ORIGINAL ARTICLE

Chromosomal Mapping and Phenotypic Characterization of Hereditary Otosclerosis Linked to the *OTSC4* Locus

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Objective: To perform chromosomal mapping and clinical analysis of hereditary otosclerosis linked to the fourth locus for otosclerosis (*OTSC4*) in an Israeli family.

Design: Pedigree study.

Setting: A genetics of hearing loss research laboratory, a clinical genetics laboratory, a center for speech and hearing, and an otolaryngology department at a university and medical centers in Israel.

Subjects: An Israeli family of which 24 members were ascertained and a pedigree was constructed; 12 members had otosclerosis.

Interventions: Confirmation of otosclerosis by surgery (3 subjects) and by audiologic evaluation, medical history, and family history (9 subjects), and whole-genome scanning to identify the chromosomal region of the mutant locus.

Main Outcome Measures: Chromosomal location of the otosclerosis locus.

Results: Linkage to the 16q21-23.2 interval was identified and confirmed with a logarithm of odds (LOD) score of 3.97 at $\theta = 0$. The new locus for otosclerosis was designated *OTSC4*. The *OTSC4* interval of 9 to 10 megabase includes several genes involved in the immune system and bone homeostasis that may be good candidates for genes otosclerosis.

Conclusion: The elucidation of the *OTSC4* gene may disclose the etiology of the disorder, and the functional and structural analysis of the protein may open new options for diagnosis, treatment, and prevention of otosclerosis.

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OTOSCLEROSIS IS A COMMON disorder of the otic capsule of the human temporal bone. An unknown trigger initiates bone remodeling, characterized by an active phase of resorption of mature bone and deposition of a spongy vascularized bone, which eventually results in an inactive phase of a dense sclerotic mass.[1-4] Invasion of otosclerotic foci into the stapediovestibular joint leads to fixation of the stapes in the oval window, resulting in clinical otosclerosis, which occurs in about 0.2% to 1% of white individuals.[5]

Otosclerosis is characterized by progressive conductive hearing impairment ranging up to 60 dB, which might develop into mixed or even sensorineural hearing loss (SNHL). The hearing loss (HL) first affects the low frequencies. This first stage is thought to be caused by the presence of highly cellular fibrous tissue that characterizes the spongiotic phase. As the pathologic changes progress to a stage of localized bony fixation of the anterior part of the footplate, it is thought to result in a moderate conductive HL spanning all frequencies. The HL increases to moderately severe when the diffuse bony ankylosis involves the entire circumference of the annular ligament, completely preventing the motion of the stapes in the oval window.[2,6]

The cause of SNHL in otosclerosis is unknown. Cochlear otosclerotic foci adjacent to the basilar membrane might constrict the cochlear lumen, with distortion of the basilar membrane, leading to inhibition of the traveling sound wave and therefore adding a sensorineural component to the HL.[7] Another possible explanation is that the SNHL is caused by hydrolytic enzymes secreted into the perilymph by an impaired blood supply to the stria vascularis, due to vascular shunts.[8,9] An additional recent hypothesis, based on immunostaining of ion transport molecules in temporal bones of patients with

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histologic otosclerosis, suggested that defective potassium ion recycling causes a decrease in the endocochlear potential, leading to cochlear otosclerosis.[10] A sensorineural component may also be a result of the Carhart notch effect that is characteristic of otosclerosis.[11]

Controversy exists as to whether pure SNHL can be caused by isolated cochlear otosclerosis without stapedial involvement, since histologic evidence of pure cochlear otosclerosis is very rare.[12] Nevertheless, it is quite common for SNHL to develop as the disease progresses. Long-term follow-up shows that approximately 10% of individuals with otosclerosis ultimately develop severe to profound SNHL.[9]

The age at onset for otosclerosis is usually 20 to 40 years, and in most cases both ears are involved, but HL is often asymmetric.[13] A definitive diagnosis of otosclerosis can be made only by surgery, but existence of family history of the disease, as well as some other clinical characteristics, are considered sufficient to diagnose otosclerosis in a family where only some members were surgically confirmed.[2,14,15] Both environmental and genetic factors have been implicated in otosclerosis. A 2:1 female preponderance suggests hormonal involvement as well.[16] An association between mutations in the collagen gene *COL1A1*, which underlies osteogenesis imperfecta, and otosclerosis was found in a small percentage of cases.[17] In some other cases of otosclerosis, a collagen autoimmune mechanism was suggested because antibodies against collagen II and IX were present.[18] Otosclerosis is inherited in an autosomal dominant fashion with reduced penetrance, estimated at 40%.[1,19] Thus far, 4 locations have been reported for otosclerosis genes: chromosomes 15q25-q26 (*OTSC1*),[20] 7q34-36 (*OTSC2*),[21] 6p21.3-22.3 (*OTSC3*),[22] and 3q22-24 (*OTSC5*),[23] although none has been cloned.

METHODS

SUBJECTS

A 5-generation pedigree of an Israeli family, of Yemenite Jewish origin, was constructed (**Figure 1**). The disorder was transmitted from generation to generation, and an autosomal dominant age-dependent inheritance with reduced penetrance was suggested. The project was approved by the Helsinki (IRB [institutional review board]) committees at Tel Aviv University, Tel Aviv, Israel; the Chaim Sheba Medical Center, Tel Hashomer, Israel; the National Helsinki Committee for Human Genetic Research of the Israel Ministry of Health; and by the University of Washington, Seattle (IRB protocol 99-110). Twenty-four individuals, both affected and unaffected, were ascertained. Particular emphasis was given to medical history regarding subjective degree of HL, age at onset, evolution of HL, symmetry of the hearing impairment, hearing aids, presence of tinnitus, medication, noise exposure, pathologic changes in the ear, and other relevant clinical manifestations. Inclusion criteria for the affected group included (1) surgically confirmed otosclerosis or family history of otosclerosis, (2) HL with a conductive component characteristic of otosclerosis, and (3) normal-appearing tympanic membrane. Blood was drawn when subjects signed Helsinki committee–approved consent forms, for both DNA extraction and establishment of lymphoblastoid cell lines, and genomic DNA was extracted from the 24 family members.

AUDIOMETRY

Pure-tone audiometry was performed with a portable audiometer (model MA-40; Maico Diagnostics, Eden Prairie, Minn) in the person's home, in a closed but not soundproof room, for all 24 members of the family. Air conduction was performed at 250, 500, 1000, 2000, 4000, and 8000 Hz; and bone conduction at 500, 1000, 2000, and 4000 Hz. Two affected individuals (IV:6 and IV:10) and 1 unaffected individual (V:1) underwent pure-tone and speech audiometry at the Speech and Hearing Center, Hadassah Hebrew University Hospital and Medical School, Jerusalem, Israel, in a sound-treated room. Very similar audiometric curves were obtained from the Hearing Center when compared with the results obtained with the portable audiometer. Severity of HL was classified according to American Speech-Language-Hearing Association guidelines as follows: less than 16 dB, normal hearing; 16 to 25 dB, slight HL; 26 to 40 dB, mild HL; 41 to 55 dB, moderate HL; 56 to 70 dB, moderately severe HL; 71 to 90 dB, severe HL; and greater than 90 dB, profound HL.[24]

LINKAGE EXCLUSION

To determine the chromosomal location of the otosclerosis locus in the family, linkage to the known otosclerosis loci was first examined on chromosomes 15q25-q26, 7q34-36, and 6p21.3-22.3 by means of the linked markers *D15S1004*, *D15S652*,[20] *D7S684*, *D7S2513*,[21] and *D6S291*.[22] A DNA sequencing system (Silver Sequence; Promega Corp, Madison, Wis) and radioactive labeling with 2′-deoxycytidine 5′-triphosphate labeled with α–phosphorus 32 were used for band detection. Two-point logarithm of odds (LOD) scores were calculated by means of LIPED software, version 1.21 (1995; Jurg Ott, The Rockefeller University, New York, NY [http://linkage.rockefeller.edu/ott/liped.html]).

LINKAGE ANALYSIS

A genome-wide search for linkage was performed by the Laboratory of DNA Analysis at the Institute of Life Sciences, Hebrew University of Jerusalem. A total of 400 microsatellites spaced at 10-centimorgan intervals were analyzed. Polymerase chain reaction product electrophoresis and detection was performed with an automated DNA analyzer (model 3700; Applied Biosystems, Foster City, Calif). Sizing and genotyping were performed with GENESCAN and GENOTYPER software (Applied Biosystems). Linkage was evaluated with LIPED software. Autosomal dominant inheritance with 80% penetrance was assumed.[1,4,18] Gene frequency was set at 0.0001. Equal male and female recombination frequency was assumed. Marker frequencies were set arbitrarily at 0.2. Regions with LOD scores greater than 1.5 were further genotyped with additional microsatellite markers, which were chosen from the University of California, Santa Cruz (UCSC), Genome Browser (http://genome.ucsc.edu/). The *OTSC4* gene symbol was approved by the HUGO Gene Nomenclature Committee (http://www.gene.ucl.ac.uk/nomenclature/).

RESULTS

SUBJECTS

No reliable information could be obtained regarding hearing status of generations I and II, although they are shown in the pedigree to demonstrate family relationships in the subsequent generations. All 24 ascertained individuals underwent audiometric evaluation. The hearing for 12 members was within normal limits (data not shown).

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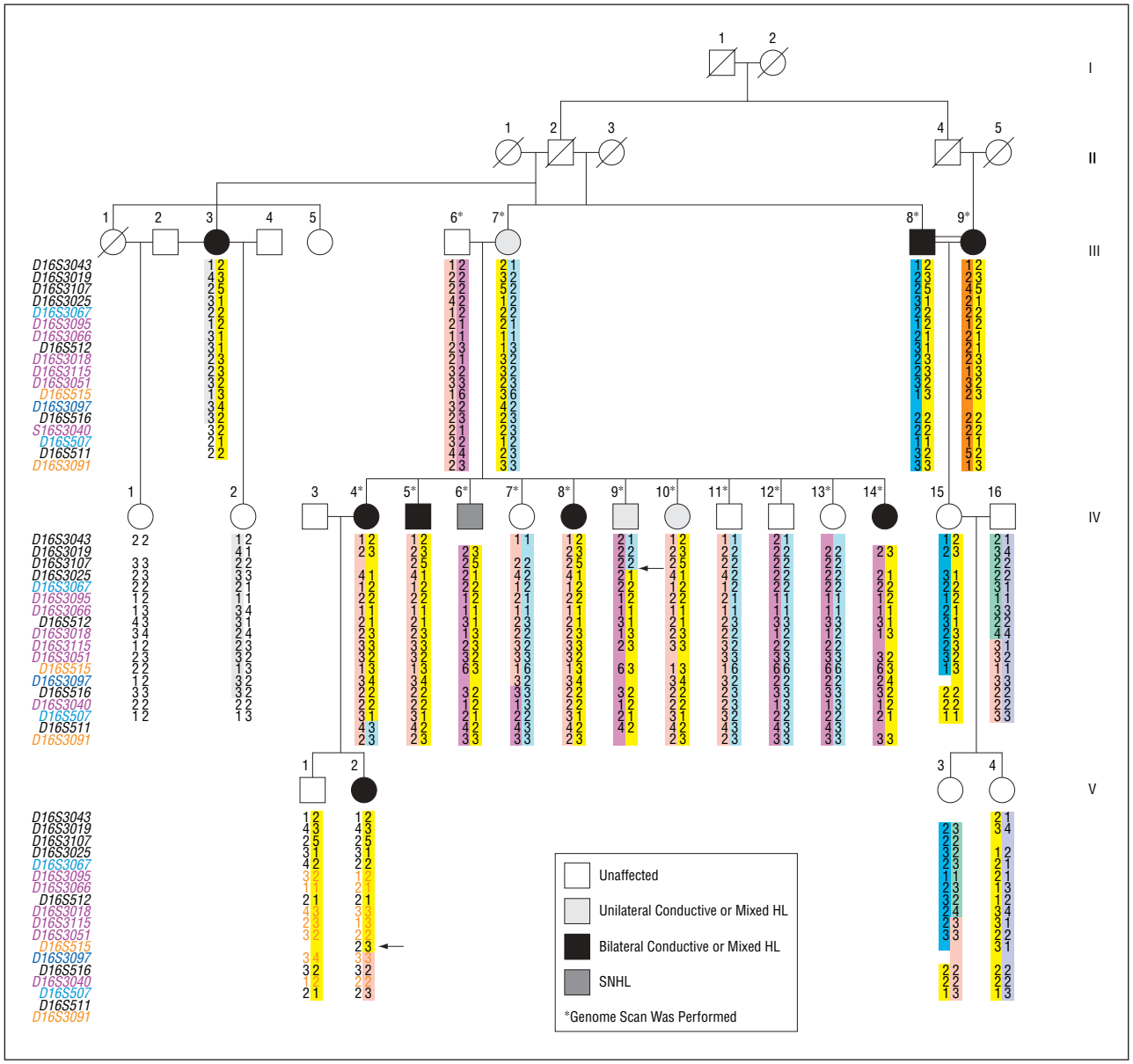

Figure 1. Pedigree of the family studied with haplotypes of the region linked to the fourth locus for otosclerosis (*OTSC4*) on chromosome 16q22.3-23.1. Circles and squares represent females and males, respectively. A double horizontal line between male and female demonstrates a consanguineous marriage. Lines through symbols indicate deceased individuals (generations I and II and individual III:1), for whom hearing status is not known. The yellow haplotype segregates with the mutation. Arrows at the haplotypes of IV:9 and V:2 indicate proximal and distal recombinations, respectively, flanking the linked region.

Twelve members of the family manifested different forms of HL characteristic of otosclerosis (Figure 1), with a 2:1 female preponderance (8 females and 4 males), which is concordant with the incidences published elsewhere.[16,25] In 3 individuals (IV:4, IV:8, and IV:14) otosclerosis was surgically confirmed. The other affected family members were included according to the criteria described in the "Methods" section. One affected individual, IV:6, met only 2 of the 3 criteria presented, and therefore the analysis was repeated with him being considered affected or unaffected.

AUDIOMETRY

All of the otosclerotic members of the family experienced progressive HL, beginning in their late 20s to their 40s. There was a large variability between affected individuals in age at onset, type of HL, shape of audiogram, and symmetry of HL. There were no reports of tinnitus.

AGE AT ONSET

Most of the women in the family started to have difficulties in hearing in their late 20s to early 30s, and the men claimed to have noticed their HL in their late 30s to early 40s.

SYMMETRY OF HL

Of the 12 affected members of the family, 9 had bilateral HL and 3 (2 females and 1 male) had unilateral HL. No differences in severity or shape of audiogram were observed between individuals with bilateral vs unilateral HL.

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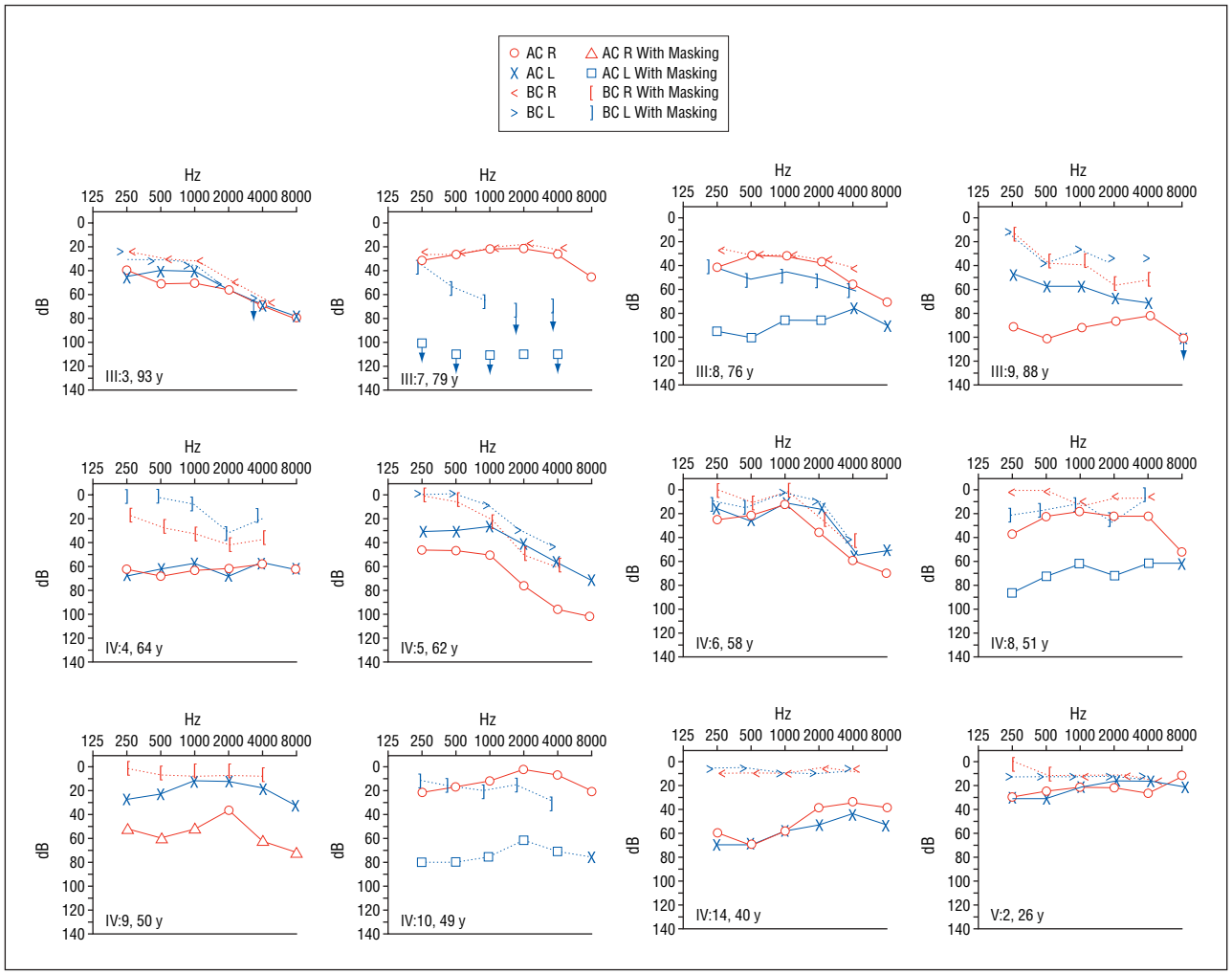

Figure 2. Audiograms of the 12 affected individuals of the family. Note the large interaural and intersubject variability regarding type, severity, and shape of audiogram. AC indicates air conduction; BC, bone conduction.

SEVERITY AND TYPE OF HL

There was a large variation in severity and type of HL of the affected family members (**Figure 2**). Severity of HL ranged from mild to profound. Even though the youngest affected individual (individual V:2 at 26 years old) had mild HL and one of the oldest (individual III:7 at 79 years old) had the most severe HL in the family, in between there was little correlation between age and severity (**Figure 3**). Comparison of severity of HL between females and males showed higher degrees of HL for females in all age ranges, even though considerable variability was seen within the sexes (Figure 3).

Considerable intersubject and interaural variability in type of audiogram was seen. Conductive HL was observed in individuals IV:8, IV:9, IV:14, and V:2. Mixed HL was present in individuals III:3, III:8, III:9, IV:4, IV:5, and IV:10, and individuals III:3, III:8, and IV:6 had SNHL in at least 1 ear. Individuals IV:4, IV:8, and IV:10 showed elevations in bone conduction thresholds characteristic of Carhart effect (Figure 2).

AUDIOMETRIC CONFIGURATION

A large variability existed in the shape of the audiogram as well as in the other aspects of HL. Individuals III:7, III:9, IV:4, IV:8, and IV:10 had flat audiograms. Individuals III:3, III:8, III:9, IV:5, and IV:6 had sloping audiograms; tent-shaped audiograms were seen for individuals IV:8 and IV:9; and individuals III:8, IV:14, and V:2 had rising audiograms (Figure 2). In general, most of the sloping audiograms were seen in males and most of the females had flat or rising audiograms (Figure 2 and Figure 3).

IMMITTANCE TESTING

Normal compliance of the tympanic membrane (type A tympanometry) was found bilaterally in individuals IV:6, IV:14, and V:1, while individual IV:4 had normal compliance in the right ear and high compliance (type A deep [AD]) in the left ear. Because otosclerosis causes fixation of the stapes, low compliance of the middle ear is expected. Type A deep (AD) tympanometry, which reflects low stiffness, seems to be a paradoxical result in otosclerosis. It is noteworthy that similar findings were reported in a small percentage of subjects with confirmed otosclerosis in other populations as well, and that an overlap of compliance results was noted between otosclerotic and normal ears.[2,26,27] Acoustic reflexes were present bilaterally in all frequencies tested in individual V:1,

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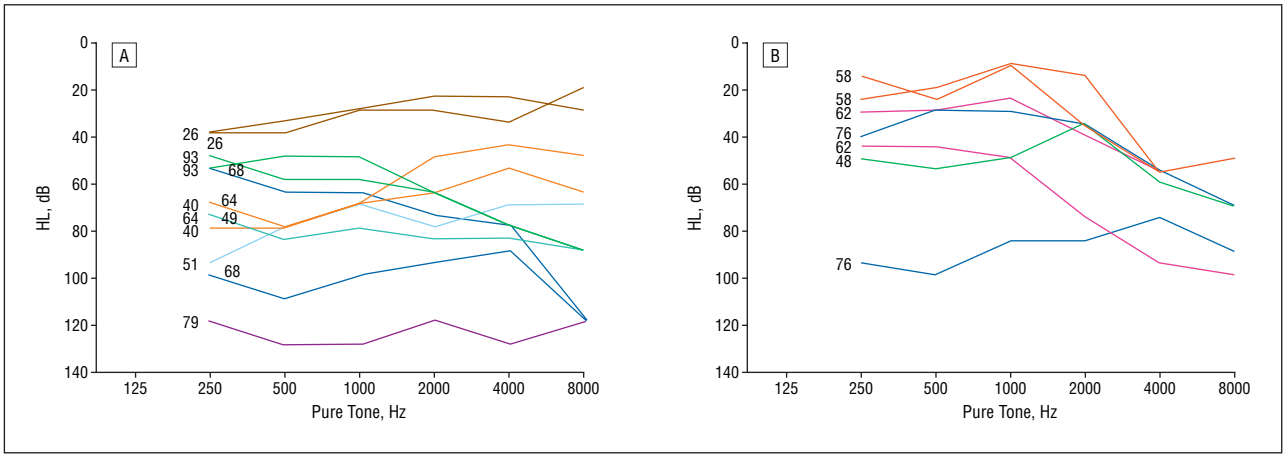

Figure 3. Pure-tone audiograms of affected family members. All sclerotic ears are represented. Colors distinguish between different members; age in years is shown for each person. A, Otosclerotic ears of females (age range, 26-93 years). Six women had bilateral and 2 had unilateral otosclerosis, but data for only 13 ears are presented because a 51-year-old woman (IV:8) had one ear operated on successfully. B, Otosclerotic ears of men (age range, 48-76 years). There were more otosclerotic ears in women than men, hearing loss (HL) was more severe in women than in men at certain age ranges, and HL in women began at a younger age.

Table 1. Exclusion of *OTSC1*, *OTSC2*, and *OTSC3* Loci by Genotyping With Markers From Respective Chromosomal Regions

	2-Point LOD Scores at θ =						
Locus and Markers	**0**	**0.001**	**0.05**	**0.1**	**0.2**	**0.3**	**0.4**
OTSC1							
D15S1004	−4.28	−1.83	−0.18	0.06	0.19	0.15	0.05
D15S652	−4.35	−1.43	0.15	0.32	0.35	0.24	0.08
OTSC2							
D7S684	−4.41	−4.20	−1.25	−0.70	−0.25	−0.08	−0.01
D7S2513	−4.41	−4.06	−0.97	−0.45	−0.06	0.04	0.02
OTSC3							
D6S291	−4.41	−1.09	0.51	0.69	0.68	0.49	0.19

Abbreviations: LOD, logarithm of odds; *OTSC1*, *OTSC2*, and *OTSC3*, first, second, and third respective loci for otosclerosis.

who had normal hearing at the age of 30 years. In individual IV:6, acoustic reflexes were obtained in frequencies of 500 to 2000 Hz but not in 4000 Hz, which was compatible with his high-tone SNHL. Acoustic reflexes were absent in individuals IV:4 and IV:14, as was expected according to their conductive HL. Close linkage to the known otosclerosis loci on chromosomes 15q25-q26, 7q34-36, and 6p21.3-22.3 was excluded with negative 2-point LOD scores (**Table 1**).

LINKAGE ANALYSIS

Fifteen members of the family underwent a whole-genome scan. Four hundred polymorphic markers were evaluated. Three possible regions of linkage were identified, on chromosomes 2q37.1 (marker D2S206; LOD score, 1.92 at θ=0.1), 16q22.3-23.1 (marker D16S515; LOD score, 1.78 at θ=0.1), and 20q13.12 (marker D20S119; LOD score, 1.69 at θ=0). Further genotyping of the family members with 2 additional microsatellite markers in each region did not support linkage to chromosomes 2 and 20 and gave an additional 2-point LOD score of 1.92 at θ=0 in the 16q21-23.2 interval (**Table 2**), leading to further genotyping of the region with DNA derived from all 24 family members. Two-point LOD scores were calculated for the haplotype and for 17 markers in the chromosomal region 16q21-16q24.1 with the penetrance set at 80% (**Table 3**). Hearing individual IV:15 had the otosclerosis haplotype and probably falls in the incomplete penetrance category. Because there was approximately a 10-year difference between sexes in the cutoff age at onset in this family (in favor of males), all male individuals younger than 35 years and all female individuals younger than 25 years were excluded from the statistical calculations (male subject V:1 was 29 years old and female subjects V:3 and V:4 were 22 and 20 years old, respectively). The highest 2-point LOD score of 3.97 at θ=0 was obtained for the haplotype, and LOD scores of 3.75 at θ=0 and 3.72 at θ=0 were obtained for markers *D16S515* and *D16S3025*, respectively.

Because individual IV:6 had a high-tone SNHL that is not common in otosclerosis, LOD scores were calculated twice, once considering him affected and once unaffected. In both calculations, LOD scores higher than 3 were obtained (3.97 and 3.20 at the haplotype, respectively; **Table 4**).

Haplotypes were constructed as demonstrated in Figure 1. Flanking markers for genotyping were defined by recombination in individuals IV:9 (*D16S3107*) and V:2 (*D16S3097*). A genetic map of the candidate re-

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Table 2. Linkage Analysis of the 3 Chromosomal Loci With LOD Scores Greater Than 1.5, Obtained by the Genome Scan*

Chromosome and Marker	2-Point LOD Score at θ = 0	0.001	0.05	0.1	0.2	0.3	0.4
2q37.1							
D2S362	−4.44	−4.36	−1.73	−1.10	−0.50	−0.20	−0.05
D2S206	−99.99	0.49	1.91	**1.92**	1.60	1.12	0.53
D2S345	1.19	1.18	1.03	0.88	0.60	0.33	0.10
20q13.12							
D20S107	−0.03	−0.03	0.05	0.09	0.09	0.06	0.02
D20S119	**1.69**	1.69	1.52	1.34	0.98	0.61	0.25
D20S891	−4.14	−2.42	−0.73	−0.44	−0.18	−0.06	−0.01
16q22.3-23.1							
D16S3106	−0.18	−0.18	−0.15	−0.12	−0.07	−0.03	−0.01
D16S515	−99.99	0.33	1.76	**1.78**	1.49	1.04	0.50
D16S507	**1.92**	1.91	1.72	1.53	1.11	0.68	0.24

Abbreviation: LOD, logarithm of odds.
*LOD scores greater than 1.5 are shown in boldface. For each chromosomal region, the middle marker was genotyped in the genome scan, and the flanking markers were chosen to confirm or reject linkage to the locus. Note that linkage was confirmed only on chromosome 16.

Table 3. Two-Point LOD Scores Between *OTSC4* Locus and Chromosome 16q22.1-23.1 Haplotype and Markers With Penetrance Set at 80%*

Marker	Distance, Mb†	2-Point LOD Score at θ = 0	0.001	0.05	0.1	0.2	0.3	0.4	Zmax	θmax
D16S-Hap		**3.97**	**3.97**	**3.62**	**3.26**	**2.49**	**1.67**	**0.79**	**3.97**	**0**
D16S3043	65.2	0.37	0.37	0.34	0.30	0.27	0.13	0.06	0.37	0
D16S3019	65.8	−1.79	0.48	1.91	1.92	1.62	1.15	0.58	1.92	0.1
D16S3107	67.3	−2.03	−0.22	1.23	1.28	1.06	0.68	0.25	1.28	0.1
D16S3025	68.2	3.72	3.72	3.38	3.02	2.26	1.46	0.61	3.72	0
D16S3067	68.8	0.42	0.42	0.38	0.35	0.26	0.17	0.08	0.42	0
D16S3095	69.6	1.73	1.73	1.56	1.38	1.00	0.60	0.21	1.73	0
D16S512	73.7	3.18	3.17	2.88	2.56	1.89	1.18	0.41	3.18	0
D16S3018	73.9	3.52	3.57	3.22	2.91	2.24	1.52	0.72	3.52	0
D16S3115	74.2	1.83	1.83	1.63	1.42	0.99	0.54	0.12	1.83	0
D16S3051	75.0	2.56	2.55	2.33	2.09	1.58	1.02	0.42	2.56	0
D16S515	76.2	3.75	3.74	3.42	3.08	2.36	1.59	0.75	3.75	0
D16S3097	77.1	−1.59	0.02	1.47	1.51	1.28	0.89	0.41	1.51	0.1
D16S516	78.8	1.72	1.72	1.53	1.32	0.89	0.44	0.04	1.72	0
D16S3040	79.3	2.30	2.30	2.16	1.99	1.57	1.05	0.44	2.30	0
D16S507	79.8	−1.89	−0.36	1.18	1.29	1.14	0.77	0.27	1.29	0.1
D16S511	81.4	−2.13	−0.03	1.44	1.50	1.30	0.93	0.47	1.50	0.1
D16S3091	82.7	0.20	0.20	0.17	0.14	0.08	0.04	0.01	0.20	0

Abbreviations: LOD, logarithm of odds; Mb, megabase; *OTSC4*, fourth locus for otosclerosis; θmax, recombination fraction in the maximum LOD score (Zmax).
*LOD scores calculated for the entire haplotype are shown in boldface type.
†Distances between markers are taken from the University of California, Santa Cruz, Genome Browser, July 2003 version.

gion showing a portion of the markers genotyped and distances between markers is presented in **Figure 4**. Distances and information regarding genes in the region were taken from the UCSC Genome Browser July 2003 version. The region contains 74 known genes and 28 predicted genes.

COMMENT

The hearing impairment in the affected members of the family in this study was diagnosed as otosclerosis on the basis of several of the following criteria: family history of confirmed otosclerosis, age at onset, normal results of otoscopy, progressive conductive to mixed HL, and audiogram showing a characteristic Carhart effect. The disease has also been implicated in certain cases of pure SNHL, and even though such a diagnosis cannot be made safely without exploratory tympanotomy, we could relegate individual IV:6 to this category because of family history and a similar audiogram configuration to that of some of his brothers. In addition, a large variability existed among the affected members of the family in all aspects of HL, so it is not inevitable that cochlear otosclerosis is one of the various forms of the disease in the family.

Sex differences in distribution of the disease, age at onset, and audiogram configuration were observed. The 2:1

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Table 4. Comparison Between Highest Haplotype LOD Scores and Highest LOD Scores for Each Marker Considering Individual IV:9 Affected vs Not Affected, With Penetrance Set at 80%*

	Individual IV:9 Affected		Individual IV:9 Not Affected	
Marker	Zmax	θ	Zmax	θ
D16S-Hap	**3.97**	**0**	**3.20**	**0**
D16S3043	0.37	0	0.37	0
D16S3019	1.92	0.1	1.34	0.1
D16S3107	1.28	0.1	0.70	0.1
D16S3025	3.72	0	2.94	0
D16S3067	0.42	0	0.42	0
D16S3095	1.73	0	1.73	0
D16S512	3.18	0	2.40	0
D16S3018	3.52	0	2.74	0
D16S3115	1.83	0	1.83	0
D16S3051	2.56	0	1.78	0
D16S515†	3.75	0	2.97	0
D16S3097	1.51	0.1	1.51	0.1
D16S516	1.72	0	1.72	0
D16S3040	2.30	0	1.53	0
D16S507	1.29	0.1	0.75	0.15
D16S511	1.50	0.1	0.93	0.15
D16S3091	0.20	0	0.20	0

Abbreviations: LOD, logarithm of odds; Zmax, maximum LOD score.
*LOD scores calculated for the entire haplotype are shown in boldface type.
†Marker from genome scan that identified chromosome 16 linked region.

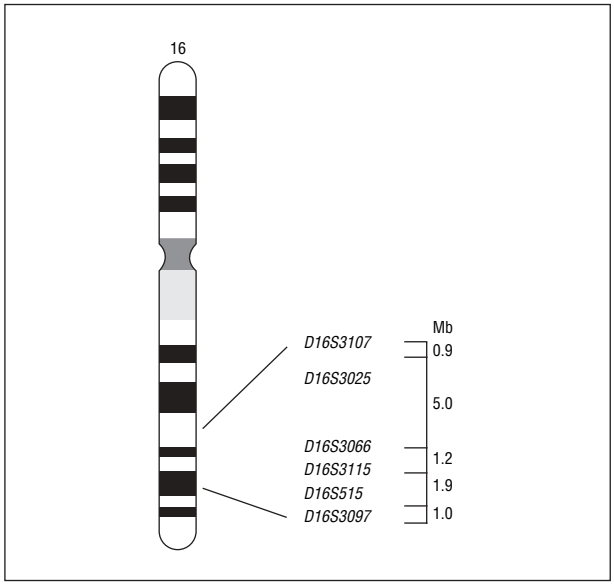

Figure 4. Location of the fourth locus for otosclerosis (*OTSC4*) on chromosome 16. Megabase (Mb) distances between markers are taken from the University of California, Santa Cruz, Genome Browser, July 2003 version.

female preponderance is concordant with the incidences published elsewhere.[16,25] Age at onset was higher in males than females in the family. All women became aware of hearing problems before the age of 40 years and in some cases before the age of 30 years, whereas the men noticed loss in hearing after the age of 40 years. These findings may correlate with the female-male ratio, as the differences in sex distribution might be explained by endocrinologic factors and endocrine activity, such as puberty or pregnancy, which might cause otosclerosis to increase rapidly.[16] At the age of 29 years, individual IV:14 had her first hearing test after her first child was born, and individual IV:4 underwent stapes surgery on the right ear in her early 40s after having 4 children, when she noticed a deterioration in hearing. Individual V:2, who was not aware of hearing problems and showed a mild HL when tested, was 26 years old and pregnant with her fourth child. The males may have had an age at onset similar to that of the females, but in the absence of the hormonal factors, the progression was slower and the HL was noticed later. Furthermore, greater degrees of HL were observed in the females of the family than the males in all age ranges. This was reported in other populations as well,[28] and the explanation may also be related to the maturation history of women leading to more severe expression.

The variability of audiogram configuration and severity of HL in the family may represent different stages of otosclerosis. A mild conductive rising audiogram, exhibited by member V:2, is characteristic of the first stage and is thought to be caused by the presence of cellular fibrous tissue. The second stage, with localized bony fixation of the anterior part of the footplate, is thought to result in moderate conductive HL, as was the case with individuals IV:9 and IV:14. The final stage, fixation of the entire circumference of the annular ligament, is considered to result in a moderately severe conductive to mixed HL.[2] Individuals IV:4, IV:8, and IV:10 had this kind of audiogram. Histologic studies have shown that in some patients with stapes fixation, a second focus of otosclerosis can be found in the cochlea that is probably responsible for the sensorineural component. A cochlear focus might cause profound mixed HL to SNHL, as was the case with individuals III:7, III:8, and III:9. In rare cases there was evidence of isolated cochlear otosclerosis,[12] which may begin with a high-tone loss, depending on the otosclerotic locus in the cochlea. Individual IV:6 was thought to have this form of otosclerosis. Individuals IV:4, IV:8, and IV:10 had a characteristic elevation in bone conduction with a notch at 2000 Hz, characterized as the Carhart effect.

In the present study we have mapped the fourth otosclerosis locus, *OTSC4*, to the long arm of chromosome 16 in an extended Israeli family. All 12 affected members of the family inherited the otosclerosis haplotype. Individual IV:6, diagnosed as having SNHL, inherited the same haplotype pattern as that of family members with otosclerosis, suggesting that his HL may be due to mutations in the same gene. Nevertheless, we calculated LOD scores labeling him as both affected and unaffected. The calculated LOD scores confirmed linkage to chromosome 16q22.1-23.1 for both options. Unaffected individual IV:15 inherited the disease haplotype. At the age of 50 years, after having had 5 children, it is extremely unlikely that she will develop otosclerosis, as the onset of the disease in females in this family was in the late 20s to early 30s and was reported to be first noticed or deteriorating during or after pregnancies. Therefore, in view of the prevalence of reduced penetrance associated with otosclerosis, we predict that individual IV:15 is bearing

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the mutation but that the phenotype is not expressed due to reduced penetrance. In the families in which the 3 *OTSC* loci, *OTSC1-3*, were mapped, there were 3, 2, and 1 individuals, respectively, without HL but with the otosclerosis haplotypes. They were considered to reflect reduced gene penetrance as well.[20-22]

Because there are diverse hypotheses regarding the cause of otosclerosis, many genes in the maximal region may fit into at least 1 of the theories suggested. Among the proteins encoded by these genes are a few members of the cadherin superfamily, cadherin 1 and cadherin 3. Cadherins are mostly transmembrane proteins that mediate cell recognition and cell-cell adhesion, with unique cadherin domains or ectodomains consisting of sequence motifs that are rich in negatively charged amino acids.[29] The perturbed bone homeostasis in otosclerosis might involve a disturbance in cell signaling and/or tissue cohesion involving a cadherin. Furthermore, cadherin proteins are expressed in connective tissues, in bone, and in the cochlea.[30-34]

Conserved oligomeric Golgi 8 (*COG8*) and *COG4* are 2 genes in the 16q21.1-23.1 region that belong to the COG group of multiprotein complexes. These complexes are key determinants of the Golgi apparatus structure and are actively involved in intracellular membrane trafficking.[35] The COG complexes are expressed in the immune system,[33] and mutations in COG genes might be compatible with the morphologic findings of an inflammatory process within the temporal bone at distinct stages of otosclerosis.[4]

Several members of the DEAD (Asp-Glu-Ala-Asp) box proteins, DEAD (Asp-Glu-Ala-Asp) box polypeptide (DDX) 19, DDX28, and DEAH (Asp-Glu-Ala-His) box polypeptide (DHX) 38 are encoded by genes included in the *OTSC4*-linked region. These proteins are implicated in many cellular processes involving RNA, including transcription, translation, RNA export and turnover, and ribosome and spliceosome assembly.[36,37] Some members of the family are involved in cellular growth and division. Several of the DEAD box proteins are reported to be expressed in the immune system, cartilage, and fibrous tissue,[33,38-40] all with possible relation to otosclerosis.

A number of the genes coding for zinc finger (ZNF) proteins, ZNF19, ZNF23, zinc and ring finger protein (ZNRF) 1, and zinc finger protein 1 homologue (ZFP1) are also included in the *OTSC4*-linked region. Zinc finger proteins are multifunctional proteins with both transcriptional and posttranscriptional functions.[41] Their broad expression pattern includes tissues involved in different stages of otosclerosis, such as the inner ear, the immune system, and fibrous tissue.[33]

CONCLUSIONS

The different stages of otosclerosis are directly correlated with the type and severity of HL and audiogram configuration. The present investigation is unique, to our knowledge, in that it shows considerable intersubject and interaural variability in the same family. The activity of the otosclerotic lesion could provide convincing explanations for all these differences.

Identification of the causative gene for *OTSC4* and the protein it encodes will shed light on the etiology of otosclerosis and will enable identification of factors involved in the process of the development of the disease, in addition to expanding genetic counseling and providing potential targets for therapeutic intervention.

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