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Identification of a new locus for autosomal dominant non-syndromic hearing impairment (*DFNA7*) in a large Norwegian family

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Hereditary hearing impairment affects about 1 in 1000 newborns. In most cases hearing loss is non-syndromic with no other clinical features, while in other families deafness is associated with specific clinical abnormalities. Analysis of large families with non-syndromic and syndromic deafness have been used to identify genes or gene locations that cause hearing impairment. The present report describes a large Norwegian family with autosomal dominant non-syndromic, progressive high tone hearing loss with linkage to 1q21-q23. A maximum LOD score of 7.65 ($\theta = 0.00$) was obtained with the microsatellite marker *D1S196*. Analysis of recombinant individuals maps the deafness gene (*DFNA7*) to a 22 cM region between *D1S104* and *D1S466*. The region contains several attractive candidate genes. This report supports the idea of extensive genetic heterogeneity in hereditary hearing impairment and represents the first localization of a deafness gene in a Norwegian family.

INTRODUCTION

Genetic epidemiological studies have suggested that clinically significant hearing loss affects one in 1000 infants, and it is estimated that more than one half of these cases is due to genetic factors (1–3). Non-syndromic hearing loss accounts for more than 70%. The other 30% are syndromic and affected individuals have a specific pattern of clinical features.

Although progressive hearing loss in mid- and late adulthood is considered multifactorial with involvement of genetic and environmental factors, childhood or adolescent non-syndromic hearing loss is often inherited as a simple Mendelian trait. In ~2–3% of the reported families, the disorder is inherited according to an X-linked recessive pattern, in 75% as an autosomal recessive and in ~20% as an autosomal dominant trait (1). Only a small percentage is due to mitochondrial mutations.

However, linkage studies have demonstrated the tremendous genetic heterogeneity in both autosomal dominant (ADD) and autosomal recessive (ARD) inherited forms. To date 19 different loci have been identified in ADD and ARD, but new loci are rapidly being mapped (Hereditary Hearing Loss Homepage: http://hgins.uia.ac.be/u/dnalab/hhh.html). Overlapping locations are reported for *DFNB1* and *DFNA3*, which may indicate that the same gene can be involved in both autosomal recessive and autosomal dominant deafness (4).

Little is known about the genes involved in ADD and ARD, and so far only the *DFN3* gene has been cloned and characterized (5).

We present a new locus (*DFNA7*) involved in autosomal dominant progressive high tone hearing loss. After exclusion of linkage to previously described loci for both recessive and dominant hereditary hearing loss (*DFNA1–DFNA6*, *DFNA8* and *DFNB1–DFNB8*), *DFNA7* was localized to chromosome 1q21-q23 in one extended Norwegian family.

RESULTS

Family material

The pedigree of the whole family is shown in Figure 1 with the included family members indicated by a dot. The progressive sensorineural hearing loss in the family, beginning in the high frequencies is inherited according to an autosomal dominant pattern. Transmission through affected males makes mitochondrial inheritance unlikely, and the occurrence of several instances of male to male transmissions exclude an X-linked inheritance pattern. We have performed genetic analysis of 42 family members of whom 22 had hearing loss. In the linkage study we only included individuals above the age of 10.

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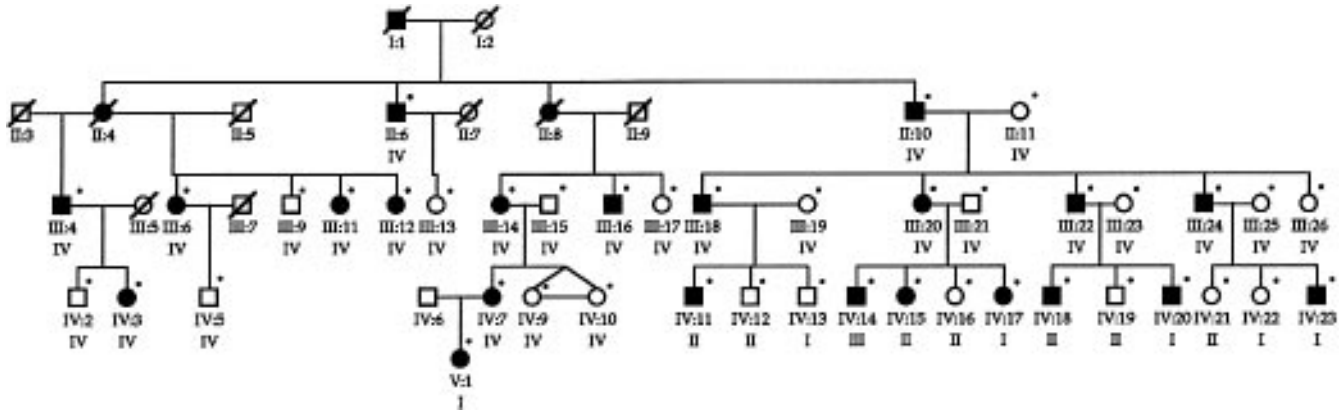


Figure 1. The pedigree for the Norwegian deafness family. The 42 family members included in the study are indicated with a dot and have their corresponding liability class number below their pedigree number. Liability class number I (10–15 years), II (16–20 years), III (21–25 years) and IV (>25 years) have the penetrance values of 0.5, 0.75, 0.90 and 0.99 respectively.

Table 1. Two-point linkage analysis between the *DFNA7* mutation and chromosome 1 markers

	Recombination fraction θ							(θ)	Z_{max}
Marker	0.0	0.01	0.05	0.1	0.2	0.3	0.4		
ApoA2	–1.455	3.320	4.139	4.144	3.498	2.483	1.222	0.072	4.19
D1S104	0.858	6.069	6.253	5.882	4.729	3.288	1.611	0.034	6.29
D1S426	4.139	4.073	3.807	3.460	2.720	1.910	1.014	0.0	4.14
D1S194	5.756	5.659	5.262	4.746	3.644	2.434	1.112	0.0	5.76
D1S196	7.653	7.530	7.027	6.370	4.954	3.385	1.644	0.0	7.65
D1S210	7.165	7.048	6.570	5.948	4.610	3.135	1.508	0.0	7.17
D1S218	4.250	4.168	3.835	3.409	2.522	1.599	0.662	0.0	4.25
D1S416	3.736	3.675	3.433	3.132	2.479	1.711	0.841	0.0	3.74
D1S466	–0.738	2.996	3.332	3.168	2.509	1.705	0.865	0.047	3.33
D1S238	–8.908	0.380	2.375	2.934	2.844	2.140	1.083	0.136	3.01

Linkage analysis

Initially, possible linkage to previously published loci for non-syndromic deafness was investigated in a core pedigree of 20 persons. To test for linkage to the loci *DFNA1–DFNA6, DFNA8* and *DFNB1–DFNB8*, genetic markers flanking these loci were analyzed. All loci could be excluded with LOD scores below –2 (results not shown). A genome search using a set of polymorphic microsatellite markers with 20 cM resolution was then initiated, starting on chromosome 1 (6). A total of 13 markers were tested until the first suggestion of linkage was detected with the markers *D1S194* and *D1S196*.

Nine additional markers spanning the region around *D1S194* and *D1S196* were subsequently included in the analysis, as illustrated in Figure 2. The pedigree was extended to include all available family members, a total of 42. Longitudinal studies in this family have demonstrated considerable variability in age of onset and rate of progression of the hearing loss. The linkage was therefore performed assuming age dependent liability classes (see Materials and Methods). The family members included in the extended analysis, have their liability classes assigned in Figure 1. Absence of recombination was observed with several markers (Table 1), and in two-point linkage analysis a maximal LOD score of 7.65 at θ = 0.0 was obtained with *D1S196*. Recombinants were obtained with *D1S104* (centromeric) and with *D1S466* (telomeric), limiting the candidate region to approximately 22 cM (1q21-q23) (Fig. 3). Multipoint linkage analysis was also performed, and a maximal LOD score of 9.68 was reached in the region from *D1S426* to *D1S416* (results not shown).

DISCUSSION

Linkage analysis in the Norwegian family resulted in the identification of a seventh locus (*DFNA7*) for non-syndromic ADD located in 1q21-q23. The candidate region is limited to a 22 cM region by recombinants in the family (Fig. 3). As indicated in Figure 3, individual IV:7 shows recombination between *D1S104* and *D1S426*, which is confirmed in her daughter V:1, limiting the centromeric border of the region to *D1S104*. Individual III:4 is recombinant between *D1S416* and *D1S466*, which is also found in his daughter IV:3, setting the telomeric border to *D1S466*. Both recombination events are confirmed with flanking markers. Recombination is also seen between markers *D1S466* and *D1S238* as shown by III:16 and IV:14. The disease segregating allele size for the latter marker is 288. Although individual IV:14 is 288–292 for this marker, his mother (III:20) who is affected has 288–292 and his father (III:21) is 284–288. It is therefore clear that IV:14 received the 292 allele with the

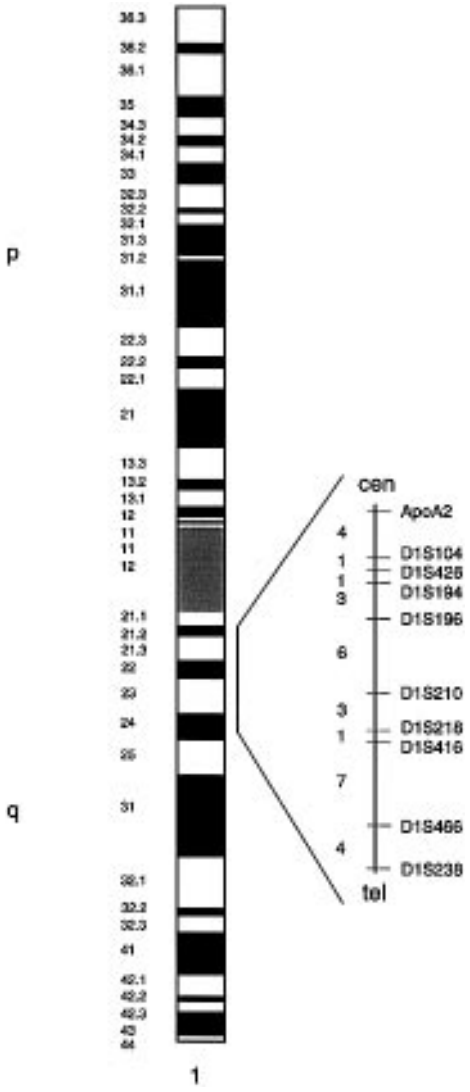


Figure 2. Localization and distances between the markers on 1(q21-q23).

disease and the 288 allele on the normal chromosome. The rest of the affected individuals in the family (total of 16) share the same disease haplotype. However, it should be noted that individual IV:22, who is a non-affected female of 14.5 years has inherited the disease haplotype. She has the full disease associated haplotype, spanning markers on both sides of the disease locus, demonstrating that the haplotype is not the consequence of recombination. She is still quite young, thus it remains to be seen if she will become affected or whether she demonstrates the existence of incomplete penetrance. She was tested at age 14.5 year and had normal hearing. None of the other unaffected individuals in the analysis shows the disease associated haplotype. Most affected family members experienced decreased hearing before they were 20 years. One family member (III:18), however, had a late occurrence of hearing loss. Aged 20, at conscription, a minor hearing loss was found, and he was exempted from noise exposure during the military service. He experienced hearing problems from age 22, and at age 26 his first audiogram recorded showed a 60 dB hearing loss. Some age variation of the onset and progression of hearing impairment is therefore present in his family (Fig. 4). In the majority of the affected family members with successive audiograms recorded, the hearing loss was ≥45 dB by age 15 (Fig. 4). There is however, no evidence of reduced penetrance.

Another locus on chromosome 1 has previously been reported (*DFNA2*) (7). *DFNA2* and *DFNA7* must involve independent genes because of the 120 cM distances between these loci, furthermore, we have previously excluded the *DFNA2* location in our family.

Several candidate genes are located in 1q21-q23. Recently, a gene involved in X-linked deafness (*DFN3*) has been identified as *POU3F4* (8,9). *POU3F4* is a member of the POU (Pit-1, Oct-1, Unc-86) domain family of genes which contains DNA binding motifs and act as transcription factors (10). Interestingly, there is also a POU domain gene called *POU2F1* or *OCT1* (11,12) located only 0.8 cM from the *D1S196* marker which gave the highest LOD score. The expression of *OCT1* in the cochlea of rat during embryogenesis has been demonstrated, which is consistent with its contribution to inner ear phenotypic development (13). *OCT1* is therefore an obvious candidate gene for *DFNA7*. Other transcriptional factors, like LMX1 (14) and USF1 (15) are reported to be encoded from the candidate region.

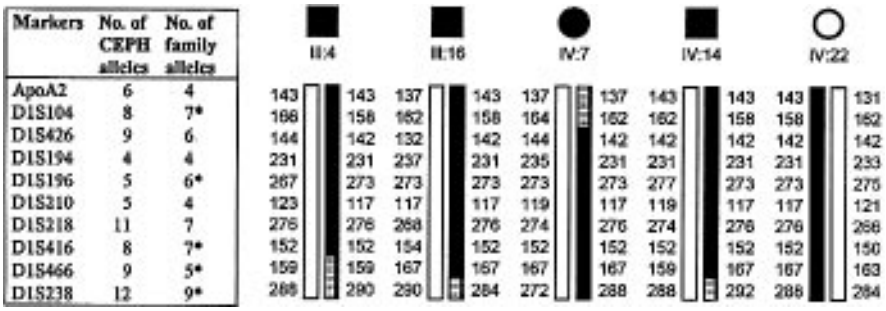
Hereditary sensory-motor neuropathy of Charcot-Marie-Tooth type 1B (CMT1B) is associated with sensorineural hearing loss (16). Recently, mutations underlying CMTB1 have been located in the *myelin protein zero* (P_0) gene which is located in the candidate region (17,18). It is possible that isolated hearing loss is an allelic manifestation of mutations in the P_0 gene. Such mutations might be of a different kind than those leading to CMT. A mutation search in P_0 is ongoing. The relatively large genetic interval presented here probably contains many more candidate genes yet to be discovered. Reports of more families with hearing deficit linked to *DFNA7* may enable us to narrow down the genetic interval and thereby facilitate the investigation of a few relevant candidate genes.

Our findings support the idea of extensive genetic heterogeneity among genes involved in autosomal dominant deafness. These results represent the first localization of a gene involved in hearing impairment in a Norwegian family. There is also evidence for genetic heterogeneity within the Norwegian population, which is assumed to be relatively isolated in genetic terms. Another large Norwegian family with ADD, supposedly unrelated to the family reported here, was described by Teig (19). Linkage to 1q21–23 or to other previously described deafness loci (*DFNA1–6, DFNA8* and *DFNB1–8*), has been excluded (results not shown). In a third family with ADD we have also excluded linkage to the *DFNA7* locus. Efforts are presently being made to locate the gene responsible for hearing loss in these families.

MATERIALS AND METHODS

Family material

Forty-two family members in four generations were available for linkage studies (Fig. 1). Chromosome analysis of cultured lymphocytes from one hearing impaired male showed normal male karyotype: 46,XY. Family members had pure tone audiometry performed repeatedly for up to 26 years. The sensorineural hearing loss was of post-lingual onset. Variation in development of hearing loss before age 15 is shown in Figure 4. In all cases the hearing loss progressed from the high frequency region. The



Markers	No. of CEPH alleles	No. of family alleles
ApoA2	6	4
D1S104	8	7*
D1S426	9	6
D1S194	4	4
D1S196	5	6*
D1S210	5	4
D1S218	11	7
D1S416	8	7*
D1S466	9	5*
D1S238	12	9*

Figure 3. Haplotype analysis in the key family members, showing the region between the markers ApoA2 and D1S238 (1q21-q23). The filled bars show the disease segregating haplotype as it is segregating in 16 out of 22 affected persons from the family. Individuals III:4, III:16, IV:7, IV:14 show recombinations compared to the original disease segregating haplotype. Individual IV:22 however, who has normal hearing at age 14.5, is a non recombinant carrying the full disease associated haplotype. On the left is the markers with both the corresponding number of CEPH alleles and the number of alleles segregating in the Norwegian family. *indicates that there are alleles observed in the family which have not been reported for the CEPH samples.

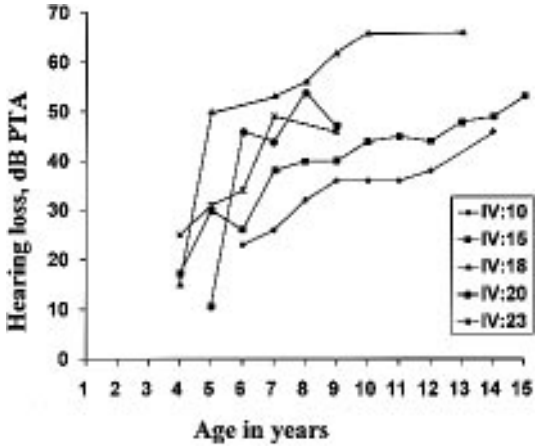


Figure 4. Progression of sensorineural hearing loss with increasing age in five representative affected siblings and first cousins. The hearing loss is expressed as pure tone average (PTA) across 500 Hz, 1 kHz, 2 kHz, and 4 kHz, both ears together. Labeling of each curve is according to the family pedigree (Fig. 1). The majority of affected family members had developed a ≥ 1145 dB hearing loss by age 15.

audiograms thus initially had a sharply sloping pattern affecting the high frequencies, approaching a gently sloping pattern in mid-life (20). No vestibular dysfunction could be found in any hearing impaired person.

Genetic analysis

Venous blood samples were taken after informed consent from co-operative family members and genomic DNA was extracted by standard techniques. Polymorphic microsatellite markers were chosen primarily from the Généthon maps (6), but some were provided by Dr Wadelius, Uppsala, Sweden. PCR amplifications were done on 75 ng of genomic DNA using ^{33}P labelled dCTP. PCR conditions were 28 cycles of 95°C for 50 s, 55°C for 50 s and 72°C for 25 s and a final extension 72°C for 7 min. The PCR products were separated on 6% or 8% denaturing polyacrylamide gels and visualised by audioradiography after 1–3 days exposure. An M13 sequencing ladder was used as the standard for fragment size determination.

Linkage analysis

Twopoint LOD were performed using the Linkage Package 5.1 (21). Autosomal dominant inheritance was assumed with a disease frequency of 0.001. A high variability regarding the onset of hearing loss has earlier been demonstrated in this family. Therefore, the linkage analysis was performed assuming age dependent liability classes with penetrance values of 0.5, 0.75, 0.90 and 0.99 for age groups 10–15, 16–20, 21–25, and >25 years, respectively. Recombination frequencies were assumed to be equal in both females and males, and phenocopy rate was set to 0. The marker allele frequencies were estimated from the family and spouses using the ILINK program (21). Marker allele frequencies in the *DFNA7* family did not significantly differ from the ones observed in the CEPH sample (GDB), but as indicated in Figure 3 by an asterisk, we found unreported alleles for five of the markers. Multipoint linkage analysis was performed using the LINKMAP option of the FASTLINK package (22).

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