

SHORT REPORT

A novel locus for autosomal dominant non-syndromic hearing loss, DFNA31, maps to chromosome 6p21.3

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Background: Non-syndromic hearing loss is the most genetically heterogeneous trait known in humans. To date, 51 loci for autosomal dominant non-syndromic sensorineural hearing loss (NSSHL) have been identified by linkage analysis.

Objective: To investigate the genes involved in a Dutch family with NSSHL.

Methods: Linkage analysis in a large Dutch pedigree with progressive bilateral loss of the mid and high frequencies, in which a novel dominant locus for postlingual NSSHL (DFNA31) has been identified.

Results: DFNA31 was found to be located in a 7.5 cM region of chromosome 6p21.3 between D6S276 (telomeric) and D6S273 (centromeric), with a maximum two point LOD score of 5.99 for D6S1624. DNA sequencing of coding regions and exon/intron boundaries of two candidate genes (*POU5F1*, *GABBR1*) in this interval did not reveal disease causing mutations.

Conclusions: Haplotype analysis indicated that the genetic defect in this family does not overlap the DFNA13 and DFNA21 regions that are also located on 6p. Identification of the disease gene will be of major importance in understanding the pathophysiology of hearing impairment.

The most common sensory defect in humans is hearing loss. About one in 650 children suffers from hearing impairment before speech acquisition.¹ This prelingual deafness has a genetic cause in 60% of the cases.^{2,3} In nearly all cases it is monogenic and has an autosomal recessive (75%), autosomal dominant (20–25%), or X linked (1–4%) inheritance pattern.⁴ More common is postlingual hearing loss, which affects 10% of the population by the age of 60 and 50% by the age of 80.⁵ In most cases the aetiology is multifactorial, resulting from a combination of genetic and environmental factors. This complex trait is called presbycusis and is the commonest form of adult onset hearing loss. Monogenic forms of postlingual progressive hearing impairment are also seen. In these families, the onset is earlier than presbycusis, and the inheritance is nearly always autosomal dominant. Lists of all currently known genes and loci for non-syndromic hereditary hearing impairment can be obtained from the hereditary hearing loss homepage (<http://www.uia.ua.ac.be/dnalab/hhh>). Identification of genes for hereditary hearing impairment is an important step towards clarifying the biology of hearing and deafness at a molecular level.

METHODS

Nomenclature

Gene symbols used in this article follow the recommendations of the HUGO gene nomenclature committee.⁶

Family data

In this study we investigated a four generation Dutch family with autosomal dominant sensorineural hearing impairment. Informed consent was obtained from all study participants and from the parents of minors. A clinical history interview of members of this family was undertaken by one of the investigators, with special emphasis on identifying potential environmental causes of hearing loss or on evidence of syndromic forms of deafness. Otoscopic examination was done, along with pure tone audiometry for air conduction (125–8000 Hz) and bone conduction (250–4000 Hz).

Blood samples were collected from 39 pedigree members. DNA was extracted from leucocytes according to standard methods. Of the study subjects from whom DNA samples were obtained, 12 had hearing impairment, 15 were unaffected, and 12 had an unclear diagnosis or were too young to have symptoms of hearing impairment (fig 1).

Clinical features

Clinical investigations of this family have been reported by Ensink *et al.*⁷ Briefly, hearing impairment in the family is, apart from presbycusis, most pronounced at 1 to 2 kHz. On the basis of audiograms and clinical investigations, 12 individuals were classified as affected and included in the linkage analysis. The estimated age of onset varied between frequencies, ranging from five years in the low frequencies to 12 years in the high frequencies. Fifteen persons above the age of 12 were classified as unaffected. In the remaining 12 persons, the clinical diagnosis was unclear because of an atypical audiometric pattern or because the age of onset was not yet reached. These subjects were not included in LOD score calculations and are not represented in fig 1.

Genotyping and linkage analysis

Twenty microsatellite markers were used to analyse the first 13 DFNA loci. Markers for the refinement of the critical interval were taken from the Génethon human linkage map.⁸ The order of these markers was established on the basis of the NCBI physical map (<http://www.ncbi.nlm.nih.gov>). All the genotyping was done by polymerase chain reaction (PCR) and polyacrylamide gel electrophoresis, using standard procedures. Two point linkage analysis was conducted for each marker using the MLINK program from the LINKAGE package, version 5.1.⁹ Two point LOD scores between the deafness locus and each marker were calculated under a fully penetrant autosomal dominant mode of inheritance, setting the disease allele frequency to 0.00001 and considering marker allele frequencies as being equal to each other. We used the observed numbers of alleles in the pedigree (N) in the LOD score calculations and set the allele frequencies at 1/N.

Candidate gene analysis

Primers were designed for the amplification of coding regions and exon–intron boundaries of *POU5F1* (MIM 164177) and

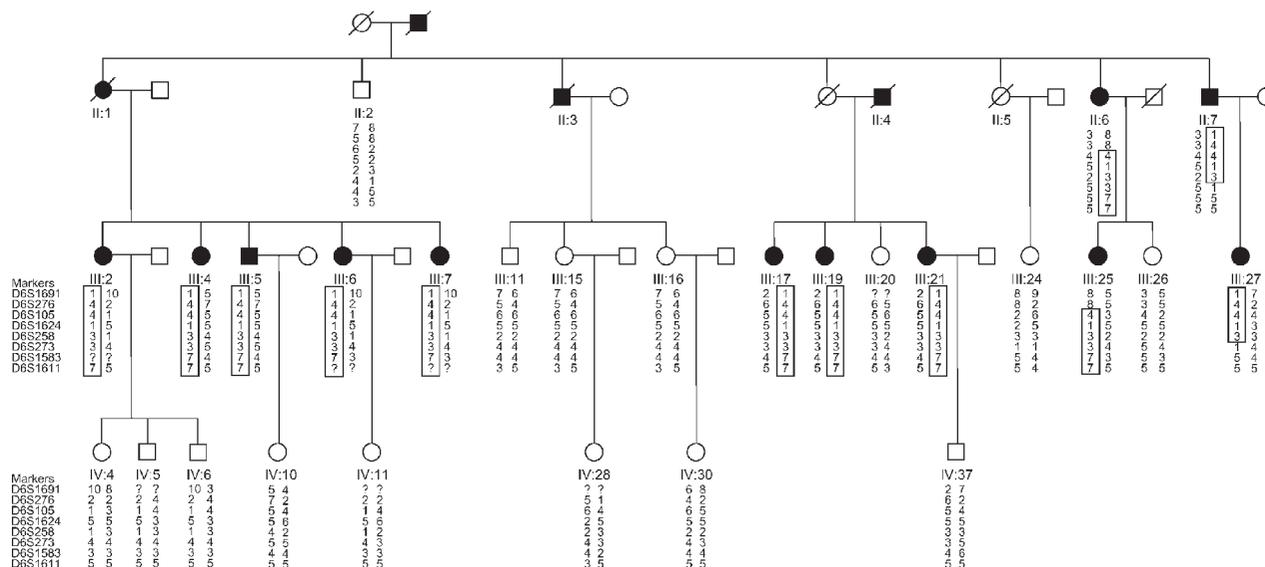


Figure 1 Pedigree of the family with autosomal dominantly inherited hearing impairment. Filled symbols represent individuals with hereditary hearing impairment and empty symbols represent individuals with normal hearing. In order to allow detailed comparisons of genotypes and phenotypes, the numbering of family members is the same as in the previous clinical description of this family.⁷ Haplotypes for the markers of table 1 are given below each symbol. Question marks indicate unknown alleles. The haplotype segregating with the disease allele appears in rectangles. In 12 family members the clinical diagnosis was unclear because of an atypical audiometric pattern or because the age of onset was not yet reached. These subjects were not included in LOD score calculations and are not represented in this figure.

GABBR1 (MIM 603540). Sequencing of the PCR products was done using standard procedures and analysed in an automated DNA sequencer ABI PRISM 3100 (Applied Biosystems).

RESULTS

Microsatellite markers for known DFNA loci were analysed in numerical order. DFNA1 to DFNA12 were excluded by means of linkage analysis. Markers linked to DFNA13¹⁰ showed positive LOD scores, suggesting linkage. Additional STRP markers in this chromosomal region were analysed, confirming linkage with maximum two point LOD scores up to 5.99 (table 1), and delineating a candidate region between markers D6S276 and D6S273. At the time of analysis, the most comprehensive genetic map of the human genome was the Généthon human linkage map.⁸ On this map, the key recombinant markers—D6S276 and D6S273—were located, respectively, at position 44.9 cM and 45.4 cM of chromosome 6p, suggesting that the candidate region was very small (0.5 cM). The telomeric key recombinant marker of the DFNA13 region, D6S1666, had the same genetic position as D6S273 (45.4 cM).¹¹ For this reason we concluded that the family was linked to DFNA13, and most probably had a mutation in the

COL11A2 gene, responsible for hearing impairment in several families linked to the DFNA13 region.¹¹

DISCUSSION

We analysed the complete coding region of the *COL11A2* gene but did not find a disease causing mutation, which was puzzling. Subsequently, tremendous progress was made in the accuracy of available genetic and physical maps, as a result of the human genome project. These newly developed physical maps revealed serious errors in the Généthon map of chromosome 6p21.3. It became clear that the current family was linked to a region non-overlapping and telomeric of the DFNA13 locus, and a new locus name (DFNA31) was obtained from the HUGO nomenclature committee. A physical map of the 6p21.3 region based on recent data from the National Center for Biotechnology Information and containing all relevant genetic markers is given in fig 2. The new map shows that the candidate region for the current family does not include the *COL11A2* gene, explaining why no disease causing mutation was found in the *COL11A2* gene. Because of the large physical distance between *COL11A2* and DFNA31, a positional effect of the *COL11A2* gene is highly unlikely, and *COL11A2* can be excluded as the gene for DFNA31. In addition, the size of the candidate region, flanked by D6S276 and D6S273, was larger than previously anticipated on the basis of the Généthon genetic map, and spans 7.5 million base pairs (fig 2).

A third locus for non-syndromic hearing impairment, DFNA21, has been localised to chromosome 6p.¹² DFNA21 is flanked by the marker D6S1691 at the centromeric site (de Brouwer A *et al*, unpublished data), and as a result does not overlap DFNA13 or DFNA31. The localisation of different genes for non-syndromic hearing impairment in close proximity is not surprising. Non-syndromic hearing impairment is the most genetically heterogeneous disease known. Currently, there are close to 100 different localisations, and new genes are localised at a rapid pace (for an overview, see the hereditary hearing loss homepage, <http://www.uia.ua.ac.be/dnalab/hhh>). It can be expected that, just by chance, some of these genes will be localised closely together.

Table 1 Results of two point linkage analysis between hearing impairment and 6p21 genetic markers

Marker	Recombination fraction						
	0.00	0.01	0.05	0.10	0.20	0.30	0.40
D6S1691	-inf	4.52	4.81	4.57	3.70	2.55	1.20
D6S276	-inf	3.92	4.20	3.98	3.18	2.16	0.97
D6S105	4.07	3.99	3.67	3.25	2.40	1.49	0.56
D6S1624	5.99	5.88	5.41	4.80	3.49	2.05	0.55
D6S258	4.25	4.17	3.84	3.40	2.48	1.51	0.56
D6S273	-inf	4.46	4.73	4.47	3.56	2.38	1.04
D6S1583	-inf	4.28	4.56	4.31	3.43	2.31	1.03
D6S1611	-inf	2.57	2.87	2.65	1.86	0.91	0.10

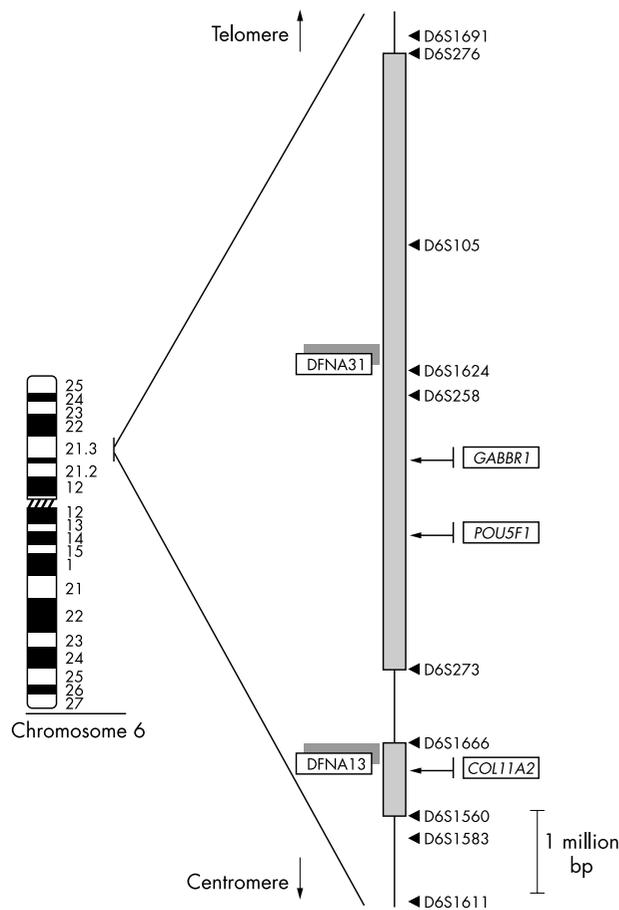


Figure 2 Ideogram of chromosome 6, with a schematic physical map of the 6p21.3 region, showing the markers used in this study. The *COL11A2* gene and the two analysed candidate genes are indicated. The marker order and intermarker distances are based on maps at NCBI (<http://www.ncbi.nlm.nih.gov>). bp, base pairs.

Another example is the DFNA2 locus, for which three different genes have been implicated.¹³

The 7.5 cM DFNA31 interval includes most of the histone gene cluster and a part of the MHC region.^{14–16} Within the entire MHC region, more than 200 genes have so far been identified, making it one of the most gene dense and complex regions of the genome.¹⁷ Because HLA genes have their function in immunological processes, a specific pathophysiological function in hearing impairment is not very likely. Many of the other genes in our region are ones that encode for histones. They are not considered good candidate genes for hearing impairment because of their elementary function. We did find two genes in the DFNA31 region that are very interesting as possible candidates. The first of these, *GABBR1*, encodes for the β receptor of the neurotransmitter GABA, which is an important neurotransmitter in the sound perception system and was therefore a good candidate gene for hearing impairment. A second candidate gene is the *POU5F1* gene, a transcription factor that contains a POU domain.¹⁸ Two POU genes have already been reported to cause hearing impairment—*POU3F4*¹⁹ and *POU4F3*²⁰; these are responsible for DFN3 and DFNA15, respectively. However, DNA sequencing of coding regions and exon-intron boundaries of the two genes did not reveal a disease causing mutation.

The ultimate cloning of the gene causing DFNA31 will provide further insights into our understanding of the molecular pathophysiology of late onset hearing loss.

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