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Main Article

No evidence of linkage between 7q33–36 locus (OTSC2) and otosclerosis in seven British Caucasian pedigrees

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Abstract

Background: The aetiology of otosclerosis is complex, and probably involves an interaction between genes and environmental factors. Previous studies have revealed genetic linkage with a number of chromosome regions, including position 7q33–36.

Aim: To confirm whether linkage exists between otosclerosis and chromosome region 7q33–36.

Materials and methods: Seven multiply affected families were ascertained. Deoxyribonucleic acid from members of these families was extracted, and six markers were genotyped to cover a 16 cM region at 7q33–36. Both parametric and non-parametric multipoint linkage analyses were performed.

Results: Parametric multipoint linkage analysis excluded any linkage at 7q33–36 (logarithm of odds score < -4.0). Non-parametric linkage analysis also failed to confirm any linkage (non-parametric linkage < 1.66). When tested individually, pedigree four was the only one to show a significant non-parametric linkage score between D7s684 and D7s2513 (non-parametric linkage $= 1.96$).

Conclusion: No linkage was detected between otosclerosis and the 7q33–36 region. This could be explained by the study's lack of power, due to the limited number of families available.

Key words: Otosclerosis; Genetics; Genetic Linkage; Lod Score; Chromosomes; Human; Pair 7

Introduction

Otosclerosis is a disorder of bone unique to the human ear. It occurs equally in males and females, with a mean age of onset in the third decade; 90 per cent of affected individuals are under 50 years of age at the time of diagnosis.[1,2]

Temporal bone studies document two forms of otosclerosis. The first, histological otosclerosis, occurs in about 2.5 per cent of the white population.[3] The observed otosclerotic foci do not impair stapedial movement and therefore remain clinically inconsequential. The second form, clinical otosclerosis, has a lower prevalence (0.2–1 per cent),[1] interferes with ossicular chain motility, and leads to conductive and/or mixed hearing loss. Both ears are involved in approximately 85 per cent of patients,[4] and about 10 per cent of otosclerosis patients will develop a profound sensorineural hearing loss across all frequencies,[5,6] with limited benefit from conventional hearing aids. More recently, cochlear implantation has allowed some relief from the auditory isolation that such patients suffer.

A genetic basis to otosclerosis has been proposed. Autosomal dominant inheritance with incomplete penetrance[7–9] has been suggested, although a complex aetiology is likely which probably involves an interaction between genetic and environmental factors. This lack of knowledge is an important obstacle in the development of better therapies and prevention strategies for this disease. Furthermore, there is some controversy regarding the size of the genetic contribution to the aetiology, due to lack of a positive family history in 40–50 per cent of cases.[10,11]

Recent studies have suggested the possibility of a viral aetiology 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.[12]

To date, five autosomal dominant otosclerosis loci have been reported. The first is OTSC1 on chromosome 15q25–26, with a logarithm of odds score of 3.4[13] (this score is a measure of the likelihood of genetic linkage between the markers used and the disease locus, where a logarithm of odds score of 3 is evidence of linkage and that of -2 excludes any linkage). The other reported loci are: OTSC2 on chromosome 7q33–36, with a logarithm of odds score of 3.54;[14] OTSC3 on chromosome 6p21–22.8,

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with a logarithm of odds score of 3.83,[15] OTSC4 on chromosome 16q21–23.2, with a logarithm of odds score of 3.97;[16] and OTSC5 on chromosome 3q22–24, with a logarithm of odds score of 3.46.[17] All of these loci were revealed by conducting a whole genome linkage screen on a single extended pedigree, although none of the corresponding genes have yet been cloned, nor have any of these linked regions been replicated in other pedigrees.

The OTSC2 locus was first linked to otosclerosis in a single Belgian family.[14] However, when nine additional families (seven Belgian and two Dutch) were recruited and analysed by the same researchers, linkage was excluded at this locus in seven pedigrees, and logarithm of odds scores of 1.91 and 0.74 ($p > 0.05$) were obtained in two Belgian families. This result was considered to demonstrate significant evidence of genetic heterogeneity.[18] Furthermore, the OTSC2 locus, together with OTSC1 (15q25–26), was excluded in a linkage study conducted on four extended Italian pedigrees.[19]

Here, we report a study of OTSC2 in a collection of seven families from north-west England.

Methods

Families

The study group comprised a total of 61 individuals (25 male, 36 female) from seven multicase families of Caucasian origin (Figure 1). These families were ascertained through the department of otolaryngology of the Manchester Royal Infirmary, UK. Twenty-six of the study group (nine males, 17 females) were diagnosed as affected, based on otoscopic and tuning fork examination and on audiometric analysis; of these cases, 13 were confirmed surgically. The remaining 35 patients, with no clinical findings on examination, were grouped as unaffected individuals. For the patients who had not undergone surgery, the clinical diagnosis of otosclerosis was based on audiological data, which were analysed using an algorithm described elsewhere.[20]

Ethical approval for the study was obtained from the Manchester Health Authority ethics committee, and all individuals gave informed consent prior to blood collection. The genomic deoxyribonucleic acid (DNA) was extracted from the samples using the DNAce Maxiblood purification system (Bioline, London, UK).

Microsatellite marker genotyping

Six highly informative microsatellite markers (D7S509, D7S495, D7S2560, D7S684, D7S2513 and D7S2426) were genotyped to cover a 16 cM region at 7q33–36. Marker information and distances between them were taken from the website of the Marshfield Medical Research Foundation.[21] The genetic distance separating the markers was D7S509 – 1.4 cM – D7S495 – 0.6 cM – D7S2560 – 1.9 cM – D7S684 – 4.0 cM – D7S2513 – 8.8 cM – D7S2426. The microsatellites were amplified by polymerase chain reaction, using fluorescently labelled primers. The polymerase chain reactions were carried out in 96-well microtitre plates, and the pooled samples of the microsatellite markers were electrophoresed using an Abi Prism® 3100 genetic analyser (Applied Biosystems, California, USA) electrophoresis platform. Allele assignment and genotyping were carried out using Genotyper® 3.7 software, and all results were checked manually.

Statistical analysis

The Simlink program[22] was used to estimate the statistical power of detecting linkage, given the family history information on the seven pedigrees. This was done by simulating the mean maximum logarithm of odds scores, assuming both an autosomal dominant inheritance with a gene frequency of 0.3 per cent and 50 per cent penetrance, and an autosomal recessive inheritance model with a gene frequency of 5 per cent and 50 per cent penetrance. Locus homogeneity and heterogeneity were presumed with different recombination fraction levels. The probability of a maximum logarithm of odds score greater than 3.0 gave the probability that the pedigree, or set of pedigrees, would be sufficient to demonstrate linkage.

To minimise the errors and identify inconsistencies under Mendelian inheritance, the genotype data were screened using the Gas 2.0[23] and PedCheck software programs.[24] The Splink version 1.5 software package[25] was used to calculate the allele frequencies.

Non-parametric linkage analysis was carried out with the 'score all' function of the Genehunter 2.1 software program.[26] A parametric analysis model of inheritance was chosen in compliance with previous studies, suggesting an autosomal-dominant model of inheritance with a gene frequency of 0.03 and a penetrance of 50 per cent.

For screening purposes, a p value of 0.05 or less, which was calculated by multipoint analysis via the Genehunter software program, was considered as significant evidence for linkage. A logarithm of odds score of less than -2.0 is customarily accepted as conclusive evidence for the exclusion of linkage.[27]

Results

The results of the parametric multipoint linkage analysis are shown in Figure 2. This analysis excluded any linkage in chromosome 7q at the region between D7S495 and D7S2426, according to the parameters used (logarithm of odds < -2.0). To eliminate any bias resulting from an inaccurate model of inheritance, a non-parametric linkage analysis was performed in which no model of inheritance was implemented. Non-parametric linkage analysis failed to confirm any linkage to the region in question (non-parametric linkage < 1.66) (Figure 3). Parametric and non-parametric linkage analyses were conducted for each pedigree individually. Pedigree four was the only one to show a significant non-parametric linkage score at the locus D7S2513 (non-parametric linkage $= 1.96$) (Figure 4); however, no significant association was seen on parametric analysis (Figure 5).

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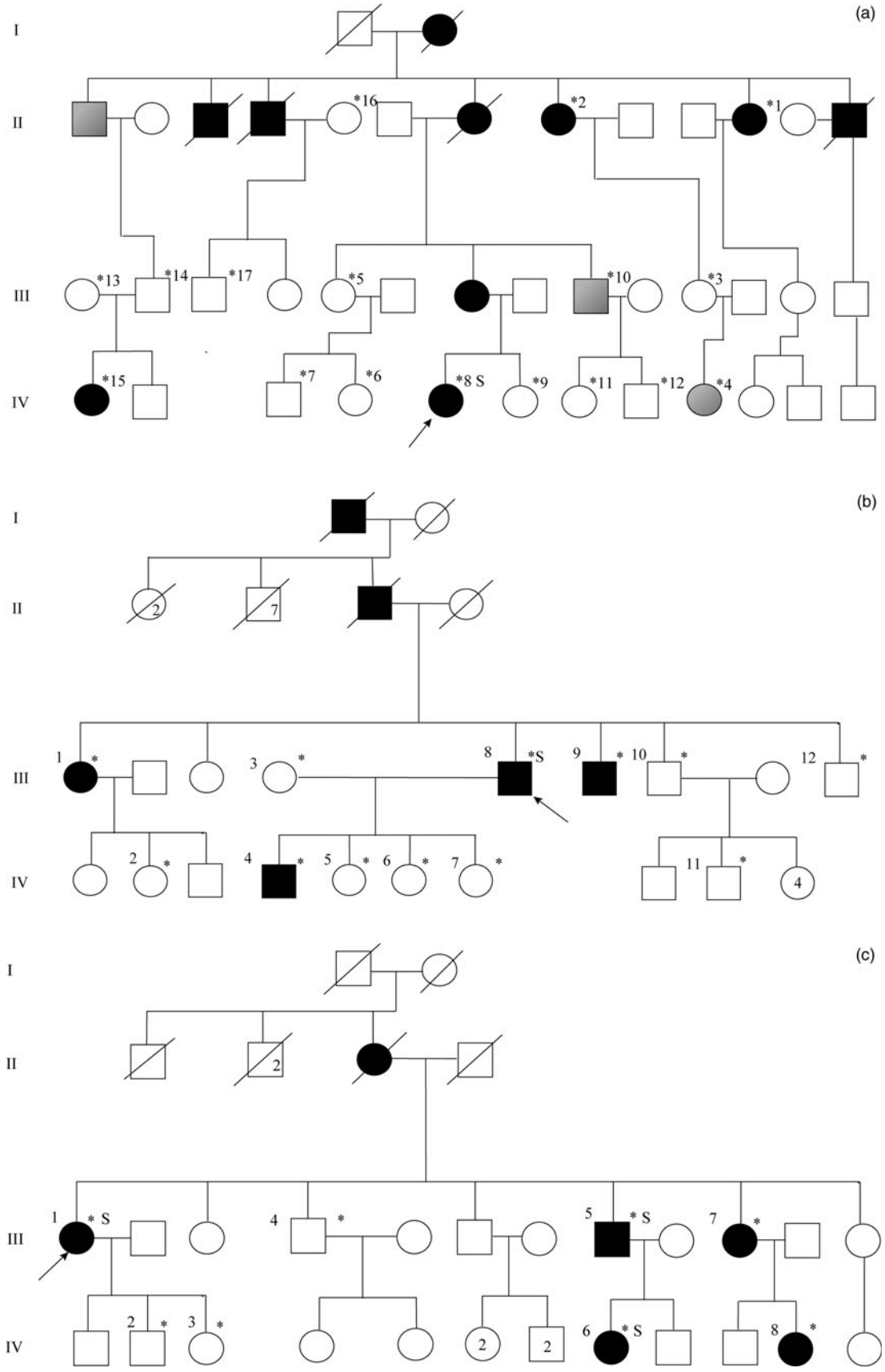

FIG. 1

Pedigrees for families (a) 1, (b) 2, (c) 3, (d) 4, (e) 5, (f) 6 and (g) 7. Only family members whose deoxyribonucleic acid was analysed are numbered. S = surgically confirmed otosclerosis; symbols marked by an arrow = index cases; black symbols = affected individuals, greysymbols = possibly affected individuals, diagonal line = deceased members and an asterisk *indicates those individuals from whom blood was collected.

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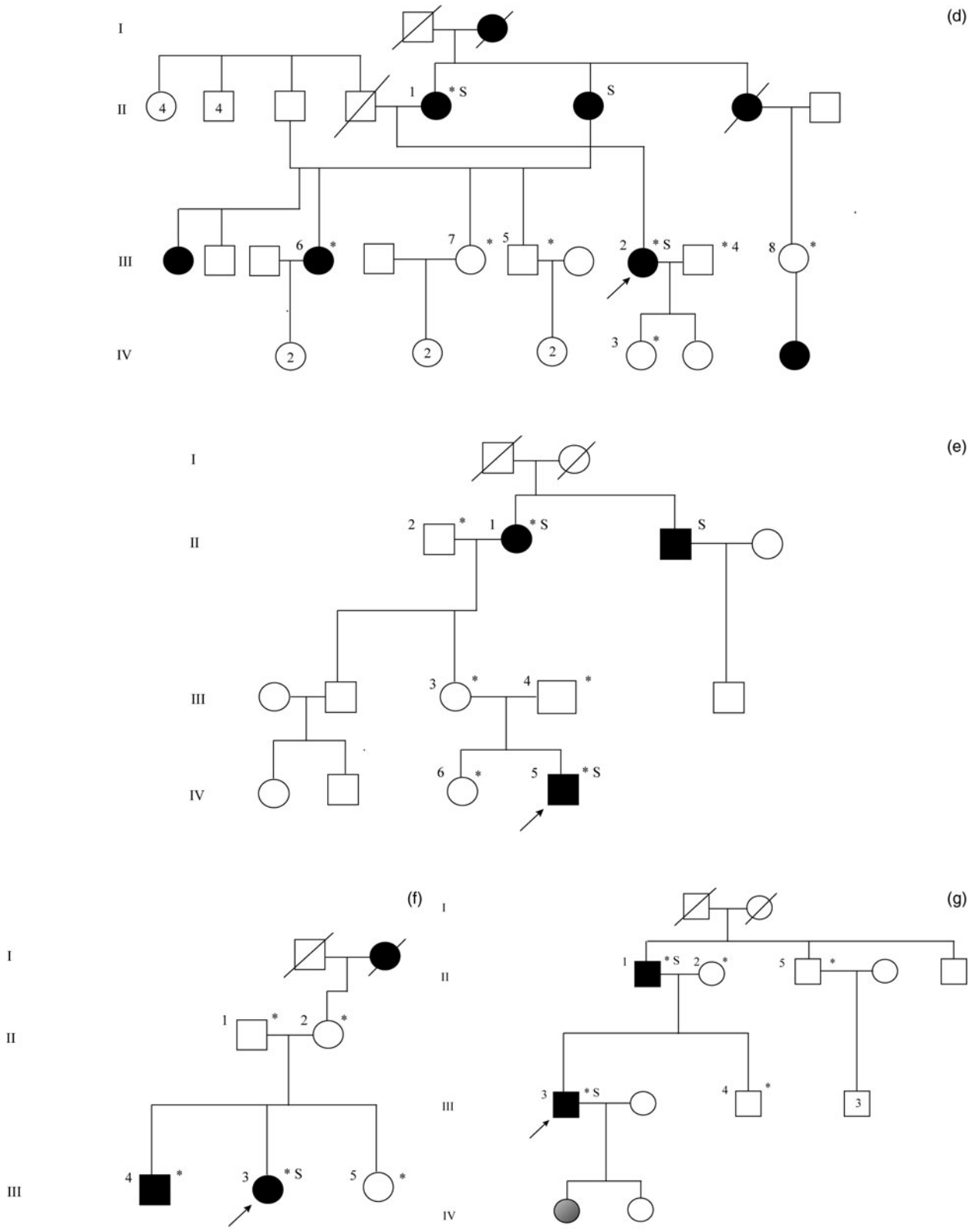

FIG. 1
Continued

Discussion

This study investigated linkage analysis in a series of seven British otosclerosis families. The results excluded linkage of otosclerosis to the 7q33–36 region in a Caucasian population; they also raise the issue of the power of the pedigrees in detecting linkage.

Our inability to replicate any of the reported linkage can be explained by the presence of several

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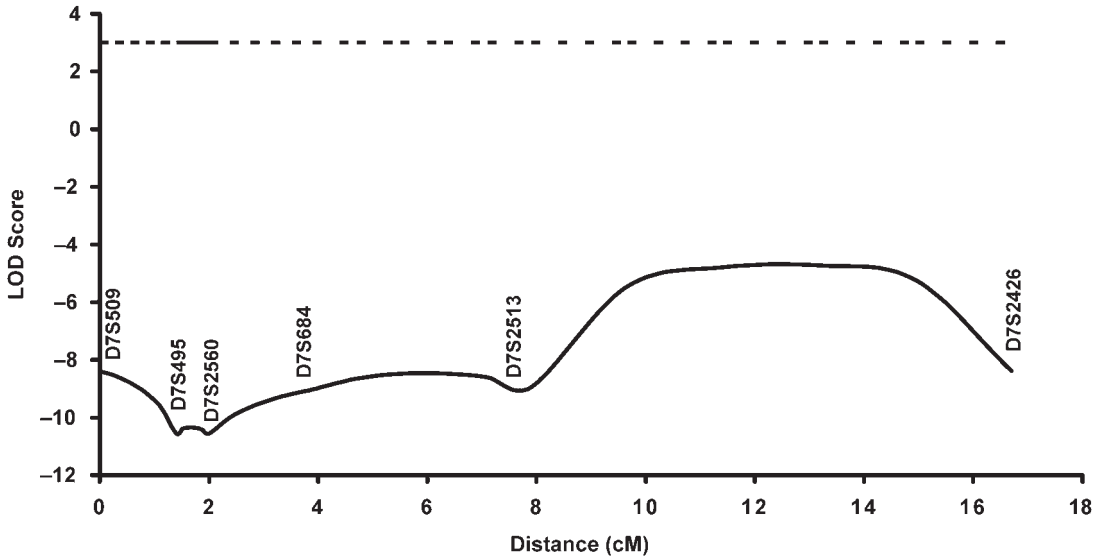

FIG. 2
Parametric multipoint linkage analysis logarithm of odds (LOD) score for the pedigrees.

genes responsible for the disease in some but not all pedigrees (i.e. genetic heterogeneity), or by the high chance of false positive results due to multiple testing. The reported linked loci were revealed previously by conducting a whole genome linkage screen in only a single pedigree, involving a large number of markers to cover the genome and thus involving considerable multiple testing. Although adding more markers to the region of initial linkage and producing higher logarithm of odds scores increased the validity of these results, the chances of false positive results remain considerable. Furthermore, the logarithm of odds scores reported did not exceed 3.97 in the best cases;[16] such scores indicate an area of possible linkage that needs further exploration and confirmation.

Otosclerosis is considered a complex disease, to which genetically susceptible individuals may be predisposed by several environmental factors. Studying the genetic basis of this disease is difficult for many reasons. Firstly, there is a high frequency of phenocopies, and incudo-malleolar fixation and tympanosclerosis can result in identical audiometric profiles. Distinguishing different aetiologies of otosclerosis requires surgical exploration. The majority of studies are not restricted to surgically diagnosed otosclerosis, and thus may include some false cases.

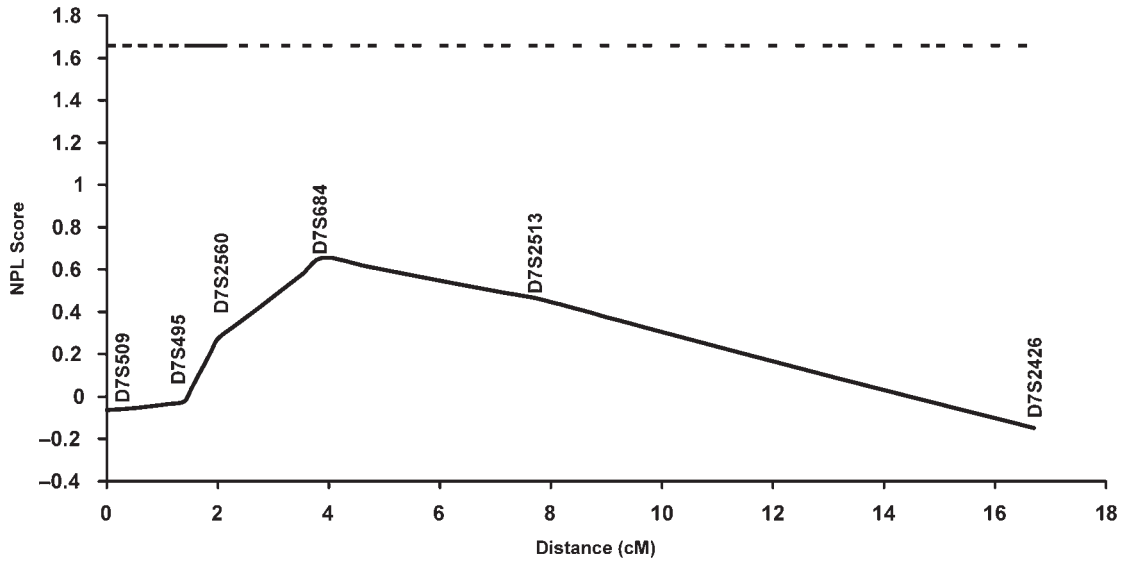

FIG. 3
Logarithm of odds scores for non-parametric linkage analysis (NPL).

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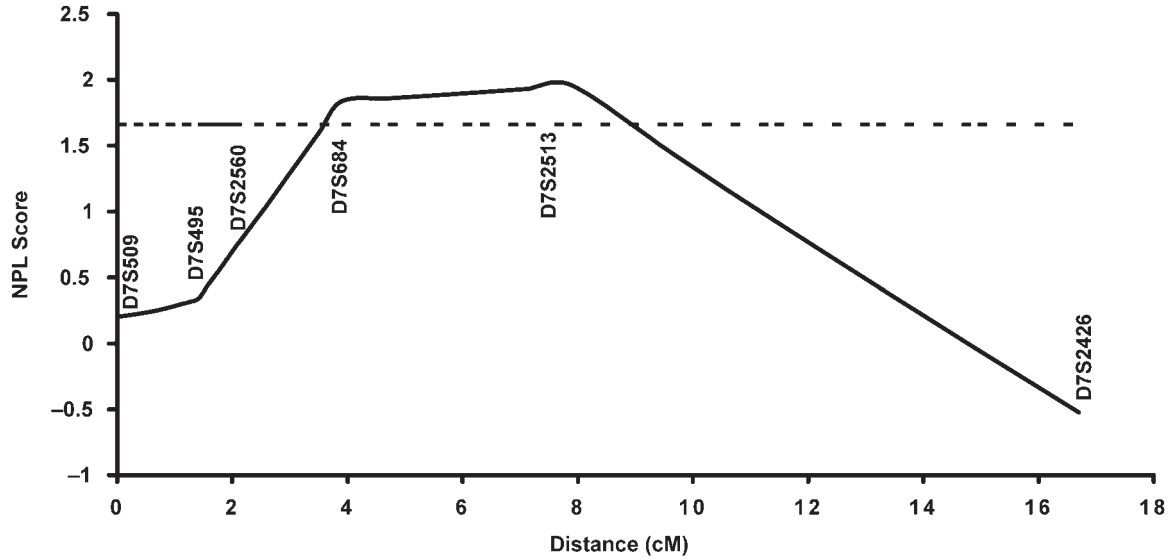

FIG. 4
Logarithm of odds scores for non-parametric linkage analysis (NPL) of pedigree 4.

A second reason for the difficulty in otosclerosis genetic research is that the exact model of inheritance is not accurately known. However, several older studies[7,8,28] suggested that otosclerosis is inherited as an autosomal dominant disease with 40 per cent penetrance;[29] the disease gene frequency reported at that time ranged between 0.0001 and 0.005. These were set as standard linkage parameters for all studies that investigated linkage to otosclerosis, except for penetrance, which ranged between 40 and 90 per cent.[13–15] The wide ranges of disease gene penetrance and frequency are likely to have an effect on the reported logarithm of odds scores. Furthermore, despite the wide acceptance of the dominant model of inheritance, another mode has also been reported.[30] Therefore, it is more appropriate to investigate linkage without implementing any mode of inheritance and by using a non-parametric linkage analysis.

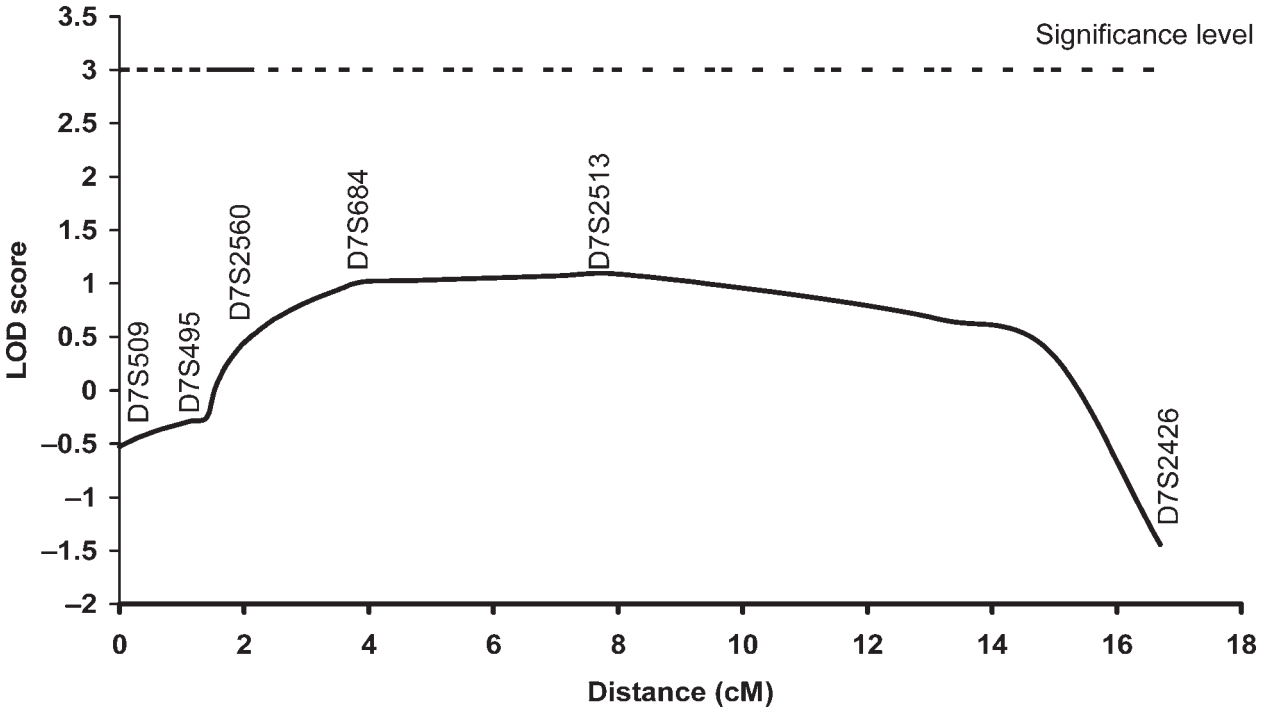

FIG. 5
Logarithm of odds (LOD) score for parametric linkage analysis of pedigree 4.

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- **This study excluded linkage between otosclerosis and the previously reported q33–36 region on the long arm of chromosome seven**
- **Only three studies, including the present one, have investigated linkage in a series of pedigrees; the remaining studies have relied on a single pedigree**
- **Power calculations were not conducted for any of the previous pedigrees or series of pedigrees used to investigate linkage to otosclerosis**
- **Extensive collaboration will be needed in order to conduct a non-parametric linkage analysis on more extended pedigrees of sufficient power, including only surgically confirmed cases**

As with other complex diseases, several loci are usually involved in determining disease susceptibility. These loci are usually of small to moderate effect; hence, a large number of extended pedigrees with many affected individuals is needed in order to detect a significant effect. Large families with multiple cases of otosclerosis are very rare and difficult to ascertain. Only three studies, including the present one, have investigated linkage in a series of pedigrees; the remaining studies have relied on a single pedigree. This raises the question of the power of such studies to detect linkage to loci making a moderate or small genetic contribution. Power calculations were not conducted for any of the pedigrees or series of pedigrees used to investigate linkage to otosclerosis.

Conclusion

We believe that these reported loci need to be analysed using non-parametric linkage analysis on more extended pedigrees of sufficient power, and including only surgically confirmed cases. Such a task would require extensive collaboration. Further investigation of reported loci represents a good start in confirming or excluding these areas; this might be achieved by conducting a pedigree disequilibrium test analysis on trios (i.e. healthy parents with surgically confirmed offspring), as extended pedigrees may be difficult to ascertain. Alternatively, a more practical approach may be to collect a large number of affected, sporadic cases and perform high density single nucleotide polymorphism (SNP) (sequence variation, occuring when a single nucleotide in the genome is altered) based whole genome screening by association.

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