Original Article

Characteristics of 43 multiple auricular deformity case families and auricle morphology in 463 microtia patients in South China

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Background: Earlier studies have suggested that microtia is a genetic disease with a worldwide incidence of microtia is between 0.83/10,000 and 17.40/10,000. For microtia, auricle morphology is the most crucial characteristic. However, no studies have been performed to characterize the genetic similarity of microtia and auricle morphology similarity. For the sporadic patients, the relationship between the gestational age of parents and the incidence of microtia is unclear. To obtain the characteristics of auricular deformity multiple case family (AD-MCF) and clarify the relationship between genetic similarity and auricle morphology similarity in AD-MCF.

Methods: This study included 463 AD patients who were diagnosed by Sun Yat-sen Memorial Hospital, Sun Yat-sen University, from 2013 to 2019. Among these patients, 116 are from 43 MCF and the other 347 patients are sporadic. For the patients from families, the disease status of the four generations of immediate family members and the family tree map were collected to analyze the similarity of auricle shape in family members. A score evaluated the similarity of auricle shape according to the structure of the residual ear and the similarity in the morphology of each auricle. Moreover, the population distribution of AD and the gestational age of patients were further analyzed.

Results: From 2013 to 2019, a total of 463 patients were diagnosed as microtia in our hospital. There were 427 patients with unilateral disease and 36 patients with bilateral disease. Among them, 116 patients were from 34 families and 9 *de novo* families. The total scores of patients in different genetic difference levels were compared and were found significantly different (P<0.001). Moreover, 58.14% of families were consistent with the law of chromosomal recessive genetic diseases. Importantly, we found that the gestational age of father in microtia *de novo* families is 30.94±0.75, and mother in *de novo* is 28.39±0.73 that is significantly higher than the gestational ages of parents from microtia families with P value =0.0001.

Conclusions: The auricle similarity between family members is positively related to the genetic distance between family members. The microtia patients are potentially associated with the gestational ages of parents.

Keywords: Characteristics of the family; auricular deformity multiple case family (AD-MCF); auricular morphology; microtia; South China

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Introduction

Microtia is a congenital disease of the ear, manifested as auricular deformity (AD), which is also the main reason for the patient's consultation. The auricle shape determines the subtype of microtia. Microtia commonly involves the external canal and middle ear, which can hence influence hearing. Four microtia subtypes were observed currently (1). The shape of microtia malformation is the result of the combined action of multiple pathogenic factors, as demonstrated in many studies (2-5). However, no research on the relationships between shapes of residual ears has been performed currently to which may benefit the discovery of the mechanism of the formation of AD.

Besides the shape of residual ears, many researchers suggest that congenital diseases may be related to the gestational age of the parents (6-8). Besides, Jacobs *et al.* discovered the time-dependent nature of somatic events in the etiology of cancer and potentially other late-onset diseases (9). However, no study has been performed to construct the relationship between AD and the gestational age of the parents.

Moreover, the incidence of microtia is not consistent across countries and regions, which may due to the differences on the heritability of populations and definition of microtia (10-16). According to Alasti and Luquetti, the global incidence of microtia varies from 0.83/10,000 to 17.40/10,000 (17,18). Microtia incidence of pedigrees ranges from 2.9% to 33.3% (18). The prevalence of microtia was about 20–40% higher in men than in women (*Table 1*). However, no study has been performed to discover the incidence of microtia in China, especially in South China.

In this study, 43 families AD and AD-MCF were collected by Sun Yat-sen Memorial Hospital of Sun Yat-sen University from South China, which was involved by 116 affected. The AD and AD-MCF patients in families were studied to improve the understanding of morphological characteristics of AD. Moreover, this study has collected parents of each MCF patient to investigate the contribution of parents gestational age in appearing of AD-MCF.

Methods

Study population and data collection

Patients with microtia deformities were collected from Sun Yat-sen Memorial Hospital of Sun Yat-sen University from January 2013 to January 2019. All subjects in this study are Chinese citizens. The necessary information of the patients include age, address, medical history, the auricular morphological characteristics of the patient and the affected side, the family history, their parents reproductive age, occupation, body mass index (BMI), medical history such as previous medical history, symptoms, place of residence, contact phone number, etc. The health information of the patient family was recoded for four generations of immediate family members, which had auricle shape and other diseases.

Assessment of AD

In this study, patients with microtia were classified into four types according to the degree of deformity. Classic examples of these four types of microtia were shown in *Figure 1A*. The detailed descriptions of them were shown in *Table 2*. The type I microtia usually means hidden ear, wind ear, or cup-shaped ear that are with the recognizable anatomy of the auricle. The type II microtia with characteristics that the anatomical structure of the auricle was recognizable, the auricle and triangular socket were merged, the shape of the auricle was significantly narrowed, and the ear cavity was narrowed considerably. The type II of microtia is often divided into type II-A and Type II-B according to the auricle cartilage curling degree and clinical treatment methods. The patients with type IIA were seen having the upper auricle with a broad transverse diameter and more folding cartilage.

In comparison, the patients with type II B have the narrow upper part of the auricle, less amount of folded cartilage. For type II B patients, no auricle cartilage can be used for stretching, or even after stretching, and the cartilage auricle was not dilated significantly. Microtia

Table 1 Studies about the incidence of microtia

Author	Topic	Prevalence (per 10,000)	M/F	U/B	R/L	MCF (%)
Gary M. Shaw	Epidemiologic Characteristics of Anotia and Microtia in California, 1989–1997	2.50	1.27/1	–	–	–
Mark A. Canfield	Epidemiologic Features and Clinical Subgroups of Anotia/Microtia in Texas	2.86	–	3.50/1	–	–
Mathias B	Descriptive epidemiology of anotia and microtia, Hawaii, 1986–2002	3.79	1.67/1	3.95/1	1.77/1	–
John Harris	The epidemiology of anotia and microtia	French: 0.76; Swedish: 2.35	1.33/1	1.01/1	1.17/1	–
Castilla EE	Prevalence Rates of Microtia in South America	17.4	–	–	–	–
Klockars T	Inheritance of microtia in the Finnish population	–	1.80/1; 1.86/1*; 1.75/1#	11.33/1; 12.43/1*; 10.00/1#	1.82/1; 1.81/1*; 1.86/1#	20.65
Our	–	–	3.13/1; 4.18/1*; 1.58/1#	11.86/1; 10.47/1*; –#	2.78/1; –*; –#	9.43
Du, Jiamei	Psychological status of congenital microtia patients and relative influential factors: analysis of 410 cases	–	3.27/1	–	–	–
Smahel Z	Craniofacial changes in unilateral microtia	–	–	–	–	4
Isabel Llano-Rivas	Microtia: A Clinical and Genetic Study at the National Institute of Pediatrics in Mexico City	–	2/1	2.22/1	2/1	33.33

*, sporadic; #, MCF. MCF, multiple case family; M, male; F, female; U, unilateral; B, bilateral; R, right; L, left.

type III is represented by the unrecognizable anatomical structure of the auricle, and irregular shape of the residual ear, which is mainly similar to peanut-shaped, boat-shaped, cord-shaped, or sausage-shaped. Microtia Type IV denotes the affected ear with small skin tags or scattered hill-like bulges, the auricle completely missing, and no local anatomical traces. Patients who belong to at least one type of microtia malformation were included in this study.

Assessment of similarity of residual auricles

The similarity of residual auricles was evaluated by Structural Similarity Score Method (SSSM) that is similar to Qing He and Gaute Floer Johnsen (19,20). The comparison is on the shape of auricle morphology. For four types of AD, the similarity evaluations focus on different respects. The Type I AD is on the similarities of concealed ears, stroke ears, and cup-shaped ears. The similarity of the type IIA AD is on cartilage extension length and remained auricle structure. For the type IIB, the resemblance is on cartilage margin length, auricle width, the remained height and auricle structure. The similarity of type III is defined as the presence of cartilage in the upper auricle, middle cartilage, and the lower cartilage, the shape of each cartilage, the width and length of cartilage, and the external ear canal. The comparison of the type IV auricles is on the presence of residual ear, residual ear shape, and residual ear volume.

The residual ears of patients were compared with their parents. SSSM is used to evaluate the similarity of anatomical structures. Structural Similarity Score Method (SSSM) is to evaluate the structure and the shape similarity of microtia auricle shape (*Figure 1B,C*). The similarity was scored by SP% = (CS – US)/AS, where SP is the Similarity point of structure, the US is the number of Unshared structure, CS is the number of Common structure, AS is the number of total structure (*Figure 1B*). The observed structure of the ear includes upper cartilage of the ear, middle cartilage of the ear, lower cartilage of the ear, earlobe, and external acoustic

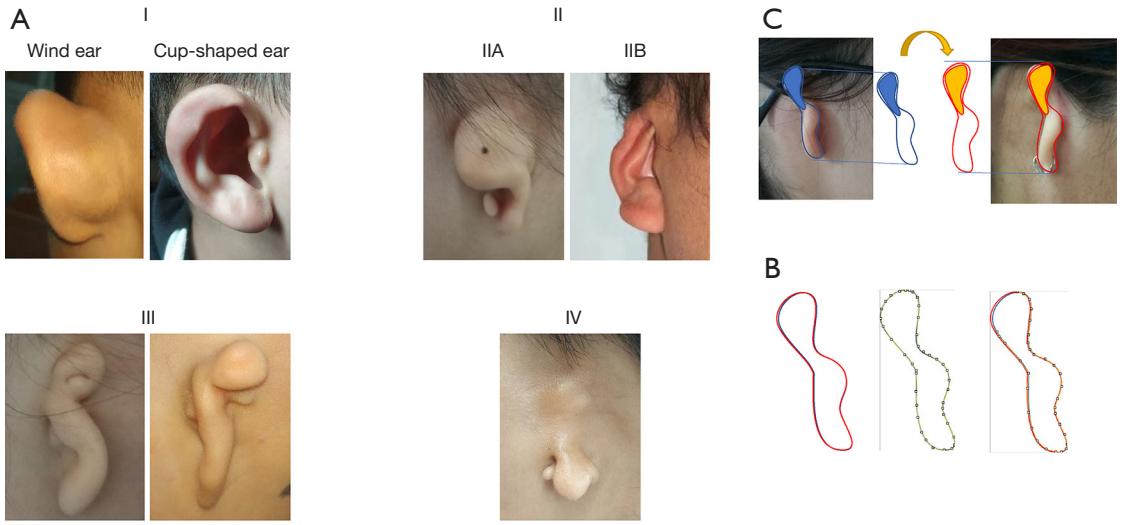

Figure 1 Classic examples of these four types of microtia and Structural Similarity Score Method (SSSM). (A) The type I microtia with the recognizable anatomy of the auricle. The type II of microtia is often divided into type II-A and Type II-B. Type IIA were observed having the upper auricle with a broad transverse diameter and more folding cartilage. Type II B has a narrow upper part of the auricle, less amount of folded cartilage. Microtia type III is represented by the unrecognizable anatomical structure of the auricle, and irregular shape of the residual ear, which is mainly similar to peanut-shaped, boat-shaped, cord-shaped, or sausage-shaped. Microtia Type IV denotes the affected ear with small skin tags or scattered hill-like bulges, the auricle completely missing, and no local anatomical traces. (B) Methods of the similarity point of structure, the blue structure is the proband's microtia auricle cartilage, which includes the middle and lower cartilage of the auricle, and the orange structure is the mother's microtia auricle cartilage, which consists of the middle and lower cartilage of the auricle, both With earlobe (lower part of blue line), then the number of All structures is 3, the number of Common structure is 3, the number of Unshared structure is 0, and AV% = (CS – US)/AS = (3–0)/3 =100%. (C) Draw the outline of the microtia auricle of the proband and other patients in the family with red and blue lines, and use Image J to measure the length of the protagonist's microtia auricle outline, and scale the contour line to the highest point and the lowest point. After the points were overlapped, the overlap length of the microtia auricle contours of other patients and the proband was measured with Image J to calculate the percentage of overlap.

meatus. SP –100% is considered as 0 point, SP between –99% and –60% is considered as 1 point, SP between –59% and –20% is considered 2 points, SP between –19% and 20% is considered 3 points, SP between 21% and 60% is considered 4 points , SP between 61% and 100% is considered 5 points. Higher the score means higher the similarity. The shape similarity score (0–5) was used to score each retained structure of microtia auricle shape. Then we used the software (ImageJ-win64) to calculate the proportion of contour line overlap (*Figure 1C*) by calculating coincidence degree. The coincidence degree between 0 and 15% is considered as 0 points, between 16% and 30% is considered as 1 point, between 31% and 45% is considered 2 points, between 46% and 60% is considered 3 points, between 61% and 80% is considered 4 points, and between 81% and 100% is considered 5 points. The higher the similarity of microtia auricle shape, the higher the score. The morphological characteristics of the auricles in the family were further compared with the genetic pattern (*Table 2*).

Statistical analysis

All normal distribution data were expressed as mean ± standard deviation (SD). All non-normally distributed values were expressed as medians, and the proportions were expressed as percentages. Comparisons between groups were performed using independent sample *t*-test and Chi-square test, and multivariate logistic regression analysis. All statistical analyses using SPSS 21.0 $P<0.05$ was considered statistically significant.

Table 2 Residual ear structure similarity score table

Items	Grading items	Point
Type I		
Cryptotia	Cranioauricular angle	
	Auricle circumference	
Protruding ears	Cranioauricular angle	
	Auricle circumference	
Cup ear	Cranioauricular angle	
	Auricle circumference	
Type II		
Type IIA	Residual ear cartilage similarity	
	Auricle structure	
Type IIB	Residual ear cartilage similarity	
	Residual ear shape similarity	
	Auricle structure	
Type III	Auricle structure	
	Residual ear cartilage similarity	
	Residual ear shape similarity	
Type IV	Residual ear cartilage similarity	
	Residual ear shape similarity	

Results

Patients with microtia and general auricle morphology

A total of 484 patients diagnosed with microtia were included in this study. From them, 21 patients with non-auricular deformities were excluded. Finally, a total of 463 patients with microtia met the criteria of AD, of which 351 were male patients (75.81%) and 112 female patients (24.19%). There were 427 patients (92.22%) with the unilateral onset and 36 patients (7.78%) with bilateral onset. The ratio of single to dual was 11.86:1, P<0.001. Among the patients with unilateral disease, 113 patients had left ear lesions (26.46%), 314 patients had right ear lesions (73.54%), and the ratio of left and right ears was 1:2.704, P<0.001.

The patients [463] in this study were from 456 families, which include 116 patients from 43 families with two or more microtia patients in each family. Thus, the multiple case family rate is 43/456 (9.43%). Among the 43 multiple-case families, 87 patients have AD auricular morphology. All of them were unilateral, and all were type III.

Patients were classified according to the auricle morphology and analyzed according to the types of auricular morphology. Eight (1.73%), 23 (4.97%), 427 (92.22%), and 5 (1.08%) patients are belonged to type I, type II, type III, and type IV auricular morphology, respectively. One of the

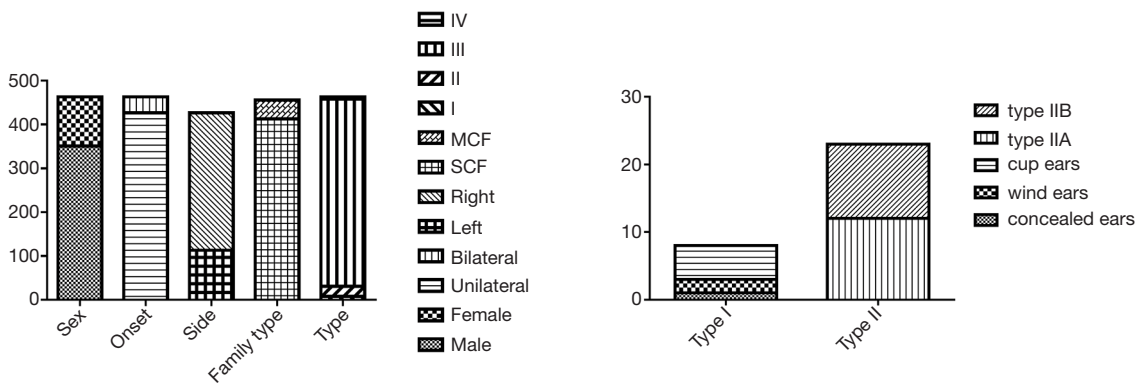

Figure 2 General situation of AD patients and auricle morphology. A total of 463 patients with 351 were male patients (75.81%) and 112 female patients (24.19%). There were 427 patients (92.22%) with the unilateral onset and 36 patients (7.78%) with bilateral onset. One hundred thirteen patients had left ear lesions (26.46%); Three-hundred and fourteen patients had right ear lesions (73.54%). The multiple-case family rate is 43/456 (9.43%). Eight (1.73%), 23 (4.97%), 427 (92.22%), and 5 (1.08%) patients belonged to type I, type II, type III, and type IV auricular morphology. One of the patients in type I was found with concealed ears (0.22%), 2 cases were wind ears (0.43%), and 5 were cup ears (1.08%). Among type II patients, 12 were type IIA patients (2.59%), and 11 were type IIB patients (2.38%). AD, auricular deformity.

Table 3 Characteristics of the genealogical tree with AD-MCF

Characteristics of families	Number	Percentage (%)
Patient with first-degree relatives		
Yes	28	44.19
De novo (yes)	9	20.93
De novo (no)	19	44.19
No	15	34.88
Total	43	100
Patients with AD		
Male	71	61.21
Female	45	38.79
Total	116	100
Relationship with AD and relatives		
Two successive generations (yes)	9	20.93
Two successive generations (no)	25	58.14
First-degree relative (yes)	10	23.26
Second-degree relatives	4	9.30
Third-degree relatives	6	13.95
First-degree relative (no)	15	34.88
Second-degree relatives only	6	13.95
Third-degree relatives only	9	20.93
De novo	9	20.93
Total	43	100

AD-MCF, auricular deformity multiple case family.

Table 4 Gender differences between AD-MCF and Sporadic patients

Gender	AD-MCF	Sporadic patients	Total
Male	71	280	351
Female	45	67	112
Total	116	347	463

P<0.001. AD-MCF, auricular deformity multiple case family.

Table 5 Gender differences between the *de novo* and other MCF

Gender	*De novo*	Other MCF	Total
Male	6	65	71
Female	12	33	45
Total	18	98	116

P=0.03, P<0.05. MCF, multiple case family.

patients in type I was found with concealed ears (0.22%), 2 cases were wind ears (0.43%), and 5 were cup ears (1.08%). Among type II patients, 12 were type IIA patients (2.59%), and 11 were type IIB patients (2.38%) (*Figure 2*).

AD-MCF genealogy characteristics analysis

There are 43 families with MCF. Among them, 28 families (65.12%) have probands with first-degree relatives (including parent-child relationship and sibling relationship), which includes 19 (44.19%) families having parent-child relationships. There were 15 families (34.88%) of probands who did not have first-degree relatives. Families having no earlier AD patients were named as *de novo* families. Nine (20.93%) *de novo* families were found in this study (*Table 3*).

Moreover, 116 patients from 43 families include 71 males (61.21%) and 45 females (38.79%). Additionally, a total of 347 sporadic patients with 280 males and 67 females took part in this study. These results showed that females with AD-MCF have a higher incidence than sporadic patients (P<0.001) (*Table 4*).

Among 43 MCF families, 9/43 (20.93%) AD-MCF families included 21 patients in the parent-child relationship, which were 14 males and 7 females (male to female ratio 2:1) and without maternal genetic characteristics. Additionally, 25/43 MCF (58.14%) families were atavism with 51 males and 26 females, among which 10/43 (23.26%) were in first-degree relatives. The remained families were involved by 4/43 (9.30%) families with patients with first and second-degree relatives, which included 12 patients (8 males and 4 females). There were 6/43 (13.95%) families with patients in second-degree relatives, which were involved by 14 patients (9 males and 5 females).

A total of 18 patients were found in the 9/43 (20.93%) *de novo* families, including 6 males and 12 females, with a male to female ratio of 1:2. The incidence of *de novo* females was higher than that of other types of families, with statistical differences, P=0.03, P<0.05 (*Table 5*).

Characteristics of parental reproductive age in patients with AD-MCF

Twenty-five out of 43 families in atavism included patients with AD-MCF. In these families, the childbearing ages of the parents were tracked, which include 84 persons. Among them, 4/43 (9.30%) families (including 10 patients) with first- and second-degree relatives among patients. The average childbearing ages of father and mothers

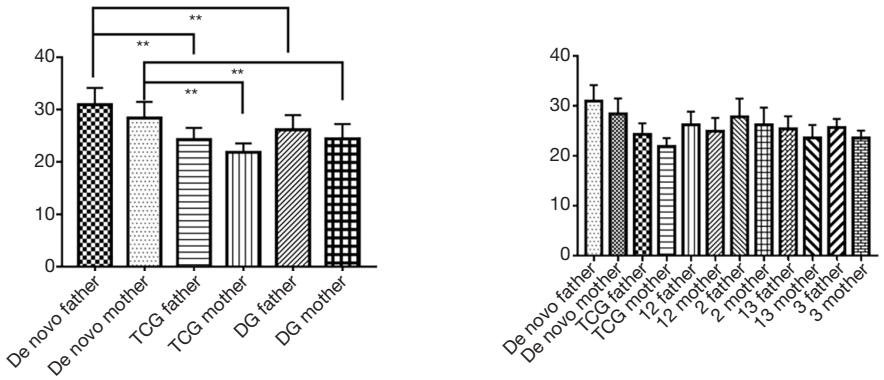

Figure 3 Childbearing age of each family. The average childbearing ages of fathers and mothers in families with first- and second-degree relatives were 26.2±0.84, 24.9±0.85, respectively. The reproductive ages of fathers and mothers in families with only second-degree relatives were 27.82±1.09 and 26.18±1.04 (n=11). There were 6/43 (13.95%) families with first- and third-degree relatives among patients, among which the father's reproductive age was 25.37±0.58 (n=19), and the mother's reproductive age was 23.58±0.60 (n=19). Additionally, 9/43 (20.93%) families with only third-degree relatives, among which father's reproductive age was 25.67±0.58 (n=9), and mother's reproductive age was 23.56±0.50 (n=9). Moreover, the average childbearing ages of 9 families with patients in two consecutive generations, fathers and mothers were 24.29±0.49 (n=18), and 21.86±0.37 (n=18), respectively. For 9 *de novo* families, the average childbearing age of father was 30.94±0.75, n=18, and the average childbearing age of mother was 28.39±0.73, n=18, which were significantly higher than the average childbearing age of other types of families (father P=0.0001, mother P=0.0001, both P<0.01). **, P<0.01.

were 26.2±0.84 (n=10) and 24.9±0.85 (n=10), respectively. There were 6/43 (13.95%) families with only second-degree relatives among patients. For these families, the reproductive ages of fathers and mothers were 27.82±1.09 and 26.18±1.04 (n=11), respectively. There were 6/43 (13.95%) families with first- and third-degree relatives among patients, among which the father's reproductive age was 25.37±0.58 (n=19), and the mother's reproductive age was 23.58±0.60, (n=19). Additionally, 9/43 (20.93%) families with only third-degree relatives, among which father's reproductive age was 25.67±0.58 (n=9), and mother's reproductive age was 23.56±0.50, (n=9). Moreover, 9 families with patients in two consecutive generations, which were involved by 18 parents with known childbearing age. For these families, the average childbearing ages of fathers and mothers were 24.29±0.49 (n=18), and 21.86±0.37 (n=18), respectively.

For 9 *de novo* families, the average childbearing age of father was 30.94±0.75, n=18, and the average childbearing age of mother was 28.39±0.73, n=18, which were significantly higher than the average childbearing age of other types of families (father P=0.0001, mother P=0.0001, both P<0.01) (*Figure 3*). Thus, the childbearing ages of parents are a potential factor contributing to AD-MCF in *de novo* families.

AD-MCF residual ear morphology and parental performance

Out of 116 patients, 87 patients from 43 families have detailed auricular morphology information. All these patients were unilateral and had type III category, having both earlobe and middle cartilage. Among 87 patients, 76 (87.36%) had the lower cartilage, and 11 (12.64%) were without the lower cartilage. For these patients, residual ear morphological similarity scores were calculated using probands as controls. Except for the probands, 42 patients from 34 families were trace remaining ear patterns, 9 families of 3 generations, and above could not get the specific shape of auricle morphology. There were 32 first-degree patients with a total score of 14.09±2.22 [*Figure 4A* (see panels A1 and A3)], the structure score was 4.84±0.63, the residual ear similarity score was 4.66±0.83, and the cartilage shape similarity score was 4.59±0.84 (*Figure 4B*). For nine second-degree patients, the total score is 9.90±2.85 [*Figure 4A* (see panels A2 and A4)] with the structural score remained 3.80±1.03, the residual ear similarity score was 3.30±0.95, and the cartilage morphology similarity score was 2.80±1.23 (*Figure 4B*). For one third-degree patient with a total score of 7 (*Figure 4A,B*) with the structural score 3, the residual ear similarity score was 2, and the cartilage

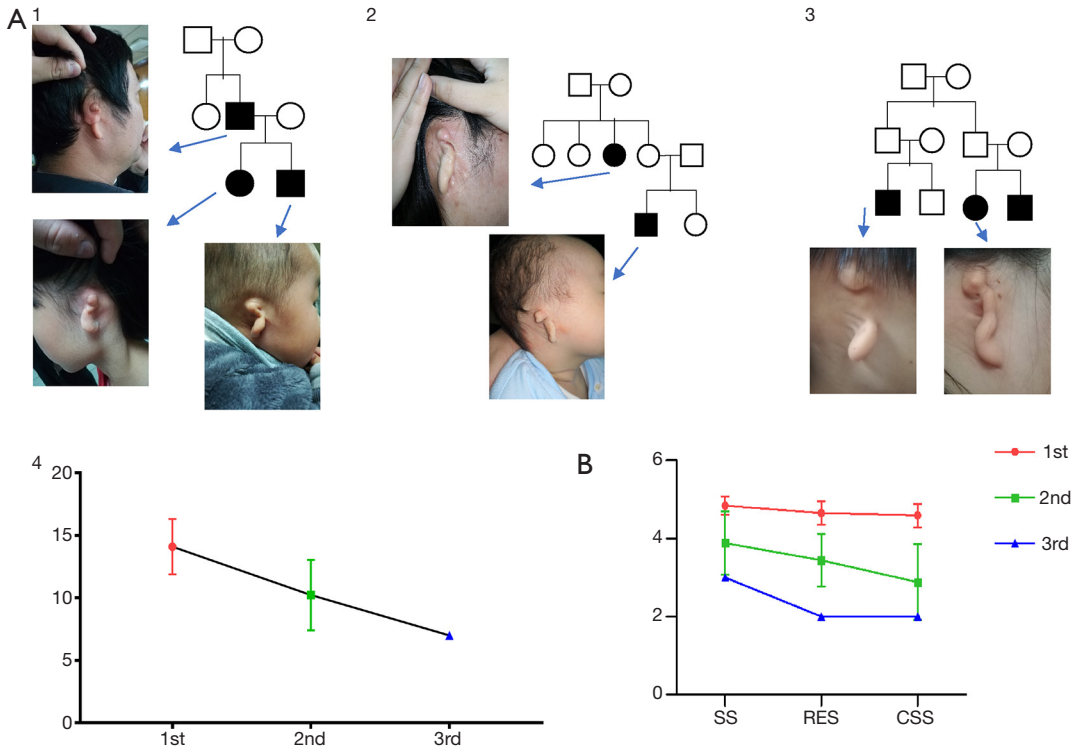

Figure 4 The score of families. (A): (A1) first-degree relatives; (A2) second-degree relatives; (A3) tertiary relatives; (A4) the closer the relatives are, the more similar the auricles are. (B) Scores of relatives of different auricle structures at all levels of kinship. SS, structure scores; RES, residual ear scores; CSS, cartilage shape scores.

morphology similarity score was 2 (*Figure 4B*). The total scores of patients in different genetic difference levels were compared and were found significantly different (P<0.001).

Discussion

The purpose of this study was to investigate the incidence of AD visits in Southern China, the rate of AD-MCF, and the similarity of residual ear morphology in order to obtain more information about the characteristics of AD families and the changes of residual ear morphology between patients in the same family. To the best of our knowledge, this was the first study based on the characteristics of AD pedigrees in southern China and residual ear morphology. The results of this study supply added scientific insights into the aspects of AD families and the shape of remaining ears in Southern China.

This study showed that patients with microtia complained of AD, and there were statistical differences in type, gender, and left and right ears. Microtia was manifested as auricle deformities, narrowing or atresia of the external acoustic meatus, and middle ear deformities. Among them, AD was the most visible manifestation. It can occur on its own or concurrently with other syndromes, multi-finger syndrome, cleft lip and palate, facial asymmetry, kidney deformity, heart defects, and dysplasia of the eyes, ears, and spine (12,14,21). This study found that from 2013 to 2019, a total of 484 patients diagnosed with microtia were diagnosed in Sun Yat-sen memorial hospital, Sun Yat-sen University, of which 21 non-AD patients (4.34%), and the remaining 463 were AD patients (95.66%). Unilateral incidence was more than the bilateral rate; the male to female ratio was 3.13:1, and the ratio of left and right ears in the unilateral rate was 1:2.704, significant statistical differences between the two groups (P<0.001). Type III was the most common type of treatment, accounting for 92.22% (P<0.05). There was a clear tendency for AD to be the chief complaint among patients, and there were significantly more patients with type III than other types of patients, which may type III with the highest rate or the most significant impact on the patient's life leads to a higher proportion of consultations than other types. The number of patients with type I and

type II was small, and their research needs to be further expanded and investigated.

This study found that patients with two or more patients in the third generation of diseases caused by genetic factors were Multiple Case Family (MCF). Although the microtia was mostly sporadic, the incidence of MCF was reported to be 2.9% to 33.3% (11,12,22). Forty-three families were named as AD-MCF, and two patients in each of the seven families were confirmed before the follow-up. That is, 463 cases of microtia were from 456 families, and MCF accounted for 43/456 (9.43%), consistent with earlier reports. Among the 43 MCF patients, there were 116 patients, 71 males (61.21%), 45 females (38.79%), and 347 sporadic patients, 280 males, and 67 females. Significant differences between the two groups (P<0.001) (*Table 2*).

On the other hand, the lower incidence in females may be related to the decrease in the rate of female patients with sporadic patients who used long hair to cover the deformed auricles. In MCF, because more than one patient in the family was more likely to pay attention to the patient, the patient would accompany by probands. The authors were more comfortable with tracking down more female patients. There were more female patients in *de novo* families than in other types of families, which may be due to the statistical difference caused by the limited sample in this study.

De novo parents have a higher childbearing age than other types of families. De novo may be caused by parents who are carriers of *de novo* heterozygotes by forming germ cells with *de novo* mutations, or they may have cross dislocations in the process of generating germ cells or *de novo* mutations in embryo development. De novo mutations are more likely to occur in older parental pregnancies (6-8). Given this phenomenon, we compared the reproductive ages of the parents of patients in each family line. In our study, we found that both childbearing age of parents from the *de novo* family were significantly older than other types of families (*Figure 3A*). Goldmann *et al.* (7) found that the number of DNM in offspring increases not only with the age of the father, but also with the age of the mother, and certain genomic regions also show an enrichment of DNM from the mother. Halldorsson *et al.* (6) pointed out that the risk of mutations at the intersections increased with the increase of parental reproductive age, and there was a more apparent relationship with the mother's reproductive age. As the childbearing age increases, *de novo* mutations were more likely to occur during meiosis during germ cell production. This study found that the average childbearing age of fathers in *de novo* families is 30.94 years and mothers 28.39 years, which significantly higher than other types of families. As the parents' reproductive age increases, the risk of DNM increases, and the risk of genetic mutations corresponding to AD also increase. The characteristics of relevant family mutations need to be analyzed to verify this content.

AD-MCF microtia residual ears developed from the structure of 1st, 2^{nd,} and 3rd hillocks were not present. The development of the auricle begins in the fifth week of the embryo. A part of the parotid arch will develop into the auricle, and the auricle was formed approximately from the fifth week to the ninth week of the embryo (5). At the 6th week of the embryo, the 6th hillocks appeared after ectoderm and mesenchyme were activated and proliferated in the mandible and hyoid bone. The 1st, 2nd, and 3rd hillocks appear at the tail of the mandibular arch, and later form the tragus, the ears of the cochlea, and the upper part of the outer ear. The 4th, 5th, and 6th hillocks appear at the head of the hyoid bone and develop into the ears, earlobe (3,5). The 6th hillocks proliferate to form a raised auricle (2,4), and the first-gill fissure was sunken inward to construct the external ear canal. The abnormal development of each structure will affect the shape of the auricle. Different pathogenic factors may affect the development of various structures of the auricle, and the occurrence of microtia malformation AD may be the result of the joint action of multiple pathogenic factors. Exquisite residual ears were present in the middle lobe, in the middle cartilage, in the lower cartilage 76 ears (87.36%), non-existent 11 ears (12.64%). The cartilage, tragus, cochlea, and external auditory canal were not present in the upper part of the residual ear. The factors that cause AD-MCF will affect the proliferation of 1st, 2nd, and 3rd hillocks, and even prevent them from forming during the embryonic period. In the structure developed by 4th, 5th, 6th hillocks, the middle cartilage and earlobe were present in each patient, while the lower cartilage was not developed in some patients, which may be caused by pathogenic factors in the AD-MCF to the middle cartilage. And earlobe formation was weak. That is, when the auricle was formed, the development of the 1st, 2nd, and 3rd hillocks were most inhibited, the 5th hillock was minorly affected, and the 4th hillock was less affected than other parts.

First-degree relatives have higher similarity in auricle shapes. The auricle morphology of AD may be affected by several factors such as heredity, chromosomal aberrations, and internal and external environments. Among them, genetic factors were essential factors, and family clustering appears in some families. This part of the study found

that microtia deformities came to the hospital in patients with type III. The majority of visits may be due to the higher incidence of type III than other phenotypes, or the impact of type III on patients and their families were more significant than different phenotypes, and the prevalence of type IV was low. In AD-MCF, the shape of residual ears increases with the degree of kinship. The closer the degree of relationship is, the closer the genotype is, the closer the degree of relationship is, other factors such as other genes and living environment were added. AD was a multifactorial congenital disease. Second-degree relatives were more likely to develop AD genetic traits, which may be related to the proximity of more pathogenic genes. The morphology of residual ears in AD patients was diverse and may be related to their polygenic effects. Among the various structures, cartilage morphology has the most significant impact on the shape of the residual ear. Even if the earlobe develops well, different cartilage morphology will cause the overall shape of the auricle to be larger, and will cause a pull on the earlobe to change the earlobe Deformation directly may cause the microtia deformity auricle deformity gene or other factors to inhibit cartilage cell proliferation and differentiation.

The pattern of auricle morphology of microtia deformities in different families was not clear. We hope that through more family reviews, we can find the role of genetic factors and the pattern of auricle morphology in families.

Conclusions

Microtia was more common in men than in women and was more common unilaterally. The proportion of MCF was about 9.43%, which has a family aggregation. *De novo* families that cannot define the genetic method for the time being account for 20.93% and AD-MCF in two consecutive generations accounted for 20.93%. Patients with a high incidence of microtia malformations have type III AD, and the upper cartilage was absent. The causative factors may causes abnormal development of hillocks 1st, 2nd, and 3rd, but the specific factors need further study.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/atm.2020.03.212). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Written informed consent was obtained from all the subjects and their parents involved in this study for participation in and publication of this study, and all procedures were approved by the Ethical Committee of Sun Yat-sen Memorial Hospital of Sun Yat-sen University and were following the Declaration of Beijing.

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