Microtia: A Combined Approach by Genetics and Audiology

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Introduction

Microtia is a condition in which the external portion of the ear (the auricle) is malformed. In the strictest definition, there is also narrowing or absence of the external auditory canal (external auditory meatus). Microtia varies in severity from barely discernible to an external ear with major structural changes. Hearing loss is a common feature that can be associated with microtia. There are different types of hearing loss – conductive, sensorineural, or mixed – depending on which part of the ear is not working as it should.

Objectives

The present study was designed with the following aims: (i) identification of the genetic etiology and patterns of inheritance of microtia for proper genetic counseling; (ii) early detection and identification of associated hearing impairment for proper management including use of a hearing aid and surgical intervention.

Patients and methods

Twenty children with microtia ranging in age between 1 and 15 years (mean: 6.2 ± 3.68 years) irrespective of sex were included in this study: 14 males and six females. All children were subjected to a full assessment of medical history, a general examination, an ENT examination, tympanometry, pure tone audiometry, and an auditory steady-state response test for patients not responding to a conventional audiometric test. Karyotyping, fluorescence in-situ hybridization (FISH) for Treacher Collins cases, radiological investigations, and fundus examination were also performed.

Results

Syndromic microtia was more frequent than nonsyndromic microtia. Treacher Collins syndrome was the most clinically diagnosed syndrome, followed by Goldenhar's syndrome. There was one case of Down's syndrome and another single case of Johnson–McMillin syndrome. Meatal atresia and preauricular tags were frequently present in the microtia cases, whereas middle ear and inner ear anomalies were only found in some cases. The most common presenting symptom of microtia is hearing loss. Its degree and type differ according to the severity of the disease and frequencies affected. In total, 88.5% (23 ears) have CHL and 11.5% (three ears) have mixed hearing loss.

Karyotyping was performed for 10 cases; nine cases were normal, whereas one case was abnormal (47XY,+21) (Down's syndrome), which represents 5% of all cases studied. FISH was performed for four cases of Treacher Collins syndrome using a probe for chromosome 5 with gene map locus 5q31q33, but no deletion was found in the chromosome 5 Treacher Collins–Franceschetti 1 (TCOF1) gene.

Conclusion

Genetic predisposition for both autosomal dominant and autosomal recessive inheritance seems to be a strong determinant factor in the etiology of microtia than the environmental one. As for Treacher Collins, which is the most frequently clinically diagnosed syndrome in the current study, the FISH study showed that the 5q31-q33 locus may not carry the causative mutation as no single case was positive for this locus. Hearing impairment, of the conductive type, is the most frequent symptom that leads parents to seek medical advice and genetic counseling.

Keywords:
audiometry, fluorescence in-situ hybridisation, genetics, hearing loss, microtia, Treacher Collins

Introduction

The ear is composed of three parts and each of these parts has a slightly different developmental process [1]. The external portion of the ear is composed of the auricle, the external auditory meatus, and the external layer of the eardrum [2]. The most visible portion of the ear is the

auricle. It is formed by a series of auricular hillocks that surround the first pharyngeal groove during the sixth week of gestation [2,3]. Microtia occurs when the tissues that form the auricle fail to develop [3].

Microtia can be grouped into four types according to the level of affection. Type I: the external ear is small and the auricle retains most of its normal structure. The external auditory meatus is usually present. Type II: the external ear is moderately anomalous. The auricle can be hook, S, or question mark shaped in appearance. Type III: the external ear is a rudimentary soft tissue structure with no cartilage; the auricle does not have a normal appearance. Type IV: anotia, where all external ear structures are absent [4].

Although the etiology of microtia is poorly understood, both environmental and genetic factors have been implicated [5], high maternal or paternal age [6], multiple births [5], and also a wide variety of chromosomal abnormalities, such as trisomies of chromosomes 13, 18 [7], and 22 [8]. Congenital aural atresia occurs in ~66% of all patients who have a terminal deletion of 18q. The extent and nature of the chromosome 18 deletions has been studied by array-CGH. A critical region of 5 Mb that was deleted in all patients with congenital aural atresia has been identified on 18q22.3–18q23. This chromosomal region can be considered a candidate region for aural atresia [9]. Chromosomal translocations affecting the 6p24 region have been associated with orofacial clefting and bilateral microtia [10]. A family with an autosomal dominant syndrome characterized by microtia grade II, eye coloboma, and incomplete perforation of the nasolacrimal duct was reported [11].

Oculoauriculovertebral (OAV) spectrum is a heterogeneous disorder involving the first and second branchial arch derivatives. The OAV spectrum is broad, ranging from isolated microtia [12] to Goldenhar's syndrome (GS) characterized by hemifacial microsomia, ocular abnormalities, and vertebral defects. Hemifacial microsomia is a congenital asymmetry of the lower face that is defined as a condition affecting primarily aural, oral, and mandibular development [13]. Additional characteristics, including developmental delay, cardiovascular, pulmonary, and gastrointestinal malformations, can also be found in patients with the OAV spectrum. In a Turkish population with GS, microtia was found in 52% of patients [14].

Around 15–60% of patients with microtia have additional abnormalities [15]. The associated malformations are more common in the bilateral cases. The most common accompanying dysmorphic features are facial cleft, facial asymmetry, microphthalmia, renal abnormalities, cardiac defects, polydactyly, and vertebral anomalies [16]. For some of the microtia-associated syndromes, the developmental defects are known, often at the molecular level. Trials conducted to classify these syndromes include a description in brief of some of the most common syndromes or syndromes with a known molecular basis [17].

Hearing loss is the most common symptom of microtia. Microtia and atresia may be associated with deformities of the middle and inner ear that will affect exactly the level of hearing loss experienced. Some of the deformities encountered include malformations of the ossicles and incomplete development of mastoid bone leading to poor air circulation in the middle ear. Depending on which parts of the ear are affected, typically a child with microtia and atresia will have a moderate hearing loss (40–70 dB) in the affected ear. Sensorineural hearing loss is caused by a fault in the inner ear or the auditory nerve (the nerve that carries the electrical signals from the cochlea to the brain). Sensorineural hearing loss is unusual in children with microtia. This type of hearing loss may indicate another abnormality or an associated syndrome. Mixed hearing loss may also be present [18].

Aims

The present study was designed with the following aims:

(1) Identification of the genetic etiology and patterns of inheritance of microtia for proper genetic counseling.
(2) Early detection and identification of associated hearing impairment for proper management including use of a hearing aid and surgical intervention.

Patients and methods

After approval was obtained from the ethics committee, written informed consent was obtained from all patients entering the trial.

Patients studied

This work included 20 children with microtia, either isolated or syndromic. They were referred from the

Hearing and Speech Institute and Clinic for Children with Special Needs, National Research Center, during the period from January 2008 through June 2009, seeking management of their problem. Their ages ranged from 1 to 15 years (mean: 6.2 ± 3.68 years), irrespective of sex. Fourteen were males and six were females.

All cases were subjected to the following:

(1) A thorough assessment of medical history and pedigree construction and analysis of three consecutive generations. The following factors were considered risk factors in the history: consanguinity, maternal age, multiple births, smoking, premature delivery, diabetes mellitus of the mother, also intrauterine infections, drug intake during pregnancy, and family history.
(2) A full general examination with a special focus on checking for the presence of dysmorphic features such as facial asymmetry, meatal atresia, skin tags, preauricular tags, eyes anomalies, dental anomalies, renal anomalies, heart anomalies, limb anomalies, vertebral column anomalies, and genital anomalies.
(3) Complete ENT examination.
(4) Investigations in the form of a computed tomography scan to exclude other associated inner ear anomalies, abdominal ultrasonography when indicated, echo when indicated, fundus examination when indicated, radiography for the limbs when indicated, and IQ when indicated.
(5) Cytogenetic and fluorescence in-situ hybridization (FISH) analysis:
  (a) Karyotyping for some selected cases: chromosomal analysis from peripheral blood lymphocytes was carried out using the standard G-banding technique [19,20]. Karyotyping was performed according to the International System for human Cytogenetic Nomenclature (ISCN) [21].
  (b) FISH for Treacher Collins cases: the FISH technique was used for the diagnosis of some selected cases (Treacher Collins cases) where chromosomal abnormalities could not be detected using the usual G-banding technique such as microdeletion syndromes according to Pinkle et al. [22]. FISH was performed for detection and localization [ON MDS 5q-(5q31; 5q33), dual color probe and hTERT (5p15), triple color probe]. The probe used was a locus-specific probe for 5q- with two critical regions: critical region 1 (5q33), spectrum orange and critical region 2 (5q31), spectrum green. The experiment was (5q31), conducted according to Pinkle et al. [22] and the manufacturer's instructions.
(6) Audiological measurements:
  (a) Tympanometry and acoustic reflex threshold measurements (if possible) were performed using an acoustic immittance meter interacoustics model AZ26 USA with a 220 Hz probe tone.
  (b) Hearing threshold determination was performed using a clinical audiometer interacoustics model AC40 USA.
  For children younger than 5 years of age or uncooperative children who could not perform (i), the following was carried out:
  Evoked potentials measurements: auditory steady-state response (ASSR) testing, which is nonbehavioral and also a frequency-specific test. AC/ASSR and BC/ASSR thresholds could be recorded reliably in children with normal hearing and conductive hearing losses. AC/ASSR and BC/ASSR were performed using the evoked potential system GSI Audera device (Grason-Stadler), chloral hydrate was used to sedate the children at a dose of 0.5 mg/kg. The children remained asleep during the test and the electrode site was cleaned with alcohol. Electrodes were placed on the high forehead (Fz) in the noninverting position and the ipsilateral mastoid in the inverting position. A third electrode placed on the contralateral mastoid served as a ground.

Stimulus parameters

The ASSR thresholds were recorded using the default settings of the GSI Audera evoked potential. The AC/ASSR and BC/ASSR were evoked with tones modulated in amplitude and frequency with a relative AM/FM phase difference of 08. The tones were 10% frequency modulated and 100% amplitude modulated (0.5, 1, 2, and 4 kHz modulated at 74, 81, 88, and 95 Hz, respectively). High modulation rates were used to ensure that a satisfactory signal-to-noise ratio would exist for detection of responses during sleep or sedation.

Air-conduction/auditory steady-state response and bone-conduction/auditory steady-state response

For AC stimuli, a single modulated carrier frequency was presented per trial through EAR TIP-50 (New Eagle, PA, USA) insert earphones calibrated in dBHL. The AC/ASSR initial stimulation intensity was determined by PTA threshold, starting from 10 dB

above this threshold. If the PTA threshold was absent at maximum intensities, the AC/ASSR stimulation commenced at the maximum intensities of the Audera equipment. BC stimuli were presented through a Radioear B-71 bone oscillator (New Eagle, PA, USA) that was held in place on the mastoid of each child by a headband. The initial BC stimulation commenced at the level of the corresponding AC threshold. Contralateral AC masking was presented through insert earphones at a fixed value of 50 dBHL. ASSR thresholds were established for each test frequency by increasing or decreasing the stimulus presentation level in 10 dB steps. Once an approximate minimum response level was established, the threshold was defined as the softest level at which a statistically significant response could be achieved. The presence or absence of a response was determined automatically using a statistical measure known as phase coherence squared (PC^2). Each PC^2 value is evaluated to determine the probability that a given distribution of phases could have arisen for a trial where no stimulus was present. If this probability was sufficiently small ($P < 0.03$), a response was considered to be present ($P < 0.03$ is the default criterion for the GSI Audera system).

Statistical analysis

The data collected were coded, tabulated, and statistically analyzed using the SPSS program software (version 18.0; SPSS Inc., Chicago, Illinois, USA).

Results

The present study included 20 patients ranging in age from 1 to 15 years (mean age: 6.2 ± 3.68 years).

In terms of the demographic data of the study group, it was found that the incidence of microtia increased when maternal age was beyond 35 years. Sixty percent of the children were born to mothers older than 35 years of age, whereas the rest of the children were born to mothers younger than 35 years of age. It was also found that the incidence of microtia increased with multiple births as 70% of the children were born to mothers with multiple births. Consanguinity played a role in the current study as 65% of the patients with microtia were born to consanguineous parents. It was also found that the number of males exceeded females in the study (14 male cases and six female cases). In terms of laterality, 70% of the patients with microtia were unilateral and 13% were bilateral. Among the unilateral cases, right-sided microtia was present in nine (65%) children and left-sided microtia was present in five (35%) children.

Our study showed that syndromic microtia was more frequent than nonsyndromic type (65 vs. 35%). Treacher Collins syndrome (TCS) was the most clinically diagnosed syndrome among microtia cases, followed by GS, whereas there was only one case of Down's syndrome and another single case of Johnson–McMillin syndrome. Thirty-five percent of cases were isolated and 10% of cases were sporadic (Table 1).

Meatal atresia and preauricular tags were frequently present in microtia cases (70 and 40%, respectively), whereas middle ear and inner ear anomalies were only found in some cases of microtia (30 and 20%, respectively). The percentage of occurrence of preauricular tags and meatal atresia is higher than the percentage of middle and inner ear anomalies (Fig. 1).

The most common congenital anomalies associated with microtia are eye and mandibular anomalies, followed by dental and genital anomalies and heart, limb, spine, and renal anomalies (Fig. 2).

Table 1 Classification of microtia cases

	N
Syndromic microtia	
Treacher Collins syndrome	5
Goldenhar's syndrome	4
Johnson–McMillin syndrome	1
Down's syndrome	1
Sporadic	2
Total	13
Nonsyndromic microtia	
Isolated	7
Total	7

Figure 1
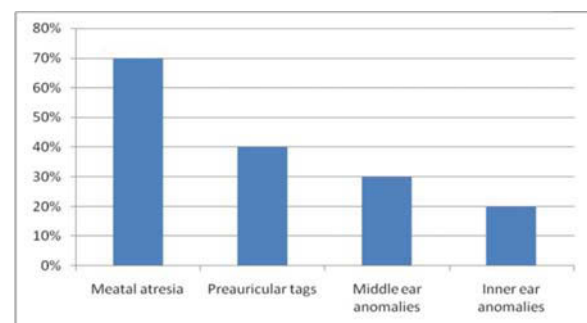
Percentage of associated ear anomalies.

Figure 2
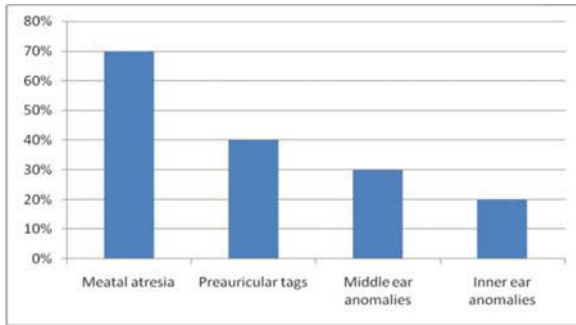
Frequency of congenital anomalies associated with microtia cases.

Table 2 Cytogenetic and fluorescence in-situ hybridization analysis of the studied cases

Tests	Number of cases	Result
Chromosomal analysis using G banding	10	Normal: 9 cases
	Treacher Collins: 3	Abnormal: 1 case
	Others: 7	(47XY) (Down's syndrome)
FISH analysis for 3 Treacher Collins cases for chromosome 5	3	No deletion

FISH, fluorescence in-situ hybridization.

Cytogenetic and fluorescence in-situ hybridization analysis

Karyotyping was performed for 10 children; nine were normal, whereas one case was abnormal *(47XY,+21)* (Down's syndrome), which represents 5% of all studied cases. FISH was performed for three patients with TCS using a probe for chromosome 5 with gene map locus *5q31-q33*, but no deletion was found in the chromosome 5 Treacher Collins–Franceschetti 1 (TCOF1) gene (Table 2).

Figure 3 shows the spread and karyotype in a normal female child.

Figure 4 shows the metaphase of a case with Treacher Collins hybridized with the locus-specific identifiers (LSI) *5q33* locus (two red signals) and the (locus-specific identifiers) *LSI 5q31* locus (two green signals), indicating no deletion of both critical regions.

Audiometric measurements

Twenty children were subjected to threshold determination using air-conducted and bone-conducted stimuli. Thirteen children could respond behaviorally to the behavioral pure tone audiometry. Ten children had unilateral microtia and the other three children had bilateral microtia. A total of 16 ears were examined ears. AC and BC thresholds and the different scores in reliable children are shown in Table 3.
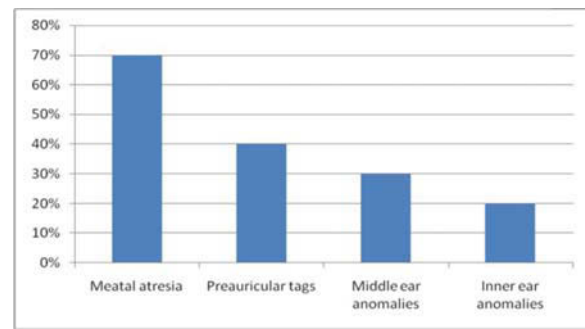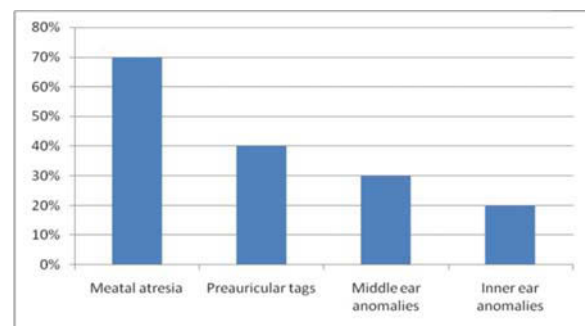
Three ears in this group showed a response to BC stimuli at high intensities, indicating mixed-type hearing loss. Seven children showed an unreliable response during the behavioral pure tone audiometric test either because of young age or other causes; thus, they were subjected to AC/ASSR and BC/ASSR measurements. A total of 10 ears were tested (four children had unilateral microtia and the other three had bilateral microtia); the test results are

Figure 3

Spread and karyotype showing a normal female.

Figure 4

Metaphase of a case with Treacher Collins syndrome hybridized with the LSI *5q33* locus (two red signals) and the *LSI 5q31* locus (two green signals).

presented in Table 4, which shows the results of ASSR at 500, 1000, 2000, and 4000 Hz.

Discussion

Chromosomal abnormalities occur in 6–16% of cases of microtia [24,25]. Common chromosomal abnormalities associated with microtia include

Table 3 Air-conduction/PTA and bone-conduction/PTA thresholds and the difference score in reliable children (*n* = 16 ears)

Frequency (Hz)	AC/PTA (dBHL) (mean ± SD)	BC/PTA (dBHL) (mean ± SD)
500	65.5 ± 5.73	15 ± 5.3
1000	65.0 ± 5.53	20.50 ± 6.4
2000	56.25 ± 5.91	22.5 ± 7.6
4000	65.25 ± 6.09	25.0 ± 5.6

AC, air-conduction; BC, bone-conduction.

Table 4 Air-conduction/auditory steady-state response and bone-conduction/auditory steady-state response thresholds and the difference score in unreliable children with CHL (*n* = 10 ears)

Frequency (Hz)	AC/ASSR (dBHL) (mean ± SD)	BC/ASSR (dBHL) (mean ± SD)
500	63 ± 5.96	25 ± 5.4
1000	53.5 ± 6.87	23.5 ± 6.0
2000	55 ± 10.40	23.1 ± 7.2
4000	45.5 ± 15.50	22.5 ± 7.7

AC, air-conduction; ASSR, auditory steady-state response; BC, bone-conduction.

trisomy 21, trisomy 18, trisomy 13, and the deletion complexes *18q-, 18p-, and 5p- [24,26,27]*. This was not the case in our work (perhaps because of the limited number of isolated and sporadic cases studied). Karyotyping was performed for 10 children; nine were normal, whereas one was abnormal (47XY,+21) (Down's syndrome), which represents 5% of all cases in this study. Isolated microtia cases in our study represented 45% of the studied cases. However, microtia was an isolated condition in 65% of the cases [24,28,29]; in contrast, other studies have reported isolated rates of less than 50% [25,30].

In our study, TCS (most frequently diagnosed) manifestations included the following: microtia, coloboma of lower eye lid, downward slanting of the eyes, facial asymmetry, micrognathia, cleft palate, brachydactyly, and syndactyly. This is in agreement with other studies of TCS that described similar associated anomalies [31–33]. One out of five cases of TCS showed limbal dermoid. Interestingly, limbal dermoid was a new finding (not described previously in any other case of TCS reported in the literature) among the patients with TCS in our study.

TCS is an autosomal dominant, craniofacial disorder caused by a mutation in the TCS gene, TCOF1 [31]. The parents of all our TCS patients studied were clinically free, even from minor manifestations. This could be because of either a dominant new mutation or germline mosaicism. In this study, FISH was performed for four patients with TCS using a probe for chromosome 5 with the gene map locus *5q31-q33*, which indicated no mutation in the chromosome *5 TCOF1 gene*.

Balestrazzi *et al.*[34] were the first to describe a de-novo balanced translocation involving chromosome 5 in a girl with the TCS; Jabs *et al.*[35] studied linkage with chromosome 5 markers in eight families with this disorder. They reported positive scores with four loci that mapped to *5q31.3-q33.3*.

Dixon *et al.*[36] also reported linkage of the TCS locus to markers in the 5q31-q34 region. Dixon *et al.*[37] refined the localization by linkage studies using FISH. They concluded that the gene is in 5q32-q33.2. Dixon *et al.* [38] further refined the assignment to 5q32-q33.1. This was further confirmed by the study of Arn *et al.*[39], who reported a case of TCS with its locus assigned to *5q31.3*. In 1996, the gene that underlies TCS was mapped to human chromosome *5q31.33.2* and named Treacher Collins–Franceschetti syndrome 1 (TCOF1). In 1997, the protein product of TCOF1 was identified as a low-complexity protein and named treacle [40]. Within the coding region of TCOF1, 51 mutations have been found that result in a truncated treacle protein [41].

In addition, Mogass *et al.*[42] reported that Treacher is found on human chromosome 5q32–33. Forty percent of the cases of TCS are because of clear inheritance of a mutation in the coding region, but the remaining 60% arise from de-novo mutations [33]. To date, more than 50 mutations have been identified in the TCOF1 gene; most of them are insertions or deletions [43]. This would, therefore, explain why the FISH study in our TCS cases failed to identify this specific *5q32-q33.1*, which could either be because of an as yet unidentified new mutation or ethnic variation.

In our study, GS manifestations among the studied cases included the following: microtia, preauricular tags, cheek tags, conductive hearing loss, facial asymmetry, micrognathia, coloboma of the upper eye lid, hemivertebrae, scoliosis of the upper dorsal spine, and hypoplastic kidney. This is in agreement with other studies of GS that described similar associated anomalies [44,45]. However, other research groups described the following anomalies that we could not identify in our cases: macrostomia or pseudomacrostomia, cleft lip or palate, delayed dental development, cardiac abnormalities such as coarctation of the aorta, ventricular septal defect,

tetralogy of Fallot, and patent ductus arteriosis, cerebral malformation, mental retardation, and renal ectopia [44,45]. Two out of four patients with GS showed high-arched palate.

We found only one case that was highly, clinically, compatible with Johnson–McMillin syndrome. This was a boy with microtia, CHL, micrognathia, squint, facial hypoplasia, facial nerve palsy, and multiple truncal cafe-au-lait spots who was born to a mother with the same manifestations. Our patient seems to be identical to the autosomal dominant disorder mentioned by Johnson et al.[46], who described a mother and son with microtia, CHL, micrognathia, facial nerve palsy, multiple truncal cafe-au-lait spots, and mild developmental delay. The son had hypotrichosis, whereas the mother also had hyposmia, increased tendency toward development of caries, and growth retardation. Interestingly, squint is a new finding in Johnson–McMillan syndrome in our study.

In our study, a single patient with Down's syndrome was identified who had bilateral microtia, which represents 5% of chromosomal abnormalities among our studied cases. Chromosomal abnormalities occur in 6–16% of cases of microtia [24,25].

The most common presenting symptom of microtia is hearing loss, which ranges from mild to severe according to the severity of the disease and frequencies affected [47].

Thirteen children could respond behaviorally to the conventional pure tone audiometry. Ten cases children had unilateral microtia and the other three had bilateral microtia. A total of 16 ears were examined ears. The AC/PTA thresholds were 65.5 ± 5.73, 65.0 ± 5.53, 56.25 ± 5.91, and 65.25 ± 6.09 dBnHL and BC/PTA thresholds were 15 ± 5.3, 20.50 ± 6.4, 22.5 ± 7.6, and 25.0 ± 5.6 dBnHL (for frequencies 500, 1000, 2000, and 4000 Hz, respectively).

The rest of the seven children who gave an unreliable response during the behavioral pure tone audiometric test were subjected to AC/ASSR and BC/ASSR measurements as ASSR provides an accurate estimation of frequency-specific AC thresholds for varying degrees of hearing loss and has the added advantage of objective response detection by statistical tests [48]. More recently, the use of BC/ASSR has also become available on clinical systems [49].

A total of 10 ears were ASSR tested (four children had unilateral microtia and the other three had bilateral microtia). The AC/ASSR thresholds were 63 ± 5.96, 53.5 ± 6.87, 55 ± 10.40, and 45.5 ± 15.50 dBnHL and BC/ASSR thresholds were 25 ± 5.40, 23.5 ± 6.0, 23.1 ± 7.2, and 22.5 ± 7.7 dBnHL (for frequencies 500, 1000, 2000, and 4000 Hz, respectively). Notably, the mean AC/ASSR thresholds in the tested ears confirmed a typical conductive hearing loss configuration – that is, slightly elevated thresholds sloping toward lower frequencies (500 Hz). Swanepoel et al.[49] reported the same finding in children with slightly elevated thresholds sloping toward the lower frequencies (250, 500 Hz). BC/ASSR could be detected at all frequencies in all tested ears. The overall average of BC/ASSR thresholds indicated normal hearing in the presence of a typical conductive hearing loss configuration with elevated BC/ASSR thresholds sloping toward lower frequencies.

After reviewing the resulting data from PTA and ASSR measurements, it was obvious that the prevailing type of hearing loss was the conductive type, 88.5% (23 ears), whereas 11.5% (three ears) had mixed-type HL.

This is in agreement with the results of Jahrsdoerfer and Jacobsont [50], who found that bilateral atresia is usually accompanied by a 45–65-dB conductive hearing loss. They also indicated that Treacher Collins patients present with maximum conductive hearing loss often compounded by a high-frequency sensory component. A symmetrical rising configuration is most prevalent, but often accompanied by a decrease in hearing sensitivity in the 4000–8000-Hz range.

The use of auditory evoked potential techniques, such as auditory brainstem response and ASSR, may be the only way to determine hearing sensitivity in difficult-to-test populations such as infants, young children, and individuals with multiple handicaps. Auditory evoked potentials are used only to estimate pure tone hearing thresholds as behavioral audiometry remains the gold standard in the classification of hearing loss with AC and BC tones. Frequency-specific AC and BC thresholds enable differentiation between sensorineural, conductive, and mixed hearing losses. This distinction is necessary for planning an appropriate medical intervention [49].

Conclusion
Genetic predisposition for both autosomal dominant and autosomal recessive inheritance seems to be a strong determinant factor, in the etiology of

microtia, than the environmental one. Consanguinity, advanced maternal age at conception, and high parity are known risk factors.

In terms of the TCS, which is the most frequently clinically diagnosed syndrome in the current study, the FISH study showed that the *5q31-q33* locus may not carry the causative mutation as no single case was positive for this locus.

Hearing impairment, conductive type, is the most frequent symptom that leads parents to seek medical advice and genetic counseling.

Recommendations

Early identification of microtia (with its associated symptoms and signs) and skilled ability to delineate new findings or new syndromes are of paramount importance for proper genetic counseling for anxious families. This would enable an early intervention using a properly selected suitable hearing aid and initiation of speech therapy at the optimum time for proper language development.

An accurate and precise diagnosis would facilitate proper timing of initiation of reconstructive plastic surgery to preserve a child's self-esteem.

Conflicts of interest
None declared.

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