Published in final edited form as:

Laryngoscope. 2010 December ; 120(12): 2489–2493. doi:10.1002/lary.21159.

A Novel Mutation in *COCH* – Implications for Genotype-Phenotype Correlations in DFNA9 Hearing Loss

Michael S. Hildebrand, PhD^{1,*}, Luke Gandolfo, BSc^{2,*}, A. Eliot Shearer, BSc¹, Jennifer A. Webster, BA³, Maren Jensen, BSc⁴, William J. Kimberling, PhD^{4,5}, Dietrich Stephan, PhD^{3,6,7}, Patrick L.M. Huygen, PhD⁸, Richard J.H. Smith, MD^{1,9,*}, and Melanie Bahlo, PhD^{2,*}

¹Department of Otolaryngology - Head and Neck Surgery, University of Iowa, Iowa City, IA, USA

²Bioinformatics Division, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

³Neurogenomics Division, Translational Genomics Research Institute, Phoenix, AZ, USA

⁴Department of Genetics, Boys Town National Research Hospital, Omaha, NE, USA

⁵Department of Ophthalmology and Visual Sciences, University of Iowa Carver School of Medicine, Iowa City, IA, USA

⁶Arizona Alzheimer's Consortium, Phoenix, AZ, USA

⁷Banner Alzheimer's Institute, Phoenix, AZ, USA

⁸Department of Otorhinolaryngology, Radboud University Nijmegen Medical Centre, 6500 HB Nijmegen, The Netherlands

⁹Interdepartmental PhD Program in Genetics, Department of Otolaryngology, University of Iowa, Iowa City, IA, USA

Abstract

Objective/Hypothesis—To determine the cause of autosomal dominant hearing loss segregating in an American family.

Study Design—Family study.

Methods—Otologic and audiometric examination was performed on affected family members. Genome wide parametric multipoint linkage mapping using a dominant model was performed with Affymetrix 50K GeneChip data. Direct sequencing was used to confirm the causative mutation.

Results—In American family 467 segregating autosomal dominant nonsyndromic hearing loss (ADNSHL), a novel heterozygous missense mutation (c.362T>C; p.F121S) was identified in the *COCH* gene. This mutation was also associated with vestibular dysfunction typical of other DFNA9 families. However, affected family members also exhibited memory loss and night blindness.

Conclusions—The novel *COCH* mutation affects the functionally important LCCL domain where most DFNA9 mutations have been localized. The onset of the hearing loss, in the second or third decade of life, is earlier than in most DFNA9 families. The progression of hearing loss and vestibular dysfunction in the American family is typical of other DFNA9 families with mutations in this domain. Memory loss and night blindness have not been previously reported in DFNA9 families.

*Corresponding authors: Dr. Richard J.H. Smith, MD, Department of Otolaryngology – Head and Neck Surgery, University of Iowa, 5270 CBRB Building, Iowa City, IA 52242, USA, Tel: +319 335 6501, Fax: +613 353 5869, richard-smith@uiowa.edu, Dr. Melanie Bahlo, PhD, Bioinformatics Division, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia, bahlo@wehi.edu.au.

*These authors contributed equally to this work

No researchers involved in this study report a conflict of interest.

Keywords

DFNA9; *COCH* gene; hearing loss; vestibular dysfunction; missense mutation

INTRODUCTION

It is estimated that globally 4 out of every 10,000 children born have profound hearing loss ¹. Seventy percent of hearing loss cases are non-syndromic (NSHL) and of these ~80% are inherited in an autosomal recessive mode (ARNSHL) and ~20% in an autosomal dominant mode (ADNSHL). Hearing loss is a genetically heterogeneous disorder since 55 genes and 134 genetic loci have been implicated in ARNSHL and ADNSHL ².

Dominantly inherited hearing loss at the DFNA9 locus on chromosome 14q12 is also progressive and often accompanied by vestibular dysfunction. It is the third most common type of ADNSHL worldwide with 12 *COCH* mutations reported in 12 families ^{3,4}. Mutation of the *COCH* gene that encodes the cochlin protein is responsible for the hearing loss and vestibular impairment at this locus. The LCCL domain of cochlin is the most frequent site of DFNA9-causing mutation although several pathogenic variants have been reported in the second von Willebrand factor A (vWFA) domain ^{5,6}.

The function of the cochlin protein, the most highly expressed protein in the human and mouse inner ear, remains unclear. A role in structural integrity or antimicrobial activity has been predicted based on the protein structure ⁷. The structural role is supported by histopathological analyses showing abundant aggregation of homogeneous acellular eosinophilic deposits in human and mouse inner ear ⁸, and a recent study demonstrating cytotoxicity and misfolding of mutant cochlin protein *in vitro* and *in vivo* ⁹.

In this study we report genetic and phenotypic analysis of an American family segregating ADNSHL. A novel mutation and unique genotype-phenotype correlations are identified and described in this family. This report provides further evidence that mutations at the DFNA9 locus are a frequent cause of ADNSHL.

MATERIALS AND METHODS

Family

Family 467 is a four-generation American family segregating apparent ADNSHL (Fig.1A). Affected individuals appear in every generation consistent with dominant inheritance. Audiologic testing was completed to document the degree of hearing loss in all affected family members (Fig.1B). Vestibular function was assessed in some family members by electronystagmography (ENG) and caloric testing. A syndromic presentation was excluded through examination by an otolaryngologist and clinical geneticist.

All participating family members gave written informed consent. Ten milliliters of whole blood was obtained from each family member by venipuncture and genomic DNA was extracted as described previously ¹⁰. Human research institutional review boards at the Boystown National Research Hospital, Omaha, Nebraska, USA and the University of Iowa, Iowa City, Iowa, USA approved all procedures.

SNP Genotyping and Linkage Analysis

Genomic DNA from individuals III-1, III-2, III-6, IV:1, IV:2, IV:3, IV:4, IV:6, IV:7 and IV:8 (Fig.1A) was genotyped for 50,000 SNPs using Affymetrix 50K XBA GeneChips at the Translational Genomics Research Institute (TGEN, Phoenix, Arizona). Genotypes were

determined using the BRLMM genotyping algorithm^{13, 14}. A subset of 8,636 single nucleotide polymorphisms (SNPs) with high heterozygosity spaced approximately 0.3 cM apart across the genome were chosen from the 50K XBA set to satisfy the linkage equilibrium requirements of the Lander-Green algorithm for linkage analysis¹⁷. The selection and assembly of the data files were performed with linkdatagen (<http://bioinf.wehi.edu.au/software>)¹¹. Mendelian inheritance errors were identified and removed with linkdatagen, and MERLIN¹⁶ was used to remove genotyping errors identified based on inferred unlikely double recombination events between tightly linked markers.

An autosomal dominant, genome wide parametric linkage analysis was performed with MERLIN since males and females appeared equally affected. Haplotypes inferred with Merlin were imported into HaploPainter¹⁸.

PCR and Sequencing

The *COCH* gene was amplified using previously reported gene-specific primers³. Amplification reactions were cycled using a standard protocol on a GeneMate Genius thermocycler (ISC BioExpress, UT, USA). Sequencing was completed with BigDye™ v3.1 Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA), according to the manufacturer's instructions. Sequencing products were read using an ABI 3730s sequencer (Perkin Elmer, Waltham, MA). All sequencing chromatograms were compared to published cDNA sequence; nucleotide changes were detected using Sequencher v4.5 (Gene Code Corporation, Ann Arbor, MI).

PolyPhen Analysis

Non-synonymous sequence variants were analyzed using Polymorphism Phenotyping (PolyPhen; <http://genetics.bwh.harvard.edu/pph2/>) to predict the impact of the amino acid substitution on the structure and function of the protein.

RESULTS

Clinical Data

Nine members of American family 467 were diagnosed with sensorineural hearing loss (SNHL) by otological and audiometric analysis. The self-reported age-of-onset of hearing loss was in the second or third decade. The audioprofiles show a general trend of initial moderate-to-severe high frequency (> 2000 Hz) hearing loss that progresses to become profound (Fig.1B). Following the initial high frequency loss, low and mid frequency loss develops with advancing age, and the audioprofile flattens. The hearing loss is not reported to fluctuate. Numerous family members have been provided with amplification through the use of hearing aids. Cochlear implantation was not reported. Balance problems, vertigo, positional nystagmus, dizziness, tinnitus, night blindness and memory loss have been reported or diagnosed in affected family members. The vertiginous symptoms are in the form of frequent but intermittent attacks that have been treated with antivert, diuretics, hydrochlorothiazide, valium or dietary salt restriction with minimal success. Surgical implementation of an endolymphatic shunt has been considered but not performed in one family member. To our knowledge this is the first report of night blindness and memory loss in DFNA9 patients.

Linkage Mapping

Genome wide linkage analysis of 50K GeneChip data detected 315 Mendelian errors. The highest parametric LOD score for family 467 of 2.71 was achieved for a region on chromosome 14 (Fig.2A). The region spanned approximately 17 cM flanked by markers SNP_A-1710588 (rs1461549) and SNP_A-1710576 (rs9322932) at chromosomal position

14q12-q13.1 (24.8–33.8 Mb). Haplotype analysis revealed that the critical region coincided with the region identified in the linkage analysis (Fig.2B). The critical region overlaps the DFNA9 locus.

Mutation Analysis

The critical region in family 467 overlapped the known ADNSHL locus DFNA9. Direct sequencing of the 11 exons of the *COCH* gene revealed a novel heterozygous missense mutation (c.362T>C) that substitutes a serine residue for a phenylalanine (p.F121S) at the C-terminal end of the LCCL domain (Fig.3A). The F121 residue is highly conserved across species (Fig.3B) and PolyPhen analysis (<http://genetics.bwh.harvard.edu/pph/>) predicted that the substitution of a serine was ‘probably damaging’ with a highest possible score of 1.0. The c.362T>C mutation segregated with the affected members of family 467 (Fig.1A) and was not observed in 129 (258 chromosomes) CEPH (Centre d’Etude du Polymorphisme Humain) control individuals.

DISCUSSION

Of the thirteen DFNA9 mutations now reported in the *COCH* gene, eleven affect residues in the LCCL (Limulus factor C, Coch-5b2 and Lgl1) domain. The p.F121S mutation affects the last of 93 amino acids (28–121) that make up the LCCL domain, a unique fold comprising a centrally located α -helix wrapped by two β -sheets⁷. Structural and mutagenesis studies have suggested that the LCCL domain interacts with an unidentified binding partner that may link cochlin to other components of the extracellular matrix⁷.

Disruption of cochlear architecture and increased susceptibility to autoimmune disease have been proposed as mechanisms underlying the pathogenesis of DFNA9¹². The former possibility has received recent support from a study showing that mutation of the LCCL domain leads to misfolding of the cochlin protein and associated cytotoxicity *in vitro* and *in vivo*⁹. Four human DFNA9-causing mutations (P51S, V66G, G88E and W117R) were introduced into mouse cochlin and the mutant proteins were shown to form stable dimers *in vitro*. The presence of these stable mutant dimers was shown to stabilize wild-type cochlin and lead to its oligomerization, supporting involvement of a dominant negative mechanism in DFNA9. Further, this misfolding and increased stability was shown to induce cytotoxicity of fibrocytes in culture or in the mouse spiral ligament when mutant cochlin was introduced. This cytotoxicity was functionally significant as mice receiving direct cochlear injection of mutant cochlin had reduced auditory thresholds of ~40 dB up to 4 weeks following treatment⁹. It is possible that the p.F121S mutation identified in this study may also lead to cochlin misfolding. Similar experiments to those described above using a cochlin protein substituted for the highly conserved F121 residue may confirm this potential role.

The progression of hearing loss in American family 467 is consistent with that of other DFNA9 families segregating mutations in the LCCL domain of cochlin – high frequency hearing impairment progresses over several decades to become severe-to-profound across all frequencies. However onset in the second or third decade in this family is earlier than in most other DFNA9 families for whom hearing impairment begins in the fourth or fifth decade of life. An additional phenotypic difference is the reported night blindness in one family member and memory loss in multiple family members. It is unclear whether these other clinical features are related to the DFNA9 mutation. To explore this possibility it would be necessary to perform additional clinical tests on all affected family members, including dark adaptation, peripheral field testing, electroretinography and neurological testing.

Consistent with possible involvement of *COCH* in the ocular and neurological symptoms noted in this family is the expression pattern of cochlin mRNA in the relevant tissues. In their original identification and characterization of the human *COCH* gene, Robertson and colleagues showed high mRNA expression in fetal cochlea and low expression in fetal brain and eye by Northern blot ¹³. In a subsequent study the same group showed that *Coch* mRNA was more widely expressed in the adult mouse, with message detected by Northern blot at high level in cochlea and spleen, and lower levels in eye, lung, cerebellum/medulla, cerebrum and thymus tissue ¹⁴.

Recently, the expression of alternate *COCH* mRNA splice variants was characterized in the inner ear and other tissues of the rat ¹⁵. Three isoforms were characterized, two that encode full-length cochlin and one shorter variant that encodes only the LCCL domain and the N-terminal region of the first vWFA domain. This short variant was only expressed in inner ear while the full-length variants were detected in a variety of tissues including inner ear, eye, cerebrum, and cerebellum ¹⁵. Importantly, the LCCL domain is encoded by all three isoforms, indicating that the p.F121S mutation in family 467 would affect all *COCH* variants. Therefore differences in expression levels of *COCH* isoforms between tissues may explain the variability in DFNA9 phenotypes.

CONCLUSION

We have identified and characterized a new American DFNA9 family carrying a novel p.F121S mutation in *COCH*. Family 467 is the sixth American DFNA9 family to be identified, suggesting that *COCH* mutations are relatively common in the US ADNSHL population. Novel genotype-phenotype correlations were observed in this family, information requisite for prognosis and genetic counseling of patients. The results of our study suggest that mutation at the DFNA9 locus is a common cause of non-syndromic hearing loss and that screening of the *COCH* gene should be considered in the American ADNSHL population.

Acknowledgments

The authors sincerely thank the family for their participation in this study. RJH Smith is the Sterba Hearing Research Professor, University of Iowa College of Medicine, who supported the project with National Institutes of Health (NIH)-NIDCD grant R01 DC003544. M. Bahlo is supported by an Australian National Health and Medical Research Council (NHMRC) Career Development Award. M. Hildebrand is supported by an NHMRC Postdoctoral Training Fellowship. AE. Shearer is supported by a Doris Duke Clinical Research Fellowship.

Funding: NIH NIDCD grant R01 DC003544 (RJHS), Australian National Health and Medical Research Council (NHMRC) Career Development Award (MB), NHMRC Overseas Biomedical Postdoctoral Training Fellowship (MH) and a Doris Duke Clinical Research Fellowship (AS).

Abbreviations

<i>COCH</i>	cochlin gene
ADNSHL	autosomal dominant non-syndromic hearing loss
SNHL	sensorineural hearing loss
LCCL	Limulus factor C, Coch-5b2 and Lgl1 domain
vWFA	von Willebrand factor A domain

REFERENCES

1. Morton NE. Genetic epidemiology of hearing impairment. *Ann N Y Acad Sci.* 1991; 630:16–31. [PubMed: 1952587]
2. Van Camp, G.; Smith, RJ. Hereditary Hearing Loss Homepage. 2010. <http://hereditaryhearingloss.org/>
3. Hildebrand MS, Tack D, Deluca A, et al. Mutation in the COCH gene is associated with superior semicircular canal dehiscence. *Am J Med Genet A.* 2009; 149A:280–285. [PubMed: 19161137]
4. Robertson NG, Lu L, Heller S, et al. Mutations in a novel cochlear gene cause DFNA9, a human nonsyndromic deafness with vestibular dysfunction. *Nat Genet.* 1998; 20:299–303. [PubMed: 9806553]
5. Yuan HJ, Han DY, Sun Q, et al. Novel mutations in the vWFA2 domain of COCH in two Chinese DFNA9 families. *Clin Genet.* 2008; 73:391–394. Epub 2008 Feb 2027. [PubMed: 18312449]
6. Street VA, Kallman JC, Robertson NG, Kuo SF, Morton CC, Phillips JO. A novel DFNA9 mutation in the vWFA2 domain of COCH alters a conserved cysteine residue and intrachain disulfide bond formation resulting in progressive hearing loss and site-specific vestibular and central oculomotor dysfunction. *Am J Med Genet A.* 2005; 139A:86–95. [PubMed: 16261627]
7. Liepinsh E, Trexler M, Kaikkonen A, et al. NMR structure of the LCCL domain and implications for DFNA9 deafness disorder. *EMBO J.* 2001; 20:5347–5353. [PubMed: 11574466]
8. Robertson NG, Cremers CW, Huygen PL, et al. Cochlin immunostaining of inner ear pathologic deposits and proteomic analysis in DFNA9 deafness and vestibular dysfunction. *Hum Mol Genet.* 2006; 15:1071–1085. [PubMed: 16481359]
9. Yao J, Py BF, Zhu H, Bao J, Yuan J. The role of protein misfolding in DFNA9 hearing loss. 2010; 285(20):14909–14919.
10. Grimberg J, Nawoschik S, Belluscio L, McKee R, Turck A, Eisenberg A. A simple and efficient non-organic procedure for the isolation of genomic DNA from blood. *Nucleic Acids Res.* 1989; 17:8390. [PubMed: 2813076]
11. Bahlo M, Bromhead CJ. Generating linkage mapping files from Affymetrix SNP chip data. *Bioinformatics.* 2009; 25:1961–1962. [PubMed: 19435744]
12. Boulassel MR, Tomasi JP, Deggouj N, Gersdorff M. COCH5B2 is a target antigen of anti-inner ear antibodies in autoimmune inner ear diseases. *Otol Neurotol.* 2001; 22:614–618. [PubMed: 11568667]
13. Robertson NG, Khetarpal U, Gutierrez-Espeleta GA, Bieber FR, Morton CC. Isolation of novel and known genes from a human fetal cochlear cDNA library using subtractive hybridization and differential screening. *Genomics.* 1994; 23:42–50. [PubMed: 7829101]
14. Robertson NG, Skvorak AB, Yin Y, et al. Mapping and characterization of a novel cochlear gene in human and in mouse: a positional candidate gene for a deafness disorder, DFNA9. *Genomics.* 1997; 46:345–354. [PubMed: 9441737]
15. Sekine K, Ikezono T, Matsumura T, et al. Expression of cochlin mRNA splice variants in the inner ear. *Audiol Neurotol.* 2010; 15:88–96. Epub 2009 Aug 2004. [PubMed: 19657184]

A **B** **Figure 1.**

Pedigree and audiograms of American family 467.

A. Family 467; genotypes for individuals carrying the c.362T>C mutation are shown. Open symbols = unaffected; filled symbols = affected; diagonal line = deceased. B. Audiograms of affected members of family 467. Age at time of audiogram are shown in brackets.

Chromosome 14

Figure 2.

Linkage mapping results for family 467. A. Graph showing LOD score for the region on chromosome 14. LOD scores of 0 (blue line) and 3 (pink line) are indicated. B. Haplotype analysis results of family 467. Affymetrix SNP markers are shown.

Figure 3. Mutation analysis results for family 467. A. Novel *COCH* mutation in family 467. B. Multisequence alignment of the cochlin protein showing high conservation of the F121 residue across species.