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Phenotypic Sub-Grouping in Microtia Using a Statistical and a Clinical Approach

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Abstract

The clinical presentation of microtia varies widely from minimal morphological abnormalities to complete absence of the ear. In this study we sought to identify and characterize sub-groups of microtia using a statistical and a clinical approach. Photographs of 86 ears were classified in relation to all the external ear components. We used cluster analysis and rater's clinical opinion to identify groups with similar phenotypes in two separate analyses. We used Cramer's Phi coefficient of association to assess the similarity among the clinician's groupings as well as among the statistical sub-phenotypic groups and each of the clinician's groupings. The cluster analysis initially divided the 86 ears into a more and a less severe group. The less severe group included two sub-groups that included ears classified as normal and a group that had very few anomalous components. The group of 48 more affected ears all had abnormalities of the helix crus; antihelix-stem, -superior crus and -inferior crus; and antitragus. These were further divided into 4 sub-phenotypes. There was a moderate degree of association among the raters' groupings (Cramer's Phi: 0.64 to 0.73). The statistical and clinical groupings had a lower degree of association (Cramer's Phi : 0.49 to 0.58). Using standardized characterization of structural abnormalities of the ear we identified six distinct phenotypic groups; correlations with clinicians' groupings were moderate. These clusters may represent groups of ear malformations associated with the same etiology, similar time of insult or target cell population during embryonic development. The results will help inform investigations on etiology.

Keywords

microtia; phenotype; ear morphology

INTRODUCTION

The term “microtia” is used to describe a wide range of malformations of the external ear. To date, the etiopathogenesis is largely unknown; however it is likely multifactorial as different teratogens cause microtia, and some mouse models support a polygenic model. In a multifactorial model, the phenotypic variability of ear anomalies is often a consequence of etiology heterogeneity, although cases with the same etiology also demonstrate clinical variability.

Careful phenotyping can aid in the identification of the genetic causes of Mendelian and complex diseases. The existing classification systems for microtia describe in general terms the malformation of the ear and do not specifically account for the various features of the ear. Thus they do not sufficiently distinguish among distinct subphenotypes and may be unable to capture the diversity observed among affected individuals [Luquetti et al., 2012]. In order to investigate the etiology of microtia, we sought to identify a more specific method for characterizing the microtia phenotype. First we developed the “Phenotypic Assessment Tool for Microtia (PAT-Microtia)” to facilitate the description and recording of anomalies of the different parts of the external ear using the definitions by the Elements of Morphology group [Hunter et al., 2009]. As part of that study, we validated the PAT-Microtia by examining the interrater reliability for ratings of each component of the ear using photographs of typical and microtic ears [Luquetti et al., 2013].

In the current study, we explore a statistical and a clinical method to identify specific microtia sub-phenotypes and the morphology elements of these sub-groups using the data obtained from the PAT-Microtia tool. We investigated sub-phenotypes identified by cluster analysis and those identified by clinicians using the gestalt approach and assessed whether the sub-phenotypes are comparable between clinicians as well as to the groups identified by the cluster analysis.

METHODS

1. Sample Selection and Ear Phenotype Characterization

We used standardized photographs of ears and the ratings of their morphology obtained in our previous study [Luquetti et al., 2013] where we identified individuals with a diagnosis of microtia using the Craniofacial Clinic database at Seattle Children’s Hospital. Medical charts were reviewed in a random order and individuals who had a confirmed diagnosis of microtia were included, regardless of whether the individuals’ microtia was isolated or associated with multiple anomalies. All patients seen through the Craniofacial Clinic have standardized photographs taken as part of their clinic visits. We selected 68 patients who met the inclusion criteria and had adequate photographic documentation of their ears. Both sides were included as separate photographs for the two individuals with bilateral microtia.

Sixteen unaffected contralateral ears from individuals with unilateral microtia were included as controls. All photographs were cropped and oriented in a left profile view. Thus, the final dataset of 86 ear photographs from 68 individuals included 70 ears with microtia and 16 unaffected ears.

As previously described, the original photographic data set was classified by six raters from pediatrics (n=2), plastic surgery (n=2), and otolaryngology (n=2) who were members of our Craniofacial Center at the time of the study and had a minimum of one year of training in the management of children with microtia. Raters were asked to independently identify the presence or absence of specific external ear components, as well as auricular and preauricular pits and tags, and to assign a Marx classification score. Although this classification system is not the one recommended by the Elements of Morphology group [Hunter et al., 2009], the Marx system was used in the previous study system and is commonly used in the clinical setting, thus we used this data to verify how the microtia types I to IV (Figure 1) correlate to the sub-phenotypes identified by the statistical and clinical approach as explained in detail below. Raters were blinded from each other's ratings.

2. Identification of Sub-Phenotypes

Statistical Sub-Phenotyping—We selected 11 categories, which included ear structures with high interrater reliability [defined as intra-class correlation (ICC) ≥ 0.70] in our previous study [Luquetti et al., 2013] as well as those structures that were considered to be informative about the overall phenotype by the clinical raters. All ear structures were categorized as absent or present. For the purposes of this project, ratings were designated according to the rating assigned by at least four of the six raters. We achieved consensus during an in-person meeting for the small subset of ears for which there was not a plurality of 4 raters with the same rating.

Clinical Sub-Phenotyping—We provided each of four raters (2 pediatricians, 1 plastic surgeon and 1 otolaryngologist) from the prior study with a printed set of 86 photographs (5×7 inches). Independently, each of the four raters identified groups of ears that appeared to be most similar based on their clinical opinion and using the following guidelines: a) groups should be based on similarities in the phenotype and not on surgical considerations; b) raters should create between 5 and 10 groups; c) each group must have at least two images. If there were ears that raters believed were unique, they could place them in a “miscellaneous” group; and d) raters should generate a description of the characteristics that captures the features associated with each group.

3. Data Analysis

We provide descriptive statistics for the sex and age of the patients, prevalence of each ear feature (e.g.: helix, stem of the antihelix) and the correlations among the ear components for the 86 ears. For the statistical sub-phenotyping we used the Partitioning Around Medoids (PAM) method which is a robust variation of K-means clustering that is based on medians rather than means to characterize cluster centers (“medoids”) and uses measures of dissimilarity of an observation from each medoid to assign observations to clusters

[Kaufman et al., 2005]. We found the cluster solution for 2 through 6 clusters (with more than 6 clusters, cluster sizes were too small to be useful). We present the results in a tabular dendrogram and used descriptive statistics to characterize each cluster of the 6-cluster solution, relating to both the component features and the Marx classification. Marx grading was used as a way to verify if the cluster solution was able to capture degrees of severity, it was not used as a variable in the cluster analysis.

For the clinical sub-phenotyping, we used Cramer's Phi coefficient of association [Cramér, 1946] to assess the similarity among the rater's groupings. We also assessed the associations among the statistical sub-phenotypic groups and each of the rater's clinical groupings using Cramer's Phi.

RESULTS

Ear Phenotype Characterization

The mean age of the patients was 6.1 years (standard deviation 4.4, range 2 weeks to 17 years) and 70.1% were male. The severity of microtia was similar between males and females. Grade I microtia was not associated with malformations of the helix, lobe, tragus or antitragus (Figure 1). Tags were present in similar proportions for all types of microtia (Table I).

All malformations of the auricular structures were highly correlated, varying from 0.68 to 0.95, except for malformations of the lobe, for which correlations with the other malformations were less than or equal to 0.25. The presence of preauricular pits and tags demonstrated low correlations with the malformations of the auricular structures ranging from -0.01 to 0.29. The presence of pretragal ectopia was negatively correlated with all ear structures (ranging from -0.17 to -0.04) except the lobe (0.18), and only weakly correlated with the presence of tags and pits (0.32 and 0.09, respectively; Table II).

Statistical Sub-Phenotyping

The statistical analyses using the PAM clustering method produced a nearly hierarchical sequence of partitions of the ears. The initial split into two clusters included a group of 37 ears with very few of the features present and a group of 49 ears that were affected with many of the features. Subsequent partitioning led to refinements of these two groups, and one ear crossed over between the initial two clusters (from B1.2 to A2). The 6-cluster solution included 37 subjects that were further divided into a group of 30 (A1) with very few anomalous components, which included the 16 ears classified as "normal" and a group of 8 ears (A2) all of whom had antihelix-superior crus present but very few other features. The 48 more affected ears all with abnormalities of the crus helix, antihelix-stem, antihelix-superior crus, antihelix-inferior crus, and antitragus which were further divided into 4 sub-phenotypes distinguished by presence or absence of other features: group B1.1.1 (n=25) characterized by universal presence of helix; group B1.1.2 (n=4) without helix; group B1.2 (n=10) with preauricular tags; and group B2 (n=9) with preauricular pits (Figure 2 and Table III).

Clinical Sub-Phenotyping

There was a moderate degree of association among the raters' groupings, as indicated by Cramer's Phi coefficients of 0.64 to 0.73 (Table IV). Thirty two ears were assigned to the same group and named either "peanut ear" or "classic peanut" by all raters and an additional eight ears were also included in this group by three of the four raters. These data indicate that this previously described phenotype was independently recognized by the raters.

Using the Cramer's Phi measure, we observed similar magnitude of association among the statistical and clinical groupings for all four raters (Cramer's Phi= 0.49 to 0.58).

DISCUSSION

We identified six distinct phenotypic clusters using detailed, standardized characterization of structural abnormalities in our exploratory statistical approach. To our knowledge, this is the first study to apply such methods to ear anomalies. The identification of these clusters may help us refine description of phenotypic subgroups. They may represent groups of ear malformations that are associated with the same etiology, similar time of embryonic insult or target the same cell population during embryonic development.

The investigation of the relationships among malformations of specific parts of the ear and their correlation with malformations of nearby structures may provide insight into the pathogenesis of microtia and related malformations such as auricular and preauricular pits and tags. In our study, while the correlation between ear components was high for most ear structures, it was not 100%. For example, not all individuals with an abnormality of the crux helix had a helix abnormality. This likely explains some of the observed variation among ears within morphological clusters. The low correlation of the lobe with the other ear structures could be related to the relatively simple structure of the lobe. Thus any remnant of the ear could be rated as "lobe", even when it may have originated entirely from another structure. In fact, among the 70 microtic ears only 5 were rated as having abnormal or absent lobe. It is also possible that the superior part of the ear is more frequently affected in microtia than the inferior part where the lobe is located. Likewise, it may be that other features are misappropriated – when for example some structure that looks like a tragus may actually be a tag in the place of a missing tragus, such mislabeling might result in non-differential misclassification and result in a dilution of the accuracy of sub-phenotyping.

Pretragal ectopia is considered to be a duplication of the ear (polyotia) or of the pretragal region and most of the ear is preserved in this type of malformation. It is considered microtia by some clinical groups. In our series, two of the four ears with pretragal ectopia did not have any other ear malformations. This raises a question about whether ears with isolated pretragal ectopia should be considered within the spectrum of microtia.

The moderate to low overlap among raters' gestalt grouping of microtic ears might have happened because raters assigned different weights for different malformations or had different interpretations about the correlation that certain structures appeared to have to one other. In contrast, the type of ear named as "peanut" by the clinicians seemed to be a distinctive phenotype and as a result most ears in this category (32) were sorted by all raters

in the same group. Nonetheless, raters had variable numbers of ears that were classified in the “peanut” group (ranging from 41 to 51 ears), indicating that there is still some heterogeneity in each clinician’s idea of what constitutes a peanut ear. In the statistical sub-phenotyping, the ears named as “peanut” by the clinicians were part of clusters B1.1.1, B1.2 and B2; the differences among them being related to the absence of helix and lobe and presence of tags, pits and pretragal ectopia. This might indicate that the clinician (gestalt) approach may rely more on overall form, shape and placement of various structures that were not captured by the numerical clustering method which only took into account the presence or absence of specific features and for which weights were not assigned in order to minimize biases.

We observed fairly homogeneous groups in the subjective evaluation of the photographs in each cluster, which validates the cluster approach. In addition, when we compared the six morphological clusters to the Marx classification (assigned by clinicians to the ear photos), we observed that ears classified as grade I microtia were only identified in the less severely affected cluster (cluster A), whereas grades III and IV only in the cluster with the more severely affected ears (B). Ears classified as grade II microtia appeared in almost all clusters.

There are some limitations to this study. First, we included photos of ears from children that were identified as having microtia by providers at our craniofacial center; it was not possible to include typically formed, small ears, which are also sometimes considered as microtia since the ears could not be measured in the photos. We did not have photos of the ears of the parents of the affected child to account for family “normal” variation, although minor variations might represent a microform of microtia and thus difficult to account for in this type of analysis. We also did not distinguish between isolated forms of microtia, and forms associated with multiple congenital anomalies. It is possible that we could identify additional patterns with added information about associated features such as branchial remnants or renal anomalies, and will consider this in future studies. Our primary goal was related to identification of ear sub-phenotypes, therefore clusters may look very different if defined for other purposes-such as candidacy for specific surgical approaches for ear reconstruction; this evaluation was beyond the scope of this study.

We hope that identifying ear malformation phenotypic subgroups will enable the identification of genetic or teratogenic associations by having more homogeneous groups of affected individuals. We plan to use the clusters identified in this study in future investigations on etiology.

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Figure 1. Photos of the 86 ears distributed in the six identified clusters: A1, A2, B1.1.1, B1.1.2, B1.2 and B2. Marx classification of each ear, performed in the prior study, are shown as underscore below wach image. (Images courtesy of Photographer Erik Stuhaug)

Sex and frequency of abnormalities of the external ear by Marx classification based on consensus ratings from 6 clinicians in our prior study [3].

Table 1

	Normal N=16 n (%)	Grade I N=14 n (%)	Grade II N=10 n (%)	Grade III N=44 n (%)	Grade IV N=2 n (%)	Total N=86 n (%)
Sex						
Female	3 (18.8)	3 (24.1)	3 (30.0)	13 (29.5)	2 (100.0)	24
Male	13 (81.3)	11 (78.6)	7 (70.0)	31 (70.5)	0 (0.0)	62
Absence of						
Helix	0 (0.0)	0 (0.0)	1 (10.0)	40 (90.9)	2 (100.0)	44
Crus helix	0 (0.0)	0 (0.0)	6 (60.0)	44 (100.0)	2 (100.0)	53
Antihelix, Stem	0 (0.0)	0 (0.0)	3 (30.0)	44 (100.0)	2 (100.0)	50
Antihelix, Superior crus	0 (0.0)	3 (21.4)	7 (70.0)	44 (100.0)	2 (100.0)	58
Antihelix, Inferior crus	0 (0.0)	0 (0.0)	7 (70.0)	44 (100.0)	2 (100.0)	54
Incisura	0 (0.0)	2 (14.3)	3 (30.0)	44 (100.0)	2 (100.0)	52
Lobe	0 (0.0)	0 (0.0)	0 (0.0)	3 (6.8)	2 (100.0)	5
Tragus	0 (0.0)	0 (0.0)	1 (10.0)	41 (93.2)	2 (100.0)	45
Antitragus	0 (0.0)	0 (0.0)	3 (30.0)	44 (100.0)	2 (100.0)	50
Presence of						
Pretragal ectopia	0 (0.0)	2 (14.3.0)	1 (10.0)	1 (2.3)	0 (0.0)	4
Tags*	0 (0.0)	3 (21.4)	4 (40.0)	8 (18.2)	1 (50.0)	16
Pits*	0 (0.0)	0 (0.0)	1 (10.0)	9 (20.5)	0 (0.0)	10

* Auricular and preauricular

Heatmap illustrating the correlation between abnormalities of each component of the external ear (n=86)

Table II

		Abnormality of Ear Component										Presence of abnormality		
		Helix	Crus helix	AH, Stem	AH, Supercus	AH, Inf crus	Incisura	Tragus	Antitragus	Lobe	Pits*	Tags*	Pretragalect	
Abnormality of Ear Component	Helix	1.00	0.76	0.82	0.68	0.79	0.78	0.84	0.82	0.25	0.22	0.06	-0.11	
	Crus helix	0.76	1.00	0.93	0.91	0.88	0.83	0.83	0.88	0.20	0.29	0.14	-0.05	
	AH, Stem	0.82	0.93	1.00	0.84	0.91	0.91	0.89	0.95	0.22	0.24	0.11	-0.14	
	AH, Sup crus	0.68	0.91	0.84	1.00	0.83	0.73	0.75	0.79	0.18	0.27	0.16	-0.07	
	AH, Inf crus	0.79	0.88	0.91	0.83	1.00	0.81	0.81	0.86	0.20	0.21	0.13	-0.17	
	Incisura	0.78	0.83	0.91	0.73	0.81	1.00	0.85	0.95	0.21	0.23	0.09	-0.04	
	Tragus	0.84	0.83	0.89	0.75	0.81	0.85	1.00	0.89	0.14	0.28	-0.01	-0.12	
	Antitragus	0.82	0.88	0.95	0.79	0.86	0.95	0.89	1.00	0.22	0.24	0.05	-0.14	
	Lobe	0.25	0.20	0.22	0.18	0.20	0.21	0.14	0.22	1.00	-0.09	0.01	0.18	
	Pits*	0.22	0.29	0.24	0.27	0.21	0.23	0.28	0.24	-0.09	1.00	-0.08	0.09	
Presence of abnormality	Tags*	0.06	0.14	0.11	0.16	0.13	0.09	-0.01	0.05	0.01	-0.08	1.00	0.32	
	Pretragal ect	-0.11	-0.05	-0.14	-0.07	-0.17	-0.04	-0.12	-0.14	0.18	0.09	0.32	1.00	

Table III

Statistical sub-phenotyping: identified clusters and prevalence (%) of the ear features by the six clusters solution.

Cluster solutions	An=37		Bn=49					
	An=37		B1n=40			B2 n=9		
	An=37		B1.1n=29			B1.2n=11		
2-clusters								
3-clusters								
4-clusters								
5-clusters								
6-clusters								
Absence of	A1n=30	A2n=7+I *	B1.1n=29		B1.2n=11-I *		B2n=9	
Helix	A1n=30	A2n=8	B1.1.1n=25		B1.1.2n=4		B1.2n=10	
	%	%	%	%	%	%	%	%
Crus helix	3.1	0.0	100.0	0.0	0.0	90.0	88.9	
Antihelix, Stem	0.0	44.4	100.0	100.0	100.0	100.0	100.0	
Antihelix, Superior crus	0.0	11.1	100.0	100.0	100.0	100.0	100.0	
Antihelix, Inferior crus	0.0	100.0	100.0	100.0	100.0	100.0	100.0	
Incisura	6.3	33.3	100.0	100.0	100.0	100.0	100.0	
Lobe	9.4	0.0	100.0	100.0	100.0	100.0	100.0	
Tragus	0.0	0.0	15.4	0.0	0.0	10.0	0.0	
Antitragus	0.0	0.0	96.2	75.0	80.0	100.0	100.0	
Presence of	3.1	0.0	100.0	100.0	100.0	100.0	100.0	
Pretargal ectopia	6.3	11.1	3.8	0.0	0.0	0.0	0.0	
Tags *	9.4	33.3	0.0	0.0	0.0	100.0	0.0	
Pits *	0.0	11.1	0.0	0.0	0.0	0.0	100.0	
Marx classification (n)								
Normal (16)	100.0	0.0	0.0	0.0	0.0	0.0	0.0	
Grade I (14)	78.6	21.4	0.0	0.0	0.0	0.0	0.0	
Grade II (10)	30.0	50.0	0.0	10.0	10.0	10.0	0.0	
Grade III (44)	0.0	0.0	54.5	6.8	18.2	20.5	0.0	
Grade IV (2)	0.0	0.0	50.0	0.0	50.0	0.0	0.0	

* Between the 4 and 5-cluster solutions, one ear crossed over from cluster B1.2 to a new cluster A2.

Table IV

Clinical sub-phenotyping: association between groupings of the ears by the raters using Cramer's Phi

	Rater 1	Rater 2	Rater 3	Rater 4
Rater 1	1	0.64	0.65	0.73
Rater 2	0.64	1	0.69	0.72
Rater 3	0.65	0.69	1	0.72
Rater 4	0.73	0.72	0.72	1