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

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ABSTRACT

**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 (ADNSSHL) have been identified by linkage analysis.

**Methods:** Here we report the mapping of a novel autosomal dominant deafness locus on the long arm of chromosome 14 at 14q11.2-q12, DFNA53, by studying a large multi-generational Chinese family with post-lingual, high-frequency hearing loss that progresses to involve all frequencies.

**Results:** A maximum multi-point 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.

**Conclusions:** 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 for deafness in this family.

**Key words:** Autosomal dominant non-syndromic hearing loss; Linkage analysis; microsatellite; chromosome 14
INTRODUCTION

Hearing loss is the most common sensory defect in humans. Approximately 1 in 1000 children is born with pre-lingual 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 and is usually transmitted as an autosomal dominant trait. To date, at least 50 loci for autosomal dominant non-syndromic sensorineural hearing loss (ADNSSHL) 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 for progressive hearing loss.[5] Thus for late-onset of deafness, identification of genes through human mapping studies remains an important approach toward 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.

MATERIALS AND 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 and physical examination of family members was 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.[6] Otoscopic examination was performed, along with air conduction thresholds measurement 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. The history of motor development and Romberg testing were obtained on all family members and vestibular function was further evaluated by ice water caloric using Frenzel’s glasses in selected patients. Individuals from 3 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 purposes of linkage analysis.

**Genotyping and linkage analysis**
A genome wide screening was initially performed 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 performed using the MLINK program from the LINKAGE package ver5.1.[9] under an autosomal dominant mode of inheritance with 90% penetrance in the heterozygote, setting the disease allele frequency to 0.001 and considering marker allele frequencies equal to each other. 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. Multi-point 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. Polymerase chain reaction (PCR) was performed using genomic DNA obtained from affected and unaffected members of the family. 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.

**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 (table1).
**Table 1** Phenotypic evaluation of deaf individuals in generations III, IV and V of the family

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age</th>
<th>Gender</th>
<th>Degree of hearing loss&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Audiogram shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>III-23</td>
<td>67</td>
<td>M</td>
<td>Profound</td>
<td>Residual</td>
</tr>
<tr>
<td>III-25</td>
<td>63</td>
<td>M</td>
<td>Profound</td>
<td>Residual</td>
</tr>
<tr>
<td>III-32</td>
<td>59</td>
<td>M</td>
<td>Severe</td>
<td>Sloping</td>
</tr>
<tr>
<td>III-35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40</td>
<td>F</td>
<td>Profound</td>
<td>Residual</td>
</tr>
<tr>
<td>IV-38</td>
<td>50</td>
<td>M</td>
<td>Moderate-severe</td>
<td>Sloping</td>
</tr>
<tr>
<td>IV-44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40</td>
<td>F</td>
<td>Profound</td>
<td>Residual</td>
</tr>
<tr>
<td>IV-47</td>
<td>39</td>
<td>F</td>
<td>Moderate-severe</td>
<td>Sloping</td>
</tr>
<tr>
<td>IV-49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33</td>
<td>M</td>
<td>Profound</td>
<td>Residual</td>
</tr>
<tr>
<td>IV-50</td>
<td>36</td>
<td>M</td>
<td>Moderate</td>
<td>Flat</td>
</tr>
<tr>
<td>IV-55</td>
<td>37</td>
<td>F</td>
<td>Moderate-severe</td>
<td>Sloping</td>
</tr>
<tr>
<td>IV-71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20</td>
<td>F</td>
<td>Moderate</td>
<td>Flat</td>
</tr>
<tr>
<td>V-73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27</td>
<td>F</td>
<td>Moderate</td>
<td>Sloping</td>
</tr>
<tr>
<td>V-82</td>
<td>14</td>
<td>F</td>
<td>Moderate</td>
<td>Sloping</td>
</tr>
<tr>
<td>V-85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17</td>
<td>F</td>
<td>Mild</td>
<td>Sloping</td>
</tr>
<tr>
<td>V-91</td>
<td>14</td>
<td>M</td>
<td>Moderate</td>
<td>Sloping</td>
</tr>
<tr>
<td>V-92</td>
<td>21</td>
<td>M</td>
<td>Moderate</td>
<td>Sloping</td>
</tr>
</tbody>
</table>

<sup>a</sup>Audiograms were categorized based on the classification of Liu and Xu.[11]

<sup>b</sup>Caloric testing was performed.

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 gender 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 Analyzer (Otodynamics UK 1996), showed cochlear dysfunction.
Linkage analysis
Genomic scanning at 10-cM intervals identified a region on the long arm of chromosome 14 that yielded a LOD 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, D14S608, with a maximum two point lod score of 3.8 at theta = 0 for marker D14S608 (table 2) and a maximum multi-point lod score of 5.4 at D14S1280 (fig 2). Haplotypes were then constructed using the affected 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 (figs1 and 3).

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

<table>
<thead>
<tr>
<th>Markers</th>
<th>Position</th>
<th>Position</th>
<th>Recombination Fraction (θ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Marshfield map¹</td>
<td>Physical map²</td>
<td>0 0.001 0.01 0.05 0.1 0.15 0.2 0.3 0.4</td>
</tr>
<tr>
<td>D14S581</td>
<td>21.51</td>
<td>22288622</td>
<td>-1.55 -1.55 1.43 -0.71 -0.34 -0.18 -0.11 -0.06 -0.04</td>
</tr>
<tr>
<td>D14S1041</td>
<td>23.2</td>
<td>23472695</td>
<td>0.92 0.92 0.91 0.87 0.79 0.69 0.58 0.35 0.14</td>
</tr>
<tr>
<td>D14S1280</td>
<td>25.87</td>
<td>24645913</td>
<td>1.36 1.36 1.33 1.18 1.01 0.83 0.66 0.35 0.12</td>
</tr>
<tr>
<td>D14S615</td>
<td>28.01</td>
<td>26506789</td>
<td>2.97 2.96 2.91 2.65 2.3 1.94 1.59 0.91 0.36</td>
</tr>
<tr>
<td>D14S608</td>
<td>28.01</td>
<td>26839334</td>
<td>3.76 3.75 3.66 3.27 2.78 2.3 1.84 0.98 0.34</td>
</tr>
<tr>
<td>D14S975</td>
<td>31.13</td>
<td>28669559</td>
<td>-0.18 -0.18 -0.18 -0.16 -0.14 -0.12 -0.11 -0.07 -0.03</td>
</tr>
</tbody>
</table>

¹Sex-average Kosambi cM map distance from the Marshfield genetic map.
²Sequence-based physical map distance in bases according to Human Genome Project-Santa Cruz.

Candidate genes analyses
The interval for DFNA53 overlaps the region containing DFNA9. We analyzed 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. Another potential functional candidate, the EFS gene, encodes a docking protein which plays a central coordinating role in signaling related to cell adhesion, was also screened with no deafness related sequence change 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.
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 localized 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 studies[14] 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][17][18][19][20][21] Interestingly, all the reported mutations causing 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 exhibit a spectrum of vestibular dysfunctions, ranging from lack of symptoms to 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][17][18][19][20][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 analyzed the coding regions and exon/intron boundaries of the COCH gene by direct sequencing, in affected individuals with no mutation detected. There are several types of mutations 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 promoter 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, mutations 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 pre-lingual 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 DFNB35 loci are telomeric to the DFNA53 locus. Taken together, this points to a 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 characterized genes according to the annotation in the Human Genome Project- Santa Cruz database (www.Genome.ucsc.edu).

Typically, autosomal dominant HL is distinguished by post-lingual onset of hearing loss, compared to the pre-lingual onset of deafness observed in autosomal recessive cases. DFNA53 is characterized by hearing loss affecting high-frequency that begins in the second decade of life, 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 (HHI) that progresses to involve all frequencies is characteristic of age related hearing loss (ARHL), 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 nonsyndromic HHI genes associated with progressive hearing loss, will provide critical insights into an understanding of the molecular pathophysiology of late-onset hearing loss.
COMPETING INTERESTS STATEMENTS: The authors declare that they have no competing financial interests.

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ACKNOWLEDGEMENTS

We would like to thank the family for its contribution to this study. This work was supported by NIH grant DC 05575 (to X.ZL).
FIGURE LEGENDS

**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.

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

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


