DFNA49, a novel locus for autosomal dominant non-syndromic hearing loss, maps proximal to DFNA7/DFNM1 region on chromosome 1q21–q23

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Key points

- Autosomal dominant inheritance accounts for about 20% of the cases of hereditary non-syndromic sensorineural hearing loss (NSSHL)—that is, hearing loss not associated with other clinical features. So far, 36 loci have been mapped in familial cases that segregate autosomal dominant NSSHL (DFNA), and 17 genes have been identified.

- Here we report the location of a novel autosomal dominant deafness locus on 1q21–q23, DFNA49, found by studying a large Spanish family with non-syndromic, progressive mid-frequency hearing loss of post-lingual onset. A maximum lod score of 6.02 at \( \theta = 0 \) was obtained for markers D1S3784 and D1S3785. Analysis of recombinant haplotypes placed the deafness locus within a 4 cM region defined by markers GDB:190880 and D1S3788.

- This genetic interval is proximal to and does not overlap with the previously identified loci, DFNA7 and DFNM1, on 1q21–q23.

- Screening of candidate genes within the DFNA49 interval (KCNJ9, KCNJ10, ATP1A2 and CASQ1) did not reveal the mutation causing this deafness.

Where possible, results from previous audiological tests were collected.

Genotyping and linkage analysis

A wide genome scan was performed with 394 microsatellite markers distributed with an average spacing of 10 cM (ABI Prism Linkage Mapping Set 2; Applied Biosystems, Foster City, CA, USA). Markers for the exclusion of all known DFNA loci and for fine mapping of the critical interval were taken from the Ge
énthon human linkage map and from the Marshfield chromosome 1 map (http://research.marshfieldclinic.org/genetics). Additional short tandem repeats (STRs) in the DFNA49 region were identified by inspection of publicly available sequence data (NCBI: http://www.ncbi.nlm.nih.gov). Flanking primers were designed for polymerase chain reaction (PCR) amplification of these sequences.

Abbreviations: ADNSSHL, autosomal dominant non-syndromic sensorineural hearing loss; NSSHL, non-syndromic sensorineural hearing loss; PCR, polymerase chain reaction; STRs, short tandem repeats.
STRs. The amplicons were confirmed to be polymorphic microsatellite markers and deposited into the GDB database (http://www.gdb.org), being assigned the following D numbers: D1S3780 (GDB: 11511382), D1S3783 (GDB: 11511388), D1S3784 (GDB: 11511390), D1S3785 (GDB: 11511392), and D1S3786 (GDB: 11511394). The order of markers used in this work was established by integrating genetic and physical maps (NCBI).

Fluorescently labelled alleles were analysed in an ABI Prism 310 automated DNA sequencer (Applied Biosystems). Linkage analysis was performed using the Linkage 5.1 software package. 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 to be equal to each other.

**DNA sequencing analysis**

Four candidate genes, KCNJ9 (MIM600932), KCNJ10 (MIM6002208), ATP1A2 (MIM182340) and CASQ1 (MIM114250) in the DFNA49 interval were screened by heteroduplex analysis and direct sequencing of PCR products generated from genomic DNA of affected subjects. Primers were designed to amplify each exon and adjacent intron-exon boundaries. PCR was performed by standard procedures as previously described. Heteroduplex analysis was carried out in mutation detection enhancement gels (BioWhittaker, Rockland, ME, USA) according to the manufacturer’s protocol. Sequences of PCR products were analysed in an automated DNA sequencer (ABI Prism 310; Applied Biosystems).

**RESULTS AND DISCUSSION**

**Clinical features**

Affected subjects of the family present a symmetrical, bilateral, and progressive nonsyndromic sensorineural hearing loss. The hearing loss appears in the first decade of life in affected members. The earliest clinical evidence of hearing loss in the family was obtained from individual III:12 at the age of 8 years. Initially, the hearing loss in this family is moderate for low and mid frequencies and mild for high frequencies (4000–8000 Hz), so the affected members show in this stage a gently upsloping audiometric profile. Later, it progresses to moderate in the 125–250 Hz and 4000–8000 Hz ranges and to severe in the 500–2000 Hz range (U shaped audiometric profile) in the fourth decade. Linear regression analysis, based on all available audiograms from affected subjects, showed a 0.7 dB/year age linked progression of the hearing loss at all frequencies. Affected subjects of the family did not exhibit either tinnitus or clinical features suggestive of vestibular dysfunction.

**Linkage analysis**

Linkage to previously published loci responsible for autosomal dominant deafness (DFNA) was investigated in a core pedigree of 26 persons (subject IV:3 is 20 months old, clearly below the age of deafness onset, and therefore he was not included in the linkage analysis) obtaining negative results. A wide genome search was then performed to map the deafness locus, using a set of 394 microsatellite markers with an average spacing of 10 cM. Interestingly, the two point lod scores obtained for markers D1S498 (3.01 at theta = 0) and D1S484 (3.72 at theta = 0.05) on chromosome 1q21–q23
were suggestive of linkage to this region (fig 2). We then
tested additional markers spanning this region, and evidence
of linkage to markers D1S3780, D1S3783, D1S1167, and
D1S2707 was found, with maximum two point lod scores of
6.02 at theta = 0 for markers D1S3784 and D1S3785 (table 1).
Extensive alterations of the disease gene frequency or the
allele frequencies of microsatellite markers did not change
the conclusions of the analysis. The inspection of recombin-
ant haplotypes in subjects III:9 and II:8 placed the hearing
loss locus between the proximal marker GDB: 190880 and the
distal one D1S3786, which define a critical interval of about
4 cM for the novel locus, DFNA49 (fig 1 and 2). All the
patients in the family share the same disease haplotype,
which is also carried by the 20 month old child IV:3. Periodic
audiometric examination of this subject will be performed to
precisely determine when the hearing impairment begins in
this family.

Another dominant locus for hearing loss (DFNA7) was
mapped to a 22 cM region on 1q21–q23 between D1S104 and
D1S466 in a Norwegian family (fig 2). The DFNA7 interval
has been reported to include the DFNM1 locus, a dominant
modifier that suppresses the DFNB26 phenotype. The
DFNA7/DFNM1 region had previously been excluded for
linkage in our family. As shown in fig 2, a genetic distance of
6 cM separates this interval and the one defined for DFNA49.
It should also be noted that the audiometric response
associated with DFNA7 and DFNA49 in the reported families
is clearly different. DFNA7 patients show a sharply sloping
audiogram affecting the high frequencies, instead of the U
shaped audiometric pattern found in the DFNA49 affected
subjects.

Candidate gene analysis
No genes for syndromic deafness have been mapped to
DFNA49 critical interval. This interval spans 0.9 Mb and
includes 23 known genes and several predicted or poorly
characterised genes according to the annotation in the NCBI
database. Of these, we selected ATP1A2, CASQ1, KCNJ10 and
KCNJ9 for screening of mutations (fig 2).

ATP1A2 consists of 23 exons and encodes the NaK-ATPase
alpha2 subunit, which is responsible for the catalytic activity
of the enzyme. In mouse cochlea, the alpha2 isofrom is expressed in
the spiral structures: ligament, limbus, and ganglion, where
it is believed to play an important role in the development

Figure 2  Physical and genetic maps of the chromosome 1q21–q23 region showing the localisation of DFNA49 and DFNA7/DFNM1 critical
intervals. cM, centimorgan; kb, kilobase. KCNJ9 (OMIM #600932); KCNJ10 (OMIM #602208); ATP1A2 (OMIM #182340) and CASQ1 (OMIM
#114250). For clarity, the genetic and physical distances are not represented to scale.
and maintenance of the fluid and electrolyte balance. \textit{CASQ1} has 11 exons\(^{37}\) and codes for calsequestrin, a calcium binding protein found in the cytoplasm of outer hair cells where it is thought to store and release calcium from membrane bound intracellular storage sites.\(^{38}\) Calcium is believed to play a major signalling role in outer hair cells by controlling metabolism, cytoskeletal integrity, cell shape, and cell excitability. \textit{KCNJ10} consists of two exons\(^{39}\) and encodes an inwardly rectifying K+ channel subunit that is strongly expressed in the cochlear stria vascularis, where it is crucially involved in the generation of the endocochlear potential,\(^{18,19}\) and in the satellite cells that surround the neurones and axons of the cochlear and vestibular ganglia.\(^{20}\) Knockout mice for \textit{KCNJ10} present a profound deafness and severe structural degeneration of the cochlea.\(^{21,22}\) \textit{KCNJ9} has three exons\(^{33}\) and codes for a K+ channel with structural and functional similarity to \textit{KCNJ10},\(^{40}\) although its expression has not been reported in the inner ear so far.

The exons and flanking regions of the four genes were investigated by heteroduplex and DNA sequencing in two affected subjects (I-1 and II-2). These analysis only revealed the non-pathogenic polymorphisms, 1097T\(\rightarrow\)CT in exon 3 of \textit{KCNJ9} (NCBI SNP cluster id: rs3001040) and the IVS1-\textit{R}\textsuperscript{C} intronic change in \textit{KCNJ10}, which was present in several control individuals with normal hearing. This last polymorphism allowed us to verify the presence in affected subjects of the two intact copies of \textit{KCNJ10} exon 2, which include the entire coding region, so excluding the deletion of, or insertions in, this exon as the cause of deafness (data not shown).

Reports of more families with hearing deficit linked to DFNA49 may enable a further refinement of the critical interval facilitating the identification of the responsible gene. We are now searching for novel candidate genes at the DFNA49 interval.

**ACKNOWLEDGEMENTS**

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**Table 1**

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\(h\) = 14; \(a\) = 10

**REFERENCES**

rectifying potassium channel, KAB-2 (Kir4.1), in cochlear stria vascularis of inner ear: its specific subcellular localization and correlation with the formation of endocochlear potential. J Neurosci 1997;17:4711–21.


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Susceptibility to Crohn’s disease may lurk in enzyme mutation

A preliminary study of genetic polymorphisms affecting transforming enzymes in the gut has singled out for the first time microsomal epoxide hydrolase as a likely candidate for susceptibility to Crohn’s disease (CD).

Homozgyous Tyr 113 substitution for His 113 in exon 3 of the microsomal epoxide hydrolase (EPXH) gene was the only one of seven variants in a series of genes coding for detoxifying enzymes with a significantly higher frequency in patients with CD than controls (47% vs 21%, respectively). Tyr/Tyr genotype was also more common within the patient group than in the control group (allele frequency 0.67 vs 0.61, respectively), and the odds of having the Tyr 113 allele were almost three times higher. This variant and another, in exon 4 of the same gene, were not associated with disease site, disease onset, fistulas, or history of bowel resection.

The variants were identified by PCR-RFLP (restriction fragment length polymorphism). They included those in cytochrome P-450 1A1C (CYP1A1 3’ flanking region, CYP1A1 exon 7); glutathione S-transferases mu-1, pi-1, and theta-1 (GSTM1, GSTP1, GSTT1); and epoxide hydrolases (EPXH) in exons 3 and 4. Screening was performed on 151 consecutive outpatients at a hospital clinic in the Netherlands and age and sex matched healthy controls; all were Caucasian.

Reactive oxygen species and their toxic metabolites have been implicated in inflammation of the gut in CD. Detoxifying enzymes, such as glutathione S-transferases and epoxide hydrolases, may also have a role, so polymorphisms affecting their activity may affect risk of developing CD.