LETTER TO JMG

A novel locus for autosomal dominant non-syndromic deafness, DFNA53, maps to chromosome 14q11.2-q12

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Background: Non-syndromic hearing loss is among the most genetically heterogeneous traits known in humans. To date, at least 50 loci for autosomal dominant non-syndromic sensorineural hearing loss (ADNSSL) have been identified by linkage analysis.

Objective: To report the mapping of a novel autosomal dominant deafness locus on the long arm of chromosome 14 at 14q11.2-q12, DFNA53, in a large multigenerational Chinese family with post-lingual, high frequency hearing loss that progresses to involve all frequencies.

Results: A maximum multipoint LOD score of 5.4 was obtained for marker D14S1280. The analysis of recombinant haplotypes mapped DFNA53 to a 9.6 cM region interval between markers D14S581 and D14S1021. Four deafness loci (DFNA9, DFNA23, DFNB5, and DFNB35) have previously been mapped to the long arm of chromosome 14. The critical region for DFNA53 contains the gene for DFNA9 but does not overlap with the regions for DFNB5, DFNA23, or DFNB35. Screening of the *COCH* gene (DFNA9), *BOCT*, *EFS*, and *HSPC156* within the DFNA53 interval did not identify the cause of deafness in this family.

Conclusions: Identifying the DFNA53 locus is the first step in isolating the gene responsible for hearing loss in this large multigeneration Chinese family.

Hearing loss is the most common sensory defect in humans. Approximately 1 in 1000 children is born with prelingual deafness, and it is estimated that in developed countries, 60% of these cases are genetic in origin.^{1,2} Post-lingual hearing loss is even more common and it affects 10% of the population by the age of 60, and 50% by the age of 80.^{1,3} Age related late onset hearing loss (presbycusis) is also a heterogeneous trait with many suspected causes.⁴ Genetic factors, including diabetes and mitochondrial mutations, and environmental factors, such as noise exposure, may contribute to the trait. In addition, monogenic forms of progressive post-lingual hearing impairment beginning in adolescence or adult life contribute to this important cause of morbidity. In these families, the hearing loss is usually non-syndromic, progressive, and may affect a particular range of frequencies. It is usually transmitted as an autosomal dominant trait. To date, at least 50 loci for autosomal dominant non-syndromic sensorineural hearing loss (ADNSSL) have been mapped and 20 genes coding for a wide variety of proteins have been identified (Hereditary hearing loss homepage: <http://dnalab-www.uia.ac.be/dnalab/hhh/>); some of these genes have a known function, but for the majority the underlying mechanisms leading to hearing loss are still elusive. Despite recent progress in identifying genes underlying deafness in humans, there are still relatively few mouse models of progressive hearing loss.⁵

Thus for late onset of deafness, the identification of genes through human mapping studies remains an important approach to clarifying the biology of hearing and deafness at a molecular level. In the present study, we describe the mapping of a novel DFNA locus (DFNA53) on the long arm of chromosome 14 at 14q11.2-q12, segregating in a large Chinese family with post-lingual and progressive hearing loss. The genetic interval of DFNA53 contains the gene for DFNA9 but does not overlap with the regions for DFNB5, DFNA23, or DFNB35.

METHODS

Family data

We investigated a six generation family from the northern part of China with autosomal dominant sensorineural hearing impairment. Informed consent was obtained from all study participants and from the parents of minors. The clinical history was taken and a physical examination carried out on the family members by one of the investigators, with special emphasis on identifying potential environmental causes of hearing loss such as infections, trauma, and exposure to ototoxic drugs including aminoglycoside and noise, or for evidence of syndromic forms of deafness.⁶

Otoscopic examination was done, and air conduction threshold measurements made at 250 Hz, 500 Hz, 1 kHz, 2 kHz, 4 kHz, 6 kHz, and 8 kHz. Bone conduction thresholds were determined to exclude a conductive component in patients with hearing loss. Oto-immittance measurements were obtained in all subjects. A history of motor development was obtained and Romberg testing done in all family members, and vestibular function was further evaluated by ice water calorics using Frenzel's glasses in selected patients.

Individuals from three generations were available for testing (fig 1). Blood samples were collected and DNA was extracted by standard techniques. Of the 48 individuals in whom DNA was obtained, 16 were classified as affected for the purposes of linkage analysis.

Genotyping and linkage analysis

A genome-wide screen was initially carried out by the Center for Inherited Disease Research (CIDR) with 351 microsatellite markers (Marshfield screening set 9) distributed with an average spacing of 10 cM intervals throughout the genome. Additional markers were chosen from the Marshfield maps (<http://research.marshfieldclinic.org/genetics/>) in the regions on chromosome 14 where markers yielded LOD scores greater than 1.5, and were typed in our laboratory by polymerase chain reaction (PCR) and polyacrylamide gel electrophoresis, using standard procedures.⁷ The genotyping data was screened for incompatibilities with PEDCHECK ver1.1.⁸ Two point linkage analysis was undertaken using the MLINK program from the LINKAGE package version 5.1⁹ under an

Abbreviations: ADNSSL, autosomal dominant non-syndromic sensorineural hearing loss

Figure 1 Pedigree and haplotype analysis of the Chinese family which segregates with the DFNA53 locus. Black symbols represent affected subjects. Haplotypes are represented by bars, with the haplotype associated with hearing loss in black.

autosomal dominant mode of inheritance with 90% penetrance in the heterozygote, setting the disease allele frequency to 0.001 and considering the marker allele frequencies to be equal. At-risk individuals without hearing loss who were under the age of 15 were considered to have an unknown phenotype with respect to the trait. Multipoint linkage analysis was carried out using the program SIMWALK v2.0.¹⁰

Candidate genes analysis

Four candidate genes—*COCH* (NM_004086), *BOCT* (AJ243653), *EFS* (NM_005864), and *HSPC156* (AF161505)—in the DFNA53 interval were screened for

mutation. Position, sequences of coding regions, and intron-exon junctions of the genes were determined using either the web-browser interface to the genomic sequence at UCSC (<http://genome.ucsc.edu/>) or Ensembl (<http://www.ensembl.org/>).

Direct sequencing was used for mutation analysis. PCR was carried out using genomic DNA obtained from affected and unaffected family members. Primers were designed to amplify each exon and adjacent intron-exon boundaries. The resulting PCR products were gel purified and sequenced using the ABI PRISM BigDye terminator cycle sequencing reaction kit. The automated sequencer (ABI 3100) was used for direct sequencing.

Table 1 Phenotypic evaluation of deaf individuals in generations III, IV, and V of the family

Subject	Age (years)	Sex	Degree of hearing loss*	Audiogram shape
III-23	67	M	Profound	Residual
III-25	63	M	Profound	Residual
III-32	59	M	Severe	Sloping
III-35†	40	F	Profound	Residual
IV-38	50	M	Moderate-severe	Sloping
IV-44†	40	F	Profound	Residual
IV-47	39	F	Moderate-severe	Sloping
IV-49†	33	M	Profound	Residual
IV-50	36	M	Moderate	Flat
IV-55	37	F	Moderate-severe	Sloping
IV-71†	20	F	Moderate	Flat
V-73†	27	F	Moderate	Sloping
V-82	14	F	Moderate	Sloping
V-85†	17	F	Mild	Sloping
V-91	14	M	Moderate	Sloping
V-92	21	M	Moderate	Sloping

*Audiograms were categorised using the classification of Liu and Xu.¹¹

†Caloric testing was done.

RESULTS

Clinical features

The clinical history and audiological finding of the hearing loss in family members clearly revealed an autosomal dominant form of post-lingual, bilateral sensorineural hearing loss. The reported onset of hearing problems in most cases was in the second decade of life with subsequent gradual progression from mild to profound hearing loss involving the high frequencies in the majority of patients. The earliest clinical evidence of hearing loss in the family was obtained from individuals V-82 and V-91 at the age of 14 years. Affected individuals had sloping, flat, or residual audiograms (table 1).

Hearing loss was variable, not only between generations but also within generations, demonstrating the substantial variation in phenotypic expressivity of DFNA53. There was no evidence for an effect of sex on progression, and six affected subjects of the family underwent caloric testing with normal results (table 1). Distortion product otoacoustic emission (DPOAE) testing in all the affected individuals, using the IL096 DP analyser (Otodynamics UK 1996), showed cochlear dysfunction.

Figure 2 Multipoint linkage analysis for markers on chromosome 14. The DFNA53 genetic interval overlaps with that of DFNA9 (D14S54–D14S597; markers not shown).

Linkage analysis

Genomic scanning at 10 cM intervals identified a region on the long arm of chromosome 14 that yielded a LOD (logarithm of odds) score >3.0 and exclusion of the remainder of the genome. We then tested additional markers spanning the region, and positive LOD scores were obtained for the markers D14S1041, D14S1280, D14S615, and D14S608, with a maximum two point LOD score of 3.8 at $\theta=0$ for marker D14S608 (table 2) and a maximum multipoint LOD score of 5.4 at D14S1280 (fig 2). Haplotypes were then constructed using affected subjects only to identify the recombination events. The position of the deafness locus, DFNA53, was delimited by analysis of recombinant haplotypes in subjects III:25 and V:92. This analysis placed the DFNA53 locus between the proximal marker D14S581 and the distal marker D14S1021, which defines a critical interval of 9.6 cM (figs 1 and 3).

Candidate genes analyses

The interval for DFNA53 overlaps the region containing DFNA9. We analysed the coding regions and exon–intron boundaries of the *COCH* gene for DFNA9 and did not find a disease causing mutation, apparently excluding it as a causative gene. Three other genes in the critical region were identified as candidate genes because of their presence in the Inner Ear Gene Expression Database (<http://www.mgh.harvard.edu/depts/coreylab/index.html>) and were screened for mutation (fig 3). These include *BOCT*, a brain-type organic cation transporter, also known as *SLC22A17* (solute carrier family 22, member 17). Given the cochlea's function as a neurosensory organ, and its need to maintain the unique ionic homeostasis of endolymph, it is not surprising that several genes encoding ion transporters have been associated with hearing impairment in mouse models or deafness in humans. We excluded *SLC22A17* as a DFNA53 causative gene, as no disease causing mutation was found by sequencing the coding sequences and exon–intron boundaries. We also screened another potential functional candidate, the *EFS* gene, which encodes a docking protein that plays a central coordinating role in signalling related to cell adhesion, but no deafness related sequence change was found. Finally, sequencing of the exons and flanking regions of *HSPC156* (*STXB6*), encoding a syntaxin binding protein, only revealed one intronic change in the gene, but no segregation between the development of deafness and the sequence change was found.

Table 2 Two point LOD scores between 14q microsatellite markers and DFNA53

Markers	Marshfield map* position	Physical map† position	Recombination fraction (θ)									
			0	0.001	0.01	0.05	0.1	0.15	0.2	0.3	0.4	
D14S581	21.51	22288622	-1.55	-1.55	1.43	-0.71	-0.34	-0.18	-0.11	-0.06	-0.04	
D14S1041	23.2	23472695	0.92	0.92	0.91	0.87	0.79	0.69	0.58	0.35	0.14	
D14S1280	25.87	24645913	1.36	1.36	1.33	1.18	1.01	0.83	0.66	0.35	0.12	
D14S 615	28.01	26506789	2.97	2.96	2.91	2.65	2.3	1.94	1.59	0.91	0.36	
D14S608	28.01	26839334	3.76	3.75	3.66	3.27	2.78	2.3	1.84	0.98	0.34	
D14S975	31.13	28669559	-0.18	-0.18	-0.18	-0.16	-0.14	-0.12	-0.11	-0.07	-0.03	

*Sex-average Kosambi cM map distance from the Marshfield genetic map.

†Sequence based physical map distance in bases according to Human Genome Project-Santa Cruz.
LOD, log of odds.

DISCUSSION

Here, we report identification of DFNA53, a locus for ADNSSHL on the long arm of chromosome 14 at 14q11.2-q12. Four other loci for hearing loss have previously been localised on the long arm of chromosome 14: DFNA9, DFNA23, DFNB5, and DFNB35. DFNA9 is the only DFNA type of hearing loss with concomitant vestibular impairment.¹²⁻¹³ Its characteristic histopathological features had been established in temporal bone studies¹⁴ long before it was found to map to the long arm of chromosome 14 at 14q12-q13,¹⁵ and *COCH* was subsequently identified as the gene causing the trait.¹⁶ Molecular analysis of cases of DFNA9 have identified several families worldwide with only six different mutations in the *COCH* gene.¹⁶⁻²¹ Interestingly, all the reported mutations causing the DFNA9 type deafness

disorder affect the LCCL domain of the protein. Most DFNA9 mutations affected conserved structural elements of the LCCL fold and disrupted the proper folding of this domain.²¹ In DFNA9 patients, onset of hearing loss appears to be in early adult life with significant loss in the fourth or fifth decade of life. High frequencies are involved initially, but with progression there is involvement of the lower frequencies. In addition to cochlear involvement, DFNA9 patients also show a spectrum of vestibular dysfunctions, ranging from lack of symptoms to the presence of vertigo. Affected individuals have mucopolysaccharide depositions in the channels of the cochlear and vestibular nerves. Accumulation of deposits in vestibular and cochlear nerve channels would lead to strangulation and progressive degeneration of the dendrites, and loss of cochlear and vestibular neurones.¹⁴

The critical region of the DFNA53 locus contains the gene for DFNA9. Despite some variability in the degree and extent of vestibular involvement, there is consensus on the concomitance of the sensorineural deafness and vestibular disorder in the DFNA9 families.¹⁶⁻²¹ Moreover, from a study in a Japanese population, it was concluded that mutations in the *COCH* gene are responsible for a significant fraction of patients with autosomal dominant inherited hearing loss accompanied by vestibular symptoms, but not for dominant hearing loss without vestibular dysfunction.²⁰ Although, there is some similarity in the audiograms of DFNA53 patients in comparison with those of patients with DFNA9, none of the affected members (14 to 67 years of age) in the DFNA53 family showed vestibular dysfunction. We have also analysed the coding regions and exon-intron boundaries of the *COCH* gene by direct sequencing in affected individuals with no detected mutation.

There are several types of mutation that the DNA sequencing alone would not be able to detect. These include any large deletions or duplications. The mutation could also be located in the promotor region, the 3' or 5' untranslated regions of the *COCH* gene, which we did not screen. However, so far, all the reported mutations causing DFNA9 type deafness occur in exons 4 and 5 of *COCH*, which encode the LCCL domain of the protein. Based on the clinical features of DFNA53 patients, mutation type in DFNA9, and our mutation screening data, we exclude *COCH* as the DFNA53 causative gene. DFNA23, identified in a Swiss German kindred, is another dominant locus on the long arm of chromosome 14 at 14q22-q22. The hearing impaired members presented with prelingual neurosensory and conductive hearing loss.²²

Two recessive loci for hearing loss have also been reported on the long arm of chromosome 14: DFNB5 mapped to the long arm of chromosome 14 at 14q12 in a consanguineous Indian kindred with severe to profound hearing impairment,²³ and DFNB35 was mapped to 14q24.1-14q24.3 in an inbred kindred from Pakistan.²⁴ The DFNA23, DFNB5, and

Figure 3 Schematic physical and genetic maps of the 14q12-14q13 chromosomal region showing the localisation of DFNA53 and DFNA9, DFNA23, DFNB5, and DFNB35 candidate intervals. The analysed genes are indicated. The marker order and intermarker distances are based on maps at UCSC (<http://genome.ucsc.edu/>). For clarity, the physical distances are not represented to scale. bp, base pairs.

DFNB35 loci are telomeric to the DFNA53 locus. Taken together, this points to the probable presence of a novel gene.

The human chromosome region containing DFNA53 shares conserved synteny with a segment of mouse chromosome 12, but no mutation causing hearing impairment in the mouse has been mapped to this region. This interval spans a physical distance of about 6.4 Mb, and includes ~60 known genes and a series of predicted or poorly characterised genes according to the annotation in the Human Genome Project–Santa Cruz database (www.Genome.ucsc.edu).

Typically, autosomal dominant hearing loss is distinguished by post-lingual onset of hearing loss, compared with the prelingual onset of deafness observed in autosomal recessive cases. DFNA53 is characterised by hearing loss affecting high frequencies that begins in the second decade of life and progresses to severe deafness involving all frequencies. This hearing phenotype is also observed in other previously identified ADNSSHL loci, including DFNA2, DFNA5, DFNA7, and DFNA9. High frequency hearing loss that progresses to involve all frequencies is characteristic of age related hearing loss, also known as presbycusis. Identifying the DFNA53 locus is the first step in isolating the gene responsible for hearing loss in this large multi-generation Chinese family. The ultimate cloning of the gene causing DFNA53, as well as other non-syndromic high frequency hearing loss genes associated with progressive hearing loss, will provide critical insights into an understanding of the molecular pathophysiology of late onset hearing loss.

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REFERENCES

- Morton NE. Genetic epidemiology of hearing impairment. *Ann NY Acad Sci* 1991;**630**:16–31.
- Marazita ML, Ploughman LM, Rawlings B, Remington E, Arnos KS. Genetic epidemiological studies of early-onset deafness in the US school-age population. *Am J Med Genet* 1993;**46**:486–91.
- Liu XZ, Xu LR, Hu Y, Nance WE, Sismanis A, Zhang SL, Xu Y. Epidemiological studies on hearing impairment with reference to genetic factors in Sichuan, China. *Ann Otol Rhinol Laryngol* 2001;**110**:356–63.
- Schuknecht HF, Gacek MR. Cochlear pathology in presbycusis. *Otol Rhinol Laryngol* 1993;**102**:1–16.
- Vreugde S, Erven A, Kros CJ, Marcotti W, Fuchs H, Kurima K, Wilcox ER, Friedman TB, Griffith AJ, Balling R, Hrabé De Angelis M, Avraham KB, Steel KP. Beethoven, a mouse model for dominant, progressive hearing loss DFNA36. *Nat Genet* 2002;**30**:257–8.
- Liu XZ, Li RX, Ying H, Nance WE, Sismanis A, Zhang SL, Ying X. Epidemiological studies on hearing impairment with reference to genetic factors in Sichuan, China. *Ann Otol Rhinol Laryngol* 2001;**110**:356–63.
- Liu XZ, Blanton SH, Bitner-Glindzicz M, Pandya A, Landan L, MacArdle B, Rajput K, Bellman S, Webb BT, Ping X, Smith RJ, Nance WE. Haplotype analysis of the USH1D locus and genotype-phenotype correlations. *Clin Genet* 2001;**60**:58–62.
- O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet* 1998;**63**:259–66.
- Lathrop GM, Lalouel JM, Juliet C, Ott J. Strategies for multilocus linkage analysis in humans. *Proc Nat Acad Sci USA* 1984;**81**:3443–6.
- Sobel E, Lange K. Descent graphs in pedigree analysis: applications to haplotyping, location scores, and marker-sharing statistics. *Am J Hum Genet* 1996;**58**:1323–37.
- Liu X, Xu L. Nonsyndromic hearing loss: an analysis of audiograms. *Ann Otol Rhinol Laryngol* 1994;**103**:428–33.
- Huygen PLM, Verhagen WIM. Peripheral vestibular and vestibulo-ocular dysfunction in hereditary disorders. A review of the literature and a report on some additional findings. *J Vestib Res* 1994;**4**:81–104.
- Bom SJH, Kunst HPM, Huygen PLM, Cremers FP, Cremers CW. Non-syndromal autosomal dominant hearing impairment: ongoing phenotypical characterization of genotypes. *Br J Audiol*. 1999;**33**: 33548).
- Khetarpal U, Schuknecht HF, Gacek RR, Holmes LB. Autosomal dominant sensorineural hearing loss. Pedigrees, audiologic findings, and temporal bone findings in two kindreds. *Arch Otolaryngol Head Neck Surg* 1991;**117**:1032–42.
- Manolis EN, Yandavi N, Nadol JB, Eavey RD, McKenna M, Rosenbaum S, Khetarpal U, Halpin C, Merchant SN, Duyk GM, MacRae C, Seidman CE, Seidman JG. A gene for non-syndromic autosomal dominant progressive postlingual sensorineural hearing loss maps to chromosome 14q12-13. *Hum Mol Genet* 1996;**5**:1047–50.
- Robertson NG, Lu L, Heller S, Merchant SN, Eavey RD, McKenna M, Nadol JB, Miyamoto R, Linthicum FH, Mubianca Neto JF, Hudspeth AJ, Seidman CE, Morton CC, Seidman JG. Mutations in a novel cochlear gene cause DFNA9, a human nonsyndromic deafness with vestibular dysfunction. *Nat Genet* 1998;**20**:299–303.
- de Kok YJM, Bom SJH, Brunt TM, Kemperman MH, van Beusekom E, van der Velde-Visser SD, Robertson NG, Morton CC, Huygen PLM, Verhagen WIM, Brunner HG, Cremers CWRJ, Cremers FPM. A Pro51 Ser mutation in the COCH gene is associated with late onset autosomal dominant progressive sensorineural hearing loss with vestibular defects. *Hum Mol Genet* 1999;**8**:361–6.
- Fransen E, Verstreken M, Verhagen WJ, Wuyts FL, Huygen PL, D'Haese P, Robertson NG, Morton CC, McGuire WT, Smith RJ, Declau F, Van de Heyning PH, Van Camp G. High prevalence of symptoms of Meniere's disease in three families with a mutation in the COCH gene. *Hum Mol Genet* 1999;**8**:1425–9.
- Kamarinos M, McGill J, Lynch M, Dahl H. Identification of a novel COCH mutation, I109N, highlights the similar clinical features observed in DFNA9 families. *Hum Mutat* 2001;**17**:351–6.
- Usami S, Takahashi K, Yuge I, Ohtsuka A, Namba A, Abe S, Fransen E, Pathy L, Otting G, Van Camp G. Mutations in the COCH gene are a frequent cause of autosomal dominant progressive cochleo-vestibular dysfunction, but not of Meniere's disease. *Eur J Hum Genet* 2003;**11**:744–8.
- Nagy I, Horvath M, Trexler M, Repassy G, Pathy L. A novel COCH mutation, V104del, impairs folding of the LCCL domain of cochlin and causes progressive hearing loss. *J Med Genet* 2004;**41**:e9.
- Salam AA, Hafner FM, Linder TE, Spillmann T, Schinzel AA, Leal SM. A novel locus (DFNA23) for prelingual autosomal dominant nonsyndromic hearing loss maps to 14q21-q22 in a Swiss German kindred. *Am J Hum Genet* 2000;**66**:1984–8.
- Fukushima K, Ramesh A, Srisailapathy CR, Ni L, Chen A, O'Neill M, Van Camp G, Coucke P, Smith SD, Kenyon JB. Consanguineous nuclear families used to identify a new locus for recessive non-syndromic hearing loss on 14q. *Hum Mol Genet* 1995;**4**:1643–8.
- Ansar M, Din MA, Arshad M, Sohail M, Faiyaz-Ul-Haque M, Haque S, Ahmad W, Leal SM. A novel autosomal recessive non-syndromic deafness locus (DFNB35) maps to 14q24.1-14q24.3 in large consanguineous kindred from Pakistan. *Eur J Hum Genet* 2003;**11**:77–80.