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

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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.

H earing loss is a common form of sensory impairment, affecting millions of people world wide.[1] 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.[2] Non-syndromic hearing loss is characterised by extensive genetic heterogeneity. More than 70 loci have been mapped[3] 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
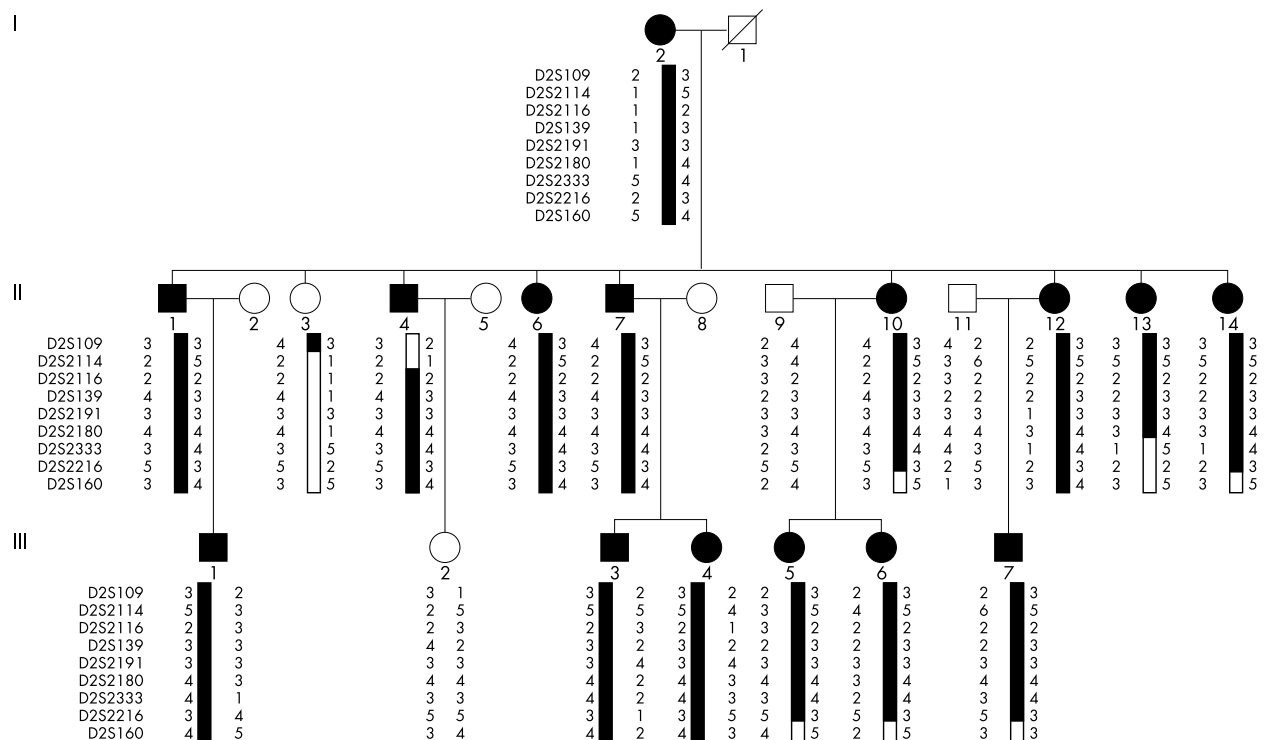

Figure 1 Haplotypes of nine DNA markers on chromosome 2p12 in the branch of the family examined. The blackened bars indicate the haploidentical region in affected subjects that define the critical DFNA43 region between flanking markers D2S2114 and D2S2333.
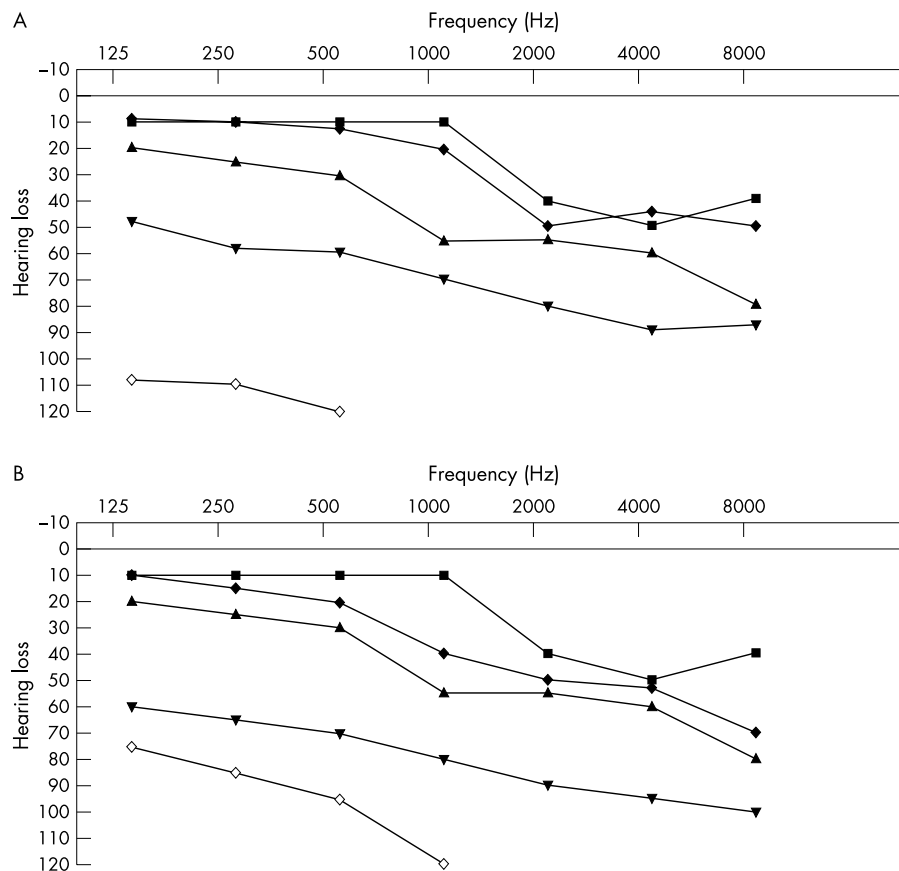
Figure 2 Audiograms of affected subjects from three generations (■ III.7, 24 years old; ◆ III.1, 35 years old; ▲ II.7, 54 years old; ▼ II.14, 74 years old; ◇ I.2, 96 years old), left ear (A) and right ear (B).

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 MgCl$_2$ (25 mmol/l), 1.5 μl dNTPs mix (2.5 mmol/l), 1 μl primer mix (5 μmol/l), and 0.6 U of Ampli*Taq* 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.[4] Two point lod scores between the disease gene and each marker ware 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.[5–7] Multipoint analysis was performed with the VITESSE computer program.[8]

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 PCRs 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

Table 1 Lod scores

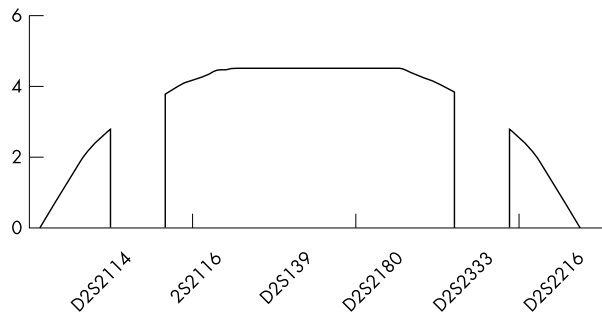| Markers	Lod score at θ =						
	0.0	0.01	0.05	0.1	0.2	0.3	0.4
D2S109	−∞	−3.83	−1.23	−0.29	0.36	0.45	0.30
D2S2114	−∞	2.15	2.60	2.57	2.16	1.52	0.74
D2S2116	2.41	2.37	2.21	1.99	1.53	1.01	0.42
D2S139	**4.21**	4.45	4.16	3.78	2.96	2.04	0.98
D2S2180	2.71	2.67	2.49	2.25	1.74	1.16	0.50
D2S2333	−∞	2.15	2.60	2.57	2.16	1.52	0.7
D2S2216	−∞	2.15	2.60	2.57	2.16	1.52	0.74
D2S160	−∞	−4.13	−1.51	−0.54	0.16	0.31	0.22

Figure 3 Multipoint lod score analysis for the region 2p12. The maximum multipoint lod score within the interval of ~9.6 cM between markers D2S2114 and D11S2333 was 4.52.

a dip at 4000 Hz, similar to a noise induced hearing loss. The hearing impairment was slowly progressive, involving at first the high frequencies and extending to all frequencies by the fifth to sixth decade of life. The level of impairment became severe at later ages. Tympanometry and stapedial reflexes were normal. The hearing impairment was cochleopathic, with presence of recruitment and with tinnitus as a variable manifestation. In comparison with other genetic non-syndromic conditions, there was little intrafamilial variability and differences could be mainly attributed to 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://dnalab-www.uia.ac.be/dnalab/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 (θ=0) 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 DS2333 (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).[9] 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-

tions in CTNNA2. Then, the gene coding regions were sequenced without finding any variation.

DISCUSSION
We performed a genome search in a three generation Italian family, in which a non-syndromic hearing impairment was transmitted as an autosomal dominant trait. We found linkage with markers located on chromosome 2p12. The DFNA43 critical region does not overlap with any other locus for non-syndromic hearing loss previously mapped on chromosome 2 (DFNA16, DFNB9, DFNB27).[10–12] In an effort to identify the DFNA43 gene, we tested the cadherin associated protein (α-catenin) gene (CTNNA2). Several pieces of evidence pointed to CTNNA2 as a DFNA43 candidate gene. Nonsense and frameshift mutations in the cadherin-like protein CDH23 have been identified in Usher syndrome 1D patients (USH1D, MIM 601067),[13 14] while missense mutations can cause severe to profound hearing loss (DFNB12).[15] Moreover, mutations in two cadherin related genes were reported in the deaf mouse mutants waltzer[16] and Ames-waltzer.[17] Cadherins are thought to be involved in cell-cell interactions within the inner ear, but the presence of disorganised stereocilia in waltzer and Ames-waltzer mutants also suggests involvement in the lateral or tip links joining adjacent stereocilia.[16] Proteins such as vezatin or harmonin could also have a role in these linkage complexes.[3] The activity of cadherins in mediating homophilic cell-cell Ca^{2+} dependent association would rely on their anchorage to the cytoskeleton via the catenins.[18 19] However, mutation screening of CTNNA2 gene coding regions in DFNA43 patients was negative, apparently excluding it as a candidate. To date, no other gene mapping in the region appears a suitable candidate for this disease. The analysis of new families linked to the DFNA43 locus, as well as a more complete expression map of the region will help the task of isolating the DFNA43 gene.

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