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Localization of a gene for otosclerosis to chromosome 15q25–q26

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Among white adults otosclerosis is the single most common cause of hearing impairment. Although the genetics of this disease are controversial, the majority of studies indicate autosomal dominant inheritance with reduced penetrance. We studied a large multi-generational family in which otosclerosis has been inherited in an autosomal dominant pattern. Five of 16 affected persons have surgically confirmed otosclerosis; the remaining nine have a conductive hearing loss but have not undergone corrective surgery. To locate the disease-causing gene we completed genetic linkage analysis using short tandem repeat polymorphisms (STRPs) distributed over the entire genome. Multipoint linkage analysis showed that only one genomic region, on chromosome 15q, generated a lod score >2.0. Additional STRPs were typed in this area, resulting in a lod score of 3.4. STRPs *FES* (centromeric) and *D15S657* (telomeric) flank the 14.5 cM region that contains an otosclerosis gene.

INTRODUCTION

Clinical otosclerosis (MIM 166800) has a prevalence of 0.2–1% among white adults, making it the single most common cause of hearing impairment (1,2). The disease is characterized by isolated endochondral bone sclerosis of the labyrinthine capsule that leads to hearing loss. Auditory impairment is heralded by the appearance of otosclerotic foci that invade the stapedio-vestibular joint (oval window) and interfere with free motion of the stapes (3). Mean age of onset is in the third decade and 90% of affected persons are under 50 years of age at the time of diagnosis (2,4). Long-term follow-up suggests that ~10% of these persons ultimately develop a profound sensorineural hearing loss across all frequencies (4,5). This loss may reflect the effect of either mechanical or toxic damage to the inner ear caused by otosclerotic foci that invade the cochlear endosteum and encroach on the membranous labyrinth. Although the sensorineural component of the hearing loss cannot be corrected, stapes microsurgery has proven to be a highly successful means to restore the normal conduction mechanism and can improve hearing thresholds by as much as 50 dB (5).

The etiology of otosclerosis is unknown and its genetics is poorly understood. Reports of an inherited disease that probably represents otosclerosis date to the mid 19th century, when Toynbee described a familial pattern of conductive hearing loss (6). In his catalogue of 1837 he noted that thickening of the anterior two thirds of the stapedial footplate resembled ivory originating from the vestibular surface of the labyrinth (7). In 1876 Magnus documented a family in which the father and seven of 13 children had conductive hearing impairment, verified in one child to be due to ankylosis of the stapes (8). Eighteen years later Politzer coined the term ‘otosclerosis’, in reference to a ‘disease that has its seat in the labyrinthine capsule [and] leads, through new formation and growth of osseous tissue, to ankylosis of the stapes in the fenestra ovalis’ (7,9).

The majority of current epidemiological studies indicate autosomal dominant inheritance, a conclusion first reached by Albrecht in 1922 (10). This observation was supported by Larsson’s analyses of 262 probands, in which he also recognized that penetrance is incomplete (11,12). In a detailed study of 150 probands Morrison calculated the fractions of first, second and third degree relatives and noted that the observed ratios are consistent with autosomal dominant inheritance with 40% penetrance (13). Other studies have confirmed these findings (14,15) and although detailed mathematical calculations by Larsson (11) and Gapany-Gapanavicius (16) suggest that other modes of transmission are unlikely, Baurer and Stein have postulated digenic recessive inheritance based on a study of 94 families (17). Hernandez-Orozzo and Cortney also invoked digenic inheritance, but of an X-linked dominant gene and an autosomal recessive gene (18).

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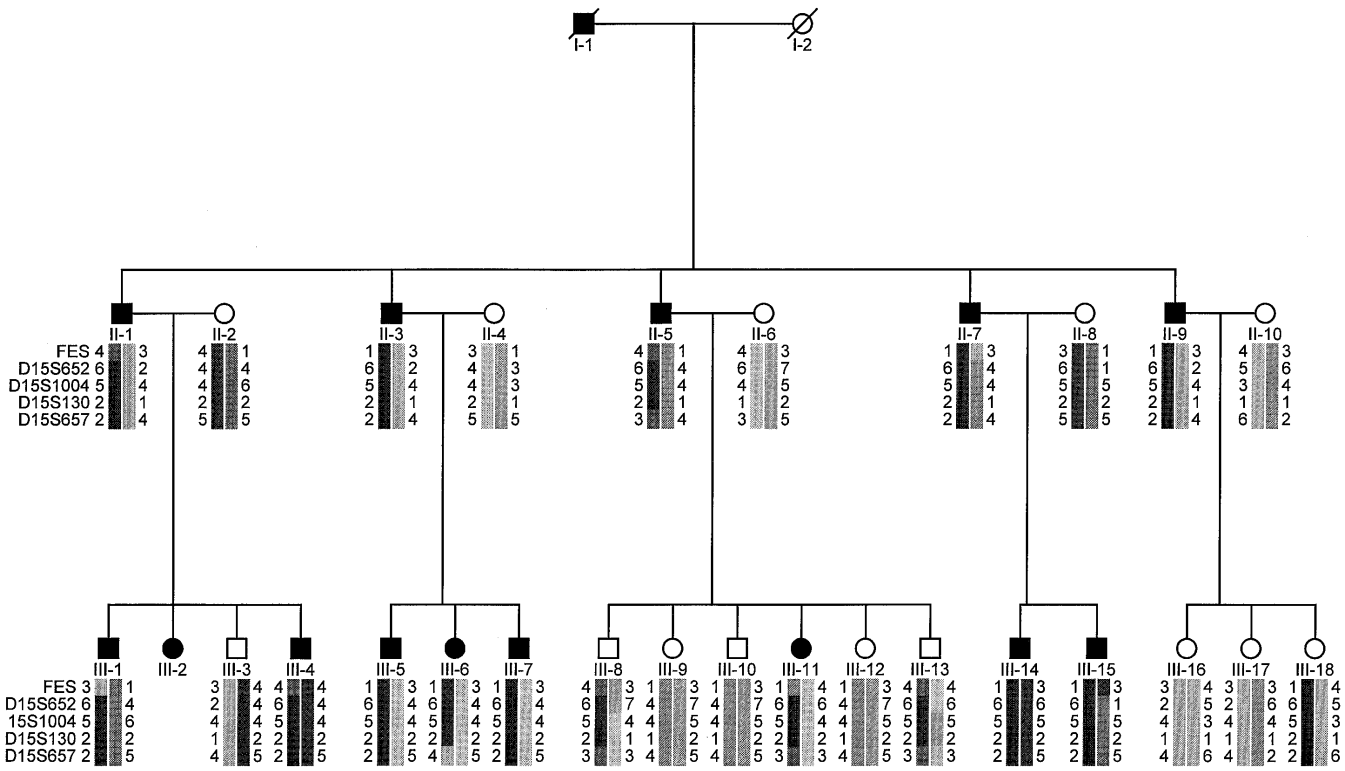

Figure 1. Pedigree used to localize a gene for otosclerosis to chromosome 15q25–q26. Individuals 39, 41 and 47 carry the disease haplotype but do not express the phenotype. □, male; ○, female; ■, affected male.

Adding to the inheritability controversy is the lack of a positive family history in 40–50% of cases (19–21). These isolated cases have been hypothesized by Morrison and Bundey to represent an autosomal recessive form of otosclerosis in which the heterozygotic state is identifiable only by histological examination of the temporal bones (22). Based on this assumption, they calculated the predicted prevalence of histological disease at 6–7%, a figure in close agreement with the observed value of 10% (23–25).

Recent studies have also suggested the possibility of a viral etiology in the pathogenesis of otosclerosis. In particular, polyclonal and monoclonal antibodies against mumps, rubella and, most commonly, measles viruses have been used to demonstrate antigenic expression in otosclerotic foci (26). By PCR amplification a 115 bp fragment of the measles nucleocapsid gene has also been identified in temporal bone specimens from persons with otosclerosis but not in histologically negative controls (27). Based on these data, it has been hypothesized that the middle ear mucosa becomes infected via the eustachian tube, whereupon viral particles invade the bone of the labyrinth via either lymphatic or pericapillary spaces, ultimately causing otosclerotic foci to develop (28).

A causal role for measles in otosclerosis, however, has not been proven. The disparate ethnic-based epidemiological data of these two diseases suggest that the presence of viral material in otosclerotic foci could represent a secondary pathophysiological event (29–32). In contrast, evidence for genetic factors as a cause of otosclerosis, possibly in combination with other factors, is strong. Clearly, our understanding of this disease will require identifying those genes essential for its pathogenesis. This report is the first to demonstrate linkage in otosclerosis and describes the localization of an otosclerosis gene to chromosome 15q25–q26.

RESULTS

Clinical data

Among individuals in this family with otosclerosis the hearing impairment varies, older persons typically having a greater loss than younger persons, reflecting the time-dependent development of a sensorineural component superimposed on the characteristic conductive loss (Fig. 1 and Table 1; 4,5). The degree of conductive loss as assessed by Rinne testing ranged from ~20 (negative Rinne at 256 Hz) to 45 dB (negative Rinne at 256, 512 and 1024 Hz) and did not show the same age-related increase in severity (33,34). Five persons had surgically confirmed otosclerosis and in three there was a persistent conductive hearing loss in the non-operated ear (individuals 14, 24 and 51; individual 13 had had bilateral stapedectomies). Nine persons with conductive hearing loss had not undergone surgery.

Table 1. Individual phenotypes

Individual	Age	Air conduction	Rinne test 256 Hz	512 Hz	1024 Hz	Surgery	Affectation
II-1	68	Severe	–			No	+
II-3	64	Severe	–			No	+
II-4	58	Normal	+	+	+	No	–
II-5	62	Moderate	–	–	–	No	+
II-7	57	Mild (L), moderate (R)	–	–	– (R)	No	+
II-9	47	Mild (R), moderate (L)	–	– (L)		No	+
III-1	42	Normal (R), mild (L)	–	–	+	Bilateral stapedectomies	+
III-2	38	Normal (R), moderate (L)	– (L)	– (L)	– (L)	Right stapedectomy	+
III-3	35	Normal	+	+		No	–
III-4	29	Mild (R), moderate (L)	–	–	–	No	+
III-5	34	Normal (R), moderate (L)	– (L)	– (L)		Right stapedectomy	+
III-6	25	Moderate to severe (L, R)	–	–	–	No	+
III-7	18	Normal (R), mild (L)	+	+		Right stapedectomy	+
III-8	30	Normal	+	+		No	–
III-9	27	Normal	+	+		No	–
III-10	25	Normal	+	+		No	–
III-11	24	Mild to moderate (L, R)	–	–	+	No	+
III-12	19	Normal	+	+		No	–
III-13	16	Normal	+	+		No	–
III-14	30	Mild (L), moderate (R)	–	– (R)	– (R)	No	+
III-15	28	Normal (L), moderate (R)	–	– (R)	– (R)	Left stapedectomy	+
III-16	14	Normal	+	+		No	–
III-17	13	Normal	+	+		No	–
III-18	13	Normal	+	+		No	–

Table 2. Genome-wide screen

Interval	Z_{max}
D3S2427–D3S2418	1.1
D8S264–D8S1145	1.5
D15S111–D15S1014	2.4
D16S2616–D16S764	1.4

Table 3. Two point lod scores

STRP	Z_{max}	θ
FES	0	50
D15S652	2.9	0
D15S1004	2.5	0
D15S130	2.8	0
D15S657	0.9	15

Genetic mapping

Based on a possible relationship between minor collagens and inner and middle ear pathology, linkage to the genes encoding collagens II, V, VII, IX and XI was tested and excluded (*COL2A1*, 12q13.11–q13.12; *COL5A1*, 9q34.2–q34.3; *COL7A1*, 3p21; *COL8A1*, 3q12–q13.1; *COL8A2*, 1p32.3–p34.3; *COL9A1*, 6q12–q13; *COL9A2*, 1p32.3–p33; *COL11A2*, 6p21.3–p22). A genome-wide linkage study using 160 genetic markers evenly dispersed over the human genome was then performed. Multipoint linkage analysis (three point rolling lods) identified four regions that produced lod scores >1; only a single region generated a lod score >2 (*D15S111–D15S1014* interval, $Z = 2.3$ at *D15S1004*; Table 2). Additional short tandem repeat polymorphisms (STRPs) in this area were typed and haplotypes were reconstructed (Fig. 1). In the *FES–D15S657* interval the calculated maximum multipoint lod score between *D15S652* and *D15S657* was 3.4 at *D15S1004* (Fig. 2 and Table 3).

Flanking markers are defined by individuals 1, 3, 13 and 45 (*FES*, centromeric) and individuals 3 and 26 (*D15S657*, telomeric). Therefore, the 14.5 cM region between *FES* and *D15S657* harbors an otosclerosis gene. Three persons (individuals 39, 41 and 47) without a conductive deficit appear to have inherited the

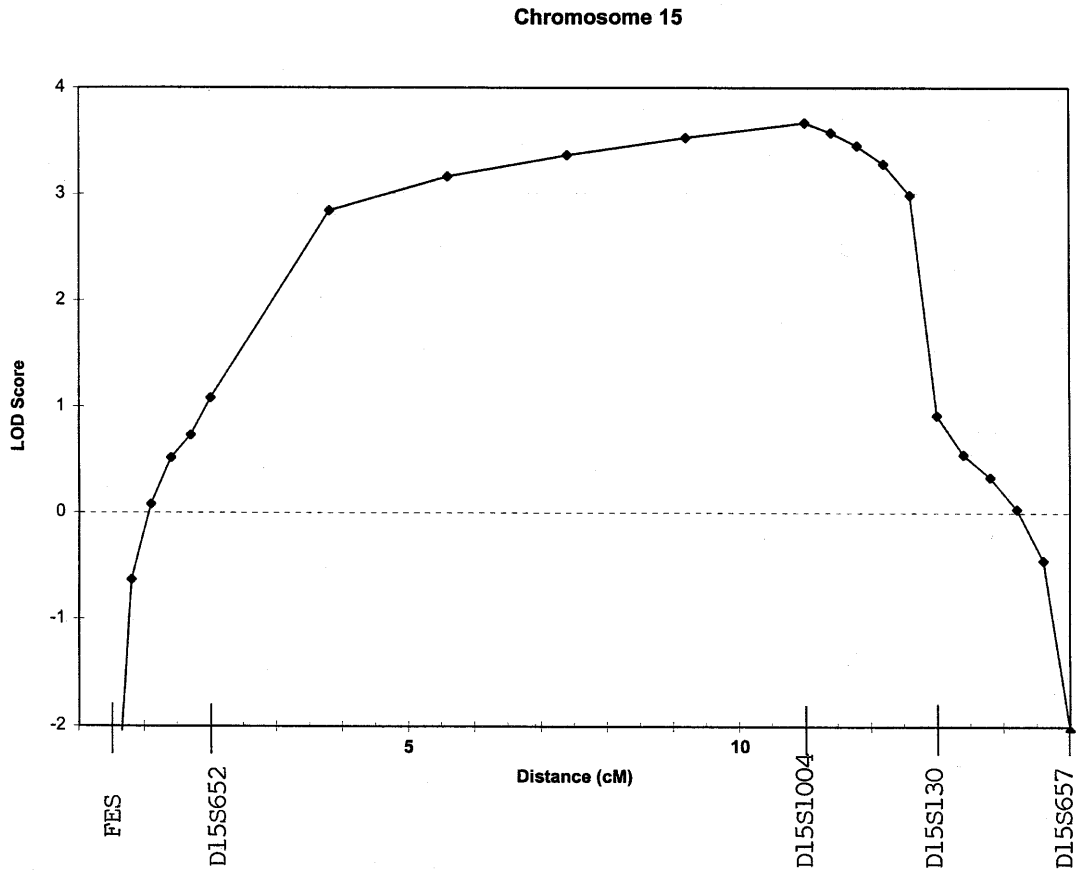

Figure 2. Multipoint linkage analysis over the chromosome 15 candidate region. Z_{max} = 3.4 at *D15S1004*.

affected haplotype. This finding presumably could reflect reduced gene penetrance, although individuals 39 and 47 are too young to make this claim confidently (Table 1).

DISCUSSION

This linkage finding is in compliance with the body of older studies suggesting that otosclerosis is inherited as an autosomal dominant disease with reduced penetrance. However, it must be emphasized that these results pertain to only a single family. Other studies suggest heterogeneity (15) and additional data sets will be required to determine the extent to which this locus (*Otosclerosis1*) is responsible for otosclerosis.

With respect to this study, the most interesting gene that has been mapped to the *FES–D15S657* interval is *aggrecan*, the quantitatively major non-collagenous component of the extracellular matrix of cartilage (35). This gene is an excellent candidate for a major role in the pathogenesis of otosclerosis for four reasons: (i) aggrecan is a complicated protein for which there are several different alleles; (ii) the bony labyrinth of the inner ear develops from a cartilaginous precursor in which aggrecan is expressed; (iii) mutations in the homologous mouse mutant produce hearing impairment; and (iv) glycosaminoglycan side chains bind to aggrecan and can be utilized by microorganisms for binding to target cells.

Structural studies of aggrecan have shown that it consists of an extended multidomain core of ~220–250 kDa to which are covalently attached glycosaminoglycan (GAG) side chains (36). Two globular domains, G1 and G2, comprise the N-terminus and a third globular region, G3, makes up the C-terminus. The latter is a complex structure that contains two variably spliced exons which generate products with or without complement-regulatory protein-like and epidermal growth factor-like domains (36). The protein also has two internal blocks of highly repetitive sequence, 11 repeats of a six amino acid sequence and 19 repeats of a 19 amino acid sequence (37), suggesting that several different alleles of *aggrecan* may exist. Recently 13 different alleles in the coding region have been identified, which represent an unusual example of an expressed variable number of tandem repeat polymorphism (37). Conceivably some of these alleles could be more frequent in select populations and may play a greater role in the pathogenesis of otosclerosis, an important consideration given the epidemiological data on this disease (25,31,32).

The role of aggrecan as an essential component in the formation of bony labyrinth of the inner ear can be inferred from studies on the *Agc*/*Agc* mouse, which carries mutations in the homologous mouse gene. A complex phenotype is seen in the homozygous mouse that is characterized, in part, by marked hearing loss, as measured by auditory evoked potentials (38). Although this mouse model also exhibits cartilage abnormalities in the development of the inner ear that are not part of the otosclerosis phenotype, these experiments do implicate aggrecan as a protein that is expressed in the bony labyrinth and can be associated with hearing loss.

Most compelling, in view of the possibility of a viral role in the pathogenesis of otosclerosis (26,27), is the fact that GAGs are often involved in cell–cell interactions and cell proliferation (39). In addition, they have receptor-like activity for certain biologically active substances. The most ubiquitous GAGs, heparan sulfates, are bound to aggrecan and are used by pathogenic microorganisms as diverse as viruses, protozoa and bacteria to gain entry into host cells (40).

In summary, aggrecan maps to the otosclerosis candidate internal, exists in several different allelic forms, is expressed in the cartilaginous/bony labyrinth, is associated with hearing deficits in animal models and binds GAGs that often serve as target receptors for pathogenic viruses. These facts suggest that otosclerosis may be a xenogenetic disease that requires a specific host genotype and exposure to a specific viral pathogen for the disease phenotype to be expressed.

MATERIALS AND METHODS

Clinical data

The family in this study was ascertained through the University of Madras and visited in their home in Southern India. Historical information was obtained by questionnaire and review of the medical records. No consanguinity was recorded. All individuals were examined thoroughly by an otolaryngologist (R.I.S.Z.) and none had features to suggest a syndromic form of hearing loss. Audiometry was performed using a portable Maico MA25 audiometer. The presence of an air–bone gap was assessed by tuning fork tests at 256, 512 and 1024 Hz (33,34), as battery powered audiometers capable of measuring bone conduction were not available. Responses to the Rinne test were recorded as + (air conduction > bone conduction, normal) or – (bone conduction > air conduction) for each fork. By audiometric criteria hearing losses were graded as mild (26–40 dB), moderate (41–55 dB), moderately severe (56–70 dB), severe (71–90 dB) or profound (>90 dB) (41).

Genotyping

Genomic DNA was extracted from whole blood following a standard protocol (42), quantitated by spectrophotometric readings at OD_{260} and diluted to 30 ng/µl. PCR amplification of STRPs was performed using 30 ng DNA in a 15.0 µl reaction mixture containing 1.5 µl buffer (100 mM Tris–HCl, pH 8.8, 500 mM KCl, 15 mM $MgCl_2$, 0.01% w/v gelatin), 1 µl each 10 mM dCTP, dGTP and dTTP, 1 µl 0.1 mM unlabeled dATP supplemented with 0.4 µl [^{35}S]dATP (1000 Ci/nmol, 10 µCi/ml), 7.5 pmol forward and reverse primers and 0.2 U Taq polymerase, with the remainder being double distilled H_2O. Each reaction was overlaid with mineral oil and 25 cycles of amplification were completed at 94°C for 30 s, 55°C for 30 s and 72°C for 30 s. Reaction products were resolved on 6% polyacrylamide gels (7.7 M urea) and visualized by autoradiography. Initially STRPs linked to the minor collagens were tested for linkage to the disease gene. The genome-wide screen was completed with STRP screening set version 8A (43). Haplotyping was done with additional STRPs mapped to chromosome 15q25–q26.

Linkage analysis

Lod scores were calculated using FASTLINK (44). The frequency of the otosclerosis gene was set at 0.005 and the disease was assumed to be 40% penetrant and dominant (16–18). Multipoint calculations were performed using three or four point rolling lods.

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