184 | KM06-227 | AP07-66 | OP07-270 | CP07-187 | CC07-269 | 19126 | 19149 | 19135 |
| 28,630,354 | D14S262 | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 204 | 200 | 200 |
| 29,749,271 | D14S975 | 171 | 171 | 171 | 171 | 171 | 171 | 171 | 171 | 171 | 171 | 171 |
| 30,341,868 | D14S1021 | 262 | 262 | 262 | 262 | 262 | 262 | 262 | 262 | 268 | 262 | 262 |
| 30,413,441 | COCH c.151C>CT | C | T | T | T | T | T | T | T | T | T | T |
| 30,799,447 | D14S257 | 171 | 179 | 179 | 179 | 179 | 179 | 179 | 179 | 179 | 179 | 179 |
| 30,898,090 | D14S1071 | 285 | 281 | 281 | 281 | 281 | 281 | 281 | 281 | 281 | 281 | 281 |
| 31,281,164 | D14S1040 | 234 | 234 | 234 | 234 | 234 | 234 | 234 | 234 | 234 | 234 | 234 |
| 31,537,191 | D14S1034 | 173 | 169 | 169 | 169 | 169 | 169 | 169 | 169 | 169 | 169 | 169 |
| 32,485,191 | D14S1060 | 207 | 201 | 201 | 201 | 201 | 201 | 201 | 201 | 201 | 201 | 201 |
| 33,528,945 | D14S70 | 214 | 214 | 214 | 214 | 214 | 214 | 214 | 214 | 210 | 214 | 214 |

**Table 3.6 Phenotypic Features of Affected Family 2071 Individuals. Shown first are family members with the deletion, and at the bottom, those without the deletion (pedigree shown in Figure 3.7).**

| Family Member | Variant | Hearing Status | Gender | Ear Affected | Noise Exposure | High Frequency | Mid Frequency | Low Frequency | Age | Test-Age |
|---|---|---|---|---|---|---|---|---|---|---|
| III-12 | 806_808-delCCT | Affected | Male | Bilateral | None recorded | Profound | Moderate | Normal | 70 | 68 |
| III-14 | 806_808-delCCT | Affected | Female | N/A | None recorded | N/A | N/A | N/A | 60 | N/A |
| III-6 | 806_808-delCCT | Affected | Male | Bilateral | None recorded | Profound | Moderate | Mild/Moderate | 67 | 57 |
| III-4 | 806_808-delCCT | Affected | Male | Bilateral | None recorded | Profound | Moderate | Mild | 66 | 63 |
| IV-10 | 806_808-delCCT | Affected | Female | Bilateral | None recorded | Profound | Moderate | Mild | 39 | 37 |
| V-1 | 806_808-delCCT | Affected | Female | Bilateral | None recorded | Severe | Moderate | Mild | 20 | 18 |
| III-2 | 806_808-delCCT | Affected | Female | N/A | None recorded | N/A | N/A | N/A | 64 | N/A |
| II-2 | 806_808-delCCT | Affected | Female | Bilateral | None recorded | Profound | Moderate-Severe | Moderate-Severe | 94 | 90 |
| IV-1 | 806_808-delCCT | Affected | Female | Bilateral | None recorded | Severe | Moderate | Mild | 40 | 39 |
| IV-2 | 806_808-delCCT | Affected | Female | Bilateral | None recorded | Severe | Moderate | Mild | 38 | 33 |
| IV-4 | 806_808-delCCT | Affected | Female | Bilateral | None recorded | Moderately Severe | Moderate | Mild | 43 | 41 |
| IV-5 | 806_808-delCCT | Affected | Female | Bilateral | None recorded | Profound | Moderate | Moderate | 34 | 32 |
| IV-7 | 806_808-delCCT | Affected | Male | Bilateral | Yes | Moderate | Moderate | Mild | 44 | 40 |
| II-3 | No deletion | Affected | Male | Bilateral | None recorded | Moderately Severe | Moderate | Mild | 86 | 83 |
| III-10 | No deletion | Affected | Female | N/A | None recorded | N/A | N/A | N/A | 55 | N/A |
| III-9 | No deletion | Affected | Male | Bilateral, left ear worse | None recorded | Moderate (Right) Severe (Left) | Severe (Left) | Severe (Left) | 71 | 67 |
| IV-8 | No deletion | Affected | Female | Left Ear | None recorded | Mild | Moderate-Severe | Moderate | 46 | 40 |

N/A Information Not Available

**Table 3.7 Audiology Testing Results of Affected Family 2071 Individuals. Shown first are family members with the deletion, and then those without the deletion (pedigree shown in Figure 3.7).**

Clinical features of family members with 806_808delCCT

| Family Member | Age | Sex | Hearing Threshold (dB) | | | | | | | | | | Test-Age |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 500 Hz | | 1000 Hz | | 2000 Hz | | 4000 Hz | | 8000 Hz | | |
| | | | Right | Left | Right | Left | Right | Left | Right | Left | Right | Left | |
| III-12 | 70 | Male | 30 | 35 | 50 | 40 | 60 | 60 | 85 | 90 | 90 | 90 | 63 |
| III-14 | 60 | Female | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| III-6 | 67 | Male | 65 | 55 | 70 | 65 | 65 | 65 | 80 | 80 | 110 | 110 | 62 |
| III-4 | 66 | Male | 50 | 45 | 70 | 70 | 90 | 110 | 100 | 110 | 95 | 100 | 63 |
| IV-10 | 39 | Female | 45 | 30 | 55 | 40 | 70 | 60 | 90 | 90 | 100 | 100 | 30 |
| V-1 | 20 | Female | 45 | 40 | 60 | 50 | 70 | 70 | 85 | 90 | 100 | 100 | 18 |
| III-2 | 64 | Female | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| II-2 | 94 | Female | 110 | 75 | 115 | 80 | 120 | 100 | 120 | 100 | 100 | 100 | 90 |
| IV-7 | 44 | Male | 25 | 30 | 55 | 40 | 55 | 50 | 60 | 75 | 60 | 60 | 40 |
| IV-1 | 40 | Female | 30 | 30 | 35 | 35 | 45 | 45 | 70 | 70 | 85 | 70 | 39 |
| IV-2 | 38 | Female | 25 | 25 | 30 | 35 | 35 | 35 | 60 | 65 | 90 | 90 | 33 |
| IV-4 | 43 | Female | 30 | 25 | 35 | 40 | 50 | 50 | 50 | 45 | 70 | 60 | 41 |
| IV-5 | 34 | Female | 50 | 50 | 60 | 55 | 60 | 55 | 85 | 85 | 90 | 90 | 32 |
| Clinical Features of Affected Family Members w/out deletion | | | | | | | | | | | | | |
| III-10 | 55 | Female | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| III-9 | 71 | Male | 10 | 80 | 10 | 75 | 10 | 70 | 50 | 80 | 30 | 80 | 67 |
| II-3 | 86 | Male | 30 | 30 | 35 | 50 | 40 | 50 | 60 | 70 | 60 | 70 | 83 |
| IV-8 | 46 | Female | 10 | 60 | 15 | 50 | 10 | 35 | 5 | 40 | 15 | 40 | 44 |

**N/A No information was available**

**Table 3.8 *KCNQ4* Variants Used to Create the Intragenic *KCNQ4* Haplotype**

| *KCNQ4* Sequencing Variants | Exon/Intron | Genomic Position | Pathogenic |
|---|---|---|---|
| c.34690A>AT | Exon 3 | 41056458 | No |
| 35184G>GC | Intron 5 | 41056958 | No |
| 35224A>AG | Intron 5 | 41056992 | No |
| c.35905T>TC | Exon 5 | 41057674 | No |
| c.35934_35936del | Exon 5 | 41057702 | Yes |
| c.47646T>TG | Exon 10 | 41069414 | No |

**Table 3.9 Audiological Summary of Family 2102 Individuals With & Without the p.R93X Nonsense Mutation.**

| Family Member | Variant | Status | Gender | Ear Affected | High Frequency | Mid Frequency | Low Frequency | Age | Test-Age |
|---|---|---|---|---|---|---|---|---|---|
| IV-1 | p.R93X | Affected | Male | Bilateral | Moderate | Moderate | Mild | 43 | 40 |
| III-1 | p.R93X | Affected | Male | Bilateral | Moderate | Moderate | Moderate | 71 | 68 |
| IV-5 | p.R93X | Affected | Female | Bilateral | Moderate | Moderate | Moderate | 40 | 12 |
| III-2 | No Variant Detected | Unaffected | Female | None | N/A | N/A | N/A | 59 | N/A |

**N/A Information Not Available**

# Chapter 4: Discussion

The aim of this thesis was to determine the genetic etiology of late onset autosomal dominant (AD) hearing loss in 28 Newfoundland families. This was investigated by screening the genomic DNA of 28 probands for mutations in four genes known to cause AD hearing loss, specifically *KCNQ4*, *COCH*, *TECTA,* and *MYO1A*. All 28 families were first genotyped to exclude the p.A716T mutation in *WFS1*, a mutation previously found to cause AD hearing loss in the Newfoundland population, as well as the full *GJB2* gene and the del13S1830 mutation in *GJB6*.

## Family 2094 Hearing Loss Caused by *COCH* Mutation

Of the 28 probands, one was found to have a mutation in *COCH*. *COCH* encodes cochlin, and has previously been shown to be prominently expressed in a ribbon-like pattern in the basilar membrane of the cochlea, providing evidence that it is involved in the structural regulation of that membrane (Kommareddi et al. 2007). Cochlin's exact role, however, remains unknown. *COCH* maps to chromosome 14q12-13. Cochlin is predicted to be 550 amino acids long and is highly conserved. It comprises a short predicted signal peptide, an N-terminal factor C homology domain, and two von Willebrand factor A-like domains.

Seven missense mutations within *COCH* that cause hearing loss have previously been reported: six of these cause a very recognizable phenotype characterized by a late

onset, progressive hearing loss associated with parallel vestibular decline. All six are found in the Factor C Homologous (FCH) domain of the cochlin protein (Kemperman et al. 2005). Of the FCH domain mutations, three originate in North America (p.V66G, p.G88E, and p.W117R); one is a founder mutation (p.P51P/S) present in many Dutch and Belgian families, one originated in Australia (p.I109N), and one in Japan (p.A119T). This audiological phenotype of late-onset high-frequency hearing loss caused by these mutations is in close correlation with the audiological phenotype of many of the 28 AD Newfoundland probands being studied in this research project and was the primary reason for choosing this gene for screening.

In Newfoundland Family 2094 a known mutation causing a heterozygous C→T base change was found in the proband. This resulted in the substitution of a conserved proline residue for a serine residue at amino acid position 51 (p.P51P/S) (Figure 3.2). All seven affected members of Family 2094 shared the mutation, while an unaffected relative did not harbor the mutation (Figure 3.1). Individuals harboring the p.P51P/S variant suffer from a late onset, progressive, high-frequency hearing loss with an obvious onset from ~40 years of age onwards (Bischoff, 2005). All affected family members share the same audiological profile (Figure 3.3), matching the pattern of hearing loss seen in previous cases (de Kok et al. 1999). The progressive nature of hearing loss in Family 2094 also matches previously reported cases of p.P51P/S carriers (de Kok et al. 1999).

This mutation is also associated with vestibular dysfunction, such as motion sickness and vertigo, which in most cases develops to complete vestibular areflexia or vestibular hyporeflexia (Verhagen et al. 2001; Bischoff et al. 2005). Onset of progressive

vestibular failure presents earlier, from the fourth into the sixth decade of life, declines more rapidly, and is eventually more complete than the associated hearing impairment (Bischoff et al. 2005). Five out of seven Family 2094 members have so far experienced these vestibular problems.

In 2009, Hildebrand et al. reported a p.P51P/S case with the very rare disease Superior Semicircular Canal Dehiscence (SSCD), which is characterized by the absence of bone overlying the superior semicircular canal, creating a third labyrinthine window. It was suggested that individuals with *COCH* mutations like p.P51P/S should be given a CT scan to screen for SSCD (Carey et al. 2007). Of the Family 2094 individuals who have had CT scans; V-2, V-6, and IV-1, none were diagnosed with SSCD, though it has been suggested that diagnosis of SSCD often requires the doctor to be specifically looking for the defect (Hildebrand et al. 2009). This therefore represents both a clinical and a scientific opportunity to learn more about this possible association. Carriers could undergo a CT scan to look for this rare defect, and if found, that individual can have surgery done, as SSCD's severe vestibular symptoms can be fully corrected with surgery (Carey et al. 2007). This would also add to the current literature on the possible association between these two rare disorders.

Previous studies have also demonstrated a link between *COCH* mutations like p.P51P/S and vertical corneal striae. Two families carrying the p.P51P/S mutation were found to have a 93 % and a 78 % prevalence of these striae, respectively; age of onset is late 40s to late 50s (Bischoff et al. 2007). In Family 2094, V-6 was reported to have a corneal scratch in the right eye. This was first reported at the age of 31, which falls close

to the previously reported age of onset for corneal striae in p.P51P/S carriers. This scratch was not reported to be due to any physical injury to the eye, though no other medical information is available. Clinical follow-up is currently ongoing; this may be further evidence of a link between vertical corneal striae and hearing loss mutations within *COCH*, like p.P51P/S.

## Confirmation of p.P51P/S as a Dutch Founder Mutation

The p.P51P/S mutation has been reported in ten Belgian, seven Dutch, and one American family and is widely accepted to be a Dutch founder mutation (de Kok et al. 1999; Fransen et al. 2001). This study is only the second reported occurrence of this mutation outside of Europe. An opportunity to confirm this Newfoundland p.P51P/S occurrence as a Dutch founder mutation arose at the 2010 Association for Research in Otolaryngology MidWinter conference. While attending this conference, I met with Dr. Hannie Kremer, a scientist working in the Netherlands, who has published on the p.P51P/S vestibular phenotype (Bischoff et al. 2005). Dr. Kremer offered to contribute Dutch p.P51P/S DNA, allowing me to confirm the Dutch founder hypothesis. Using DNA from this Dutch family and Newfoundland Family 2094, a series of microsatellite markers were genotyped to develop ancestral haplotypes (Figure 3.5). These haplotypes demonstrate allelic sharing between the two families for five contiguous markers close to the *COCH* gene, spanning a total genetic distance of ~2.1 Mb (Figure 3.4; Table 3.5). The probability of these two families sharing this five marker haplotype by chance is low,

and therefore, it is likely that these two families are distantly related and originate from a common ancestor in the Netherlands. The genealogy of this shared ancestry was not followed up on, as the objective was to provide further support to the current Dutch founder hypothesis for p.P51P/S DNA, and because no further genealogy on these families is available. However, individuals in Family 2094 could be researched using the Newfoundland Genealogy Database and the Heritability Analytics Infrastructure (Population Therapeutics Research Group, St. John's, Newfoundland & Labrador) to trace the genealogy of Family 2094. If successful, this would provide the opportunity to increase the power of this linkage association, and possibly directly link it to the Dutch family with which it shares an ancestral haplotype. This avenue warrants further investigation. It is interesting to note that mutation carriers in Family 2094 possess an uninterrupted Newfoundlander ancestry, going back at least six generations. Up until recently, p.P51P/S had never been seen outside of central Europe (Fransen et al. 2001). Its recent discovery in a United States family (Hildebrand et al. 2009), and now in a family living here in Newfoundland, Canada, could be evidence of an ancient origin. If true, this would have strong implications for cochleovestibular diagnostic screening of p.P51P/S and other mutations within the *COCH* gene, as the older a mutation's origin, the more likely it is to be widespread throughout all populations and ethnicities.

## Family 2071 Hearing Loss Caused By Novel *KCNQ4* Deletion

*KCNQ4* (Potassium Voltage-Gated Channel 4) encodes the protein potassium voltage-gated channel subfamily KQT member 4. This protein is part of a family that forms channels to transport positively charged potassium ions between neighboring cells. More importantly for hearing, the potassium channels that this protein forms are thought to play an indispensable role in the regulation of neuronal excitability, particularly in the sensory cells of the cochlea (inner ear), where they are expressed (Kubisch et al. 1999). Expression gradients of *KCNQ4* in spiral ganglion and in these cochlear hair cells correlate very closely with progressive hearing loss (Beisel et al. 2001).

Twelve mutations have been reported in *KCNQ4* (DFNA2): ten missense and two deletions. The missense mutations are believed to cause hearing loss beginning at a young age (Hilgert et al. 2009). The deletions are thought to cause a milder phenotype, have an older age of onset and primarily affect perception of high frequencies (Topsakal et al. 2005). While both late-onset and early-onset hearing loss can be caused by mutations in *KCNQ4,* this gene is also associated with age-related hearing loss. One research team has demonstrated that several SNPs associated with age-related hearing loss in two independent Caucasian populations were all located in the same 13 kb region in the middle of the *KCNQ4* gene (Van Eyken et al. 2006).

The first *KCNQ4* deletion (c.211del13) was discovered in a Belgian family. Affected individuals lacked 13 nucleotides between positions 211 and 224. This resulted in a frame-shift after Gly70 (p.fsX71), followed by 63 novel amino acids and a premature stop codon (Coucke et al. 1999). Consequently, the protein is truncated before the first

transmembrane domain and is rendered nonfunctional (Nie, 2008; Figure 3.11). More recently, the second deletion was found in a Japanese family, and is a 1 bp deletion (c.211delC). Similarly to the previous case, a truncated, nonfunctional *KCNQ4* protein is generated (Kamada et al. 2006). The milder high frequency phenotype of cases reported to harbor these two *KCNQ4* deletions correlates closely with the phenotype of several of the 28 Newfoundland probands under study in this research project, and so *KCNQ4* was chosen as a functional candidate gene to screen for potential causative mutations.

The proband of Newfoundland Family 2071, III-12 (Figure 3.7), was found to carry a novel 3bp heterozygous deletion in exon 5 of *KCNQ4* (Figure 3.8). Upon sequencing other Family 2071 individuals, thirteen affected individuals were found to share the 3 bp deletion. This is the third deafness causing deletion found in *KCNQ4*, and the first outside of exon 1 (Coucke et al. 1999; Kamada et al. 2006). Audiology reports of deletion carriers demonstrate a high-frequency, late-onset hearing loss (Figure 3.9), supporting the current genotype-phenotype correlation that *KCNQ4* deletions associate with a late-onset and milder hearing impairment (high-frequency loss) than corresponding *KCNQ4* missense mutations (Kamada et al. 2006).

This deletion predicts an in-frame removal of a serine residue at amino acid position 269 within the P-loop domain of the KCNQ4 protein (Figure 3.11). Interestingly, the P-loop domain is a mutational hotspot where ten missense mutations causing early onset hearing loss have previously been described (Coucke et al. 1999; Kubisch et al. 1999; Talebizadeh et al. 1999; Van Hauwe et al. 2000; Kamada et al. 2006).

In order to determine the level of variant sharing among deletion carriers and between all individuals of Family 2071, and to verify whether further hearing loss patterns might be seen, we next constructed an intragenic haplotype using commonly occurring variants within and flanking exon 5 of *KCNQ4*. While these markers did not singularly provide any additional interesting information regarding affected individuals not harboring the deletion, all deletion carriers shared this deafness associated haplotype. Additionally, of 90 ethnically matched controls, and of all unaffected relatives, none shared this deafness associated haplotype. This haplotype, used primarily as a method of further investigation and mutation confirmation, contrasts sharply with the aforementioned p.P51P/S haplotype, which was constructed to demonstrate ancestral linkage between Newfoundland Family 2094 and a Dutch family, and which can be taken as evidence for p.P51P/S being a Dutch founder mutation.

While this deletion is not seen in four Family 2071 members, these four individuals present a distinctly different audioprofile compared to deletion carriers (Figure 3.10; Table 3.6; Table 3.7). The cause of hearing loss for these four individuals is likely due to several separate genetic or environmental predispositions, thus making them phenocopies. Environmental factors could also be the cause of hearing loss in these individuals. The medical records show no indication of noise exposure or physical injury to the ears in these four individuals. To rule out a different mutation on the same gene as the cause of hearing loss in these individuals, they were screened for all exons within *KCNQ4*. No additional mutations were detected, and they did not share any particular variants or SNPs at the locus when observed in the intragenic haplotype.

A further avenue of research for Family 2071 would be functional studies. Because *KCNQ4* is strongly expressed in the sensory cells of the cochlea (Kubisch et al. 1999; Beisel et al. 2001), studies on deletion carriers should be conducted to investigate whether the 3 bp deletion negatively affects the potassium ion channels formed by *KCNQ4,* whether these ion channels remain structurally and functionally intact, and whether potassium ions are able to effectively move through these channels to complete the mechanoelectrical transduction pathway. This could potentially shed further light on the molecular pathways underlying this hearing loss mutation and could provide added credence to the current genotype-phenotype correlation.

## Family 2102 Hearing Loss Caused By *MYO1A* Mutation

*MYO1A* encodes the protein myosin 1a, which is present in the inner ear and plays a role in human hearing (Donaudy et al. 2003). Donaudy et al. (2003) postulated that *MYO1A* plays a role in ion transport. More recently, Hilgert & Smith (2009) present a slightly different hypothesis. At the brush border surface of intestinal epithelial cells, myosin 1a is a major component of the actin-rich cytoskeleton, where it is involved in membrane trafficking. It could serve the same function in the inner ear, because the cytoskeleton of the intestinal cells and the inner ear cells (hair cells and supporting cells) are very similar. The specific expression pattern in the inner ear has not yet been established, by may provide further clues in the future (Hilgert & Smith, 2009).

*MYO1A* was chosen as a candidate gene for the same reason as all the functional candidate genes in this study: a phenotype correlation between individuals with hearing loss due to mutations previously found within *MYO1A* and the audioprofile seen in some of the 28 Newfoundland probands under study. While most of the 28 probands suggest a late-onset hearing loss, some are reported as early-onset, but still progressive and autosomal dominant. Four mutations in *MYO1A* have been associated with an early-onset AD, progressive, phenotype (Donaudy et al. 2003). These four mutations were all found in Italian probands.

A heterozygous nonsense mutation was discovered at amino acid position 93 within exon 4 of *MYO1A* in the Family 2102 proband IV-3 (Figure 3.12). This nonsense mutation, p.R93X substitutes an arginine residue for a stop signal in the motor domain (Figure 3.13). All three affected Family 2102 individuals shared the mutation, and reported their first hearing loss at 5 years of age, with hearing coming and going, but progressively deteriorating to severe hearing loss (Figure 3.14).

While this mutation is not novel, it is only the second reported case world-wide. The p.R93X nonsense mutation was first reported in a very small southern Italian family. p.R93X was present in the male proband who suffered from moderate to severe bilateral hearing loss. This is the same audioprofile seen in Family 2102, though time of onset was not available for this Italian family. The proband received the mutant allele from his mother. The mother stated that she has normal hearing, although no audiological evaluation of any kind was carried out. A healthy brother of the proband did not carry the p.R93X mutant allele and did not possess any form of hearing loss (Donaudy et al. 2003).

Several possibilities could explain the Italian mother's supposed normal hearing: 1) The mother does in fact have the same hearing loss as her son, 2) this family segregates an AR form of deafness and digenic inheritance is responsible for this hearing loss phenotype, or 3) the trait is not 100 % penetrant. While many types of hearing loss are caused by a mutation in one single gene, digenic inheritance requires the interaction of two genes for phenotypic expression. If the mother does not have hearing loss, despite carrying the p.R93X mutation, she may not have hearing loss because this trait is an AR form of hearing loss and segregates in a digenic manner. She would not inherit the second disease causing mutation in the putative second unknown gene. Additionally, if the trait is not fully penetrant then this too could explain the mother's normal hearing. Detection of the p.R93X mutation is simple and does not require sequencing because the mutation is easily identified by digestion of PCR products, as the mutant allele destroys an A*va*II restriction site. A further avenue of research for Family 2102 would be to attain DNA from the affected Italian individuals previously reported to create an ancestral haplotype, as done previously for Family 2094. It could then be determined whether or not Family 2102, from Newfoundland, share a common ancestor with the previously reported Italian family (Donaudy et al. 2003).

## Candidate Gene *TECTA*

*TECTA* encodes the protein Alpha-tectorin. The tectorial membrane is an extracellular matrix that covers the Organ of corti sensory epithelium in the ear. Sound waves induce a vertical movement of the basilar membrane, and this movement evokes a deflection of stereocilia against the tectorial membrane. Alpha-tectorin is one of the major noncollageneous components of the tectorial membrane. Mutations in the *TECTA* gene have been shown to be responsible for ADNSHL, with audioprofiles similar to some of the 28 Newfoundland probands under study (Verhoeven et al. 1998).

Exons sequenced included 5, 9-14, 17-18, and 20. However, no hearing loss mutations were discovered among the 28 AD Newfoundland probands. This does not rule out the possibility that hearing loss in one or more of these Newfoundland probands if caused by mutations in *TECTA* within an exon that was not sequenced in this research project. A total of 13 exons were not sequenced, and this gap represents an opportunity for further research, as *TECTA* remains a strong candidate gene for hearing loss in Newfoundland families.

## Non-Founder Mutations in a Founder Population

As previously discussed, the province of Newfoundland & Labrador is a founder population, due to its cultural and geographic isolation. Since the second major wave of

settlement, taking place in the late 18th and early 19th centuries, little immigration or outmigration has occurred. These original colonists have contributed to several founder mutations that cause specific diseases. For example, an exon 8 deletion in *MSH2*, a gene causing hereditary non-polyposis colorectal cancer, has been found in 5 different Newfoundland families (N=74 carriers) (Frogatt et al. 1996; Stuckless et al. 2006), and the c.782+3delGAG mutation found in the deafness gene *TMPRSS3* has been reported in 2 different Newfoundland families (Ahmed et al. 2004; Young et al. unpublished data).

This study sought to potentially identify AD hearing loss founder mutations in Newfoundland. No founder mutations were identified. All three of the mutations identified in this study - p.P51P/S, p.Ser269del, and p.R93X in the genes *COCH, KCNQ4,* and *MYO1A* respectively - were each reported in one family only, after screening 68 Newfoundland & Labrador hearing loss probands. We identified three separate mutations in three separate genes causing hearing loss with three separate phenotypes. Additionally, these mutations were found to cause hearing loss in three Newfoundland families that are geographically separated from one another. So while the genetic history and nature of Newfoundland's population, combined with previous successes (Young et al. 2001; Ahmed et al. 2004) implies that the future discovery of hearing loss founder mutations in Newfoundland & Labrador is still a possibility, the results of this study unexpectedly point to Newfoundland's genetic diversity, rather than its homogeneity. This isn't the first time this has happened. An increased level of genetic diversity was noted when, for example, nine mutations in six genes were detected in 21 families with Bardet-Biedl syndrome (Webb et al. 2009). I speculate here that the solved

families in this study, 2094, 2071, and 2102, all descend from original but separate groups of Newfoundland colonists. These colonists would likely have been from several different areas of Ireland or England. The geographical isolation of their origin home towns could account for the potential genetic diversity seen in the identification of these three separate hearing loss mutations. These findings, therefore, do not marginalize the efficacy of the Newfoundland population in the search for novel gene discovery or for founder mutations.

The clinical and diagnostic utility of these identified mutations is significant for the families in question, as they are now able to screen new family members, receive much improved genetic counseling, and hopefully, benefit from improved treatment options. However, identification of a Newfoundland founder mutation would be of increased clinical and diagnostic utility. Any mutation that is prevalent throughout a large portion of native Newfoundlanders is screened for at birth, and would thus be identified in all future cases born in Newfoundland. The aforementioned benefits would therefore apply to a much wider range of people than a single family, as is the case for non-founder mutations. This study, therefore, does not reduce the strong clinical and diagnostic potential that can be fulfilled through the discovery of Newfoundland founder mutations, nor does it point to an inefficacy of the Newfoundland population as a medium of novel gene discovery.

## A Changing Landscape of Gene Identification Methodology

Despite the increase in hearing loss gene identification, many deafness causing genes and loci remain undiscovered. High density SNP arrays, which are a type of DNA microarray used to detect polymorphisms across large portions of a genome have been successfully applied in a new approach to find hearing loss genes. Shahin et al. (2010) applied SNP array-based homozygosity mapping of families with a high degree of consanguinity. Homozygosity mapping is a powerful method of localizing genes for autosomal recessive disorders. Using this approach, Shahin et al. (2010) identified five genome regions likely to harbor novel genes for pre-lingual non-syndromic hearing loss in six Palestinian kindreds. This approach is currently being investigated for AR hearing disorders in the Newfoundland population. Traditionally this method has been limited to families who share a recent common ancestor, but Hildebrandt et al. (2009) have recently demonstrated that this technique can be used on outbred populations. They performed homozygosity mapping on 72 single affected individuals of 54 kindreds ascertained worldwide using a 250 K SNP array. This discovery could potentially open up further opportunities for novel gene discovery in Newfoundland & Labrador as many specialty clinics have access to cohorts of individuals from out-bred populations.

A second technological advance having huge implications on novel gene discovery is the advent of next-generation sequencing (Schuster, 2008). Next-generation sequencing allows for the easy production of millions of DNA sequence reads in a single run. Next-generation sequencing instruments can generate as much data in 24 hours as several hundred traditional DNA capillary sequencers, but are operated by a single person

(Schuster, 2008). A recent study of non-syndromic hearing loss used targeted genome capture combined with next-generation sequencing to analyze 2.9 Mb of the *DFNB79* interval on chromosome 9q34.3 (Rehman et al. 2010). Rehman et al. (2010) detected a nonsense mutation in the predicted gene *C9orf75*, which they renamed taperin (*TPRN*). A nonsense mutation is a change in DNA sequence that results in a premature stop codon, leading to an incomplete, and usually nonfunctional, protein product. Rehman et al. (2010) next performed immunolocalization experiments on the *TPRN* protein in a mouse cochlea, and saw prominent expression in the taper region of hair cell stereocilia.

A third strategy showing recent success (Meyer et al. 2007) and strong promise for gene identification, specifically for AD hearing loss, is the use of AudioGene Audioprofiling. Audiogene is a computer program that uses a machine-learning approach to analyze audioprofiles as a method of prioritizing genes for mutation screening in small families segregating AD hearing loss. The audiogene dataset has recently been expanded to include a total of 16 DFNA loci, including COCH, *KCNQ4,* and *TECTA* (Hildebrand et al. 2009). This could be very useful in the investigation of Newfoundland families segregating AD hearing loss, and many of these families are small with limited recorded data. Hildebrand et al. (2009) performed an experiment where a series of audiograms were analyzed by a panel of hearing loss experts, and concurrently by AudioGene. The accuracy of matching the audiograms with the genotypic cause was 55 % for the human experts, and 88 % for AudioGene (Hildebrand et al. 2008). Furthermore, as the size of this database increases, so too will its predictive capacities. For now, it represents a promising avenue for Newfoundland AD hearing loss research at almost zero cost, and

should be the next strategy employed for mutation detection in the 25 remaining Newfoundland AD probands.

These are just some examples of recent advances in technology leading to novel gene discovery, and as these technologies become more common, their use will be instrumental in new investigative disease causing studies not just in the Newfoundland population, but all over the world. The ability of next-generation sequencing to perform so many 'reads' so quickly will be invaluable to future hearing loss studies in Newfoundland. Screening candidate genes and potentially mutated chromosomal regions will be significantly easier, allowing for the 'quick' discovery of novel (and existing) causative hearing loss mutations in Newfoundland probands.

## Limitations of this Study

Despite the discoveries discussed above, the candidate gene approach undertaken in this study does present various limitations in the search for hearing loss mutations. These limitations vary widely, and while mentioned above throughout this thesis, a short summary of them will serve to illuminate the path forward both in filling any gaps, and in approaching mutation detection in various untried ways up to this point.

Firstly, it is important to note that mutations in *WFS1* may not be more common than other hearing loss mutation just because they are more commonly reported in Newfoundland. The reason the p.A716T mutation in *WFS1*is so widely reported is due in large part to its pathognomonic character. A pathognomonic sign is a particular sign

whose presence is characteristic for a certain disorder beyond any doubt. The phenotype of p.A716T mutation in *WFS1* is one such pathognomonic trait. Local audiologists are able to both distinguish this pattern of hearing loss and recognize surnames of the extended family thus far affected, and contact us directly when they have patients that may be related to these originally reported families (Young et al. 2001). Clinical application like this makes it likely that the high frequency of reported *WFS1*-related hearing loss is an overstatement, not describing the true situation (Tranebjaerg 2008).

It is also possible that one or more of the families under investigation may be incorrectly classified as AD due to a lack of sufficient data. Many individuals in these pedigrees (Figure 2.1) are ascertained through relatives' word of mouth, and so it is entirely possible that digenic inheritance, penetrance, or even mitochondrial mutations may be obscuring the proper ascertainment of inheritance pattern and thus the search for causative hearing loss mutations. These possibilities should undoubtedly be investigated in future studies on the 25 remaining AD families.

The possible presence of larger genomic abnormalities at work is another limitation of this study, which did not search for any such possible occurrence. This study investigates genes through targeted gene sequencing. However, larger genomic abnormalities have been shown to cause hearing loss phenotypes (Lisenka et al. 2003; Shaffer et al. 2006). Genomic abnormalities, therefore, may account for hearing loss in some of the Newfoundland families under investigation. Large genomic rearrangements, deletions, inversions, etc. can cause and affect the degree and severity of diseases, and such large-scale anomalies are not detected through traditional DNA sequencing methods

(Lisenka et al. 2003; Idbaih et al. 2010). The possible presence of such abnormalities should be investigated in future studies.

The presence of phenocopies in Family 2071 is also a relevant pit-fall in this study of AD hearing loss families. While phenocopies are common, they are also often difficult to prove beyond any doubt. A routine aspect of the clinical ascertainment is a request of patients and family members to fill out a detailed hearing loss questionnaire. This includes specific questions about noise exposures, head injuries, usage of drugs known to be ototoxic, etc. In this study, the cause of hearing loss in Family 2071's putative phenocopies is unknown. These unanswered questions obscure the full picture of hearing loss in Family 2071. The continued investigation of this family is, therefore, essential to gain a more complete understanding of hearing loss in this family and to confirm beyond any doubt that several individuals within Family 2094 are phenocopies.

Lastly, the approach taken in this study is also a limitation unto itself. This study was a "targeted" candidate gene approach which focused only on genes previously associated with hearing loss. While this improved the likelihood of detecting hearing loss mutations, it restricted the chance of identifying potential "genetic surprises" regarding genotype-phenotype. And while several exons within *KCNQ4, COCH, TECTA,* and *MYO1A* not previously associated with hearing loss were bi-directionally sequenced, this approach was limited by the two year time-frame of a master's thesis.

# Chapter 5: Summary

The aim of this thesis was to determine the genetic etiology of AD hearing loss in 28 large, multi-generational Newfoundland families. Probands were first screened for hearing loss alleles previously reported in the Newfoundland population: specifically the full *GJB2* gene, the del13S1830 mutation in *GJB6GJB2, GJB6,* and the p.A716T in *WFS1.* The next step was a systematic functional candidate gene search for genes and mutations from the primary literature and the NCBI database. Genotype-phenotype evaluation of potential candidate genes, and frequency of mutations found previously, helped to narrow the list down to four likely functional candidate genes: *COCH, KCNQ4, TECTA,* and *MYO1A.* These genes all code for proteins that play an important role in human hearing, and harbor hearing loss mutations recurrent in Caucasian populations. Once a mutation was identified, we were then able to highlight common patterns among the phenotypes of Newfoundland probands and the phenotypes of known mutations in AD deafness causing genes. Figure 2.2 illustrates the progression of this study from the stage of experimental design to the discovery of novel and known causative mutations.

In Newfoundland Family 2094 a known mutation (p.P51P/S) within *COCH* was discovered to be the cause of hearing loss. The p.P51P/S mutation causes a late-onset progressive high-frequency hearing loss, and is associated with severe vestibular defects, such as vertigo and motion sickness. Using DNA from a Dutch p.P51P/S family, and Family 2094, an ancestral haplotype was created through successful fragment analysis, confirming Family 2094's p.P51P/S transition to be a Dutch founder mutation. However,

no other Newfoundland families were explained by this mutation. No further genealogy was done to elaborate on this shared ancestry.

In Newfoundland Family 2071, a novel 3 bp deletion in exon 5 of *KCNQ4* has been found to be the cause of hearing loss. This discovery also provides further evidence of the current genotype-phenotype correlation, whereby deletions in *KCNQ4* cause a milder, later onset, high-frequency loss in patients compared to *KCNQ4* missense mutations (Nie, 2008). This mutation was not detected in any additional Newfoundland probands.

In Newfoundland Family 2102, the cause of hearing loss was identified to be a nonsense mutation in exon 4 of *MYO1A* (p.R93X). All affected individuals are reported to have suffered from hearing loss from the age of five, with hearing coming and going but progressively deteriorating. Again, this mutation was not reported in any additional Newfoundland hearing loss families. No hearing loss causing mutations were discovered within the *TECTA* gene, but sequencing of this gene should be completed in the future to rule it out completely as a candidate gene for hearing loss.

In the introduction, a table was presented indicating all known deafness related genes and their mutations within the Newfoundland population. An updated version of this table is seen in Table 5.1. Of the 28 Newfoundland families suffering from AD hearing loss at the beginning of this study, three families have now been solved. Further research on the remaining 25 unsolved Newfoundland families is of paramount importance. Genomic DNA from four of these 24 families has recently been sent for a

genome wide scan (GWS). The data gained from this GWS will inevitably enable further successful identification of the genetic cause of hearing loss in these families. AudioGene Audioprofiling also presents a promising avenue of detecting further mutations within the remaining 25 probands, and should be investigated. The determination of these hearing loss causing mutations must remain of critical importance to researchers and clinicians alike. With a greater understanding of the genetic mutations causing various families' hearing loss, comes a greater understanding of the pathogenic mechanisms and a greater chance of improved treatment options and screening abilities.

**Table 5.1 Deafness Genes Identified in Newfoundland Population at End of This Study**

| Gene | Mutation | # of NL Families | Literary Reference |
|---|---|---|---|
| *KCNQ4* | c.806_808delCCT | 1 | Young et al. Unpublished |
| *COCH* | c.151C>CT | 1 | De Kok et al. 1999 |
| *MYO1A* | c.2435C>CT | 1 | Donaudy et al. 2003 |
| *GJB2* | c.35delG | 7 | Denoyelle et al. 1997 |
| *GJB6* | D13S1830 | 3 | del Castillo et al. 2002 |
| *TMPRSS3* | c.207delC | 1 | Ahmed et al. 2004 |
| *TMPRSS3* | c.782+3delGAG | 2 | Ahmed et al. 2004 |
| *PCDH15* | c.1978T>A | 1 | Ahmed et al. 2003 |
| *WFS1* | c.2146G>A | 1 | Young et al. 2001; Bespolova et al. 2001; Sivakumaran & Lesperance, 2002 |

# Literature Cited


Ahmed ZM, Riazuddin S, Riazuddin S, Wilcox ER. 2003. The molecular genetics of Usher syndrome. Clinical Genetics 63:431-444.

Ahmed ZM, Xiaoyan CL, Powell SD, Riazuddin S, Young TL, Ramzan K, Ahmad Z, Luscombe S, Dhillon K, MacLaren L, Ploplis B, Shotland LI, Ives E, Riazuddin S, Friedman TB, Morell RJ, Wilcox ER. 2004. Characterization of a new full length TEMPRSS3 isoform and identification of mutant alleles responsible for nonsyndromic recessive deafness in Newfoundland and Pakistan. BMC Medical Genetics5:24.

Asher JH, Friedman TB. 1990. Mouse and hamster mutants as models for Waardenburg syndromes in humans. J Med Genet 27:618-626.

Bates DE. 2003. Aminoglycoside ototoxicity. Drugs Today (Barc) 39:277–85.

Bear JC, Nemec TF, Kennedy JC, Marshall WH, Power AA, Kolonel VM, Burke GB. 1987. Persistent Genetic Isolation in Outport Newfoundland. Am. J. Hum. Genet. 27:807-830.

Bennett D. 2002. On the trail of French Ancestors. St.John's NL:Robinson Blackmore.

Bespalova IN, Van Camp G, Bom SJH, Brown DJ, Cryns K, DeWan AT, Erson AE, Flothmann K, Kunst HPM, Kurnool P, Sivakumaran TA, Cremers C, Leal SM, Burmeister M, Lesperance MM. 2001. Mutations in the Wolfram syndrome 1 gene (*WFS1)* are a common cause of low frequency sensorineural hearing loss. Human Molecular Genetics 10(22): 2501-2508.

Beisel KW, Nelson NC, Delimont DC, Fritzsch B. 2001. Longitudinal gradients of KCNQ4 expression in spiral ganglion and cochlear hair cells correlate with progressive hearing loss in DFNA2. Brain Res. Mol. Brain Res. **82**(1-2): 137–49

Bischoff A, Huygen P, Kemperman M, Pennings RJE, Bom SJH, Verhagen WIM, Admiraal RJC, Kremer H, Cremers CRJW. 2005. Vestibular Deterioration Precedes Hearing Deterioration in the P51S *COCH* Mutation (DFNA9): An Analysis in 74 Mutation Carriers. Otology & Neurotology 26:918-925.

Bischoff A, Pauw R, Huygen P, Aandekerk A, Kremer H, Cremers C, Cruysberg J. 2007. Vertical Corneal Striae in Families with Autosomal Dominant Hearing Loss: DFNA/COCH. American Journal of Ophthalmology 143:847-852.

Bitner-Glinzicz M. Hereditary deafness and phenotyping in humans. 2002. British Medical Bulletin 63:73-94.

Brown SDM, Hardisty-Hughes RE, Mburu P. 2008. Quiet as a mouse: dissecting the molecular and genetic basis of hearing. Nature Genetics 9:277-290.

Cambron N. 2006. Speech Recognition Ability in Cochlear Implant Users 65 and Older. Seminar Hearing 27(4):345-347.

Carey JP, Migliaccio A, Minor LB. 2007. Semicircular Canal Function Before and After Surgery for Superior Canal Dehiscence. Otology & Neurotology 28:356-364.

Casano RA, Johnson DF, Bykhovskaya Y, Torricelli F, Bigozzi M, Fischel-Ghodsian N. 1999. Inherited susceptibility to aminoglycoside ototoxicity: genetic heterogeneity and clinical implications. Am J Otolaryngol. 20: 151–6.

Chen A, Wayne S, Bell A, Ramesh A, Srisailapathy CR, Scott DA, Sheffield VC, Van Hauwe P, Zbar RI, Ashley J, Lovett M, Van Camp G, Smith RJ. 1997. New gene for autosomal recessive non-syndromic hearing loss maps to either chromosome 3q or 19p. Am. J. Med. Genetics 5;71(4):467-471.

Coucke PJ, Van Hauwe P, Kelley PM, Kunst H, Schatteman I, Van Velzen D, Meyers J, Ensink RJ, Verstreken M, Declau F, Marres H, Kastury K, Bhasin S, McGuirt WT, Smith RJH, Cremers CWRJ, Van de Heyning P, Willems PJ, Smith SD, Van Camp G. 1999. Mutations in the *KCNQ4* gene are responsible for autosomal dominant deafness in four DFNA2 families. Human Molecular Genetics 8(7): 1321-1328.

Cremers CW, Marres HA, Van Rijn PM. 1991. Nonsyndromal profound genetic deafness in childhood. Ann N U Acad Sci. 630:191-196.

De Kok YJM, Bom S, Brunt T, Kemperman MH, Van Beusekom E, Van Der Velde-Visser SD, Robertson NG, Morton CC, Huygen PLM, Verhagen WIM, Brunner HG, Cremers CWRJ, Cremers PM. 1999. A Pro51Ser mutation in the *COCH* gene is associated with late onset autosomal dominant progressive sensorineural hearing loss with vestibular defects. Human Molecular Genetics 8(2):361-366.

Del Castillo I, Villamar M, Moreno-Pelayo MA, Del Castillo FJ, Alvarez A, Telleria D, Menendez I, Moreno F. 2002. A Deletion Involving the Connexin 30 Gene in Nonsyndromic Hearing Impairment. N. Engl. J. Med. 346(4):243-249.

Donaudy F, Ferrara A, Esposito L, Hertzano R, Ben-David O, Bell RE, Melchionda S, Zelante L, Avraham KB, Gasparini P. 2003. Multiple Mutations of *MYO1A*, a

Cochlear-Expressed Gene, in Sensorineural Hearing Loss. Am. J. Hum. Genet. 72:1571-1577.

Fitzgibbons PJ, Gordon-Salant S. 2010. Age-related differences in discrimination of temporal intervals in accented tone sequences. Hearing Research 264:41-47.

Fransen E, Verstreken M, Bom S, Lemaires F, Kemperman MH, De Kok YJM. 2001. A common ancestor for *COCH* related cochleovestibular (DFNA9) patients in Belgium and The Netherlands bearing the P51S mutation. J Med Genet 38:61-64.

Friedman T, Griffith A. 2003. Human Non-syndromic Sensorineural Deafness. Annu. Rev. Genomics of Human Genetics 4:341-402.

Frogatt NJ, Brassett C, Koch DJ, Evans DG, Hodgson SV, Ponder BA, Maher ER. 1996. Mutation screening of MSH2 and MLH1 mRNA in hereditary non-polyposis colon cancer syndrome. J. Med. Genet. 33(9):726-730.

Gates GA, Mills JH. 2005. Presbyacusis. Lancet 366:1111-1120.

Gorlin RJ, Toriello HV, Cohen M. Hereditary Hearing Loss and its Syndromes. 1995. Oxford University Press NY.

Gratton MA, Vazquez AE. 2003. Age-related hearing loss: current research. Curr. Opin. Otolaryngol. Head Neck Surg. **11**:367–371.

Grifa A, Wagner CA, D'Ambrosio L, Melchionda S, Bernardi F, Lopez-Bigas N, Rabionet R, Arbones M, Monica MD, Estivill X, Zelante L, Lang F, Gasparini P. 1999. Mutations in GJB6 cause nonsyndromic autosomal dominant deafness at DFNA3 locus.

Griffith AJ, Friedman TB. 2002. Autosomal and X-Linked Auditory Disorders. In Keats BJ, Popper AN, Fay RR (Eds.), Genetics and Auditory Disorders. New York Inc: Springer-Verlag 121-228.

Hancock WG. 1989. Soe longe as there comes noe women: Origins of the English settlement in Newfoundland. St.John's NL: Breakwater.

Hildebrand MS, Tack D, DeLuca A, Ae Hur I, Van Rybroek JM, McMordie SJ, Muilenburg A, Hoskinson DP, Van Camp G, Pensak ML, Storper IS, Huygen PLM, Casavant TL, Smith RJH. 2009. Mutation in the *COCH* Gene is Associated With Superior Semicircular Canal Dehiscence. Am. J. Med. Genet. 149A:280-285.

Hildebrand MS, Tack D, McMordie SJ, DeLuca A, Ae Hur I, Nishimura C, Huygen P, Casavant TL, Smith RJH. 2008. Audioprofile-directed screening identifies novel mutations in *KCNQ4* causing hearing loss at the DFNA2 locus. Genet. Med. 10(11):797-804.

Hildebrandt F, Heeringa SF, Ruschendorf F, Attanasio M, Nurnberg G, Becker C, Seelow D, Huebner N, Chernin G, Vlangos CN, Zhou W, O'Toole JF, Hoskins BE, Wolf MTF, Hinkes BG, Chaib H, Ashraf S, Schoeb DS, Ovunc B, Allen SJ, Vega-Warner V, Wise E. 2009. A Systematic Approach to Mapping Recessive Disease Genes in Individuals from Outbred Populations. PLoS Genetics 5(1):1-10.

Hildebrand MS, Tack D, McMordie SJ, DeLuca A, Hur IA, Nishimura C, Huygen P, Casavant TL, Smith RJ. 2008. Audioprofile-directed screening identifies novel mutations in KCNQ4 causing hearing loss at the DFNA2 locus. Genetic Med. 10:797-804.

Hildebrand MS, DeLuca AP, Taylor KR, Hoskinson DP, Hur IA, Tack D, McMordie SJ, Huygen P, Casavant TL, Smith RJH. 2009. A Contemporary Review of AudioGene Audioprofiling: A Machine Based Candidate Gene Prediction Tool for Autosomal Dominant Nonsyndromic Hearing Loss. Laryngoscope 119:2211-2215.

Hilgert N, Smith R, Van Camp G. 2009. Forty-six genes causing nonsyndromic hearing impairment: Which ones should be analyzed in DNA diagnostics? Mutation Research 681:189-196.

Hilgert N, Smith R, Van Camp G. 2009. Function and expression pattern of nonsyndromic deafness genes. Curr. Mol. Med. 9(5):546-564.

Idbaih A, Dalmasso C, Kouwenhoven M, Jeuken J, Carpaentier C, Gorlia T, Kros JM, French P, Teepen J, Broet P, Delattre O, Mokhtari K, Sanson M, Delattre JY, Van der Bent M, Hoang-Xuan K. 2010. Genomic aberrations associated with outcome in anaplastic oligodendral tumors treated within the EORTC phase III trial 26951. J. Neurooncology DOI 10.1007/s11060-010-0380-9.

Jentsch TJ. 2000. Neuronal KCNQ potassium channels: physiology and role in disease. Nature Reviews Neuroscience 1:21-30.

Kamada F, Kure S, Kudo T, Suzuki Y, Oshima T, Ichinohe A, Kojima K, Niihori T, Kanno J, Narumi Y, Narisawa A, Kati K, Aoki Y, Ikeda K, Kobayashi T, Matsubara Y. 2006. A novel *KCNQ4* one-base deletion in a large pedigree with hearing loss: implication for the genotype-phenotype correlation. J Hum Genet 51:455-460.

Keats BJ, Berlin C. 2002. Introduction and Overview: Genetics in Auditory Science and Clinical Audiology. In Keats BJ, Popper AN and Fay RR (Eds.), Genetics and Auditory Disorders (pp. 1-22). New York, Inc: Springer-Verlag.

Kemperman MH, De Leenheer EM, Huygen PL, Van Duijnhoven G, Morton CC, Robertson NG, Cremers FP, Kremer H, Cremers CW. 2005. Audiometric, vestibular,and genetic aspects of a DFNA9 family with a G88E COCH mutation. Otol. Neurotol. 26:926–933.

Kodera K, Adachi T, Sidara J, Kachi K. 1994. The effects of hearing aid frequency responses on speech discrimination score. Nippon Jibiinkoka Gakkai Kaiho 97(9):1669-1674.

Kommareddi P, Nair TS, Raphael Y, Telian SA, Kim AH, Arts HA, El-Kashlan H, Carey TE. 2007. Cochlin Isoforms and Their Interaction with CTL2 (SLC44A2) in the Inner Ear. Journal of the Association for Research in Otolaryngology 8:435-446.

Kubisch C, Schroeder BC, Friedrich T, Lutjohann B, El-Amraouni A, Marlin S, Petit C, Jentsch TJ. 1999. KCNQ4, a novel potassium channel expressed in sensory outer hair cells, is mutated in dominant deafness. Cell 96:437–446.

Leon PE, Bonilla JA, Sanchez JR, Vanegas R, Villalobos M, Torres L, Leon F, Howell AL, Rodriguez JA. 1981. Low Frequency Hereditary Deafness in Man with Childhood Onset. Am. J. Hum. Genet. 33:209-214.

Leon PE, Raventos H, Lynch E, Morrow J, King MC. 1992. The gene for an inherited form of deafness maps to chromosome 5q31. Proc. Natl. Acad. Sci. 89:5181-5184.

Leung J, Wang NW, Yeagle JD, Chinnici J, Bowditch S, Francis HW, Niparko JK. 2005. Predictive Models for Cochlear Implantation in Elderly Candidates. Arch. Otol. Head Neck Surg. 131:1049-1054.

Lisenka E, Vissers LM, Vries B, Osoegawa K, Janssen IM, Feuth T, Choy CO, Straatman H, Van der Vliet W, Huys E, Van Rijk A, Smeets D, Van Ravenswaaij-Arts CMA, Knoers NV, Van der Burgt I, Jong PJ, Brunner HG, Van Kessel AG, Schoenmakers EF, Veltman JA. 2003. Array-Based Comparative Genomic Hybridization for the Genomewide Detection of Submicroscopic Chromosomal Abnormalities. Am. J. Hum. Genet. 73:1261-1270.

Liu XZ, Yuan Y, Yan D, Ding EH, Ouyang XM, Fei Y, Tang W, Yuan H, Chang Q, Du LL, Zhang X, Wang G, Ahmad S, Kang DY, Lin X, Dai P. 2009. Digenic inheritance of non-syndromic deafness caused by mutations at gap junction proteins Cx26 and Cx31. Human Genetics 125:53-62.

Lynch ED, Lee MK, Morrow JE, Welcsh PL, Leon PE, King MC. 1997. Nonsyndromic Deafness DFNA1 Associated with Mutation of a Human Homolog of the Drosophila Gene diaphanous. Science 278:1315-1318.

Mazzoli M, Van Camp G, Newton V, Giarbini N, Declau F, Parving A. 2003. Recommendations for the Description of Genetic and Audiological Data for Families with Nonsyndromic Hereditary Hearing Impairment. Audiological Medicine 1:148-150.

McAlister PV. 1990. The effects of hearing aids on speech discrimination in noise by normal-hearing listeners. Journal of Rehabilitation Research 27(1):33-42.

Meyer NC, Nishimura CJ, McMordie S, Smith RJH. Audioprofiling identifies *TECTA* and *GJB2*-related deafness segregating in a single extended pedigree. 2007. Clin. Genetics 72:130-137.

Morell RJ, Kim HJ, Hood LJ, Goforth L, Friderici K, Fisher R, Van Camp G, Berlin C, Oddoux C, Ostrer H, Keats B, Friedman TB. 1998. Mutations in the Connexin 26 Gene (GJB2) Among Ashkenazi Jews with Nonsyndromic Recessive Deafness. The New Eng. Jour. Med. 339(21):1500-1505.

Morton CC, Nance WE. 2006. Newborn Hearing Screening – A Silent Revolution. N. Engl. J. Med. 354:2151-2164.

Nebel A, Filon D, Faerman M, Soodyall H, Oppenheim A. 2005. Y chromosome evidence for a founder effect in Ashkenazi Jews. European Journal of Human Genetics 13:388-391.

Neuhausen SL. 2000. Founder populations and their uses for breast cancer genetics. Breast Cancer Research 2:77-81.

Nie L. 2008. *KCNQ4* mutations associated with nonsyndromic progressive sensorineural hearing loss. Curr Opin Otolaryngol Head Neck Surg 16:441-444.

Parfrey PS, Davidson WS, Green JS. 2009. Clinical and genetic epidemiology of inherited renal disease in Newfoundland. Kidney Int. 61:1925-1934.

Park HJ, Cho HJ, Baek JI, Yosef TB, Kwon TJ, Griffith AJ, Kim UK. 2010. Evidence for a founder mutation causing DFNA5 hearing loss in East Asians. Journal of Human Genetics 55:59-62.

Poole CR, Cuff RH. 1994. Settlement. Encyclopedia of Newfoundland & Labrador. St. John's Newfoundland Boulder Publications Ltd.:Portugal Cove, Newfoundland 133-142.

Rehman AU, Morell RJ, Belyantseva IA, Khan SY, Boger ET, Shahzad M, Ahmed ZM, Riazuddin S, Khan SN, Riazuddin S, Friedman TB. 2010. Targeted Capture and Next-Generation Sequencing Identifies C9orf75, Encoding Taperin, as the Mutated Gene in Nonsyndromic Deafness DFNB79. The American Journal of Human Genetics 86:378-388.

Saihan Z, Webster A, Luxon L, Bitner-Glindzicz M. 2009. Update on Usher Syndrome. Current Opinion in Neurology 22(1): 19-27.

Schaffer LG, Kashork CD, Saleki R, Rorem E, Sundin K, Ballif BC, Bejjani BA. 2006. Targeted genomic microarray analysis for identification of chromosome abnormalities in 1500 consecutive clinical cases. Journal of Pediatrics 149(1):98-102.

Schuknecht HF, Gacek MR. 1993. Cochlear pathology in presbyacusis. Ann. Otol. Rhinol. Laryngol. 102(1-2):1-16.

Schuster SC. 2008. Next-generation sequencing transforms today's biology. Nature Methods 5(1):16-18.

Shahin H, Walsh T, Rayyan AA, Lee MK, Higgins J, Dickel D, Lewis K, Thompson J, Baker C, Nord AS, Stray S, Gurwitz D, Avraham KB, King MC, Kanaan M. 2010. Five novel loci for inherited hearing loss mapped by SNP-based homozygosity profiles in Palestinian families. European Journal of Human Genetics 18:407-413.

Smith R, Van Camp G. 2007. Deafness and Hereditary Hearing Loss Overview. Gene Reviews University of Washington, Seattle.

St. John JC, Facucho-Oliveira J, Jiang Y, Kelly R, Salah R. 2010. Mitochondrial DNA transmission, replication, and inheritance: a journey from the gamete through the embryo and into offspring and embryonic stem cells. Hum. Reprod. 16(5):488-509.

Strachan T, Read A. 2003. Human Molecular Genetics. Garland Science/Taylor & Francis Group; 3rd edition London, New York.

Stuckless S, Parfrey P, Woods MO, Cox J, Fitzgerald WG, Green JS, Green RC. 2006. The phenotypic expression of three *MSH2* mutations in large Newfoundland families with Lynch syndrome. Familial Cancer 6:1-12.

Talebizadeh Z, Kelley PM, Askew JW, Beisel KW, Smith SD. 1999. Novel Mutation in the KCNQ4 Gene in a Large Kindred With Dominant Progressive Hearing Loss. Human Mutation 14:493-501.

Tassabehji M, Read AP, Newton VE, Harris R, Balling R, Gruss P, Strachan T. 1992. Waardenburg's syndrome patients have mutations in the human homologue of the *Pax-3* paired box gene. Nature 355:635-636.

Topsakal V, Pennings RJ, te Brinke Hamel B, Huygen PLM, Kremer H, Cremers CWRJ. 2005. Phenotype determination guides swift genotyping of a DFNA2/KCNQ4 family with a hot spot mutation (W276S). Otology & Neurotology 26:52-58.

Toriello HV, Reardon R, Gorlin RJ. 2004. Hereditary Hearing Loss and its Syndromes. Oxford University Press Inc., Oxford.

Tranebjærg L. 2008. Wolframin 1-related Disease and Hearing. In: Kõks S and Vasar E (eds.). Wfs1 protein (wolframin): emerging link between the emotional brain and endocrine pancreas. Kerala, India, Research Signpost.

Van Camp G, Smith RJH. Hereditary Hearing Loss Homepage. May 2010. URL: http://hereditaryhearingloss.org. Last updated May 10, 2010.

Van Camp G, Smith RJH. Nonsyndromic hearing impairment: unparalleled heterogeneity. 1997. Am. J. Hum. Genet. 60:758-764.

Van Eyken E, Van Laer L, Fransen E, Topsakal V, Lemkens N, Laureys W, Nelissen N, Vandevelde A, Wienker T, Van De Heyning P, Van Camp G. 2006. KCNQ4: A Gene for Age-Related Hearing Impairment? Human Mutation 27(10):1007-1016.

Van Graefe A. 1858. Exceptionelles Verhalten des Gesichtsfeldes bei Pigmententartung der Netzhaut. Arch. Ophthamol. 4:250-253.

Van Hauwe P, Coucke PJ, Ensink RJ, Huygen P, Cremers C, Van Camp G. 2000. Mutations in the *KCNQ4* K+ Channel Gene, Responsible for Autosomal Dominant Hearing Loss, Cluster in the Channel Pore Region. Am. J. Med. Genet. 93:184-187.

Verhagen W, Bom S, Huygen M, Fransen E, Van Camp G, Cremers CWRJ. 2000. Familial Progressive Vestibulocochlear Dysfunction Caused by a *COCH* Mutation (DFNA9). Arch Neurol. 57:1045-1047.

Verhoeven K, Van Laer L, Kirschofer K, Legan PK, Hughes DC, Schatteman I, Verstreken M, Van Hauwe P, Coucke P, Chen A, Smith RJH, Somers T,

Offeciers E, Van de Heyning, Richardson GP, Wachtler F, Kimberling WJ, Willems PJ, Govaerts PJ, Van Camp G. 1998. Mutations in the human a-tectorin gene cause autosomal dominant non-syndromic hearing impairment. Nature Genetics 19:60-62.

Wayne S, Robertson NG, DeClau F, Chen N, Verhoeven K, Prasad S, Tranebjarg L, Morton CC, Ryan AF, Van Camp G, Smith RJH. 2001. Mutations in the transcriptional activator *EYA4* cause late-onset deafness at the DFNA10 locus. Hum. Molec. Genet. 10(3):195-200.

Webb MP, Dicks EL, Green JS, Moore SJ, Warden GM, Gamberg JS, Davidson WS, Young TL, Parfrey PS. 2009. Autosomal recessive Bardet-Beidl syndrome: first-degree relatives have no predisposition to metabolic and renal disorders. Kidney Int. 76:215-223.

Weil D, Blanchard S, Kaplan J, Guilford P, Gibson F, Walsh J, Mburu P, Varela A, Leviliers J, Weston MD, Kelley PM, Kimberling WJ, Wagenaar M, Levi-Acobas F, Larget-Piet D, Munnich A, Steel KP, Brown SDM, Petit C. 1995. Defectice myosin VIIA gene responsible for Usher syndrome type 1B. Nature 374:60-61.

Willems P. 2001. Genetic Causes of Hearing Loss. Mechanisms of Disease 342(15):1101-1109.

World Health Organization. 2002. Active ageing: a policy framework. A contribution of the World Health Organization to the Second United Nations World Assembly on Ageing. Madrid http://whqlibdoc.who.int/hq/2002/WHO_NMH_NPH_02.8.pdf.

Wu T, Marcus DC. 2002. Age-Related Changes in Cochlear Endolymphatic Potassium and Potential in CD-1 and CBA-CaJ Mice. Journal of the Association for Research in Otolaryngology 04:353-362.

Yang T, Gurrola JG, Wu H, Chiu SM, Wangemann P, Snyder PM, Smith RJH. 2009. Mutations in KCNJ10 Together with Mutations of SLC26A4 Cause Digenic Nonsyndromic Hearing Loss Associated with Enlarged Vestibular Aqueduct Syndrome. American Journal of Human Genetics 84:651-657.

Young TL, Ives E, Lynch E, Person R, Snook S, MacLaren L, Cator T, Griffin A, Fernandez B, Lee MK, King MC. 2001. Non-syndromic progressive hearing loss *DFNA*38 is caused by heterozygous missense mutation in the Wolfram syndrome gene *WFS1*. Human Molecular Genetics 10(22): 2509-2514.

Yueh B, Shekelle P. 2007. Quality Indicators for the Care of Hearing Loss in Vulnerable Elders. Journal of the American Geriatrics Society 55(S2):35-39.

**Appendix A:** PCR Primer Sequences and Expected PCR Product Sizes.

| Gene | Amplified Exon | Primer Name | Primer Sequence | Size of Fragment (bp) |
|---|---|---|---|---|
| KCNQ4 | 1A | 4700-1AF<br>4700-1AR | agttggagtcggaaagagca<br>CGCAAACTCACATGAAGACG | 567 |
| KCNQ4 | 1B | 4700-1BF<br>4700-1BR | AGCCATGCGTCTCTGAGC<br>ctgggagatcagggcttagg | 584 |
| KCNQ4 | 2 | 4700-2R<br>4700-2F | ccagggaattccaattctga<br>gaagcctctttccaccttca | 456 |
| KCNQ4 | 3 | 4700-3F<br>4700-3R | ggaatcgtcaagtccaggaa<br>agggtcagagtcgggattg | 358 |
| KCNQ4 | 4 | 4700-4F<br>4700-4R | tactcccaatcccgactctg<br>ttagacctcgcctcctgcta | 486 |
| KCNQ4 | 5 | 4700-5F<br>4700-5R | tgggaggagctgagaaagaa<br>tgagtcaggagtcacgatgg | 351 |
| KCNQ4 | 6+7 | 4700-6&7F<br>4700-6&7R | ccctcatgatcaggctccta<br>gtcagcacacagggttgaca | 554 |
| KCNQ4 | 8 | 4700-8F<br>4700-8R | ccacaactggaccaaggact<br>aaggacactccaggctctga | 356 |
| KCNQ4 | 9 | 4700-9F<br>4700-9R | tccaccctgtcctattctgg<br>aaggcaggtctgagagagga | 397 |
| KCNQ4 | 10 | 4700-10F<br>4700-10R | catccttgttccatcccaag<br>ccaaagacggtccatcagtt | 494 |
| KCNQ4 | 11 | 4700-11F<br>4700-11R | ctggtggtttggcatacaag<br>ggctggtctcaaactcctga | 287 |
| KCNQ4 | 12 | 4700-12F<br>4700-12R | tccatctcatccctgtttctg<br>ggcctcagacttcattcagg | 392 |
| KCNQ4 | 13 | 4700-13F<br>4700-13R | ggtgccttctccttcatcag<br>cgggtttatgggaatgtctg | 394 |
| KCNQ4 | 14A | 4700-14AF<br>4700-14AR | ctagccaagctccacctttc<br>GCCTCTGAGAAGTCCCTCAGT | 383 |
| KCNQ4 | 14B | 4700-14BF<br>4700-14BR | GACCTGCTGTTGGGCTTCTA<br>gctgctgctgccctctgt | 418 |
| TECTA | 5 | 5422-5F<br>5422-5R | accctgactcggctatgaaa<br>ccattacccagcggagagat | 480 |
| TECTA | 9A | 5422-9AF<br>5422-9AR | gggcagaccgtgtctttatc<br>ACTCCAGGAAGGAGCTGTTG | 497 |
| TECTA | 9B | 5422-9BF<br>5422-9BR | GCTTGTGCGGCTTCTACAAT<br>acctggaagggaagtcctga | 488 |
| TECTA | 10A | 5422-10AF<br>5422-10AR | gcactcacaaacacacatgc<br>AAGGTGAGGTAGTGGCCGTA | 496 |
| TECTA | 10B | 5422-10BF<br>5422-10BR | CTTCTGGGTGGACCTGGACT<br>tttctttggattccggacct | 499 |

**Appendix A:** PCR Primer Sequences and Expected PCR Product Sizes (cont).

| Gene | Amplified Exon | Primer Name | Primer Sequence | Size of Fragment (bp) |
|---|---|---|---|---|
| TECTA | 11A | 5422-11AF<br>5422-11AR | ctgctcaaacttccctctgg<br>AAGGCAGCGTTCTGGTTG | 499 |
| TECTA | 11B | 5422-11BF<br>5422-11BR | ACCCTGATGATGACCTGGAG<br>tcagttccaaagtgcatatcct | 497 |
| TECTA | 12 | 5422-12BF<br>5422-12BR | tgcctttcatctccctgagt<br>cgaacacgacgctcttcata | 415 |
| TECTA | 13A | 5422-13AF<br>5422-13AR | catttgagttgaggccgttt<br>AGTAGACGGGCGAAATGATG | 494 |
| TECTA | 13B | 5422-13BF<br>5422-13BR | CGTCGCAACGTGATTCAG<br>acctggtcactgtgtgtgga | 470 |
| TECTA | 14 | 5422-14F<br>5422-14R | cagaatggagtcgttgagacag<br>aggcattcctcattcacacc | 499 |
| TECTA | 17 | 5422-17F<br>5422-17R | atgcccaggttactgctttg<br>gcagatcaccctgaagttgg | 493 |
| TECTA | 18 | 5422-18F<br>5422-18R | gccatttctccactttcagg<br>tagggcatcaaagacaaacg | 354 |
| TECTA | 20 | 5422-20F<br>5422-20R | gcatttctgccatttatggtg<br>gatgattccagtccggtcac | 381 |
| MYO1A | 3 | 5379-3F<br>5379-3R | gcctctggctgtggatatgt<br>acgcaggttcatccactctc | 399 |
| MYO1A | 4 | 5379-4F<br>5379-4R | gcccagtctgctccaagtag<br>tggagggtcaggtctaggtc | 297 |
| MYO1A | 6+7 | 5379-6&7F<br>5379-6&7R | tgagccctagaccctcttcc<br>gttgggaagtctccttgacg | 500 |
| MYO1A | 10 | 5379-10F<br>5379-10R | atgaatccattagggcaagg<br>aggcagaaagcagaaatcaaa | 495 |
| MYO1A | 11+12 | 5379-11&12F<br>5379-11&12R | caccagtgtctcaggcagtt<br>tcatcctccctactctgctca | 490 |
| MYO1A | 18 | 5379-18F<br>5379-18R | gcaccgtgtgcagcatag<br>ttcacccagcttcagcagat | 395 |
| MYO1A | 22 | 5379-22F<br>5379-22R | actcaggtctttgcgtggtt<br>gcagactgaggaaactcttgg | 281 |
| MYO1A | 25 | 5379-25F<br>5379-25R | ggtgctgatgtcttggtcct<br>caaacacagcctgccatct | 374 |
| COCH | 2+3 | 4086-2&3F<br>4086-2&3R | tctgtgtcctctctcctctgc<br>atgggagaaacaggtgagca | 499 |
| COCH | 4 | 4086-4F<br>4086-4R | ctggaatggtatggaagggta<br>tatccaggagaaccgtgaaa | 463 |
| COCH | 5 | 4086-5F<br>4086-5R | agcgagacgccatcaaataa<br>ccatcaaggttaaagaggctga | 395 |

**Appendix A:** PCR Primer Sequences and Expected PCR Product Sizes (cont).

| Gene | Amplified Exon | Primer Name | Primer Sequence | Size of Fragment (bp) |
|---|---|---|---|---|
| COCH | 12A | 4086-12AF<br>4086-12AR | tttgccactctcgtcacaat<br>TTTGCCTAAATGGCTGTTGA | 492 |
| COCH | 12B | 4086-12BF<br>4086-12BR | GATGTCATCAGAGGCATTTGT<br>CCTGAACCATGTTAAAGAGCTG | 488 |
| COCH | 12C | 4086-12CF<br>4086-12CR | CACTGCTGAGGCTTCATAATCA<br>CCTGAACCATGTTAAAGAGCTG | 243 |
| COCH | 12D | 4086-12DF<br>4086-12DR | TCTGGATATAGAAAGGAGACCTGT<br>cagattggtctttccacatga | 384 |

**Appendix B:** Mutations Previously Found Within the Four Selected Candidate Genes *KCNQ4, COCH, TECTA,* and *MYO1A*.

| Mutation | Protein Domain | Exon | Reference |
|---|---|---|---|
| *KCNQ4*-p.W276S | Pore Region | 5 | Coucke et al. 1999. |
| *KCNQ4*-p.L274H | Pore Region | 5 | Kubisch et al. 1999. |
| *KCNQ4*-p.L281S | Pore Region | 6 | Talebizadeh et al. 1999. |
| *KCNQ4*-p.G285S | Pore Region | 6 | Kubisch et al. 1999. |
| *KCNQ4*-p.G285C | Pore Region | 6 | Coucke et al. 1999. |
| *KCNQ4*-p.G321S | S6 Transmembrane | 7 | Coucke et al. 1999. |
| *KCNQ4*-p.G455H | Pore Region | N/A | Van Laer et al. 2006. |
| *KCNQ4*-p.Q71fsX138 | Transmembrane | 1 | Kamada et al. 2006. |
| *KCNQ4*-p.FS71 | N-Terminal Cytoplasmic | 1 | Coucke et al. 1999. |
| *COCH*-p.V104del | FCH | 5 | Nagy et al. 2004. |
| *COCH*-p.P51P/S | FCH | 4 | Fransen et al. 1999. |
| *COCH*-p.V66G | FCH | 4 | Robertson et al. 1998. |
| *COCH*-p.G87W | FCH | 5 | Collin et al. 2006. |
| *COCH*-p.G88E | FCH | 5 | Robertson et al. 1998. |
| *COCH*-p.I109N | FCH | 5 | Kamarinos et al. 2001. |
| *COCH*-p.W117R | FCH | 5 | Robertson et al. 1998. |
| *COCH*-p.A119T | FCH | 5 | Usami et al. 2003. |
| *COCH*-p.C542F | vWFA2 | 12 | Street et al. 2005. |
| *TECTA*-p.N864K | N/A | 9 | Hutchin et al. 2005. |
| *TECTA*-p.C1057S | Zona Adhesion | 10 | Balciuniene et al. 1999. |
| *TECTA*-p.C1352Y | N/A | 11 | Hutchin et al. 2005. |
| *TECTA*-p.C1509G | vWFD4 | 13 | Pfister et al. 2004. |
| *TECTA*-p.C1619S | Zona Adhesion | 14 | Alloisio et al. 1999. |
| *TECTA*-p.L1820F | Zona Pellucida | 17 | Verhoeven et al. 1998. |
| *TECTA*-p.G1824D | Zona Pellucida | 17 | Verhoeven et al. 1998. |
| *TECTA*-p.C1837G | Zona Pellucida | 17 | Moreno-Pelayo, 2001. |
| *TECTA*-p.Y1870C | Zona Pellucida | 18 | Verhoeven et al. 1998. |
| *TECTA*-p.R2021H | Zona Pellucida | 20 | Iwasaki et al. 2002. |
| *TECTA*-p.F119fsX131 | N/A | 5 | Hutchin et al. 2005. |
| *MYO1A*-p.R93X | Motor Domain | 3 | Donaudy et al, 2003 |
| *MYO1A*-p.V306M | N/A | 10 | Donaudy et al, 2003 |
| *MYO1A*-p.E385D | Motor Domain | 12 | Don+audy et al, 2003 |
| *MYO1A*-p.G662E | N/A | 18 | Donaudy et al, 2003 |
| *MYO1A*-p.G674D | N/A | 18 | Donaudy et al, 2003 |
| *MYO1A*-p.S797F | N/A | 22 | Donaudy et al, 2003 |

**Appendix B:** Mutations Previously Found Within the Four Selected Candidate Genes *KCNQ4, COCH, TECTA,* and *MYO1A* (cont).

| Mutation | Protein Domain | Exon | Reference |
|---|---|---|---|
| *MYO1A*-p.349-350insCTT | N/A | 4 | Donaudy et al, 2003 |

**Appendix C:** *COCH* Microsatellite Marker Primer Sequences and Expected PCR Product Sizes.

| Marker Name | Primer Name | Primer Sequence | Size of Fragment (bp) | 5' Modifications |
|---|---|---|---|---|
| **D14S262** | D14S262-F<br>D14S262-R | GCAGTGGACTGATGCTCC<br>CCATGAAACTGGTCCCG | 200 | 6FAM |
| **D14S975** | D14S975-F<br>D14S975-R | CATACACAGACACACGGAGA<br>TGCCAAATAATCAGTTTTGC | 174 | 6FAM |
| **D14S1021** | D14S1021-R<br>D14S1021-F | AGTCGTGTATCCTGGGCAT<br>GCGCTGGTGTGAATCTTTA | 266 | 6FAM |
| **D14S257** | D14S257-F<br>D14S257-R | CAGTGAGCCATGACTGTG<br>TTGGTAAAGTGGTAAAAGGC | 182 | 6FAM |
| **D14S1071** | D14S1071-F<br>D14S1071-R | AGTGATCCACCCACCTTC<br>GGCTCAACTACGTGTTGCT | 279 | 6FAM |
| **D14S1040** | D14S1040-F<br>D14S1040-R | GGCACTATGAAACCAATTTTAAC<br>GGCCTGCTGAATCAGA | 231 | PET |
| **D14S1034** | D14S1034-F<br>D14S1034-R | CGTAGATGCTCCAAATCCTAC<br>TAGACAAATCGCTGGTCACT | 176 | 6FAM |
| **D14S1060** | D14S1060-F<br>D14S1060-R | GTTAAAATGGGCCACAATAAAT<br>CTGTTATGTATCAGACCAACCC | 193-219 | 6FAM |
| **D14S70** | D14S70-F<br>D14S70-R | ATCAATTTGCTAGTTTGGCA<br>AGCTAATGACTTAGACACGTTGTAG | 214-224 | 6FAM |
| **D14S1014** | D14S1014-F<br>D14S1014-R | AGCTATTCAGGTCAAAAAGGTC<br>AATCCCTACCCTTGTGGTG | 236-246 | 6FAM |

**Appendix D:** Fragment Analysis of Microsatellite Markers Flanking the *COCH* gene.

| Sample | Marker | Allele 1 | Allele 2 |
|---|---|---|---|
| OP06-115 | D14S1021 | 262 | 262 |
| JC07-184 | D14S1021 | 262 | 262 |
| KM06-227 | D14S1021 | 262 | 262 |
| JS07-182 | D14S1021 | 262 | 274 |
| 19126 | D14S1021 | 268 | 268 |
| 19135 | D14S1021 | 262 | 268 |
| 19149 | D14S1021 | 262 | 268 |
| AP07-66 | D14S1021 | 262 | 274 |
| OP07-270 | D14S1021 | 262 | 274 |
| CP07-187 | D14S1021 | 262 | 268 |
| CC07-269 | D14S1021 | 262 | 268 |
| OP06-115 | D14S975 | 163 | 171 |
| JC07-184 | D14S975 | 163 | 171 |
| KM06-227 | D14S975 | 167 | 171 |
| JS07-182 | D14S975 | 171 | 171 |
| 19126 | D14S975 | 163 | 171 |
| 19135 | D14S975 | 167 | 171 |
| 19149 | D14S975 | 167 | 171 |
| AP07-66 | D14S975 | 163 | 171 |
| OP07-270 | D14S975 | 163 | 171 |
| CP07-187 | D14S975 | 163 | 171 |
| CC07-269 | D14S975 | 165 | 171 |
| OP06-115 | D14S1040 | 234 | 234 |
| JC07-184 | D14S1040 | 232 | 234 |
| KM06-227 | D14S1040 | 234 | 234 |
| JS07-182 | D14S1040 | 232 | 234 |
| 19126 | D14S1040 | 214 | 234 |
| 19135 | D14S1040 | 232 | 234 |
| 19149 | D14S1040 | 232 | 234 |
| AP07-66 | D14S1040 | 232 | 234 |
| OP07-270 | D14S1040 | 232 | 234 |
| CP07-187 | D14S1040 | 232 | 234 |
| CC07-269 | D14S1040 | 232 | 234 |
| OP06-115 | D14S1071 | 277 | 281 |
| JC07-184 | D14S1071 | 275 | 281 |
| KM06-227 | D14S1071 | 281 | 281 |
| JS07-182 | D14S1071 | 277 | 285 |
| 19126 | D14S1071 | 277 | 281 |
| 19135 | D14S1071 | 275 | 281 |
| 19149 | D14S1071 | 275 | 281 |
| AP07-66 | D14S1071 | 285 | 281 |
| 19149 | D14S1060 | 201 | 207 |

**Appendix D:** Fragment Analysis of Microsatellite Markers Flanking the *COCH* Gene (cont).

| Sample | Marker | Allele 1 | Allele 2 |
|---|---|---|---|
| OP07-270 | D14S1071 | 285 | 281 |
| CP07-187 | D14S1071 | 275 | 281 |
| CC07-269 | D14S1071 | 275 | 281 |
| OP06-115 | D14S1034 | 169 | 173 |
| JC07-184 | D14S1034 | 169 | 177 |
| KM06-227 | D14S1034 | 169 | 173 |
| JS07-182 | D14S1034 | 173 | 175 |
| 19126 | D14S1034 | 169 | 177 |
| 19135 | D14S1034 | 169 | 175 |
| 19149 | D14S1034 | 169 | 175 |
| AP07-66 | D14S1034 | 169 | 175 |
| OP07-270 | D14S1034 | 169 | 175 |
| CP07-187 | D14S1034 | 169 | 169 |
| CC07-269 | D14S1034 | 169 | 179 |
| OP06-115 | D14S262 | 200 | 200 |
| JC07-184 | D14S262 | 200 | 200 |
| KM06-227 | D14S262 | 200 | 200 |
| JS07-182 | D14S262 | 200 | 202 |
| 19126 | D14S262 | 204 | 204 |
| 19135 | D14S262 | 200 | 204 |
| 19149 | D14S262 | 200 | 204 |
| AP07-66 | D14S262 | 200 | 202 |
| OP07-270 | D14S262 | 200 | 202 |
| CP07-187 | D14S262 | 200 | 198 |
| CC07-269 | D14S262 | 200 | 204 |
| OP06-115 | D14S257 | 179 | 177 |
| JC07-184 | D14S257 | 179 | 191 |
| KM06-227 | D14S257 | 179 | 179 |
| JS07-182 | D14S257 | 171 | 177 |
| 19126 | D14S257 | 179 | 179 |
| 19135 | D14S257 | 179 | 191 |
| 19149 | D14S257 | 179 | 191 |
| AP07-66 | D14S257 | 179 | 171 |
| OP07-270 | D14S257 | 179 | 171 |
| CP07-187 | D14S257 | 179 | 191 |
| CC07-269 | D14S257 | 179 | 191 |
| OP06-115 | D14S1060 | 201 | 207 |
| JC07-184 | D14S1060 | 201 | 191 |
| KM06-227 | D14S1060 | 201 | 191 |
| JS07-182 | D14S1060 | 201 | 197 |
| 19126 | D14S1060 | 201 | 205 |
| 19135 | D14S1060 | 201 | 207 |

**Appendix E:** Medical Hearing Loss Questionnaire

# Newfoundland and Labrador

# Hearing Loss Study

## Medical Information Questionnaire

The information we are asking you to provide in this questionnaire could help to find the cause of the hearing loss in your family. Please don't be discouraged from completing the questionnaire if you do not know the answers to all the questions - just fill in as much as you can. Any information you provide will be beneficial.
We are always available to answer questions and we can complete the questionnaire with you over the phone, if you prefer.

Adapted from:

THE HARVARD CENTRE
FOR HEREDITARY HEARING LOSS

**Appendix E:** Medical Hearing Loss Questionnaire (cont)

## SECTION 1 - GENERAL INFORMATION

1. Your Name_____ Date of Birth_____

   Address_____

   Home Phone_____ Work Phone_____

   E-mail Address (if you have one) _____

2. To your knowledge, are your parents related, even distantly? ☐ Yes ☐ No ☐ Don't Know

   (This may sound like a strange question, but in a genetic study, we ask it of everyone)

**Please answer the following questions as best you can.** If you have seen the doctor, please give us the name and address, if possible. If you think you have seen them but you are not sure of their names, or when you saw them, just indicate approximate date, for example, "*saw an audiologist 10 years ago in Grand Falls*". If you have not had an appointment with the medical person listed, tick no and move to the next question. Any information you can provide will be helpful.

3. Have you ever visited any of the following doctors?

   (Don't Know)

   - An ENT Doctor? (Ear, Nose and Throat) ☐ Yes ☐ No ☐ D/K

     If yes, where did you see them:_____

   - An Audiologist? (Person performing hearing tests) ☐ Yes ☐ No ☐ D/K

     If yes, where did you see them:_____

   - An Eye Doctor? (Ophthalmologist) ☐ Yes ☐ No ☐ D/K

     If yes, where did you see them:_____

   - A Genetics Doctor? (Geneticist) ☐ Yes ☐ No ☐ D/K

     If yes, where did you see them:_____

   - A doctor who treats diseases of the nervous system? (Neurologist) ☐ Yes ☐ No ☐ D/K

     If yes, where did you see them:_____

   - A Heart Doctor? (Cardiologist) ☐ Yes ☐ No ☐ D/K

     If yes, where did you see them:_____

4. Have you ever been admitted to hospital? If yes, please give name of hospital and approximate date(s) of admisison.

   _____

   _____

**Appendix E:** Medical Hearing Loss Questionnaire (cont)

## SECTION II – MEDICAL HISTORY

**Please answer as many of the following questions as you can about your hearing loss**. If you don't know the answer to the question, write don't know or d/k next to it and go to the next question. Any information you can provide will be helpful.

**A. Hearing History** - Please **circle** the term that best describes your hearing at the present time:

5. Left Ear: normal less than normal

   Right ear: normal less than normal

If hearing is less than normal, what is used to improve hearing?

6. Left Ear: nothing hearing aid cochlear implant other _____

   Right Ear: nothing hearing aid cochlear implant other _____

7. Were you born with hearing loss?..... ☐ Yes ☐ No

   If yes, tick affected ear..... ☐ Right ☐ Left

   If no, when did it start?..... ☐ During Childhood ☐ During Teen Years ☐ During Adulthood

8. Did your hearing loss begin during or soon after:

| | | | (Not Applicable) |
|---|---|---|---|
| - being pregnant..... | ☐ Yes | ☐ No | ☐ N/A |
| - an airplane flight..... | ☐ Yes | ☐ No | ☐ N/A |
| - scuba diving..... | ☐ Yes | ☐ No | ☐ N/A |
| - intravenous antibiotic treatment..... | ☐ Yes | ☐ No | ☐ N/A |
| - chemotherapy for cancer..... | ☐ Yes | ☐ No | ☐ N/A |
| - a severe infection, such as meningitis?..... | ☐ Yes | ☐ No | ☐ N/A |
| - exposure to a **sudden** loud noise..... | ☐ Yes | ☐ No | ☐ N/A |
| - **prolonged** exposure to loud noise..... | ☐ Yes | ☐ No | ☐ N/A |
| - an ear infection..... | ☐ Yes | ☐ No | ☐ N/A |
| - ear surgery (including insertion of T-tubes)... | ☐ Yes | ☐ No | ☐ N/A |
| - injury to the head or the ear..... | ☐ Yes | ☐ No | ☐ N/A |

## **Appendix E:** Medical Hearing Loss Questionnaire (cont)

**Pattern of Hearing Loss.** Please tell us which ear has hearing loss by answering the following questions.

9. Your hearing is:

| | | | (Not Applicable) |
|---|---|---|---|
| - Stable (has not changed much over several years). ... | Right | Left | N/A |
| - Fluctuating (sometimes better, sometimes worse)..... | Right | Left | N/A |
| - Slowly progressing (getting worse over years)..... | Right | Left | N/A |
| - Rapidly progressing (getting worse over weeks/months)..... | Right | Left | N/A |
| - Sudden hearing loss ..... | Right | Left | N/A |

**B. Patient's Medical History.** Have you ever had any of the following:

| | | | (Don't Know) |
|---|---|---|---|
| 10. - Scarlet fever..... | Yes | No | D/K |
| - Measles or German measles (circle which one)..... | Yes | No | D/K |
| - Mumps..... | Yes | No | D/K |
| - Meningitis (brain infection)..... | Yes | No | D/K |
| - Tuberculosis (TB)..... | Yes | No | D/K |
| - Repeated ear infections..... | Yes | No | D/K |
| - Premature graying of hair before age 30..... (Not just at the temples) | Yes | No | D/K |
| - Kidney problems..... | Yes | No | D/K |
| - Diabetes mellitus ("sugar diabetes")..... | Yes | No | D/K |
| - Thyroid problems (goiter, under active, overactive).... | Yes | No | D/K |
| - Depression or "nerves"..... | Yes | No | D/K |

11. Is there anything else which you think we should know about your medical history?

**Appendix E:** Medical Hearing Loss Questionnaire (cont)

**Faculty of Medicine, Schools of Nursing and Pharmacy of Memorial University of Newfoundland; Health Care Corporation, St. John's; Newfoundland Cancer Treatment and Research Foundation**

## Consent to Take Part in Health Research

**TITLE**: The Genetics of Hereditary Deafness in Newfoundland

**INVESTIGATOR(S)**:

**SPONSOR**:

**You have been asked to take part in a research study. It is up to you to decide whether to be in the study or not. Before you decide, you need to understand what the study is for, what risks you might take and what benefits you might receive. This consent form explains the study.**

**The researchers will:**

- **discuss the study with you**
- **answer your questions**
- **keep confidential any information which could identify you personally**
- **be available during the study to deal with problems and answer questions**

**If you decide not to take part or to leave the study this will not affect your health care.**

**1. Introduction/Background:**

Some forms of deafness are hereditary. That means that they are inherited in families as a result of an altered gene. A gene is a piece of genetic material (DNA) which is passed from parents to children. If we can identify the genes that are altered in each family, it would allow us to better understand the process of hearing. We might also learn what goes wrong in some forms of deafness and how it might be treated.

**2. Purpose of study:**

Our goal is to identify the genes involved in hereditary deafness in families.

**3. Description of the study procedures and tests:**

If you agree to take part in this study, you will be asked to:

- Tell us about your hearing and the hearing of other members of your family, and other related aspects of your health.
- Have your hearing tested by a registered audiologist.
- Have a blood sample drawn for DNA testing.
- Complete a hearing loss questionnaire.

We might also want to review your medical records related to your deafness.

Initials: _____

## **Appendix E:** Medical Hearing Loss Questionnaire (cont)

4. **Length of time:**
   If you take part in this study, the interview will last 30-60 minutes including the blood sampling. The hearing test will take about 30 minutes. It will be arranged at a time that is convenient for you. The research may take us several years but you will not have to be involved again. We will keep you informed of our findings.

5. **Possible risks and discomforts:**
   The only discomfort is that of giving a blood sample.

6. **Benefits:**
   It is not known whether this study will benefit you.

7. **Liability statement:**
   **Signing this form gives us your consent to be in this study. It tells us that you understand the information about the research study. When you sign this form, you do not give up your legal rights. Researchers or agencies involved in this research study still have their legal and professional responsibilities.**

8. **Compensation:**
   In the event that you suffer injury as a direct result of taking part in this study, necessary medical treatment not covered by provincial health care insurance will be available at no additional cost to you.

9. **Confidentiality:**
   Unless required by law, only the researchers may have access to any confidential documents pertaining to your participation in this study that may identify you by name. Furthermore, your name will not appear in any report or article published as a result of this study.

10. **Genetic Studies:**
    In order to interpret the results of genetic research, we need to have correct information about parents. Sometimes the research shows new information about birth parents. This could happen in the case of an adoption or a mistake in the identity of a mother or father. This information will not be given to anyone including you or other family members.

**Appendix E:** Medical Hearing Loss Questionnaire (cont)

11. **Future use of tissue/DNA samples.**

In order to preserve a valuable resource, your (tissue/DNA) samples may be stored at the end of this research project. It is possible that these samples may be useful in a future research project dealing with hereditary deafness which may or may not be related to the current research project. **Any future research would have to be approved by a Research Ethics Board (REB).**

Please tick **one** of the following options:

| | |
|---|---|
| | I agree that my (tissue/DNA) samples can be used for any approved research project but only if I am contacted again to give consent for the new project. |
| | I agree that my (tissue/DNA) sample can be used for any approved research project without contacting me again, but **only if my name* cannot be linked, in any way, to the sample.** |
| | Under no circumstances may my sample by used for future research. **My sample must be destroyed** at the end of this present project. |

*Includes name, MCP number or any other identifying information.

The DNA sample from this study will be stored in St. John's, Newfoundland and Seattle, Washington for an indefinite period of time.

**11. Contact Information:**

If you have any questions about taking part in this study, you can meet with the investigator who is in charge of the study at this institution. That person is:

Or you can talk to someone who is not involved with the study at all, but can advise you on your rights as a participant in a research study. This person can be reached through:

**Initials:**_____

## **Appendix E:** Medical Hearing Loss Questionnaire (cont)

**Signature Page**

**Study title:** **The Genetics of Hereditary Deafness in Newfoundland**

**Name of principal investigator:** **Dr. Terry-Lynn Young**

**To be filled out and signed by the participant:**

| | Please check as appropriate: | |
|---|---|---|
| I have read the consent [and information sheet]. | Yes { } | No { } |
| I have had the opportunity to ask questions/to discuss this study. | Yes { } | No { } |
| I have received satisfactory answers to all of my questions. | Yes { } | No { } |
| I have received enough information about the study. | Yes { } | No { } |
| I have spoken to Dr. Young or her research assistant and she has answered my questions. | Yes { } | No { } |
| I understand that I am free to withdraw from the study<br>• at any time<br>• without having to give a reason<br>• without affecting my future care | Yes { } | No { } |
| I understand that it is my choice to be in the study and that I may not benefit. | Yes { } | No { } |
| I agree that the study doctor or investigator may read the parts of my hospital records which are relevant to the study. | Yes { } | No { } |
| I agree to take part in this study. | Yes { } | No { } |

_____ _____

Signature of participant Date

_____ _____

Signature of witness Date

**To be signed by the investigator:**

I have explained this study to the best of my ability. I invited questions and gave answers. I believe that the participant fully understands what is involved in being in the study, any potential risks of the study and that he or she has freely chosen to be in the study.

_____ _____

Signature of investigator Date

Telephone number: _____

**Assent of minor participant (if appropriate):**

_____ _____

Signature of minor participant Date

_____ _____

Relationship to participant named above Age

Initials: _____

# INVESTIGATION OF THE GENETIC CAUSE OF HEARING LOSS IN 28 AUTOSOMAL DOMINANT FAMILIES WITHIN THE NEWFOUNDLAND FOUNDER POPULATION

DAVID A. McCOMISKEY

**Memorial University**

# Investigation of the Genetic Cause of Hearing Loss in 28 Autosomal Dominant Families within the Newfoundland Founder Population

**Masters of Science (Medicine)**

**David A. McComiskey**
**September 2010**

## Abstract

The purpose of this study was to determine the genetic cause of hearing loss in 28 Newfoundland families with Autosomal Dominant hearing loss. AD hearing loss is highly genetically heterogeneous, and is mainly associated with a late onset, progressive phenotype. After a comprehensive literature search, genotype-phenotype evaluations, and a functional candidate gene approach, all 28 probands were sequenced to identify mutations in four genes known to cause autosomal dominant hearing loss, *COCH*, *KCNQ4, TECTA,* and *MYO1A*. First, a known Dutch founder mutation within exon 4 of *COCH*, c.151 C>CT, was found in a Newfoundland proband of Family 2094. All affected family members (n=7) shared this mutation, while unaffected members did not. This is only the second family found to harbor this mutation outside of Europe. This mutation is strongly associated with severe vestibular decline. Affected Family 2094 members carrying the mutation do present vestibular decline in the form of vertigo and balance difficulties. As this mutation is considered to be a Dutch founder mutation, DNA samples from a Dutch p.P51P/S family were genotyped and compared with Family 2094 genotypes. Fragment analysis confirmed haplotype sharing of five markers closely bordering the c.151 C>CT mutation between Newfoundland and Dutch mutation carriers. Second, a novel 3bp deletion in exon 5 of *KCNQ4* was found in 13 affected members of Family 2071. While the mutation was not seen in four other affected family members, audiology test results suggest that these four individuals are phenocopies. Sequencing of the full *KCNQ4* gene was done in all individuals, to rule out another mutation on the same gene. Further investigation, through the construction of an intragenic haplotype, did

not point to any further hearing loss associated variants within *KCNQ4,* and confirmed that all deletion carriers share a common hearing loss haplotype and deletion. Third, a nonsense mutation was found in exon 4 of *MYO1A* in the proband of Newfoundland Family 2102. This is a C→T nucleotide substitution (c.2435 C>CT) that causes a change (p.R93X) in the motor domain of myosin 1A. Of four individuals in Family 2102, three were found to carry the p.R93X mutation, while one unaffected sibling was not. This mutation has been reported once before in a small Italian family. No mutations were discovered in the *TECTA* gene. When each of the causative mutations in *COCH, KCNQ4, and MYO1A* was detected, additional Newfoundland hearing loss probands were screened, to rule out the possibility of a founder mutation. In no case were additional mutation carriers identified. While no founder mutations were discovered in this study, the genetic cause of hearing loss was identified in three families.

## Acknowledgments

I thoroughly enjoyed this graduate program. It was full of rewarding experiences and I am sad to see it end. I would like to take this opportunity to thank those who aided me and without whom I likely never would have succeeded. First, I would like to thank the lab staff, including Mr. Dante Galutria, Mr. Jim Houston, and Ms. Annette Greenslade, for their patient and unwavering encouragement. Secondly, I would like to thank my fellow students in the lab, including Lance Doucette, Nelly Abdelfatah, and Jessica Squires for their optimism, encouragement, and happy dispositions. Third, I thank Carol Negrijn, for her invaluable clinical, medical, and computer knowledge. And finally, I wish to thank my supervisor, Dr. Terry-Lynn Young, and my thesis committee members Dr. Ban Younghusband and Dr. Jane Green for pushing me to push myself, in order to bring out the best in myself, and the best in my research. The things I've learned and the bonds I've forged during my time in "The Young Lab" will never be forgotten.

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# List of Abbreviations & Symbols

**ABI** Applied Biosystems International

**AD** Autosomal Dominant

**ADNSHL** Autosomal Dominant Non-Syndromic Hearing Loss

**AHC** Auditory Hair Cell

**AR** Autosomal Recessive

**C9orf75** C9 open reading frame 75

**cM** Centimorgan

**COCH** Coagulation Factor C Homolog

**CP** Cytoplasmic

**CT** Computed Tomography

**dB** Decibels

**DFNA** Autosomal dominant deafness gene

**DFNB** Autosomal recessive deafness gene

**DFN** X-Linked deafness gene

**$dH_2O$** De-ionized water

## List of Abbreviations & Symbols (cont)

**DIAPH1** Protein diaphanous homolog 1

**dNTP** Dideoxynucleotide Triphosphate

**DMF** Deionized Formamide

**DMSO** Dimethyl Sulfoxide

**DNA** Deoxyribonucleic acid

**EDTA** Ethylenediaminetetraacetic acid

**EtOH** Ethanol

**EYA4** Eyes absent homolog 4

**FCH** Factor C Homologous Domain

**GS500 (-250) LIZ** GeneScan 500 (-250) LIZ Size Standard

**GJB2** Gap junction protein, beta 2

**GJB6** Gap junction protein, beta 6

**GWS** Genome Wide Scan

**HIC** Human Investigations Committee

**Hz** Hertz

**KCNQ4** Potassium Voltage-Gated Channel 4

## List of Abbreviations & Symbols (cont)

**kD** Kilodalton

**L** Liter

**LD** Linkage Disequilibrium

**Mb** Megabase

**$MgCl_2$** Magnesium Chloride

**mM** Millimolar

**MP3** MPEG-1 Audio Layer 3

**MSH2** MutS homolog 2

**MYO1A** Myosin IA

**MYO7A** Myosin VIIA

**ng** Nanogram

**OMIM** Online Mendelian Inheritance in Man

**PAX3** Paired box gene

**PCR** Polymerase Chain Reaction

**rpm** revolutions per minute

## List of Abbreviations & Symbols (cont)

**SSCP** Single strand conformation polymorphism

**SNP** Single Nucleotide Polymorphism

**Taq** Thermus aquaticus DNA Polymerase

**TBE** Tris/Borate/EDTA Buffer

**TECTA** Tectorin Alpha

**TMPRSS3** Transmembrane protease 3, serine 3

**TORCH** Toxoplasmosis, rubella, cmv, and herpes

**TPRN** Taperin

**U** Units

**$\mu$l** Microlitre

**$\mu$M** Micromolar

**USA** United States of America

**USH** Usher Syndrome

**WFS1** Wolfram syndrome 1 (wolframin)

**WS** Waardenburg Syndrome

# List of Appendices

# Chapter 1: Introduction

## Purpose

The aim of this research project is to determine the genetic etiology of autosomal dominant (AD) hearing loss in 28 Newfoundland families.

## Overview

Hearing loss is the most common sensory disorder in humans. For example, one in every 500 newborns has hearing loss (Morton & Nance, 2006). The prevalence of hearing loss increases dramatically with age, and by puberty, the number of affected persons doubles (Morton & Nance, 2006). Hearing loss is even more prevalent in adults, as 60 % of people older than 70 years have a hearing loss of 25 dB or more (Gratton & Vazquez, 2003).

Hearing loss is a multi-factorial disorder caused by both genetic and environmental factors. Genetic factors account for 50 % of all hearing loss cases, while environmental factors cause 25 %. The remaining 25 % are classified as being of unknown etiology (Willems, 2001). Environmental causes of hearing loss include exposure to high sound decibel levels, head trauma, prematurity, neonatal hypoxia, low birth weight, prenatal infections from "TORCH" organisms (i.e., **to**xoplasmosis, **r**ubella, **C**MV, and **h**erpes), and postnatal infections like bacterial meningitis (Willems, 2001; Bitner-Glindzicz, 2002).

Approximately 30 % of genetic cases are syndromic: the phenotype includes other signs and symptoms throughout the body in addition to deafness. Over 400 genetic syndromes include some degree of hearing loss (Gorlin et al. 1995; Nie et al. 2008). Two examples are Usher syndrome (USH): hearing loss accompanied by retinitis pigmentosa, and Pendred syndrome: a hearing loss disorder accompanied by goiter, which is a swelling in the thyroid gland. However, the vast majority, around 70%, of inherited hearing disorders are non-syndromic (Cremers et al. 1991; Van Camp et al. 1997). Worldwide, within non-syndromic cases, 88 % of the hearing loss genes identified cause autosomal recessive (AR) hearing loss, 11 % AD, and the remaining 1% either mitochondrial or X-linked (Smith & Van Camp, 2007).

The five factors used to describe hearing loss are age of onset, sound frequencies affected (low, middle or high), degree of hearing loss (measured in dBs), affected part of the auditory system (conductive, sensorineural or mixed), and configuration (unilateral, or bilateral).

Hearing loss has a high degree of genetic heterogeneity. A large number of mutations within many different genes cause similar hearing loss phenotypes. As of May 2010, the Hereditary Hearing Loss Homepage listed 141 non-syndromic deafness loci that have been mapped and 50 genes that have been identified. Twenty-two of the 50 known genes harbor mutations that cause AD hearing loss (Table 1.1), 33 cause AR hearing loss, and 2 cause X-linked hearing loss (Van Camp G, Smith RJH http://hereditaryhearingloss.org). Loci for non-syndromic hearing loss are denoted 'DFNA' for AD inheritance, 'DFNB' for AR inheritance and 'DFN' for X-linked

inheritance (Griffith & Friedman, 2002). Some genes cause both AD and AR hearing loss. For example, Grifa et al. (1999) found a C→T change in gap junction protein, beta 6 (*GJB6*) that resulted in the substitution of a highly conserved threonine residue for a methionine at amino acid position 5 (p.T5M), resulting in nonsyndromic AD hearing loss. While this *GJB26* mutation causes AD hearing loss, Del Castillo et al. (2002) identified a 342 kb deletion in *GJB6* by studying 422 unrelated subjects from Spain and Cuba with an AR pattern of inheritance.

## Pedigrees

When studying hearing loss, or any hereditary disorder, family members are visualized on a pedigree chart, which in this study shows all known hearing loss phenotypes presented at the time of clinical and audiological testing. This allows easier identification of the inheritance pattern and of the relationships among haplotypes. A haplotype is a combination of alleles that are transmitted together. When a causative mutation is found, alleles of linked markers are assessed in order to develop a haplotype, or pedigree that illustrates shared genetic variants between family members. These haplotypes are then compared between members of the same family or between members of different families that share the same mutation. A common haplotype with the same mutation suggests a common ancestor for that mutation. Furthermore, a haplotype can point to associations between different variants that may be combining to affect the phenotype.

## Audiograms

Audiograms are graphs of the minimal level of sound that a given person can hear at various frequencies (Figure 1.1; Figure 1.2). They are produced using an audiometer, a machine that tests hearing by exposing patients to a range of sounds at different pitches and decibel (dB) levels. During hearing tests, separate audiograms are obtained for each ear. Each line on the audiogram represents one ear. The y-axis measures sound intensity in units of dB, which increases logarithmically. The x-axis of the audiogram measures the frequency, or pitch, of a sound in Hz (Hertz). Low pitch sounds have low frequencies (< 500 Hz), medium pitch sounds have medium frequencies (500 - 2000 Hz), and high pitch sounds have high frequencies (> 2000 Hz). Hearing loss is characterized by intensity, which can be mild, moderate, severe or profound, and by which frequency is affected, such as low, middle or high.

An individual with normal hearing can detect sounds between 0 dB and 20 dB. The minimum level of hearing, 0 dB, is equivalent to a barely audible whisper. Those affected with hearing loss, however, have a higher than normal minimum hearing level. This means that any given sound intensity must be greater than 0 dB for them to hear it. People with a mild degree of hearing loss can only hear sound at intensities between 20 – 40 dB for the frequencies of 500 – 4000 Hz. Individuals with moderate hearing loss can only hear sound from 40-70 dB, and those with severe hearing loss can only hear sound between 70-95 dB in intensity. Lastly, those with profound hearing loss cannot detect sound at all unless it is 95 dB or greater (such as the sound produced by an .MP3 player at maximum volume; Mazzoli et al. 2003).

Figure 1.1 shows a series of simple audiograms: audiogram A shows an individual with normal hearing, B an individual with moderate bilateral (affecting both ears) hearing loss, and C an individual with severe bilateral hearing loss. However, audiograms are often not so simple to read. Figure 1.2 shows two additional audiograms: audiogram A shows an individual with moderate to mild hearing loss in the left ear and normal hearing in the right ear (unilateral), and B shows an individual with bilateral hearing loss sloping to moderate and profound at the higher frequencies. This audioprofile is typical of presbyacusis, or age-related hearing loss. 40 % of the population older than 65 years of age is affected, and 80 % of hearing loss cases occur in elderly people (Gates & Mills, 2005). It is now generally accepted that presbyacusis is most often caused by age-related declines in the auditory system, such as loss or deterioration of sensory cells within the cochlea. Moreover, impaired temporal processing is associated with age-related factors that affect neural synchrony of hearing (Schuknecht et al. 1993; Friedman 2003; Wu et al. 2003; Fitzgibbons et al. 2010). Temporal processing refers to the processing of acoustic stimuli over time. Temporal processing allows us to distinguish speech from background noise, as the decibel levels of the background noise varies over time.

Another common and important characteristic of presbyacusis, and of any sensorineural hearing loss, is the level of speech discrimination a patient demonstrates. Hearing a sound does not always translate into properly distinguishing speech. Tests are also performed to determine a patient's speech discrimination. The measure of speech discrimination is often a percentage, and describes the ability of a patient to correctly

identify words when the sound is loud enough for them to comfortably hear. When a patient has low speech discrimination, a hearing aid will successfully amplify sound in the patient's ear, but will not necessarily improve speech perception. The amplified sound remains gibberish to the patient because he/she is unable to identify the words. (McAlister, 1990; Kodera et al. 1994). A cochlear implant, a surgically implanted electronic device that provides sound to profoundly deaf or severely hard of hearing individuals, has been found in many cases to markedly improve speech discrimination (Leung et al. 2005; Cambron, 2006; Yueh & Shekelle, 2007).

## Autosomal Dominant Hearing Loss

Autosomal dominant non-syndromic hearing loss (ADNSHL) accounts for approximately 15 % of inherited hearing loss (Hildebrand et al. 2008). To date, 59 loci for ADNSHL have been identified, along with 22 causally related genes (Table 1.1; Van Camp G, Smith RJH. Hereditary Hearing Loss Homepage. http://hereditaryhearingloss.org). The majority of AR hearing loss cases are caused by mutations in just a few genes, most notably gap junction protein beta 2 (*GJB2*) and *GJB6*. This contrasts sharply with AD hearing loss, which is significantly more genetically heterogeneous (Griffith & Friedman, 2002), making cost-effective screening for diagnosis in a clinical setting highly problematic. Mutations within the genes wolfram syndrome 1 (*WFS1*), cochlin (*COCH*), potassium voltage-gated channel 4 (*KCNQ4*), and tectorin alpha (*TECTA*) are marginally more frequently reported in comparison to the

other reported causative genes. The audioprofile sometimes provides clues to the underlying causative gene. For example, *WFS1* harbors mutations found in 75 % of families segregating for AD, non-syndromic hearing loss that initially affect only the low frequencies (Young et al. 2001; Bespolova et al. 2001).

ADNSHL is often characterized by a post-lingual, late-onset, progressive phenotype that affects mainly adults. Post-lingual hearing loss is much more frequent than pre-lingual hearing loss, and affects 10 % of the population by the age of 60 (Van Camp et al. 1997). This most often results from damage to auditory hair cells (AHCs) or their innervation (Gates & Mills, 2005). For example, one late-onset progressive hearing loss associated gene is eyes absent homolog 4 (*EYA4*), a member of the vertebrate *Eya* family of transcriptional activators. Mutations in this gene were found in Belgian and USA families, and create premature stop codons leading to post-lingual, progressive, AD hearing loss. *EYA4* was subsequently shown to be critical in the continued function of the mature organ of Corti, an organ in the cochlea that contains the AHCs (Wayne et al. 2001).

## Critical Considerations When Researching Autosomal Dominant Hearing Loss

Of the 28 families researched in this study that are classified as having an AD pattern of inheritance, one or more may have been incorrectly classified due to a lack of sufficient data. Many individuals in these pedigrees (Figure 2.1) are ascertained through

relatives' word of mouth. For this reason, it is important to discuss the role that different factors may be playing in confusing the ascertainment of individuals and thus the search for causative hearing loss mutations.

Digenic inheritance is when a phenotype is expressed only if an interaction between two mutant alleles in two separate genes occurs (Strachan & Read, 2003). Digenic inheritance does not cause AD hearing loss, but digenic inheritance may play a role in the hearing loss of one of the families under investigation in this study. For example, Chen et al. (1997) reported a small consanguineous family with three affected and three unaffected members. Two regions shared by the three affected individuals were identified, one on 3q21.3-q25.2 (LOD = 2.78) and 19p13.3-p13.1 (LOD = 2.78). LOD (Logarithm (base 10) of odds) is a statistical test used to determine the likelihood of obtaining test data if two loci are linked compared to the likelihood of observing the data by chance. Chen et al. (1997) speculated that two non-allelic recessive mutations accounted for the profound congenital deafness in this family. In a Chinese family, Liu et al. (2009) demonstrated through DNA sequencing that mutations in *GJB2* and *GJB3* interact to cause hearing loss in digenic heterozygotes. To support this, they discovered overlapping expression patterns of *GJB2* and *GJB3* in the cochlea, along with co-assembly of the *GJB2* and *GJB3* proteins when co-transfected in human embryonic kidney (HEK) cells (Liu et al. 2009). And a third example was seen recently when mutations within ATP sensitive inward rectifier potassium channel 10 (*KCNJ10*) and solute carrier family 26, member 4 (*SLC26A4*) were found to cause digenic non-syndromic hearing loss associated with enlarged vestibular aqueduct syndrome (EVA)

(Yang et al. 2009). Mutations in *SLC26A4* were previously shown to cause Pendred syndrome (PS), a genetic disorder leading to hearing loss and goiter with occasional hypothyroidism. Many individuals with an EVA/PS phenotype had only one disease-causing variant in *SLC26A4.* Yang et al. (2009) identified double heterozygosity in affected individuals from two separate families. These patients carry single mutations in both *KCNJ10* and *SLC26A4*, and the mutation in *SLC26A4* has been previously associated with the EVA/PS phenotype. The *KCNJ10* mutation reduces potassium conductance activity, which is critical for generating and maintaining proper ion homeostasis in the ear. To add further support to their digenic interaction hypothesis, Yang et al. (2009) demonstrated haploinsufficiency of *Slc26a4* in *Slc26a4+/-* mouse mutants resulted in reduced protein expression of *Kcnj10* in the inner ear.

One important term to keep in mind when researching AD hearing loss is penetrance. Penetrance refers to the proportion of individuals with a mutation who exhibit clinical symptoms. For example, if a mutation in a gene responsible for a type of AD hearing loss is 95 % penetrant, then 95 % of individuals with the mutation will exhibit symptoms, while 5 % will not during their lifetime. Penetrance is often expressed as a frequency at different ages because, for many hereditary diseases, onset of symptoms is age-related (Strachan & Read, 2003). This is particularly important because AD hearing loss is often late-onset and progressive. For this reason, a family's inheritance pattern could appear to be sporadic, when in fact the disorder segregates autosomal dominantly, and the individuals under study simply haven't yet presented the hearing loss phenotype, as the age of onset varies widely and can range well into 50 years of age. A

related but distinct potential problem is expressivity. Expressivity refers to variations of a phenotype for a particular genotype. When a condition has highly variable signs and symptoms, it can be difficult to diagnose.

Mitochondrial inheritance could also be confusing the ascertainment of the families investigated in this study. Mitochondrial inheritance is the inheritance of a trait encoded in the mitochondrial genome, and is always of maternal origin. It is therefore often also called maternal inheritance. When a woman harbors a mitochondrial mutation, and her egg cells are forming an ovary, these egg cells contain a random distribution of both normal and mutated copies of the mitochondrial gene (St. John et al. 2010). Therefore, all children of this mother may inherit some mutated mitochondria, but if the number of mutated mitochondria reaches a critical level, termed the "threshold effect", then an adverse phenotype is seen (St. John et al. 2010; Van Camp G, Smith RJH. Hereditary Hearing Loss Homepage). These mitochondrial hearing loss mutations can be late-onset, or if the carrier is administered certain antibiotics, the phenotype will be "drawn out". This is the case with mutations in MT-RNR1, which are known to cause non-syndromic deafness (Casano et al. 1999; Bates 2003). Mitochondrial mutations are beyond the scope of this study, but have previously been shown to cause late-onset hearing loss that is comparable to the phenotypes of Families 2093, 2112, and 2125 investigated here (Casano et al. 1999). While there are no incidences of male to male transmission in these families, additional clinical ascertainment could potentially reveal a mitochondrial inheritance pattern. This study primarily targets only genes and mutations known to be associated with AD hearing loss, but the possibility of maternally inherited

mutations causing hearing loss in the above mentioned families should not be ruled out, and should be investigated in future studies.

As a result of random genetic drift in the founder population of Newfoundland, there is an elevated incidence of particular rare disorders, such as Bardet-Biedl syndrome (Webb et al. 2009). This makes the founder population of Newfoundland ideal for the study of genetic disorders and increases the chance of detecting novel causative genes and mutations. However, due to the nature of Newfoundland as a genetic isolate, some potential pitfalls arise. One potential pitfall is the uncertainty in inheritance ascertainment. For example, assortative mating could confuse the ascertainment of families and therefore the search for hearing loss mutations. Assortative mating occurs when sexually reproducing organisms choose to mate with individuals that are similar (positive assortative mating) or dissimilar (negative assortative mating) to themselves in some specific way. One family under investigation in this study (Family 2069) is a potential example of positive assortative mating (see p.42, bottom pedigree, $5^{th}$ generation). This is critically important. Positive assortative mating could result in both parents carrying a mutation that causes hearing loss. This may, however, simply be a result of studying a genetic disorder in a highly isolated population, and it is possible that this mating took place not because both individuals were affected by hearing loss, but instead due simply to the low level of mating choice in small out-port community. Either way, our current inheritance classification of Newfoundland Family 2069 could possibly be incorrectly stated as AD, and our candidate gene selection would then be based on unfounded and false assumptions. It is important to keep this possibility under

unfounded and false assumptions. It is important to keep this possibility under consideration, and to investigate and screen for commonly occurring recessive mutations in the proband of Family 2069 as well as dominant mutations. Pseudo-dominance should also be taken into account. This is the situation where the inheritance of an AR trait mimics an AD pattern, and due to the limited extent of clinical ascertainment in these families' histories, it is possible that one of these AD families is in fact affected by an AR mutation segregating in a pseudo-dominant fashion.

Another critical factor to judge when researching genetic hearing loss is the possible presence of phenocopies. A phenocopy is an affected individual who has the same disease, but due to a different cause, as relatives affected with the genetic condition under study. Hearing loss is a very common type of sensory loss in humans. Many types of environmental and genetic factors account for hearing loss so individuals within families affected with hearing loss can be afflicted due to a plethora of different reasons (Griffith & Friedman 2002). For example, a study of heterozygous *WFS1* mutations in two low frequency sensorineural hearing loss families showed that these two families' hearing loss were linked to adjacent but non-overlapping loci on 4p16, DFNA6 and DFNA14 (Van Camp et al. 1999). Upon further study, it was found that an individual in the DFNA6 family who had a recombination event excluding the DFNA14 candidate region was actually a phenocopy. The cause of hearing loss in this phenocopy was reported as unknown, but as a consequence they were able to determine that DFNA6 and DFNA14 are allelic (Bespalova et al. 2001).

Throughout this study genes are investigated through targeted gene sequencing. However, it must be mentioned that it is possible larger genomic abnormalities may account for hearing loss in some of the Newfoundland families under investigation (Lisenka et al. 2003; Shaffer et al. 2006). Large genomic rearrangements, deletions, inversions, etc. can cause and affect the degree and severity of diseases, and these large-scale anomalies are not detected through traditional DNA sequencing methods (Lisenka et al. 2003; Idbaih et al. 2010).

## The Pioneering of Hearing Loss Gene Discovery

The first genes to be implicated in hearing loss were found in the syndromic disorders. Syndromic forms of hearing loss are classified by their associated symptoms. For example, Waardenburg syndrome (WS) is the most common cause of AD syndromic hearing loss. WS is characterized by varying degrees of hearing loss associated with pigmentation anomalies and neural crest defects. It was first described in 1951, but it took several decades to identify the causative genes. Asher and Friedman (1990) studied mice and hamsters with four mutations causing phenotypes similar to those seen in human WS patients. They used the chromosomal locations and syntenic relationships associated with three of these four mutant mouse genes to predict human chromosomal locations for the causative WS gene. Synteny is the situation whereby organisms of relatively recent divergence show similar blocks of genes in the same relative positions in the genome. Asher and Friedman (1990) predicted four possible locations for the causative gene, and

one turned out to be correct. In 1992, mutations causing WS were discovered in the paired box gene (*PAX3*) gene (on chromosome 2q) (Tassabehji et al. 1992). A second common cause of AR syndromic hearing loss is Usher syndrome (Toriello et al. 2004). Usher syndrome was first described in 1858 when Van Graefe reported the case of a deaf and "dumb" male patient presenting with retinal pigment degeneration, who had two similarly affected brothers (Van Graefe, 1858). This was the first syndrome to demonstrate that phenotypes, in this case deafness and blindness, could be inherited in tandem. Usher syndrome is characterized by profound congenital hearing impairment, retinitis pigmentosa, and vestibular dysfunction. It has three clinical types, denoted as I, II, and III, in decreasing order of severity (Saihan et al. 2009). In 1995 one of several causative genes for Usher Syndrome Type 1 was discovered. Weil et al. (1995) chose myosin 7A (*MYOVIIA*) as a functional candidate gene, based on observations that cytoskeletal abnormalities seen in Usher syndrome patients are also seen in mouse mutants with myosin mutations. Two different premature stop codons, a six bp deletion, and two different missense mutations were detected in five unrelated families. In one family, these mutations were identified in both alleles, and resulted in the absence of a functional protein and subsequent Usher syndrome (Weil et al. 1995). Currently, 10 different types of Usher syndrome have been recognized, with more than 100 pathogenic mutations alone for the two most common molecular forms, Usher 1B (USH1B) and Usher 2a (USH2A; Ahmed et al. 2003; Saihan et al. 2009).

The first ADNSHL family investigated was from the small town of Taras, Costa Rica. The hearing loss was described as low frequency AD with a post-lingual age of

onset at 10 years of age (Leon et al. 1981). Leon et al. (1992) performed linkage analysis to determine that the causative gene was linked to markers defining a 7 cM critical region on chromosome 5q31. Genetic markers are DNA sequences with a known location on a chromosome, and are useful in linkage analysis because they are easily identifiable, associated with a specific locus, and highly polymorphic. LOD scores for linkage of deafness to markers in the 7 cM region showed a score of 13.55 at markers D5S2119 and D5S2010 (Leon et al. 1992). This was not only the first AD critical region described, but the first autosomal non-syndromic hearing loss gene to be mapped altogether. It was not until 1997, 26 years after first being reported, that the region was narrowed down further. Positional cloning, sometimes referred to as reverse genetics, is the cloning of an area known to be associated with a disease. It involves the isolation of overlapping DNA segments that progress along the chromosome toward a candidate gene. Lynch et al. (1997) performed fine mapping using positional cloning techniques to narrow the critical region to 1 cM. This revealed protein diaphanous homolog 1 (*DIAPH1*), a previously unidentified human gene. *DIAPH1*, in this case, is a positional candidate gene, a gene identified based upon a determined critical region. This differs from functional candidate genes, which are known to play a role in the disease pathology, or have been previously shown to harbor mutations that cause a disease, like the p.A716T mutation in *WFS1* in Newfoundland (Young et al. 2001; Bespalova et al. 2001). Lynch et al. (1997) sequenced the positional candidate gene *DIAPH1* in all affected family members using Single Strand Conformation Polymorphism (SSCP) analysis. SSCP is the electrophoretic separation of single-stranded nucleic acids based on differences in sequences which bring about a

different secondary structure and thus a measureable difference in mobility through a gel. The causative mutation, a G→T substitution, was now revealed, and *DIAPH1* was also found to be highly expressed in the cochlea (Lynch et al. 1997). Lynch et al. (1997) speculate that the protein this gene encodes, protein diaphanous homolog 1, plays a role in the regulation of actin polymerization in the hair cells of the cochlea.

## Founder Populations & Mutations

No summary of hearing loss associated genes and mutations would be complete without mentioning the importance of founder populations, the founder effect, and founder mutations. A founder population is a small subpopulation that has been isolated due to geography, culture, religion or a combination of these. This subpopulation has a significantly decreased amount of genetic diversity, causing certain genetic traits to either vanish or become very abundant in further generations. When a founder population is isolated individuals in later generations are likely to share many genes, because a mutation in a founder will be passed on to a large proportion of the population in subsequent generations (Nurhousen, 2000). Founder populations, therefore, possess much promise in determining the genes involved in genetic diseases. As there is little genetic heterogeneity, the majority of the individuals with a given disease will carry the same gene mutation. For example, the Ashkenazi Jews were a reproductively isolated population in Europe for roughly a thousand years, with very little out-migration or inter-marriage with other groups (Nebel et al. 2005). As a result of this event, the *GJB2*

mutation c.167delT was found to be highly prevalent in the Ashkanazi Jewish population (Morell et al. 1998). Other examples of founder populations include the Canadian province of Québec, which was established by as few as 2600 individuals, the United States Amish population, and the population of Pingelap, a small island in Micronesia.

A founder mutation is a mutation found as an allele and shared by several individuals from a founder population and derived from a single ancestor. For example, *COCH* p.P51P/S mutation carriers are considered to have originated from a common ancestor (de Kok et al. 1999). A second example was recently seen when Park et al. (2010) investigated the 3-bp deletion in intron 7 (c.991-15_991-13del) of DFNA5, and identified a conserved haplotype between a Korean family and a Chinese family segregating the deletion in DFNA5, suggesting that this deletion represented a founder mutation originating from a common ancestor.

## Colonization of Newfoundland: A Founder Population

The island of Newfoundland makes up a large part of the Canadian province of Newfoundland & Labrador. It is the most easterly landmass on the North American continent. In 1497 the European explorer Giovanni Gabotto (John Cabot) "discovered" Newfoundland, though Vikings had landed earlier. Europeans voyaged across the North Atlantic from England, Scotland, Ireland, France and Portugal to harvest the rich fish stocks. When each fishing season ended they returned to their countries, as permanent

those considering permanent settlement, and with a lack of basic supplies even a one year stay would have been very difficult (Poole & Cuff, 1994). This deterrence lessened throughout the 17th century, however, as small groups of English, Scottish, and Irish settlers set sail from western England in 1610 and throughout the 17th century. These colonists excluded other nations from fishing off of Newfoundland's east coast, but were discouraged in their settlement by the English government, who saw their presence as a threat to the monopoly control that Western England fishing centers had established. Meanwhile, fisherman from France dominated the island's south coast and northern peninsula (Bennett, 2002). Throughout the 1600s, the French began to permanently settle, but in 1713, with the Treaty of Utrecht, the English gained control of the south and north shores of the island. Permanent settlement increased rapidly by the late 18th century, peaking in the early years of the 1800s.

The colonization of Newfoundland began in earnest in the early 19th century mainly from Southwest England and Southeast Ireland. Fisherman brought their families to the island, intending to settle permanently, and these families are the founders of much of today's Newfoundland population. Ninety percent of Newfoundland's population descends from roughly 30 000 founders (Parfrey et al. 2009). Families settled in small inlets along Newfoundland's coast in groups of one or two families. These communities developed in geographical and cultural isolation, and can be characterized by large families, and a strong founder effect (Bear et al. 1987). This isolation also directly led to many generations of interbreeding (Poole & Cuff, 1994; Hancock, 1989).

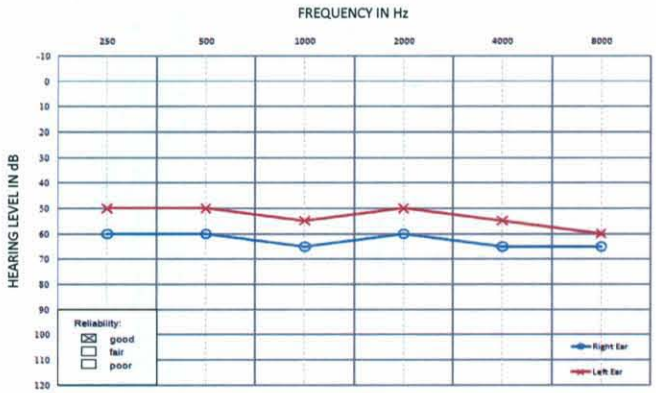
families, and a strong founder effect (Bear et al. 1987). This isolation also directly led to many generations of interbreeding (Poole & Cuff, 1994; Hancock, 1989).

Large extended pedigrees from genetic isolates have been instrumental in the identification of genetic causes of hereditary disorders. Several founder mutations have been identified in Newfoundland. For example, an exon 8 deletion in *MSH2*, a gene causing hereditary non-polyposis colorectal cancer, has been found in five different Newfoundland families (N=74 carriers), and an intron 5 splice site mutation (c.942+3A>T) has been found in 12 different Newfoundland families (N=151 carriers) (Frogatt et al, 1996; Stuckless et al. 2006). A third example is a founder mutation (c.782+3delGAG) found in the deafness gene *TMPRSS3* in two different Newfoundland families (Ahmed et al. 2004; Young et al. unpublished data). These are just a few examples of founder mutations identified in Newfoundland, and serve to highlight the importance of genetically isolated Newfoundland families in the study of hearing loss. At the beginning of this study, only six hearing loss associated genes had been identified in the Newfoundland population (Table 1.2). There is a possibility that a founder mutation exists and could be found in some of these 28 Newfoundland families. When a founder mutation is identified, the prevalence of this mutation in different worldwide populations can be compared, and better estimates of risk for individuals in the founder population can be calculated. The presence of founder mutations in Newfoundland could thus have strong clinical implications in terms of improved diagnosis and the ability to routinely screen individuals if a founder mutation is common enough in the population.

with Bardet-Biedl syndrome (Webb et al. 2009). So while many Newfoundlanders can trace their roots to roughly 30 000 founders, it is critical to keep in mind that these founders came from different towns and different regions. The current population of Newfoundland & Labrador, according to a 2006 census, is 505 469. A map of Newfoundland & Labrador is seen in Figure 1.3.

A)

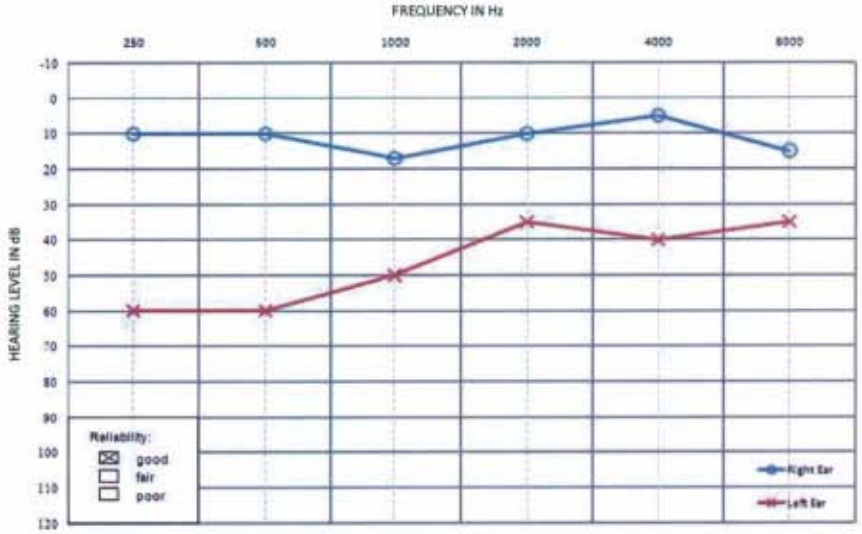

B)




C)


**Figure 1.1 Examples of Audiograms. A) Audiogram of an individual with normal hearing in both ears. B) Audiogram of an individual with moderate hearing loss in both ears. C) Audiogram of an individual with severe hearing loss in both ears.**

A)




B)

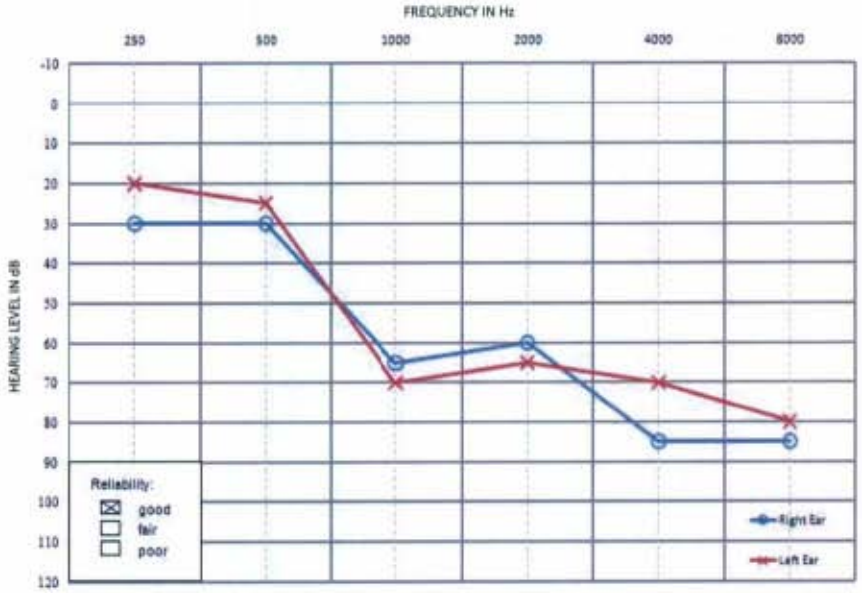



**Figure 1.2 Examples of More Complex Audiograms A) Audiogram of an individual with moderate hearing loss in the low frequencies sloping upwards to mild hearing loss in the mid- to high-frequencies for the left ear only. Hearing in the right ear is normal. B) Audiogram of an individual with progressive hearing loss showing a mild loss at low frequencies which slopes downwards to a severe bilateral loss in the mid- to high-frequencies.**

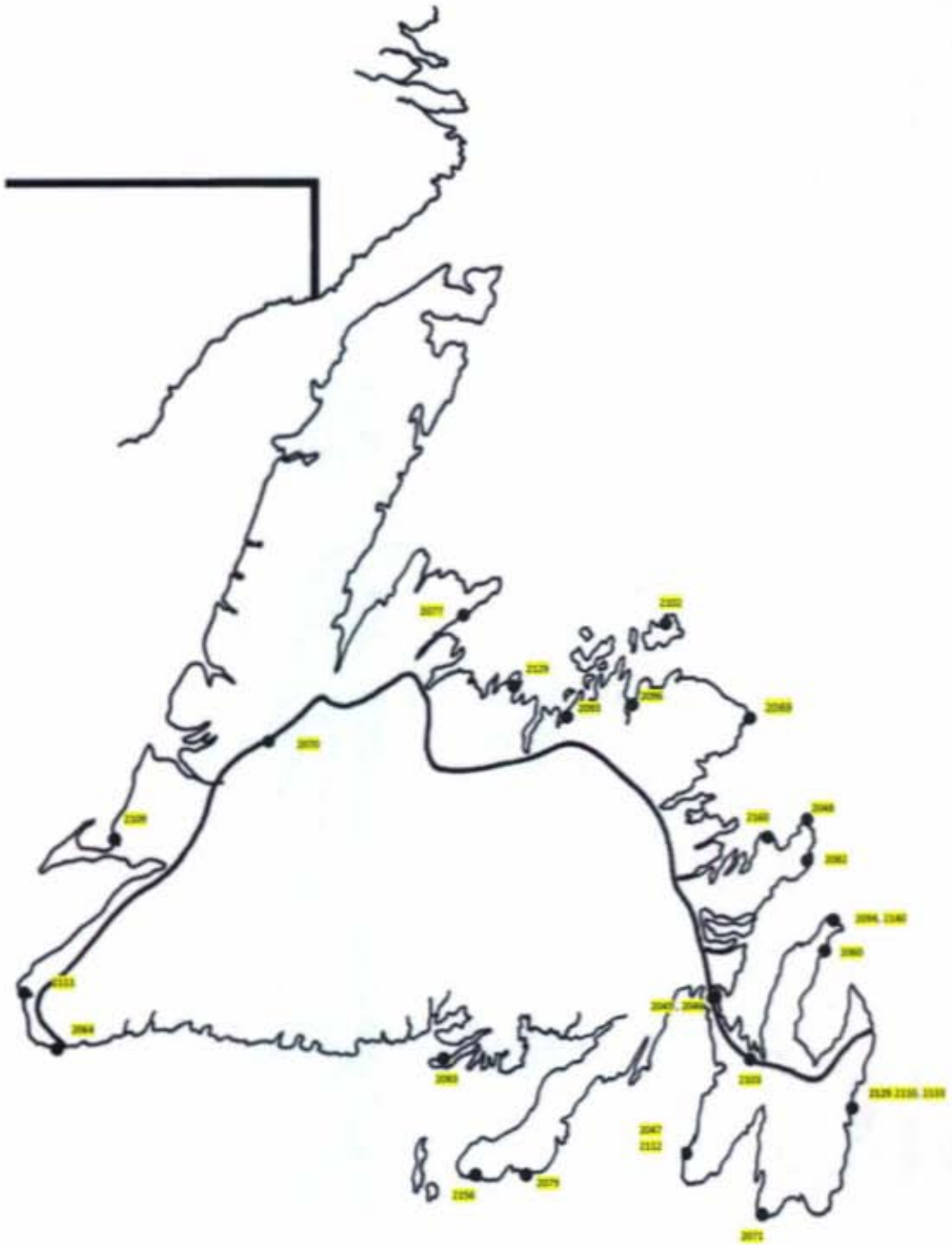


**Figure 1.3 Map of the Island of Newfoundland. AD Families under investigation in this study are indicated with their geographic location.**

**Table 1.1 AD Non-Syndromic Deafness Genes Identified Worldwide to Date (Van Camp G, Smith RJH. Hereditary Hearing Loss Homepage. http://hereditaryhearingloss.org, May 2010)**

| AD Deafness Genes | Protein Encoded | Function |
|---|---|---|
| *CRYM* | Mu-crystallin homolog | Ion Homeostasis |
| *DIAPH1* | Protein diaphanous homolog 1 | Hair Bundle, Cytoskeletal Formation |
| *GJB3* | Gap junction beta-3 protein | Ion Homeostasis |
| *KCNQ4* | Potassium voltage-gated channel subfamily KQT member 4 | Ion Homeostasis |
| *MYH14* | Myosin-14 | Unknown |
| *DFNA5* | Non-syndromic hearing impairment protein 5 | Unknown |
| *WFS1* | Wolframin | Ion Homeostasis |
| *TECTA* | Alpha-tectorin | Extracellular Matrix |
| *COCH* | Cochlin | Extracellular Matrix |
| *EYA4* | Eyes absent homolog 4 | Transcription Factor |
| *COLL11A2* | Collagen, type XI, alpha 2 | Extracellular Matrix |
| *POU4F3* | POU domain, class 4, transcription factor 3 | Transcription Factor |
| *MYH9* | Myosin, heavy chain 9, non muscle | Hair Bundle, Motor Protein |
| *ACTG1* | Actin, gamma 1 | Hair Bundle, Cytoskeletal Formation |
| *MYO6* | Myosin-6 | Hair Bundle, Motor Protein |
| *TFCP2L3* | Grainyhead-like 2 | Transcription Factor |
| *MYO1A* | Myosin-Ia | Unknown |
| *GJB2* | Gap junction beta-2 protein | Ion Homeostasis |
| *GJB6* | Gap junction beta-6 protein | Ion Homeostasis |
| *MYO7A* | Myosin VIIa | Hair Bundle, Motor Protein |
| *TMC1* | Transmembrane channel-like protein 1 | Unknown |
| *CCDC50* | Coiled-coil domain-containing protein 50 | Hair Bundle, Cytoskeletal Formation |

**Table 1.2 Deafness Genes Identified in the Newfoundland Population at Beginning of This Study.**

| Gene | Mutation | # of NL Families | Literary Reference |
|---|---|---|---|
| *GJB2* | c.35delG | 7 | Denoyelle et al. 1997 |
| *GJB6* | D13S1830 | 3 | Del Castillo et al. 2002 |
| *TMPRSS3* | c.207delC | 1 | Ahmed et al. 2004 |
| *TMPRSS3* | c.782+3delGAG | 2 | Ahmed et al. 2004 |
| *PCDH15* | c.1978T>A | 1 | Ahmed et al. 2003 |
| *WFS1* | c.2146G>A | 1 | Young et al. 2001; |

# Chapter 2: Methods & Materials

## Human Subjects & Pedigrees

This study is one part of a larger study to determine the genetic cause of hearing loss in Newfoundland & Labrador. Family members were recruited through the Newfoundland Provincial Genetics Program, and a province-wide ascertainment drive. Informed consent was obtained from all participants, granting researchers permission to access medical records and family history. Blood samples were collected and genomic and mitochondrial DNA was extracted from peripheral leukocytes from participants. Audiological tests were performed to determine the type of hearing loss of each subject and to confirm normal hearing in unaffected subjects. Audiograms are available for all individuals marked with an asterisk in Figure 2.1, and for each of these individuals several audiograms are available at different test ages, with new ones routinely being collected. DNA from several Dutch individuals was provided by Dr. Hannie Kremer of the Radboud University Medical Centre in Nijmegen, Netherlands. This project was approved by The Human Investigations Committee (HIC) (Research Ethics Board of Memorial University, Newfoundland & Labrador) (# 01.186).

So far, 128 probands have been recruited to the study. All probands in the study were routinely screened for mutations previously identified to cause hearing loss in the Newfoundland population. Of these, 28 probands are members of multiplex families with a family history of hearing loss consistent with AD inheritance, and were chosen for this

study (Figure 2.1). Inheritance patterns were determined through an extensive family history questionnaire, and pedigrees were electronically stored using the computer program Progeny. Many individuals' hearing loss was determined by word of mouth from family members. In these cases, the age of onset and the degree and severity of hearing loss are not known. For this reason, the determination of an AD pattern of inheritance is not certain in some cases, but these families were deemed to likely have an AD form of hearing loss, and it was therefore worth testing them for potential AD hearing loss mutations. Due to the extent of genealogy work possible in Newfoundland up to this point, it is important to keep other potential forms of inherited hearing loss, such as mitochondrial inheritance, in mind when searching for causative mutations.

## Experimental Design: Functional Candidate Gene Mutation Screening

Genomic DNA from probands (n=28) was screened using a functional candidate gene approach. A comprehensive literature search was done to collect information on all AD hearing loss genes. One recent review (Hilgert et al. 2009) discussed in depth the genes causing AD hearing loss. It describes how each gene associated with AD hearing loss functions in the ear, and what types of hearing loss they cause. For each of these genes, the mutations found both worldwide and within Caucasian populations are described in detail. Many of these genes may be causative in Newfoundland families (Table 2.1).

This literature search formed the basic foundation from which genotype-phenotype evaluation was performed. Potential candidate genes were investigated for specific case-by-case details on the hearing loss phenotype each mutation caused, and in what population and ethnicity they were reported. These phenotypes were then cross checked with the phenotypes of the 28 families to further narrow down the functional candidate gene list to four: *COCH, KCNQ4, TECTA,* and *MYO1A* (see Figure 2.2). This approach is a form of audioprofiling, a method of categorizing phenotypic data to make genotypic correlations. The audiological data of several members in a family, or in this case several probands from different families, associates with a specific unknown genotype as a function of time (Meyer et al. 2007). From this, we have drawn correlations to the overall phenotype of the group of probands and used this as a foundation for selecting candidate genes previously reported to cause hearing loss with a similar phenotype.

Information on all known hearing loss mutations in these four candidate genes was next collated, including which domain and exon each mutation was reported in (Appendix A). This allowed the identification of the exons most likely to harbor causative mutations in the Newfoundland probands. For example, the majority of mutations reported in *COCH* are reported in the factor c homologous (FCH) domain, spanning exons 4 and 5, and so this area of *COCH* was screened first.

*COCH* has a total of 12 exons, and causes a late-onset, progressive hearing loss most often associated with vestibular dysfunction (Kemperman et al. 2005), which correlates with several AD Newfoundland families. *COCH* is important in maintaining

structural support within the cochlea (Kommareddi et al. 2007). Exons screened were 2-5, and 12. Two deletions within *KCNQ4* have been reported to cause a late-onset, progressive hearing loss (Coucke et al. 1999; Kamada et al. 2006), matching the phenotype of many AD Newfoundland probands. This gene is critical in ion homeostasis with the ear (Kubisch et al. 1999), and is coded by 14 exons, all of which were screened. Missense mutations within *TECTA*, a second gene important in structural support within the auditory system, have been shown to cause late onset hearing loss (Verhoeven et al. 1998). Coded by 23 exons in total, exons 5, 9-14, 17, 18, and 20 were screened. The last candidate gene, *MYO1A* is thought to play a role in sound processing through ion transport (Donaudy et al. 2003), and again, causes a late-onset, progressive hearing loss phenotype. Coded by a total of 28 exons, exons 3, 4, 6, 7, 10-12, 18, and 22 were screened.

While the more "targeted" candidate gene approach outlined above is likely to lead to the identification of mutations, it does restrict the chance of identifying potential "genetic surprises" regarding genotype-phenotype. This method allows for a complete investigation of possible genetic mutations in a given set of promising candidate genes within the two-year time frame of a Master's thesis. However, exons within these candidate genes that have never before been associated with hearing loss mutations are bi-directionally sequenced in this study, and so there is potential for "genetic surprises".

Because Newfoundland is a founder population, it is possible that families would share the same mutation, particularly if those families are from the same geographic area. When a mutation is discovered in an AD Newfoundland proband, all otosclerosis, AD,

and AR Newfoundland probands (n=68) are subsequently screened for that mutation. When appropriate, apparent founder mutations are confirmed by haplotype analysis, to determine the level of sharing for a selection of linked microsatellite markers between families with the same mutation (see Figure 2.2).

## General Strategy for PCR and Sequencing of Candidate Genes

Both forward and reverse strands of specific exon PCR products in each gene were bi-directionally sequenced, along with all intron/exon boundaries to ensure the entire coding region of each exon was covered. PCR primers were designed using Primer 3 software (v. 0.4.0, http://frodo.wi.mit.edu/primer3/). These primer sequences can be found in Appendix B.

## DNA Preparation, PCR Thermocycling, and Electrophoresis

DNA was extracted from whole blood and diluted to10 ng/µl. This blood was stored at 4 °C (performed by research assistant). 1 µL of diluted (stock) DNA was added to 2 µL 10X PCR Buffer (containing $MgCl_2$), 0.4 µL dNTPs (10 mM), 0.08 µL KapaTaq DNA Polymerase (5 U/µL) (Kapa Biosystems, Boston, MA), 12.92 µL of distilled $dH_2O$, 1.0 µL of forward primer (10 µM) and 1.0 µL of reverse primer (10 µM), as per standard PCR protocol. The amount of $dH_2O$ was reduced to add betaine or Dimethyl Sulfoxide (DMSO) when necessary to achieve a successful amplified PCR product. This mix was

centrifuged and addeqd to wells in 20 µL aliquots in a 96-well PCR plate, where it was then sealed, centrifuged, and placed in the GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA). PCR products were electrophoresed on a 1% agarose gel (1.5 g agarose/100 mL TBE) stained with SybrSafe and viewed under UV light on a Kodiak GEL LOGIC 100 Molecular Imaging system (Rochester, NY, Version 4.01, 2005).

## Preparation for ABI Cycle Sequencing

Sephacryl S-300HR was resuspended and 300 µL aliquots were added to wells on a Millipore Multi-screen HTS plate, which was placed over a corresponding 96-well waste plate to catch flow-through. Plates were then balanced and centrifuged at 3000 rpm for 5 minutes. Flow-through was discarded and PCR products were added to wells on the Multi-screen HTS plate. The Multi-screen HTS plate was then positioned and placed over a clean PCR plate, balanced, and centrifuged at 3000 rpm for 5 minutes. The flow-through product collected in the PCR plate contains the purified PCR products.

Successfully amplified PCR products, visualized as bands of the correct size (using a 100 bp marker) when electrophoresed on an agarose gel, were cycle sequenced using the following reaction mix: 0.5 µL of Big Dye Terminator V. 3.1 Sequencing Mix, 2 µL of 5X sequencing buffer, 0.32 µL of Primer (10 µM), 1 µL of purified PCR product DNA template, and 16.18 µL of $dH_2O$, per the Big Dye Terminator V. 3.1 protocol (Applied Biosystems, Foster City, CA). This equals a total reaction volume of 20 µL per

well in a sequencing plate. The resulting plate was centrifuged briefly, loaded onto the thermal cycler, and subjected to a thermal cycling program according to ABI BDT V. 3.1 protocol (Applied Biosystems, Foster City, CA).

Upon completion, 5 µL of 125 mM EDTA followed by 65 µL of 95% Ethanol (EtOH) was added to each reaction well. Plates were briefly centrifuged and then incubated overnight in the dark at ambient temperature. The plate was then centrifuged at 3000 x g for 30 minutes, inverted to decant EtOH, and briefly centrifuged while inverted at 200 rpm for 4 – 5 seconds with folded paper towels placed underneath the sequencing plate to absorb residual ethanol. 150 µL of 70 % EtOH was added to each sample, and the plate was centrifuged at 3000 g for 15 minutes. The plate was again inverted to decant ethanol and spun at 200 rpm for 4 – 5 seconds over a paper towel. Samples were left to air dry in the dark at room temperature for 10 - 15 minutes. 15 µL of Hi-Di Formamide was subsequently added to each well and the plate was vortexed and centrifuged briefly. The final mix was denatured at 95 °C for 2 minutes on a thermal cycler. Once denatured, samples were kept on ice until placed in the ABI 3130 XL DNA Analyzer.

## Automated Sequencing Using the ABI 3130 XL

Automated sequencing was performed using either the ABI 3130 XL DNA Analyzer (Applied Biosystems, Foster City, CA) available in the lab or the ABI 3730 DNA Analyzer in the Genomic & Proteomics Facility, at CREAIT, Memorial University of Newfoundland. The raw sequence data were initially analyzed for quality using

Sequencing Analysis software (Version 5.2, Applied Biosystems, Foster City, CA). High quality sequences were imported into Mutation Surveyor (Version 3.0, Softgenetics, State College, PA). Mutation Surveyor identifies DNA sequence variants in the sample sequence DNA by comparing it to a reference gene sequence.

## Tracing Variants Through Families: Genotype & Haplotype Analysis

Sequencing variants were traced through the pedigrees to see if they co-segregated with hearing loss. Haplotype analysis was performed when necessary. Table 3.4 is a list of microsatellite markers used to characterize the p.P51P/S haplotype shared between a Newfoundland family and a Dutch family. This was done to confirm the founder hypothesis for the *COCH* mutation p.P51P/S (de Kok et al. 1999), identified in this study in a Newfoundland proband. Markers were selected based on location as well as degree of heterozygosity in order to confirm haplotype sharing between the two families, which provided further evidence that p.P51P/S is a Dutch founder mutation.

Initial setup for genotyping required running PCR under standard conditions. Each reaction mix contained 8.5 µL of Hi-Di Formamide, 0.5 µL Genotyping Size Standard *GS500* (*-250*) *LIZ,* and 1 µL of DNA, per manufacturers standard protocol (Applied Biosystems, Foster City, CA). Post-PCR products were electrophoresed on a 1% agarose gel (1 g agarose/100 mL TBE, pH 8.0) containing 4.0 µL of SybrSafe 10-000 X concentrate in DMSO (Invitrogen, Eugene, OR) and viewed under UV light on a

Kodiak GEL LOGIC 100 Molecular Imaging system (Rochester, NY, Version 4.01, 2005). The PCR product was then diluted based on its band intensity to a suitable concentration. Optical plates containing these samples were briefly vortexed and then centrifuged at 1250 rpm for 10 seconds, denatured on a thermal cycler, and immediately loaded onto the ABI 3130 XL DNA Analyzer for genotyping. The PCR products from the fluorescently labeled primers were detected by the ABI Prism 3130 XL DNA Analyzer and genotyped using GeneMapper Software (ABI Prism, Version 4.0).

GeneMapper assisted in making allele calls at each marker for each individual, which were then compared with other individuals and families. Once a pedigree was constructed using the software Progeny (Progeny Software LLC, Delray Beach, FL), markers were integrated for each selected individual. Allele calls, SNPs, and/or common variants were then inputted into each individual's data set to create a pedigree illustrating the segregation of different haplotypes (Progeny Software LLC, Delray Beach, FL; Figure 3.5; Figure 3.10).

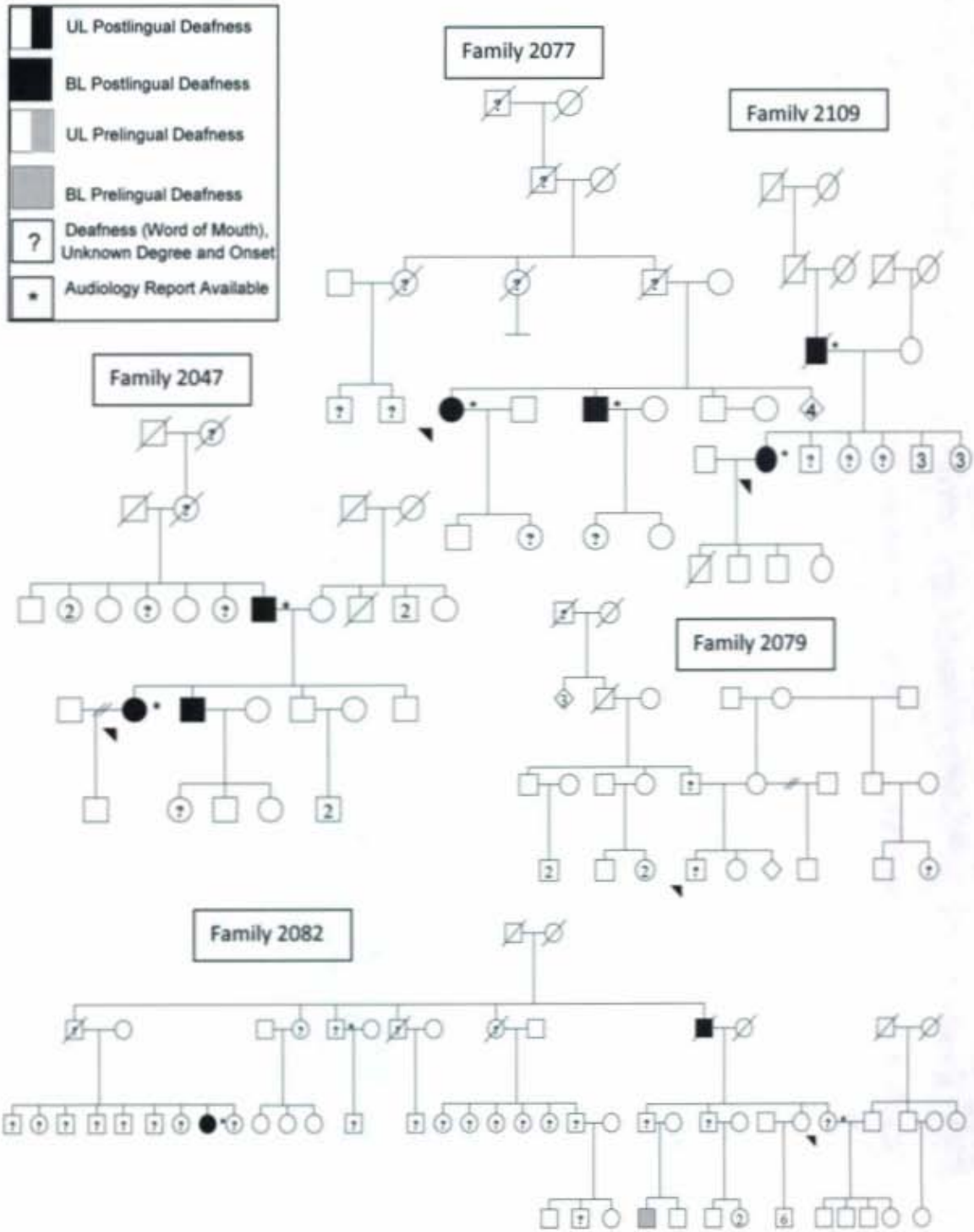



**Figure 2.1 28 Newfoundland Families with AD hearing loss**

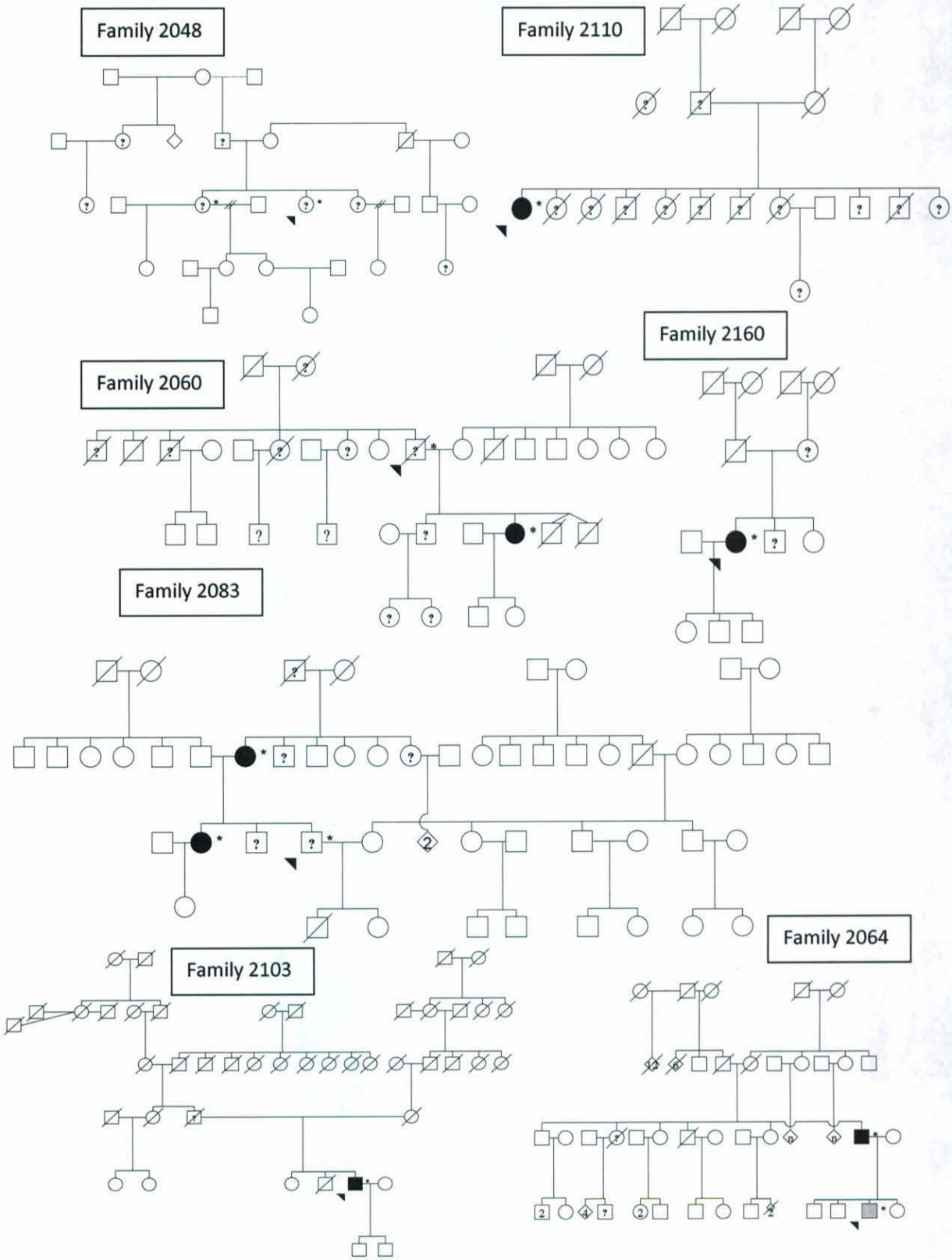



**Figure 2.1 28 Newfoundland Families with AD hearing loss (cont).**

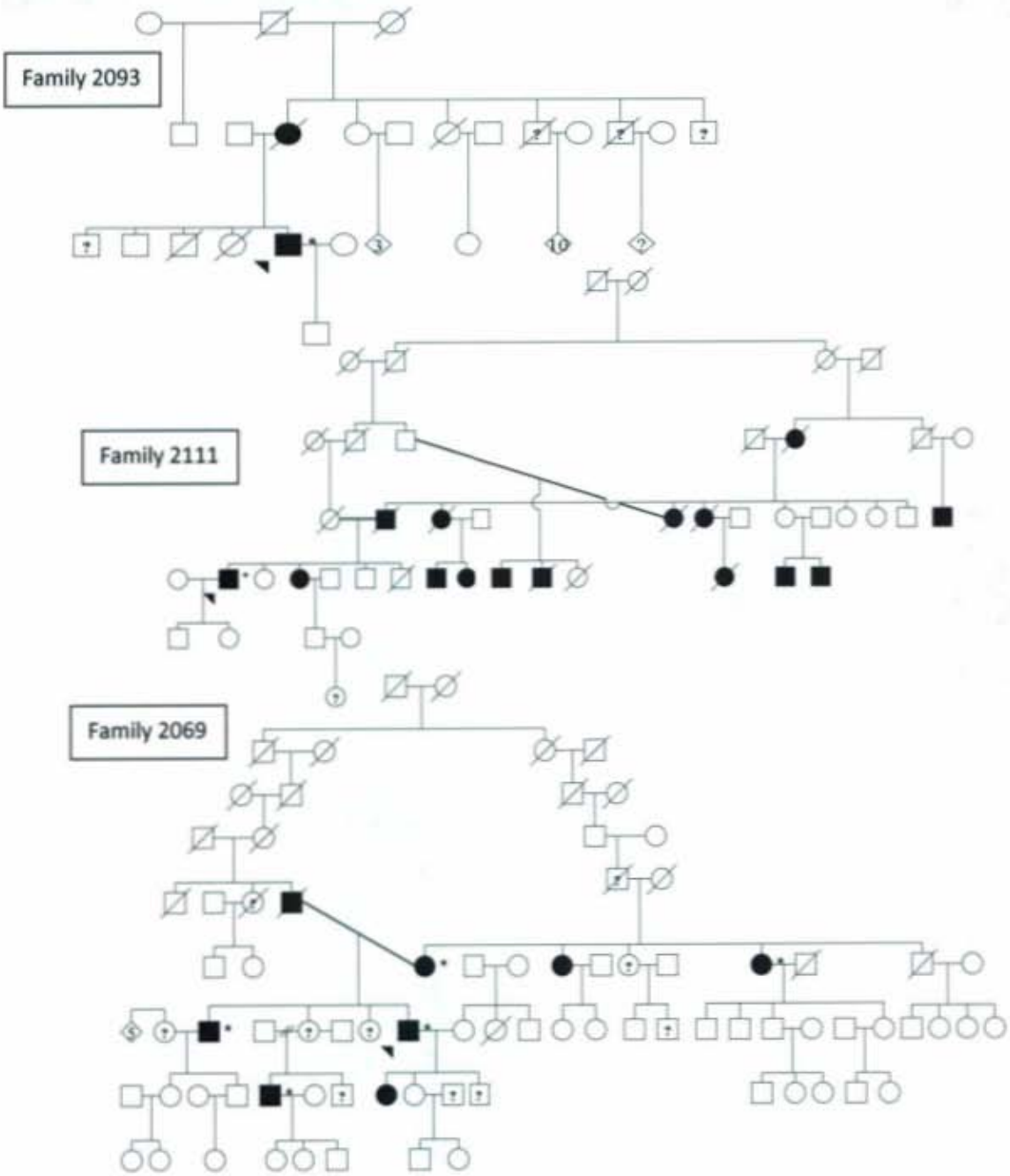



**Figure 2.1 28 Newfoundland Families with AD hearing loss (cont).**

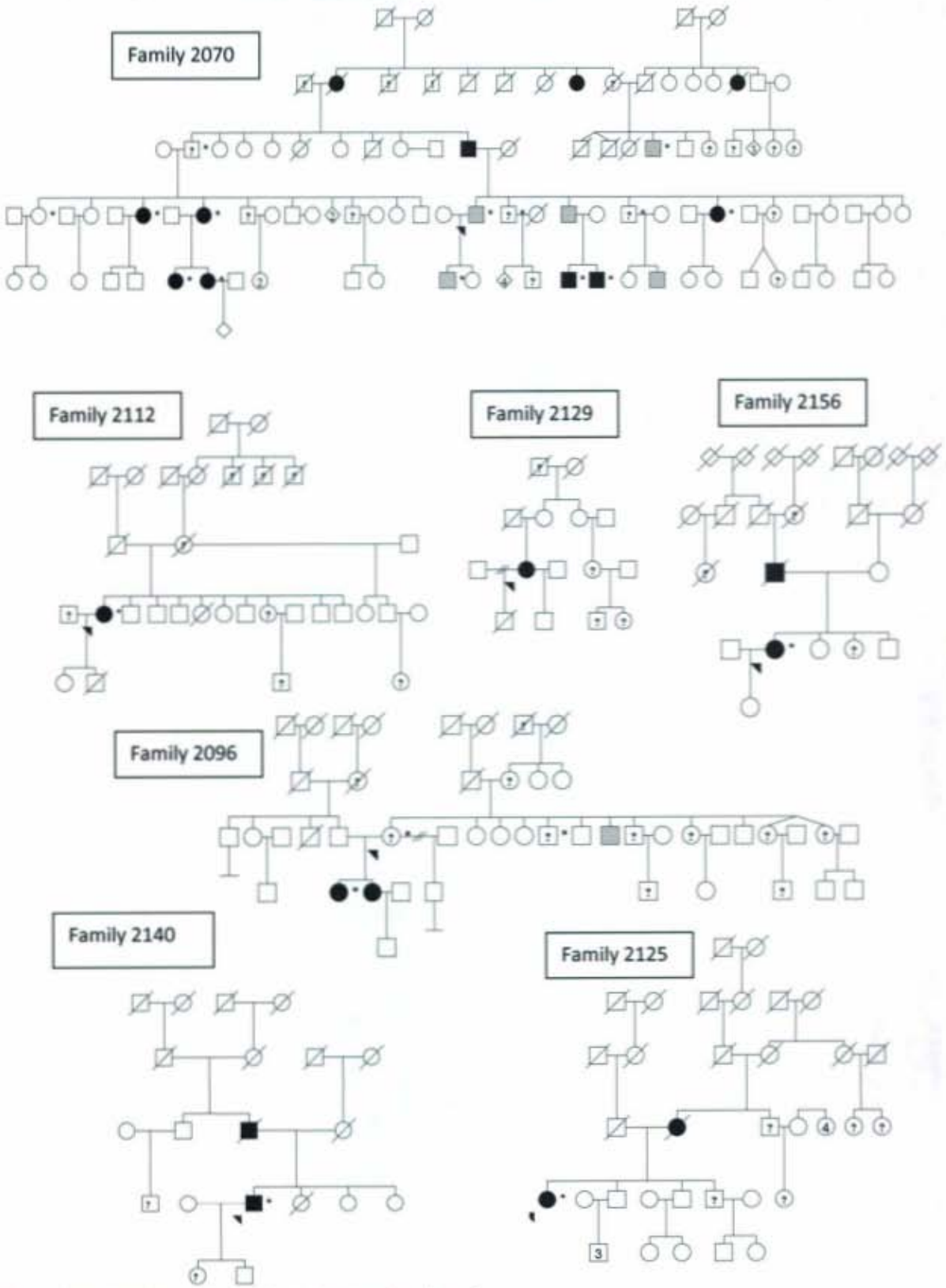



**Figure 2.1 28 Newfoundland Families with AD hearing loss (cont).**

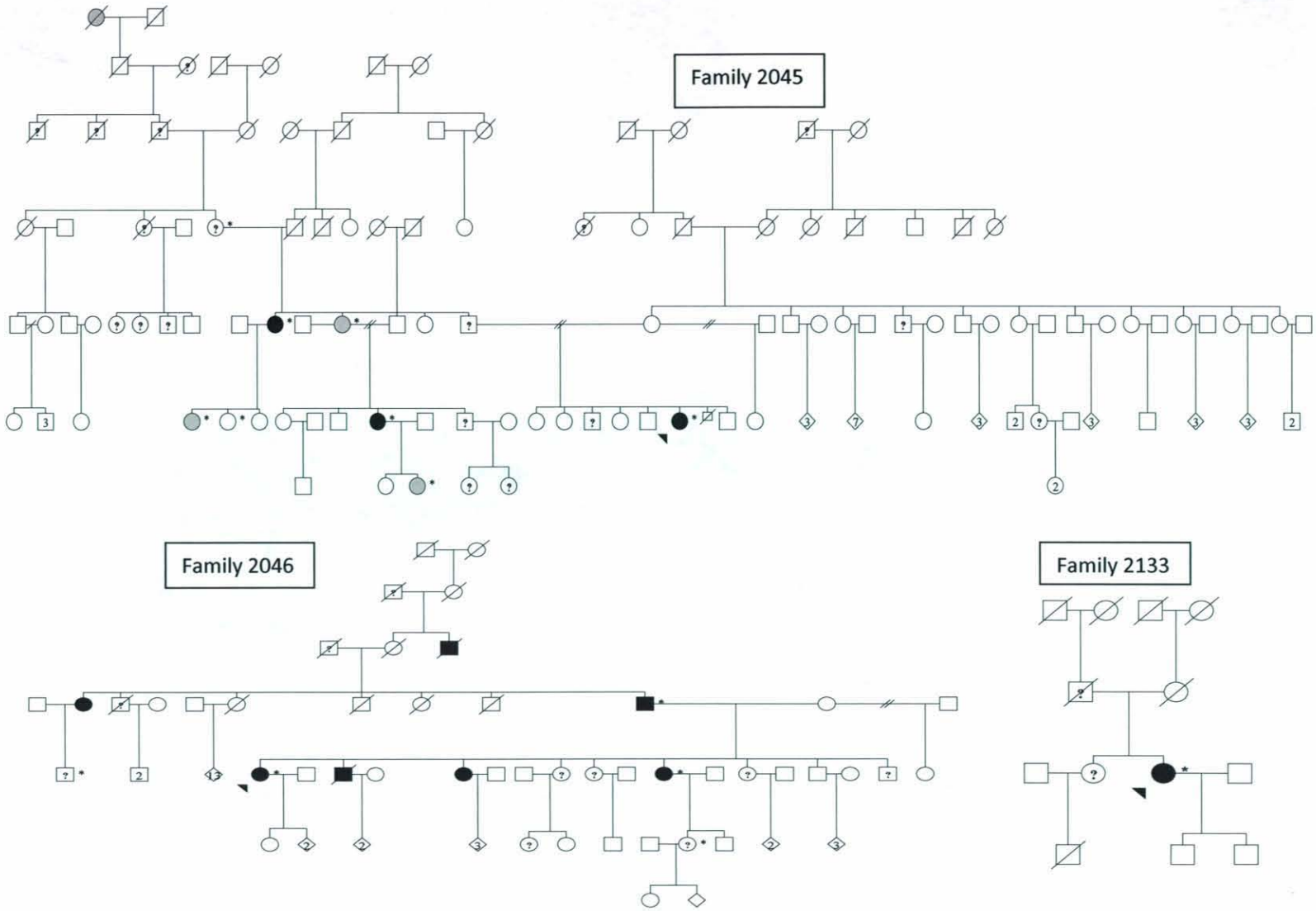



**Figure 2.1 28 Newfoundland Families with AD hearing loss (cont).**

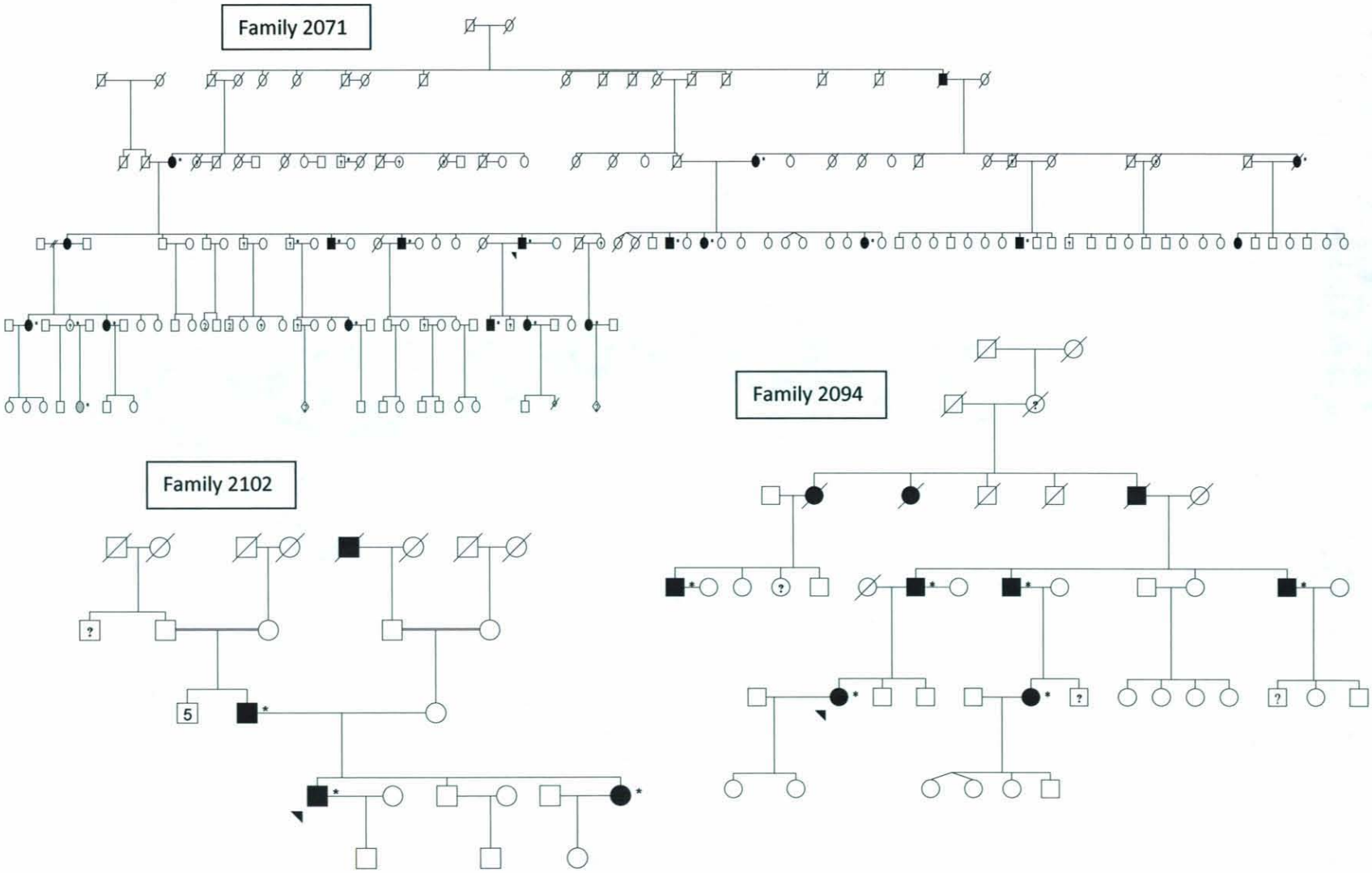



**Figure 2.1 28 Newfoundland Families with AD hearing loss (cont).** Causative hearing loss mutations were identified in these three families.

**Figure 2.2 Flow Chart Demonstrating Experimental Design and Progression**

**Table 2.1 Candidate genes for Newfoundland Families Having Late-Onset AD Hearing Loss (Adapted from Hilgert et al. 2009).**

| Candidate Gene | Number of Mutations Found Worldwide | Number of Mutations Found in Caucasians | Function | Original Reference |
|---|---|---|---|---|
| *KCNQ4* | 12 | 8 | Ion Homeostasis | Kubisch et al. 1999 |
| *COCH* | 12 | 8 | Extracellular Matrix | Robertson et al. 1998 |
| *MYO1A* | 8 | 8 | Unknown | Donaudy et al. 2003 |
| *TECTA* | 8 | 7 | Extracellular Matrix | Verhoeven et al. 1998 |
| *ACTG1* | 6 | 6 | Hair Bundle, Cytoskeletal Formation | Zhu et al. 2003; Van Wijk et al. 2003 |
| *EYA4* | 6 | 4 | Transcription Factor | Wayne et al. 2001 |
| *MYH14* | 5 | 5 | Unknown | Donaudy et al. 2004 |
| *MYO6* | 5 | 5 | Hair Bundle, Motor Protein | Melchionda et al. 2001 |
| *MYO7A* | 5 | 4 | Hair Bundle, Motor Protein | Liu et al. 1997 |
| *ESPN* | 4 | 4 | Hair Bundle, Cytoskeletal Formation | Naz et al. 2004 |
| *DFNA5* | 4 | 2 | Unknown | Van Laer et al. 1998 |
| *GJB3* | 3 | 1 | Ion Homeostasis | Xia et al. 1998 |
| *POU4F3* | 3 | 2 | Transcription Factor | Vahava et al. 1998 |
| *TMC1* | 2 | 2 | Unknown | Kurima et al. 2002 |
| *COL11A2* | 2 | 2 | Extracellular Matrix | McGuirt et al. 1999 |
| *CRYM* | 2 | 0 | Ion Homeostasis | Abe et al. 2003 |
| *TFCP2L3* | 1 | 1 | Transcription Factor | Peters et al. 2002 |

**Table 2.1 Candidate Genes for Newfoundland Families Having Late-Onset AD Hearing Loss (Adapted from Hilgert et al. 2009) (cont).**

| | | | | |
|---|---|---|---|---|
| *MYH9* | 1 | 1 | Hair Bundle, Motor Protein | Lalwani et al. 2000 |
| *CCDC50* | 1 | 1 | Hair Bundle, Cytoskeletal Formation | Modamio-Hoybjor et al. 2007 |
| *DIAPH1* | 1 | 0 | Hair Bundle, Cytoskeletal Formation | Lynch et al. 1997 |

# Chapter 3: Results

## Overview

The purpose of this study was to determine the genetic etiology of hearing loss in 28 autosomal dominant hearing loss Newfoundland probands using a functional candidate gene approach. Candidate genes *COCH, TECTA, KCNQ4,* and *MYO1A* were chosen primarily because they have a higher frequency of mutations in autosomal dominant families (Hilgert et al. 2009). The full *GJB2* gene and the del13S1830 mutation in *GJB6,* which underlies the majority of congenital deafness worldwide, were first excluded in all 28 probands. A heterozygous mutation in *WFS1* - a gene now known to cause both syndromic and non-syndromic deafness (Young et al. 2001; Bespalova et al. 2001) - previously found to cause autosomal dominant hearing loss (p.A716T) in a large Newfoundland family (Young et al. 2001) was also excluded in all 28 probands. Figure 2.2 illustrates the research projects experimental progression.

Results of candidate gene screening revealed three distinct mutations causing hearing loss in three separate Newfoundland families. First, a C→T base change in exon 4 of *COCH* in the proband of Family 2094 resulted in the substitution of a conserved proline residue for a serine residue at amino acid position 51 (p.P51P/S). Second, a novel 3bp heterozygous deletion in exon 5 of *KCNQ4* was found in the proband of Family 2071. Third, a nonsense mutation was discovered at amino acid position 93 within exon 4 of *MYO1A*. This nonsense mutation, p.R93X, is due to a C→T nucleotide change, and was found in the proband of Newfoundland Family 2102.

## Family 2094

Within *COCH,* exons 2, 3, 4, 5, and 12 were sequenced in all 28 AD hearing loss families. Of the 28 probands, one proband was identified with a C→T base change in exon 4 of *COCH.* The proband is a member of a family (Family 2094) with four generations of documented hearing loss. The complete pedigree documents 44 individuals and extends back six generations. A partial pedigree is seen in Figure 3.1. Twelve family members have been diagnosed with AD hearing loss. A summary of audiology reports and a phenotype summary are found in Tables 3.2 and 3.3 respectively. The proband (V-2, Figure 3.1), is a 39-year-old female presenting with hearing loss. Reports showed a bilateral hearing loss sloping to moderate loss at high-frequencies (Figure 3.3). Three more audiology tests were conducted over the next three years, showing a gradual worsening of high-frequency hearing loss.

The proband is heterozygous for the C→T base change in exon 4 of *COCH,* which substitutes a highly conserved proline residue for a serine residue at amino acid position 51 (p.P51P/S) (Figure 3.2). Upon identification of p.P51P/S in the proband, DNA from all available individuals was amplified and sequenced for exon 4. Seven out of seven with documented hearing loss harbored the C→T transition; one unaffected (IV-10) did not.

## Search for a Vestibular Phenotype in Family 2094 Mutation Carriers

Previous studies of mutation carriers of p.P51P/S show severe vestibular phenotypes associated with hearing loss (de Kok et al. 1999), so medical and audiological records for all available family members were reviewed (see Figure 3.1). The proband (V-2) has not yet had any episodes of vertigo or associated vestibular problems. She had a Computed Tomography (CT) scan at 40 years of age which did not detect any abnormalities. The probands cousin, V- 6, complained of episodes of dizziness and vertigo at age 32. She has had two CT scans at ages 36 and 38, which did not detect any abnormalities. Her hearing loss was first reported at age 35 as mild hearing loss in the high-frequency range. This cousin also first reported a scratch on her left cornea at age 31. IV-1 has stated he has had balance problems when walking at night since his early 50's. He had a CT scan at age 62, which detected no abnormalities. His hearing loss was diagnosed at age 49 as moderate in the high-frequency range. IV-5 reported balance problems and episodes of dizziness from the age of 50. Audiology testing at age 57 showed a moderate bilateral loss that over the following 15 years progressed to severe hearing loss across all frequencies. IV-7 presented the typical p.P51P/S hearing loss phenotype, and also had occupational noise exposure. IV-10 is an unaffected woman who has not reported any vestibular problems and is not affected with hearing loss. IV-11 reported spinning dizziness and unsteadiness, along with balance problems in his early 30s. His hearing loss was first documented at age 49, and further audiology tests over the following 15 years show bilateral loss beginning in the high-frequencies and then

flattening out later in life to profound hearing loss across all frequencies. III-6 had documented hearing loss from middle age, but no other data was available.

Because Newfoundland is a founder population, and this study is focused on the identification of founder mutations, all otosclerosis, AD, and AR probands in the Newfoundland hearing loss study (n=40) were screened for this mutation, but no additional cases were found.

## Identification of a Dutch Founder Mutation

As p.P51P/S is widely believed to be a Dutch founder mutation (de Kok et al. 1999; Fransen et al. 2001), we genotyped the seven affected Family 2094 members, along with three Dutch p.P51P/S carriers, for seven microsatellite markers closely flanking the *COCH* gene, (Fransen et al. 2001) in order to construct an ancestral haplotype. Affected Family 2094 individuals and the Dutch affected individuals share a contiguous five-microsatellite-marker haplotype at markers D14S257, D14S1071, D14S1040, D14S1034, and D14S1060: 179-281-234-169-201. *COCH* sits ~0.4 Mb downstream of marker D14S257. These markers closely flank the *COCH* gene and constitute a total minimum shared region of ~2.1 Mb on chromosome 14q12 (Figure 3.4; Table 3.5). The full hearing loss haplotype shared among Newfoundland and Dutch families is shown in their respective pedigrees in Figure 3.5. Unaffected individual IV-10 does not share this haplotype. Further genotyping was conducted for additional microsatellite markers

upstream of D14S1060. Markers D14S70 and D14S1014 both displayed allelic disparity between the two families, demonstrating the relatively short length of this putative ancestral haplotype (Table 3.5).

## Family 2071

Of the 28 probands, one proband was identified to have a novel 3 bp deletion, p.Ser269del in exon 5 of *KCNQ4* (DFNA2A). The proband is a member of a family (Family 2071) with four generations of documented hearing loss. The complete pedigree documents 97 individuals, extends back five generations, and reports no cases of consanguinity (Figure 3.7). Twenty-four family members have been diagnosed with AD non-syndromic hearing loss. A summary of audiology reports for participating individuals is found in Table 3.7, with phenotype data shown in Table 3.6.

The proband (III-12, Figure 3.7) is a 62-year-old male presenting with a bilateral hearing loss sloping to moderate loss at mid-frequencies and profound at high-frequencies. The proband harbors a novel heterozygous 3 bp deletion, p.Ser269del, in exon 5 of *KCNQ4* (Figure 3.8). All available family members were sequenced for exon 5. Thirteen members with documented hearing loss shared p.Ser269del. No unaffected family members (n=18) carried the deletion, and of 90 ethnically matched population controls, none carried the deletion. While p.Ser269del is not seen in four members with hearing loss, these four individuals present a distinctly different audioprofile (Figure 3.9;

Table 3.6). Audiology reports of affected relatives with the novel deletion show a high-frequency, late onset hearing loss (Figure 3.9).

This deletion predicts an in-frame removal of a serine residue at amino acid position 269 within the P-loop domain of the KCNQ4 protein (Figure 3.10). We next constructed an intragenic haplotype using commonly occurring SNPs and variants within and surrounding exon 5 of *KCNQ4*. This was done to determine whether or not any interesting and possibly causative variants within *KCNQ4* were shared by affected individuals of Family 2071, and to determine the level of sharing among of intragenic SNPs and variants among deletion carriers. No markers brought any additional interesting information, and no family members without the deletion, or without hearing loss, shared this hearing loss haplotype.

Upon discovering this novel deletion, all otosclerosis, AD, and AR probands in the Newfoundland hearing loss study (n=68) were screened to determine whether or not any additional Newfoundland families shared the mutation, thereby suggesting a possible founder mutation. However, no additional cases outside of Family 2071 were detected. All exons (1-14) were sequenced in the 28 AD probands for this gene.

## Family 2102

A third proband was identified with a C→T base change in exon 4 of *MYO1A* (DFNA48). The proband is a member of a family (Family 2102) with four generations of

documented hearing loss. The complete pedigree documents 22 individuals, extends back five generations, and indicates two consanguineous marriages in early generations (Figure 3.12). Three family members have been diagnosed with AD non-syndromic hearing loss from age 5 with progressive deterioration. A summary of audiology reports is found in Table 3.9. The proband (IV-1) is heterozygous for a C→T nucleotide change, which substitutes a highly conserved arginine residue for a stop signal at amino acid position 98 in exon 4 of *MYO1A* (Figure 3.13). DNA from four available family members was sequenced for exon 4 of *MYO1A.* Three members with documented hearing loss (III-1; IV-1; IV-5) shared the mutation, and one unaffected (III-2) did not. All affected individuals first reported their hearing loss near the age of five, with hearing coming and going but progressively deteriorating (Figure 3.14).

Again, all otosclerosis, AD, and AR probands in the Newfoundland hearing loss study (n=68) were screened to determine if this was a Newfoundland founder mutation. No additional cases were found. Exons 3-4, 6-7, 10-12, 18, and exon 22 were sequenced.

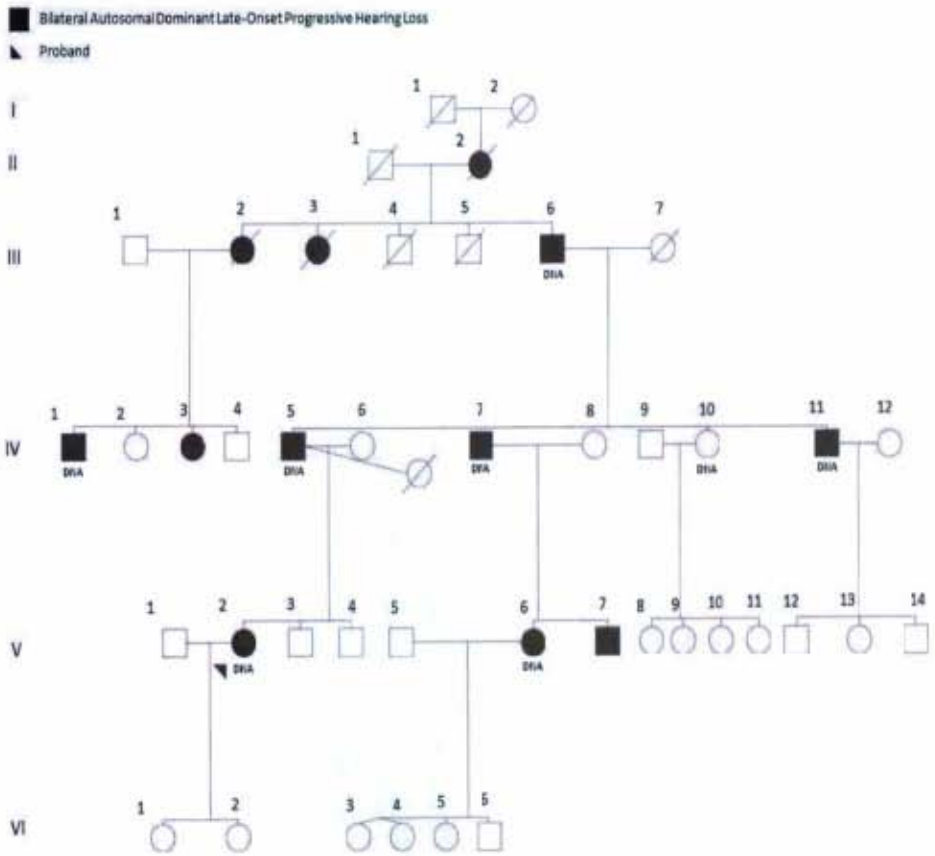



**Figure 3.1 A Six Generation Newfoundland Family (2094) Segregating an Autosomal Dominant Form of Late-Onset Progressive Hearing Loss (partial pedigree). The proband (PID V-2: arrow) was first found to carry the p.P51P/S mutation. DNA from all available affected relatives were screened and also carry the p.P51P/S mutation. Unaffected individual IV-10 does not carry the mutation.**

c.151C>CT:p.P51P/S
PID V-2
Family 2094

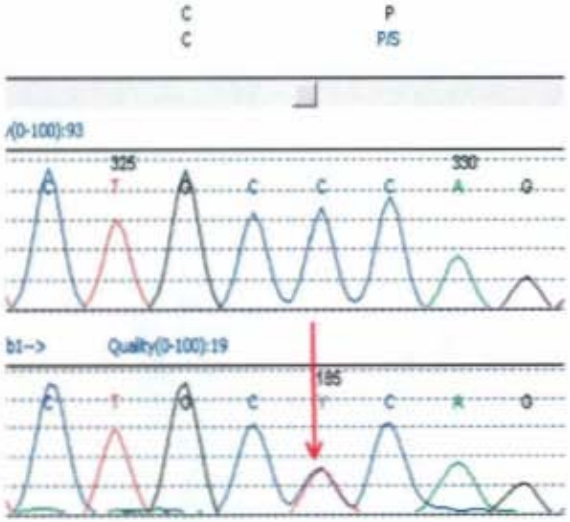



**Figure 3.2 Electropherogram of the Substitution Mutation in *COCH* (c.151C>CT:pP51P/S) Identified in All Affected Family Members. The top trace is from the proband (PID IV-2); the bottom trace is from a reference sequence (obtained from NCBI; NM_004086).**

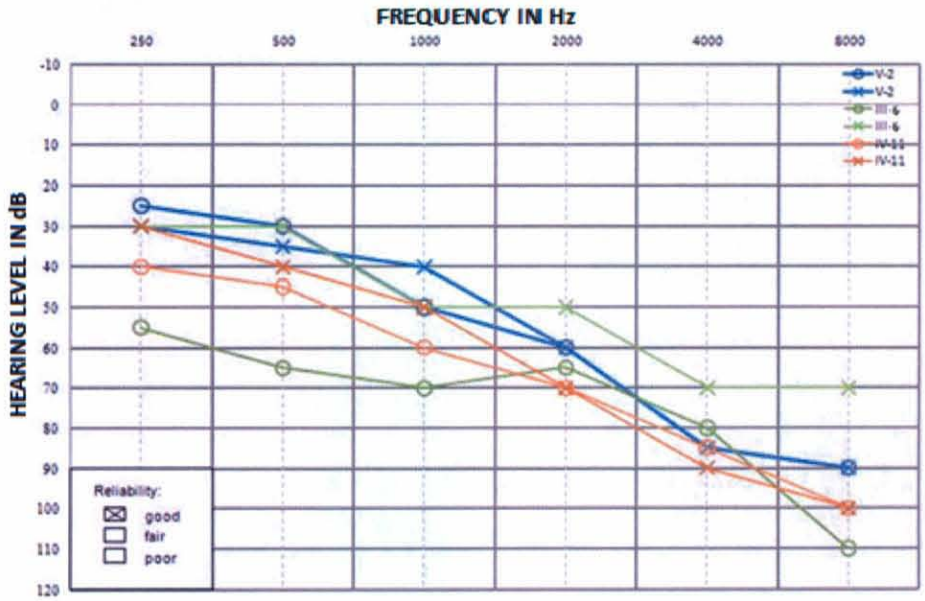



**Figure 3.3 Hearing Loss Phenotype of *COCH* p.P51P/S carriers III-6 , IV-11, and V-2 in Newfoundland Family 2094 (Figure 3.1). Hearing loss is most pronounced in the high frequencies. *Audiology reports were randomly selected, and the same trend is observed for for remaining Family 2094 members, which can be seen in Table 3.2.**

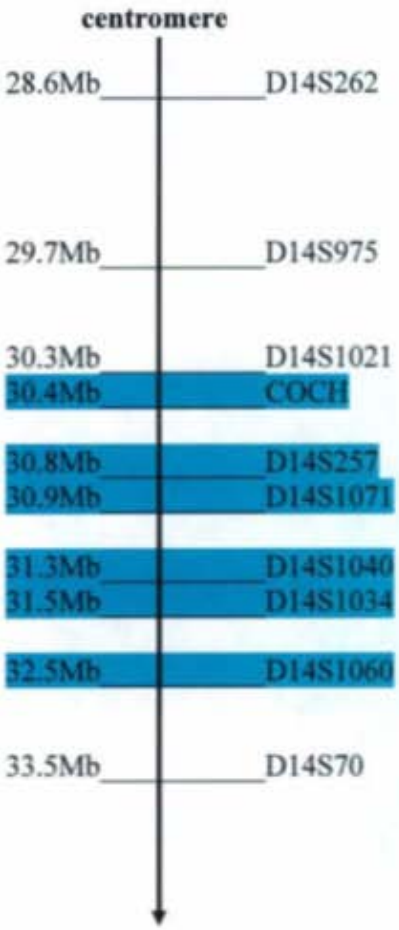



**Figure 3.4 Genetic Map of Markers Used to Construct the p.P51P/S Deafness Haplotype for Newfoundland & Dutch Carriers. Yellow denotes minimum shared region between families. Markers start from centromere. Markers selected from Fransen et al (2001).**

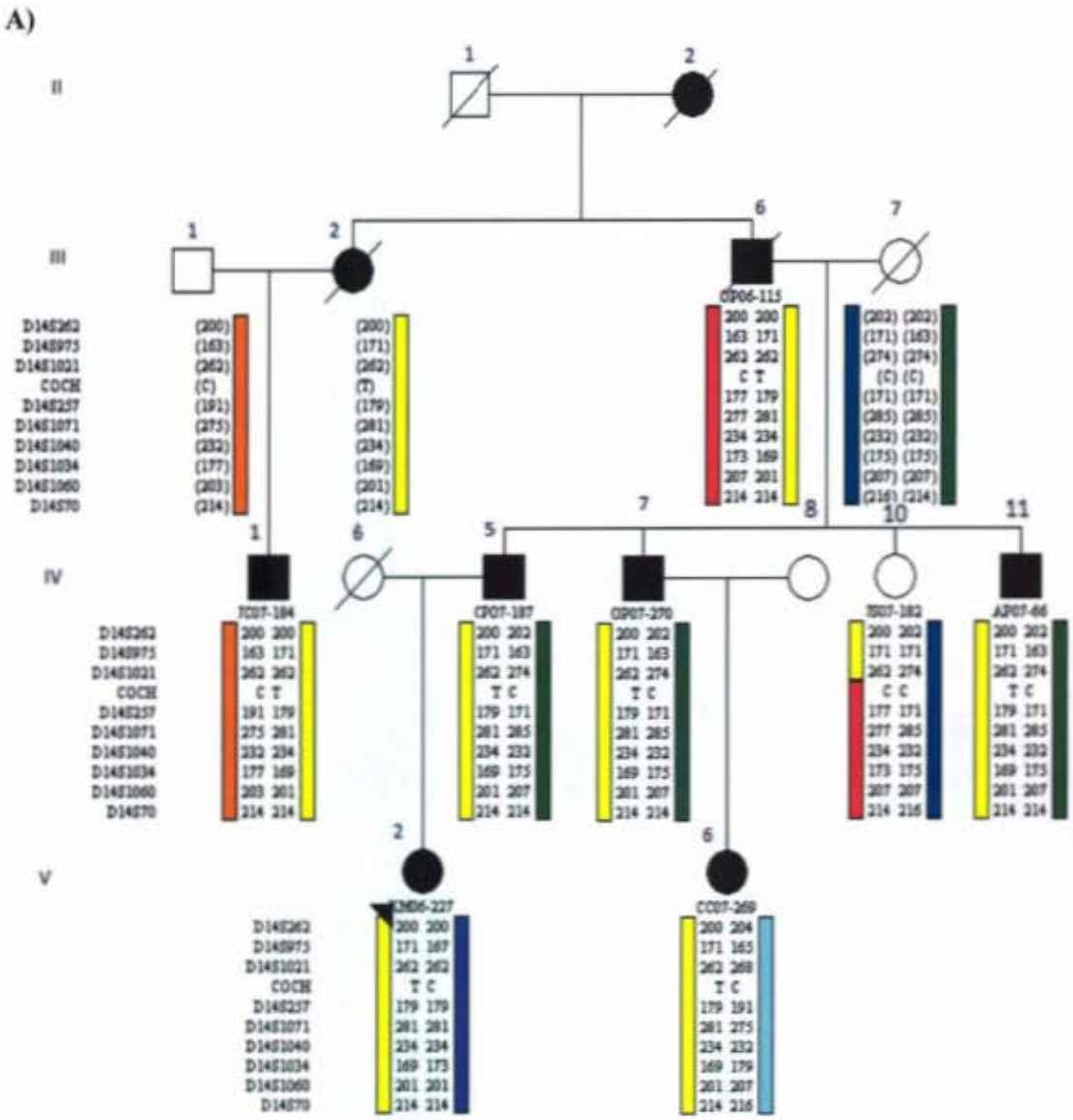



**Figure 3.5 p.P51P/S Family Haplotypes. A) Haplotype for Newfoundland p.P51P/S Family 2094 (partial pedigree). Sharing between families is seen for markers D14S257, D14S1071, D14S1040, D14S1034, and D14S1060, spanning a minimum shared region of ~2.1 Mb.**

B)

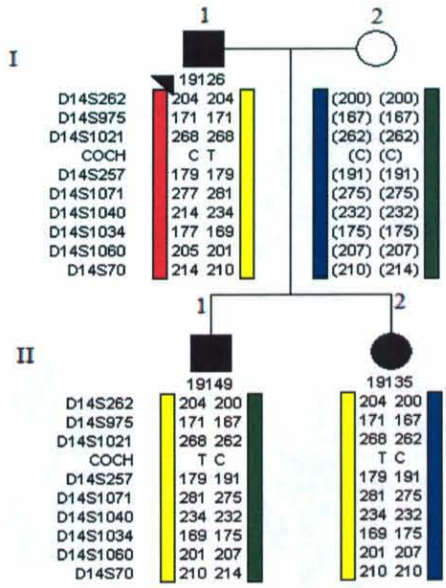
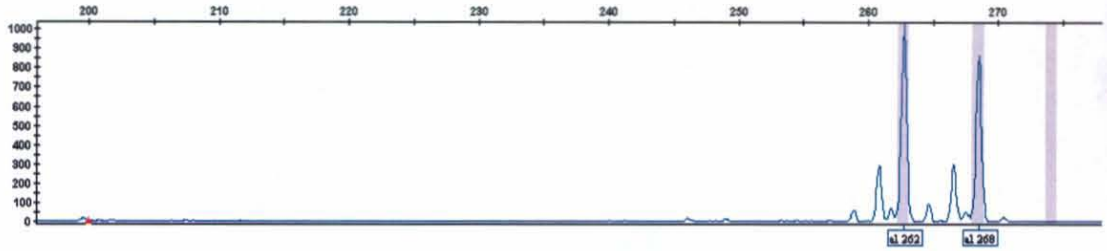
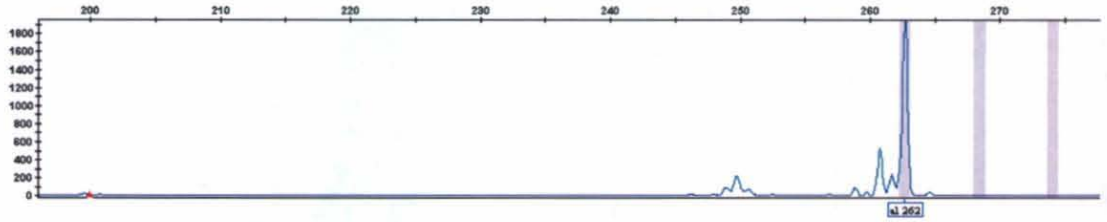



**Figure 3.5 p.P51P/S Family Haplotypes. B) Haplotype for Dutch p.P51P/S family (partial pedigree); associated haplotype is colored yellow. Sharing between families is seen for markers D14S257, D14S1071, D14S1040, D14S1034, and D14S1060, spanning a minimum shared region of ~2.1 Mb.**

A) II-1 (Figure 3.5B)




B) IV-1 (Figure 3.1)




C) IV-10 (Figure 3.1)

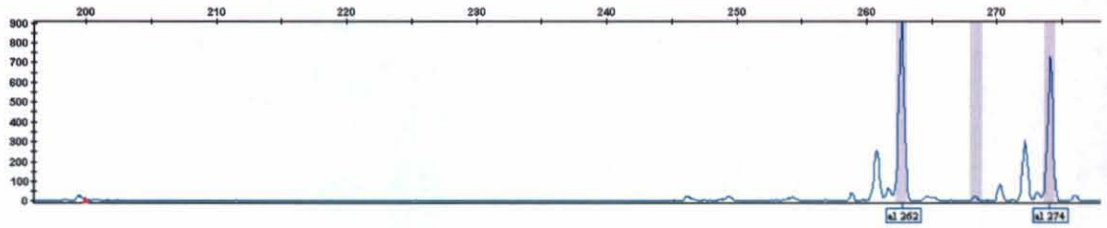



**Figure 3.6 Genotype Examples From Fragment Analysis of Newfoundland and Dutch Families. Homozygosity and Heterozygosity at a marker is described in GeneMapper by the number of times a strong vertical peak is present. If only one strong vertical peak is present, the person is a homozygote; if more than one strong vertical peaks are seen, the person is heterozygous. The numbers in the boxes under these vertical peaks represent the genotypes, or alleles, for that individual. A) II-1 is a Dutch individual (Figure 3.5B) and is a heterozygote (262, 268) for marker D14S1021. B) Family 2094 individual IV-1 (Figure 3.1) is a homozygote (262) for marker D14S1021. C) Family 2094 individual IV-10 (Figure 3.1) is a heterozygote (262, 274) for marker D14S1021.**

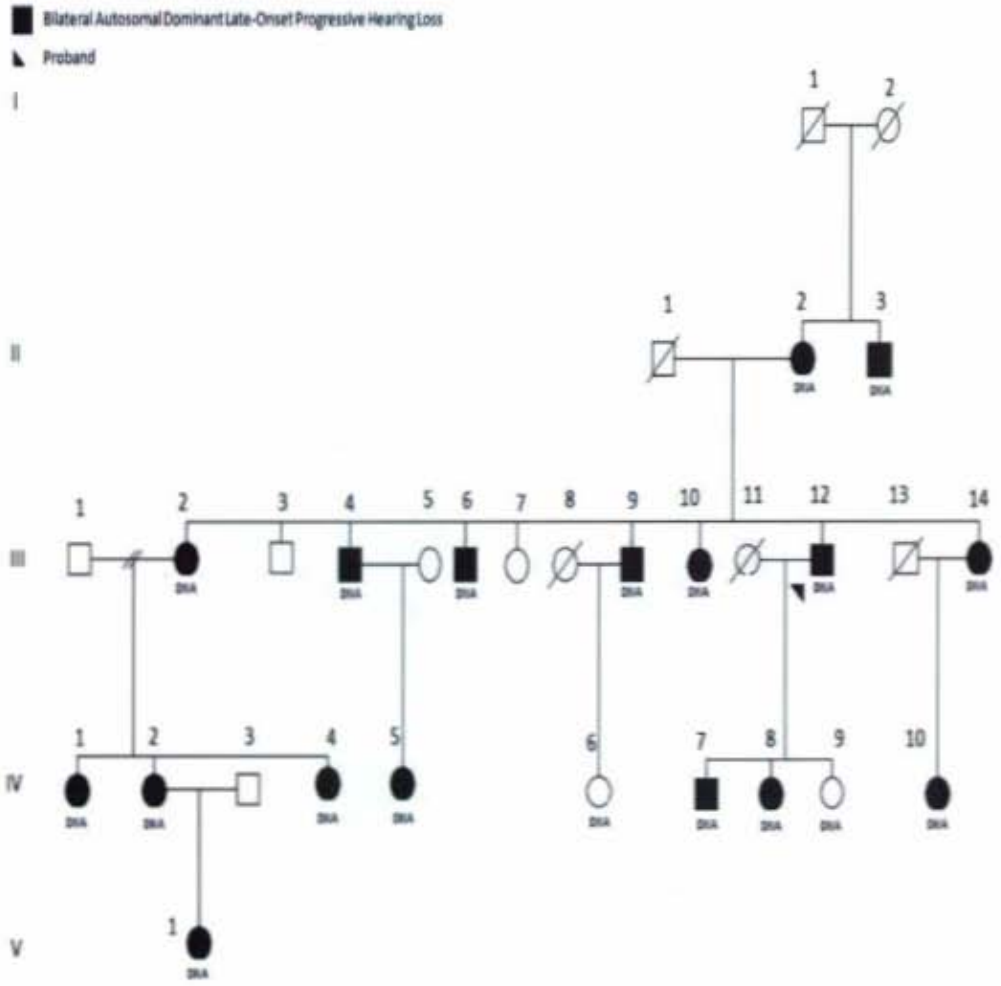



**Figure 3.7 Family 2071. A five generation Newfoundland family affected with autosomal dominant, late-onset, progressive hearing loss (partial pedigree); the 3bp deletion in *KCNQ4* (DFN2A) was first detected in the proband (arrow).**

p.Ser269del
PID III-12
Family 2071

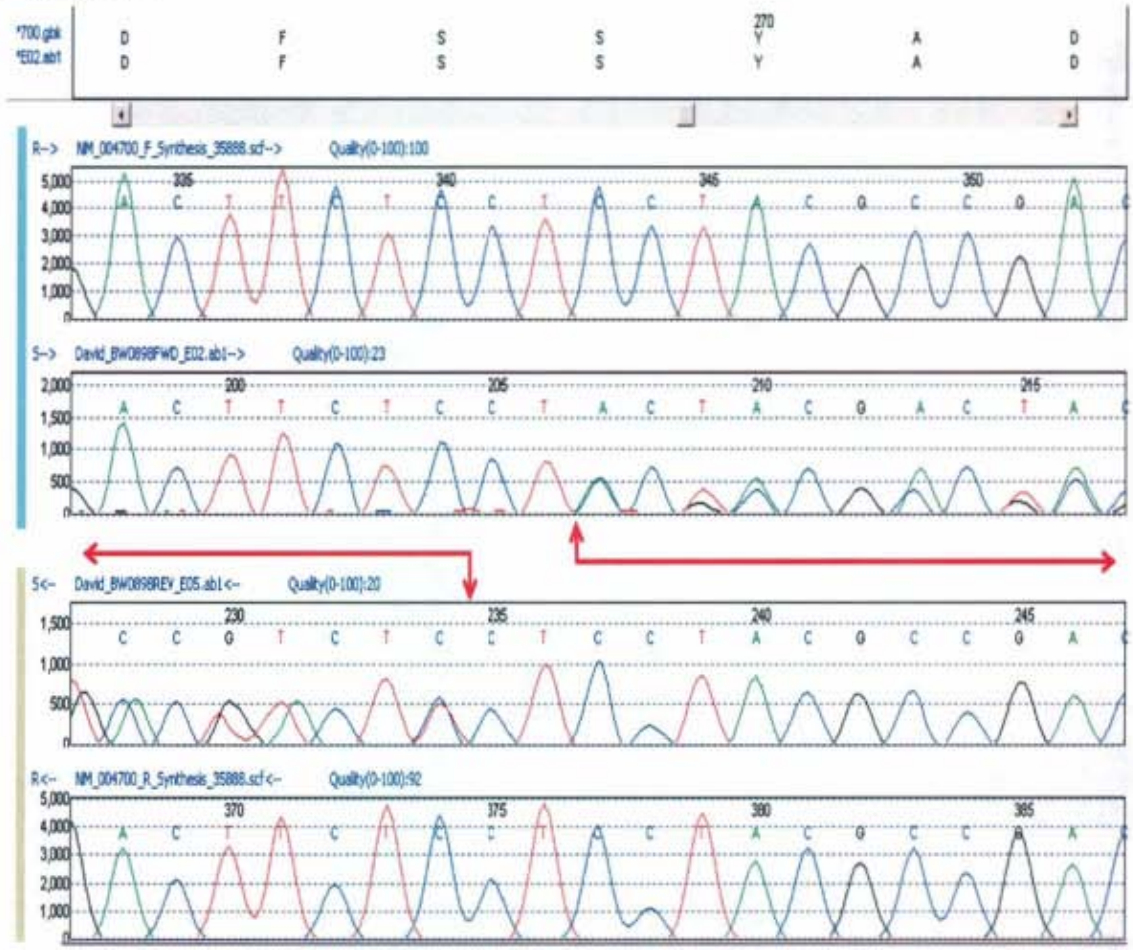
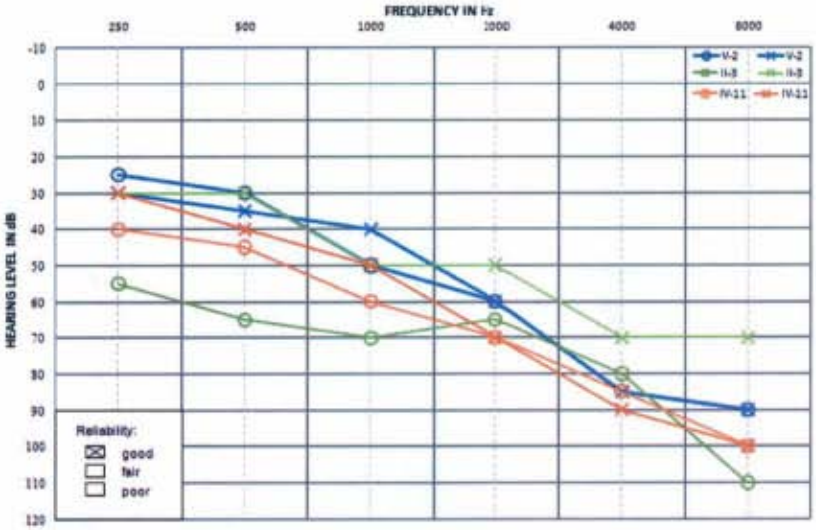



**Figure 3.8 Electropherogram of the 3 bp Deletion in *(*p.Ser269del) Identified in 13 Affected Family 2071 Individuals. The heterozygous deletion causes a 3 bp shift in the Electropherogram of the affected proband's forward and reverse strands, causing the above bidirectional pattern. The top and bottom traces are from a reference sequence, while the middle two traces are the forward and reverse strand traces of the proband III-12 (obtained from NCBI; NM_004700). The bottom trace is from the proband (PID III-12).**

A




B

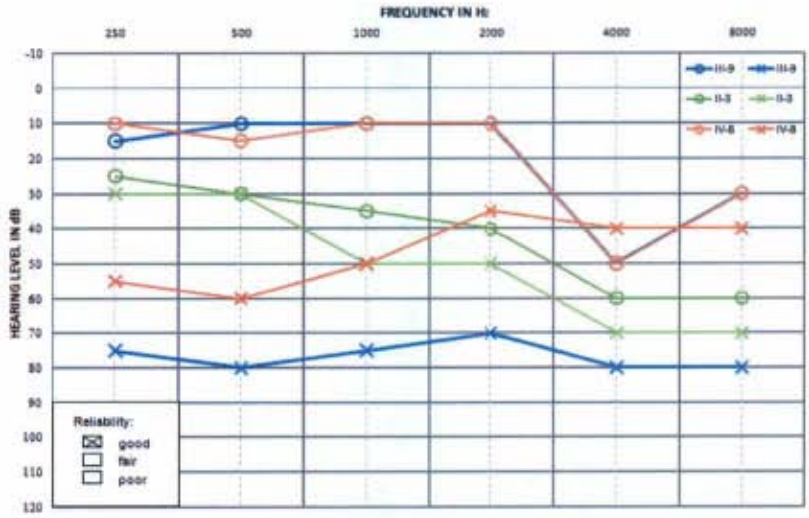



**Figure 3.9 Audiological Summary of Family 2071 Family Members. A: Hearing loss phenotype of *KCNQ4* deletion carriers III-12, V-1, and IV-1 (Figure 3.7) Remaining 2071 deletion carriers all present the same trend observed above, and can be observed in Tables 3.6 & 3.7. Audiology reports were randomly selected. Hearing loss is most pronounced in the high frequencies. B: Hearing loss phenotype of affected family members not carrying the deletion: III-9, II-3, and IV-8.**

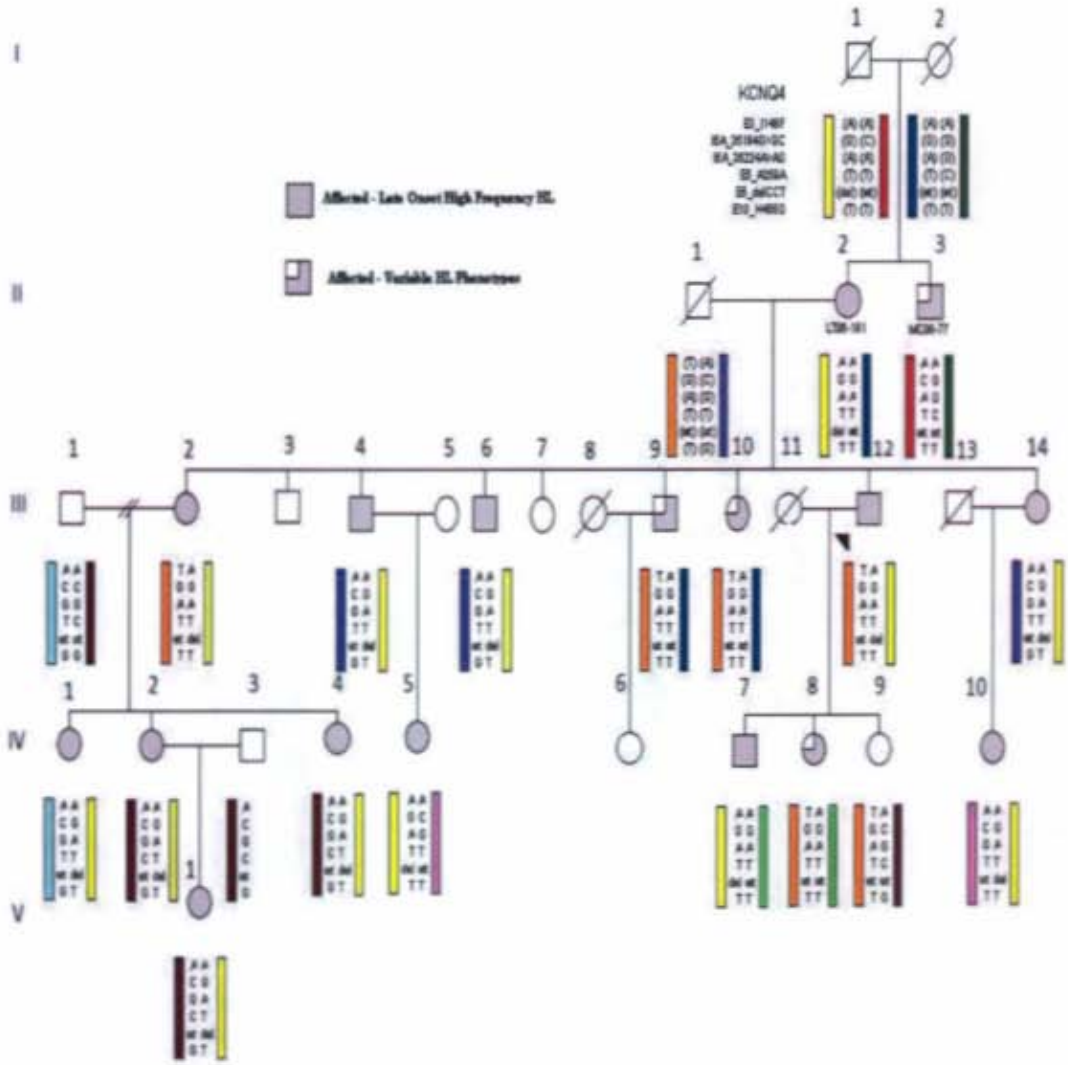


**Figure 3.10 Family 2071 Pedigree With Haplotype. The deafness associated haplotype is shown in yellow. The proband (III-12) and 13 first and second degree relatives harbor the *KCNQ4* deletion. Normal hearing individuals do not carry the deletion.**

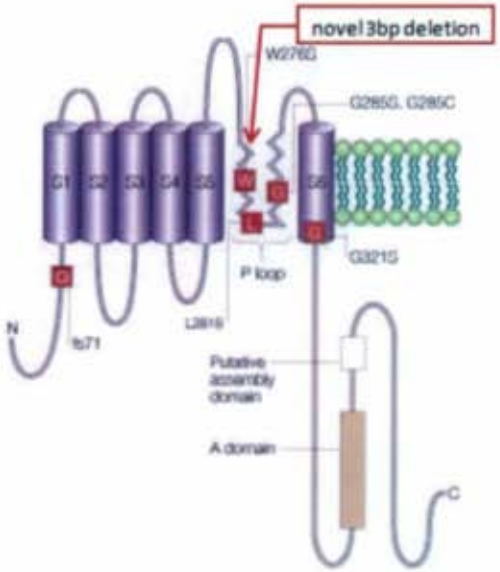



**Figure 3.11 Structure of *KCNQ4*. Arrow denotes novel 3bp deletion discovered in Newfoundland Family 2071. Also shows previously reported missense and deletion mutations found to cause hearing loss. Adapted (Jentsch, 2000).**

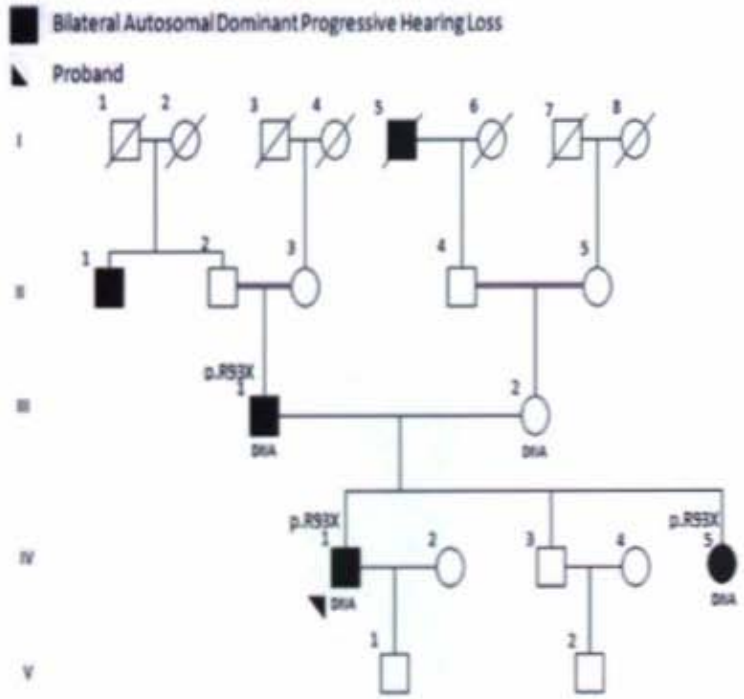



**Figure 3.12 Pedigree of Newfoundland Family 2102. A five generation family originating from Fogo Island, Newfoundland, segregating an apparent AD form of late onset progressive hearing loss. DNA not available for individuals I-5 and II-1.**

p.R93X
PID IV-1
Family 2102

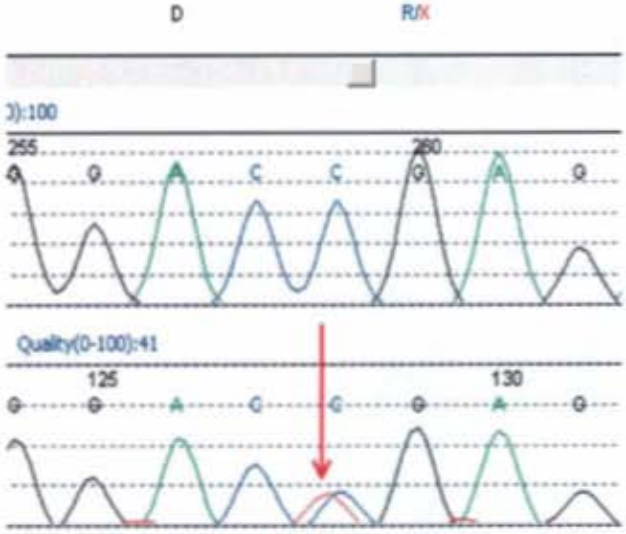



**Figure 3.13 Electropherogram of p.R93X Mutation in *MYO1A*. Individual IV-1 of Family 2102 (Figure 3.12).**

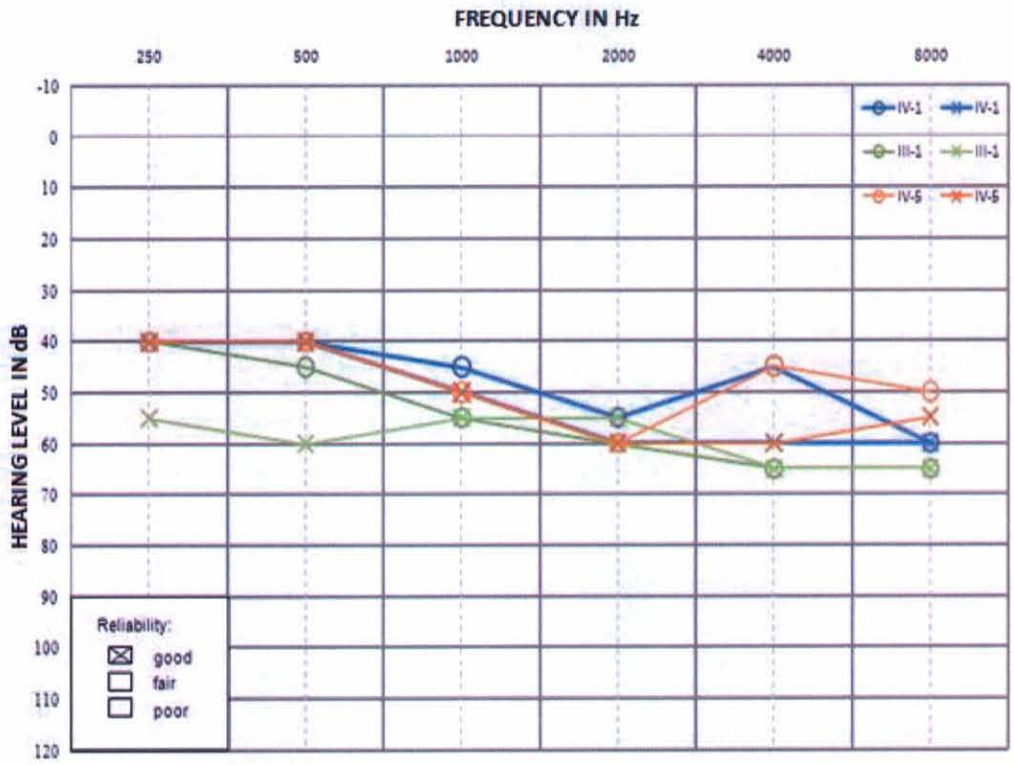



**Figure 3.14 Hearing Loss Phenotype of Newfoundland Family 2102 *MYO1A* Nonsense Mutation Carriers IV-1, III-1, and IV-5 (Figure 3.12). Onset of hearing loss is 5 years of age.**

**Table 3.1 Candidate Genes Screened For Mutations in Newfoundland Families Having Late-Onset Autosomal Dominant Hearing Loss.**

| Candidate Gene | Function | Number of Mutations Found in Caucasian Populations | Number of Mutations Associated with Progressive ADHL |
|---|---|---|---|
| *KCNQ4* | Ion Homeostasis | 8 | 2 |
| *COCH* | Extracellular Matrix | 8 | 6 |
| *MYO1A* | Unknown | 8 | 4 |
| *TECTA* | Extracellular Matrix | 7 | 7 |

**Table 3.2 Audiology Summary For Family 2094 Family Members (pedigree shown in Figure 3.1).**

| Family Member | Age | Onset (yrs) | Sex | Hearing Threshold (dB) | | | | | | | | | | Test Age |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 500 Hz | | 1000 Hz | | 2000 Hz | | 4000 Hz | | 8000 Hz | | |
| | | | | Right | Left | Right | Left | Right | Left | Right | Left | Right | Left | |
| V-2 | 42 | | Female | 30 | 35 | 50 | 40 | 60 | 60 | 85 | 90 | 90 | 90 | 63 |
| III-6 | 98 | | Male | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| IV-1 | 71 | | Male | 65 | 55 | 70 | 65 | 65 | 65 | 80 | 80 | 110 | 110 | 62 |
| IV-5 | 74 | | Male | 50 | 45 | 70 | 70 | 90 | 110 | 100 | 110 | 95 | 100 | 63 |
| IV-7 | 73 | | Female | 45 | 30 | 55 | 40 | 70 | 60 | 90 | 90 | 100 | 100 | 30 |
| IV-11 | 66 | | Female | 45 | 40 | 60 | 50 | 70 | 70 | 85 | 90 | 100 | 100 | 18 |
| V-6 | 37 | | Female | 10 | 10 | 20 | 10 | 30 | 25 | 35 | 30 | 25 | 20 | 35 |

N/A No information was available

**Table 3.3 Phenotype Summary of Family 2094 Individuals (pedigree shown in Figure 3.1).**

| Family Member | Variant | Status | Recorded Vestibular Defects? | Gender | Ear Affected | Noise Exposure | High Frequency | Mid Frequency | Low Frequency | Age | Test-Age |
|---|---|---|---|---|---|---|---|---|---|---|---|
| V-2 | p.P51P/S | Affected | None | Female | Bilateral | None | Profound | Severe | Mild | 42 | 39 |
| III-6 | p.P51P/S | Affected | N/A | Male | Bilateral | None | N/A | N/A | N/A | 98* | 55 |
| IV-1 | p.P51P/S | Affected | Unsteadiness | Male | Bilateral | None | Profound | Severe | Moderate | 71 | 53 |
| IV-5 | p.P51P/S | Affected | Dizziness, Unsteadiness | Male | Bilateral | None | Profound | Profound | Moderate | 74 | 57 |
| IV-7 | p.P51P/S | Affected | Dizziness | Female | Bilateral | Yes | Profound | Severe | Mild | 73 | 55 |
| IV-11 | p.P51P/S | Affected | Unsteadiness | Female | Bilateral | None | Profound | Severe | Mild | 66 | 49 |
| V06 | p.P51P/S | Affected | Vertigo | Female | Bilateral | None | Mild | Mild | Normal | 37 | 35 |
| JS07-182 | No Variant Detected | Not Affected | N/A | Female | N/A | None | N/A | N/A | N/A | N/A | N/A |

N/A Information Not Available
* III-6 died in 2006

**Table 3.4 Physical Location of Markers Used to Create the p.P51P/S Deafness Haplotype. The markers were taken from Fransen et al, 2001.**

| Marker/Gene | Genomic Starting Position on 14q12 (bp) | Type of Nucleotide Repeat | Heterozygosity |
|---|---|---|---|
| D14S262 | 28,630,354 | Dinucleotide | 0.57 |
| D14S975 | 29,749,271 | Dinucleotide | - |
| D14S1021 | 30,341,868 | Dinucleotide | - |
| COCH | 30,413,441 | N/A | N/A |
| D14S257 | 30,799,447 | Dinucleotide | 0.69 |
| D14S1071 | 30,898,090 | Dinucleotide | 0.72 |
| D14S1040 | 31,281,164 | Dinucleotide | 0.73 |
| D14S1034 | 31,537,191 | Dinucleotide | 0.75 |
| D14S1060 | 32,485,191 | Dinucleotide | 0.79 |
| D14S70 | 33,528,945 | Dinucleotide | 0.76 |

**Table 3.5 Haplotype Sharing Across Markers Nearby the *COCH* Gene Between Newfoundland Family 2094 and a Dutch p.P51P/S Family.**

| Location On 14q12 (bp) | Marker | Family 2094 | | | | | | | | Family Dutch | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | JS07-182 | OP06-115 | JC07-184 | KM06-227 | AP07-66 | OP07-270 | CP07-187 | CC07-269 | 19126 | 19149 | 19135 |
| 28,630,354 | D14S262 | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 204 | 200 | 200 |
| 29,749,271 | D14S975 | 171 | 171 | 171 | 171 | 171 | 171 | 171 | 171 | 171 | 171 | 171 |
| 30,341,868 | D14S1021 | 262 | 262 | 262 | 262 | 262 | 262 | 262 | 262 | 268 | 262 | 262 |
| 30,413,441 | COCH c.151C>CT | C | T | T | T | T | T | T | T | T | T | T |
| 30,799,447 | D14S257 | 171 | 179 | 179 | 179 | 179 | 179 | 179 | 179 | 179 | 179 | 179 |
| 30,898,090 | D14S1071 | 285 | 281 | 281 | 281 | 281 | 281 | 281 | 281 | 281 | 281 | 281 |
| 31,281,164 | D14S1040 | 234 | 234 | 234 | 234 | 234 | 234 | 234 | 234 | 234 | 234 | 234 |
| 31,537,191 | D14S1034 | 173 | 169 | 169 | 169 | 169 | 169 | 169 | 169 | 169 | 169 | 169 |
| 32,485,191 | D14S1060 | 207 | 201 | 201 | 201 | 201 | 201 | 201 | 201 | 201 | 201 | 201 |
| 33,528,945 | D14S70 | 214 | 214 | 214 | 214 | 214 | 214 | 214 | 214 | 210 | 214 | 214 |

**Table 3.6 Phenotypic Features of Affected Family 2071 Individuals. Shown first are family members with the deletion, and at the bottom, those without the deletion (pedigree shown in Figure 3.7).**

| Family Member | Variant | Hearing Status | Gender | Ear Affected | Noise Exposure | High Frequency | Mid Frequency | Low Frequency | Age | Test-Age |
|---|---|---|---|---|---|---|---|---|---|---|
| III-12 | 806_808-delCCT | Affected | Male | Bilateral | None recorded | Profound | Moderate | Normal | 70 | 68 |
| III-14 | 806_808-delCCT | Affected | Female | N/A | None recorded | N/A | N/A | N/A | 60 | N/A |
| III-6 | 806_808-delCCT | Affected | Male | Bilateral | None recorded | Profound | Moderate | Mild/Moderate | 67 | 57 |
| III-4 | 806_808-delCCT | Affected | Male | Bilateral | None recorded | Profound | Moderate | Mild | 66 | 63 |
| IV-10 | 806_808-delCCT | Affected | Female | Bilateral | None recorded | Profound | Moderate | Mild | 39 | 37 |
| V-1 | 806_808-delCCT | Affected | Female | Bilateral | None recorded | Severe | Moderate | Mild | 20 | 18 |
| III-2 | 806_808-delCCT | Affected | Female | N/A | None recorded | N/A | N/A | N/A | 64 | N/A |
| II-2 | 806_808-delCCT | Affected | Female | Bilateral | None recorded | Profound | Moderate-Severe | Moderate-Severe | 94 | 90 |
| IV-1 | 806_808-delCCT | Affected | Female | Bilateral | None recorded | Severe | Moderate | Mild | 40 | 39 |
| IV-2 | 806_808-delCCT | Affected | Female | Bilateral | None recorded | Severe | Moderate | Mild | 38 | 33 |
| IV-4 | 806_808-delCCT | Affected | Female | Bilateral | None recorded | Moderately Severe | Moderate | Mild | 43 | 41 |
| IV-5 | 806_808-delCCT | Affected | Female | Bilateral | None recorded | Profound | Moderate | Moderate | 34 | 32 |
| IV-7 | 806_808-delCCT | Affected | Male | Bilateral | Yes | Moderate | Moderate | Mild | 44 | 40 |
| II-3 | No deletion | Affected | Male | Bilateral | None recorded | Moderately Severe | Moderate | Mild | 86 | 83 |
| III-10 | No deletion | Affected | Female | N/A | None recorded | N/A | N/A | N/A | 55 | N/A |
| III-9 | No deletion | Affected | Male | Bilateral, left ear worse | None recorded | Moderate (Right) Severe (Left) | Severe (Left) | Severe (Left) | 71 | 67 |
| IV-8 | No deletion | Affected | Female | Left Ear | None recorded | Mild | Moderate-Severe | Moderate | 46 | 40 |

N/A Information Not Available

**Table 3.7 Audiology Testing Results of Affected Family 2071 Individuals. Shown first are family members with the deletion, and then those without the deletion (pedigree shown in Figure 3.7).**

| Clinical features of family members with 806_808delCCT | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Family Member | Age | Sex | Hearing Threshold (dB) | | | | | | | | | | Test-Age |
| | | | 500 Hz | | 1000 Hz | | 2000 Hz | | 4000 Hz | | 8000 Hz | | |
| | | | Right | Left | Right | Left | Right | Left | Right | Left | Right | Left | |
| III-12 | 70 | Male | 30 | 35 | 50 | 40 | 60 | 60 | 85 | 90 | 90 | 90 | 63 |
| III-14 | 60 | Female | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| III-6 | 67 | Male | 65 | 55 | 70 | 65 | 65 | 65 | 80 | 80 | 110 | 110 | 62 |
| III-4 | 66 | Male | 50 | 45 | 70 | 70 | 90 | 110 | 100 | 110 | 95 | 100 | 63 |
| IV-10 | 39 | Female | 45 | 30 | 55 | 40 | 70 | 60 | 90 | 90 | 100 | 100 | 30 |
| V-1 | 20 | Female | 45 | 40 | 60 | 50 | 70 | 70 | 85 | 90 | 100 | 100 | 18 |
| III-2 | 64 | Female | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| II-2 | 94 | Female | 110 | 75 | 115 | 80 | 120 | 100 | 120 | 100 | 100 | 100 | 90 |
| IV-7 | 44 | Male | 25 | 30 | 55 | 40 | 55 | 50 | 60 | 75 | 60 | 60 | 40 |
| IV-1 | 40 | Female | 30 | 30 | 35 | 35 | 45 | 45 | 70 | 70 | 85 | 70 | 39 |
| IV-2 | 38 | Female | 25 | 25 | 30 | 35 | 35 | 35 | 60 | 65 | 90 | 90 | 33 |
| IV-4 | 43 | Female | 30 | 25 | 35 | 40 | 50 | 50 | 50 | 45 | 70 | 60 | 41 |
| IV-5 | 34 | Female | 50 | 50 | 60 | 55 | 60 | 55 | 85 | 85 | 90 | 90 | 32 |
| Clinical Features of Affected Family Members w/out deletion | | | | | | | | | | | | | |
| III-10 | 55 | Female | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A | N/A |
| III-9 | 71 | Male | 10 | 80 | 10 | 75 | 10 | 70 | 50 | 80 | 30 | 80 | 67 |
| II-3 | 86 | Male | 30 | 30 | 35 | 50 | 40 | 50 | 60 | 70 | 60 | 70 | 83 |
| IV-8 | 46 | Female | 10 | 60 | 15 | 50 | 10 | 35 | 5 | 40 | 15 | 40 | 44 |

**N/A No information was available**

**Table 3.8 *KCNQ4* Variants Used to Create the Intragenic *KCNQ4* Haplotype**

| *KCNQ4* Sequencing Variants | Exon/Intron | Genomic Position | Pathogenic |
|---|---|---|---|
| c.34690A>AT | Exon 3 | 41056458 | No |
| 35184G>GC | Intron 5 | 41056958 | No |
| 35224A>AG | Intron 5 | 41056992 | No |
| c.35905T>TC | Exon 5 | 41057674 | No |
| c.35934_35936del | Exon 5 | 41057702 | Yes |
| c.47646T>TG | Exon 10 | 41069414 | No |

**Table 3.9 Audiological Summary of Family 2102 Individuals With & Without the p.R93X Nonsense Mutation.**

| Family Member | Variant | Status | Gender | Ear Affected | High Frequency | Mid Frequency | Low Frequency | Age | Test-Age |
|---|---|---|---|---|---|---|---|---|---|
| IV-1 | p.R93X | Affected | Male | Bilateral | Moderate | Moderate | Mild | 43 | 40 |
| III-1 | p.R93X | Affected | Male | Bilateral | Moderate | Moderate | Moderate | 71 | 68 |
| IV-5 | p.R93X | Affected | Female | Bilateral | Moderate | Moderate | Moderate | 40 | 12 |
| III-2 | No Variant Detected | Unaffected | Female | None | N/A | N/A | N/A | 59 | N/A |

**N/A Information Not Available**

# Chapter 4: Discussion

The aim of this thesis was to determine the genetic etiology of late onset autosomal dominant (AD) hearing loss in 28 Newfoundland families. This was investigated by screening the genomic DNA of 28 probands for mutations in four genes known to cause AD hearing loss, specifically *KCNQ4*, *COCH*, *TECTA,* and *MYO1A*. All 28 families were first genotyped to exclude the p.A716T mutation in *WFS1*, a mutation previously found to cause AD hearing loss in the Newfoundland population, as well as the full *GJB2* gene and the del13S1830 mutation in *GJB6*.

## Family 2094 Hearing Loss Caused by *COCH* Mutation

Of the 28 probands, one was found to have a mutation in *COCH*. *COCH* encodes cochlin, and has previously been shown to be prominently expressed in a ribbon-like pattern in the basilar membrane of the cochlea, providing evidence that it is involved in the structural regulation of that membrane (Kommareddi et al. 2007). Cochlin's exact role, however, remains unknown. *COCH* maps to chromosome 14q12-13. Cochlin is predicted to be 550 amino acids long and is highly conserved. It comprises a short predicted signal peptide, an N-terminal factor C homology domain, and two von Willebrand factor A-like domains.

Seven missense mutations within *COCH* that cause hearing loss have previously been reported: six of these cause a very recognizable phenotype characterized by a late

onset, progressive hearing loss associated with parallel vestibular decline. All six are found in the Factor C Homologous (FCH) domain of the cochlin protein (Kemperman et al. 2005). Of the FCH domain mutations, three originate in North America (p.V66G, p.G88E, and p.W117R); one is a founder mutation (p.P51P/S) present in many Dutch and Belgian families, one originated in Australia (p.I109N), and one in Japan (p.A119T). This audiological phenotype of late-onset high-frequency hearing loss caused by these mutations is in close correlation with the audiological phenotype of many of the 28 AD Newfoundland probands being studied in this research project and was the primary reason for choosing this gene for screening.

In Newfoundland Family 2094 a known mutation causing a heterozygous C→T base change was found in the proband. This resulted in the substitution of a conserved proline residue for a serine residue at amino acid position 51 (p.P51P/S) (Figure 3.2). All seven affected members of Family 2094 shared the mutation, while an unaffected relative did not harbor the mutation (Figure 3.1). Individuals harboring the p.P51P/S variant suffer from a late onset, progressive, high-frequency hearing loss with an obvious onset from ~40 years of age onwards (Bischoff, 2005). All affected family members share the same audiological profile (Figure 3.3), matching the pattern of hearing loss seen in previous cases (de Kok et al. 1999). The progressive nature of hearing loss in Family 2094 also matches previously reported cases of p.P51P/S carriers (de Kok et al. 1999).

This mutation is also associated with vestibular dysfunction, such as motion sickness and vertigo, which in most cases develops to complete vestibular areflexia or vestibular hyporeflexia (Verhagen et al. 2001; Bischoff et al. 2005). Onset of progressive

vestibular failure presents earlier, from the fourth into the sixth decade of life, declines more rapidly, and is eventually more complete than the associated hearing impairment (Bischoff et al. 2005). Five out of seven Family 2094 members have so far experienced these vestibular problems.

In 2009, Hildebrand et al. reported a p.P51P/S case with the very rare disease Superior Semicircular Canal Dehiscence (SSCD), which is characterized by the absence of bone overlying the superior semicircular canal, creating a third labyrinthine window. It was suggested that individuals with *COCH* mutations like p.P51P/S should be given a CT scan to screen for SSCD (Carey et al. 2007). Of the Family 2094 individuals who have had CT scans; V-2, V-6, and IV-1, none were diagnosed with SSCD, though it has been suggested that diagnosis of SSCD often requires the doctor to be specifically looking for the defect (Hildebrand et al. 2009). This therefore represents both a clinical and a scientific opportunity to learn more about this possible association. Carriers could undergo a CT scan to look for this rare defect, and if found, that individual can have surgery done, as SSCD's severe vestibular symptoms can be fully corrected with surgery (Carey et al. 2007). This would also add to the current literature on the possible association between these two rare disorders.

Previous studies have also demonstrated a link between *COCH* mutations like p.P51P/S and vertical corneal striae. Two families carrying the p.P51P/S mutation were found to have a 93 % and a 78 % prevalence of these striae, respectively; age of onset is late 40s to late 50s (Bischoff et al. 2007). In Family 2094, V-6 was reported to have a corneal scratch in the right eye. This was first reported at the age of 31, which falls close

to the previously reported age of onset for corneal striae in p.P51P/S carriers. This scratch was not reported to be due to any physical injury to the eye, though no other medical information is available. Clinical follow-up is currently ongoing; this may be further evidence of a link between vertical corneal striae and hearing loss mutations within *COCH*, like p.P51P/S.

## Confirmation of p.P51P/S as a Dutch Founder Mutation

The p.P51P/S mutation has been reported in ten Belgian, seven Dutch, and one American family and is widely accepted to be a Dutch founder mutation (de Kok et al. 1999; Fransen et al. 2001). This study is only the second reported occurrence of this mutation outside of Europe. An opportunity to confirm this Newfoundland p.P51P/S occurrence as a Dutch founder mutation arose at the 2010 Association for Research in Otolaryngology MidWinter conference. While attending this conference, I met with Dr. Hannie Kremer, a scientist working in the Netherlands, who has published on the p.P51P/S vestibular phenotype (Bischoff et al. 2005). Dr. Kremer offered to contribute Dutch p.P51P/S DNA, allowing me to confirm the Dutch founder hypothesis. Using DNA from this Dutch family and Newfoundland Family 2094, a series of microsatellite markers were genotyped to develop ancestral haplotypes (Figure 3.5). These haplotypes demonstrate allelic sharing between the two families for five contiguous markers close to the *COCH* gene, spanning a total genetic distance of ~2.1 Mb (Figure 3.4; Table 3.5). The probability of these two families sharing this five marker haplotype by chance is low,

and therefore, it is likely that these two families are distantly related and originate from a common ancestor in the Netherlands. The genealogy of this shared ancestry was not followed up on, as the objective was to provide further support to the current Dutch founder hypothesis for p.P51P/S DNA, and because no further genealogy on these families is available. However, individuals in Family 2094 could be researched using the Newfoundland Genealogy Database and the Heritability Analytics Infrastructure (Population Therapeutics Research Group, St. John's, Newfoundland & Labrador) to trace the genealogy of Family 2094. If successful, this would provide the opportunity to increase the power of this linkage association, and possibly directly link it to the Dutch family with which it shares an ancestral haplotype. This avenue warrants further investigation. It is interesting to note that mutation carriers in Family 2094 possess an uninterrupted Newfoundlander ancestry, going back at least six generations. Up until recently, p.P51P/S had never been seen outside of central Europe (Fransen et al. 2001). Its recent discovery in a United States family (Hildebrand et al. 2009), and now in a family living here in Newfoundland, Canada, could be evidence of an ancient origin. If true, this would have strong implications for cochleovestibular diagnostic screening of p.P51P/S and other mutations within the *COCH* gene, as the older a mutation's origin, the more likely it is to be widespread throughout all populations and ethnicities.

## Family 2071 Hearing Loss Caused By Novel *KCNQ4* Deletion

*KCNQ4* (Potassium Voltage-Gated Channel 4) encodes the protein potassium voltage-gated channel subfamily KQT member 4. This protein is part of a family that forms channels to transport positively charged potassium ions between neighboring cells. More importantly for hearing, the potassium channels that this protein forms are thought to play an indispensable role in the regulation of neuronal excitability, particularly in the sensory cells of the cochlea (inner ear), where they are expressed (Kubisch et al. 1999). Expression gradients of *KCNQ4* in spiral ganglion and in these cochlear hair cells correlate very closely with progressive hearing loss (Beisel et al. 2001).

Twelve mutations have been reported in *KCNQ4* (DFNA2): ten missense and two deletions. The missense mutations are believed to cause hearing loss beginning at a young age (Hilgert et al. 2009). The deletions are thought to cause a milder phenotype, have an older age of onset and primarily affect perception of high frequencies (Topsakal et al. 2005). While both late-onset and early-onset hearing loss can be caused by mutations in *KCNQ4,* this gene is also associated with age-related hearing loss. One research team has demonstrated that several SNPs associated with age-related hearing loss in two independent Caucasian populations were all located in the same 13 kb region in the middle of the *KCNQ4* gene (Van Eyken et al. 2006).

The first *KCNQ4* deletion (c.211del13) was discovered in a Belgian family. Affected individuals lacked 13 nucleotides between positions 211 and 224. This resulted in a frame-shift after Gly70 (p.fsX71), followed by 63 novel amino acids and a premature stop codon (Coucke et al. 1999). Consequently, the protein is truncated before the first

transmembrane domain and is rendered nonfunctional (Nie, 2008; Figure 3.11). More recently, the second deletion was found in a Japanese family, and is a 1 bp deletion (c.211delC). Similarly to the previous case, a truncated, nonfunctional *KCNQ4* protein is generated (Kamada et al. 2006). The milder high frequency phenotype of cases reported to harbor these two *KCNQ4* deletions correlates closely with the phenotype of several of the 28 Newfoundland probands under study in this research project, and so *KCNQ4* was chosen as a functional candidate gene to screen for potential causative mutations.

The proband of Newfoundland Family 2071, III-12 (Figure 3.7), was found to carry a novel 3bp heterozygous deletion in exon 5 of *KCNQ4* (Figure 3.8). Upon sequencing other Family 2071 individuals, thirteen affected individuals were found to share the 3 bp deletion. This is the third deafness causing deletion found in *KCNQ4*, and the first outside of exon 1 (Coucke et al. 1999; Kamada et al. 2006). Audiology reports of deletion carriers demonstrate a high-frequency, late-onset hearing loss (Figure 3.9), supporting the current genotype-phenotype correlation that *KCNQ4* deletions associate with a late-onset and milder hearing impairment (high-frequency loss) than corresponding *KCNQ4* missense mutations (Kamada et al. 2006).

This deletion predicts an in-frame removal of a serine residue at amino acid position 269 within the P-loop domain of the KCNQ4 protein (Figure 3.11). Interestingly, the P-loop domain is a mutational hotspot where ten missense mutations causing early onset hearing loss have previously been described (Coucke et al. 1999; Kubisch et al. 1999; Talebizadeh et al. 1999; Van Hauwe et al. 2000; Kamada et al. 2006).

In order to determine the level of variant sharing among deletion carriers and between all individuals of Family 2071, and to verify whether further hearing loss patterns might be seen, we next constructed an intragenic haplotype using commonly occurring variants within and flanking exon 5 of *KCNQ4*. While these markers did not singularly provide any additional interesting information regarding affected individuals not harboring the deletion, all deletion carriers shared this deafness associated haplotype. Additionally, of 90 ethnically matched controls, and of all unaffected relatives, none shared this deafness associated haplotype. This haplotype, used primarily as a method of further investigation and mutation confirmation, contrasts sharply with the aforementioned p.P51P/S haplotype, which was constructed to demonstrate ancestral linkage between Newfoundland Family 2094 and a Dutch family, and which can be taken as evidence for p.P51P/S being a Dutch founder mutation.

While this deletion is not seen in four Family 2071 members, these four individuals present a distinctly different audioprofile compared to deletion carriers (Figure 3.10; Table 3.6; Table 3.7). The cause of hearing loss for these four individuals is likely due to several separate genetic or environmental predispositions, thus making them phenocopies. Environmental factors could also be the cause of hearing loss in these individuals. The medical records show no indication of noise exposure or physical injury to the ears in these four individuals. To rule out a different mutation on the same gene as the cause of hearing loss in these individuals, they were screened for all exons within *KCNQ4*. No additional mutations were detected, and they did not share any particular variants or SNPs at the locus when observed in the intragenic haplotype.

A further avenue of research for Family 2071 would be functional studies. Because *KCNQ4* is strongly expressed in the sensory cells of the cochlea (Kubisch et al. 1999; Beisel et al. 2001), studies on deletion carriers should be conducted to investigate whether the 3 bp deletion negatively affects the potassium ion channels formed by *KCNQ4,* whether these ion channels remain structurally and functionally intact, and whether potassium ions are able to effectively move through these channels to complete the mechanoelectrical transduction pathway. This could potentially shed further light on the molecular pathways underlying this hearing loss mutation and could provide added credence to the current genotype-phenotype correlation.

## Family 2102 Hearing Loss Caused By *MYO1A* Mutation

*MYO1A* encodes the protein myosin 1a, which is present in the inner ear and plays a role in human hearing (Donaudy et al. 2003). Donaudy et al. (2003) postulated that *MYO1A* plays a role in ion transport. More recently, Hilgert & Smith (2009) present a slightly different hypothesis. At the brush border surface of intestinal epithelial cells, myosin 1a is a major component of the actin-rich cytoskeleton, where it is involved in membrane trafficking. It could serve the same function in the inner ear, because the cytoskeleton of the intestinal cells and the inner ear cells (hair cells and supporting cells) are very similar. The specific expression pattern in the inner ear has not yet been established, by may provide further clues in the future (Hilgert & Smith, 2009).

*MYO1A* was chosen as a candidate gene for the same reason as all the functional candidate genes in this study: a phenotype correlation between individuals with hearing loss due to mutations previously found within *MYO1A* and the audioprofile seen in some of the 28 Newfoundland probands under study. While most of the 28 probands suggest a late-onset hearing loss, some are reported as early-onset, but still progressive and autosomal dominant. Four mutations in *MYO1A* have been associated with an early-onset AD, progressive, phenotype (Donaudy et al. 2003). These four mutations were all found in Italian probands.

A heterozygous nonsense mutation was discovered at amino acid position 93 within exon 4 of *MYO1A* in the Family 2102 proband IV-3 (Figure 3.12). This nonsense mutation, p.R93X substitutes an arginine residue for a stop signal in the motor domain (Figure 3.13). All three affected Family 2102 individuals shared the mutation, and reported their first hearing loss at 5 years of age, with hearing coming and going, but progressively deteriorating to severe hearing loss (Figure 3.14).

While this mutation is not novel, it is only the second reported case world-wide. The p.R93X nonsense mutation was first reported in a very small southern Italian family. p.R93X was present in the male proband who suffered from moderate to severe bilateral hearing loss. This is the same audioprofile seen in Family 2102, though time of onset was not available for this Italian family. The proband received the mutant allele from his mother. The mother stated that she has normal hearing, although no audiological evaluation of any kind was carried out. A healthy brother of the proband did not carry the p.R93X mutant allele and did not possess any form of hearing loss (Donaudy et al. 2003).

Several possibilities could explain the Italian mother's supposed normal hearing: 1) The mother does in fact have the same hearing loss as her son, 2) this family segregates an AR form of deafness and digenic inheritance is responsible for this hearing loss phenotype, or 3) the trait is not 100 % penetrant. While many types of hearing loss are caused by a mutation in one single gene, digenic inheritance requires the interaction of two genes for phenotypic expression. If the mother does not have hearing loss, despite carrying the p.R93X mutation, she may not have hearing loss because this trait is an AR form of hearing loss and segregates in a digenic manner. She would not inherit the second disease causing mutation in the putative second unknown gene. Additionally, if the trait is not fully penetrant then this too could explain the mother's normal hearing. Detection of the p.R93X mutation is simple and does not require sequencing because the mutation is easily identified by digestion of PCR products, as the mutant allele destroys an A*va*II restriction site. A further avenue of research for Family 2102 would be to attain DNA from the affected Italian individuals previously reported to create an ancestral haplotype, as done previously for Family 2094. It could then be determined whether or not Family 2102, from Newfoundland, share a common ancestor with the previously reported Italian family (Donaudy et al. 2003).

## Candidate Gene *TECTA*

*TECTA* encodes the protein Alpha-tectorin. The tectorial membrane is an extracellular matrix that covers the Organ of corti sensory epithelium in the ear. Sound waves induce a vertical movement of the basilar membrane, and this movement evokes a deflection of stereocilia against the tectorial membrane. Alpha-tectorin is one of the major noncollageneous components of the tectorial membrane. Mutations in the *TECTA* gene have been shown to be responsible for ADNSHL, with audioprofiles similar to some of the 28 Newfoundland probands under study (Verhoeven et al. 1998).

Exons sequenced included 5, 9-14, 17-18, and 20. However, no hearing loss mutations were discovered among the 28 AD Newfoundland probands. This does not rule out the possibility that hearing loss in one or more of these Newfoundland probands if caused by mutations in *TECTA* within an exon that was not sequenced in this research project. A total of 13 exons were not sequenced, and this gap represents an opportunity for further research, as *TECTA* remains a strong candidate gene for hearing loss in Newfoundland families.

## Non-Founder Mutations in a Founder Population

As previously discussed, the province of Newfoundland & Labrador is a founder population, due to its cultural and geographic isolation. Since the second major wave of

settlement, taking place in the late 18th and early 19th centuries, little immigration or outmigration has occurred. These original colonists have contributed to several founder mutations that cause specific diseases. For example, an exon 8 deletion in *MSH2*, a gene causing hereditary non-polyposis colorectal cancer, has been found in 5 different Newfoundland families (N=74 carriers) (Frogatt et al. 1996; Stuckless et al. 2006), and the c.782+3delGAG mutation found in the deafness gene *TMPRSS3* has been reported in 2 different Newfoundland families (Ahmed et al. 2004; Young et al. unpublished data).

This study sought to potentially identify AD hearing loss founder mutations in Newfoundland. No founder mutations were identified. All three of the mutations identified in this study - p.P51P/S, p.Ser269del, and p.R93X in the genes *COCH, KCNQ4,* and *MYO1A* respectively - were each reported in one family only, after screening 68 Newfoundland & Labrador hearing loss probands. We identified three separate mutations in three separate genes causing hearing loss with three separate phenotypes. Additionally, these mutations were found to cause hearing loss in three Newfoundland families that are geographically separated from one another. So while the genetic history and nature of Newfoundland's population, combined with previous successes (Young et al. 2001; Ahmed et al. 2004) implies that the future discovery of hearing loss founder mutations in Newfoundland & Labrador is still a possibility, the results of this study unexpectedly point to Newfoundland's genetic diversity, rather than its homogeneity. This isn't the first time this has happened. An increased level of genetic diversity was noted when, for example, nine mutations in six genes were detected in 21 families with Bardet-Biedl syndrome (Webb et al. 2009). I speculate here that the solved

families in this study, 2094, 2071, and 2102, all descend from original but separate groups of Newfoundland colonists. These colonists would likely have been from several different areas of Ireland or England. The geographical isolation of their origin home towns could account for the potential genetic diversity seen in the identification of these three separate hearing loss mutations. These findings, therefore, do not marginalize the efficacy of the Newfoundland population in the search for novel gene discovery or for founder mutations.

The clinical and diagnostic utility of these identified mutations is significant for the families in question, as they are now able to screen new family members, receive much improved genetic counseling, and hopefully, benefit from improved treatment options. However, identification of a Newfoundland founder mutation would be of increased clinical and diagnostic utility. Any mutation that is prevalent throughout a large portion of native Newfoundlanders is screened for at birth, and would thus be identified in all future cases born in Newfoundland. The aforementioned benefits would therefore apply to a much wider range of people than a single family, as is the case for non-founder mutations. This study, therefore, does not reduce the strong clinical and diagnostic potential that can be fulfilled through the discovery of Newfoundland founder mutations, nor does it point to an inefficacy of the Newfoundland population as a medium of novel gene discovery.

## A Changing Landscape of Gene Identification Methodology

Despite the increase in hearing loss gene identification, many deafness causing genes and loci remain undiscovered. High density SNP arrays, which are a type of DNA microarray used to detect polymorphisms across large portions of a genome have been successfully applied in a new approach to find hearing loss genes. Shahin et al. (2010) applied SNP array-based homozygosity mapping of families with a high degree of consanguinity. Homozygosity mapping is a powerful method of localizing genes for autosomal recessive disorders. Using this approach, Shahin et al. (2010) identified five genome regions likely to harbor novel genes for pre-lingual non-syndromic hearing loss in six Palestinian kindreds. This approach is currently being investigated for AR hearing disorders in the Newfoundland population. Traditionally this method has been limited to families who share a recent common ancestor, but Hildebrandt et al. (2009) have recently demonstrated that this technique can be used on outbred populations. They performed homozygosity mapping on 72 single affected individuals of 54 kindreds ascertained worldwide using a 250 K SNP array. This discovery could potentially open up further opportunities for novel gene discovery in Newfoundland & Labrador as many specialty clinics have access to cohorts of individuals from out-bred populations.

A second technological advance having huge implications on novel gene discovery is the advent of next-generation sequencing (Schuster, 2008). Next-generation sequencing allows for the easy production of millions of DNA sequence reads in a single run. Next-generation sequencing instruments can generate as much data in 24 hours as several hundred traditional DNA capillary sequencers, but are operated by a single person

(Schuster, 2008). A recent study of non-syndromic hearing loss used targeted genome capture combined with next-generation sequencing to analyze 2.9 Mb of the *DFNB79* interval on chromosome 9q34.3 (Rehman et al. 2010). Rehman et al. (2010) detected a nonsense mutation in the predicted gene *C9orf75*, which they renamed taperin (*TPRN*). A nonsense mutation is a change in DNA sequence that results in a premature stop codon, leading to an incomplete, and usually nonfunctional, protein product. Rehman et al. (2010) next performed immunolocalization experiments on the *TPRN* protein in a mouse cochlea, and saw prominent expression in the taper region of hair cell stereocilia.

A third strategy showing recent success (Meyer et al. 2007) and strong promise for gene identification, specifically for AD hearing loss, is the use of AudioGene Audioprofiling. Audiogene is a computer program that uses a machine-learning approach to analyze audioprofiles as a method of prioritizing genes for mutation screening in small families segregating AD hearing loss. The audiogene dataset has recently been expanded to include a total of 16 DFNA loci, including COCH, *KCNQ4,* and *TECTA* (Hildebrand et al. 2009). This could be very useful in the investigation of Newfoundland families segregating AD hearing loss, and many of these families are small with limited recorded data. Hildebrand et al. (2009) performed an experiment where a series of audiograms were analyzed by a panel of hearing loss experts, and concurrently by AudioGene. The accuracy of matching the audiograms with the genotypic cause was 55 % for the human experts, and 88 % for AudioGene (Hildebrand et al. 2008). Furthermore, as the size of this database increases, so too will its predictive capacities. For now, it represents a promising avenue for Newfoundland AD hearing loss research at almost zero cost, and

should be the next strategy employed for mutation detection in the 25 remaining Newfoundland AD probands.

These are just some examples of recent advances in technology leading to novel gene discovery, and as these technologies become more common, their use will be instrumental in new investigative disease causing studies not just in the Newfoundland population, but all over the world. The ability of next-generation sequencing to perform so many 'reads' so quickly will be invaluable to future hearing loss studies in Newfoundland. Screening candidate genes and potentially mutated chromosomal regions will be significantly easier, allowing for the 'quick' discovery of novel (and existing) causative hearing loss mutations in Newfoundland probands.

## Limitations of this Study

Despite the discoveries discussed above, the candidate gene approach undertaken in this study does present various limitations in the search for hearing loss mutations. These limitations vary widely, and while mentioned above throughout this thesis, a short summary of them will serve to illuminate the path forward both in filling any gaps, and in approaching mutation detection in various untried ways up to this point.

Firstly, it is important to note that mutations in *WFS1* may not be more common than other hearing loss mutation just because they are more commonly reported in Newfoundland. The reason the p.A716T mutation in *WFS1*is so widely reported is due in large part to its pathognomonic character. A pathognomonic sign is a particular sign

whose presence is characteristic for a certain disorder beyond any doubt. The phenotype of p.A716T mutation in *WFS1* is one such pathognomonic trait. Local audiologists are able to both distinguish this pattern of hearing loss and recognize surnames of the extended family thus far affected, and contact us directly when they have patients that may be related to these originally reported families (Young et al. 2001). Clinical application like this makes it likely that the high frequency of reported *WFS1*-related hearing loss is an overstatement, not describing the true situation (Tranebjaerg 2008).

It is also possible that one or more of the families under investigation may be incorrectly classified as AD due to a lack of sufficient data. Many individuals in these pedigrees (Figure 2.1) are ascertained through relatives' word of mouth, and so it is entirely possible that digenic inheritance, penetrance, or even mitochondrial mutations may be obscuring the proper ascertainment of inheritance pattern and thus the search for causative hearing loss mutations. These possibilities should undoubtedly be investigated in future studies on the 25 remaining AD families.

The possible presence of larger genomic abnormalities at work is another limitation of this study, which did not search for any such possible occurrence. This study investigates genes through targeted gene sequencing. However, larger genomic abnormalities have been shown to cause hearing loss phenotypes (Lisenka et al. 2003; Shaffer et al. 2006). Genomic abnormalities, therefore, may account for hearing loss in some of the Newfoundland families under investigation. Large genomic rearrangements, deletions, inversions, etc. can cause and affect the degree and severity of diseases, and such large-scale anomalies are not detected through traditional DNA sequencing methods

(Lisenka et al. 2003; Idbaih et al. 2010). The possible presence of such abnormalities should be investigated in future studies.

The presence of phenocopies in Family 2071 is also a relevant pit-fall in this study of AD hearing loss families. While phenocopies are common, they are also often difficult to prove beyond any doubt. A routine aspect of the clinical ascertainment is a request of patients and family members to fill out a detailed hearing loss questionnaire. This includes specific questions about noise exposures, head injuries, usage of drugs known to be ototoxic, etc. In this study, the cause of hearing loss in Family 2071's putative phenocopies is unknown. These unanswered questions obscure the full picture of hearing loss in Family 2071. The continued investigation of this family is, therefore, essential to gain a more complete understanding of hearing loss in this family and to confirm beyond any doubt that several individuals within Family 2094 are phenocopies.

Lastly, the approach taken in this study is also a limitation unto itself. This study was a "targeted" candidate gene approach which focused only on genes previously associated with hearing loss. While this improved the likelihood of detecting hearing loss mutations, it restricted the chance of identifying potential "genetic surprises" regarding genotype-phenotype. And while several exons within *KCNQ4, COCH, TECTA,* and *MYO1A* not previously associated with hearing loss were bi-directionally sequenced, this approach was limited by the two year time-frame of a master's thesis.

# Chapter 5: Summary

The aim of this thesis was to determine the genetic etiology of AD hearing loss in 28 large, multi-generational Newfoundland families. Probands were first screened for hearing loss alleles previously reported in the Newfoundland population: specifically the full *GJB2* gene, the del13S1830 mutation in *GJB6GJB2, GJB6,* and the p.A716T in *WFS1.* The next step was a systematic functional candidate gene search for genes and mutations from the primary literature and the NCBI database. Genotype-phenotype evaluation of potential candidate genes, and frequency of mutations found previously, helped to narrow the list down to four likely functional candidate genes: *COCH, KCNQ4, TECTA,* and *MYO1A.* These genes all code for proteins that play an important role in human hearing, and harbor hearing loss mutations recurrent in Caucasian populations. Once a mutation was identified, we were then able to highlight common patterns among the phenotypes of Newfoundland probands and the phenotypes of known mutations in AD deafness causing genes. Figure 2.2 illustrates the progression of this study from the stage of experimental design to the discovery of novel and known causative mutations.

In Newfoundland Family 2094 a known mutation (p.P51P/S) within *COCH* was discovered to be the cause of hearing loss. The p.P51P/S mutation causes a late-onset progressive high-frequency hearing loss, and is associated with severe vestibular defects, such as vertigo and motion sickness. Using DNA from a Dutch p.P51P/S family, and Family 2094, an ancestral haplotype was created through successful fragment analysis, confirming Family 2094's p.P51P/S transition to be a Dutch founder mutation. However,

no other Newfoundland families were explained by this mutation. No further genealogy was done to elaborate on this shared ancestry.

In Newfoundland Family 2071, a novel 3 bp deletion in exon 5 of *KCNQ4* has been found to be the cause of hearing loss. This discovery also provides further evidence of the current genotype-phenotype correlation, whereby deletions in *KCNQ4* cause a milder, later onset, high-frequency loss in patients compared to *KCNQ4* missense mutations (Nie, 2008). This mutation was not detected in any additional Newfoundland probands.

In Newfoundland Family 2102, the cause of hearing loss was identified to be a nonsense mutation in exon 4 of *MYO1A* (p.R93X). All affected individuals are reported to have suffered from hearing loss from the age of five, with hearing coming and going but progressively deteriorating. Again, this mutation was not reported in any additional Newfoundland hearing loss families. No hearing loss causing mutations were discovered within the *TECTA* gene, but sequencing of this gene should be completed in the future to rule it out completely as a candidate gene for hearing loss.

In the introduction, a table was presented indicating all known deafness related genes and their mutations within the Newfoundland population. An updated version of this table is seen in Table 5.1. Of the 28 Newfoundland families suffering from AD hearing loss at the beginning of this study, three families have now been solved. Further research on the remaining 25 unsolved Newfoundland families is of paramount importance. Genomic DNA from four of these 24 families has recently been sent for a

genome wide scan (GWS). The data gained from this GWS will inevitably enable further successful identification of the genetic cause of hearing loss in these families. AudioGene Audioprofiling also presents a promising avenue of detecting further mutations within the remaining 25 probands, and should be investigated. The determination of these hearing loss causing mutations must remain of critical importance to researchers and clinicians alike. With a greater understanding of the genetic mutations causing various families' hearing loss, comes a greater understanding of the pathogenic mechanisms and a greater chance of improved treatment options and screening abilities.

**Table 5.1 Deafness Genes Identified in Newfoundland Population at End of This Study**

| Gene | Mutation | # of NL Families | Literary Reference |
|---|---|---|---|
| *KCNQ4* | c.806_808delCCT | 1 | Young et al. Unpublished |
| *COCH* | c.151C>CT | 1 | De Kok et al. 1999 |
| *MYO1A* | c.2435C>CT | 1 | Donaudy et al. 2003 |
| *GJB2* | c.35delG | 7 | Denoyelle et al. 1997 |
| *GJB6* | D13S1830 | 3 | del Castillo et al. 2002 |
| *TMPRSS3* | c.207delC | 1 | Ahmed et al. 2004 |
| *TMPRSS3* | c.782+3delGAG | 2 | Ahmed et al. 2004 |
| *PCDH15* | c.1978T>A | 1 | Ahmed et al. 2003 |
| *WFS1* | c.2146G>A | 1 | Young et al. 2001; Bespolova et al. 2001; Sivakumaran & Lesperance, 2002 |

# Literature Cited


Ahmed ZM, Riazuddin S, Riazuddin S, Wilcox ER. 2003. The molecular genetics of Usher syndrome. Clinical Genetics 63:431-444.

Ahmed ZM, Xiaoyan CL, Powell SD, Riazuddin S, Young TL, Ramzan K, Ahmad Z, Luscombe S, Dhillon K, MacLaren L, Ploplis B, Shotland LI, Ives E, Riazuddin S, Friedman TB, Morell RJ, Wilcox ER. 2004. Characterization of a new full length TEMPRSS3 isoform and identification of mutant alleles responsible for nonsyndromic recessive deafness in Newfoundland and Pakistan. BMC Medical Genetics5:24.

Asher JH, Friedman TB. 1990. Mouse and hamster mutants as models for Waardenburg syndromes in humans. J Med Genet 27:618-626.

Bates DE. 2003. Aminoglycoside ototoxicity. Drugs Today (Barc) 39:277–85.

Bear JC, Nemec TF, Kennedy JC, Marshall WH, Power AA, Kolonel VM, Burke GB. 1987. Persistent Genetic Isolation in Outport Newfoundland. Am. J. Hum. Genet. 27:807-830.

Bennett D. 2002. On the trail of French Ancestors. St.John's NL:Robinson Blackmore.

Bespalova IN, Van Camp G, Bom SJH, Brown DJ, Cryns K, DeWan AT, Erson AE, Flothmann K, Kunst HPM, Kurnool P, Sivakumaran TA, Cremers C, Leal SM, Burmeister M, Lesperance MM. 2001. Mutations in the Wolfram syndrome 1 gene (*WFS1)* are a common cause of low frequency sensorineural hearing loss. Human Molecular Genetics 10(22): 2501-2508.

Beisel KW, Nelson NC, Delimont DC, Fritzsch B. 2001. Longitudinal gradients of KCNQ4 expression in spiral ganglion and cochlear hair cells correlate with progressive hearing loss in DFNA2. Brain Res. Mol. Brain Res. **82**(1-2): 137–49

Bischoff A, Huygen P, Kemperman M, Pennings RJE, Bom SJH, Verhagen WIM, Admiraal RJC, Kremer H, Cremers CRJW. 2005. Vestibular Deterioration Precedes Hearing Deterioration in the P51S *COCH* Mutation (DFNA9): An Analysis in 74 Mutation Carriers. Otology & Neurotology 26:918-925.

Bischoff A, Pauw R, Huygen P, Aandekerk A, Kremer H, Cremers C, Cruysberg J. 2007. Vertical Corneal Striae in Families with Autosomal Dominant Hearing Loss: DFNA/COCH. American Journal of Ophthalmology 143:847-852.

Bitner-Glinzicz M. Hereditary deafness and phenotyping in humans. 2002. British Medical Bulletin 63:73-94.

Brown SDM, Hardisty-Hughes RE, Mburu P. 2008. Quiet as a mouse: dissecting the molecular and genetic basis of hearing. Nature Genetics 9:277-290.

Cambron N. 2006. Speech Recognition Ability in Cochlear Implant Users 65 and Older. Seminar Hearing 27(4):345-347.

Carey JP, Migliaccio A, Minor LB. 2007. Semicircular Canal Function Before and After Surgery for Superior Canal Dehiscence. Otology & Neurotology 28:356-364.

Casano RA, Johnson DF, Bykhovskaya Y, Torricelli F, Bigozzi M, Fischel-Ghodsian N. 1999. Inherited susceptibility to aminoglycoside ototoxicity: genetic heterogeneity and clinical implications. Am J Otolaryngol. 20: 151–6.

Chen A, Wayne S, Bell A, Ramesh A, Srisailapathy CR, Scott DA, Sheffield VC, Van Hauwe P, Zbar RI, Ashley J, Lovett M, Van Camp G, Smith RJ. 1997. New gene for autosomal recessive non-syndromic hearing loss maps to either chromosome 3q or 19p. Am. J. Med. Genetics 5;71(4):467-471.

Coucke PJ, Van Hauwe P, Kelley PM, Kunst H, Schatteman I, Van Velzen D, Meyers J, Ensink RJ, Verstreken M, Declau F, Marres H, Kastury K, Bhasin S, McGuirt WT, Smith RJH, Cremers CWRJ, Van de Heyning P, Willems PJ, Smith SD, Van Camp G. 1999. Mutations in the *KCNQ4* gene are responsible for autosomal dominant deafness in four DFNA2 families. Human Molecular Genetics 8(7): 1321-1328.

Cremers CW, Marres HA, Van Rijn PM. 1991. Nonsyndromal profound genetic deafness in childhood. Ann N U Acad Sci. 630:191-196.

De Kok YJM, Bom S, Brunt T, Kemperman MH, Van Beusekom E, Van Der Velde-Visser SD, Robertson NG, Morton CC, Huygen PLM, Verhagen WIM, Brunner HG, Cremers CWRJ, Cremers PM. 1999. A Pro51Ser mutation in the *COCH* gene is associated with late onset autosomal dominant progressive sensorineural hearing loss with vestibular defects. Human Molecular Genetics 8(2):361-366.

Del Castillo I, Villamar M, Moreno-Pelayo MA, Del Castillo FJ, Alvarez A, Telleria D, Menendez I, Moreno F. 2002. A Deletion Involving the Connexin 30 Gene in Nonsyndromic Hearing Impairment. N. Engl. J. Med. 346(4):243-249.

Donaudy F, Ferrara A, Esposito L, Hertzano R, Ben-David O, Bell RE, Melchionda S, Zelante L, Avraham KB, Gasparini P. 2003. Multiple Mutations of *MYO1A*, a

Cochlear-Expressed Gene, in Sensorineural Hearing Loss. Am. J. Hum. Genet. 72:1571-1577.

Fitzgibbons PJ, Gordon-Salant S. 2010. Age-related differences in discrimination of temporal intervals in accented tone sequences. Hearing Research 264:41-47.

Fransen E, Verstreken M, Bom S, Lemaires F, Kemperman MH, De Kok YJM. 2001. A common ancestor for *COCH* related cochleovestibular (DFNA9) patients in Belgium and The Netherlands bearing the P51S mutation. J Med Genet 38:61-64.

Friedman T, Griffith A. 2003. Human Non-syndromic Sensorineural Deafness. Annu. Rev. Genomics of Human Genetics 4:341-402.

Frogatt NJ, Brassett C, Koch DJ, Evans DG, Hodgson SV, Ponder BA, Maher ER. 1996. Mutation screening of MSH2 and MLH1 mRNA in hereditary non-polyposis colon cancer syndrome. J. Med. Genet. 33(9):726-730.

Gates GA, Mills JH. 2005. Presbyacusis. Lancet 366:1111-1120.

Gorlin RJ, Toriello HV, Cohen M. Hereditary Hearing Loss and its Syndromes. 1995. Oxford University Press NY.

Gratton MA, Vazquez AE. 2003. Age-related hearing loss: current research. Curr. Opin. Otolaryngol. Head Neck Surg. **11:**367–371.

Grifa A, Wagner CA, D'Ambrosio L, Melchionda S, Bernardi F, Lopez-Bigas N, Rabionet R, Arbones M, Monica MD, Estivill X, Zelante L, Lang F, Gasparini P. 1999. Mutations in GJB6 cause nonsyndromic autosomal dominant deafness at DFNA3 locus.

Griffith AJ, Friedman TB. 2002. Autosomal and X-Linked Auditory Disorders. In Keats BJ, Popper AN, Fay RR (Eds.), Genetics and Auditory Disorders. New York Inc: Springer-Verlag 121-228.

Hancock WG. 1989. Soe longe as there comes noe women: Origins of the English settlement in Newfoundland. St.John's NL: Breakwater.

Hildebrand MS, Tack D, DeLuca A, Ae Hur I, Van Rybroek JM, McMordie SJ, Muilenburg A, Hoskinson DP, Van Camp G, Pensak ML, Storper IS, Huygen PLM, Casavant TL, Smith RJH. 2009. Mutation in the *COCH* Gene is Associated With Superior Semicircular Canal Dehiscence. Am. J. Med. Genet. 149A:280-285.

Hildebrand MS, Tack D, McMordie SJ, DeLuca A, Ae Hur I, Nishimura C, Huygen P, Casavant TL, Smith RJH. 2008. Audioprofile-directed screening identifies novel mutations in *KCNQ4* causing hearing loss at the DFNA2 locus. Genet. Med. 10(11):797-804.

Hildebrandt F, Heeringa SF, Ruschendorf F, Attanasio M, Nurnberg G, Becker C, Seelow D, Huebner N, Chernin G, Vlangos CN, Zhou W, O'Toole JF, Hoskins BE, Wolf MTF, Hinkes BG, Chaib H, Ashraf S, Schoeb DS, Ovunc B, Allen SJ, Vega-Warner V, Wise E. 2009. A Systematic Approach to Mapping Recessive Disease Genes in Individuals from Outbred Populations. PLoS Genetics 5(1):1-10.

Hildebrand MS, Tack D, McMordie SJ, DeLuca A, Hur IA, Nishimura C, Huygen P, Casavant TL, Smith RJ. 2008. Audioprofile-directed screening identifies novel mutations in KCNQ4 causing hearing loss at the DFNA2 locus. Genetic Med. 10:797-804.

Hildebrand MS, DeLuca AP, Taylor KR, Hoskinson DP, Hur IA, Tack D, McMordie SJ, Huygen P, Casavant TL, Smith RJH. 2009. A Contemporary Review of AudioGene Audioprofiling: A Machine Based Candidate Gene Prediction Tool for Autosomal Dominant Nonsyndromic Hearing Loss. Laryngoscope 119:2211-2215.

Hilgert N, Smith R, Van Camp G. 2009. Forty-six genes causing nonsyndromic hearing impairment: Which ones should be analyzed in DNA diagnostics? Mutation Research 681:189-196.

Hilgert N, Smith R, Van Camp G. 2009. Function and expression pattern of nonsyndromic deafness genes. Curr. Mol. Med. 9(5):546-564.

Idbaih A, Dalmasso C, Kouwenhoven M, Jeuken J, Carpaentier C, Gorlia T, Kros JM, French P, Teepen J, Broet P, Delattre O, Mokhtari K, Sanson M, Delattre JY, Van der Bent M, Hoang-Xuan K. 2010. Genomic aberrations associated with outcome in anaplastic oligodendral tumors treated within the EORTC phase III trial 26951. J. Neurooncology DOI 10.1007/s11060-010-0380-9.

Jentsch TJ. 2000. Neuronal KCNQ potassium channels: physiology and role in disease. Nature Reviews Neuroscience 1:21-30.

Kamada F, Kure S, Kudo T, Suzuki Y, Oshima T, Ichinohe A, Kojima K, Niihori T, Kanno J, Narumi Y, Narisawa A, Kati K, Aoki Y, Ikeda K, Kobayashi T, Matsubara Y. 2006. A novel *KCNQ4* one-base deletion in a large pedigree with hearing loss: implication for the genotype-phenotype correlation. J Hum Genet 51:455-460.

Keats BJ, Berlin C. 2002. Introduction and Overview: Genetics in Auditory Science and Clinical Audiology. In Keats BJ, Popper AN and Fay RR (Eds.), Genetics and Auditory Disorders (pp. 1-22). New York, Inc: Springer-Verlag.

Kemperman MH, De Leenheer EM, Huygen PL, Van Duijnhoven G, Morton CC, Robertson NG, Cremers FP, Kremer H, Cremers CW. 2005. Audiometric, vestibular,and genetic aspects of a DFNA9 family with a G88E COCH mutation. Otol. Neurotol. 26:926–933.

Kodera K, Adachi T, Sidara J, Kachi K. 1994. The effects of hearing aid frequency responses on speech discrimination score. Nippon Jibiinkoka Gakkai Kaiho 97(9):1669-1674.

Kommareddi P, Nair TS, Raphael Y, Telian SA, Kim AH, Arts HA, El-Kashlan H, Carey TE. 2007. Cochlin Isoforms and Their Interaction with CTL2 (SLC44A2) in the Inner Ear. Journal of the Association for Research in Otolaryngology 8:435-446.

Kubisch C, Schroeder BC, Friedrich T, Lutjohann B, El-Amraouni A, Marlin S, Petit C, Jentsch TJ. 1999. KCNQ4, a novel potassium channel expressed in sensory outer hair cells, is mutated in dominant deafness. Cell 96:437–446.

Leon PE, Bonilla JA, Sanchez JR, Vanegas R, Villalobos M, Torres L, Leon F, Howell AL, Rodriguez JA. 1981. Low Frequency Hereditary Deafness in Man with Childhood Onset. Am. J. Hum. Genet. 33:209-214.

Leon PE, Raventos H, Lynch E, Morrow J, King MC. 1992. The gene for an inherited form of deafness maps to chromosome 5q31. Proc. Natl. Acad. Sci. 89:5181-5184.

Leung J, Wang NW, Yeagle JD, Chinnici J, Bowditch S, Francis HW, Niparko JK. 2005. Predictive Models for Cochlear Implantation in Elderly Candidates. Arch. Otol. Head Neck Surg. 131:1049-1054.

Lisenka E, Vissers LM, Vries B, Osoegawa K, Janssen IM, Feuth T, Choy CO, Straatman H, Van der Vliet W, Huys E, Van Rijk A, Smeets D, Van Ravenswaaij-Arts CMA, Knoers NV, Van der Burgt I, Jong PJ, Brunner HG, Van Kessel AG, Schoenmakers EF, Veltman JA. 2003. Array-Based Comparative Genomic Hybridization for the Genomewide Detection of Submicroscopic Chromosomal Abnormalities. Am. J. Hum. Genet. 73:1261-1270.

Liu XZ, Yuan Y, Yan D, Ding EH, Ouyang XM, Fei Y, Tang W, Yuan H, Chang Q, Du LL, Zhang X, Wang G, Ahmad S, Kang DY, Lin X, Dai P. 2009. Digenic inheritance of non-syndromic deafness caused by mutations at gap junction proteins Cx26 and Cx31. Human Genetics 125:53-62.

Lynch ED, Lee MK, Morrow JE, Welcsh PL, Leon PE, King MC. 1997. Nonsyndromic Deafness DFNA1 Associated with Mutation of a Human Homolog of the Drosophila Gene diaphanous. Science 278:1315-1318.

Mazzoli M, Van Camp G, Newton V, Giarbini N, Declau F, Parving A. 2003. Recommendations for the Description of Genetic and Audiological Data for Families with Nonsyndromic Hereditary Hearing Impairment. Audiological Medicine 1:148-150.

McAlister PV. 1990. The effects of hearing aids on speech discrimination in noise by normal-hearing listeners. Journal of Rehabilitation Research 27(1):33-42.

Meyer NC, Nishimura CJ, McMordie S, Smith RJH. Audioprofiling identifies *TECTA* and *GJB2*-related deafness segregating in a single extended pedigree. 2007. Clin. Genetics 72:130-137.

Morell RJ, Kim HJ, Hood LJ, Goforth L, Friderici K, Fisher R, Van Camp G, Berlin C, Oddoux C, Ostrer H, Keats B, Friedman TB. 1998. Mutations in the Connexin 26 Gene (GJB2) Among Ashkenazi Jews with Nonsyndromic Recessive Deafness. The New Eng. Jour. Med. 339(21):1500-1505.

Morton CC, Nance WE. 2006. Newborn Hearing Screening – A Silent Revolution. N. Engl. J. Med. 354:2151-2164.

Nebel A, Filon D, Faerman M, Soodyall H, Oppenheim A. 2005. Y chromosome evidence for a founder effect in Ashkenazi Jews. European Journal of Human Genetics 13:388-391.

Neuhausen SL. 2000. Founder populations and their uses for breast cancer genetics. Breast Cancer Research 2:77-81.

Nie L. 2008. *KCNQ4* mutations associated with nonsyndromic progressive sensorineural hearing loss. Curr Opin Otolaryngol Head Neck Surg 16:441-444.

Parfrey PS, Davidson WS, Green JS. 2009. Clinical and genetic epidemiology of inherited renal disease in Newfoundland. Kidney Int. 61:1925-1934.

Park HJ, Cho HJ, Baek JI, Yosef TB, Kwon TJ, Griffith AJ, Kim UK. 2010. Evidence for a founder mutation causing DFNA5 hearing loss in East Asians. Journal of Human Genetics 55:59-62.

Poole CR, Cuff RH. 1994. Settlement. Encyclopedia of Newfoundland & Labrador. St. John's Newfoundland Boulder Publications Ltd.:Portugal Cove, Newfoundland 133-142.

Rehman AU, Morell RJ, Belyantseva IA, Khan SY, Boger ET, Shahzad M, Ahmed ZM, Riazuddin S, Khan SN, Riazuddin S, Friedman TB. 2010. Targeted Capture and Next-Generation Sequencing Identifies C9orf75, Encoding Taperin, as the Mutated Gene in Nonsyndromic Deafness DFNB79. The American Journal of Human Genetics 86:378-388.

Saihan Z, Webster A, Luxon L, Bitner-Glindzicz M. 2009. Update on Usher Syndrome. Current Opinion in Neurology 22(1): 19-27.

Schaffer LG, Kashork CD, Saleki R, Rorem E, Sundin K, Ballif BC, Bejjani BA. 2006. Targeted genomic microarray analysis for identification of chromosome abnormalities in 1500 consecutive clinical cases. Journal of Pediatrics 149(1):98-102.

Schuknecht HF, Gacek MR. 1993. Cochlear pathology in presbyacusis. Ann. Otol. Rhinol. Laryngol. 102(1-2):1-16.

Schuster SC. 2008. Next-generation sequencing transforms today's biology. Nature Methods 5(1):16-18.

Shahin H, Walsh T, Rayyan AA, Lee MK, Higgins J, Dickel D, Lewis K, Thompson J, Baker C, Nord AS, Stray S, Gurwitz D, Avraham KB, King MC, Kanaan M. 2010. Five novel loci for inherited hearing loss mapped by SNP-based homozygosity profiles in Palestinian families. European Journal of Human Genetics 18:407-413.

Smith R, Van Camp G. 2007. Deafness and Hereditary Hearing Loss Overview. Gene Reviews University of Washington, Seattle.

St. John JC, Facucho-Oliveira J, Jiang Y, Kelly R, Salah R. 2010. Mitochondrial DNA transmission, replication, and inheritance: a journey from the gamete through the embryo and into offspring and embryonic stem cells. Hum. Reprod. 16(5):488-509.

Strachan T, Read A. 2003. Human Molecular Genetics. Garland Science/Taylor & Francis Group; 3rd edition London, New York.

Stuckless S, Parfrey P, Woods MO, Cox J, Fitzgerald WG, Green JS, Green RC. 2006. The phenotypic expression of three *MSH2* mutations in large Newfoundland families with Lynch syndrome. Familial Cancer 6:1-12.

Talebizadeh Z, Kelley PM, Askew JW, Beisel KW, Smith SD. 1999. Novel Mutation in the KCNQ4 Gene in a Large Kindred With Dominant Progressive Hearing Loss. Human Mutation 14:493-501.

Tassabehji M, Read AP, Newton VE, Harris R, Balling R, Gruss P, Strachan T. 1992. Waardenburg's syndrome patients have mutations in the human homologue of the *Pax-3* paired box gene. Nature 355:635-636.

Topsakal V, Pennings RJ, te Brinke Hamel B, Huygen PLM, Kremer H, Cremers CWRJ. 2005. Phenotype determination guides swift genotyping of a DFNA2/KCNQ4 family with a hot spot mutation (W276S). Otology & Neurotology 26:52-58.

Toriello HV, Reardon R, Gorlin RJ. 2004. Hereditary Hearing Loss and its Syndromes. Oxford University Press Inc., Oxford.

Tranebjærg L. 2008. Wolframin 1-related Disease and Hearing. In: Kõks S and Vasar E (eds.). Wfs1 protein (wolframin): emerging link between the emotional brain and endocrine pancreas. Kerala, India, Research Signpost.

Van Camp G, Smith RJH. Hereditary Hearing Loss Homepage. May 2010. URL: http://hereditaryhearingloss.org. Last updated May 10, 2010.

Van Camp G, Smith RJH. Nonsyndromic hearing impairment: unparalleled heterogeneity. 1997. Am. J. Hum. Genet. 60:758-764.

Van Eyken E, Van Laer L, Fransen E, Topsakal V, Lemkens N, Laureys W, Nelissen N, Vandevelde A, Wienker T, Van De Heyning P, Van Camp G. 2006. KCNQ4: A Gene for Age-Related Hearing Impairment? Human Mutation 27(10):1007-1016.

Van Graefe A. 1858. Exceptionelles Verhalten des Gesichtsfeldes bei Pigmententartung der Netzhaut. Arch. Ophthamol. 4:250-253.

Van Hauwe P, Coucke PJ, Ensink RJ, Huygen P, Cremers C, Van Camp G. 2000. Mutations in the *KCNQ4* K+ Channel Gene, Responsible for Autosomal Dominant Hearing Loss, Cluster in the Channel Pore Region. Am. J. Med. Genet. 93:184-187.

Verhagen W, Bom S, Huygen M, Fransen E, Van Camp G, Cremers CWRJ. 2000. Familial Progressive Vestibulocochlear Dysfunction Caused by a *COCH* Mutation (DFNA9). Arch Neurol. 57:1045-1047.

Verhoeven K, Van Laer L, Kirschofer K, Legan PK, Hughes DC, Schatteman I, Verstreken M, Van Hauwe P, Coucke P, Chen A, Smith RJH, Somers T,

Offeciers E, Van de Heyning, Richardson GP, Wachtler F, Kimberling WJ, Willems PJ, Govaerts PJ, Van Camp G. 1998. Mutations in the human a-tectorin gene cause autosomal dominant non-syndromic hearing impairment. Nature Genetics 19:60-62.

Wayne S, Robertson NG, DeClau F, Chen N, Verhoeven K, Prasad S, Tranebjarg L, Morton CC, Ryan AF, Van Camp G, Smith RJH. 2001. Mutations in the transcriptional activator *EYA4* cause late-onset deafness at the DFNA10 locus. Hum. Molec. Genet. 10(3):195-200.

Webb MP, Dicks EL, Green JS, Moore SJ, Warden GM, Gamberg JS, Davidson WS, Young TL, Parfrey PS. 2009. Autosomal recessive Bardet-Beidl syndrome: first-degree relatives have no predisposition to metabolic and renal disorders. Kidney Int. 76:215-223.

Weil D, Blanchard S, Kaplan J, Guilford P, Gibson F, Walsh J, Mburu P, Varela A, Leviliers J, Weston MD, Kelley PM, Kimberling WJ, Wagenaar M, Levi-Acobas F, Larget-Piet D, Munnich A, Steel KP, Brown SDM, Petit C. 1995. Defectice myosin VIIA gene responsible for Usher syndrome type 1B. Nature 374:60-61.

Willems P. 2001. Genetic Causes of Hearing Loss. Mechanisms of Disease 342(15):1101-1109.

World Health Organization. 2002. Active ageing: a policy framework. A contribution of the World Health Organization to the Second United Nations World Assembly on Ageing. Madrid http://whqlibdoc.who.int/hq/2002/WHO_NMH_NPH_02.8.pdf.

Wu T, Marcus DC. 2002. Age-Related Changes in Cochlear Endolymphatic Potassium and Potential in CD-1 and CBA-CaJ Mice. Journal of the Association for Research in Otolaryngology 04:353-362.

Yang T, Gurrola JG, Wu H, Chiu SM, Wangemann P, Snyder PM, Smith RJH. 2009. Mutations in KCNJ10 Together with Mutations of SLC26A4 Cause Digenic Nonsyndromic Hearing Loss Associated with Enlarged Vestibular Aqueduct Syndrome. American Journal of Human Genetics 84:651-657.

Young TL, Ives E, Lynch E, Person R, Snook S, MacLaren L, Cator T, Griffin A, Fernandez B, Lee MK, King MC. 2001. Non-syndromic progressive hearing loss *DFNA*38 is caused by heterozygous missense mutation in the Wolfram syndrome gene *WFS1*. Human Molecular Genetics 10(22): 2509-2514.

Yueh B, Shekelle P. 2007. Quality Indicators for the Care of Hearing Loss in Vulnerable Elders. Journal of the American Geriatrics Society 55(S2):35-39.

**Appendix A:** PCR Primer Sequences and Expected PCR Product Sizes.

| Gene | Amplified Exon | Primer Name | Primer Sequence | Size of Fragment (bp) |
|---|---|---|---|---|
| KCNQ4 | 1A | 4700-1AF<br>4700-1AR | agttggagtcggaaagagca<br>CGCAAACTCACATGAAGACG | 567 |
| KCNQ4 | 1B | 4700-1BF<br>4700-1BR | AGCCATGCGTCTCTGAGC<br>ctgggagatcagggcttagg | 584 |
| KCNQ4 | 2 | 4700-2R<br>4700-2F | ccagggaattccaattctga<br>gaagcctctttccaccttca | 456 |
| KCNQ4 | 3 | 4700-3F<br>4700-3R | ggaatcgtcaagtccaggaa<br>agggtcagagtcgggattg | 358 |
| KCNQ4 | 4 | 4700-4F<br>4700-4R | tactcccaatcccgactctg<br>ttagacctcgcctcctgcta | 486 |
| KCNQ4 | 5 | 4700-5F<br>4700-5R | tgggaggagctgagaaagaa<br>tgagtcaggagtcacgatgg | 351 |
| KCNQ4 | 6+7 | 4700-6&7F<br>4700-6&7R | ccctcatgatcaggctccta<br>gtcagcacacagggttgaca | 554 |
| KCNQ4 | 8 | 4700-8F<br>4700-8R | ccacaactggaccaaggact<br>aaggacactccaggctctga | 356 |
| KCNQ4 | 9 | 4700-9F<br>4700-9R | tccaccctgtcctattctgg<br>aaggcaggtctgagagagga | 397 |
| KCNQ4 | 10 | 4700-10F<br>4700-10R | catccttgttccatcccaag<br>ccaaagacggtccatcagtt | 494 |
| KCNQ4 | 11 | 4700-11F<br>4700-11R | ctggtggtttggcatacaag<br>ggctggtctcaaactcctga | 287 |
| KCNQ4 | 12 | 4700-12F<br>4700-12R | tccatctcatccctgtttctg<br>ggcctcagacttcattcagg | 392 |
| KCNQ4 | 13 | 4700-13F<br>4700-13R | ggtgccttctccttcatcag<br>cgggtttatgggaatgtctg | 394 |
| KCNQ4 | 14A | 4700-14AF<br>4700-14AR | ctagccaagctccacctttc<br>GCCTCTGAGAAGTCCCTCAGT | 383 |
| KCNQ4 | 14B | 4700-14BF<br>4700-14BR | GACCTGCTGTTGGGCTTCTA<br>gctgctgctgccctctgt | 418 |
| TECTA | 5 | 5422-5F<br>5422-5R | accctgactcggctatgaaa<br>ccattacccagcggagagat | 480 |
| TECTA | 9A | 5422-9AF<br>5422-9AR | gggcagaccgtgtctttatc<br>ACTCCAGGAAGGAGCTGTTG | 497 |
| TECTA | 9B | 5422-9BF<br>5422-9BR | GCTTGTGCGGCTTCTACAAT<br>acctggaagggaagtcctga | 488 |
| TECTA | 10A | 5422-10AF<br>5422-10AR | gcactcacaaacacacatgc<br>AAGGTGAGGTAGTGGCCGTA | 496 |
| TECTA | 10B | 5422-10BF<br>5422-10BR | CTTCTGGGTGGACCTGGACT<br>tttctttggattccggacct | 499 |

**Appendix A:** PCR Primer Sequences and Expected PCR Product Sizes (cont).

| Gene | Amplified Exon | Primer Name | Primer Sequence | Size of Fragment (bp) |
|---|---|---|---|---|
| TECTA | 11A | 5422-11AF<br>5422-11AR | ctgctcaaacttccctctgg<br>AAGGCAGCGTTCTGGTTG | 499 |
| TECTA | 11B | 5422-11BF<br>5422-11BR | ACCCTGATGATGACCTGGAG<br>tcagttccaaagtgcatatcct | 497 |
| TECTA | 12 | 5422-12BF<br>5422-12BR | tgcctttcatctccctgagt<br>cgaacacgacgctcttcata | 415 |
| TECTA | 13A | 5422-13AF<br>5422-13AR | catttgagttgaggccgttt<br>AGTAGACGGGCGAAATGATG | 494 |
| TECTA | 13B | 5422-13BF<br>5422-13BR | CGTCGCAACGTGATTCAG<br>acctggtcactgtgtgtgga | 470 |
| TECTA | 14 | 5422-14F<br>5422-14R | cagaatggagtcgttgagacag<br>aggcattcctcattcacacc | 499 |
| TECTA | 17 | 5422-17F<br>5422-17R | atgcccaggttactgctttg<br>gcagatcaccctgaagttgg | 493 |
| TECTA | 18 | 5422-18F<br>5422-18R | gccatttctccactttcagg<br>tagggcatcaaagacaaacg | 354 |
| TECTA | 20 | 5422-20F<br>5422-20R | gcatttctgccatttatggtg<br>gatgattccagtccggtcac | 381 |
| MYO1A | 3 | 5379-3F<br>5379-3R | gcctctggctgtggatatgt<br>acgcaggttcatccactctc | 399 |
| MYO1A | 4 | 5379-4F<br>5379-4R | gcccagtctgctccaagtag<br>tggagggtcaggtctaggtc | 297 |
| MYO1A | 6+7 | 5379-6&7F<br>5379-6&7R | tgagccctagaccctcttcc<br>gttgggaagtctccttgacg | 500 |
| MYO1A | 10 | 5379-10F<br>5379-10R | atgaatccattagggcaagg<br>aggcagaaagcagaaatcaaa | 495 |
| MYO1A | 11+12 | 5379-11&12F<br>5379-11&12R | caccagtgtctcaggcagtt<br>tcatcctccctactctgctca | 490 |
| MYO1A | 18 | 5379-18F<br>5379-18R | gcaccgtgtgcagcatag<br>ttcacccagcttcagcagat | 395 |
| MYO1A | 22 | 5379-22F<br>5379-22R | actcaggtctttgcgtggtt<br>gcagactgaggaaactcttgg | 281 |
| MYO1A | 25 | 5379-25F<br>5379-25R | ggtgctgatgtcttggtcct<br>caaacacagcctgccatct | 374 |
| COCH | 2+3 | 4086-2&3F<br>4086-2&3R | tctgtgtcctctctcctctgc<br>atgggagaaacaggtgagca | 499 |
| COCH | 4 | 4086-4F<br>4086-4R | ctggaatggtatggaagggta<br>tatccaggagaaccgtgaaa | 463 |
| COCH | 5 | 4086-5F<br>4086-5R | agcgagacgccatcaaataa<br>ccatcaaggttaaagaggctga | 395 |

**Appendix A:** PCR Primer Sequences and Expected PCR Product Sizes (cont).

| Gene | Amplified Exon | Primer Name | Primer Sequence | Size of Fragment (bp) |
|---|---|---|---|---|
| COCH | 12A | 4086-12AF<br>4086-12AR | tttgccactctcgtcacaat<br>TTTGCCTAAATGGCTGTTGA | 492 |
| COCH | 12B | 4086-12BF<br>4086-12BR | GATGTCATCAGAGGCATTTGT<br>CCTGAACCATGTTAAAGAGCTG | 488 |
| COCH | 12C | 4086-12CF<br>4086-12CR | CACTGCTGAGGCTTCATAATCA<br>CCTGAACCATGTTAAAGAGCTG | 243 |
| COCH | 12D | 4086-12DF<br>4086-12DR | TCTGGATATAGAAAGGAGACCTGT<br>cagattggtctttccacatga | 384 |

**Appendix B:** Mutations Previously Found Within the Four Selected Candidate Genes *KCNQ4, COCH, TECTA,* and *MYO1A*.

| Mutation | Protein Domain | Exon | Reference |
|---|---|---|---|
| *KCNQ4*-p.W276S | Pore Region | 5 | Coucke et al. 1999. |
| *KCNQ4*-p.L274H | Pore Region | 5 | Kubisch et al. 1999. |
| *KCNQ4*-p.L281S | Pore Region | 6 | Talebizadeh et al. 1999. |
| *KCNQ4*-p.G285S | Pore Region | 6 | Kubisch et al. 1999. |
| *KCNQ4*-p.G285C | Pore Region | 6 | Coucke et al. 1999. |
| *KCNQ4*-p.G321S | S6 Transmembrane | 7 | Coucke et al. 1999. |
| *KCNQ4*-p.G455H | Pore Region | N/A | Van Laer et al. 2006. |
| *KCNQ4*-p.Q71fsX138 | Transmembrane | 1 | Kamada et al. 2006. |
| *KCNQ4*-p.FS71 | N-Terminal Cytoplasmic | 1 | Coucke et al. 1999. |
| *COCH*-p.V104del | FCH | 5 | Nagy et al. 2004. |
| *COCH*-p.P51P/S | FCH | 4 | Fransen et al. 1999. |
| *COCH*-p.V66G | FCH | 4 | Robertson et al. 1998. |
| *COCH*-p.G87W | FCH | 5 | Collin et al. 2006. |
| *COCH*-p.G88E | FCH | 5 | Robertson et al. 1998. |
| *COCH*-p.I109N | FCH | 5 | Kamarinos et al. 2001. |
| *COCH*-p.W117R | FCH | 5 | Robertson et al. 1998. |
| *COCH*-p.A119T | FCH | 5 | Usami et al. 2003. |
| *COCH*-p.C542F | vWFA2 | 12 | Street et al. 2005. |
| *TECTA*-p.N864K | N/A | 9 | Hutchin et al. 2005. |
| *TECTA*-p.C1057S | Zona Adhesion | 10 | Balciuniene et al. 1999. |
| *TECTA*-p.C1352Y | N/A | 11 | Hutchin et al. 2005. |
| *TECTA*-p.C1509G | vWFD4 | 13 | Pfister et al. 2004. |
| *TECTA*-p.C1619S | Zona Adhesion | 14 | Alloisio et al. 1999. |
| *TECTA*-p.L1820F | Zona Pellucida | 17 | Verhoeven et al. 1998. |
| *TECTA*-p.G1824D | Zona Pellucida | 17 | Verhoeven et al. 1998. |
| *TECTA*-p.C1837G | Zona Pellucida | 17 | Moreno-Pelayo, 2001. |
| *TECTA*-p.Y1870C | Zona Pellucida | 18 | Verhoeven et al. 1998. |
| *TECTA*-p.R2021H | Zona Pellucida | 20 | Iwasaki et al. 2002. |
| *TECTA*-p.F119fsX131 | N/A | 5 | Hutchin et al. 2005. |
| *MYO1A*-p.R93X | Motor Domain | 3 | Donaudy et al, 2003 |
| *MYO1A*-p.V306M | N/A | 10 | Donaudy et al, 2003 |
| *MYO1A*-p.E385D | Motor Domain | 12 | Don+audy et al, 2003 |
| *MYO1A*-p.G662E | N/A | 18 | Donaudy et al, 2003 |
| *MYO1A*-p.G674D | N/A | 18 | Donaudy et al, 2003 |
| *MYO1A*-p.S797F | N/A | 22 | Donaudy et al, 2003 |

**Appendix B:** Mutations Previously Found Within the Four Selected Candidate Genes *KCNQ4, COCH, TECTA,* and *MYO1A* (cont).

| Mutation | Protein Domain | Exon | Reference |
|---|---|---|---|
| *MYO1A*-p.349-350insCTT | N/A | 4 | Donaudy et al, 2003 |

**Appendix C:** *COCH* Microsatellite Marker Primer Sequences and Expected PCR Product Sizes.

| Marker Name | Primer Name | Primer Sequence | Size of Fragment (bp) | 5' Modifications |
|---|---|---|---|---|
| **D14S262** | D14S262-F<br>D14S262-R | GCAGTGGACTGATGCTCC<br>CCATGAAACTGGTCCCG | 200 | 6FAM |
| **D14S975** | D14S975-F<br>D14S975-R | CATACACAGACACACGGAGA<br>TGCCAAATAATCAGTTTTGC | 174 | 6FAM |
| **D14S1021** | D14S1021-R<br>D14S1021-F | AGTCGTGTATCCTGGGCAT<br>GCGCTGGTGTGAATCTTTA | 266 | 6FAM |
| **D14S257** | D14S257-F<br>D14S257-R | CAGTGAGCCATGACTGTG<br>TTGGTAAAGTGGTAAAAGGC | 182 | 6FAM |
| **D14S1071** | D14S1071-F<br>D14S1071-R | AGTGATCCACCCACCTTC<br>GGCTCAACTACGTGTTGCT | 279 | 6FAM |
| **D14S1040** | D14S1040-F<br>D14S1040-R | GGCACTATGAAACCAATTTTAAC<br>GGCCTGCTGAATCAGA | 231 | PET |
| **D14S1034** | D14S1034-F<br>D14S1034-R | CGTAGATGCTCCAAATCCTAC<br>TAGACAAATCGCTGGTCACT | 176 | 6FAM |
| **D14S1060** | D14S1060-F<br>D14S1060-R | GTTAAAATGGGCCACAATAAAT<br>CTGTTATGTATCAGACCAACCC | 193-219 | 6FAM |
| **D14S70** | D14S70-F<br>D14S70-R | ATCAATTTGCTAGTTTGGCA<br>AGCTAATGACTTAGACACGTTGTAG | 214-224 | 6FAM |
| **D14S1014** | D14S1014-F<br>D14S1014-R | AGCTATTCAGGTCAAAAAGGTC<br>AATCCCTACCCTTGTGGTG | 236-246 | 6FAM |

**Appendix D:** Fragment Analysis of Microsatellite Markers Flanking the *COCH* gene.

| Sample | Marker | Allele 1 | Allele 2 |
|---|---|---|---|
| OP06-115 | D14S1021 | 262 | 262 |
| JC07-184 | D14S1021 | 262 | 262 |
| KM06-227 | D14S1021 | 262 | 262 |
| JS07-182 | D14S1021 | 262 | 274 |
| 19126 | D14S1021 | 268 | 268 |
| 19135 | D14S1021 | 262 | 268 |
| 19149 | D14S1021 | 262 | 268 |
| AP07-66 | D14S1021 | 262 | 274 |
| OP07-270 | D14S1021 | 262 | 274 |
| CP07-187 | D14S1021 | 262 | 268 |
| CC07-269 | D14S1021 | 262 | 268 |
| OP06-115 | D14S975 | 163 | 171 |
| JC07-184 | D14S975 | 163 | 171 |
| KM06-227 | D14S975 | 167 | 171 |
| JS07-182 | D14S975 | 171 | 171 |
| 19126 | D14S975 | 163 | 171 |
| 19135 | D14S975 | 167 | 171 |
| 19149 | D14S975 | 167 | 171 |
| AP07-66 | D14S975 | 163 | 171 |
| OP07-270 | D14S975 | 163 | 171 |
| CP07-187 | D14S975 | 163 | 171 |
| CC07-269 | D14S975 | 165 | 171 |
| OP06-115 | D14S1040 | 234 | 234 |
| JC07-184 | D14S1040 | 232 | 234 |
| KM06-227 | D14S1040 | 234 | 234 |
| JS07-182 | D14S1040 | 232 | 234 |
| 19126 | D14S1040 | 214 | 234 |
| 19135 | D14S1040 | 232 | 234 |
| 19149 | D14S1040 | 232 | 234 |
| AP07-66 | D14S1040 | 232 | 234 |
| OP07-270 | D14S1040 | 232 | 234 |
| CP07-187 | D14S1040 | 232 | 234 |
| CC07-269 | D14S1040 | 232 | 234 |
| OP06-115 | D14S1071 | 277 | 281 |
| JC07-184 | D14S1071 | 275 | 281 |
| KM06-227 | D14S1071 | 281 | 281 |
| JS07-182 | D14S1071 | 277 | 285 |
| 19126 | D14S1071 | 277 | 281 |
| 19135 | D14S1071 | 275 | 281 |
| 19149 | D14S1071 | 275 | 281 |
| AP07-66 | D14S1071 | 285 | 281 |
| 19149 | D14S1060 | 201 | 207 |

**Appendix D:** Fragment Analysis of Microsatellite Markers Flanking the *COCH* Gene (cont).

| Sample | Marker | Allele 1 | Allele 2 |
|---|---|---|---|
| OP07-270 | D14S1071 | 285 | 281 |
| CP07-187 | D14S1071 | 275 | 281 |
| CC07-269 | D14S1071 | 275 | 281 |
| OP06-115 | D14S1034 | 169 | 173 |
| JC07-184 | D14S1034 | 169 | 177 |
| KM06-227 | D14S1034 | 169 | 173 |
| JS07-182 | D14S1034 | 173 | 175 |
| 19126 | D14S1034 | 169 | 177 |
| 19135 | D14S1034 | 169 | 175 |
| 19149 | D14S1034 | 169 | 175 |
| AP07-66 | D14S1034 | 169 | 175 |
| OP07-270 | D14S1034 | 169 | 175 |
| CP07-187 | D14S1034 | 169 | 169 |
| CC07-269 | D14S1034 | 169 | 179 |
| OP06-115 | D14S262 | 200 | 200 |
| JC07-184 | D14S262 | 200 | 200 |
| KM06-227 | D14S262 | 200 | 200 |
| JS07-182 | D14S262 | 200 | 202 |
| 19126 | D14S262 | 204 | 204 |
| 19135 | D14S262 | 200 | 204 |
| 19149 | D14S262 | 200 | 204 |
| AP07-66 | D14S262 | 200 | 202 |
| OP07-270 | D14S262 | 200 | 202 |
| CP07-187 | D14S262 | 200 | 198 |
| CC07-269 | D14S262 | 200 | 204 |
| OP06-115 | D14S257 | 179 | 177 |
| JC07-184 | D14S257 | 179 | 191 |
| KM06-227 | D14S257 | 179 | 179 |
| JS07-182 | D14S257 | 171 | 177 |
| 19126 | D14S257 | 179 | 179 |
| 19135 | D14S257 | 179 | 191 |
| 19149 | D14S257 | 179 | 191 |
| AP07-66 | D14S257 | 179 | 171 |
| OP07-270 | D14S257 | 179 | 171 |
| CP07-187 | D14S257 | 179 | 191 |
| CC07-269 | D14S257 | 179 | 191 |
| OP06-115 | D14S1060 | 201 | 207 |
| JC07-184 | D14S1060 | 201 | 191 |
| KM06-227 | D14S1060 | 201 | 191 |
| JS07-182 | D14S1060 | 201 | 197 |
| 19126 | D14S1060 | 201 | 205 |
| 19135 | D14S1060 | 201 | 207 |

**Appendix E:** Medical Hearing Loss Questionnaire

# Newfoundland and Labrador

# Hearing Loss Study

## Medical Information Questionnaire

The information we are asking you to provide in this questionnaire could help to find the cause of the hearing loss in your family. Please don't be discouraged from completing the questionnaire if you do not know the answers to all the questions - just fill in as much as you can. Any information you provide will be beneficial.
We are always available to answer questions and we can complete the questionnaire with you over the phone, if you prefer.

Adapted from:

THE HARVARD CENTRE
FOR HEREDITARY HEARING LOSS

**Appendix E:** Medical Hearing Loss Questionnaire (cont)

## SECTION 1 - GENERAL INFORMATION

1. Your Name_____ Date of Birth_____

   Address_____

   Home Phone_____ Work Phone_____

   E-mail Address (if you have one) _____

2. To your knowledge, are your parents related, even distantly? ☐ Yes ☐ No ☐ Don't Know

   (This may sound like a strange question, but in a genetic study, we ask it of everyone)

**Please answer the following questions as best you can.** If you have seen the doctor, please give us the name and address, if possible. If you think you have seen them but you are not sure of their names, or when you saw them, just indicate approximate date, for example, "*saw an audiologist 10 years ago in Grand Falls*". If you have not had an appointment with the medical person listed, tick no and move to the next question. Any information you can provide will be helpful.

3. Have you ever visited any of the following doctors?

   - An ENT Doctor? (Ear, Nose and Throat) ☐ Yes ☐ No ☐ D/K (Don't Know)

     If yes, where did you see them:_____

   - An Audiologist? (Person performing hearing tests) ☐ Yes ☐ No ☐ D/K

     If yes, where did you see them:_____

   - An Eye Doctor? (Ophthalmologist) ☐ Yes ☐ No ☐ D/K

     If yes, where did you see them:_____

   - A Genetics Doctor? (Geneticist) ☐ Yes ☐ No ☐ D/K

     If yes, where did you see them:_____

   - A doctor who treats diseases of the nervous system? (Neurologist) ☐ Yes ☐ No ☐ D/K

     If yes, where did you see them:_____

   - A Heart Doctor? (Cardiologist) ☐ Yes ☐ No ☐ D/K

     If yes, where did you see them:_____

4. Have you ever been admitted to hospital? If yes, please give name of hospital and approximate date(s) of admisison.

   _____

   _____

**Appendix E:** Medical Hearing Loss Questionnaire (cont)

## SECTION II – MEDICAL HISTORY

**Please answer as many of the following questions as you can about your hearing loss.** If you don't know the answer to the question, write don't know or d/k next to it and go to the next question. Any information you can provide will be helpful.

**A. Hearing History** - Please **circle** the term that best describes your hearing at the present time:

5. Left Ear: normal less than normal

   Right ear: normal less than normal

If hearing is less than normal, what is used to improve hearing?

6. Left Ear: nothing hearing aid cochlear implant other _____

   Right Ear: nothing hearing aid cochlear implant other _____

7. Were you born with hearing loss? ☐ Yes ☐ No

   If yes, tick affected ear ☐ Right ☐ Left

   If no, when did it start? ☐ During Childhood ☐ During Teen Years ☐ During Adulthood

8. Did your hearing loss begin during or soon after:

| | | | (Not Applicable) |
|---|---|---|---|
| - being pregnant | ☐ Yes | ☐ No | ☐ N/A |
| - an airplane flight | ☐ Yes | ☐ No | ☐ N/A |
| - scuba diving | ☐ Yes | ☐ No | ☐ N/A |
| - intravenous antibiotic treatment | ☐ Yes | ☐ No | ☐ N/A |
| - chemotherapy for cancer | ☐ Yes | ☐ No | ☐ N/A |
| - a severe infection, such as meningitis? | ☐ Yes | ☐ No | ☐ N/A |
| - exposure to a **sudden** loud noise | ☐ Yes | ☐ No | ☐ N/A |
| - **prolonged** exposure to loud noise | ☐ Yes | ☐ No | ☐ N/A |
| - an ear infection | ☐ Yes | ☐ No | ☐ N/A |
| - ear surgery (including insertion of T-tubes) | ☐ Yes | ☐ No | ☐ N/A |
| - injury to the head or the ear | ☐ Yes | ☐ No | ☐ N/A |

## **Appendix E:** Medical Hearing Loss Questionnaire (cont)

**Pattern of Hearing Loss.** Please tell us which ear has hearing loss by answering the following questions.

9. Your hearing is:

| | | | (Not Applicable) |
|---|---|---|---|
| - Stable (has not changed much over several years) | Right | Left | N/A |
| - Fluctuating (sometimes better, sometimes worse) | Right | Left | N/A |
| - Slowly progressing (getting worse over years) | Right | Left | N/A |
| - Rapidly progressing (getting worse over weeks/months) | Right | Left | N/A |
| - Sudden hearing loss | Right | Left | N/A |

**B. Patient's Medical History.** Have you ever had any of the following:

| 10. | | | (Don't Know) |
|---|---|---|---|
| - Scarlet fever | Yes | No | D/K |
| - Measles or German measles (circle which one) | Yes | No | D/K |
| - Mumps | Yes | No | D/K |
| - Meningitis (brain infection) | Yes | No | D/K |
| - Tuberculosis (TB) | Yes | No | D/K |
| - Repeated ear infections | Yes | No | D/K |
| - Premature graying of hair before age 30 (Not just at the temples) | Yes | No | D/K |
| - Kidney problems | Yes | No | D/K |
| - Diabetes mellitus ("sugar diabetes") | Yes | No | D/K |
| - Thyroid problems (goiter, under active, overactive) | Yes | No | D/K |
| - Depression or "nerves" | Yes | No | D/K |

11. Is there anything else which you think we should know about your medical history?

## **Appendix E:** Medical Hearing Loss Questionnaire (cont)

**Faculty of Medicine, Schools of Nursing and Pharmacy of Memorial University of Newfoundland; Health Care Corporation, St. John's; Newfoundland Cancer Treatment and Research Foundation**

### Consent to Take Part in Health Research

**TITLE**: The Genetics of Hereditary Deafness in Newfoundland

**INVESTIGATOR(S)**:

**SPONSOR**:

**You have been asked to take part in a research study. It is up to you to decide whether to be in the study or not. Before you decide, you need to understand what the study is for, what risks you might take and what benefits you might receive. This consent form explains the study.**

**The researchers will:**

- **discuss the study with you**
- **answer your questions**
- **keep confidential any information which could identify you personally**
- **be available during the study to deal with problems and answer questions**

**If you decide not to take part or to leave the study this will not affect your health care.**

**1. Introduction/Background:**

Some forms of deafness are hereditary. That means that they are inherited in families as a result of an altered gene. A gene is a piece of genetic material (DNA) which is passed from parents to children. If we can identify the genes that are altered in each family, it would allow us to better understand the process of hearing. We might also learn what goes wrong in some forms of deafness and how it might be treated.

**2. Purpose of study:**

Our goal is to identify the genes involved in hereditary deafness in families.

**3. Description of the study procedures and tests:**

If you agree to take part in this study, you will be asked to:

- Tell us about your hearing and the hearing of other members of your family, and other related aspects of your health.
- Have your hearing tested by a registered audiologist.
- Have a blood sample drawn for DNA testing.
- Complete a hearing loss questionnaire.

We might also want to review your medical records related to your deafness.

Initials: _____

## **Appendix E:** Medical Hearing Loss Questionnaire (cont)

4. **Length of time:**
   If you take part in this study, the interview will last 30-60 minutes including the blood sampling. The hearing test will take about 30 minutes. It will be arranged at a time that is convenient for you. The research may take us several years but you will not have to be involved again. We will keep you informed of our findings.

5. **Possible risks and discomforts:**
   The only discomfort is that of giving a blood sample.

6. **Benefits:**
   It is not known whether this study will benefit you.

7. **Liability statement:**
   **Signing this form gives us your consent to be in this study. It tells us that you understand the information about the research study. When you sign this form, you do not give up your legal rights. Researchers or agencies involved in this research study still have their legal and professional responsibilities.**

8. **Compensation:**
   In the event that you suffer injury as a direct result of taking part in this study, necessary medical treatment not covered by provincial health care insurance will be available at no additional cost to you.

9. **Confidentiality:**
   Unless required by law, only the researchers may have access to any confidential documents pertaining to your participation in this study that may identify you by name. Furthermore, your name will not appear in any report or article published as a result of this study.

10. **Genetic Studies:**
    In order to interpret the results of genetic research, we need to have correct information about parents. Sometimes the research shows new information about birth parents. This could happen in the case of an adoption or a mistake in the identity of a mother or father. This information will not be given to anyone including you or other family members.

11. **Future use of tissue/DNA samples.**

In order to preserve a valuable resource, your (tissue/DNA) samples may be stored at the end of this research project. It is possible that these samples may be useful in a future research project dealing with hereditary deafness which may or may not be related to the current research project. **Any future research would have to be approved by a Research Ethics Board (REB).**

Please tick **one** of the following options:

| | |
|---|---|
| | I agree that my (tissue/DNA) samples can be used for any approved research project but only if I am contacted again to give consent for the new project. |
| | I agree that my (tissue/DNA) sample can be used for any approved research project without contacting me again, but **only if my name* cannot be linked, in any way, to the sample.** |
| | Under no circumstances may my sample by used for future research. **My sample must be destroyed** at the end of this present project. |

*Includes name, MCP number or any other identifying information.

The DNA sample from this study will be stored in St. John's, Newfoundland and Seattle, Washington for an indefinite period of time.

**11. Contact Information:**

If you have any questions about taking part in this study, you can meet with the investigator who is in charge of the study at this institution. That person is:

Or you can talk to someone who is not involved with the study at all, but can advise you on your rights as a participant in a research study. This person can be reached through:

**Initials:** _____

## **Appendix E:** Medical Hearing Loss Questionnaire (cont)

**Signature Page**

**Study title:** **The Geneties of Hereditary Deafness in Newfoundland**

**Name of principal investigator:** **Dr. Terry-Lynn Young**

**To be filled out and signed by the participant:**

| | Please check as appropriate: |
|---|---|
| I have read the consent [and information sheet]. | Yes { } No { } |
| I have had the opportunity to ask questions/to discuss this study. | Yes { } No { } |
| I have received satisfactory answers to all of my questions. | Yes { } No { } |
| I have received enough information about the study. | Yes { } No { } |
| I have spoken to Dr. Young or her research assistant and she has answered my questions. | Yes { } No { } |
| I understand that I am free to withdraw from the study<br>• at any time<br>• without having to give a reason<br>• without affecting my future care | Yes { } No { } |
| I understand that it is my choice to be in the study and that I may not benefit. | Yes { } No { } |
| I agree that the study doctor or investigator may read the parts of my hospital records which are relevant to the study. | Yes { } No { } |
| I agree to take part in this study. | Yes { } No { } |

_____ _____

Signature of participant Date

_____ _____

Signature of witness Date

**To be signed by the investigator:**

I have explained this study to the best of my ability. I invited questions and gave answers. I believe that the participant fully understands what is involved in being in the study, any potential risks of the study and that he or she has freely chosen to be in the study.

_____ _____

Signature of investigator Date

Telephone number: _____

**Assent of minor participant (if appropriate):**

_____ _____

Signature of minor participant Date

_____ _____

Relationship to participant named above Age

Initials: _____