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Otosclerosis: A Genetically Heterogeneous Disease Involving at Least Three Different Genes

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Otosclerosis is caused by abnormal bone homeostasis of the otic capsule, resulting in hearing impairment in 0.3%–0.4% of the white population. The etiology of the disease remains unclear and environmental as well as genetic factors have been implicated. We localized the first autosomal-dominant locus to chromosome 15 in 1998 (*OTSC1*) in an Indian family and, recently, we reported the localization of a second gene for otosclerosis to a 16 cM interval on chromosome 7q (*OTSC2*). In this study, we recruited and analyzed nine additional families (seven Belgian and two Dutch families with 53 affected and 20 unaffected subjects) to investigate the importance of these loci in autosomal-dominant otosclerosis. We completed linkage analysis with three microsatellite markers of chromosome 15 (D15S652, D15S1004, D15S657) and five microsatellite markers of chromosome 7 (D7S495, D7S2560, D7S684, D7S2513, D7S2426). In two families, results compatible with linkage to *OTSC2* were found, but in the seven remaining families *OTSC1* and *OTSC2* were excluded. Heterogeneity testing provided significant evidence for genetic heterogeneity, with an estimated 25% of families linked to *OTSC2*. These results indicate that, besides *OTSC1* and *OTSC2*, there must be at least one additional otosclerosis locus. (Bone 30:624–630; 2002) © 2002 by Elsevier Science Inc. All rights reserved.

Key Words: Otosclerosis; Genetic linkage; Genetic heterogeneity; *OTSC1*; *OTSC2*; Otic capsule.

Introduction

Otosclerosis is a common bone dysplasia that is unique to the endochondral bone layer of the otic capsule. This capsule forms from a cartilaginous substrate precursor that persists as a calcified cartilaginous matrix in which primitive bone is neither removed nor replaced. Otosclerosis affects this unique homeostasis resulting in abnormal resorption and deposition of new bone, mostly of the woven type.[4] The pathological bone has a variable appearance, with areas of differing cellularity. Gradual fixation of the stapes limits, and finally prevents, stapedial footplate motion, resulting in a progressive, conductive hearing impairment. The disease may extend to the inner ear, producing a combined (conductive and sensorineural) hearing impairment.

Otosclerosis is divided into histological and clinical types. "Histological otosclerosis" refers to a disease process without clinical symptoms or manifestations that only can be discovered by sectioning of the temporal bone at autopsy. Its prevalence has recently been estimated as 2.5% in the white population.[3] "Clinical otosclerosis" (MIM 166800), necessarily refers to the presence of otosclerosis at a site where it causes conductive and/or sensorineural hearing loss. Mean age of onset is between 20 and 40 years, and in the majority of persons (~85%) both ears are involved.[8,13] Stapes microsurgery is widely acknowledged to be the treatment of choice for otosclerosis, but nevertheless otosclerosis gives rise to considerable morbidity.[12]

The etiology of otosclerosis is poorly characterized and probably involves an interaction between genes and environment factors. This lack of knowledge is an important obstacle in the development of better therapies or prevention strategies for this disease. In 1998, we used a large Indian family segregating for autosomal-dominant otosclerosis to map the first otosclerosis locus (*OTSC1*) on chromosome 15q25–26.[14] Recently, we localized a second locus for otosclerosis (*OTSC2*) on chromosome 7q34–36 in a large Belgian family.[15] Neither gene has been cloned.

We recruited and analyzed nine additional families segregating for otosclerosis to investigate genetic heterogeneity. In seven of these families, *OTSC1* and *OTSC2* were excluded, confirming that otosclerosis, in parallel with sensorineural hereditary hearing impairment, is a genetically heterogeneous condition.

Subjects and Methods

Clinical Diagnosis

The families were ascertained through the Department of Otolaryngology of four different hospitals: family A through the St.-Vincentius Hospital, Antwerp (Belgium); families B and D through the University Hospital of Antwerp (Belgium); families C, E, F, and G through the St.-Augustinus Hospital, Antwerp (Belgium), and families H and I through the University Medical

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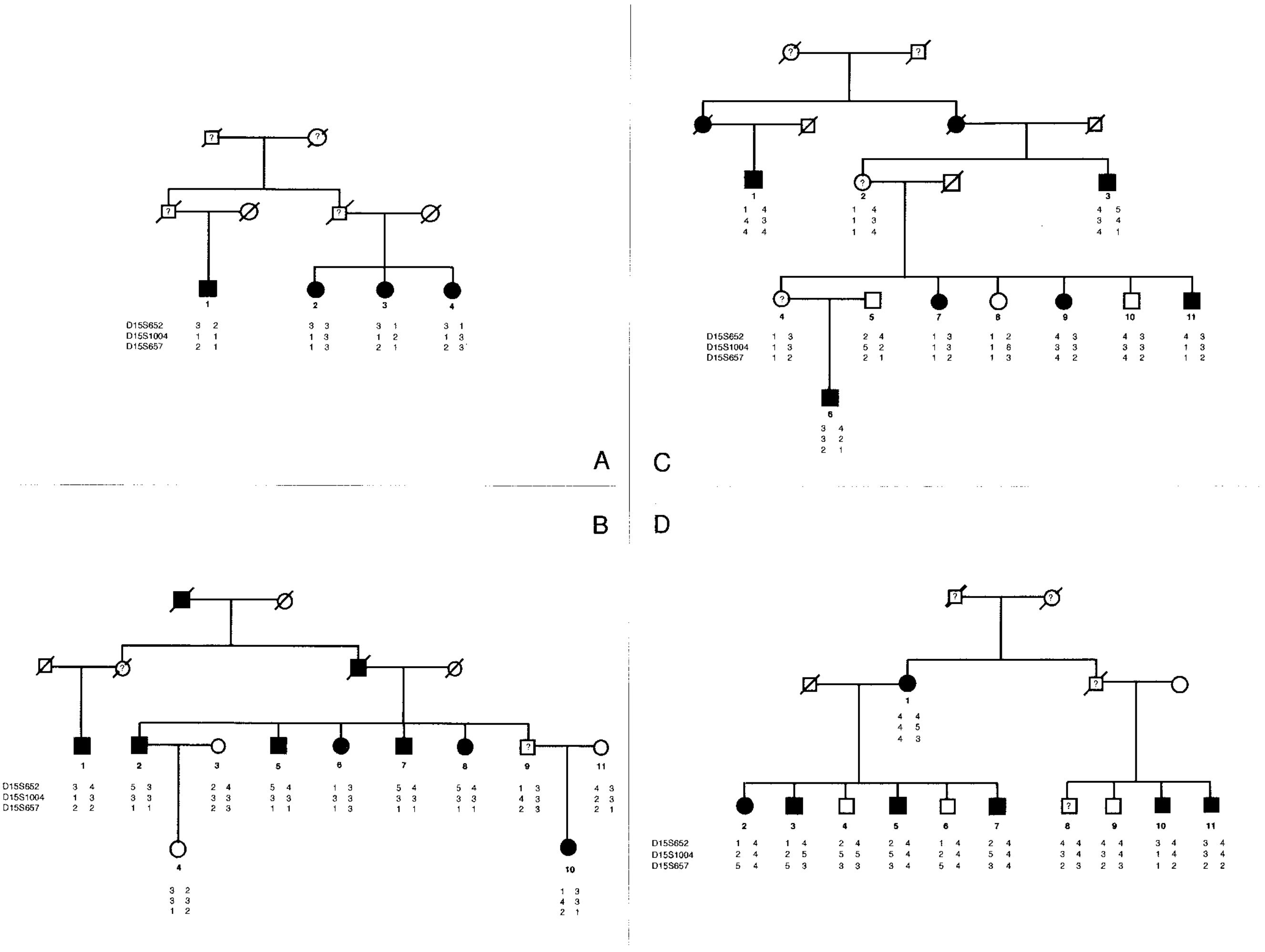

Figure 1. Pedigrees of the nine additional families (A)–(I) with autosomal-dominant otosclerosis, showing the haplotypes for the chromosome 15 markers of *OTSC1*. Only family members whose DNA was analyzed are numbered. Affected family members are represented by solid symbols, male family members by squares, and female members by circles. Deceased family members for whom we had no information on their hearing or living family members with an atypical or limited hearing impairment are indicated with a question mark.

Center St.-Radboud, Nijmegen (The Netherlands) (**Figures 1 and 2**). We performed pure-tone audiometry on 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. We also measured tympanic membrane compliance and ipsi- and contralateral stapedial reflex decay. Persons in whom stapesfixation with an otosclerotic focus was confirmed during stapes microsurgery were considered affected. In nonoperated persons, the clinical diagnosis of otosclerosis was based on audiologic data. Persons with a conductive or mixed hearing loss together with absent or immeasurable stapedial reflexes were classified as affected. Because of variability in the age of onset, only family members >50 years of age and with normal hearing were considered "unaffected." The remaining persons were given an "uncertain" affection status. Information on deceased members of the pedigree was obtained by history.

Genotyping

Blood samples from study participants were obtained after informed consent and used as a source of genomic DNA, which was isolated by standard techniques. The microsatellite markers used in the linkage analysis were D15S652, D15S1004, and D15S657 for *OTSC1* and D7S495, D7S2560, D7S684, D7S2513, and D7S2426 for *OTSC2*. Information for all markers was taken from the Genome Database (http://gdbwww.gdb.org/). The genetic distance separating the *OTSC1* markers was taken from Tomek et al.[14]: D15S652–9 cM–D15S1004–4 cM–D15S657. The genetic distance separating the *OTSC2* markers was taken from Généthon maps[5]: D7S495–0.6 cM–D7S2560–2 cM–D7S684–4.5 cM–D7S2513–8.8 cM–D7S2426 for *OTSC2*. Polymerase chain reaction (PCR) was carried out under standard conditions. One of the primers was synthesized with an M13 sequence at the 5′ end. A 5′-IRD labeled (800 nm) M13 primer was included in the PCR reaction, thus labeling the PCR product. Gel electrophoresis and pattern visualization were performed using a DNA analyzer (Model 4200, Li-Cor, Inc.).

Linkage Analysis

Multipoint lod scores were calculated using the VITESSE computer program.[2] The linkage parameters were chosen in compliance with the body of older studies,[1,6,7,10] suggesting that otosclerosis is inherited as an autosomal-dominant disease with

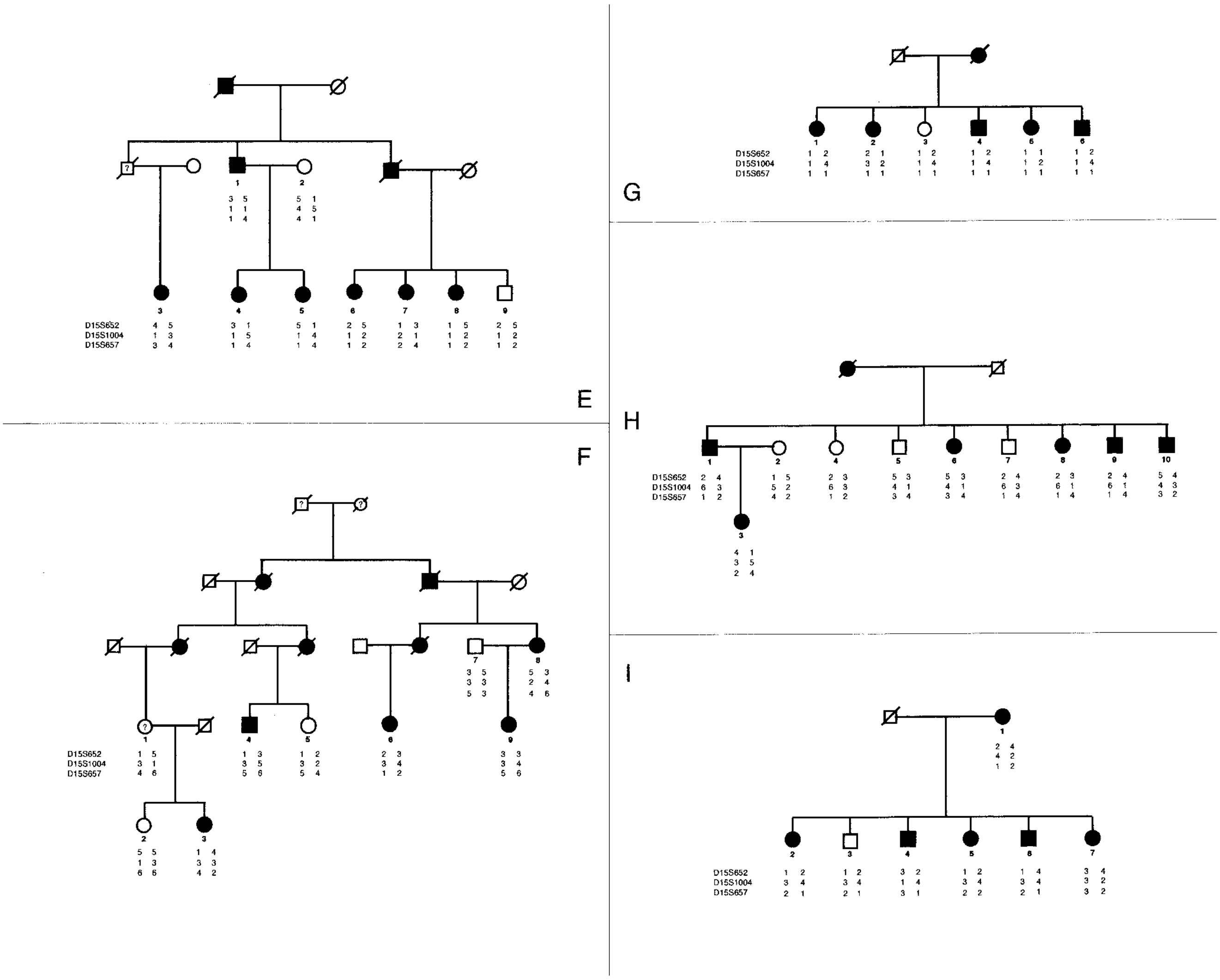

Figure 1. (*Continued*)

reduced penetrance. As standard linkage parameters, the frequency of the otosclerosis gene was set at 0.0001 and the disease was assumed to be 50% penetrant and autosomal-dominant. 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.

Multipoint lod scores were calculated using four point rolling lods in the D15S652–D15S657 interval (with markers D15S652, D15S1004, and D15S657) and in the D7S2560–D7S2513 interval (with markers D7S2560, D7S684, and D7S2513). These loci were also analyzed for linkage heterogeneity, using the Homog program.[11]

Results

Genotyping was performed on DNA from 78 persons, 53 of whom were diagnosed as affected based on audiograms and surgery. Twenty persons were labeled unaffected and five were given an uncertain diagnosis. Haplotypes were constructed, as shown in Figures 1 and 2. The graphs of the multipoint calculations in each family are presented in **Figure 3**.

OTSC1 analysis revealed no haplotype sharing by all affected subjects within each of the families analyzed. In addition, multipoint lod scores were negative in each family, providing evidence of nonlinkage in these nine families to the locus on chromosome 15. With *OTSC2*, in contrast, in Belgian families B and D, haplotype segregation was consistent with linkage to this locus. In family B, one haplotype of *OTSC2* was shared by all affected subjects. A maximum multipoint lod score of 1.91 was obtained. In family D, one haplotype of *OTSC2* was shared by all affected individuals except person 5, who inherited the normal haplotype. If the family was linked to *OTSC2*, the hearing loss in this individual may have been caused by nongenetic factors (phenocopy) or by one or more other genes. In this family, a maximum multipoint lod score of 0.74 was obtained.

To investigate the candidate loci further, heterogeneity testing was also performed using the Homog program on multipoint lod scores from the regions of interest. The original *OTSC2* family, as well as the nine families described in this study, were included

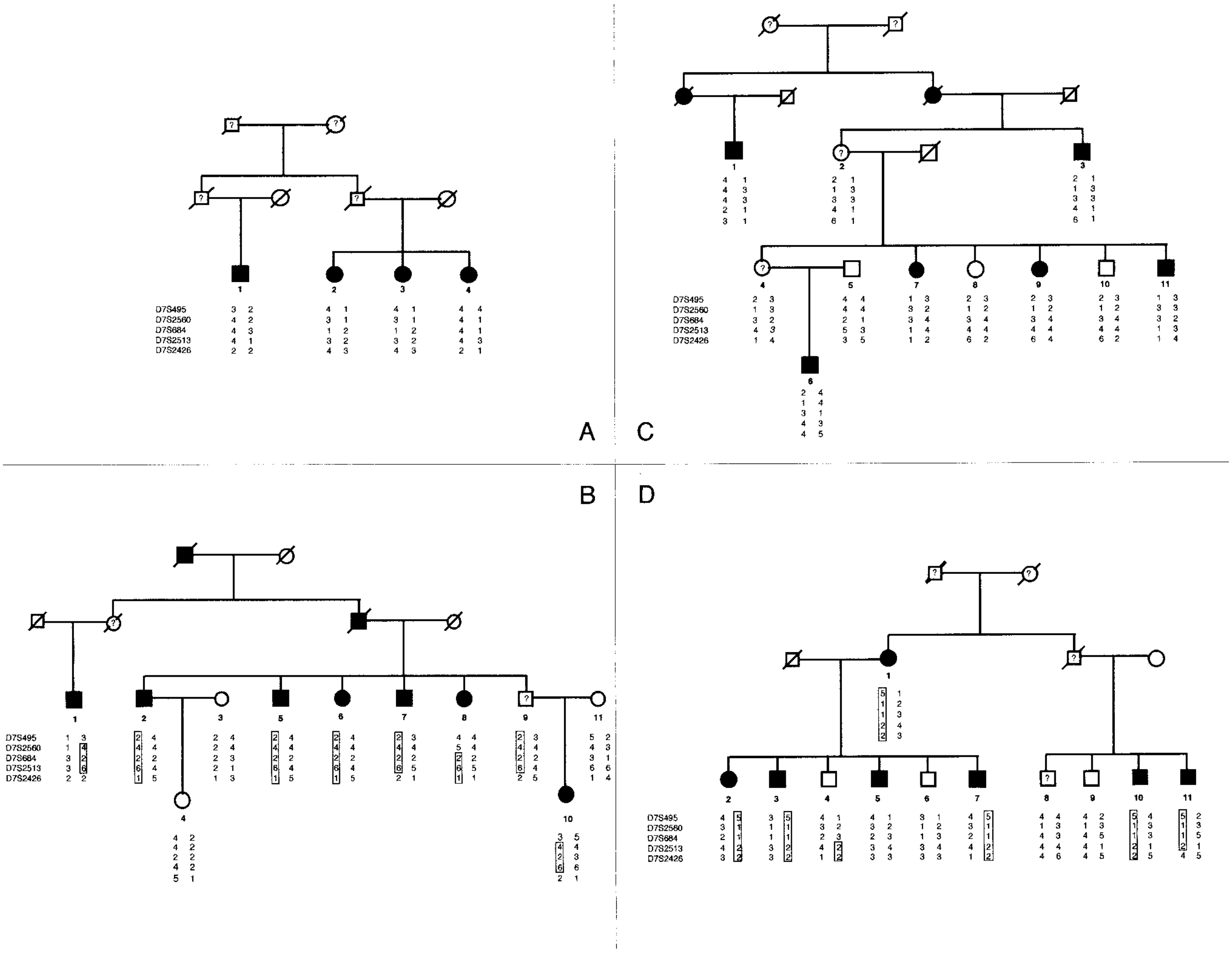

Figure 2. Pedigrees of the nine additional families (A)–(I) with autosomal-dominant otosclerosis, showing the haplotypes for the chromosome 7 markers of *OTSC2*. The haplotype probably linked to otosclerosis is boxed. Only family members whose DNA was analyzed are numbered. Affected family members are represented by solid symbols, male family members by squares, and female members by circles. Deceased family members for whom we had no information on their hearing or living family members with an atypical or limited hearing impairment are indicated with a question mark.

in the analysis. For the *OTSC1* locus, there was no evidence to support linkage under a model of heterogeneity nor under a model of homogeneity. For the *OTSC2* locus there was no evidence to support linkage under a model of homogeneity, but there was evidence of linkage under a model of heterogeneity between the markers D7S2560 and D7S2513 at $\alpha = 0.25$ (α is the proportion of families segregating the linked gene). Hypothesis testing showed significant evidence for linkage and heterogeneity (H_2) vs. linkage and homogeneity (H_1) or vs. neither linkage nor heterogeneity; (H_0) ($\chi^2 = 14.595$, 2 *df*, $p < 0.001$).

To investigate the possibility that some of the three *OTSC2* families (B and D and the original *OTSC2* family) were related, we compared interfamilial disease haplotypes. Three different linked haplotypes were found, with the exception of marker D7S2560, in which the original *OTSC2* family and family D shared a common allele variant. The frequency of this allele in the white population is 5%, making it impossible to know whether the shared allele represents identity by descent or identity by state.

Discussion

Two loci for otosclerosis have been discovered. We mapped the first locus in 1998 to a 14.5 cM interval on chromosome 15q25–26 (*OTSC1*) in a large multigenerational family of Indian origin.[14] An additional family, originating from Tunisia, has also been reported to be linked to this locus (Drira et al., "Etude génétique et localisation d'un gène de l'otospongiose chez les familles Tunisiennes," 106 Congres Français d'Oto-Rhino-Laryngologie et de Chirurgie de la Face et du Cou, Paris, 1999). Recently, we mapped the second gene for otosclerosis to a 16 cM interval on chromosome 7q34–36 (*OTSC2*) in a large Belgian family.[15]

In this study we have reported significant evidence for genetic heterogeneity based on the analysis of nine additional families segregating for otosclerosis, seven of Belgian and two of Dutch origin. This is the first report describing linkage analysis in a series of otosclerosis families. Our results suggest that *OTSC1* is probably a minor otosclerosis locus in persons of western European descent, because this locus was excluded in all families.

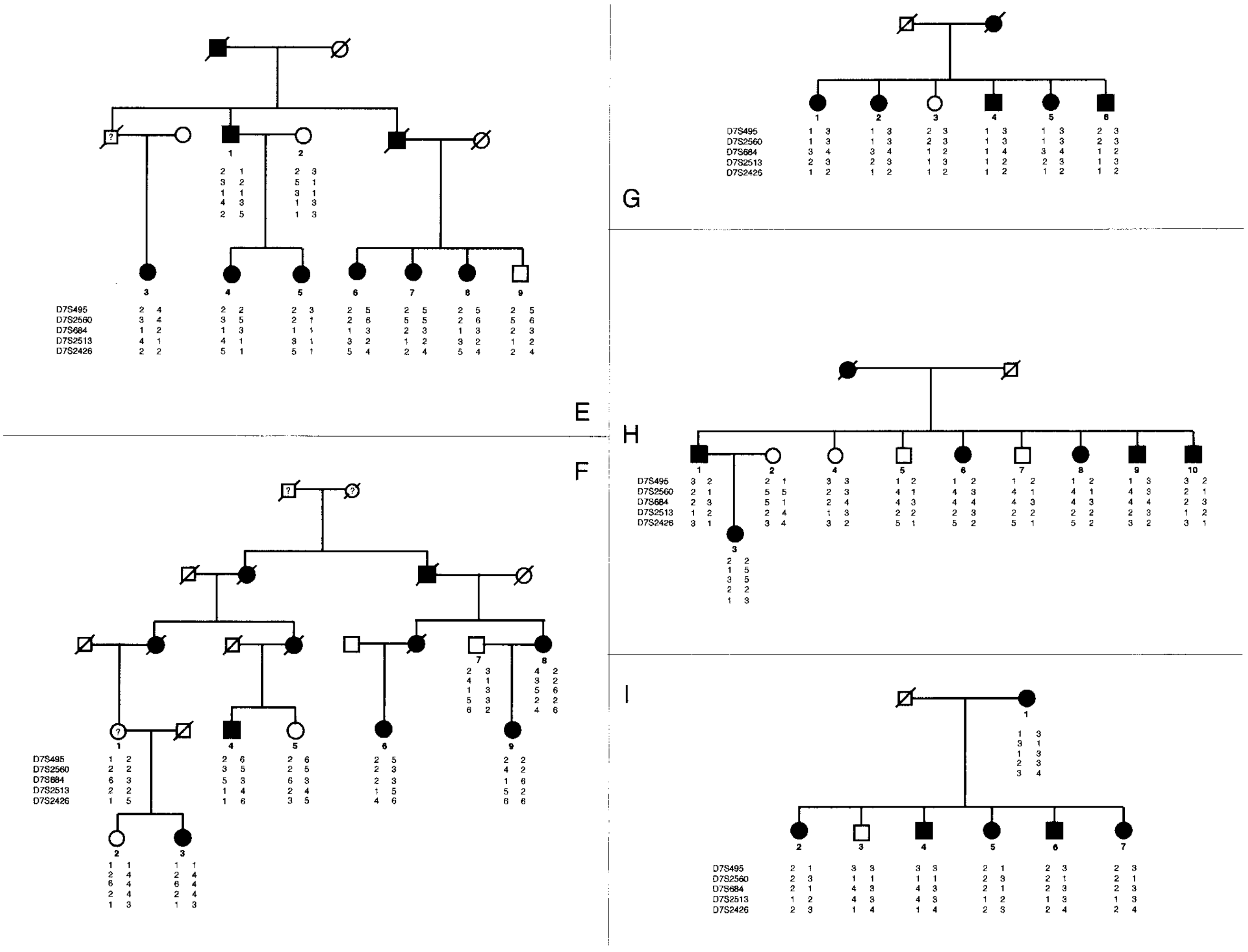

Figure 2. (*Continued*)

However, we found linkage to *OTSC2* in Belgian family B, with a lod score of 1.91. The generally accepted significance threshold for lod scores of +3, corresponding to a p value of 10^{-4}, is only valid when a complete genome search has been performed, because multiple testing for hundreds of loci is taken into account.[9] In this study, only two loci were tested and a lod score of +2 (corresponding roughly to $p = 10^{-3}$) was a reasonable significance threshold. Linkage to *OTSC2* was therefore very likely for family B. Although the Belgian family D was too small to yield statistically significant lod scores, haplotype segregation was consistent with linkage to the *OTSC2* locus, except for a single person, which may represent a phenocopy. Confirmation of linkage will therefore only be possible after the *OTSC2* gene has been identified, when this gene can be analyzed in patients from family D.

The *OTSC2* gene may play a major role in otosclerosis in western Europe, because formal heterogeneity testing using HOMOG indicated that disease-related genes in 25% of the families may be linked to the *OTSC2* region on chromosome 7q34–36. Hypothesis testing of heterogeneity given linkage vs. homogeneity given linkage (H_2 vs. H_1) was significant ($p < 0.001$), showing that, in addition to *OTSC1* and *OTSC2*, at least one other otosclerosis-causing gene must exist.

The genes responsible for otosclerosis are likely to have specific roles in bone homeostasis in the otic capsule. Little is known about this process at the molecular level and the identification of these genes is the first step in the elucidation of mechanisms of bone turnover of the otic capsule. This knowledge could lead to substantial improvements in our ability to diagnose and possibly even prevent this type of hearing impairment.

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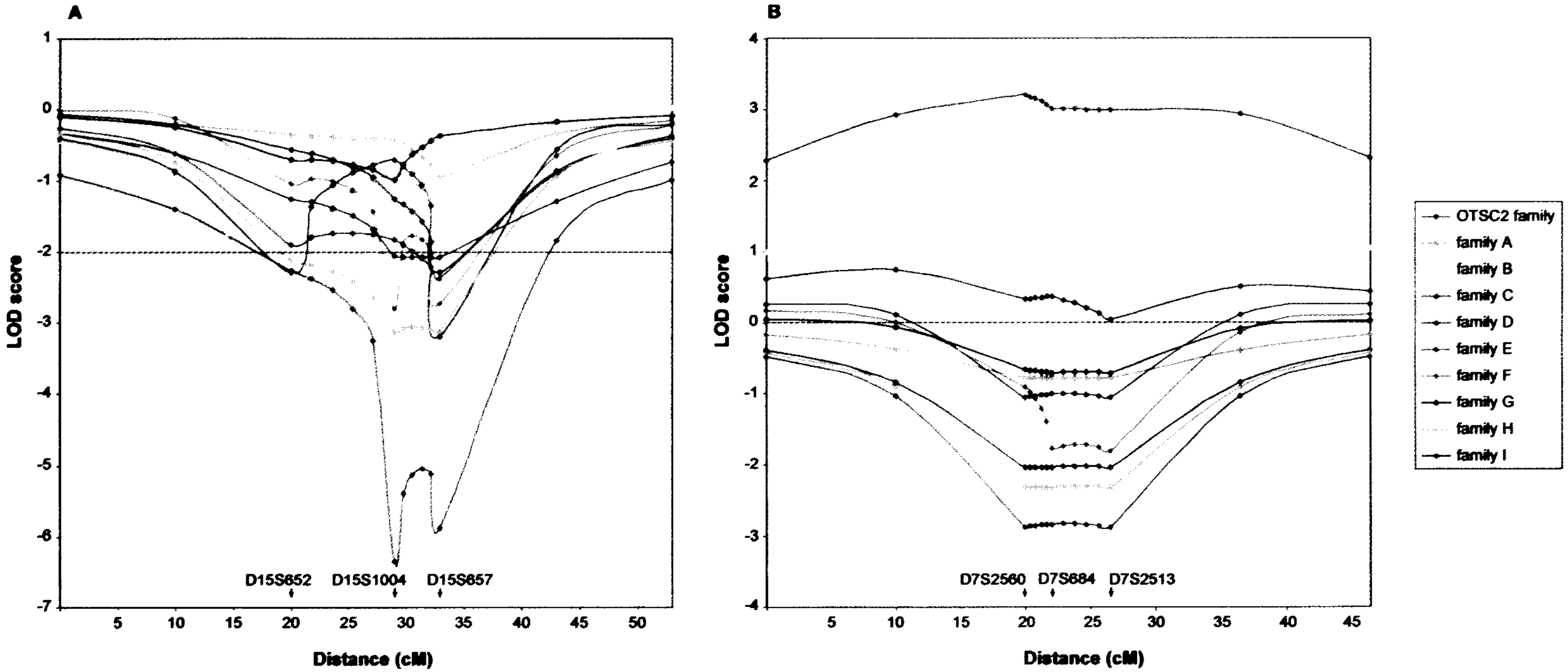

Figure 3. Graphical presentation of multipoint lod score calculations in the *OTSC1* (A) and *OTSC2* region (B). The position of selected markers in the regions of interest are indicated.

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