A Study of Cephalometric Features in Cleft Lip–Cleft Palate Families I: Phenotypic Heterogeneity and Genetic Predisposition in Parents of Sporadic Cases

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Several previous studies have indicated that unaffected parents of children with nonsyndromic cleft lip and palate show unusual craniofacial features. This study reexamines this question by applying multivariate cluster analysis to lateral cephalometric head plates from 82 individuals who are parents of sporadic cases of cleft lip with or without cleft palate (CL/P). Considerable phenotypic heterogeneity was present within the sample. Three major groupings were defined. Two of these groups showed cephalometric similarities to individuals with overt clefts, while the third showed a generalized concordance to published norms. In almost every case only one member of each parental pair showed the cleft related cephalometric phenotype, suggesting the possibility of a substantial genetic component in many cases of sporadic CL/P. However, there were several cases in which neither parent showed the phenotypic traits. Such cases may have a different etiology or a greater environmental component.

KEY WORDS: *cleft lip, cleft palate, sporadic cleft lip and palate, cephalometrics, cluster analysis*

A recurring theme in the study of oral clefts is that noncleft relatives of affected individuals may display unusual facial features that distinguish them from the general population. Central to most of these studies is the premise that such unique features reflect the expression of genetic susceptibility to clefting. Thus, Trasler (1968), citing experimental evidence from mice, suggested that the shape of the embryonic face could be a predisposing factor to clefting. Fraser and Pashayan (1970) also theorized that such predisposing factors should be evident in the human face, and they demonstrated that parents of individuals with clefts had a suite of facial features that distinguished them from a normal control group. Coccaro et al (1972), Kurisu et al (1974), Nakasima and Ichinose (1983), and Prochazkova and Tolarova (1986) expanded on these earlier results and in each case demonstrated that noncleft parents of children with clefts had facial features that differed quantitatively from those seen in parents of unaffected children. However, these studies have produced little agreement on which cephalometric variables most effectively characterize the parents. For instance, Fraser and Pashayan (1970), Coccaro et al (1972), and Kurisu et al (1974), all report decreased facial convexity in parents of children with clefts. However, this was not noted in the study by Nakasima and Ichinose (1983). Similarly, Fraser and Pashayan (1970) and Nakasima and Ichinose (1983) reported an increased facial height in parents of children with clefts while Cocarro et al (1974) and Kurisu et al (1974) do not.

This lack of agreement may stem in part from the fact that all previous studies have been conducted with at least the tacit acceptance of the multifactorial threshold model (MFT) for the transmission of cleft lip with or without cleft palate and of isolated cleft palate (CP). This model assumes that both parents contribute predisposing factors to an affected child. Therefore, when seeking to characterize these factors the parents of children with clefts are compared en masse to some control group of parents who have not produced children with clefts. The resultant characterizations make no allowance for the possibility that one parent may contribute more to the susceptibility for oral clefting than does the other.

This approach can be questioned on several grounds. First, the MFT model need not require an equal contribution to genetic liability for clefting from each parent. Second, evidence is accumulating for the influence of a major gene or genes on the inheritance of clefting (Fogh-Andersen, 1942; Marazita et al, 1984 and 1986; Melnick et al, 1986;

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Chung et al, 1987; and Eiberg et al, 1987). These authors also point out that several mathematical and logical expectations of the MFT model are not supported by the data accumulated from large population studies (Kurisu et al, 1974; Marazita et al, 1984; Melnick et al, 1986). It therefore appears that in at least some cases the MFT model is not the most parsimonious for explaining the transmission of oral clefts. Finally, if one assumes etiologic heterogeneity in the production of facial clefts, parental contribution should be minimal in some cases, heavily weighted to one parent in others, and approximately equal only in those instances where by chance each parent happens to possess the same degree of predisposing factors.

An alternate approach to the search for predisposing factors is one in which no prior assumptions of parental contribution are made. Cluster analysis or numerical taxonomy offers such a means to an end. This multivariate statistical technique identifies "natural" groupings of phenotypically similar individuals from a sample. Therefore, it allows for the possibility of etiologic and genetic heterogeneity within a given sample. The purpose of the present study was to utilize cluster analysis to search for unique cephalometric features in the noncleft parents of children affected with CL/P. It is hypothesized that the results of a cluster analysis will support the presence of both phenotypic and etiologic heterogeneity among the parents of children with clefts and that the identifiable risk factors will be unevenly distributed within parental pairs.

METHOD

Subjects

The data base for this study consists of lateral cephalometric head plates previously collected for other studies from parents of sporadic cases of CL/P. Posterior–anterior head plates were not available for this retrospective study. Isolated CP was not considered at the present time because of the possibility that it represents a distinct etiologic entity different from CL/P (Fogh-Andersen, 1942). Sporadic cases were chosen over familial ones because it was reasoned that the former should be the more heterogenous of the two. Sporadic cases probably result from the interaction of a variety of genetic and environmental causes (Crawford and Sofaer, 1987).

All edentulous individuals not wearing both upper and lower dentures were excluded from the study because of poor definition of the occlusal plane and jaw relationships. Probands were examined by a dysmorphologist for multiple congenital anomalies in order to minimize the possibility of including parents of syndromic cases in the sample. This left a total of 82 individuals including 40 males and 42 females with an average age of 29 years. There were 35 couples and 12 single parents in this sample. Because no control sample had been collected when the cephalograms were originally obtained, we relied on published normative data (Saksena et al, 1987) to provide a basis of comparison for the "at-risk" sample.

Variables

Cephalometric analysis was conducted using standard techniques. Landmarks were identified and tracings were made on acetate overlays. Eleven linear and six angular measurements were used in this study as described in Table 1 (detailed descriptions of these measurement variables are presented in Riolo et al, 1974 and Saksena et al, 1987). Measurements were made by hand and verified by repeated measurements. Values were recorded to the nearest half millimeter or half angular degree.

The choice of variables used in this study was determined by three factors: (1) The need to describe as completely as possible all anatomic regions of the head and face represented on the lateral head plate; (2) The availability of age and sex matched normal population standards; (3) The need to avoid unnecessary duplication or the use of highly correlated variables in the analysis (defined as pairs of variables with r values greater than 0.70). The latter factor is a prerequisite for the efficient application of cluster analysis, the methodology of which will be detailed shortly. When two variables were highly correlated ($r>0.70$), the choice of which one to eliminate was based on the perception of the actual anatomic information contained in the variable, the reliability with which it could be measured, and the desire

TABLE 1 Linear and Angular Cephalometric Measurements Used To Evaluate the Craniofacies in Cleft Lip and Palate Families

		Structures Evaluated
1. Nasion to Basion	N-Ba	Cranial base
2. Sella to Nasion	S-N	Anterior cranial base
3. Sella to Basion	S-Ba	Posterior cranial base
4. Nasion to Sella to Basion	N-S-Ba	Cranial base flexure
5. Anterior nasal spine to Posterior nasal spine	ANS-PNS	Palatal length
6. Articulare to Gonion	Ar-Go	Mandible ramus height
7. Gonion to Pogonion	Go-Pg	Mandibular body length
8. Articulare to Gonion to Menton	Ar-Go-Me	Mandibular angle
9. Nasion to Menton	N-Me	Facial height
10. Nasion to Anterior nasal spine	N-ANS	Upper facial height
11. Anterior nasal spine to Menton	ANS-Me	Lower facial height
12. Sella to Posterior nasal spine	S-PNS	Posterior facial height
13. Sella to Gonion	S-Go	Posterior facial height
14. Sella to Nasion to Anterior nasal spine	S-N-ANS	Maxilla position relative to cranial base
15. Maxillary plane (Var. 5) intersecting Var. 10	ANS-PNS/N-ANS	Maxilla position
16. Sella to Nasion to Pogonion	S-N-Pg	Mandible position relative to cranial base
17. Maxillary plane (Var. 5) intersecting Nasion to Pogonion	ANS-PNS/N-PG	Mandible position

Variables 4, 8, 14, 15, 16 and 17 are angular; all others are linear.

to use variables that other investigators had used in similar studies. For example, nasion-to-menton and nasion-to-pogonion are highly correlated ($r=0.95$). The former was retained because it was felt that it gave a truer estimate of anatomic facial height. In three instances, pairs of variables with intercorrelations greater than 0.70 were retained because it was felt that each made a slightly different but essential contribution to the analysis. Thus, the correlation between Ar-Go and S-Go was $r=0.82$. However, the former measures mandibular ramus length, while the latter is used to represent posterior facial height. Similarly, N-Ba correlated highly with S-N ($r=0.71$). Both were retained because they are, respectively, measures of total and anterior cranial base length. On the other hand, total anterior facial height (N-Me) and lower facial height (ANS-Me) ($r=0.82$) were both retained because previously published studies (Coccaro et al, 1972; Nakasima and Ichinose, 1983; Prochazkova and Tolarova, 1987) have indicated that both are important in differentiating relatives of clefts from normal control populations. After the data collection, raw values were adjusted to remove differences due to age or sex using the equations and methods outlined in Saksena et al (1983). Prior to clustering, all raw adjusted values were converted to Z scores to eliminate the effect of different scales of measurement from the analysis (Romesburg, 1984).

Clustering

Cluster analysis refers to a collection of multivariate statistical techniques that have been developed to overcome problems of classification and taxonomy. The theoretical underpinnings of cluster analysis have been discussed in detail elsewhere (Anderberg, 1973; Romesburg, 1984) and will not be reviewed here. Nevertheless, in very general terms, clustering techniques are used to identify groups of objects within a sample that share more attributes in common with one another than they do with objects from other groups. Thus, in theory cluster analysis provides an objective way of partitioning a sample into natural subgroupings. In practice, however, subjective decisions about choice of variables, similarity coefficients, and clustering algorithms all affect the results obtained. Therefore, cluster analysis may best be used as an heuristic device, rather than as a means of arriving at a definitive classification (Ward and Meaney, 1984). Used in this way the goal of cluster analysis is to identify groups of individuals who share a unique set of features. These groupings then provide insight into the structure of the variability within a sample.

In the present study the Clustran© clustering package (Wishart, 1987) was used to perform a hierarchical cluster analysis. Similarity between objects was calculated using euclidean distances, and clusters were formed with the unweighted pair-group method using arithmetic averages (UPGMA). This combination of methods was chosen because it is the most widely used of the many clustering techniques available (Romesburg, 1984). Hierarchical clustering operates in two stages. First, each object (individual) is compared to every other object (individual) in the sample for the preselected set of variables. The second stage occurs when the resultant "distance" matrix is iteratively sorted to find first the two most similar individuals and then the next most similar and the next, etc., until the whole sample has been sorted. Thus, larger and larger clusters are formed until at the last cycle a single all inclusive cluster exists. This process may be conveniently represented by a dendrogram, which displays the series of evermore inclusive fusions in the form of an inverted tree.

Figure 1 represents an abbreviated version of such a dendrogram. The values along the ordinate are the euclidian distances separating objects at the time they are fused into clusters. Clusters are designated by letters along the abscissa, and the number of individuals included in each cluster is shown in parentheses.

The clusters were defined directly from the dendrogram as groupings of individuals who share a high degree of similarity with one another, but who are separated from other individuals and clusters by large euclidian distances. The characteristics of these groupings were defined by subsequent analysis. Thus, once clusters were identified they were first characterized in terms of their variable means and standard deviations. Where cluster size allowed, Hotelling's T^2 was used to test for significant differences between the clusters over the entire variable set, and univariate t tests were performed in order to characterize specific differences between the groupings. If the parents of sporadic cases of CL/P are as homogeneous a group as presumed by previous investigators then few meaningful differences should exist between whatever groupings are identified by the clustering procedure. All statistical analyses other than those associated with cluster analysis were performed using the BMDP statistical package (Dixon, 1983).

Results

On examination of the dendrogram (see Fig. 1) produced by UPGMA, hierarchical cluster analysis indicates a complex pattern of variation within this sample of unaffected parents of sporadic CL/P children. Thus, 68 of the 82 cases

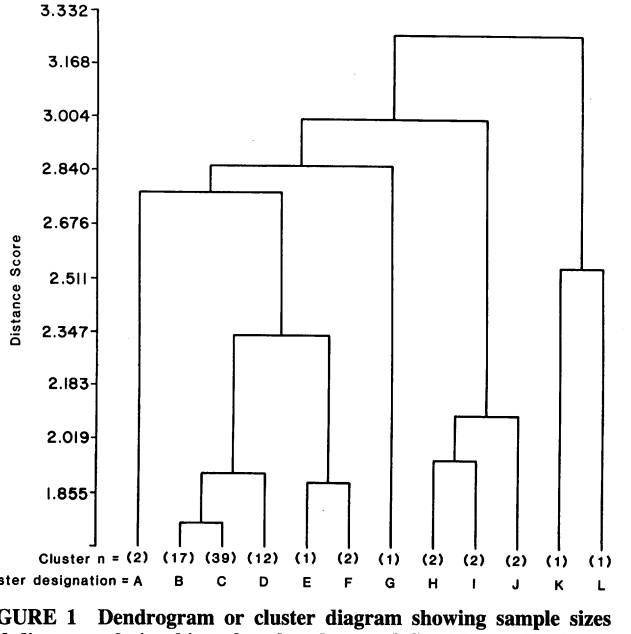

FIGURE 1 Dendrogram or cluster diagram showing sample sizes and distance relationships of twelve clusters defined by UPGMA, hierarchical cluster analysis on 82 parents of sporadic cases of CL/P.

in the sample can be found in one of three major clusters (B, C, and D, in Fig. 1). These three groups are comprised of 17, 39, and 12 individuals respectively. The remaining 14 cases in the sample show much greater diversity and remain unclustered or in groups of two until the three major clusters have fused (A and E through L, in Fig. 1). For example, cluster A is comprised of two individuals who, in spite of their similarity to one another, are so distinct from the rest of the sample that they are not fused with it until the fourth to last cycle of clustering.

The means and standard deviations for each of the 3 major clusters are presented in Table 2. Table 3 displays the results of univariate t tests comparing the means of these groups. Because this data is difficult to visualize, the relationship between the means of the three clusters are also presented graphically in Figures 2a through 2c. These "mean pattern profiles" (after Garn et al, 1984a) were generated by converting the mean values from each cluster into z scores using the normal values published by Saksena et al (1987) as population means (these norms are represented by the 0 baseline in the graph). Poznanski et al (1972) note that the linear correlation coefficient between such patterns is a measure of their overall similarity. This value, called r_z, is indicated for all possible comparisons on the graphs. Yet another way to evaluate these patterns is the standard deviation of the z score values across the entire profile. This value called z by its originators (Garn et al, 1984b) is used to assess pattern variability; the higher the value the more the pattern fluctuates from the normal mean values over its entire length. These values are also indicated on the graphs. In individuals a σ_z of 1.2 or greater is considered dysmorphic (Garn et al, 1984b). Mean pattern profiles should normally vary even less about the baseline.

Visually, it can be seen that cluster C (Fig. 2b) shows the least pronounced deviation from the normal mean values. All of the mean values in this cluster fall within ±2 standard deviations of the population means and most are within 1 SD unit. All are larger than the normal means except cranial base angle (N-S-Ba), which is slightly below the norm. The impression of limited pattern variability in this group is confirmed by the σ_z .47, which is the lowest of the three profiles. The pattern in Cluster B (Fig. 2a) is clearly different from that of C. The mean values in B fluctuate both below and above the normal values. The σ_z for this pattern profile is .94 which is twice as large as that in cluster C. The results of the univariate t tests indicate that individuals in Cluster B differ from those in Cluster C by having a smaller posterior facial height (S-Go), mandibular ramus height (Ar-Go), and mandibular length (Go-Pg), as well as a significantly flatter facial profile (ANS/PNS-N-Pg and S/N/Pg). Individuals in cluster B also have a longer cranial base, a larger mandibular angle, and greater lower facial height, although none of these differences are significant. With the exception of mandibular length, these are essentially the same differences that distinguish cluster B from the published norms.

Like cluster B, cluster D (Fig. 2c) shows mean values that fluctuate widely around the norms. The σ_z for the pattern profile in this cluster is 1.07. The univariate t tests indicate that cluster D differs significantly from C in that it is comprised of individuals who have smaller upper-posterior facial height (S-PNS), a wider cranial base angle (N-S-Ba), and flatter facial profiles (S-N-Pg). Individuals in D also display longer (anterior) cranial base measurements, greater facial height, and mandibular dimensions than seen in the other major clusters or the published norms. Both anterior and posterior facial heights are also increased relative to other groups. While cluster D shares a number of features with cluster B (such as the increased lower facial height and the flat midfacial profile), it differs in that its members have a mandible that is both significantly taller (Ar-Go) and more widely angled (Ar-Go-Me) than those seen in cluster B. Finally, it should be noted that all three clusters differ from the published norms in having longer palate (PNS-ANS) and greater facial height, particularly in the lower face. There is also a general tendency for all of the individuals in the parental sample to show larger linear measurements than are seen in the published norms (there are only five linear

TABLE 2 Values of Variables in the Three Major Clusters of Non-cleft Parents

	Cluster B (N=17)		*Cluster C (N=39)*		*Cluster D (N=12)*	
Variable	$\bar{x}$	*SD*	$\bar{x}$	*SD*	$\bar{x}$	*SD*
N-Ba	118.7	3.6	115.5	3.8	121.6	4.5
S-N	79.8	3.3	77.5	2.8	78.7	2.8
S-Ba	51.2	2.6	50.1	3.5	52.7	3.7
N-S-Ba	127.8	4.0	128.0	4.5	135.3	3.4
ANS-PNS	57.6	2.5	57.6	2.7	58.9	2.8
Ar-Go	51.8	3.1	56.7	3.4	60.0	4.1
Go-Pg	77.1	3.9	83.0	3.9	84.7	2.1
Ar-Go-Me	133.4	4.0	128.1	4.7	124.0	6.7
N-Me	131.3	4.5	129.2	5.0	136.7	4.0
N-ANS	56.4	2.7	58.5	2.6	60.4	2.7
ANS-Me	77.3	4.7	73.1	4.1	78.9	4.2
S-PNS	54.8	3.4	55.7	3.0	53.9	2.9
S-Go	83.9	3.5	88.9	4.8	93.2	4.4
S-N-ANS	84.9	3.1	87.6	3.6	84.0	2.5
ANS-PNS/N-ANS	92.8	1.9	95.8	3.3	94.3	3.1
S-N-Pg	77.5	2.0	82.9	3.2	78.3	2.6
ANS-PNS/N-Pg	84.8	2.5	90.6	2.9	88.3	2.1

TABLE 3 Results of Univariate t Tests* and Hotellings T^2 Comparing Major Clusters

Cluster Comparison	*B with C*	*B with D*	*C with D*
Variable	*P= or <*	*P= or <*	*P= or <*
N-Ans	.05	ns	ns
S-Go	ns	ns	ns
N-Ba	ns	ns	ns
S-N	ns	ns	ns
S-Ba	ns	ns	ns
S-PNS	ns	ns	.05
PNS-ANS	ns	ns	ns
Go-Pg	.0001	.01	ns
Ar-Go	.05	ns	ns
ANS-PNS/N-ANS	ns	ns	ns
ANS-PNS/N-Pg	.001	ns	ns
S/N/ANS	ns	ns	ns
S/N/Pg	.001	ns	.005
N/S/Ba	ns	ns	.05
N-Me	ns	ns	ns
ANS/Me	ns	ns	ns
Ar/Go/Me	ns	.05	ns
Hotellings T^2	.05	ns	ns

* Corrected for unequal variances when necessary.

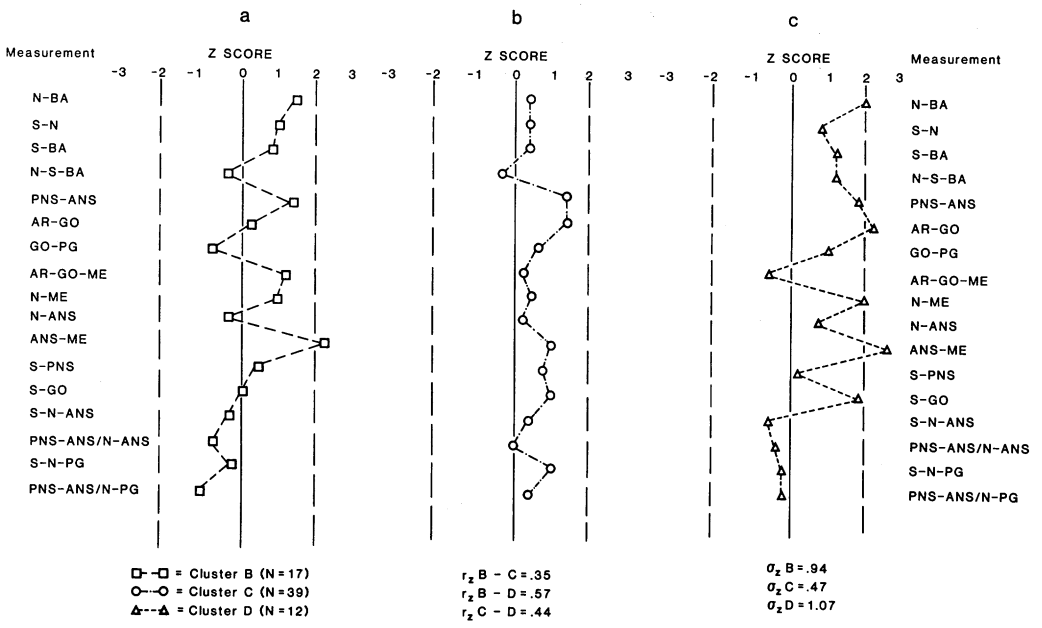

FIGURE 2 Mean pattern profiles of the three major clusters: (a) major cluster B; (b) major cluster C; (c) major cluster D. The zero baseline represents the population mean for the seventeen variables as reported by Saksena et al (1987). The value r_z is a measure of pattern similarity; 1.0 would represent a perfect correlation and 0 no correlation. The value $_z$ is a measure of pattern variability. In general, the lower this value the more normal the profile.

measurements in the three clusters that fall below the mean for the published norm). Such size differences would be consistent with a generalized populational difference in body size. In this regard it is worth noting that the normal data was collected in Philadelphia between 1948 and 1968 and has a heavy concentration of individuals with a Southern European ethnic heritage (Saksena et al, 1987). The parental data on the other hand was collected in Indiana in the mid 1970s and is comprised of individuals who are largely of Germanic or Western European ethnic background. One would suspect, therefore, that the Indiana sample would be comprised of larger individuals on the whole. Statistical tests for significant differences between published norms and cluster means were not attempted because these normal data had provided the regression equations (Saksena et al, 1983) used to adjust the raw data (to remove the effects of age and sex differences) and because only means and standard deviations were available in the published monograph (Saksena et al, 1987).

The measurements of pattern similarity (r_z) show only a moderate degree of concordance between the clusters. Not surprisingly, clusters B and D are the most similar ($r_z = .57$) and clusters B and C the least similar ($r_z = .35$). Hotelling's T^2 confirms this relationship. Hotelling's T^2 is essentially a multivariate test of significance that compares a pair of groups across a series of variables simultaneously (Timm, 1975). The null hypothesis is that overall pattern of variability across the 17 means is parallel for a given pair of clusters. Table 3 indicates that cluster B differs significantly from cluster C ($p<.05$). However, neither differs significantly from cluster D. These findings also support the view that cluster D shows characteristics that are in some way a combination of those found in the other two major clusters.

In an attempt to define the nature of the clusters further, all three were compared to an independent sample of individuals with overt clefts. It was reasoned that individuals with phenotypic features that predisposed them to produce children with clefts should show the most resemblance to such a cleft group. The cleft group consisted of 16 individuals with CL/P, all of whom had had surgical corrections (while an untreated group may have made for a more relevant comparison, such individuals are understandably hard to find). The 16 individuals were unrelated to the parental population. This was considered necessary to minimize the confounding factor of intrafamilial resemblance. Age and sex differences were removed by adjusting the data in the same fashion as was done with the parental sample (see above).

Table 4 shows the means and standard deviations for the 17 cephalometric variables in the CL/P group. Figures 3a through 3c show the mean pattern profile comparisons of the CL/P group to each of the major clusters. The similarity is most striking between cluster B and the cleft group (Fig. 3a). In fact, the pattern similarity coefficient (r_z) of .88 is higher than that between any pair of the major clusters. Cluster D also shows similarities to the cleft pattern, although not so strongly ($r_z = .68$). By contrast, cluster C shows the least similarity to the pattern profile ($r_z = .37$) of the cleft group.

The small size of the remaining clusters prevented the statistical analysis of their patterns. However, examination of Table 5 confirms the impression generated from Figure 1 that great variation exists between each of these groups, and between these groups and the two major clusters. In general, each tends to group around one or more variables with values more extreme than are seen in individuals from the major clusters. For example, the two individuals who comprise cluster A are characterized by an extreme palatal length (ANS-PNS) and a greatly reduced cranial base angle (N-S-Ba) compared to those seen in individuals from the

TABLE 4 Cephalometric Variables in the Cleft Lip and Palate or CL(P) Group

	CL(P)	*SD*
N-Ba	118.7	6.4
S-N	79.8	3.0
S-Ba	50.4	5.1
N-S-Ba	131.1	7.1
ANS-PNS	56.6	6.6
Ar-Go	54.6	6.4
Go-Pg	80.3	5.7
Ar-Go-Me	134.2	5.7
N-Me	135.0	8.1
N-ANS	56.1	4.5
ANS-Me	82.4	5.2
S-PNS	53.4	5.4
S-Go	85.8	8.0
S-N-ANS	85.4	5.7
ANS-PNS/N-ANS	91.4	8.5
S-N-Pg	77.7	4.9
ANS-PNS/N-Pg	88.1	7.2

major clusters or the published norms. Cluster G on the other hand is comprised of two individuals who share a greatly reduced cranial base, particularly in its anterior dimension. It is such unique features that prevent these "outlier" groups from fusing with each other or with the major clusters until the latter stages of the clustering process. Obviously, there are some extreme variations in facial bone size and position in these small groups. Nevertheless, about half of the outlier individuals share the flat facial profiles described for the major clusters B and D.

DISCUSSION

Hierarchical cluster analysis of parents of sporadic cases of CL/P demonstrates the presence of facial phenotypic heterogeneity within this sample. Specifically at least three groupings of parents were defined. Two of these (B and D) show wider deviations from published normal values than does the third (cluster C). This third group includes approximately half of the total sample (48 percent) and shows little pattern variability. The major difference between this latter group and the published norms seems to be in facial size, a finding that could be reasonably explained by a systematic body size difference between the population used for the normal standards (Philadelphia) and the parental sample (Indiana). In other words we propose that cluster C is comprised of individuals with large but otherwise normal craniofacial skeletons.

Clusters B and D on the other hand share an excessively large lower facial height and flat facial profile that clearly distinguishes them from the presumed normal group C (and from the published norms). While these two clusters also differ somewhat from each other in that cluster D is characterized by significantly larger mandibular measurements, both show strong similarities to the pattern profile of individuals with CL/P. We believe that this indicates that it is the parents in clusters B and D who carry the facial phenotypic risk factors associated with producing a child with CL/P.

It is also worth noting that it is unusual in these two groups for both members of a parental pair to fall into one of the "at risk" clusters. Thus, there is only one pair of parents in cluster B and only one in cluster D. By contrast, there are eight pairs of parents in cluster C. Of the 25 pairs of parents that are split between clusters, 16 have one member of the pair in the "phenotypically normal" group. These findings argue against pooling the contributions of both members of a parental pair when drawing contrasts between parents of children with clefts and normal control populations and may explain why previous studies have produced such contradictory results.

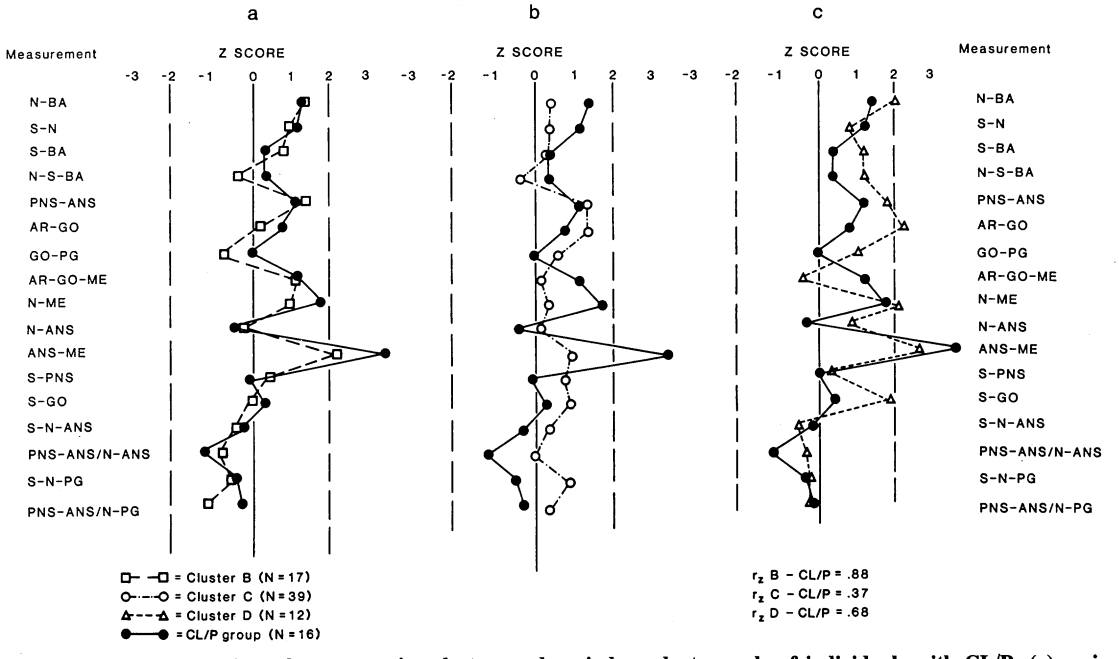

FIGURE 3 Pattern profile comparisons between major clusters and an independent sample of individuals with CL/P: (a) major cluster B compared to mean profile of cleft group; (b) major cluster C compared to cleft group; (c) major cluster D compared to cleft group. Cluster B shows the greatest similarity to the cleft profile.

TABLE 5 Values of Variables in "Outlier" Clusters

Variable	Cluster A (N=2)		Cluster E (N=1)		Cluster F (N=2)		Cluster G (N=1)		Cluster H (N=1)		Cluster I (N=2)		Cluster J (N=2)		Cluster K (N=1)		Cluster L (N=2)	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
N-Ba	113.3	3.2	118.3	2.5	116.0	—	119.6	—	113.6	—	113.1	6.4	114.5	2.8	99.0	—	110.3	4.7
S-N	76.7	0.4	80.2	1.7	76.0	—	82.1	—	74.4	—	75.1	3.9	77.0	0.7	66.5	—	71.7	1.1
S-Ba	54.3	1.7	50.3	0.3	51.0	—	51.0	—	49.1	—	45.6	3.5	47.0	0.7	44.5	—	50.5	1.5
N-S-Ba	119.3	3.2	129.0	0.0	132.0	—	126.5	—	130.0	—	137.3	1.1	134.5	3.5	126.0	—	128.7	7.4
ANS-PNS	61.7	6.0	57.6	0.1	53.0	—	58.9	—	55.7	—	51.3	1.5	60.3	0.4	49.5	—	60.9	4.1
Ar-Go	60.5	3.5	63.3	1.1	53.0	—	58.7	—	46.6	—	49.3	5.3	49.7	6.7	51.5	—	46.3	3.8
Go-Pg	82.5	5.7	88.7	6.1	90.0	—	81.2	—	72.7	—	73.5	1.8	73.0	2.1	70.5	—	81.4	1.3
Ar-Go-Me	136.0	0.0	125.5	1.4	121.0	—	151.0	—	128.0	—	113.3	1.8	140.0	1.4	130.0	—	128.3	1.1
N-Me	131.0	1.4	130.5	0.8	129.0	—	148.9	—	139.1	—	124.3	0.3	127.7	3.9	119.5	—	121.3	1.1
N-ANS	58.0	4.2	50.7	2.3	55.5	—	59.4	—	57.7	—	55.3	2.1	52.0	1.4	51.0	—	57.1	1.2
ANS-Me	75.0	6.3	79.9	1.5	74.0	—	91.3	—	85.4	—	70.4	0.7	78.5	2.8	70.0	—	68.2	3.8
S-PNS	60.3	0.3	55.7	1.8	53.0	—	56.0	—	54.0	—	52.3	3.9	46.7	2.5	49.5	—	52.0	2.1
S-Go	94.3	4.6	95.7	2.5	88.5	—	90.2	—	81.4	—	76.7	3.9	76.5	3.5	81.5	—	79.2	4.7
S-N-ANS	97.75	1.1	85.3	3.2	74.0	—	85.5	—	81.0	—	78.3	3.5	88.0	1.4	91.0	—	94.0	0.0
ANS-PNS/N-ANS	100.0	0.0	86.0	2.8	76.0	—	92.5	—	89.0	—	88.5	6.4	96.0	2.8	96.0	—	104.0	1.4
S-N-Pg	91.0	1.4	86.5	2.9	80.5	—	79.5	—	68.4	—	74.1	3.9	76.7	3.2	83.0	—	81.9	2.9
ANS-PNS/N-Pg	93.0	2.8	86.8	2.5	82.0	—	87.0	—	76.1	—	83.6	2.8	84.5	4.9	88.0	—	91.3	2.4

Most importantly, the results suggest that at least in some cases the predisposing factor(s) for CL/P is (are) being contributed by a single parent. Presuming a genetic basis for these phenotypic traits one could use such findings to argue against a multifactorial mode of inheritance in such cases. However, given the fact that so few sporadic cases later turn out to be familial (Bixler et al, 1971, reported that only 5 percent of such sporadic families eventually produce one or more additional cleft child), it is unlikely that such predisposing facial factors are the sole determinants of cleft susceptibility. Finally, the presence of several cases in which both parents share a normal phenotype suggests the possibility of a different etiology when compared to those cases in which one parent displays unusual craniofacial findings.

Conclusions

Additional studies are needed to determine whether or not similar cephalometric features can be identified among parents of familial CL/P cases and to determine if (and how) such features segregate among siblings of affected individuals. Similarly, parents and siblings of children with isolated cleft palate need to be investigated. Such studies should help to sort out the inheritance pattern of relevant cephalometric features and to determine if such features are useful in identifying individuals at greater risk for producing a child with a cleft. Such phenotypic forme frustes would have obvious utility in molecular linkage studies because it would identify potential carriers of a major gene for clefting in families with a known genetic predisposition to clefting (i.e., those families with multiple affected individuals). Indeed, it is only through such molecular studies that we are ever likely to be able to resolve the long standing debates over the etiology and inheritance of oral clefts and to provide meaningful genetic counselling to at-risk individuals.

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Commentary

The multivariate statistical approach described in this paper applies the method of cluster analysis to segregate parental cephalometric features. The premise is that unaffected parents of children with sporadic cleft lip/palate may have "unusual" or predisposing craniofacial features that distinguish them from the general population. To provide a biologically relevant basis for the application of cluster analysis to human facial features, an understanding of clinical nomenclature or biological classification is important.

Historically, the qualitative appraisal of the resemblance of organisms was subjective until numerical taxonomy revolutionalized this approach (Sokal and Sneath, 1963). In this method, as many distinguishing characteristics as possible are accumulated to give equal weight to each characteristic by evaluating similarities or differences numerically. This allows grouping of those individuals or organisms with the most characteristics in common. The availability of modern computer technology has facilitated the development of this method so that cluster analysis could be used to generate hypotheses concerning categories of structures based on aggregation and segregation. Cluster analysis covers a wide variety of techniques for delineating natural groups (clusters) into data sets. It is particularly useful in pattern recognition and the exploratory rather than explicatory analysis of large sets of numerical data. In the analysis of a data set, the choice among similarity measures, clustering criteria, and algorithms requires an intuitive approach and can be used to develop inductive generalizations. The operational objective in classification and discriminant analysis is to classify new observations whereas in cluster analysis little is known about the structure category. The object, therefore, is to discover a structural category that fits the observations or measurements and sorts them into groups in which the association is high among members of the same group. The application of cluster analysis to cephalometric variables was effectively used by Harris et al (1973) to identify the "Elder Lady." This female mummy was found in a cache of great kings and queens in 1898 in the Valley of the Kings in Egypt. By the use of cluster analysis the cephalometric features of each queen was compared with every other of the 10 queens in the Royal Mummy collection. Combinations of clustering algorithms and distance measures were used, which indicated two queens, linked at step one, as phenotypically having the most similar craniofacial morphology. Biochemical analysis of hair samples confirmed the findings from the cluster analysis, which ultimately resulted in the identification of Queen Tiye.

The authors hypothesize "that the results of a cluster analysis will support the presence of both phenotypic and etiologic heterogeneity among the parents of children with CL/P and that the identifiable risk factors will be unevenly distributed within parental pairs." The conclusion that many sporadic CL/P patients may have a genetic component that is derived from one of the parents introduces a provocative insight into the etiology of clefting. The multifactorial threshold model usually assumes that both parents contribute predisposing factors to the affected child without considering the possibility that one parent may contribute more to the susceptibillity for oral clefting than the other parent. If the facial features of noncleft relatives of individuals with CL/P can be attributed to a genetic susceptibility that predisposes to clefting, the possibility of identifying risk factors or indicators has important implications. To identify a risk factor implies a causal relationship that in human observational studies can make the case for causation strongly suggestive, but unproven. Because of the causal connotation, the term risk factor should be reserved only for those factors shown to be causal. Risk indicators or markers might therefore be preferable until the causal relationship is confirmed. By identifying an apparent high risk group, such as one or both parents of sporadic CL/P children, indicators become important for assessing and targeting populations or groups to elucidate risk factors (Beck, 1989).

The authors of this cephalometric study have attempted to group "individuals at greater risk for producing a child with a cleft." The phenotypic expression of an inheritance pattern that has utility in identifying potential carriers of a major gene for clefting in families has important implications in molecular linkage studies. The report by Moore et al (1987) and Björnsson et al (1989) indicated the X-linked mode of inheritance of cleft palate and ankyloglossia in an Icelandic family. The recognition of putative risk factors necessitates observational studies in humans and is limited by finding associations, but not causality. By utilizing recombinant DNA technology and linkage analysis or cosegregation with markers, the existence of genetic factors in the etiopathogenesis of clefting may provide clues to causality.

The interdisciplinary approach of the molecular geneticist collaborating with clinicians and craniofacial teams provides the technology for the localization of genes. With such a precise genetic identification, a clearer understand-

ing of the pathogenesis should become available. The international biomedical research community is focused on decoding the human genome so that molecular determinants for normal and abnormal craniofacial morphogenesis may well become common knowledge by the next century. The broad implications of what could or should be done with this knowledge, both at the biomedical and societal level, involve ethical and moral questions that so far have not been resolved.

Certainly, the bridging of domains among clinicians, methodologists, and the biological sciences in determining causality of birth defects converge in a mutual interest in risk assessment that is predicated on genetic predisposition. The authors have provided new insights into identifying predisposing characteristics for clefting in craniofacial morphology. The implications that this may have to genetic counseling and our understanding of inheritance patterns and causality may suggest that prospective data and previous data sets are interpreted differently in the future. The authors are to be congratulated for their rigorous work.

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