American Journal of Medical Genetics 34:574–578 (1989)

# Microtia and Associated Anomalies: Statistical Analysis

**Celia I. Kaye, Beverly R. Rollnick, Walter W. Hauck, Alice O. Martin, Joan T. Richtsmeier, and Konrad Nagatoshi**

*Section of Genetics, Lutheran General Children's Medical Center, Park Ridge, (C.I.K.), Department of Obstetrics and Gynecology, Northwestern University Medical School, Chicago (A.O.M.), and Center for Craniofacial Anomalies, Department of Pediatrics, University of Illinois College of Medicine at Chicago (B.R.R., K.N.), Illinois; Group in Biostatistics, University of California, San Francisco (W.W.H.); Department of Cell Biology and Anatomy, The Johns Hopkins University, Baltimore, Maryland (J.T.R.)*

**Terms such as oculoauriculovertebral dysplasia, Goldenhar syndrome, and hemifacial microsomia have been used to describe microtia with specific combinations of other craniofacial anomalies. Microtia is also observed with anomalies of postcranial structures. Statistical studies were performed on 297 patients with microtia and other anomalies to identify subgroups of patients representing previously described or new associations. Analysis identified 15 subgroups of patients with specific patterns of anomalies. Log-linear analyses of cranial and postcranial variables demonstrated a positive association between mandibular hypoplasia and cervical spine fusion, which was, in turn, positively associated with other spine anomalies ($P<.02$) and other skeletal anomalies ($P<.001$). Although unilateral microtia was commonly observed with mandibular hypoplasia, mandibular hypoplasia was negatively associated with bilateral microtia. Many of the associated anomalies were of structures not derived from the 1st and 2nd branchial arch neural crest. However, most associated anomalies were of structures derived from migratory cell populations or populations undergoing differentiation prior to migration between the 19th and 24th day post-fertilization (neural crest, ectodermal placode, mesoderm, surface ectoderm). These findings suggest that many different cell populations may be disturbed in the pathogenesis of microtia in association with other anomalies. The timing of the pathogenetic event may determine the specific pattern of associated anomalies.**

**KEY WORDS: oculoauriculovertebral dysplasia, hemifacial microsomia, Goldenhar anomaly**

## INTRODUCTION

Microtia occurs in newborn infants with a population frequency of 0.03%. Of these, about half are isolated malformations and the remainder occur in association with other anomalies as syndromes [Melnick and Myrianthopoulos, 1979]. A non-random association of anomalies that occurs with microtia includes mandibular hypoplasia, epibulbar dermoids, and/or anomalies of the cervical spine. Terms such as oculoauriculovertebral "dysplasia" (OAV), Goldenhar syndrome (GS), hemifacial microsomia (HFM), and others [Rollnick et al., 1987] have been used to describe combinations of these anomalies. It has been suggested [Grabb, 1965; Pashayan et al., 1970; Gorlin et al., 1976; Smith, 1982; Rollnick and Kaye, 1983; Rollnick, 1988] that these conditions represent a spectrum of severity of a single entity and that an isolated external ear anomaly may be the mildest expression of the entity.

This report represents part of a study of 297 patients with microtia and other anomalies. The phenotypic characteristics of these patients have been reported [Rollnick et al., 1987] as have pedigrees of 97 families [Rollnick and Kaye, 1983]. The goal of this component of our studies was to identify the associated anomalies found in these 297 probands and then to perform a series of statistical analyses to look for patterns within the associated anomalies. We assumed that previously described, clinically significant associations would reemerge. We wondered if new associations would also emerge that might identify new clinical problems and/or illuminate pathogenetic mechanisms.

Received for publication March 31, 1989; revision received June 21, 1989.

Address reprint requests to Celia I. Kaye, M.D., Ph.D., Department of Pediatrics, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr., San Antonio, TX, 78284.

## METHODS

### Sample

All individuals in this study were selected from the patient population of the Center for Craniofacial Anomalies, University of Illinois-Chicago (CCFA). The active data base consisting of 6,056 computer records was searched for all patients with microtia. We define microtia as a small and/or anomalous external ear. Patients with pre-auricular skin tags or sinuses, or lop, cup, or apparently low-set ears in the absence of microtia were excluded from the study. Records of all patients with microtia were reviewed by two of the authors (C.K., B.R.). Individuals with recognized Mendelian disorders, known syndromes, and chromosome abnormalities were excluded. The remaining group was composed of 297 individuals.

### Variables and Statistical Analyses

The computer record of each individual contained information on variables within 47 categories. Multiple variables were possible within each category; for example, one category was the cardiovascular system, with ventricular septal defect a variable within the category. A complete list of variables was reported previously [Rollnick et al., 1987].

We were interested in determining if the presence of one variable was positively or negatively associated with other variables. For a variable to be included in this analysis, a minimum of five occurrences within the data base of 297 was required. Given this criterion, 48 variables remained for analysis (Table I). To determine associations between the presence or absence of various anomalies, Fisher's exact test of associations was used. Because of the large number of statistical tests involved in comparison of pairs of anomalies, it was not appropriate to use the usual $P$ value of .05 to indicate statistical significance. Bonferroni's correction was used as a guide for the exact tests, and a $P$ value of less than .001 was considered statistically significant. Reported $P$ values are two-tailed tests and are not adjusted.

To further assess the large number of associations identified by Fisher's exact test, the multivariate technique of log-linear analysis was used to determine which associations remained after controlling for the primary anomalies of mandibular hypoplasia, left and right microtia. For this analysis, variables were used if 15 occurrences were observed in the data set. Eighteen variables remained for this analysis (Table II).

Because of the number of variables, not all variables were analyzed simultaneously. Instead, they were analyzed in sets of 5. Each analysis was done with a full model; a step-down procedure was used to drop nonsignificant associations. In general, results for a given variable remained consistent across analyses, both in terms of direction of associations and relative statistical significance of those associations.

To look for patterns of associations, the entire data set was inspected to identify groups of patients with identical patterns of anomalies. Fifteen such groups were noted.

TABLE I. Variables, Fisher's Exact Tests of Associations

| | |
|---|---|
| Microtia, left | Cervical vertebral anomaly |
| Microtia, right | Neck broad |
| Ear tag, left | Neck mobility abnormality |
| Ear tag, right | Spina anomaly (other) |
| External auditory canal anomaly | Skeletal anomaly (other) |
| Preauricular sinuses | Orbital asymmetry |
| Abnormal position of ear | Ocular hypertelorism |
| Abnormal shape of ear | Ocular anomaly |
| Neurocranial asymmetry | Ocular coloboma |
| Mental retardation | Ocular dermoid |
| Central nervous system anomaly | Nose anomaly |
| Fifth cranial nerve deficit | Mandibular hypoplasia |
| Seventh cranial nerve deficit | Mastoid process anomaly |
| Cranial nerve (other) deficit | Mastoid pneumatization asymmetry |
| Cleft lip | Digital anomaly (including thumb) |
| Cleft palate | Thumb anomaly, bilateral |
| Cleft lip and palate | Radial anomaly (including thumb) |
| Lateral facial cleft | Digital anomaly of feet |
| Soft palate anomaly | Cardiovascular anomaly |
| Abnormal movement soft palate | Gastrointestinal anomaly |
| Tongue anomaly | Genitourinary anomaly (male) |
| Weakness of facial musculature | Chest wall defect |
| Cranial base anomaly | Abdominal wall defect |
| Cerviacl vertebral fusion | Pigmentary abnormality |

TABLE II. Variables, Log Linear Analyses

| | |
|---|---|
| Cleft palate | Nose anomaly |
| Cleft lip | Lateral facial cleft |
| Ear tags | Fifth cranial nerve deficit |
| Mandibular hypoplasia | Seventh cranial nerve deficit |
| Ocular dermoid | Neurocranial asymmetry |
| Ear position abnormality | Cranial base and cervical spine anomaly |
| Mastoid anomaly | Genitourinary anomaly (male) |
| External auditory canal anomaly | Digital anomaly, hand (including thumb) |
| Ear shape anomaly | Central nervous system abnormality |

## RESULTS

The tests for associations between pairs of variables identified statistically significant associations between cranial variables (Table III) and between cranial and post-cranial variables (Table IV). All associations reported in Tables III and IV are positive.

Log-linear analysis of cranial versus post-cranial variables is shown in Figure 1. Analysis of cranial versus cranial variables is shown in Figure 2. In both figures, the direction of the association is shown by + or −. In both analyses, the presence of mandibular hypoplasia was negatively associated with bilateral ear involvement, reflected in the negative associations with

TABLE III. Associations of Craniofacial Anomalies in Patients With Microtia (Fisher's Exact Tests; All Associations Listed Here Are Positive)

| First anomaly | Second anomaly | *P* value |
|---|---|---|
| Mandibular hypoplasia | Mastoid anomaly | .0001 |
| Mandibular hypoplasia | Ortibal anomaly | .0001 |
| Mastoid anomaly | Orbital anomaly | .0001 |
| Nose anomaly | Orbital anomaly | .0001 |
| Soft palate anomaly | Orbital anomaly | .0001 |
| Ocular dermoid | Perauricular tags | .0001 |
| Ocular dermoid | Lateral facial cleft | .0006 |
| Cranial nerve | Mandibular hypoplasia | .0001 |

TABLE IV. Associations of Post-Cranial and/or Craniofacial Anomalies in Patients With Microtia (Fisher's Exact Tests: All Associations Listed Here Are Positive)

| First anomaly | Second anomaly | *P* value |
|---|---|---|
| Cranial base | CNS | .0004 |
| Cranial base | Fifth nerve | .0003 |
| Cervical vertebral fusion (CVF) | Ocular dermoid | .0006 |
| CVF | Fifth nerve | .0001 |
| Cervical vertebral anomaly (CVA) | Ocular dermoid | .0009 |
| CVA | Orbital anomaly | .0001 |
| CVA | Fifth nerve | .0002 |
| Cardiovascular system | Central nervous system | .0004 |
| Gastrointestinal system | Central nervous system | .0008 |
| Thumb (bilateral) | Neuroasymmetry | .0001 |
| Digital anomaly | Neuroasymmetry | .0001 |

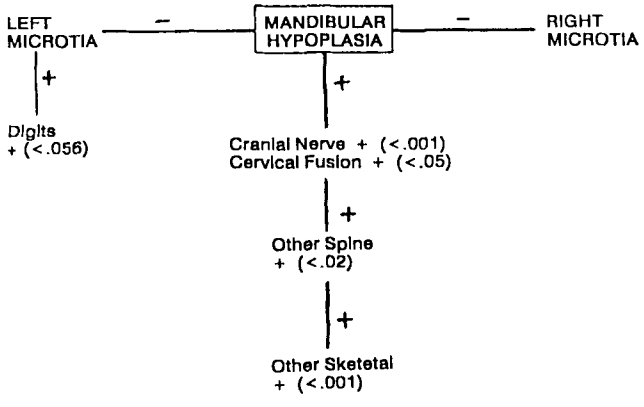


Fig. 1. Log-linear analysis of cranial versus post-cranial variables.

each of the individual microtias when controlling for the other. Of 69 patients with bilateral microtia, 46% exhibited mandibular hypoplasia. In contrast, 73% of the 228 patients with only unilateral microtia exhibited mandibular hypoplasia. There was a positive association between mandibular hypoplasia and cervical spine fusion, which was, in turn, positively associated with other spine anomalies and other skeletal anomalies.

Inspection of the data set identified 15 subgroups of patients, each with identical patterns of anomalies (Fig. 3). Individuals with unique patterns of anomalies (n = 108) were not included. The largest groups were

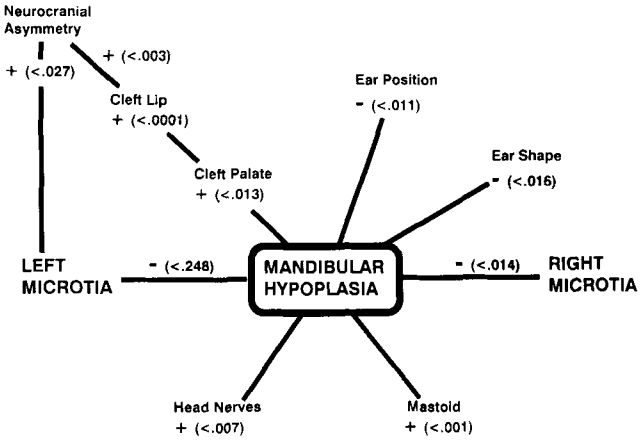


Fig. 2. Log-linear analysis of cranial versus cranial variables.

patients with microtia only (n = 51) and microtia in association with mandibular hypoplasia, without any other malformations (n = 86). Of the remaining 13 subgroups, ten (n = 43) represented microtia plus mandibular hypoplasia in association with an additional anomaly or anomalies, and three (n = 9) subgroups represented the association of microtia only with a second anomaly (cranial base, mastoid, cleft palate).

## DISCUSSION

These studies demonstrated the statistical significance of associations that have been thought to be clinically significant. Clinicians have long recognized the association of microtia and mandibular hypoplasia. This association, without other anomalies, was observed in 86/297 patients (29%). The association of cervical spine anomalies with microtia and mandibular hypoplasia has also been recognized. We identified ten patients (3%) with this pattern of anomalies. Epibulbar dermoids in association with pre-auricular nodes and mandibular hypoplasia were described by Goldenhar [1952]; we found that dermoids had a strong positive association with pre-auricular nodes, as well as with lateral facial cleft. The term oculoauriculovertebral dysplasia was coined to describe patients with microtia, cervical spine anomalies, and epibulbar dermoids [Gorlin and Pindborg, 1964]; we found a strong positive association between epibulbar dermoids and cervical spine anomalies in patients with microtia. Thus, our methods were able to identify these clinical entities as statistically significant associations among this population of patients referred to the CCFA with microtia. Other subgroups did not emerge as strong candidates for potential new conditions. Familial cases did not cluster within any subgroup or subgroups. Although each subgroup was unique in that it contained patients with identical coded anomalies, non-coded anomalies overlapped between subgroups. Therefore, we think that these other subgroups represent examples of the great variability of expression of this clinical entity.

We were intrigued by the negative association of mandibular hypoplasia with bilateral microtia in the log-

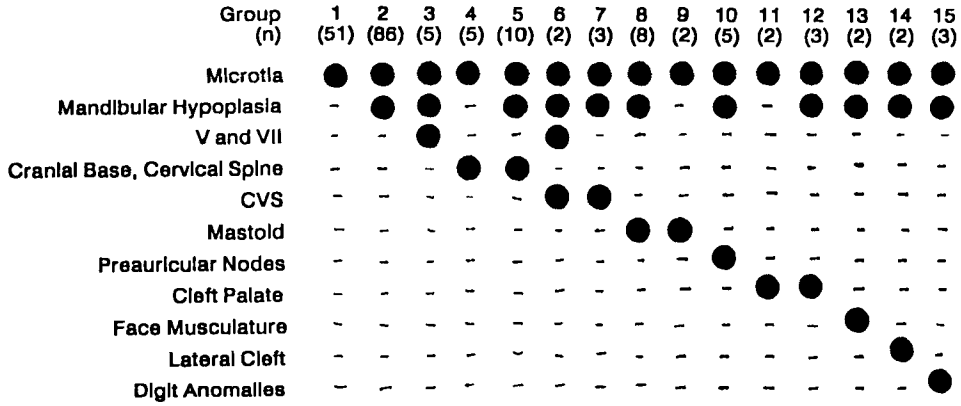


Fig. 3. Subgroups of patients with identical patterns of anomalies.

linear analyses. This finding suggests that a portion of individuals with bilateral microtia may represent a unique subset of patients. The association of mandibular hypoplasia with cervical spine anomalies, and of cervical spine anomalies with other spine and skeletal defects was also of interest. These data suggest that patients with microtia should be examined for the presence of cervical spine anomalies; when they are found, more extensive skeletal evaluations are indicated to look for clinically significant defects.

Our clinical and statistical data become more interesting when viewed in conjunction with the exciting work now being done regarding morphogenesis of the craniofacial region. The maxilla and mandible, ears, palate, and fifth and seventh cranial nerves are derived from the embryonic first and second visceral arches. Poswillo [1973] postulated that the underlying mechanism of malformations of these structures was early destruciton of neural crest cells that migrate into the visceral arches in early embyronic life. Recent studies of 13-cis-retinoic acid-induced craniofacial malformations in mice by Sulik et al. [1987a] have further clarified the mechanism of these malformations (Table V). Treatment of mice at a stage equivalent to 21 days of human gestation resulted in major effects on neural crest cell morphology, followed by abnormalities of the first and second visceral arch structures. Axial skeletal defects were also observed, which were apparently secondary to a concurrent effect on gastrulation. Later treatment resulted in craniofacial anomalies and cardiac defects reminiscent of the DiGeorge sequence. Treatment at the equivalent of 24 days resulted in cleft palate, ear malformations, anomalies of the mandible, and limb malformations. Study of the embryos at this later stage suggested that the craniofacial malformations were related to excessive cell death involving cells associated with the first and second arch ectodermal placodes [Sulik et al, 1987b]. The ectodermal placodes are areas behind

TABLE V. Retinoic Acid-Induced Carniofacial Malformations in Mice

| | | | | | |
|---|---|---|---|---|---|
| Mouse-postfertilization time | 7 d 18 hr–8 d 0 hr | 8 d 6 hr | 8 d 12 hr | 8 d 18 hr | 9 d 0 hr |
| Human-postfertilization time | 19–20 d | 21 d | 22 d | 23 d | 24 d |
| Developmental Stage | Presomite–two somites | 3–5 somites | 8–12 somites | 10–14 somites | 13–20 somites |
| Cell populations involved | Anterior neural plate 1st arch neural crest, gastrulating mesoderm, (cervical and upper thoracic) | 1st and 2nde arch neural crest, gastrulating mesoderm (lower thoracic) | Gastrulating mesoderm (lumbar and sacral), migrating hypoglossal myoblasts? | 3rd and 4th arch neural crest | Trigeminal and geniculate ganglionic placocdes, forelimb apical ectodermal ridge |
| Malformation | Anencephaly holoprosencephaly | OAV syndrome (Goldenhar, hemifacial microsomia) | Spina bifida, microglossia | DiGeorge syndrome | Mandibulofacial dysostosis (Treacher-Collins syndrome), Miller syndrome |

Modified from Sulik et al. [1987a].

the visceral arches that contribute cells to the developing cranial nerve ganglia.

These studies are helpful in clarifying the possible mechanisms for the patterns of anomalies seen in our patients. It appears that the craniofacial anomalies result from a defect in neural crest cell migration or early death of neural crest cells or placodal cells. The cervical spine anomalies may result from a concurrent defect in the cervical mesoderm. The cardiac malformations observed in our patients (ventricular septal defect, tetralogy of Fallot, and aortic defects) are those frequently seen in the DiGeorge anomaly and are also defects in structures derived from the neural crest [Lammer and Opitz, 1986]. Recent work indicates that limb anomalies probably result from a concurrent effect on the apical ectodermal ridge of the forelimb [Sulik and Dehart, 1988].

These embryologic studies, when reviewed in conjunction with our clinical data, suggest that the patterns of anomalies seen in patients with ear malformations occur because of defects that are inducible in mouse embryos by teratogens that are administered during a relatively brief period of time (21–24 days human postfertilization time). Opitz [1986] suggested that hemifacial microsomia and the Goldenhar anomaly are neurocristopathies. Our data, in conjunction with the mouse embryo studies, suggest that this hypothesis is reasonable but incomplete. Abnormalities of other cell populations must be invoked to encompass the full spectrum of associated malformations we have described. We propose that the cells involved in this pattern of malformations must include not only neural crest-derived structures, but also components of the ectodermal placodes, the mesoderm, and other surface ectoderm.

## ACKNOWLEDGMENTS

We thank Dr. K.K. Sulik for helpful discussion and review of the manuscript and Lila Markowitz for preparation of the manuscript. This work was supported in part by NIH grant DE 02872.

## REFERENCES

Goldenhar M (1952): Associations malformatives de l'oeil et de l'oreille, en particular le syndrome dermode epibulbaire-appendices auriculaires-fistula auris congenita et ses relations avec la dysostose mandibuloaciale. J Genet Hum 1:243–282.

Gorlin RJ, Pindborg JJ (1964): Oculo-auriculo-vertebral dysplasia. In "Syndromes of the Head and Neck." Ed. 1. New York: McGraw-Hill, p 419.

Gorlin RJ, Pindborg JJ, Cohen MM Jr (1976): Oculoauriculovertebral dysplasia. In "Syndromes of the Head and Neck." Ed. 2. New York: McGraw-Hill, pp 546–552.

Grabb WC (1965): The first and second branchial arch syndrome. Plast Reconstr Surg 36:485–508.

Lammer EJ, Opitz J (1986): The DiGeorge anomaly as a developmental field defect. Am J Med Genet (Suppl) 2:113–127.

Melnick M, Myrianthopoulos NC (1979): External Ear Malformations: Epidemiology, Genetics, and Natural History. New York: Alan R. Liss, Inc., for The National Foundation—March of Dimes. BD:OAS XV (9).

Opitz J (1986): Developmental field theory and observations-accidental progress? Am J Med Genet (Suppl) 2:1–9.

Pashayan H, Pinsky L, Fraser FC (1970): Hemifacial microsomia—Oculo-auriculo-vertebral dysplasia. A patient with overlapping features. J Med Genet 7:185–188.

Poswillo D (1973): The pathogenesis of the first and second branchial arch syndrome. Oral Surg 35:302–329.

Rollnick BR (1988): Oculoauriculovertebral anomaly: Variability and causal heterogeneity. Am J Med Genet (Suppl) 4:41–53.

Rollnick BR, Kaye CI (1983): Hemifacial microsomia and variants: Pedigree data. Am J Med Genet 15:233–353.

Rollnick BR, Kaye CI, Nagatoshi K, Hauck W, Martin AO (1987): Oculoauriculovertebral dysplasia and variants: Phenotypic characteristics of 294 patients. Am J Med Genet 26:361–375.

Smith DW (1982): Facio-auriculo-vertebral spectrum. In "Recognizable Patterns of Human Malformation." Ed. 3. Philadelphia: W.B. Saunders Co., pp 498–500.

Sulik KK, Dehart DB (1988): Retinoic-acid-induced limb malformations resulting from apical ectodermal ridge cell death. Teratology 37:527–537.

Sulik KK, Johnston MC, Speight HS, Smiley SJ (1987a): Retinoic acid-induced craniofacial malformations: New insights from an old teratogen. Proc Greenwood Genet Center 6:116–117.

Sulik KK, Johnston MC, Smiley SJ, Speight HS, Jarvis BE (1987b): Mandibulofacial dysostosis (Treacher Collins syndrome): A new proposal for its pathogenesis. Am J Med Genet 27:359–372.