A gene for autosomal dominant hearing impairment (DFNA14) maps to a region on chromosome 4p16.3 that does not overlap the DFNA6 locus

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Abstract

Non-syndromic hearing impairment is one of the most heterogeneous hereditary conditions, with more than 40 reported gene localisations. We have identified a large Dutch family with autosomal dominant non-syndromic sensorineural hearing impairment. In most patients, the onset of hearing impairment is in the first or second decade of life, with a slow decline in the following decades, which stops short of profound deafness. The hearing loss is bilateral, symmetrical, and only affects low and mid frequencies up to 2000 Hz. In view of the phenotypic similarities of this family with an American family that has been linked to chromosome 4p16.3 (DFNA6), we investigated linkage to the DFNA6 region. Lod score calculations confirmed linkage to this region with two point lod scores above 6. However, as haplotype analysis indicated that the genetic defect in this family is located in a 5.6 cM candidate region that does not overlap the DFNA6 region, the new locus has been named DFNA14.
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Non-syndromic hereditary hearing impairment (NSHHI) is a common genetic disorder. On the basis of age at onset, prelingual and postlingual forms are recognised. Prelingual NSHHI occurs in approximately 1 in 2000 births, is monogenic in origin in nearly all cases, and follows an autosomal recessive (75%), autosomal dominant (20-25%), or X linked (1-4%) inheritance pattern.[1]

Postlingual NSHHI is less well characterised. Although environmental factors such as noise play an important role, families with a purely monogenic inheritance have been described.[2] Usually, the mode of inheritance is autosomal dominant,[2] although mitochondrial mutations leading to postlingual NSHHI have also been described.[3 4]

Over 47 nuclear gene localisations for NSHHI have been reported.[5] These loci are named with the prefix "DFNA" for autosomal dominant, "DFNB" for autosomal recessive, and "DFN" for X linked loci, followed by a number. Nineteen autosomal dominant loci, 20 autosomal recessive loci, and eight X linked loci are referenced in the Hereditary Hearing Loss Homepage (http://dnalab-www.uia.ac.be/dnalab/hhh). However, some of these loci involve the same gene. For example, DFNA8 and DFNA12 are both caused by mutations in the α-tectorin gene.[6] Other loci, such as DFNB7[7] and DFNB11,[8] have overlapping candidate regions and a similar type of NSHHI, and are therefore suspected to be caused by a single gene.

With respect to phenotype, the autosomal recessive NSHHI families have congenital profound deafness, with the exception of two families with postlingual progressive HI linked to DFNA8[9] and DFNB13.[10] Most autosomal dominant families, in contrast, have postlingual progressive HI. The exceptions are three families linked to DFNA3[11] or DFNA8/DFNA12[6 9] that have a prelingual onset of HI. Unlike other recessive families, the HI in these three families is only moderate to severe and never profound.

When the audiometric data of the families with postlingual NSHHI are compared, these families fall into three broad categories. The first group, including families linked to DFNA2,[12] DFNA5,[13] DFNA7,[14] and DFNA9,[15] have HI that initially only affects the high frequencies and then gradually includes other frequencies. The second group, consisting of families linked to DFNA1[16] and DFNA6,[17] has a HI that is initially most pronounced in the low frequencies, although a significant difference in progression rate exists between these families. In the family linked to DFNA1, HI rapidly progresses from onset in the first decade to severe HI across all frequencies before the third decade in all family members. In the DFNA6 family, the onset of HI is generally in the second decade and progression is slow, never becoming severe in degree.

The remaining families with NSHHI form a loose third group that does not fit into the two other categories. These families have a HI that is initially most pronounced in the mid frequencies (U shaped audiogram) or that starts across all frequencies with nearly equal thresholds (flat audiogram). However, for most families in the third category, insufficient audiometric data have been published to subcategorise them further.

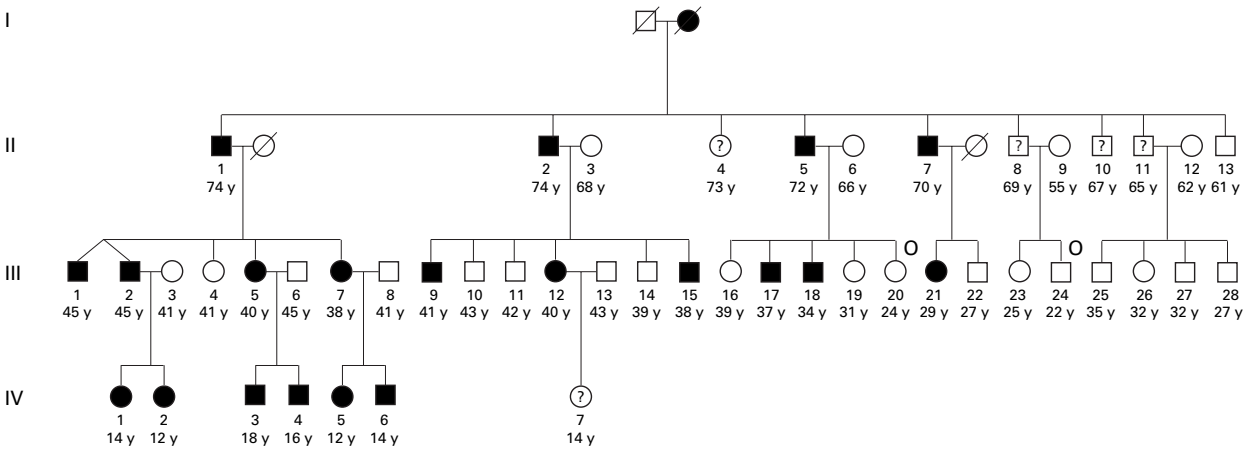

Figure 1 Pedigree of the Dutch family with progressive HI. All living family members, including spouses, were tested audiometrically. The age of the family members at the time of audiometry is given below their symbol. All family members with low frequency thresholds below the 95th centile are marked as affected. Family members with atypical audiograms or other possible causes of HI are marked with a question mark to indicate an unclear clinical diagnosis. Unaffected persons below the age of 25 were given an unknown affectedness status, indicated by a circle next to their symbol.

We have ascertained a large Dutch family with sensorineural progressive HI starting in the low and mid frequencies (up to 2000 Hz). As another family from the USA with a similar HI[17] had been linked to chromosome 4p16.3 (DFNA6), we investigated linkage to this region. We found significant linkage to chromosome 4p16 markers in our family and delineated a candidate region. However, this region did not overlap the DFNA6 region. Therefore, this new locus has been named DFNA14.

Methods

FAMILY STUDIES

All family members were interviewed regarding their medical history and family relationships and underwent an examination to exclude signs of syndromic HI. Standard audiometry (air and bone conduction) was performed and a blood sample was obtained from all participating family members.

CLINICAL DIAGNOSIS

In families with progressive NSHHI, the clinical diagnosis is not always trivial. As hearing thresholds increase significantly with age (presbyacusis) and vary between males and females, we used a methodology based on the audiometric threshold profile and clinical history to determine affected status. First, family members with suspected or identifiable causes of HI other than the familial HI were excluded. Second, hearing thresholds were compared with normal thresholds for subjects of the same age and gender.[18] When hearing between 250 and 1000 Hz was worse than the 95th centile for the general population, subjects were considered affected. The type II error of this procedure (including a genetically unaffected subject as clinically affected) is less than 5%. Subjects older than 25 years of age with hearing better than 20 dB HL or the 50th centile limit were considered unaffected.

GENETIC ANALYSIS

DNA was isolated from blood samples. Microsatellite genetic markers were analysed by polymerase chain reaction and polyacrylamide gel electrophoresis using standard procedures. Lod score calculations were performed using the LINKAGE computer programs.[19] The HI was coded as an autosomal dominant fully penetrant disease with a frequency of 1 in 10 000. Equal recombination was assumed for both sexes. We used the observed number of alleles in the pedigree (N) in the lod score calculations and set the allele frequencies at 1/N.

Results

CLINICAL STUDIES

A total of 48 family members participated in a clinical and genetic study after informed consent (fig 1). Clinical investigations were consistent with postlingual sensorineural non-syndromic HI inherited as an autosomal dominant trait. The onset of HI in most patients was in the first or second decade of life, after which hearing slowly declined over the following decades. The HI was bilateral and symmetrical, with only frequencies up to 1000 or 2000 Hz being affected. In general, hearing thresholds below 2000 Hz were worse than the 95th centile for the general population, while at 4000 and 8000 Hz, thresholds remained better than the 95th centile in most family members, even in old age. This suggested that the hereditary component of HI in this family is confined to low and mid frequencies up to 2000 Hz. There was no evidence for hearing deterioration exceeding normal presbyacusis, as thresholds did not exceed age matched normal levels at high frequencies (4000 and 8000 Hz) in most patients. Representative audiograms from affected family members of different ages are shown in fig 2.

A careful clinical selection of affected and unaffected members of the family was made as described in the Methods section. A total of 20 family members were classified as affected and 13 were classified as unaffected. In five family members (II.4, II.8, II.10, II.11, IV.7) the clinical diagnosis was unclear because of an atypical audiometric pattern or the presence of possible non-genetic causes of HI. These subjects are indicated by a question mark in fig 1. Two persons below the age of 25 (III.20,

Table 1 Results of two point linkage analysis between hearing impairment and 4p16 genetic markers

	Recombination fraction						
Marker	*0.00*	*0.01*	*0.05*	*0.10*	*0.20*	*0.30*	*0.40*
D4S3034	−∞	3.71	4.18	4.10	3.43	2.41	1.14
D4S412	−∞	2.75	3.74	3.80	3.24	2.29	1.10
D4S2957	−∞	1.39	2.38	2.54	2.21	1.52	0.65
D4S432	−∞	2.16	2.57	2.50	2.00	1.32	0.54
D4S2925	4.01	3.99	3.85	3.58	2.85	1.93	0.86
D4S3023	−∞	4.15	4.96	4.87	4.00	2.72	1.22
D4S431	6.67	6.57	6.13	5.56	4.35	3.00	1.48
D4S2935	4.09	3.99	3.62	3.13	2.15	1.19	0.37
D4S3007	−∞	0.47	1.01	1.11	0.98	0.67	0.29
D4S394	−∞	5.20	5.43	5.12	4.10	2.78	1.26
D4S2983	−∞	−1.56	−0.32	0.08	0.28	0.23	0.09
D4S3009	−∞	1.17	2.83	3.17	2.90	2.16	1.10

III.24) were considered to be too young for a reliable clinical diagnosis. These family members are indicated by a small circle next to the symbol in fig 1. Eight normally hearing spouses were given an unaffected status.

LINKAGE ANALYSIS IN THE DUTCH FAMILY

In view of the audiometric similarities of our Dutch family to an American family (DFNA6) that has been linked to chromosome 4p16.3,[17] we investigated linkage to this region in our family by analysing genetic marker D4S412. The maximum lod score for linkage to this marker was 3.8 at a recombination fraction of 0.1 (table 1). To confirm linkage, 11 other genetic markers spanning a 20 cM region in 4p16.3 were analysed. All the markers used in this study were taken from the Généthon genetic linkage map.[20] A genetic map containing all markers that were analysed is shown in fig 3. Linkage to chromosome 4p16.3 was confirmed with two point lod scores exceeding 6 (table 1).

In order to determine the genetic interval containing the genetic defect in this family, haplotype analysis was performed and crossover events providing mapping information were identified. Key crossovers delineating the telomeric boundary of the HI mutation were found in two subjects with a recombination between D4S3023 and D4S431. One person (IV.3 in figs 1 and 4) is affected, while the other (III.25 in figs 1 and 4) is unaffected at 35 years of age. A single recombination between D4S2935 and D4S3007 in an affected family member (IV.4 in figs 1 and 4) marks the centromeric boundary of the candidate region.

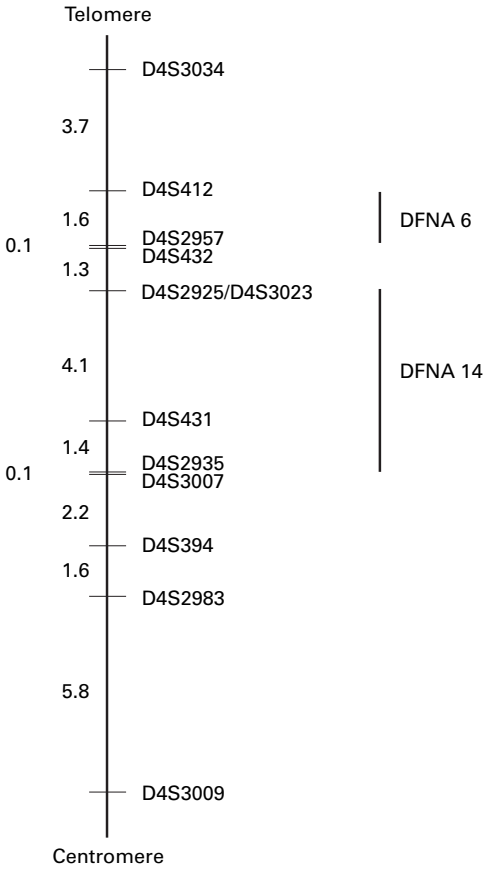

Figure 3 Genetic map of chromosome 14p16.3, showing the markers used in this study. Bars on the right indicate the linkage intervals of the DFNA6 and DFNA14 deafness loci. The marker order and intermarker distances are based on the Généthon genetic map.[20]

In combination, these recombinational events place the candidate region of the genetic defect in the Dutch family between markers D4S3023 and D4S3007, a region of 5.6 cM on the Généthon genetic map. This region is located 1.3 cM proximal to the DFNA6 region and does not overlap it (fig 3).

LINKAGE ANALYSIS IN THE AMERICAN DFNA6 FAMILY

The centromeric delineation of the DFNA6 candidate region was based only upon the result of marker D4S432 in a single affected member of the American DFNA6 family.[17] To

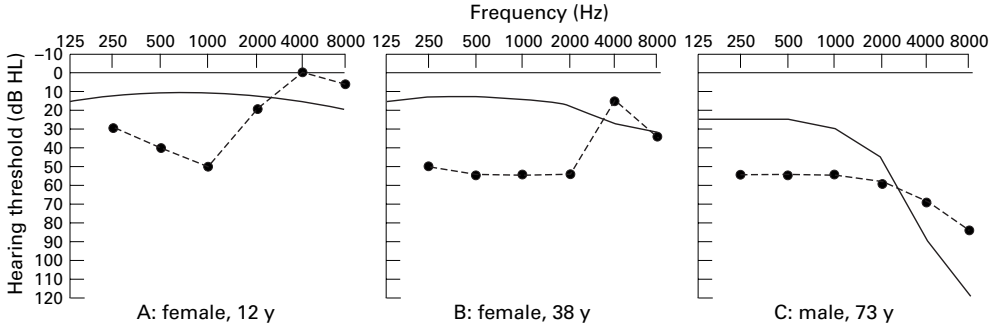

Figure 2 Typical audiograms for the right ear of affected family members. Shown are an affected female (IV.5) aged 12 (A), an affected female (III.7) aged 38 (B), and an affected male (II.2) aged 73 (C). Hearing thresholds are indicated by dotted lines, age and sex matched 95th centile values for hearing (International Organisation for Standardisation, 1984) are indicated by a solid line.

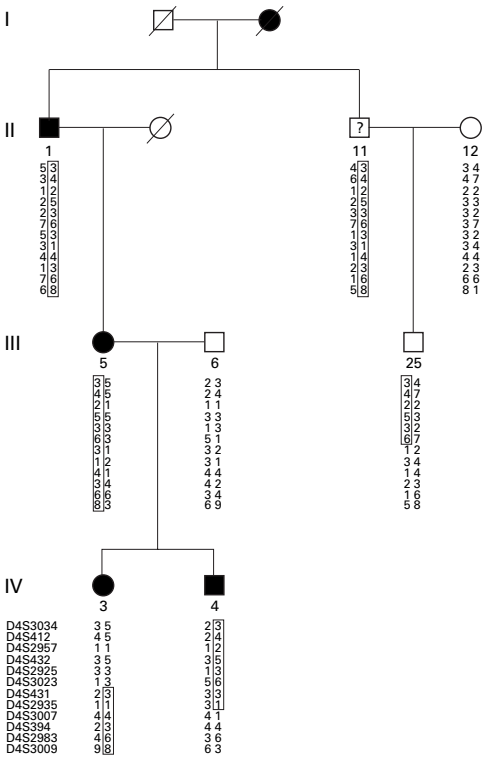

Figure 4 Haplotype analysis with 4p16 markers in key recombinants, delineating the DFNA14 candidate region. The haplotype linked to deafness is boxed. II.11 has hearing thresholds of approximately 60 dB for the low frequencies, which is typical for the family. However, as the subject suffers from several symptoms related to Menière disease, which is associated with low frequency HI, he was conservatively given an unknown affectedness status.

confirm that the DFNA6 region and the candidate region of the Dutch family do not overlap, we repeated the analysis of D4S432 in this key recombinant and analysed five additional markers centromeric to D4S432. Although markers D4S2957, D4S2925, and D4S3023 were uninformative, D4S431 and D4S2935 confirmed the DFNA6 key crossover. Subsequently, this patient was re-examined audiometrically and a new blood sample was obtained five years after the initial assessment. The new audiometric results clearly confirmed that this key recombinant was affected. Three genetic markers were retyped on the new DNA sample, giving results identical to the previous findings. In view of the evidence for separate candidate regions, the HUGO/GDB Nomenclature Committee named the new locus DFNA14.

Discussion

We report here a novel NSHHI locus (DFNA14), which is responsible for an autosomal dominant form of postlingual HI mainly affecting the low frequencies. The candidate interval of DFNA14 is a 5.6 cM region between D4S3023 and D4S3007 on distal chromosome 4p. This region does not overlap the DFNA6 gene, which is responsible for a similar low frequency HI.

The finding of separate candidate regions in these two families suggests that there are two different genes responsible for low tone HI located in the same chromosomal band. This is surprising, as the audiometric characteristics of the American DFNA6 and the Dutch DFNA14 families are specific and very similar. Furthermore, postlingual slowly progressive sensorineural HI starting in the low frequencies is very rare, and these two families are the only ones with this kind of loss reported world wide. If a single gene is responsible for DFNA6 and DFNA14, the centromeric boundary of the DFNA6 linkage interval or the telomeric boundary of the DFNA14 region may be incorrect. This is possible if one of the key recombinants is a phenocopy or non-penetrant. In the DFNA14 family, however, the telomeric boundary of the DFNA14 region is defined by two recombinations in both an affected and an unaffected subject. Only if the affected recombinant is a phenocopy and the unaffected recombinant is non-penetrant would the telomeric boundary of the DFNA14 region be wrong. The centromeric boundary of the DFNA6 region is defined by a single affected recombinant. Although a phenocopy cannot be excluded, the uncommon occurrence of this type of HI in the general population remains an argument against this hypothesis.

Another possibility is the presence of a spontaneous mutation or a typing error in one of the microsatellite markers, artificially creating a recombinant haplotype. For the telomeric boundary of the DFNA14 region, however, both recombination events were confirmed by different markers (fig 4), excluding this possibility in the DFNA14 family. However, the centromeric boundary of the DFNA6 region is defined by a single recombinant, which was shown by only a single marker (D4S432) in the initial study.[17] In this study, we repeated the genetic analysis of D4S432 for the critical DFNA6 recombinant using a new blood sample and obtained exactly the same result. In addition, five other markers were analysed. Although the two markers located between the DFNA6 and DFNA14 candidate regions (D4S2925 and D4S3023) were uninformative, the crossover was confirmed by two markers (D4S431 and D4S2935) centromeric to the uninformative markers. This analysis excludes a typing error, but a mutation in D4S432 cannot be excluded. However, the spontaneous mutation rate in dinucleotide microsatellite markers has been estimated at approximately 5×10^{-4}, making it a very infrequent event.[21]

It may be possible that a mistake in the genetic map position of the centromeric flanking marker of DFNA6 (D4S432) relative to the telomeric flanking marker of the DFNA14 region (D4S3023) incorrectly separates the DFNA6 and DFNA14 candidate regions. However, the Marshfield genetic map (http://www.marshmed.org/genetics/indexmap.html) also confirms the localisation of D4S432 telomeric to D4S3023. In addition, even if the relative order of D4S432 and D4S3023 were reversed, D4S432 would remain the centromeric flanking marker for the DFNA6 region, and this same marker would become the telomeric marker for the DFNA14 critical

region on the basis of the unaffected recombinant III.25 (fig 4). The DFNA6 and DFNA14 regions would thus remain not overlapping.

Three other possibilities should also be considered. First, the physical map may be small compared to the genetic map. Increased recombination is found on chromosome 4p16.3 and the 300-400 kb physical region near marker D4S126 in the DFNA6 candidate region[17] corresponds to a genetic distance of 3.5 cM.[22] If this high frequency of recombination extends in the centromeric direction, the 1.3 cM genetic distance between the DFNA6 and DFNA14 candidate regions could be spanned by a single large gene. Second, a gene duplication event may have occurred and two ancestrally related but discrete genes could cause DFNA6 and DFNA14. For example, mutations in either of the two closely linked collagen genes, α3(IV) or α4(IV), cause autosomal recessive Alport syndrome.[23] Third, a single gene may be responsible for both diseases, with mutations in one family in a 5' enhancer region. This type of distant effect has been hypothesised in DFN3 (X linked deafness with stapes fixation), where microdeletions 400 kb proximal to the POU3F4 gene are found in some affected persons.[24] The mechanisms by which these deletions cause an identical phenotype to mutations in the coding region of POU3F4 are currently unclear.

In conclusion, we have presented phenotypic arguments for the involvement of a single gene in DFNA6 and DFNA14, while the genetic analysis of the families argues for two genes. Whether DFNA6 and DFNA14 involve the same gene or not will ultimately be resolved by the identification of disease causing mutations.

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