



## Otosclerosis and TGF- $\beta$ 1 gene in black South Africans

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Limited literature is available on the epidemiology and genetics of otosclerosis in South African blacks, among whom it is extremely rare. We undertook this study because we had

documented and surgically confirmed cases of clinical oval window otosclerosis in this population.

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Otosclerosis is a spongifying disease of the labyrinthine capsule of unknown aetiology that occurs only in humans.<sup>1,2</sup> It affects the temporal bone, a focus of bone resorption and reactive bone formation developing into otospongiotic weak bone.<sup>1,2</sup> The prevalence of clinical otosclerosis is 0.3 - 0.4% in adult Caucasians<sup>2</sup> and is low among Japanese, Chinese, Indonesians and American Indians,<sup>3</sup> while the condition is extremely rare in blacks.<sup>1,3,4</sup> It is one of the most common causes of progressive conductive hearing loss among adult Caucasians.<sup>4</sup> Theories about the aetiology of otosclerosis have been postulated but, despite extensive research, its origin remains unclear.<sup>1</sup>

Genetically, otosclerosis is considered to be a multifactorial and complex disease.<sup>1,2,5</sup> During the embryonic formation of the otic capsule, TGF- $\beta$ 1 is considered to be a regulatory molecule that is important in the epithelial-mesenchymal interactions and epithelial induction of the chondrogenesis.<sup>5,6</sup> It is expressed in otosclerosis specimens of adult human bone consisting of stapedial footplate, suggestive of its role in bone turnover and remodelling in otosclerosis.<sup>6-8</sup>

Given the rarity of otosclerosis among blacks, we aimed to define its clinical presentation, diagnosis and genetics in black South Africans. We hypothesised that the clinical presentation would be similar to that in other races and that the TGF- $\beta$ 1 protective gene might play a role in its low incidence among blacks.

### Patients and methods

Cases from January 1993 to December 2006 were compiled from the clinical records of ENT departments at three South African universities and academic hospitals. All patients fulfilling the inclusion criteria of being indigenous black South Africans with progressive painless conductive hearing loss without infection or trauma, normal tympanic membrane and the presence of a Carhart notch on the audiogram, were included. Clinical information was obtained from the patients'

charts and any clinical communication available. Information regarding clinical presentation, demography, family history, audiometric data, operation reports and laboratory results was recorded. Audiograms were analysed based on age and sex-dependent audiometric curves according to International Organization for Standardization (ISO) standard 7029. Surgical and histological reports for all patients considered to be affected clinically were checked for confirmation.

### Genomic study

The genetic analysis was done on 116 DNA specimens obtained from black South Africans. In 10 patients with clinical otosclerosis and 106 controls, DNA was extracted from whole blood. The single nucleotide polymorph (SNP) primers were designed from intronic sequences flanking exons 1, 5 and 6 of TGF- $\beta$ 1 (rs1800472). Direct sequencing of the polymerase chain reaction (PCR) products was performed.

### Results

Our study included a total of 31 patients with clinical otosclerosis (Table I). Ages ranged from 9 to 65 years; there were 22 (71%) females and 9 (29%) males – a ratio of approximately 2:1 (Fig. 1). The mean age of onset was 41 years (females 36 years; males 45 years). They comprised several South African ethnic groups (Fig. 2): 24 (77%) Sotho, 7 Nguni, 6 Xhosa and 1 Zulu. Ten female patients had been pregnant (average of 1.2 pregnancies), and all of them indicated that pregnancy and the first symptom and presentation of otosclerosis were concurrent. The average duration of illness before presentation ranged from 1 to 14 years, with a mean of 6 years. No patients had a family history of clinical otosclerosis.

Presentation was bilateral in 27 (87%) and unilateral in 4 (12%) patients. The average air-bone gap on presentation ranged from 19 to 62 dB with a mean of 40.3 dB (Fig. 3). Most patients presented with moderate hearing loss. Tympanometric findings, recorded in 10 patients, showed type A compliance in 8 patients and type As in 2.

Clinical otosclerosis on history and presence of a Carhart notch had a 100% surgical correlation of oval window ankylosis (stapes footplate fixation) in 28 (90.3%) patients. Histological evaluation of stapes specimens confirmed otosclerosis as a cause of ankylosis (Fig. 4). Information on operative complications was not available. Patients fitted with prostheses after stapedectomy or stapedotomy were satisfied



**Table I. Cases summarised**

No.	Age (yrs)	Gender	Address	Ethnic group	Duration (yrs)	Ear	Diagnosis	Tymp. type	ABG (dB)
P1	23	F	MD	Sotho	5	Both	Clinical	A	19
P2	27	F	MD	Sotho	4	Both	Surgical	A	33
P3	23	F	MD	Sotho	1	Right	Surgical	A	22
P4	9	F	MD	Sotho	13	Both	Surgical	A	33
P5	20	M	MD	Sotho	4	Both	Surgical	A	30
P6	26	F	MD	Zulu	4	Both	Clinical	A	22
P7	49	F	MD	Sotho	6	Both	Surgical	-	37
P8	30	F	MD	Sotho	10	Right	Surgical	-	50
P9	33	F	MD	Sotho	3	Both	Surgical	-	30
P10	18	F	MD	Sotho	1	Left	Clinical	-	37
P11	24	F	MD	Sotho	14	Both	Surgical	-	40
P12	65	M	MD	Sotho	5	Both	Surgical	As	61
P13	30	F	MD	Sotho	5	Both	Surgical	As	60
P14	57	F	MD	Sotho	3	Both	Surgical	A	37
P15	55	M	MD	Sotho	6	Both	Surgical	-	30
P16	32	M	CT	Xhosa	4	Both	Surgical	-	25
P17	54	M	CT	Xhosa	10	Both	Surgical	-	50
P18	31	F	CT	Xhosa	4	Both	Surgical	-	50
P19	36	M	CT	Xhosa	3	Both	Surgical	-	48
P20	63	F	CT	Xhosa	10	Both	Surgical	-	61
P21	61	M	CT	Xhosa	2	Both	Surgical	A	60
P22	56	F	BF	Sotho	2	Left	Surgical	-	62
P23	37	F	BF	Sotho	9	Both	Surgical	-	30
P24	53	M	BF	Sotho	14	Both	Surgical	-	20
P25	50	F	BF	Sotho	4	Both	Surgical	-	40
P26	32	F	BF	Sotho	1	Both	Surgical	-	35
P27	41	F	BF	Sotho	5	Both	Surgical	-	30
P28	29	F	BF	Sotho	2	Both	Surgical	-	22
P29	50	F	BF	Sotho	5	Both	Surgical	-	20
P30	52	M	BF	Sotho	7	Both	Surgical	-	30
P31	48	F	BF	Sotho	10	Both	Surgical	-	25

P1 - 31 = Patient number; MD = University of Limpopo Medunsa Campus; CT = University of Cape Town; BF = University of the Free State; A=Type A tympanogram (normal); As = Type A with stiffness of stapes; F = Female; M = Male; dB = Decibel; Tymp. = Tympanometry type; ABG = Air-bone gap.

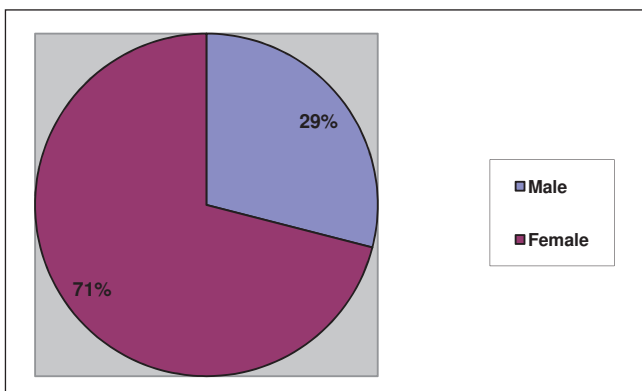


Fig. 1. The ratio of male to female subjects.

with the outcome and hearing benefit. One patient used a hearing aid because she was reluctant to have surgery. Two patients (6.5%) were satisfied with their hearing levels and opted for no treatment. No patients were on medical treatment with fluoride.

A genetic study of 116 DNA specimens was undertaken. Sequencing of the SNPs (1+2), (4+5) and (6) on 10 patients and

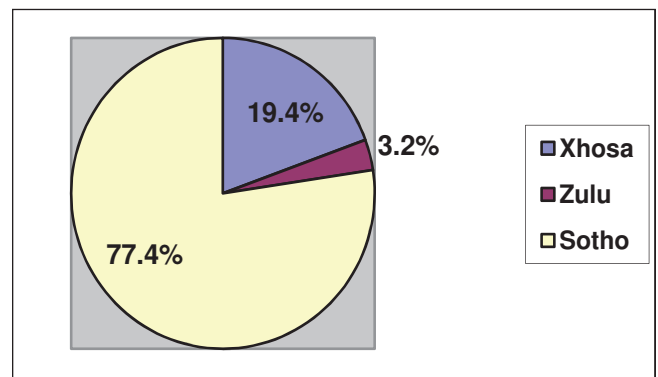


Fig 2. The ratios of ethnic groups of the subjects.

controls was done, looking for polymorphism or mutations on T263I gene associated with TGF-β1. We found that the protective haplotype SNP4-rs1800472, T263I found in European (Belgian-Dutch and French) populations as a protective gene and SNP6-rs8179181 were not polymorphs in the South African black population. However, a rare coding polymorphism on control ZAP-C 100:892 C→T at Exon6 was changing the

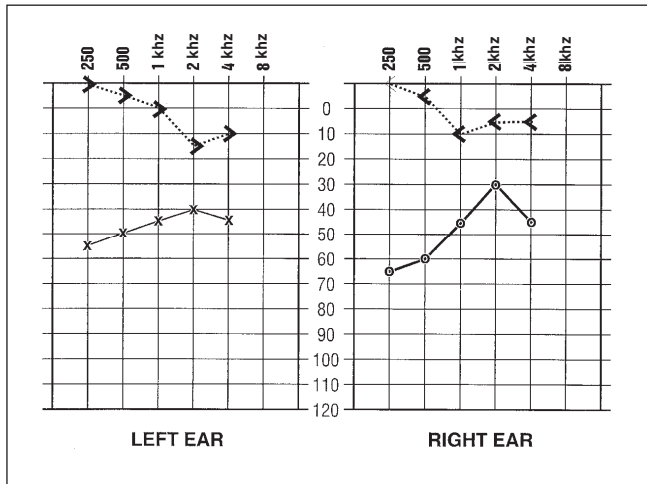


Fig. 3. An audiogram of classic conductive loss with Carhart notch between 1 kHz and 2 kHz commonly associated with otosclerosis.



Fig 4. Specimen of stapes footplate with otosclerosis (arrows).

codon CTG into TTG, coding for the amino acid leucine on position 298. This SNP is a polymorph in the South African black population but very rare in the European population. There were two SNPs at SNP2-rs1982073 at Exon1 and SNP3-rs1800471 at Exon1 which were also polymorphs in the South African black population but very rare in the European population.

## Discussion

There is a relationship between race and the incidence of otosclerosis,<sup>1,3,4,6,8-10</sup> which has been estimated to be about 7

times less common among African-American blacks than among Caucasians in the USA, and to be extremely rare or non-existent among South African blacks.<sup>3,5,6,8</sup> Our study of 31 South African black patients confirms that otosclerosis exists, although it is extremely rare;<sup>5</sup> its incidence is not yet known. Ethnic groups affected were 24 (77.4%) Sotho, 6 (19.4%) Xhosa, 1 (3.2%) Zulu and no others. Whether ethnic differences contribute to the causation of otosclerosis in this population or is an incidental finding needs to be clarified. The ages of presentation are in keeping with other studies.<sup>1,3,4,9,10</sup> Otosclerosis usually presents at around 15 - 45 years of age but can present earlier. The female:male ratio of about 2:1 is in keeping with other reports.<sup>7,11,12</sup> Females present earlier than males. The average duration of illness before presentation ranged from to 1 - 14 years, with a mean duration of 6 years. In keeping with the literature of a negative family history in 50% and variable penetrance of 40%, all patients had a negative family history of otosclerosis. Cases among blacks seem to be sporadic.<sup>1,6,10</sup>

Of our patients, 27 (87%) had bilateral and 4 (12%) unilateral disease ( $p < 0.05$ ). In other reports, more than 85% of patients had bilateral disease.<sup>1,3,4,7,12</sup> There was no significant statistical difference in severity between the left (16 - 51.6%) and the right ears (14 - 48.4%). The average air-bone gap on presentation ranged from 19 dB to 62 dB with a mean of 40.3 dB, in keeping with other studies.<sup>1,3,4</sup> Most patients presented with conductive hearing loss. The compliance in matured otosclerosis is type As.<sup>1,9,10</sup> Of the 31 patients with a clinical diagnosis of otosclerosis, 28 (90.3%) had a 100% correlation of oval window ankylosis (stapes footplate fixation) on exploratory tympanotomy. Patients were satisfied with the outcome and hearing benefits of stapedectomy or stapedotomy which, in experienced hands and appropriate patient selection, is safe and effective, with a success rate of more than 90%.<sup>1,3,7,11,12</sup> The finding in this genetic study of polymorphisms associated with TGF- $\beta$ 1 among South African blacks but which are different to the European population may be because otosclerosis is a complex functional gene condition.<sup>5</sup>

Otosclerosis can be considered as a complex disease with relatively common monogenic forms. Knowledge of these genes could lead to substantial improvements in our ability to diagnose, and possibly even prevent or treat, this type of hearing loss.<sup>2,5,8,13</sup>

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