

## Report

**A Second Gene for Otosclerosis, *OTSC2*, Maps to Chromosome 7q34-36**

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Otosclerosis due to abnormal bone homeostasis of the otic capsule is a frequent cause of hearing loss in adults. Usually, the hearing loss is conductive, resulting from fixation of the stapedial footplate, which prevents normal ossicular vibration in response to sound. An additional type of sensorineural hearing loss may be caused by otosclerotic damage to the cochlea. The etiology of the disease is unknown, and both environmental and genetic factors have been implicated. Autosomal dominant inheritance with reduced penetrance has been proposed, but large families are extremely rare. To elucidate the pathogenesis of the disease, identification of the responsible genes is essential. In this study, we completed linkage analysis in a Belgian family in which otosclerosis segregates as an autosomal dominant disease. After excluding linkage to a known locus on chromosome 15 (*OTSC1*), we found linkage on chromosome 7q, with a multipoint LOD score of 3.54. Analysis of key recombinant individuals maps this otosclerosis locus (*OTSC2*) to a 16-cM interval on chromosome 7q34-36 between markers D7S495 and D7S2426.

Hearing impairment is one of the most frequent handicaps in the Western world. It can be conductive (caused by abnormalities in the conduction of sound in the outer or middle ear) or sensorineural (resulting from defects in the perception of sound in the inner ear) or both (mixed). Otosclerosis is a common bone disorder of the otic capsule, unique to the endochondral layer of the temporal bone and known to affect human beings only (Declau and Van de Heyning 1996). Otosclerosis leads to a progressive hearing impairment characterized by conductive or mixed hearing loss. The conductive hearing loss is due to a fixation of the stapedial footplate in the oval window, and cochlear otosclerotic foci are thought to cause a sensorineural component in some cases. Although stapedial microsurgery has proved to be a successful means of reducing the conductive hearing

impairment, otosclerosis gives rise to considerable morbidity (Somers et al. 1997).

Otosclerosis is divided into histological and clinical types. The term “histological otosclerosis” is used to describe otosclerosis in the absence of clinical symptoms or manifestations. This type of disease, which is discovered by routine sectioning of temporal bones at autopsy, has a prevalence of 6%–7% (Morrison and Bunday 1970). Clinical otosclerosis (MIM 166800), in contrast, implies the presence of hearing impairment (Shambaugh 1949). Its prevalence is 0.2%–1% among white adults, and the age at onset is usually 20–40 years (Gordon 1989). In ~85% of patients, both ears are involved (Somers et al. 1994).

The etiology of otosclerosis is complex, and it probably involves an interaction between genes and environmental factors. Epidemiological studies support autosomal dominant inheritance with reduced penetrance (Albrecht 1922; Larsson 1962; Morrison 1967; Causse and Causse 1984; Ben Arab et al. 1993), although a viral involvement also has been suggested (Niedermeyer et al. 1994; Arnold et al. 1996; McKenna et al. 1996). Because the etiology of otosclerosis remains poorly characterized, no effective medical therapy has been devel-

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**Table 1**  
**Comparison of Markers for Chromosome 15q25-26 and the Known Otosclerosis Locus (OTSC1)**

MARKER	TWO-POINT LOD SCORES AT $\theta =$						
	.0	.01	.05	.1	.2	.3	.4
D15S652	-3.54	-3.16	-2.65	-2.18	-1.12	-.48	-.14
D15S1004	-5.08	-3.83	-2.35	-1.56	-.72	-.27	-.06
D15S657	-4.52	-3.50	-1.94	-1.17	-.51	-.20	-.05

oped to prevent or stabilize the disease. Large families of  $\geq 10$  affected persons are difficult to ascertain, and other factors, such as reduced penetrance and a high frequency of phenocopies, complicate efforts at linkage analysis. At present, only one locus for otosclerosis, *OTSC1* on chromosome 15q25-26, has been identified, and the responsible gene has not been cloned (Tomek et al. 1998). Otosclerosis is therefore one of the last important types of hearing impairment for which a genetic cause remains to be elucidated.

The Belgian family presented here (fig. 1) displays autosomal dominant otosclerosis and was ascertained through the Saint-Augustinus Hospital. We performed pure-tone audiometry for all family members, using air conduction at 250, 500, 1000, 2000, 4000, and 8000 Hz and bone conduction at 500, 1000, 2000, and 4000 Hz. Ten persons with surgically confirmed otosclerosis were considered affected. For the patients who had not had surgery, the clinical diagnosis of otosclerosis was based on audiological data, which we analyzed using an algorithm described elsewhere (Govaerts et al. 1998). In brief, the audiometric curves of these persons were compared with age- and sex-dependent data according to the International Organization for Standardization 7029 standard (1984). Using analysis of variance, we determined the frequency that was the most sensitive indicator of otosclerosis in this family, and we ranked family members according to their hearing loss, expressed as hearing standard deviations (HSD) at this frequency. In addition, we measured tympanic membrane compliance and ipsilateral and contralateral stapedial reflex decay. Seven additional persons, who had a conductive or mixed hearing loss that exceeded three HSDs together with absent or immeasurable stapedial reflexes, were classified as affected. Because the age at onset of otosclerotic hearing loss varies, only family members who were  $>60$  years of age and had normal hearing ( $<2$  HSDs) were considered unaffected. Only one family member (1.7 HSDs at age 70 years) and three relatives by marriage were labeled unaffected. The status of the remaining four persons was considered uncertain. Information on deceased the deceased was obtained by questioning living family members.

After the 25 participants gave informed consent, blood samples were obtained and were used as a source

of genomic DNA, which was isolated by standard techniques. All these 25 DNA samples (from family members numbered in fig. 1) were used in subsequent LOD score calculations. LOD scores were calculated using the FASTLINK computer program (Cottingham et al. 1993). The linkage parameters were chosen in compliance with the body of older studies (Larsson 1960; Morrison 1967; Gapany-Gapanavicius 1975; Donnell and Alfi 1980; Causse and Causse 1984), which suggest that otosclerosis is inherited as an autosomal dominant disease with reduced penetrance. As standard linkage parameters, the frequency of the otosclerosis gene was set at .0001, and the disease was assumed to be autosomal dominant and 90% penetrant. To allow for possible phenocopies, this chance was set at 1%, because without surgical exploration it is often difficult to exclude hearing impairment of other origins. Equal recombination frequencies between males and females were assumed. For each marker, the number of alleles in the LOD score calculations was set at the observed number of alleles in the pedigree ( $N$ ); allele frequencies were set at  $1/N$ .

First, linkage to the known otosclerosis locus on chromosome 15q25-q26 was investigated using the linked markers D15S652, D15S1004, and D15S657 (Tomek et al. 1998). This locus was excluded with negative two-point LOD scores (table 1). A genome search was then performed on 25 DNA samples, using genetic markers from the Cooperative Human Linkage Center screening set (Weber, version 6). This set contains 391 microsatellite markers covering the complete genome, with an average intermarker spacing of 10 cM and an average heterozygosity of 76%. Because we did not detect linkage with these markers, we screened another 66 markers from a second genomewide microsatellite set for fluorescence-based, semiautomated genome mapping (Reed et al. 1994) and 120 markers from the Génethon genetic linkage map (Dib et al. 1996). Although this second analysis did not generate a two-point LOD score  $>3$ , there were five regions with a positive two-point LOD score  $>1$ . At this time, 75% of the complete genome had

**Table 2**  
**Comparison of Markers for Otosclerosis and Chromosome 7 (OTSC2)**

MARKER	TWO-POINT LOD SCORES AT $\theta =$						
	.0	.01	.05	.1	.2	.3	.4
D7S509	-.54	-.06	.59	.76	.64	.35	.1
D7S495	-.26	.02	.44	.57	.48	.27	.09
D7S2560	1.21	1.18	1.07	.94	.68	.44	.22
D7S684	1.52	1.72	1.88	1.76	1.29	.74	.27
D7S2505	1.15	1.35	1.53	1.44	1.03	.56	.16
D7S2513	1.29	1.49	1.64	1.52	1.02	.45	.05
D7S2426	.11	.60	1.26	1.44	1.28	.83	.27
D7S1826	.15	.52	1.07	1.25	1.14	.77	.30

ease. However, the realization of these aims will depend, in part, on the degree of heterogeneity among affected persons. In the only two completed linkage studies, two distinct loci have been identified. In both instances, the defined candidate regions are large, and it is possible that the relevant genes will not be identified until additional families are ascertained and studied.

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## Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Cooperative Human Linkage Center, CHLC Marker Maps, <http://lpg.nci.nih.gov/html-chlc/ChlcMarkerMaps.html> (for markers used for genotyping)  
Généthon, <http://www.genethon.fr> (for markers used for genotyping)  
Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for otosclerosis [MIM 166800], *TIF1α* [MIM 603406], and *PLOD3* [MIM 603066])

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