

## Oculoauriculovertebral Anomaly: Segregation Analysis

Celia I. Kaye, Alice O. Martin, Beverly R. Rollnick, Konrad Nagatoshi, Jeannette Israel, Mark Hermanoff, Brad Tropea, Joan T. Richtsmeier, and Newton E. Morton

*Division of Growth and Development, Department of Pediatrics, The University of Texas Health Science Center at San Antonio (C.I.K.); Department of Obstetrics and Gynecology, Northwestern University Medical School (A.O.M., M.H., B.T.) and Department of Pediatrics, Center for Craniofacial Anomalies, University of Illinois College of Medicine at Chicago (B.R.R., K.N.), Chicago and Department of Pediatrics, Rush Medical College, Oak Lawn (J.I.), Illinois; Department of Cell Biology and Anatomy, The Johns Hopkins University, Baltimore, Maryland (J.T.R.); Department of Community Medicine, University of Southampton, England (N.E.M.)*

**Seventy-four families of probands with oculoauriculovertebral anomaly were evaluated, including 116 parents and 195 offspring. Relatives were examined to identify ear malformations, mandibular anomalies, and other craniofacial abnormalities. For segregation analysis using POINTER, selection of the sample was consistent with single ascertainment. Different population liabilities were used for probands and relatives, because affection was narrowly defined for probands and broadly defined for relatives. The hypothesis of no genetic transmission was rejected. The evidence favored autosomal dominant inheritance; recessive and polygenic models were not distinguishable.**

© 1992 Wiley-Liss, Inc.

**KEY WORDS:** hemifacial microsomia, Goldenhar syndrome, microtia

### INTRODUCTION

The term oculoauriculovertebral "dysplasia" (OAV) was suggested by Gorlin et al. [1963] to describe patients with microtia, mandibular hypoplasia, vertebral anomalies, and epibulbar dermoids or lipodermoids. Phenotypes overlapping OAV include hemifacial microsomia and Goldenhar "syndrome." It has been suggested that these entities represent a single disorder with great variability of expression, and that an isolated ear malformation may represent the mildest expression of the disorder [Grabb, 1965; Pashayan et al., 1970; Gorlin et

al., 1976, 1990; Smith, 1982; Rollnick and Kaye, 1983; Rollnick, 1988]. Although minimal diagnostic criteria for OAV have not been defined, most OAV patients have ear malformations. The fact that some patients thought to be affected with OAV apparently have normal ears [Grabb, 1965] raises the question of a separate disorder or a continuum which extends to normality.

Heterogeneity has complicated elucidation of the causes of the OAV phenotype. Some patients with the OAV phenotype have had exposure to thalidomide or retinoic acid [Livingston, 1965; Lammer et al., 1985; Rollnick, 1988]. Numerous chromosome abnormalities have been reported in patients with an OAV phenotype. In addition, the anomaly has been observed in other syndromes of known or unknown cause, including the branchio-oto-renal syndrome, the Townes-Brocks syndrome, and frontonasal "dysplasia" [Rollnick, 1988]. Exclusion of teratogenic exposures, chromosome abnormalities, and known syndromes results in a large group of "idiopathic," non-syndromal remaining cases. A statistical analysis of 297 such patients failed to identify any previously unrecognized distinct patterns of anomalies [Kaye et al., 1989]. Within this group of patients, there are some case reports consistent with autosomal dominant inheritance, and others with autosomal recessive inheritance [Rollnick, 1988]. It has been suggested that most cases are sporadic [Feingold, 1979].

We previously explored the hypothesis that failure to detect a clear mode of inheritance in most families with the OAV phenotype was due to incomplete definition of the phenotype associated with a single underlying disorder. We studied families of 97 probands with OAV, looking for a history of ear and mandibular anomalies that did not reach a level of severity consistent with the usual diagnosis of OAV. Mild malformations such as pretragal nodes or fistulae were counted because they are relatively rare in the general population [Melnick, 1980] but common in our probands. Of 97 probands evaluated, 44 (45%) had a family history of one or more "affected" relatives [Rollnick and Kaye, 1983]. However, many affected relatives did not appear to demonstrate

Received for publication February 25, 1991; revision received December 16, 1991.

Address reprint requests to C.I. Kaye, M.D., Ph.D., The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284-7809.

all or even most OAV manifestations; a minor ear malformation was the most common reported finding in relatives.

From the above study, we hypothesized that OAV might represent one extreme of a phenotypic continuum produced by segregation of a single gene. The present study was undertaken to determine if first degree relatives of patients with OAV were, in fact, affected with all or some of the manifestations of the disorder with higher frequency than seen in the general population. We performed segregation analysis to determine the most likely mode of inheritance, using expressions of ear or mandibular malformations to define affected individuals.

**MATERIALS AND METHODS**

All probands were patients at the Center for Craniofacial Anomalies, University of Illinois-Chicago (CCFA). Minimal diagnostic criteria for inclusion in the study as a proband were the presence of microtia of at least grade I in severity, and the presence of mandibular hypoplasia. Malformations of the external ear were graded according to a system modified from Meurman [1957] (Fig. 1). Computerized medical records were searched for all patients with microtia; 405 such patients were identified. The clinical records and photographs for each patient were reviewed by 2 of the authors (C.K., B.R.) to

TABLE I. Sibship Size (S) Distribution

S	Number of families
2	16
3	22
4	20
5	10
6	3
7	1
8	2
Total	74

determine appropriateness for inclusion in the study. Patients with chromosome abnormalities or other known syndromes were excluded; 291 patients remained after this review. Of these, 83 families agreed to participate in the study. Minimum family size for inclusion in the study was the availability of at least 2 sibs, including the proband, for examination. Nine families were excluded because of this requirement. Thus, 74 probands and families were included in the subsequent analysis.

Of the 74 families studied, both parents were available for examination in 43, a single parent was available in 30, and in one family no parent was available. Sibship size ranged from 2 to 8 (Table I). Every effort was made to examine all family members. When this was not possible, participating relatives were questioned to obtain information on unavailable relatives. In no instance was a parent or sibling reported to be affected with an ear or jaw abnormality unavailable for examination. A total of 116 parents and 195 offspring including probands was examined by a clinical geneticist. When facial asymmetry was clinically evident, cephalometric films were obtained and reviewed by a cephalometrician for coding purposes.

A relative was considered affected if he or she demonstrated: 1) an ear malformation of at least grade I microtia in severity; or 2) a mandibular anomaly; or 3) less severe abnormalities of at least 2 of the following: ear, palate, maxilla, eye, mimetic musculature, muscles of mastication (Table II). Early onset (age <20 yr) conductive hearing loss was also a criterion in this category; however, no relative was affected with this condition, and the criterion was not included in the final analysis.

For segregation analysis, the frequency of OAV in the population, and the frequencies of variables and combi-

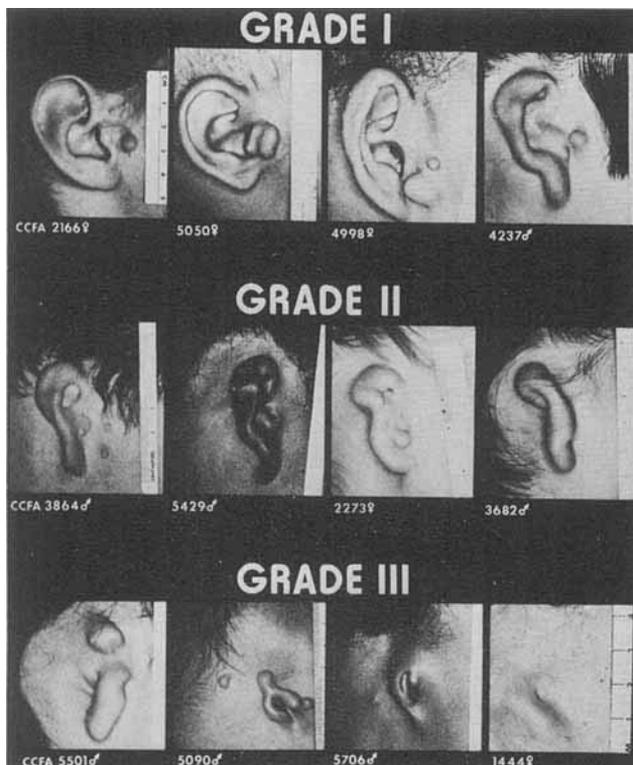


Fig. 1. Ranges of severity of microtia, including preauricular tags of cartilaginous masses, atresia of the external auditory canal, and deviations in size and shape of the external auricle ranging from variations that retain the essential morphologic characteristics of the pinna, to verticle folds of the helix, to ill-defined masses of tissue, to anotia.

TABLE II. Criteria for Affected Relative

1. At least one major ear anomaly, or
2. At least one mandibular anomaly, or
3. At least two of the following anomalies:
  - a. Minor ear anomaly
  - b. Minor palate anomaly (bifid uvula, palatal fistula, etc.)
  - c. Abnormal soft palate movement on phonation
  - d. Abnormal maxillary arch
  - e. Eye anomaly (microphthalmia, iris anomalies, ptosis, etc.)
  - f. Abnormalities of mimetic musculature or facial nerve
  - g. Abnormalities of masseter musculature or fifth nerve

nations of variables used to define "affected" were required. Population frequencies of all anomalies except mandibular hypoplasia were obtained from the literature. Melnick [1980] calculated the birth incidence of OAV at 1/26,500. This figure was based on all births in a 1 year cohort and was derived from the Collaborative Perinatal Project of the National Institute of Neurological and Communicative Disorders and Stroke [Myriantopoulos, 1985]. Control data on the frequency of microtia, preauricular tags, preauricular sinuses, and other abnormalities of the pinna were derived from Melnick and Myriantopoulos [1979], also using data from the Collaborative Perinatal Project. Control data on the frequency of fifth cranial nerve involvement and 7th cranial nerve involvement were obtained from Melnick (personal communication) and were based on the studies described above. Control data on the frequencies of eye anomalies, palate and uvula anomalies, defects of the oral cavity, maxillary anomalies, and defects in soft palate movement were derived from Myriantopoulos [1985] (Table III). All control frequencies were weighted by the racial composition of the sample.

There were no published or easily obtainable radiographic control data on mandibular hypoplasia, asymmetry, or other defects of the craniofacial skeleton. The control data used in this analysis were derived from a study by 2 orthodontic cephalometricians at CCFA. They evaluated the cephalometric radiographs of 100 late teen and early adult Caucasian patients who presented for routine orthodontic treatment. These individuals were selected at random from the cephalometric radiographic files of a large private orthodontic practice. By chance, the sample consisted of an equal number of males and females, none of whom had any facial or genetic defect. Six cases were rejected due to incorrect positioning of the ear rods. The remaining sample consisted of 94 individuals, on whom symmetry of paired facial structures was assessed (Kay and Aduss, unpublished data). Three of the 94 individuals were found to have mandibular asymmetry. This population frequency was used in the data analysis.

The probability of having at least 2 of the anomalies listed in Table III, one of the criteria used to define "affected," was calculated as

$$\begin{aligned}
 P(0) &= \text{Probability of no anomalies} \\
 P(1) &= \text{Probability of one anomaly} \\
 P(2) &= \text{Probability of at least two anomalies} \\
 n &= \text{number of anomalies analyzed} \\
 p_i &= \text{probability of the } i^{\text{th}} \text{ anomaly, } i = 1, n \\
 P(2) &= 1 - P_n(0) - P_n(1) \\
 &= 1 - \prod(1 - p_i) - \sum p_i \prod(1 - p_j) \\
 & \quad i, j, = 1, \dots, n \text{ and } i \neq j
 \end{aligned}$$

For the segregation analysis using POINTER, selection of the sample was consistent with single ascertainment, because families were selected through probands, probands were ascertained only once, and there was only one proband per family. Different population risks (or liabilities) were used for probands and relatives because affection is narrowly defined for probands and broadly defined for relatives. The population risk of

TABLE III. Population Frequencies of Minor Anomalies\*

Anomaly	Frequency, %
Minor ear	.09
Minor palate	1.07
Abnormal soft palate movement	.13
Maxillary arch	.03
Eye	2.82
Mimetic musculature or facial nerve	.08
Muscles of mastication or fifth nerve	0

\* From Melnick and Myriantopoulos (1979), Melnick (personal communication), and Myriantopoulos (1985).

OAV was used for probands ( $Lip = 0.000038$ ). For relatives, the liability was the likelihood of having either an isolated ear or mandibular anomaly, or at least 2 of the anomalies shown in Table III, based on the frequencies of these single anomalies in the general population ( $Lir = 0.0354$ ). This double liability model was selected to take into account the fact that the minor anomalies, which under the hypothesis being tested were considered indicative of gene action in relatives, are much more common than the full OAV phenotype. Therefore, to show a higher frequency of these anomalies in relatives of probands required comparison with the probability of observing these by chance in the general population.

RESULTS

The distribution of sibship size in the 74 families studied is shown in Table IV. Mating types are shown in Table V.

The results of segregation analysis are shown in Table VI. The hypothesis of no genetic transmission was rejected with a  $P$  value of  $<10^{-8}$ . By comparison of log likelihoods generated under the three genetic models (dominant, recessive, polygenic), the evidence favored autosomal dominant inheritance. Recessive and polygenic models were not distinguishable.

TABLE IV. Composition of Families Selected for Segregation Analysis

	Number of families	Number of children
Two parents	43	124
One parent	30	68
No parents	1	3
Total	74	195

TABLE V. Mating Types Studied

Mating type	n
Normal x ?	3
Affected x ?	1
? x normal	15
? x affected	11
Normal x normal	28
Normal x affected	11
Affected x normal	3
Affected x affected	1

TABLE VI. Segregation Analysis\*

Hypothesis	D	T	g	H	-2lnL + C
Dominant	(1)	2.27	.0003	(0)	0
Recessive	(0)	1.92	.097	(0)	28.13
Polygenic	—	(0)	(0)	.397	30.19
No transmission	(0)	(0)	(0)	(0)	80.39

\* D, dominance; T, displacement between homozygotes; g, gene frequency; H, polygenic heritability. No transmission:  $\chi^2 = 80.39$  ( $P < 10^{-8}$ ).

## DISCUSSION

Methods of segregation analysis have proven very powerful in confirming Mendelian and polygenic modes of inheritance for numerous traits and diseases, in identifying heterogeneity which initially obscured the mode of inheritance for a trait or disease considered to be a single entity phenotypically, and in estimating various parameters such as the proportion of sporadic cases [Morton et al., 1983].

For phenotypes that have not presented clear patterns on segregation analysis, the possibility exists that variable expressivity has not been considered adequately in definition of the "affected" phenotype. A single gene whose product enters a complex developmental pathway and emerges with phenotypic effects ranging from a severe, clinically recognizable syndrome to mild variations in the form and/or function of the same or developmentally related body parts involved in the syndrome may defy the sleuthing of segregation analysis.

Concluding that this may be the reason for controversy in determining the mode(s) of inheritance for OAV, we have taken the approach that a range of deviations from the normal form and/or function of ears, mandibles, and other craniofacial areas which are altered in persons said to be clinically affected may reflect the expression in an underlying gene. Because some of the craniofacial deviations (anomalies) are relatively common, we have imposed a criterion of "at least two" craniofacial anomalies to classify a relative as affected. Therefore, a higher underlying liability has been considered for relatives to minimize spurious results. These spurious results would have arisen if comparisons of affected relative frequencies were made to OAV frequencies which are very rare, to determine if there is a greater than chance familial occurrence. To compare family increases of affected individuals above background for our analysis, background was considered to be the joint probabilities of having at least two of the craniofacial anomalies we used to define affected in the general population.

Despite these rigid criteria, and the use of a double liability model, there was evidence for genetic transmission, most likely autosomal dominant inheritance. These results are consistent with the increased likelihood of finding craniofacial anomalies in first degree relatives of the affected, a clinical observation that prompted our approach to segregation analysis.

The mechanism by which an autosomal dominant gene might produce phenotypic effects which range in severity from non-penetrance to a severe craniofacial

anomaly syndrome is unclear. However, wide variability in expression is not unique to OAV, but is also observed in such conditions as Marfan syndrome and neurofibromatosis. Future molecular genetic studies aimed at identifying the gene for OAV will doubtless clarify the issue. Such studies will be made difficult by the incomplete penetrance and variable expressivity of the putative autosomal dominant gene. Very careful clinical evaluation of all family members will be essential. Identification of a few large families with multiple affected members will simplify such a study.

Segregation analysis is not an end in itself, but is an indication of productive directions for further research. Our results indicate that clinical observations of craniofacial anomalies in relatives of OAV probands are unlikely to be due only to heightened scrutiny, and that linkage analysis and risk calculations should take our definition of "affected" and the likelihood of autosomal dominant inheritance of OAV into consideration.

## ACKNOWLEDGMENTS

This work was supported in part by NIH grant DE 02872. The authors wish to thank Mrs. Molly West for her assistance in preparation of the manuscript.

## REFERENCES

- Feingold M (1979): Hemifacial microsomia. In Bergsma D (ed): "Birth Defects Compendium," 2nd Ed. New York: Alan R. Liss, Inc., for the National Foundation—March of Dimes, p 511.
- Gorlin RJ, Jue KL, Jacobsen V, Goldschmidt E (1963): Oculoauriculovertebral dysplasia. *J Pediatr* 63:991-999.
- Gorlin RJ, Pindborg JJ, Cohn MMJr (1976): Oculo-auriculovertebral dysplasia. In "Syndromes of the Head and Neck," Ed. 2. New York: McGraw-Hill, pp 546-552.
- Gorlin RJ, Cohen MM, Levin LS (1990): Branchial Arch and Oro-Acral Disorders. In "Syndromes of the Head and Neck," Ed. 2. New York: Oxford University Press, pp 641-649.
- Grabb WC (1965): The first and second branchial arch syndrome. *Plast Reconstr Surg* 36:485-508.
- Kaye CI, Rollnick BR, Hauck WW, Martin AO, Richtsmeier JT, Nagatoshi K (1989): Microtia and associated anomalies: Statistical analysis. *Am J Med Genet* 34:574-578.
- Lammer EJ, Chen DT, Hoar RM, Agnish ND, Benke PJ, Braun JT, Curry CJ, Fernhoff PM, Grix AW, Lott IT, Richard JM (1985): Retinoic acid embryopathy. *N Engl J Med* 313:837-841.
- Livingston G (1965): Congenital ear malformations due to thalidomide. *Proc R Soc Med* 58:493-497.
- Melnick M (1980): The etiology of external ear malformations and its relation to abnormalities of the middle ear, inner ear, and other organ systems. In Gorlin RJ (ed): "Morphogenesis and Malformation of the Ear." New York: Alan R. Liss, Inc., for the National Foundation—March of Dimes. BD:OAS XVI(4):303-331.
- Melnick M, Myriantopoulos 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).
- Meurman Y (1957): Congenital microtia and meatal atresia. *AMA Arch Otolaryngol* 66:443–463.
- Morton NE, Rao DC, Lalouel JM. (1983): "Methods in Genetic Epidemiology." New York: Karger, Vol 4, pp 62–96.
- Myriantopoulos NC (1985): "Malformations in Children From One to Seven Years: A Report From the Collaborative Perinatal Project." New York: Alan R. Liss, Inc.
- Pashayan H, Pinsky L, Fraser FC (1970): Hemifacial microsomia—
- Oculo-auriculo-vertebral dysplasia. A patient with overlapping features. *J Med Genet* 7:185–188.
- 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* 15:233–253.
- Smith DW (1982): Facio-auriculo-vertebral spectrum. In "Recognizable Patterns of Human Malformation," Ed. 3. Philadelphia: W.B. Saunders Co., pp 498–500.