SHORT REPORT

A novel locus for autosomal dominant non-syndromic deafness (DFNA41) maps to chromosome 12q24-qter

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We have studied 36 subjects in a large multigenerational Chinese family that is segregating for an autosomal dominant adult onset form of progressive non-syndromic hearing loss. All affected subjects had bilateral sensorineural hearing loss involving all frequencies with some significant gender differences in initial presentation. After excluding linkage to known loci for non-syndromic deafness, we used the Center for Inherited Disease Research (CIDR) to test for 351 polymorphic markers distributed at approximately 10 cM intervals throughout the genome. Analysis of the resulting data provided evidence that the locus designated DFNA41 maps to a 15 cM region on chromosome 12q24.32-qter, proximal to the marker D12S1609. A maximum two point lod score of 6.56 at θ=0.0 was obtained for D12S343. This gene is distal to DFNA25, a previously identified locus for dominant adult onset hearing loss that maps to 12q21-24. Positional/functional candidate genes in this region include frizzled 10, epimorphin, *RAN*, and *ZFOC1*.

Adult onset hearing loss is a heterogeneous trait with many suspected causes including progressive loss of the sensory hair cells, loss of nerve cells in the cochlea and central nervous pathways, thickening and stiffening of the basilar membrane, and atrophy of the stria vascularis.[1] Genetic or environmental factors such as diabetes, mitochondrial mutations, or environmental noise exposure may contribute to the trait. Presbycusis is the commonest form of adult onset hearing loss. Although the incidence varies greatly among populations in many eastern countries, 25-30% of the population have some degree of hearing loss by 75 years of age and 40-50% among those older than 75.[2] In some families, adult onset hearing loss clearly segregates as a monogenic autosomal dominant Mendelian trait, and these cases typically exhibit an earlier age of onset, with presentation in the third or fourth decade of life, which suggests that lifelong maintenance of the cochlea is critical. At least 40 autosomal dominant loci have been mapped to date, 15 of which have been cloned (Hereditary hearing loss homepage: http:dnalab-www.uia.ac.be/dnalab/hhh). By identifying the genes in these families, a better understanding of the causes of presbycusis will emerge.

SUBJECTS AND METHODS

We ascertained a six generation family segregating for autosomal dominant adult onset hearing loss (fig 1). The family is from the Sichuan province of mainland China. Members of four generations were available for testing. Audiograms were obtained for diagnostic purposes and DNA was obtained in order to conduct a linkage study.

This Chinese kindred was ascertained through IV.15, a 46 year old male, in an epidemiological survey of deafness.[2] Informed consent was obtained from all study participants and from parents of patients younger than 18 years. A pedigree was constructed on the basis of interviews. The clinical history interview and physical examination of members of this family were performed 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.[2] Tests to detect the presence and type of hearing loss were conducted. Air conduction thresholds were measured at 250 Hz, 500 Hz, 1 kHz, 2 kHz, 4 kHz, 6 kHz, and 8 kHz. Bone conduction thresholds were determined to ascertain whether there was any evidence for a conductive component in patients with hearing loss. Oto-immittance measurements were obtained on all subjects and all were otoscopically examined. A motor development history interview and Romberg testing were carried out in all members for evaluation of vestibular function, and ice water calorics using Frenzel's glasses was undertaken in some of the patients. In addition, where possible, previous audiological tests were collected. Venous blood samples were collected from available family members. DNA was extracted from leucocytes according to standard methods.

A genome wide scan of 351 markers using Marshfield screening set 9 was initially conducted by the Center for Inherited Disease Research (CIDR) after the known dominant loci were excluded in this family (data not shown). Additional markers were chosen from the Marshfield maps (http://research.marshfieldclinic.org/genetics/) in the regions where markers yielded lod scores greater than 1.5 and were typed in our laboratory. All the high density genotyping was performed using radioactive P^{33}dCTP incorporation with resolution of the products on 6% denaturing polyacrylamide gels.[3] The genotyping data were screened for incompatibilities with PEDCHECK version 1.1.[4] Two point linkage analysis was then conducted for each marker using the MLINK program from the LINKAGE package version 5.1.[5] Multipoint analysis and haplotype generation were performed with SimWalk2 version 2.8[6] on data converted from a LINKAGE format using Mega2 version 2.2.[7] Penetrance in the heterozygote was set to 0.9. At risk subjects without hearing loss who were under the age of 10 were considered to have an unknown phenotype with respect to the trait. In addition, all subjects with atypical audiograms were considered unknown with regard to hearing status.

RESULTS

The clinical history and audiological findings of the hearing impaired family members were consistent with an autosomal dominant form of postlingual sensorineural hearing loss. Table 1 summarises the main clinical and audiological findings of all affected subjects. Six subjects, including three deaf (V.1, V.7, and V.16) and three normal hearing (IV.14, V.6, and V.10), had a history of exposure to aminoglycoside and

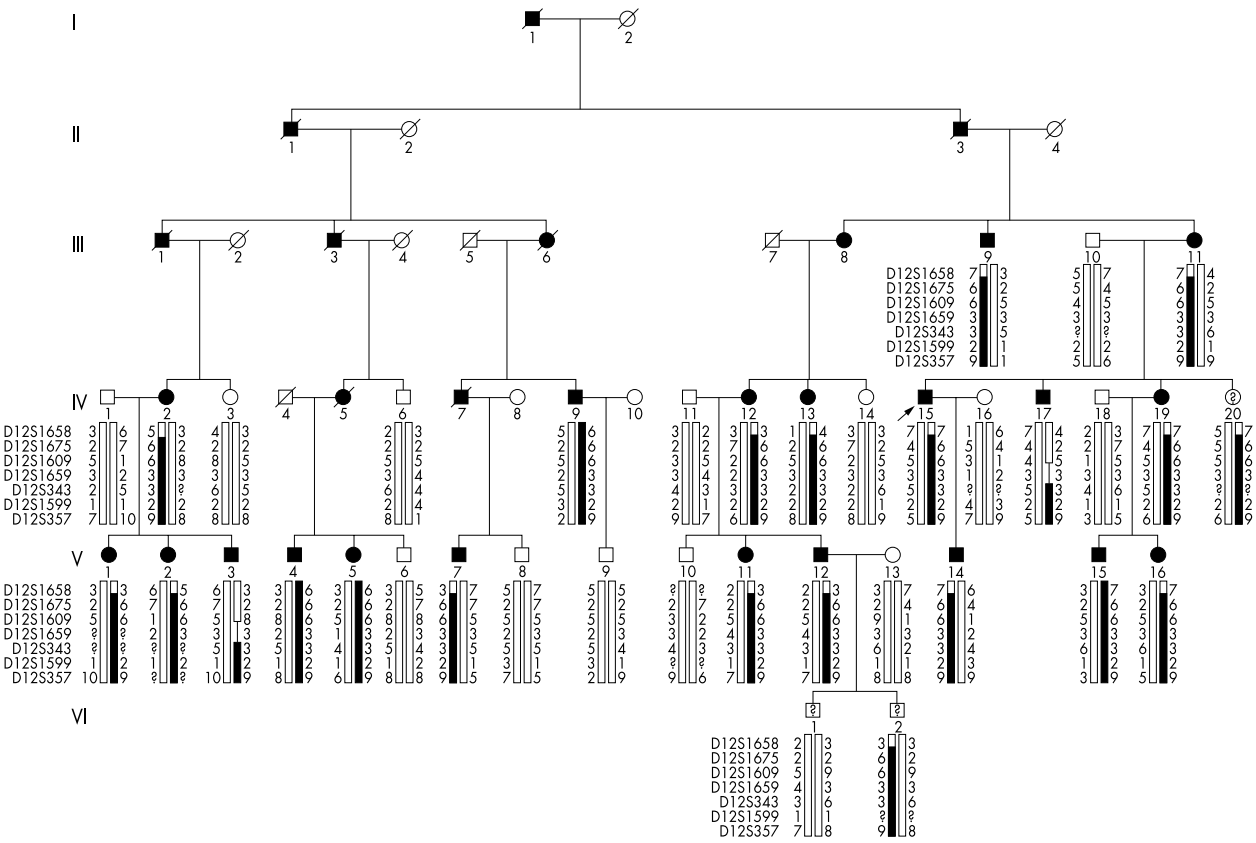

Figure 1 Pedigree of family showing segregation of the most informative markers on chromosome 12. The segregating haplotype is indicated by the black bar. A single black line indicates that a recombination has occurred but the exact position cannot be determined. Recombination between unaffected chromosomes is not depicted.

Table 1 Phenotypic evaluation of deaf subjects in the family

			Audiogram*		Exposure to	
Subjects	Age	Gender	Severity	Shape	Aminoglycoside	Noise
III.9	73	M	Severe	Flat	–	–
III.11	71	F	Severe	Flat	–	–
IV.2	54	F	Moderate	Flat	–	–
IV.9†	60	M	Severe	Flat	–	Yes
IV.12	63	F	Severe	Flat	–	–
IV.13†	56	F	Moderate	Flat	–	–
IV.15†	54	M	Severe	Flat	–	–
IV.17†	49	M	Severe	Flat	–	–
IV.19†	43	F	Severe	Ascending	–	–
V.1	30	F	Moderate	Ascending	Yes	–
V.2	18	F	Mild	Ascending	–	–
V.3†	14	M	Moderate	Flat	–	–
V.4	31	M	Severe	Sloping	–	–
V.5†	30	F	Severe	Ascending	–	–
V.7	28	M	Moderate	Sloping	Yes	–
V.11†	28	F	Moderate	Ascending	–	–
V.12†	33	M	Severe	Sloping	–	Yes
V.14	28	M	Moderate	Sloping	–	–
V.15†	18	M	Moderate	Sloping	–	–
V.16	14	F	Moderate	Ascending	Yes	–
VI.2	12	F	Moderate	Ascending	–	–

*Audiograms were categorised based on the classification of Liu and Xu.[8]
†Caloric testing was performed.

two deaf subjects (IV.9 and V.12) had a history of exposure to noise, but all denied any relationship with their hearing loss. Subjects with hearing thresholds ⩽20 dB in both ears across 500, 1000, 2000, 4000, and 6000 Hz were regarded as unaffected people. There was no obvious evidence for vestibular dysfunction or any other clinical or otological associated abnormalities. Ten deaf subjects underwent caloric testing with normal results (table 1). In most of the patients, the onset of hearing loss was in the second decade of life with a gradual progression from slight to severe loss over several decades (fig 2). In all affected subjects, audiometry showed a bilateral and symmetrical sensorineural hearing loss involving

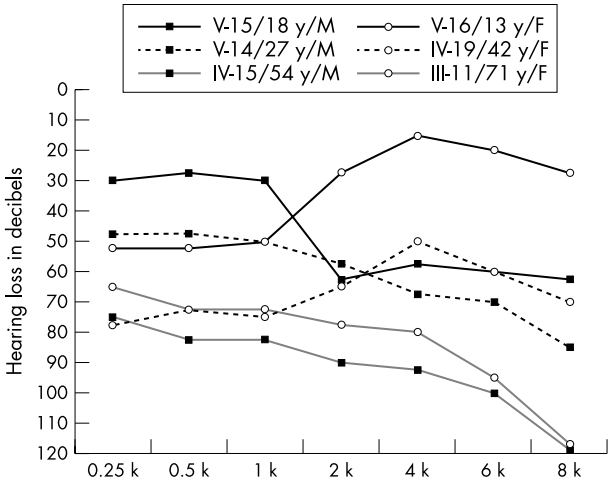

Figure 2 Results of averaging right and left ear audiograms for selected subjects to show the differences between males and females. Males are indicated by a square and females by a circle.

all frequencies. Interestingly, however, there appeared to be a gender difference in the pattern of hearing loss. Affected males initially showed a sloping audiogram (high frequencies worse than low frequencies), while young affected females typically had an ascending slope[8] (high frequencies better than low frequencies) (fig 2). These findings show that in affected males the hearing loss begins in the high frequencies, while in females the low and mid frequencies are affected initially (up to 2000 Hz). As the hearing loss progresses, the patterns of males and females tend to converge, as illustrated by the audiograms from IV.15, a 54 year old male, and III.11, a 71 year old female. One family member, IV.20, who was a 41 year old female, showed a significant audiometric asymmetry with a reported history of recurrent ear infections and was therefore classified as having an unknown phenotype for the linkage studies.

Markers on two chromosomes (1 and 12) yielded two point lod scores greater than 1.5 (data not shown). Analysis of six additional markers on chromosome 1 and 10 on chromosome 12 provided additional support for linkage only in the case of chromosome 12. Another dominant gene for deafness from a white pedigree (DFNA25) has been mapped to chromosome 12q.[9] The cytogenetic localisation was originally reported as 12q21-24; however, examination of mapping data from the Human Genome Mapping Project (http://us.ensembl.org) indicates that the flanking markers, D12S327 and D12S84, map to 12q22 and 12q23.3, respectively . Although we had previously excluded linkage in our family to two markers flanking DFNA25, we tested for linkage to additional markers in the region to exclude this locus further. DFNA25 maps to a 20 cM region on 12q21-24 between D12S327 and D12S84. In our family, a maximum two point lod score of 6.56 at $\theta=0.0$ was obtained for D12S343, which maps to 12q24.32 and is 46 cM telomeric to D12S84 (table 2). As can be seen from the two point lod scores, DFNA41 shows linkage to markers which are located over 30 cM below the proximal flanking marker for DFNA25. Unless the families differ by an inversion, DFNA25 is clearly excluded in our pedigree. A multipoint analysis of the markers clearly excludes the DFNA25 locus and places the DFNA41 locus below D12S1609, with a maximum lod score of 9.703 at D12S343 (fig 3). Inspection of haplotypes shows that all affected subjects share a common haplotype from D12S1659 and below. IV.17 has inherited a recombinant chromosome from his affected mother with a recombination between D12S1609 and D12S343. V.3 has also inherited a chromosome with a recombination between D12S1609 and D12S343. Clinical and audiological evaluation showed that both subjects had a moderate (V.3) or severe (IV.17) bilateral sensorineural hearing loss with a flat audiogram and had no history of exposure to aminoglycoside and noise (table 1). Notably, there is no recombination between DFNA41 and markers more distal in any subjects, so that a telomeric flanking marker cannot be identified to localise DFNA41 further. Currently, D12S357 is the most distal marker in the sequence database so that further typing is not possible. From inspection of her haplotype, it is clear that IV.20, who was originally thought to have an environmental form of deafness, did in fact inherit the affected haplotype from her mother. More importantly, VI.2, who is clinically normal at 6 years of age, appears to have inherited the affected haplotype from his affected father, while his 12 year old brother has inherited the unaffected haplotype.

DISCUSSION

Examination of the region between D12S1609 and 12qter in the NCBI databases identified approximately 90 potential

Table 2 Two point lod scores between the family and markers on chromosome 12

		Lod score							
Marker	cM	0.00	0.001	0.01	0.05	0.1	0.15	0.2	0.3
D12S327*	97.78	–5.36	–5.12	–4.01	–1.89	–0.80	–0.26	0.03	0.22
D12S1051*	101.98	–12.14	–10.91	–8.14	–4.81	–3.15	–2.19	–1.54	–0.72
D12S1300*	104.12	–11.28	–10.57	–7.62	–4.02	–2.52	–1.73	–1.23	–0.59
D12S1607*	107.86	–1.20	–0.97	–0.31	0.24	0.39	0.41	0.38	0.22
D12S1030*	109.47	–7.53	–6.48	–4.76	–2.76	–1.60	–0.95	–0.54	–0.11
D12S84*	117.81	–3.28	–2.61	–1.22	0.00	0.44	0.58	0.61	0.47
D12S1349	139.61	–5.19	–4.63	–3.23	–1.49	–0.39	0.15	0.44	0.61
D12S1612	140.17	–14.50	–11.30	–7.15	–3.15	–1.51	–0.69	–0.25	0.13
D12S1614	144.83	–7.95	–7.49	–5.54	–2.71	–1.34	–0.66	–0.28	0.03
D12S340	146.39	–7.26	–6.71	–4.99	–2.45	–1.24	–0.60	–0.23	0.10
D12S386	147.17	–15.69	–7.92	–3.03	0.11	1.13	1.49	1.57	1.33
D12S1658	148.24	–16.19	–10.89	–6.56	–2.81	–1.31	–0.57	–0.16	0.17
D12S1675	150.70	–8.39	–1.81	1.08	2.76	3/05	2.92	2.62	1.72
D12S1609	153.33	–3.57	1.14	3.05	4.02	4.01	3.71	3.26	2.14
D12S1659	155.94	2.53	2.53	2.49	2.32	2.09	1.83	1.55	0.97
D12S367	159.59	4.66	4.66	4.61	4.31	3.89	3.42	2.91	1.81
D12S97	160.68	2.95	2.94	2.89	2.67	2.32	2.09	1.78	1.15
D12S343	**163.55**	**6.56**	**6.56**	**6.49**	**6.14**	**5.61**	**5.01**	**4.35**	**2.89**
D12S1599	164.53	2.18	2.18	2.18	2.14	2.00	1.80	1.56	0.99
D12S1723	164.63	2.29	2.29	2.27	2.15	1.97	1.75	1.51	0.98
D12S357	168.79	5.98	5.97	5.87	5.44	4.87	4.28	3.66	2.37

*Indicates markers flanking the DFNA25 locus.

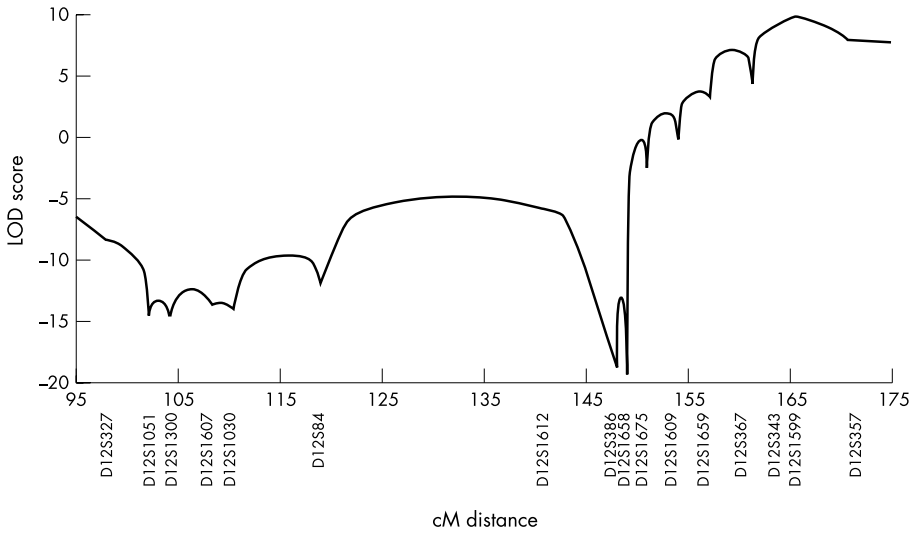

Figure 3 Multipoint map of markers on chromosome 12. The region spanning DFNA25 is excluded (D12S327-D12S84) and DFNA41 is clearly mapped to a region flanked by D12S1609 and the telomere of 12q.

transcripts, including the adenylate kinase 3 alpha-like gene, peptide/histidine transporter, frizzled 10, epimorphin, cerebellar degeneration related protein (34 kDa), *RAN*, unc-51 (*C elegans*)-like kinase 1, and a group of zinc finger proteins (26, 84, and 14). Many of the candidates were determined by the gene prediction program and, hence, the true number of candidate genes in the region is still unknown.

We searched an expression database developed from human fetal inner ear tissue to determine the pattern of some of the candidate genes (Z-Y Chen, unpublished data). Genes from this region expressed in the human inner ear include frizzled 10, epimorphin, *RAN*, and zinc finger family genes. This is the first time that frizzled 10 has been shown to be expressed in the inner ear. Frizzled genes have been shown to be involved in planar cell polarity signalling.[10] As the hair cells in the ear are polarised cells, it appears to be a good candidate gene. Epimorphin is an epithelial cell morphogen.[11] Its expression in the inner ear provides us with another candidate gene for DFNA41. *ZFOC1*, a novel zinc finger gene expressed in the inner ear, is another strong candidate for DFNA41. However, this gene currently maps to 12q14.3, placing it outside the area of interest.[12] All the genes listed here map to mouse chromosome 5, showing that the two regions between human and mouse are syntenic. Bronx waltzer (*bv*), a recessive deafness mutation, has been mapped to mouse chromosome 5.[13] There is no other obvious deaf mouse mutant mapping to the region.

Results of the linkage analysis and haplotype inspection clearly provides evidence that we have mapped a novel locus for autosomal dominant hearing loss (DFNA41) to the long arm of chromosome 12. The recombinants place our gene in a 15 cM region flanked by D12S1609 on 12q24.33 and the telomere of the q arm. Gender differences in the audiogram at initial diagnosis suggests the presence of a modifier for DFNA41. The gene causing DFNA41 may provide critical insights into an understanding of the molecular pathophysiology of late onset hearing loss.

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