Mapping of a new autosomal dominant non-syndromic hearing loss locus (DFNA43) to chromosome 2p12

E Flex, M Mangino, M Mazzoli, A Martini, V Migliosi, A Colosimo, R Mingarelli, A Pizzuti, B Dallapiccola

Hearing impairment (HI) is the most frequent sensory defect with wide genetic heterogeneity. Approximately 80% of genetic hearing loss is non-syndromic and 15-25% of exhibit autosomal dominant inheritance. We analysed an Italian three generation family in which non-syndromic hearing impairment is transmitted as an autosomal dominant trait. Onset of HI in all affected subjects occurred in the second decade of life, with subsequent gradual progression from moderate to profound loss. HI was bilateral and symmetrical, involving all frequencies. After exclusion of the known DFNA loci with markers from the Hereditary Hearing Loss Homepage (URL: http://dnalab-www.uia.ac.be/dnalab/hhh), a genome wide scan was carried out using 358 highly informative microsatellite markers. Significant linkage (Zmax=4.21, θ=0) was obtained with chromosome 2p12 markers. The results were confirmed by multipoint analysis (Zmax=4.51), using the location score method. Haplotype analysis defined a 9.6 cM disease gene interval on chromosome 2 without overlap with the other identified loci. Fine mapping and identification of candidate genes are in progress.

Hearing loss is a common form of sensory impairment, affecting millions of people world wide. Deafness can be the result of genetic or environmental causes or a combination of them. Approximately 1 out 1000 infants is affected by either severe or profound deafness at birth and during early childhood, that is, in the prelingual period. About 60% of these cases are considered to have a genetic basis, with deafness mostly occurring as a non-syndromic defect. Non-syndromic hearing loss is characterised by extensive genetic heterogeneity. More than 70 loci have been mapped and 29 genes identified so far.

We have performed genetic linkage studies in a three generation Italian family segregating an autosomal dominant form of non-syndromic hearing impairment (NSHI). A genome wide scan localised a novel NSHI locus (DFNA 43) to chromosome 2p12.

MATERIALS AND METHODS

Subjects
We examined a three generation Italian family with an autosomal dominant form of hearing loss (fig 1). Informed consent was obtained from all the enrolled patients and from...
parents of patients younger than 18 years. Physical examination was performed on all the family members. An additional clinical history interview was conducted in order to identify potential environmental causes of hearing loss, such as infections, trauma, and exposure to ototoxic drugs. 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-emittance measurements were obtained for all subjects that were otoscopically examined.

Genotyping
Genome scanning was performed using 358 microsatellite markers at a distance of ∼10 cM (ABI PRISM™ Linkage Mapping Set). PCR was performed using 50 ng of DNA in a 15 µl reaction mixture containing 1.5 µl buffer (100 mmol/l Tris HCl, pH 8.3, 500 mmol/l KCl), 1.5 µl MgCl2 (25 mmol/l), 1.5 µl dNTPs mix (2.5 mmol/l), 1 µl primer mix (5 µmol/l), and 0.6 U of AmpliTaq Gold™. PCR products were analysed on a model 3100 automated fluorescent DNA sequencer, a four colour detection system. One µl of PCR reaction was combined with 10 µl of formamide and 0.5 µl of a fluorescent size marker (ROX GS-500). Each sample was run for 30 minutes. During the electrophoresis, fluorescence detected in the laser scanning region was collected and stored using Genescan Collection software (version 1.0.1). The fluorescent data collected during the run were analysed by the Genescan Analysis program (version 3.5). Each marker was examined by the Genotyper program (version 3.7) in order to analyse inheritance patterns and prepare the allele labels for export to linkage applications.

Linkage analysis
Linkage analysis was performed by the LINKAGE 5.1 program package. Two point lod scores between the disease gene and each marker were calculated by means of the MLINK program. The phenotype was coded as a fully penetrant autosomal dominant trait, with a disease allele frequency of 0.0001. Equal recombination frequencies for male and females were assumed. The order of the marker loci and the recombination distances used for multipoint linkage analysis were based on the Généthon linkage map. Multipoint analysis was performed with the VITESSE computer program.

Candidate gene analysis
Sequence analysis of the whole cadherin associated protein (α-catenin) (CTNNA2, MIM 114025) coding region was performed. PCR primers and annealing conditions for CTNNA2 exon PCR are available on request. PCR products were purified using the Microcon 100 system (Millipore) and sequenced using a 3100 automated fluorescent DNA sequencer and the ABI Prism BigDye Terminator cycle sequencing kit. Electropherograms were analysed by the Sequencing Analysis software (version 3.7). We also analysed the intragenic marker D2S219. One primer of the microsatellite was labelled with the 6-FAM fluorochrome, which was analysed using standard conditions on a 3100 automated fluorescent DNA sequencer.

RESULTS
Subjects
We analysed an Italian three generation family in which NSHI was transmitted as an autosomal dominant trait (fig 1), characterised by bilateral symmetrical sensorineural hearing impairment starting during the second/third decade of life as

Figure 2 Audiograms of affected subjects from three generations (II.7, 24 years old; III.1, 35 years old; II.7, 54 years old; III.1, 74 years old; II.2, 96 years old), left ear (A) and right ear (B).
As shown in fig 1, three affected subjects were located the disease gene on markers at intervals of 10 cM. A genome search was then undertaken in 19 members of the family using microsatellite markers at intervals of 10 cM. A maximum lod score of 4.21 (∞) for marker D2S139 (table 1) located the disease gene on the short arm of chromosome 2. Haplotype analysis disclosed a key recombination event between D2S2114 and D2S2116 in II.4, defining the telomeric boundary of the disease interval (fig 1). The centromeric limit is determined by a crossover between D2S2180 and D2S2333, observed in subject II.13 (fig 1). These recombinations place the disease candidate region between D2S2114 and D2S2333, observed in subject II.13 (fig 1). These recombinations place the disease candidate region between markers D2S2114 and D2S2333 (multipoint lod score of 4.52, fig 3). The interval spans 9.6 cM on the Généthon genetic map, without overlap with other NSHI loci previously identified.

### Table 1 Lod scores

<table>
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<tr>
<th>Markers</th>
<th>0.0</th>
<th>0.01</th>
<th>0.05</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
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<td>−0.29</td>
<td>0.36</td>
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<td>0.30</td>
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<tr>
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<td>2.15</td>
<td>2.60</td>
<td>2.57</td>
<td>2.16</td>
<td>1.52</td>
<td>0.74</td>
</tr>
<tr>
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<td>2.37</td>
<td>2.21</td>
<td>1.99</td>
<td>1.53</td>
<td>1.01</td>
<td>0.42</td>
</tr>
<tr>
<td>D2S139</td>
<td>4.21</td>
<td>4.45</td>
<td>4.16</td>
<td>3.78</td>
<td>2.96</td>
<td>2.04</td>
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<tr>
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<td>2.67</td>
<td>2.49</td>
<td>2.23</td>
<td>1.74</td>
<td>1.16</td>
<td>0.50</td>
</tr>
<tr>
<td>D2S2333</td>
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<td>2.15</td>
<td>2.60</td>
<td>2.57</td>
<td>2.16</td>
<td>1.52</td>
<td>0.7</td>
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<tr>
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<td>1.52</td>
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<td>−0.54</td>
<td>0.16</td>
<td>0.31</td>
<td>0.22</td>
</tr>
</tbody>
</table>

The hearing impairment was slowly progressive, involving at first the high frequencies and extending to all frequencies by the high frequencies and extending to all frequencies by the age related progression (fig 2).

### Linkage analysis

Known loci for autosomal dominant hearing loss were excluded using the reference markers described on the Hereditary Hearing Loss Homepage (URL: http://dnlab-www.uia.ac.be/dnlab/hhh). A genome search was then undertaken in 19 members of the family using microsatellite markers at intervals of ∼10 cM. A maximum lod score of 4.21 (∞) for marker D2S139 (table 1) located the disease gene on the short arm of chromosome 2. Haplotype analysis disclosed a key recombination event between D2S2114 and D2S2116 in II.4, defining the telomeric boundary of the disease interval (fig 1). The centromeric limit is determined by a crossover between D2S2180 and D2S2333, observed in subject II.13 (fig 1). These recombinations place the disease candidate region between markers D2S2114 and D2S2333 (multipoint lod score of 4.52, fig 3). The interval spans 9.6 cM on the Généthon genetic map, without overlap with other NSHI loci previously identified.

### Candidate gene analysis

A genome database survey of the candidate region showed the presence of several genes. Among them is the cadherin associated protein (α-catenin) gene (CTNNA2, MIM 114025), which is expressed by the cochlea cells (Morton Fetal Cochlea, dbEST Library: ID.371). We first analysed the intragenic marker D2S2191. As shown in fig 1, three affected subjects were heterozygous. The results excluded the presence of large dele-
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