A fifth locus for otosclerosis, OTSC5, maps to chromosome 3q22–24


Otosclerosis is caused by abnormal bone homeostasis of the otic capsule leading to bony fixation of the stapedial footplate in the oval window. Because the transmission of sound waves from outer to inner ear is disturbed by this fixation, the disease is characterised by conductive hearing impairment. In some cases, an additional sensorineural component develops across all frequencies, leading to mixed hearing impairment. The conductive component of the hearing impairment can be restored by stapes replacing microsurgery; however, the sensorineural component cannot surgically be corrected.

Otosclerosis has a prevalence of 0.3–0.4% in the Caucasian population. The etiology of the disease is unknown, but epidemiological studies indicate the involvement of genetic as well as environmental factors. However, large families segregating otosclerosis are very rare, whereas there are frequent sporadic cases and smaller families with only a few affected members. Based on these findings, otosclerosis can be considered a genetically complex disease, caused by an interaction of genes and environmental factors, but with rare monogenic forms.

To date, three autosomal dominant otosclerosis loci have been reported: OTSC1 on chromosome 15q25–26, OTSC2 on chromosome 7q34–36, and OTSC3 on chromosome 6p21–22. In addition, a fourth locus, OTSC4, has been reserved by the Human Genome Organisation nomenclature committee, but this has not been published. None of the responsible genes have been cloned. In this study, we identified a large Dutch family segregating an autosomal dominant otosclerosis. After exclusion of the known loci, a genome-wide screen and linkage analysis in this family revealed the existence of a fifth otosclerosis locus, OTSC5, localised on chromosome 3q22–24.

METHODS

Clinical diagnosis

The family was identified via the Department of Otorhinolaryngology of the University Medical Center St.-Radboud Nijmegen (The Netherlands) (fig 1). Pure-tone audiometry was performed in all persons with air conduction at 125, 250, 500, 1000, 2000, 4000, and 8000 Hz, and bone conduction at 250, 500, 1000, 2000, and 4000 Hz. Tympanic membrane compliance and ipsi- and contralateral stapedial reflex decay were also measured. Individuals in whom stapes fixation with an otosclerotic focus was confirmed during microsurgery were considered affected.

To date, three autosomal dominant otosclerosis loci (OTSC1 on chromosome 15q25–26, OTSC2 on chromosome 7q34–36, and OTSC3 on chromosome 6p21–22) have been reported, while a fourth locus, OTSC4, has been reserved. None of the responsible genes have been cloned.

In this study, a fifth locus, OTSC5, was localised on chromosome 3q22–24 by linkage analysis in a Dutch pedigree segregating autosomal dominant otosclerosis. Recombinant individuals delineate a 15.5 Mb candidate interval between the markers D3S1292 centromeric and D3S1744 telomeric.

In the future, refinement of the candidate region and mutation analysis of candidate genes will lead to the identification of the disease-causing gene in this family.

Key points

- Otosclerosis, a bone disorder of the otic capsule, is the most common cause of hearing impairment among white adults with a prevalence of 0.3–0.4%. The etiology of the disease is unknown, but there are strong suggestions that genetic as well as environmental factors may be involved.
- To date, three autosomal dominant otosclerosis loci (OTSC1 on chromosome 15q25–26, OTSC2 on chromosome 7q34–36, and OTSC3 on chromosome 6p21–22) have been reported, while a fourth locus, OTSC4, has been reserved. None of the responsible genes have been cloned.
- In this study, a fifth locus, OTSC5, was localised on chromosome 3q22–24 by linkage analysis in a Dutch pedigree segregating autosomal dominant otosclerosis. Recombinant individuals delineate a 15.5 Mb candidate interval between the markers D3S1292 centromeric and D3S1744 telomeric.
- In the future, refinement of the candidate region and mutation analysis of candidate genes will lead to the identification of the disease-causing gene in this family.

Genotype analysis

Blood samples from study participants were obtained after informed consent and were used as a source of genomic DNA, which was isolated using standard techniques. The microsatellite markers used to analyse linkage to the known otosclerosis loci were D15S652, D15S1004, and D15S657 for OTSC1, D7S495, D7S2560, D7S684, D7S2513, and D7S2426 for OTSC2, and D6S5388, D6S273, D6S291, D6S1680, and D6S426 for OTSC3. Information for all markers was taken from The Genome Database (http://www.gdb.org/).

A genome-wide screen was performed by fluorescence-based semi-automated genotyping using 380 microsatellite markers selected from the final Généthon linkage map with an average heterozygosity of 0.76. For fine mapping of the candidate region additional microsatellite markers were selected from public databases. After individual PCR amplification on an MJ Research thermocycler, PCR products were pooled and size fractionated by electrophoresis on a Prism ABI 3700 DNA sequencer (Applied Biosystems). Allele sizes were determined using GENESCAN 2.1.1. and Genotyper V3.7 software (Applied Biosystems).
Linkage analysis

All genotypes were checked for Mendelian inconsistencies using LINKRUN software (Wienker, unpublished). Two-point LOD scores were calculated between each marker locus and otosclerosis under the assumption of an autosomal dominant model with 90% penetrance, a phenotype rate of 1%, a frequency of 0.0001 for the disease allele, and equal allele frequencies for each marker using LINKAGE V5.21 software. Equal recombination frequencies between males and females were assumed.

Mutation analysis

Primers were designed from intronic sequences flanking the exons of PCOLCE2 and CHST2 to amplify genomic DNA from patient and control individuals. Direct sequencing of the PCR product was performed on both forward and reverse strands on an ABI 3100 sequencer using the DyenamicTM ET Terminator Cycle Sequencing Kit (Amersham Biosciences).

RESULTS AND DISCUSSION

Clinical analysis

The family presented in this study segregates an autosomal dominant otosclerosis (fig 1) in eight of 10 affected members confirmed by stapes replacing microsurgery. In the two remaining subjects (III:11 and III:12), the presence of an air–bone gap, closing at a frequency of 2000 Hz, together with the absence of stapedial reflexes clinically confirmed the diagnosis of otosclerosis. Nine family members and one spouse had normal hearing.

Genetic analysis

Initially, genetic analysis was performed on 18 family members, of whom 10 were affected and seven unaffected, with one unaffected spouse (fig 1). Statistically significant exclusion of the three known otosclerosis loci was demonstrated (table 1). A genome-wide screen in this family revealed suggestive linkage at chromosome region 3q22–24, from which a maximum two-point LOD score of 2.98 was obtained for marker D3S1569 at θ = 0.05. Two additional unaffected subjects were identified (III:14 and III:18). After genotyping these individuals for markers in the region of interest, a maximum two-point LOD score of 3.46 was obtained for marker D3S1569 at θ = 0.05 (table 2). Haplotypes were constructed to confine the interval of the linked region. A recombination event in individuals III:5 and III:9 placed the disease locus proximal to D3S1744, while a recombination event in individuals III:12 and III:13 mapped the disease-causing gene distal to D3S1292. Hence, the maximal interval of linkage with the otosclerosis phenotype is bordered by D3S1292 (centromeric) and D3S1744 (telomeric) within a region of ~15.5 Mb, according to the last draft of the human genome sequence (Build 34 Version 1). A physical map of the candidate region is presented in figure 2.

Individual III:2, who in the linkage analysis was considered to be affected, does not carry the haplotype segregating with the disease in the other patients. Because he underwent bilateral stapes replacing microsurgery, he could be considered to be a phenocopy. In view of the high frequency of otosclerosis in the white population, the presence of a phenocopy in an otosclerosis family is not surprising. Alternatively, this could reflect an unrecognised double crossover, which is extremely unlikely, given the small intermarker distances.

The 15.5 Mb OTSC5 interval contains 59 identified genes and 45 gene predictions. Two genes were considered very good otosclerosis candidate genes: PCOLCE2 and CHST2. The PCOLCE2 (procollagen COOH-terminal proteinase enhancer protein 2) gene product is found to be a glycoprotein that binds the COOH-terminal propeptide of type I procollagen and is highly expressed in non-ossified cartilage in developing tissues. The otic capsule is unique in retaining calcified

Figure 1 Pedigree of the Dutch family with autosomal dominant otosclerosis, showing the most likely haplotypes for the chromosome 3 markers. The haplotype linked to otosclerosis is indicated with a black bar.
cartilage, known as globuli interossei, throughout life and in lacking bone remodelling. Because these factors are unique to the otic capsule they may predispose to otosclerosis. The expression pattern of PCOLCE2 suggests a possible role for this gene in the pathogenesis. The CHST2 (carbohydrate sulfotransferase 2) gene product is a Golgi-associated sulfotransferase. These sulfotransferases sulfonate glycoproteins, glycosaminoglycans, peptidyl tyrosine, and heparan sulfates and play important roles in intercellular communication. Because otosclerosis has been hypothesised to result from a disorder of the extracellular matrix of the cartilaginous rests present in the adult temporal bone, the clear relationship of CHST2 to extracellular matrix function makes this gene a strong candidate gene. However, mutation analysis of the

![Figure 2](https://www.jmedgenet.com)

**Figure 2** Localisation of OTSC5 on chromosome 3q22–24. The black bar indicates the region that contains the gene for otosclerosis.

<table>
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coding region and the intron–exon boundaries of both genes did not reveal any disease-causing mutation.

Identification of new otosclerosis loci will increase the possibility of detecting the first otosclerosis-causing gene in the near future. Identification of this first gene will probably facilitate the identification of the causative genes localised in the other known otosclerosis loci. Characterisation of these genes will give us increased knowledge regarding the underlying molecular pathway of otosclerosis and of the otic capsule in general.

ACKNOWLEDGEMENTS

We thank the family members for their cooperation, and Inka Szangolies, Regina Pospiech, and Gudrun Nürnberg for excellent technical support. KVDB holds a predoctoral research position with technical support. KVDB holds a predoctoral research position with the Instituut voor de aanmoediging van Innovatie door Wetenschap en Technologie in Vlaanderen (IWT-Vlaanderen). This research was performed in the framework of the Interuniversity Attraction Poles program P5/19 of the Belgian Federal Science Policy Office.

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This study was supported in part by NIH grant R01DC05218 (RJHS, GVC), and grants from the University of Antwerp (GVC) and from the Vlaams Fonds voor Wetenschappelijk Onderzoek (FWO) (GVC).

Conflict of interest: none declared.

Table 2 Two-point LOD scores between otosclerosis and chromosome 3 markers (OTSCS)

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REFERENCES


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\textit{J Med Genet} 2004 41: 450-453
doi: 10.1136/jmg.2004.018671