CEPHALOMETRIC SIMILARITY AMONG PARENTS OF INDIVIDUALS WITH SPORADIC ISOLATED CLEFT PALATE: IS THERE EVIDENCE FOR AN INHERITED PREDISPOSITION?

by

Edward M. Sammons

Submitted to the Graduate Faculty of the School of Dentistry in partial fulfillment of the requirements for the degree of Master of Science in Dentistry, Indiana University School of Dentistry, 1999.

Thesis accepted by the faculty of the Indiana University School of Dentistry, in partial fulfillment of the requirements for the degree of Master of Science in Dentistry.

James K. Hartsfield, Jr.

David R. Avery

Ronald R. Hathaway

Lawrence P. Garetto

Richard E. Ward
Chairman of the Committee

Date_____

ACKNOWLEDGMENTS

I would like to thank first and foremost Dr. Richard E. Ward for his continued patience and understanding throughout his tenure as chairman of my thesis committee. His commitment and caring gave me a deep respect for his knowledge and teaching.

I would also like to thank Dr. James K. Hartsfield, Jr. for his assistance on my thesis. His efforts and thoughts on my thesis and help with computer digitization are greatly appreciated.

Special thanks are extended towards the rest of the committee for their help in conducting the research involved with the thesis: Drs. Ronald R. Hathaway, David R. Avery, and Lawrence P. Garetto.

This thesis is dedicated to my wife, Christine, and to my parents James and Mary Jane Sammons. My parents have supported me throughout the years and encouraged me to always excel and persevere in all of life. My dearest Christine has been an angel in this process and has never complained and always given me strength. Thank you.

I am grateful to all my colleagues over the past few years. Special thanks to my fellow residents in Pediatric Dentistry, Drs. Brad Fulkerson and Vickie Hemann, and in Orthodontics, Drs. David Albright, Brent Callegari, Bo Young Park, Sarandeep Huja, and Fernando Martinez. These people have helped me through a long and difficult time and I appreciate it greatly.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

INTRODUCTION

The most commonly accepted genetic model for the etiology of cleft lip with or without cleft palate [CL(P)] and isolated cleft palate (CP) is multifactorial inheritance.[1-6] However, there is evidence supporting autosomal dominant and autosomal recessive inheritance suggesting the influence of major genes in clefting.[7-11] Still, other studies have proposed polygenic models with many loci determining oral cleft development with less of an environmental influence.[12,13] These studies have challenged the former model. For example, individuals other than the proband in families have been shown to have certain craniofacial characteristics that separate them from the normal population.[14] However, little investigation has been done in attempting to define similar distinguishing features in parents of sporadic CP.

The hypothesis of this study is that there will exist cephalometric differences between reference populations and parents of CP individuals. If such features can be demonstrated, it would suggest that these individuals have a phenotypic facial pattern associated with an increased risk for cleft palate. In addition, comparisons of these findings will be made to those presented in the literature for both CP and CL(P) in an attempt to clarify this often confusing and sometimes contradictory previous research.

REVIEW OF LITERATURE

PALATAL DEVELOPMENT

Differences in incidence of CP exist by gender and population. However, debate persists on the precise impact of such factors. Cleft palate occurs approximately in one in every twenty-five hundred births. Nearly all studies have concluded that there is a 2:1 female: male predilection for isolated cleft palate.[5] However, a report by Christensen et al.[15] shows a slight male predilection, 1:1.1 female: male.

Isolated palatal clefts present with variable expressivity. They may be submucosal, limited to the uvula (bifid uvula) or may be more extensive, cleaving the hard palate and the soft palate. A combination of cleft lip and palate (CLP) is most common. Rough estimates place CLP as the most common followed by CP then CL. Palatal clefts alone account for almost one-third of all clefts of the oral structures, representing one of the more commonly recognized congenital anomalies, second only to clubfoot.[16]

The development of the primary and secondary palate is a complex sequence of events that is dependent on the integral neural crest cell. Neural crest cells migrate from the embryonic neural fold to form almost all of the connective and skeletal tissues of the face. This includes all dental tissues, except enamel, cartilage, fibrous connective tissue, and bone.[16]

Development of the primary palate occurs from the merging of adjacent medial nasal placodes. This is termed the intermaxillary segment. Development is set in motion by the medial growth of the maxillary process. This forms the median of the upper lip

(philtrum) and the palatal triangular section that are destined to give rise to the four maxillary incisor teeth. The posterior aspect is the incisive foramen.[16]

Posterior to the primary palate is the location of the secondary palate, which is nine times the size of the former. The formation of the secondary palate results from the fusion of the shelves formed from each maxillary process. The closure of the palate involves an intrinsic force in the palatal shelves whose process is complicated and uncertain.[16] Fusion of the palatine shelves occurs after elimination of their epithelial covering. As the two palatine shelves merge there is adhesion of the epithelium and a midline is evident with indistinguishable epithelium. This fusion happens because DNA synthesis ceases between 24 to 36 hours before epithelial contact.[17] Because of the complexity and the numerous processes needed for completion of the palate it is to be expected that many forms of interference can cause clefting.

MECHANISMS OF CLEFT PALATE PRODUCTION

A review of the palatal closure process demonstrates some of the possible ways in which it could fail. Palatal closure can be thought of essentially as the intrinsic shelf force overcoming the resistance of the tongue and meeting in the midline. Thus a cleft could result if the resistance of the tongue was increased by mechanical stresses on the embryo following amniotic puncture. Also, genetic factors associated with a wider head and face might present a hurdle greater than the palatine shelves' ability to meet at the midline. Alternatively, the shelves might be too narrow to meet in the midline. Because of the variability present in nature, there is variation in the time at which the shelves

elevate in different embryos. If one plotted the time that the shelves came up in a large group of embryos one would expect to obtain a roughly normal frequency distribution.[18]

The point of fusion is considered the threshold, dividing the continuous distribution of shelf movement timing into two discontinuous portions; on one side normal palate development, the other depicting cleft production. Gruneberg[19] in 1952 called this threshold distribution "quasi-continuous variation." Increasing head width or decreasing shelf width would shift the threshold to the left on the multifactorial liability curve. Other genetic and environmental factors could also influence these interrelationships.

For years researchers have known of several environmental factors that can induce cleft palate. One environmental example is maternal smoking during pregnancy. Recently, in 1997, Kallen[20] investigated this association and reported statistically significant findings. An odds ratio (OR) of 1.29 (95 percent confidence interval: 1.08-1.54) was found for isolated cleft palate. The OR can be defined as the frequency with which an event (CP) occurs divided by the frequency with which it does not occur. This study utilized the largest series of oral cleft cases ever studied to date and found an OR greater for CP than for CL(P). These results indicate maternal cigarette smoking during pregnancy is associated with an increased risk for CL(P) and more so with CP. Werler et al.[21] reviewed many studies involving oral cleft prevalence, including CP, and maternal smoking. She found a lack of consistency among studies but theorized that underlying interactions between genetic factors and maternal smoking may be a possible explanation of CP production. Numerous teratogens other than cigarette smoking have been linked to increased frequency of CP. Cortisone and phenytoin can in some cases produce palatal

anomalies. Single administrations of cortisone or phenytoin to pregnant mice during days 11-14 of gestation caused reduction in fetal weight. McDevitt et al.[22] concluded from this study that a correlation between fetal weight reduction and CP incidence was evident for each drug.

ETIOLOGY

Categories

Cleft palate may be divided into two major categories: syndromic, in which the cleft is one of several congenital anomalies that appear in a non-random pattern and an isolated form in which the cleft is the only apparent major birth defect. The focus of this study will be on isolated (nonsyndromic) CP. Previous research has assumed that absence of familial occurrence indicates that the majority of nonsyndromic cases are sporadic in nature.[23]

Relation of Cleft Palate to Cleft Lip and Palate

Clefts of the primary palate and lip are both developmentally and genetically different than clefts of the secondary palate. Siblings of patients with CL(P) have an increased frequency of CL(P), but not CP. Likewise, siblings of patients of CP have an increased frequency of CP but not of CL. However, other studies have shown different results.[5] Rank and Thomson[24] found that probands with CL(P) had an increased frequency of CP. Nevertheless, given the evidence from the majority of studies it is reasonable that isolated (i.e., nonsyndromic) CP and nonsyndromic CL(P) are

etiologically distinct entities and comparisons drawn from one must be applied with caution to the other.

Evidence for a Genetic Link in Oral Clefts

The majority of oral cleft research has been on CL(P) and not isolated CP. Reviewing the studies done on CL(P) first will give background and an understanding into the less well studied genetics of CP. Recent evidence in CL(P) research suggests that the presence of one or more major gene locus (loci) conferring susceptibility to facial clefting may be present. A major gene locus located on chromosome 6 has been reported by Eiberg et al.[25] In a study by Beiraghi et al.[26] a five generation family was analyzed by short tandem repeat polymorphisms in genomic DNA linkage. Resulting data supported major gene association with nonsyndromic CLP, specifically located on the q arm of chromosome 4. Conclusive linkage could not be made, however, due to small population size and reduced penetrance observed (65 percent). Penetrance was measured from maximum likelihood methods since penetrance in the family was not 100 percent.

Studies have reported a significant association between the transforming growth factor-alpha (TGFA) locus and CL(P). Ardinger et al.[27] found an association between the TGFA locus and adjacent DNA sequences that may affect the development of a significant number of cases of CLP. Chenevix et al.[28] reported similar findings with CL(P) and Sassani et al.[29] found TGFA locus associations for the occurrence of CL. Hecht et al.[30] on the other hand, did not find linkage of the phenotype with TGFA or other markers in families they studied with CLP. Vintiner et al.[31] agreed with Hecht for families with autosomal dominant inheritance of CL(P). They studied eight families with

CL(P) inherited in an autosomal dominant manner. Their study found no linkage to TGFA. Inspite of some confusing and contradictory reports, evidence is accumulating to support the idea that, at least in some cases, single genes may be involved in susceptibility to CL(P).

Some of the first CP studies were by Fogh-Anderson[32] in 1942. He analyzed isolated CP individuals and their families and concluded that the mode of inheritance was dominant with greatly reduced penetrance. Marazita et al.[33] concluded that CP was not of multifactorial inheritance but included an autosomal major locus and multifactorial contributions. Melnick et al.[7] again refuted the multifactorial threshold inheritance but found an autosomal recessive inheritance pattern. Rollnick et al.[34] found that out of three families studied, one was autosomal dominant in transmission and the other two were X-linked recessive. More recent literature from Fitzpatrick and Farrell[12] in 1993 suggests a polygenic model with six loci for isolated CP. Also, Christensen and Mitchell[35] found multiplicative interactions between CP susceptible loci.

FACIAL DISTINCTION/ PHENOTYPE: FURTHER EVIDENCE FOR AN INHERITED PREDISPOSITION

The finding of a consistent facial phenotype in relatives of individuals with isolated clefts could support the presence of a major gene locus for susceptibility. While several studies have attempted to identify common craniofacial characteristics amongst relatives of CLP individuals, fewer studies have been done on relatives of CP cases. A review of the phenotypic evidence for genetic predisposition in both conditions provides insight on the possible link between facial development, facial shape and the relative risk

for oral clefts.

Among the earliest such studies related to CL(P) was that of Trasler[36] in 1968 who used a mouse strain (A/J) that had known susceptibility to CL and compared the early face embryology to that of another strain which never developed CL (C57BL/6J strain). It was found that one of the genetic factors involved influenced the shape of the face at the same time as formation of the lip. Facial morphology and growth differed between strains. Just before, and when the adjacent epithelia of the medial and lateral nasal processes meet and fuse at the posterior end of the nasal pit, the medial nasal processes of the A/J embryos were more prominent, diverged less and were more medially placed than these in the C57BL/6J embryos. At this juncture they postulated that these differences are causally related to the predisposition to CL in the A/J strain. The medial nasal processes do not diverge laterally as much as they do in an embryo that is not predisposed to clefting. This lack of divergence may result in decrease or failure of the epithelial fusion between the medial and lateral nasal processes and consequently a lack of consolidation at the isthmus (between nasal processes). Failure of fusion is followed by breakdown of the isthmus leading to complete CL, with complete separation of medial from lateral nasal and maxillary processes. Decreased fusion can result in varying degrees of bridging of the gap, ranging from Simonart's bands (shreds of tissue across nostril base) to an almost complete lip with broadened medial raphe and small hole in the primary palate.[37] The early facial development and morphology of the mouse embryo and human are alike and furthermore at the time of lip formation the critical areas involved are of comparable relative size.[38,39] This means it is possible that there is a class

of CL in humans which would appear to have an early morphogenesis and etiology similar to that of the A/J mouse.[40-43]

Juriloff and Trasler[44] in 1976 summarized attempts that had been made since Trasler's 1968 study to test the "face-shape" hypothesis in humans by comparing the morphology of the face of relatives and nonrelatives of human beings with CL. These studies assumed that differences in embryonic facial shape had a genetic component and that it would be present in some form in the parents of affected individuals during growth and development. Juriloff and Trasler noted little agreement among these studies.[45-47] They suggested that variance in measurement technique and methodology made comparison difficult. Juriloff and Trasler tested the usefulness of the measurement methods and new ones being developed at the time to test the predictions of the face-shape hypothesis in 3 lines of mice: L, M and C. Line L was observed to have a statistically significant difference ($p < 0.001$) compared to lines M and C with smaller inter-nasal pit distance, although difference in overall head size. One other aspect (not statistically significant) was a tendency towards reduced angle between the medial nasal processes. Of the 3 new genotypes (L, M, C) a closeness of the nasal pits and a possible lack of divergence of medial nasal placodes were shown in CL-liable genotype line L. The results were predicted by the face-shape hypothesis.

Trasler and Machado[48] studied embryonic face shape in mice in an attempt to determine if characteristics of newborn and adult face shape could be identified that were predictive of risk for clefts. A CL predisposition was found to be correlated with face shape. The CL lines could best be distinguished from non-CL lines by a short premaxilla length (PL). This held for the newborn and adult skeletal measures as well as for the

adult soft tissue PL measurement. Also, when PL was combined with three other variables (LN = length of nasal bones, ID = interorbital distance, PW = width at widest part of premaxilla), non-CL and CL lines had 100% separation using discriminant analysis. The authors then concluded that a facial complex of variables associated with CL predisposition had been found.

Nakasima et al.[49] extended previous studies in humans by utilizing cephalometric data from parents of cleft children, including those with isolated cleft palate. They studied three groups: parents of CLP children, parents of CL children, and parents of CP children. Facial characteristics were analyzed by frontal and lateral roentgenographic cephalograms. Parents of CLP, CP, and CL children showed a significantly shorter upper anterior facial height (N-ANS) and upper posterior facial height (U-PNS) compared to lower anterior facial height (ANS-M) and lower posterior facial height (Ar-Go) from the lateral view compared to controls. Shorter anterioposterior length of cranium and maxilla and a greater cranial base angle were noted in parents of CLP and CL children. From the frontal view, facial characteristics common to parents of CLP, CL, and CP children were: significantly narrower maximum head width (MHW- measured at cranium level; Euryon Left to Euryon Right) and smaller cephalic index (MHW/MHLx100) compared to controls. In addition, they had greater ratios of the following measurements to the MHW: interorbital width (OW), interzygomaticofrontal suture dimension (FW), and nasal (NW), bizygomatic (ZW), and alveolar widths (AW). The ratio of each width measurement in the upper face to the total facial height was also significantly larger for parents of CLP and CL children than for control subjects. Minor differences were observed for this ratio between parents of CP children and the controls with CP parents being slightly but not

significantly larger. Interpretation leads to a suggestion that relative to cranium width (MHW), the individuals have wide faces. For example, interorbital width to maximum head width (OW/MHW) was larger. In the data of Nakasima et al. the MHW was significantly smaller than the controls. Orbital width in and of itself is not significant. However, when divided by MHW for the ratio, the ratio is larger. This again suggests that for these individuals the ratios aforementioned and noted on Table VII are larger, meaning that these persons had wide facial features relative to the present reduced MHW. Thus, there is compelling if sometimes contradictory evidence that facial shape correlates with risk for having children with CL(P) and to a lesser extent with isolated CP. This lack of consistency in these results probably reflects the difference in methodology and study populations used by the respective researchers. It should also be realized that in the Nakasima et al. study a multifactorial threshold model was assumed because they used a mid-parental average for his data analysis.

Ward et al.[50] addressed the methodological problems in these earlier studies by first using multivariate cluster analysis to sort the parents of CL(P) individuals into groups. They demonstrated that there were distinctly different phenotypic patterns among their parents. While the majority of the parents showed no significant difference with controls, many (52 percent of parents) showed a set of cephalometric features that were highly correlated ($r = 0.88$) to those in individuals with overt clefts of the lip and palate. When this pattern was present the majority of the time (94 percent) one member of the parental pair tended to show it, while the other parent was more likely to resemble the normal controls. This suggested both phenotypic heterogeneity among the parents and a substantial genetic component contribute in the underlying facial morphology

predisposing to CL(P). In a more recent study, Ward et al.[14] identified a similar pattern of cephalometric features in suspected gene carriers amongst a large family with several generations expressing CLP. This again indicates the potential for major gene influence on facial morphology and clefting.

In a recent study by Mossey et al.[51] discriminant analysis among variables could segregate between unaffected parents of CL(P) and CP individuals. The differences were; longer mandibular and ramus length in CP parents, larger mandibular and cranial area in the CP parental group. This study utilized LA cephalograms alone and focused on parents only. They did not attempt to differentiate patterns of expression within the groups.

The documentation of significant differences between parents of CP and CL(P) cases and the fact that CP and CL(P) appear to be separate entities justifies the separate analysis of the CP groups as proposed in this study. Heterogeneity amongst parents must be examined. Statistical analysis of the parents allows gender to display the possibility of phenotypic heterogeneity.

In spite of the fact that cleft palate research has been less emphasized compared to CL(P) research there are likely to be a variety of environmental and genetic causes for sporadic cases.[52] Cleft palate, in general has been thought to reflect a multifactorial threshold model. From this concept, individuals in a population who fall beyond the threshold are thus affected and have more predisposing genes than the remaining population.[53] However, as noted previously other studies have suggested different Mendelian or polygenic models. The focus of this study will be on sporadic cases

because they allow the most efficient testing of the hypothesis, since family members have been presumed to be "non-affected."

MATERIALS AND METHODS

SUBJECTS

The study consisted of a database of previously collected posterior-anterior (PA) and lateral (LA) cephalographs on thirty individuals, from fifteen families comprising parents and siblings of sporadic cases presenting with isolated CP. Syndromic forms of CP had been excluded by way of a careful medical and dental history and exam in previous studies. As stated above, sporadic cases were used because it is the most common occurrence for isolated CP and first-degree relatives are supposedly unaffected.

Edentulous persons not in possession of fabricated removable prostheses were excluded for reasons of indeterminant jaw relations and poor definition of occlusal plane. The remaining adult population consisted of 30 individuals; fifteen parental pairs with offspring of isolated CP. Data from affected individuals were not available for analysis and not the subject of this study. Standardization via z-scores was done using reference norms published by Saksena.[54,55]

VARIABLES

A total of 60 (30 LA, 30 PA) cephalometric headplates were analyzed. The author traced all 30 LA headplates while using previously traced PA headplates for this project.

Since the present study design parallels that described by Ward et al.[14] we used the same set of (17) LA and (25) PA cephalometric variables (Tables I, II). These forty-two measurements were originally chosen to cover three portions of facial

morphology; upper, middle, and lower in three dimensions as well as to assess the size of the orbits, nasal cavity, and mandible while eliminating redundant or unreliable measurements as determined by poor landmark recognition. Cephalometric drawings illustrate the points studied (Figures I, II).

Each LA headplate was initially hand-traced and then entered into a Numonics computer digitizer (Model IPS/ TL.A, Montgomeryville, PA.). A custom designed software package "Dentofacial Planner- Version 6.2" made by Dentofacial Software (Toronto, Canada) computed the results. An analysis was created specifically for this project utilizing only relevant points, lines and angles.[56] All cephalometric tracings including PA data were made and or evaluated by the author and confirmed by an experienced observer to narrow error in measurements and landmark identification.

STATISTICAL ANALYSIS

After digitizing, the measurements were converted to z-scores by subtracting each from its appropriate age and sex matched reference mean and dividing by the reference standard deviation. Because z-scores produce quotients that are recorded in standard deviation units this treatment removed variation due to gender and age, making comparison across these categories possible. The data base used for reference norms were from Saksena.[54,55] The Statistical Package for the Social Sciences (SPSS) Version 2.5 for personal computers was used for this and all subsequent analysis.[57] Univariate analysis included t-tests between the reference means and the sample means for each variable. In this design it is assumed that the mean of a set of "z-scores" from a sample will be zero if the sample is representative of the reference population (the mean of a set

of z-scores from a normally distributed population is by definition zero). These univariate results were then compared to those reported by other investigators.

Multivariate analyses were conducted to further explore the data and in particular to investigate the possibility that phenotypically distinct subgroups could be defined within the parental sample. Because multivariate techniques are generally sensitive to large discrepancies between (small) sample size and (large) number of variables, factor analysis was used to reduce the forty-two variables into a smaller number. Factor analysis seeks to define combinations of intercorrelated variables that represent a presumed underlying factor so that a large portion of the total variation in the sample can be reduced to a smaller number of such factors.[58] Factor scores can then be used in place of the original variables where reduction in variable number is desirable.

After variable reduction, the sample was analyzed using hierarchical cluster analysis. This multivariate technique seeks to identify groups of individuals in the sample who share similar characteristics. Euclidean distance was used to measure pair-wise similarity between individuals and the Ward method[59] of sorting was used to produce clusters. A dendogram was produced representing the history of the iterative pairing beginning with the two most similar individuals in the sample at step 1 and concluding with an all inclusive cluster at step 29. The dendogram reveals groupings of individuals with similar factor scores. For the present study only the most distinct groupings were analyzed. Thus, the technique tests the sample for homogeneity. It is presumed that a population of unrelated and otherwise normal individuals should not include large subgroups with distinctly different measurement values. After clusters were

identified paired t-tests were used to compare the original z-scored variables between the clusters. This illuminated those features which differentiated the respective clusters.

In addition, pattern profiles were produced for each cluster.[60] These are graphic representations of the z-scored variables arranged by anatomical area and type of measurement. The mean z-score for each variable in each cluster is plotted against the expected population value (always zero). Thus, the degree to which the cluster mean for a given variable departs from the reference mean is depicted in negative or positive mean z-score values around the zero baseline. Combining multiple variables in the pattern profile (e.g., all the LA variables, all PA variables) provided a graphic representation of the overall pattern of the cluster.

RESULTS

TOTAL PARENT SAMPLE COMPARED WITH REFERENCE MEANS

Our hypothesis proposed that we would find differences between normals and parents of sporadic CP individuals. The results support this hypothesis. They demonstrate that overall, several of the variables from our study have means that are significantly different from the reference means (Table III, Table IV). Table III illustrates the means of the LA and PA variables for the parents of affected individuals and Table IV illustrates the p values for each variable. A pattern profile is presented in Figure 4 illustrating this divergence from the reference norms. Significantly different means ($p<0.05$) for lateral cephalometric variables included: N-ANS (upper anterior facial height), ANS-Me (lower anterior facial height), S-Ba (posterior cranial base), PNS-ANS (palatal length), ANS-PNS/N-ANS (upper facial angle), ANS-PNS/N-Pg (lower facial angle), S-N-Pg (mandible position relative to cranial base), N-S-Ba (cranial base flexure), Ar-Go-Me (mandibular angle). These significant variables are illustrated in Figure 5 (linear variables smaller than reference norms), Figure 6 (linear variables larger than reference norms), Figure 7 (angular variables smaller than reference norms), and Figure 8 (angular variables larger than reference norms).

Significant differences for frontal (PA) cephalometric variables were: MoL-MoR (orbital width measured at medial walls), ZyL-ZyR (zygomatic width), GoL-GoR (gonial width-mandibular breadth), GoNL-GoNR (gonial notch width), CRO-CNS (middle facial height), CNS-SD (vertical maxillary anterior height), ID-Me (vertical mandibular anterior height), NSR-NCR (unilateral nasal width - frontal), MxR-ZyR (zygomatic maxillary

distance), Me-GoR (mandibular corpus length-frontal). These significant variables are illustrated in Figure 9 (variables smaller than reference norms) and Figure 10 (variables larger than reference norms).

From a lateral perspective these results indicate that taken as a group, study individuals had longer lower faces, shorter upper faces, longer palatal lengths, more closed facial angles, a more obtuse cranial base angle, more retrusive mandibles and larger mandibular angles compared to reference norms. Frontal analysis demonstrated larger than normal orbital width and larger nasal width as well as smaller than normal measurements for zygomatic, gonial and gonial notch (antegonial) widths.

FACTOR ANALYSIS AND COMPARISONS OF TWO SUBGROUPS

Factor analysis collapsed the total 42 variables into 12 factors that can be interpreted as described in Table V. Using the twelve factors in conjunction with hierarchical cluster analysis two major clusters were identified (Table VI, Figure 3). These clusters are the two most distinct groupings in the sample. That is, individuals in Cluster 1 are more like one another than they are to individuals in Cluster 2. There are additional subgroups within each of the two clusters but these were not investigated for this project. Table VI illustrates the means of both clusters and Figure 11 displays these means in the form of pattern profiles of corresponding clusters. Cluster 2 is characterized relative to Cluster 1 by having longer lower facial height (ANS-Me), longer mandibular ramus length (Ar-Go), longer palatal length (PNS-ANS), less mandibular breadth (GoNL-GoNR), and a longer mid-face (CRO-CNS). Cluster 1 is distinguished from Cluster 2 by a more anterior chin position relative to cranial base (S-N-Pg), smaller facial

height (N-Me), smaller posterior facial height (S-Go), smaller cranial base angle (N-S-Ba), larger mandibular angle (Ar-Go-Me), shorter vertical maxillary height (CNS-SD) and shorter lateral mid-facial height (MxR-ZyR). Table VI highlights variables with mean difference between Cluster 1 and Cluster 2. These significant variables are illustrated in figures 12 (LA data), and 13 (PA data).

Significance values of $p < 0.05$ were obtained for several variables. These were: N-Me, ANS-Me, S-Go, PNS-ANS, Ar-Go, S-N-Pg, CRO-CNS, CNS-SD, MxR-ZyR. In addition, gender differed significantly between the two clusters. Cluster 1 consisted of 12 individuals, 9 of whom (75 percent) were female. Cluster 2 consisted of 18 individuals, only 6 (34 percent) of whom were female.

FIGURES AND TABLES

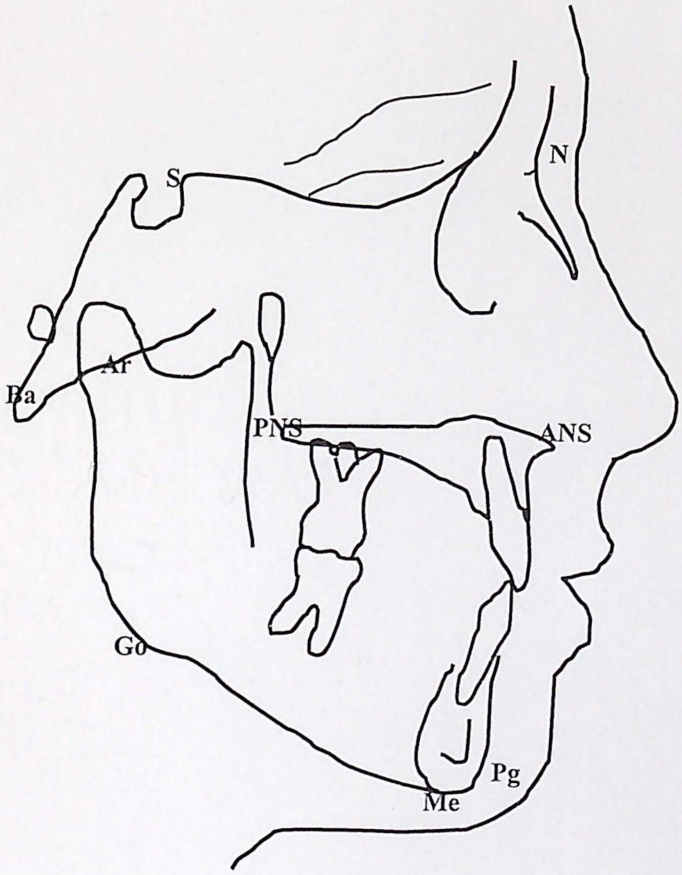


FIGURE 1. Cephalometric landmarks used to define 17 LA variables.

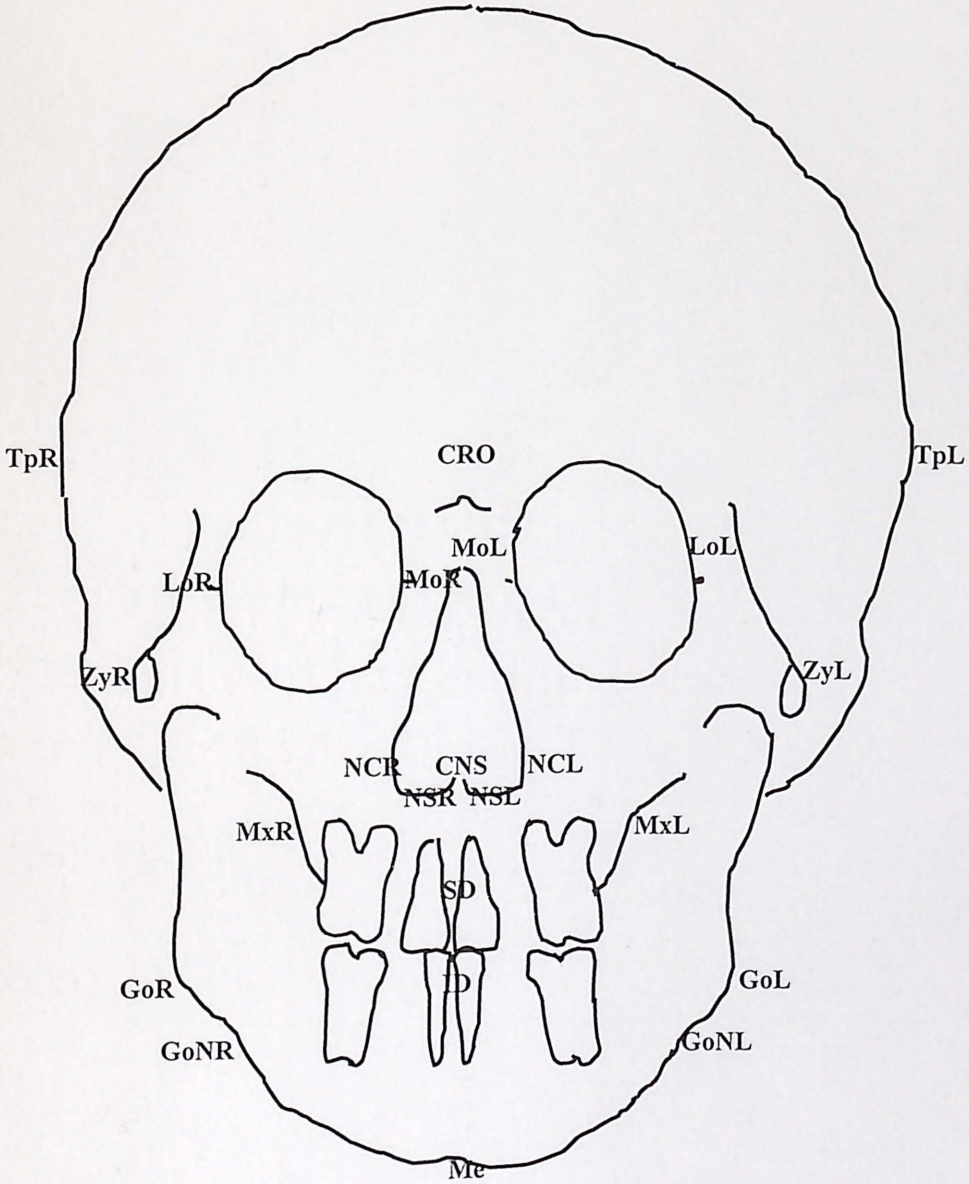


FIGURE 2. Cephalometric landmarks used to define 25 PA variables.

*Case i.d. numbers are followed with 01 indicating father (male) and 02 indicating mother (female).

FIGURE 3. Dendogram (cluster analysis).

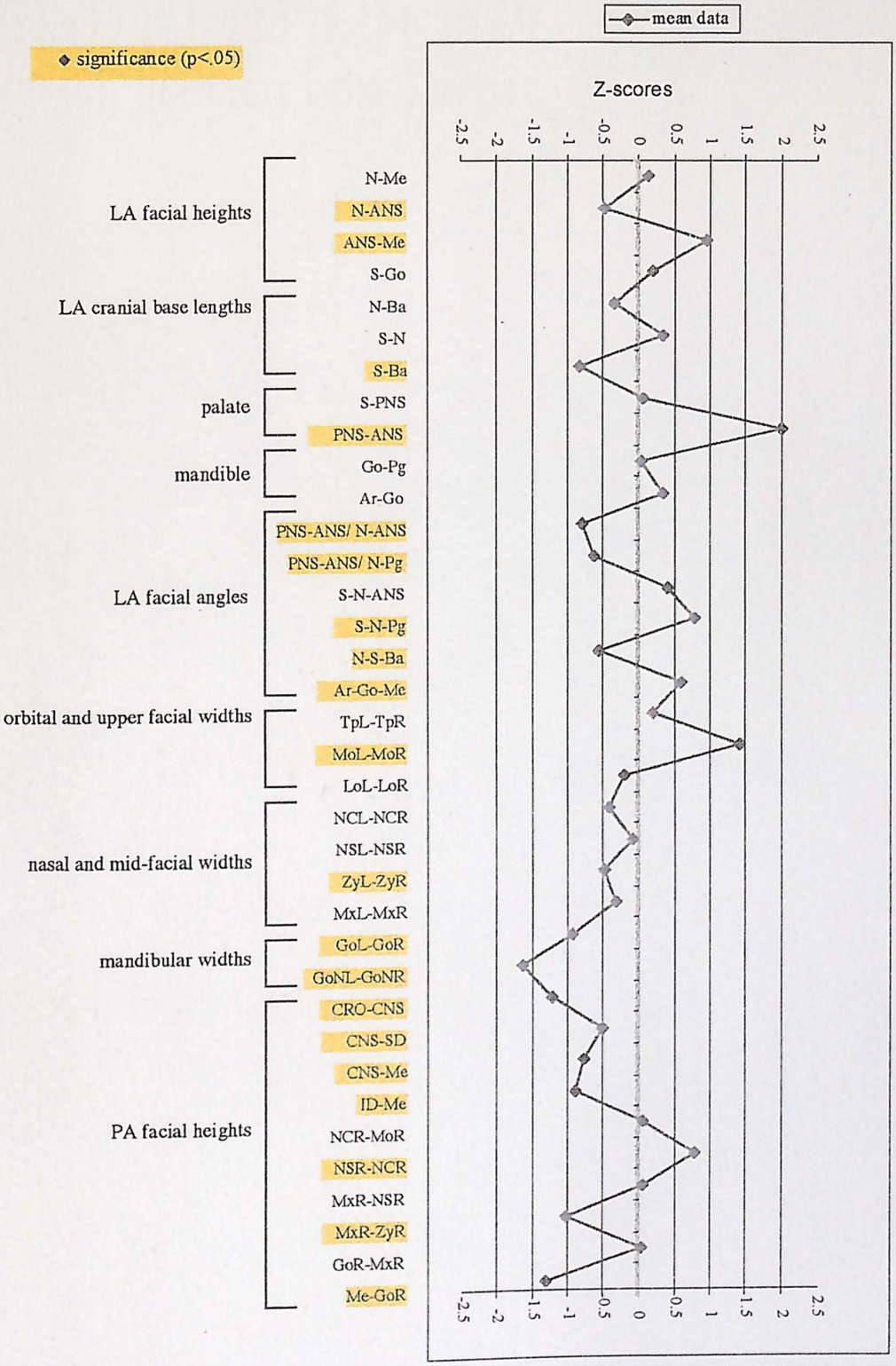
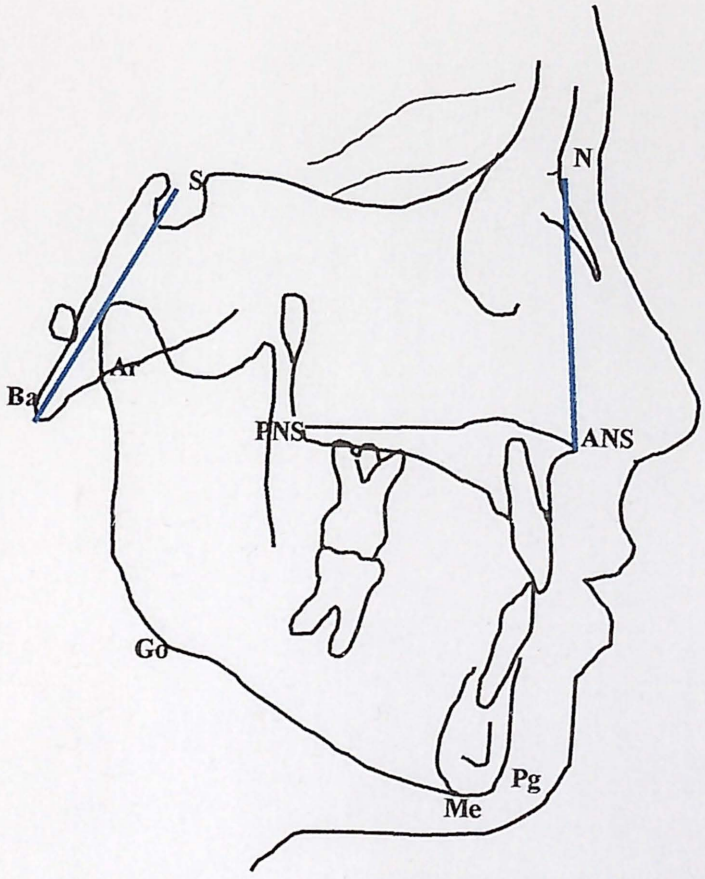
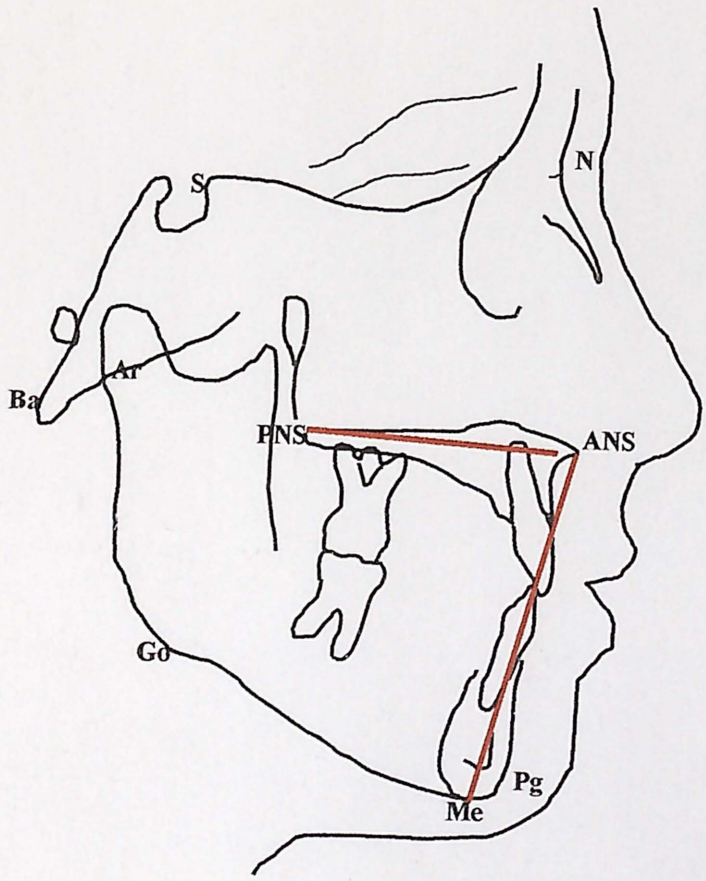


FIGURE 4. Pattern profile of mean z-scores of LA and PA variables for total sample of parents (n = 30).



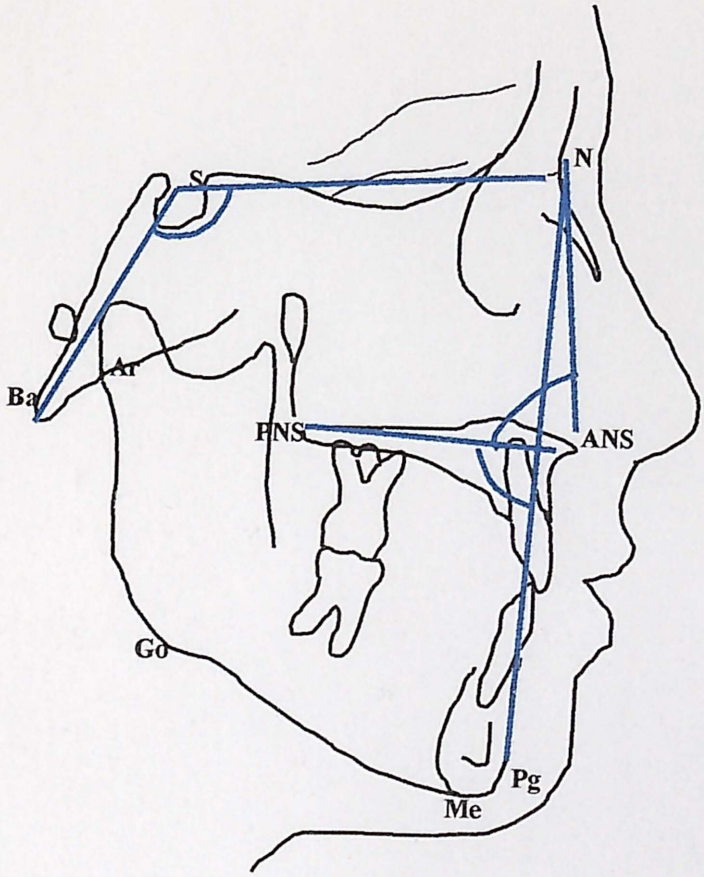
*included variables: N-ANS, S-Ba

FIGURE 5. Linear measurements that were significantly ($p < 0.05$) smaller than reference norms- LA cephalometric variables.



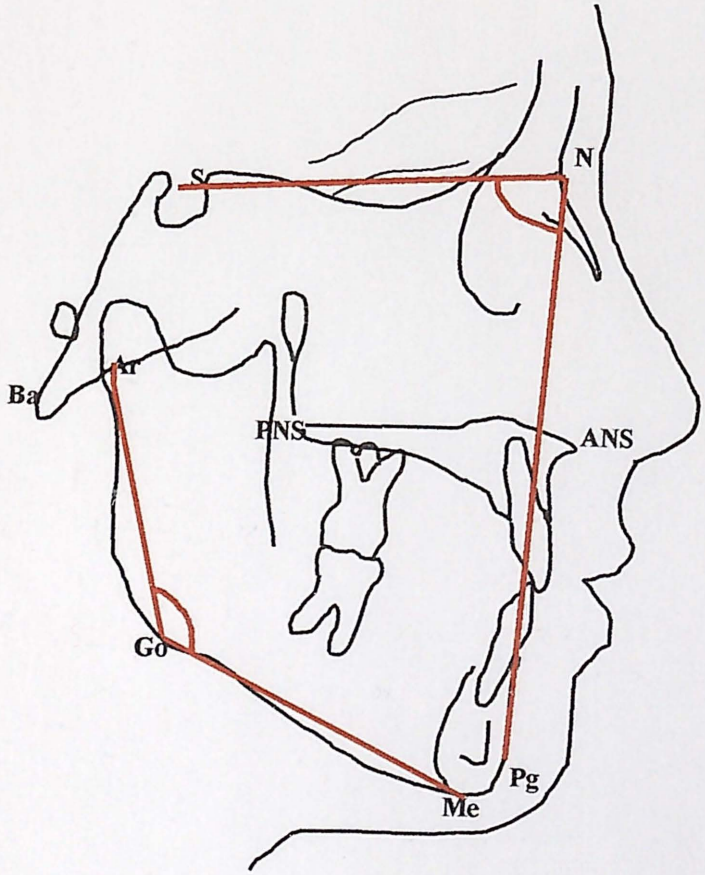
*included variables: ANS-Me, PNS-ANS

FIGURE 6. Linear measurements that were significantly ($p < 0.05$) larger than reference norms- LA cephalometric variables.



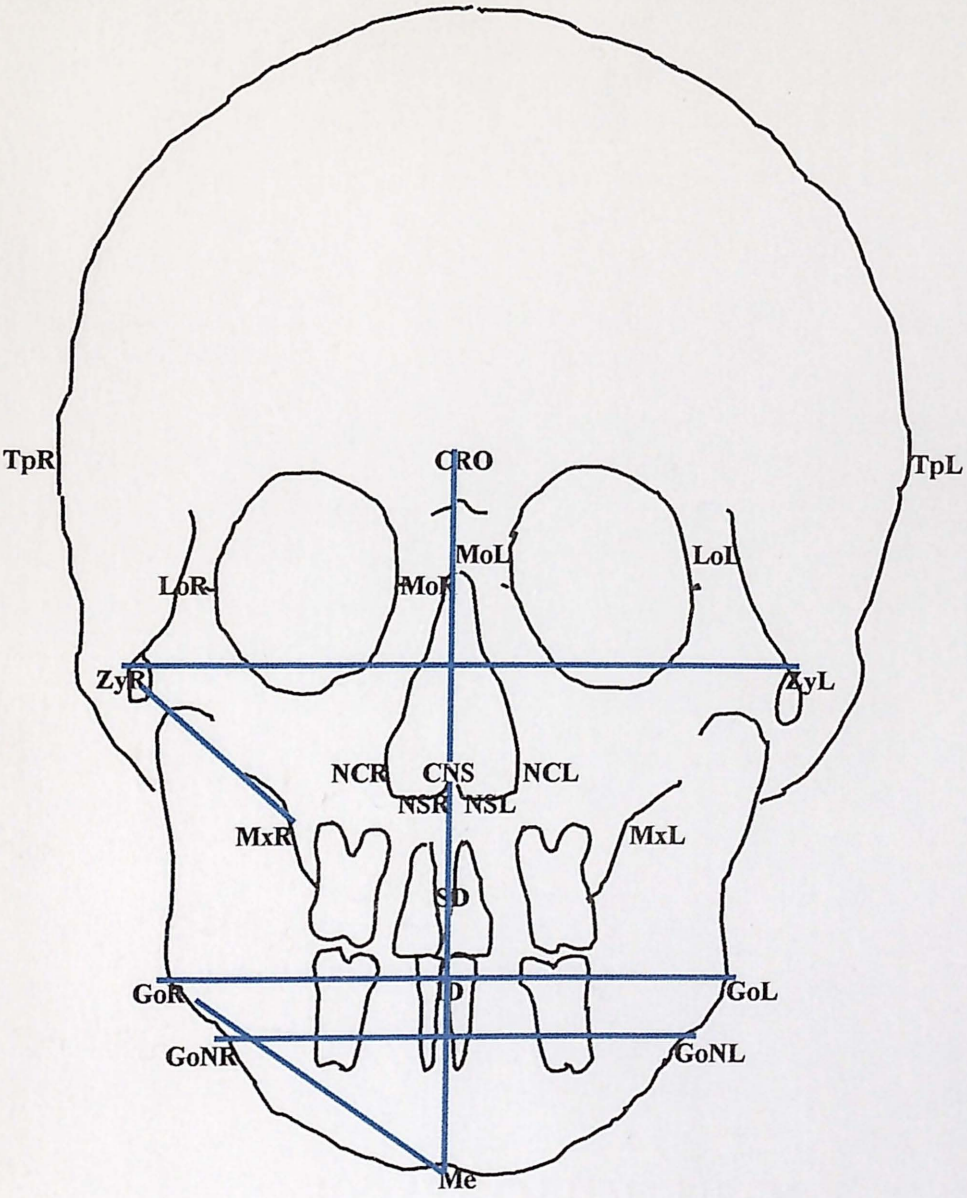
*included variables: PNS-ANS/N-ANS, PNS-ANS/N-Pg, N-S-Ba

FIGURE 7. Angular measurements that were significantly ($p < 0.05$) smaller than reference norms- LA cephalometric variables.



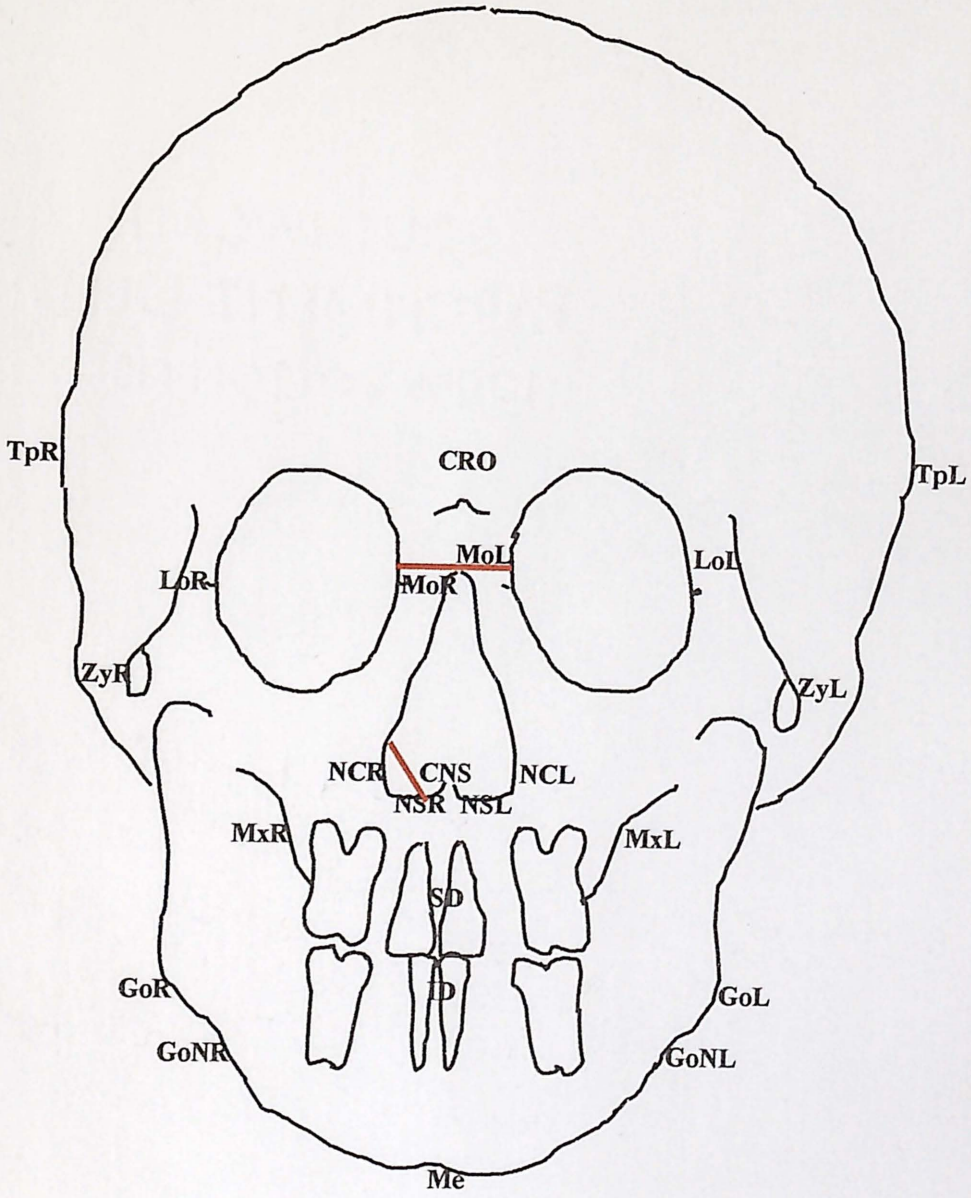
*included variables: S-N-Pg, Ar-Go-Me

FIGURE 8. Angular measurements that were significantly ($p < 0.05$) larger than reference norms- LA cephalometric variables.



*included variables: ZyL-ZyR, GoL-GoR, GoNL-GoNR, CRO-CNS, CNS-SD, CNS-Me, ID-Me, MxR-ZyR, Me-GoR

FIGURE 9. Measurements that were significantly ($p < 0.05$) smaller than reference norms- PA cephalometric variables.



*included variables: MoL-MoR, NSR-NCR

FIGURE 10. Measurements that were significantly ($p < 0.05$) larger than reference norms- PA cephalometric variables.

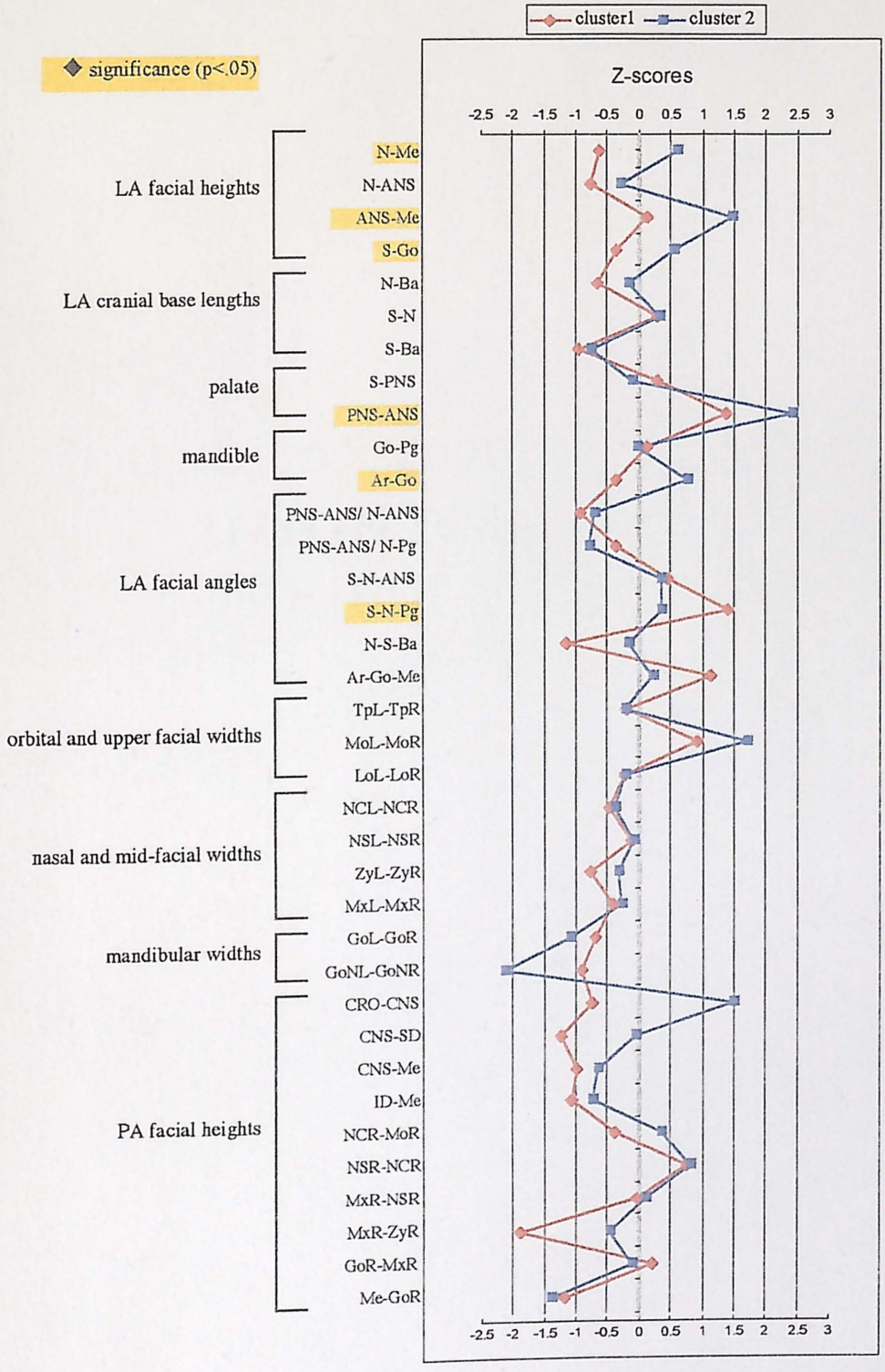
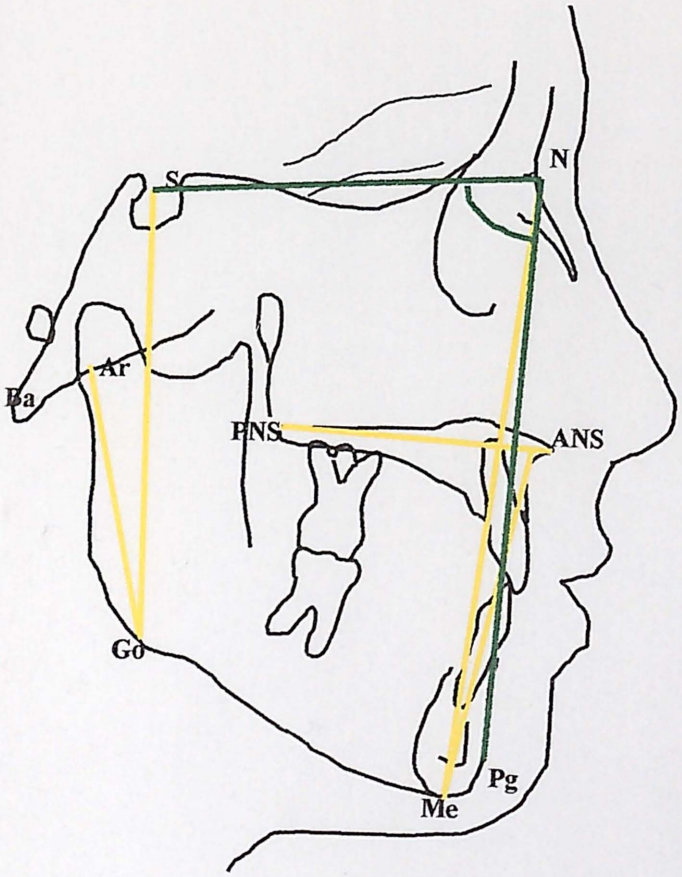
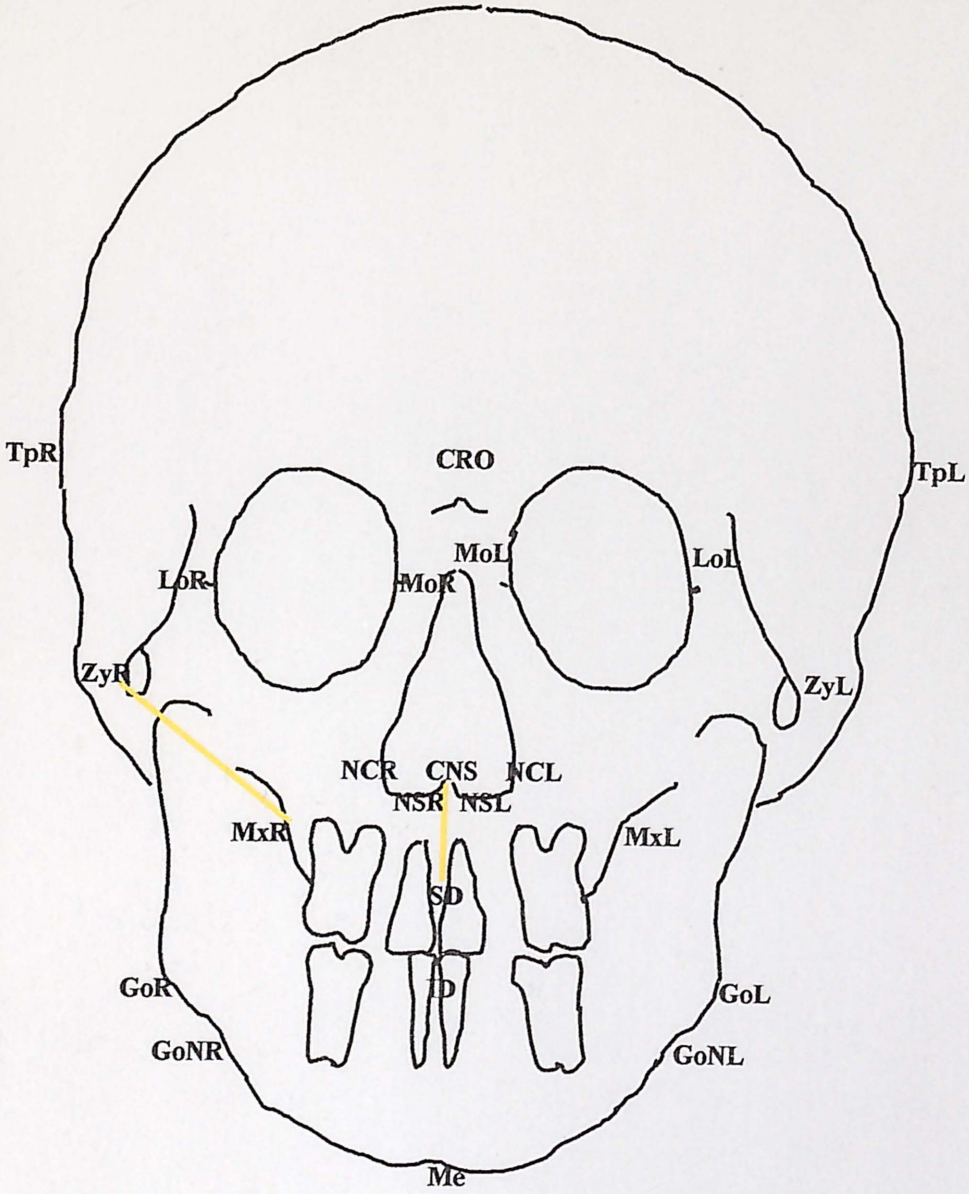


FIGURE 11. Pattern profiles of mean z-scores of Cluster 1 and Cluster 2.



*Cluster significant data: Cluster 1 smaller = yellow (N-Me, ANS-Me, S-Go, PNS-ANS, Ar-Go)
Cluster 2 smaller = green (S-N-Pg)

FIGURE 12. Statistically significant ($p < 0.05$) variables between Cluster 1 and Cluster 2- LA cephalometric data.



*Cluster significant data: Cluster 1 smaller = yellow (CNS-SD, MxR-ZyR)

FIGURE 13. Statistically significant ($p < 0.05$) variables between Cluster 1 and Cluster 2- PA cephalometric data.

TABLE I. Lateral (LA) linear and angular measurements used to evaluate CP relatives

#	description	variable	structures
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	Posterior nasal spine to Anterior nasal spine	PNS-ANS	palatal length
6	Articular to Gonion	Ar-Go	mandibular ramus length
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	maxillary position relative to cranial base
15	Maxillary plane (var. 5) intersecting var. 10	PNS-ANS/ N-ANS	upper facial angle
16	Sella to Nasion to Pogonion	S-N-Pg	mandibular position relative to cranial base
17	Maxillary plane (var. 5) intersecting Nasion to Pogonion	PNS-ANS/ N-Pg	lower facial angle

*variables 4, 8, 14, 15, 16, 17 are angular; all others are linear
*all variables are displayed in diagramatical form in Figure I

TABLE II. Posterior-Anterior (PA) measurements used to evaluate CP relatives

#	description	variable	structures
1	Medial orbital wall (left) to Nasal cavity (left)	MoL-NCL	nasal height
2	Nasal cavity (left) to Nasal shelf (left)	NCL-NSL	lateral nasal width
3	Nasal shelf (left) to Maxilla (left)	NSL-MxL	lateral maxillary width
4	Maxilla (left) to Zygoma (left)	MxL-ZyL	lateral mid-facial height
5	Maxilla (left) to Gonion (left)	MxL-GoL	lateral lower facial height
6	Gonion (left) to Menton	GoL-Me	mandibular body length
7	Menton to Gonion (right)	Me-GoR	mandibular body length
8	Gonion (right) to Maxilla (right)	GoR-MxR	lateral lower facial height
9	Maxilla (right) to Zygoma (right)	MxR-ZyR	lateral mid-facial height
10	Maxilla (right) to Nasal shelf (right)	MxR-NSR	lateral maxillary width
11	Nasal shelf (right) to Nasal cavity (right)	NSR-NCR	lateral nasal width
12	Nasal cavity (right) to Medial orbital wall (right)	NCR-MoR	nasal height
13	Medial orbital wall (left) to Medial orbital wall (right)	MoL-MoR	inner orbital width
14	Lateral orbital wall (left) to Lateral orbital wall (right)	LoL-LoR	outer orbital width
15	Zygoma (left) to Zygoma (right)	ZyL-ZyR	zygomatic width
16	Maxilla (left) to Maxilla (right)	MxL-MxR	maxillary width
17	Nasal cavity (left) to Nasal cavity (right)	NCL-NCR	total nasal width
18	Nasal shelf (left) to Nasal shelf (right)	NSL-NSR	nasal floor width
19	Gonion (left) to Gonion (right)	GoL-GoR	gonial width
20	Gonial notch (left) to Gonial notch (right)	GoNL-GoNR	gonial notch width
21	Temporal (left) to Temporal (right)	TpL-TpR	temporal width
22	Center of roof of orbit to Center nasal shelf	CRO-CNS	mid-facial height
23	Center nasal shelf to Superdentale	CNS-SD	maxillary height
24	Center nasal shelf to Menton	CNS-Me	lower facial height
25	Infradentale to Menton	ID-Me	mandibular height

*all variables are displayed in diagramatical form in Figure II

TABLE III. Mean z-scores of LA and PA variables for parents

variable	mean	standard deviation
N-Me	0.1197	1.0778
N-ANS	-0.489	.8318
ANS-Me	0.9497	1.2354
S-Go	0.192	.9686
N-Ba	-0.3423	1.2741
S-N	0.3303	1.0032
S-Ba	-0.8357	1.3829
S-PNS	0.064	1.1573
PNS-ANS	2.0103	1.2086
Go-Pg	0.037	1.2604
Ar-Go	0.3307	1.0325
PNS-ANS/ N-ANS	-0.785	1.3054
PNS-ANS/ N-Pg	-0.616	1.3650
S-N-ANS	0.408	1.1665
S-N-Pg	0.7833	1.2758
N-S-Ba	-0.55	1.3526
Ar-Go-Me	0.5887	1.4804
TpL-TpR	0.1997	1.0331
MoL-MoR	1.41	1.3156
LoL-LoR	-0.2093	.9029
NCL-NCR	-0.4067	1.5808
NSL-NSR	-0.0763	1.7856
ZyL-ZyR	-0.4923	1.0684
MxL-MxR	-0.3197	1.1141
GoL-GoR	-0.9207	1.5349
GoNL-GoNR	-1.6247	2.4429
CRO-CNS	-1.2053	1.6540
CNS-SD	-0.51	1.1299
CNS-Me	-0.7733	.8622
ID-Me	-0.8693	.8407
NCR-MoR	0.05767	1.1733
NSR-NCR	0.7953	1.4660
MxR-NSR	0.05633	.8802
MxR-ZyR	-1.03	1.3223
GoR-MxR	0.031	1.2908
Me-GoR	-1.297	1.1972

TABLE IV. Univariate t-test of parents against reference population means

variable	t	df	sig. ($p < 0.05$)
N-Me	.608	29	.548
N-ANS	-3.220	29	.003
ANS-Me	4.210	29	.000
S-Go	1.086	29	.287
N-Ba	-1.472	29	.152
S-N	1.804	29	.082
S-Ba	-3.310	29	.003
S-PNS	.303	29	.764
PNS-ANS	9.110	29	.000
Go-Pg	.161	29	.873
Ar-Go	1.754	29	.090
PNS-ANS/ N-ANS	-3.294	29	.003
PNS-ANS/ N-PG	-2.472	29	.020
S-N-ANS	1.916	29	.065
S-N-Pg	3.363	29	.002
N-S-Ba	-2.227	29	.034
Ar-Go-Me	2.178	29	.038
TpL-TpR	-1.059	29	.299
MoL-MoR	5.870	29	.000
LoL-LoR	-1.270	29	.214
NCL-NCR	-1.409	29	.169
NSL-NSR	-.234	29	.817
ZyL-ZyR	-2.524	29	.017
MxL-MxR	-1.572	29	.127
GoL-GoR	-3.285	29	.003
GoNL-GoNR	-3.643	29	.001
CRO-CNS	-3.991	29	.000
CNS-SD	-2.472	29	.020
CNS-Me	-4.913	29	.000
ID-Me	-5.664	29	.000
NCR-MoR	.269	29	.790
NSR-NCR	2.971	29	.006
MxR-NSR	.351	29	.728
MxR-ZyR	-4.266	29	.000
GoR-MxR	.132	29	.096
Me-GoR	-5.934	29	.000

TABLE V. Factors obtained via variable reduction

factor	description	variables combined in factor
1	(PA)Lower facial height,	GoR-MxR
	Width of lower mid-face	ZyL-ZyR
		CNS-Me
		TpL-TpR
		ID-Me
2	Mandibular dimensions,	GoNL-GoNR
	Height of mid-face	GoL-GoR
		Me-GoR
		CRO-CNS
3	(LA)Total facial height,	ANS-Me
	Lower facial height	N-Me
4	Nasal width	NCL-NCR
		NSL-NSR
5	Facial profile	PNS-ANS/ N-ANS
		S-N-ANS
		PNS-ANS/ N-Pg
6	Mandibular length,	Go-Pg
	Facial profile	S-N-Pg
7	Posterior facial height	Ar-Go
		S-Go
8	Mid-face width,	MoL-MoR
	Cranial base length	N-Ba
9	Palatal length, posterior	PNS-ANS
	Cranial base length	S-Ba
10	Maxilla-nasal width,	MxR-NSR
	Maxillary width	MxL-MxR
11	Nostril width	NSR-NCR
12	Upper facial height	N-ANS

TABLE VI. Mean z-scores of segregated clusters: C1, C2 and t-test of cluster means

variable	C1 (n = 12)	standard deviation	C2 (n = 18)	standard deviation	sig. p < 0.05)
SEX					.025
N-Me	-.6100	.7789	0.6061	.9811	.001
N-ANS	-0.7708	.8248	-0.311	.8041	.132
ANS-Me	0.1458	.9805	1.4856	1.1052	.002
S-Go	-0.3558	.9490	0.5572	.8152	.009
N-Ba	-0.6367	1.0921	-0.1461	1.3768	.310
S-N	0.3142	.9617	0.3411	1.0573	.944
S-Ba	-0.94	1.0394	-0.7661	1.5971	.742
S-PNS	0.3067	1.1698	-0.0978	1.1533	.357
PNS-ANS	1.3908	1.1109	2.4233	1.1144	.019
Go-Pg	0.1442	1.3478	-0.0344	1.2334	.711
Ar-Go	-0.3408	1.1328	0.7783	.6766	.002
PNS-ANS/ N-ANS	-0.9117	1.3797	-.7006	1.2869	.672
PNS-ANS/ N-PG	-0.355	1.8350	-0.7900	.9588	.402
S-N-ANS	0.4758	1.2417	0.3628	1.1481	.800
S-N-Pg	1.4075	1.4917	0.3672	.9372	.026
N-S-Ba	-1.1325	1.0227	-0.1617	1.4301	.052
Ar-Go-Me	1.1492	1.5932	0.215	1.3140	.091
TpL-TpR	-0.18	1.2088	-0.2128	.9354	.934
MoL-MoR	0.93	1.2164	1.73	1.3131	.104
LoL-LoR	-0.2192	.8846	-0.2028	.9404	.962
NCL-NCR	-0.4608	1.5522	-0.3706	1.6434	.881
NSL-NSR	-0.0917	1.9537	-0.0661	1.7231	.970
ZyL-ZyR	-0.765	1.3087	-0.3106	.8670	.261
MxL-MxR	-0.3908	.9702	-0.2722	1.2257	.781
GoL-GoR	-0.6642	.8156	-1.0917	1.8737	.465
GoNL-GoNR	-0.88	.7741	-2.1211	3.0232	.177
CRO-CNS	-0.74	.7912	1.5156	2.0018	.214
CNS-SD	-1.2192	1.3138	-0.0372	.6850	.003
CNS-Me	-0.9708	1.0695	-0.6417	.6944	.314
ID-Me	-1.0583	.7274	-0.7433	.9061	.323
NCR-MoR	-0.3883	.9262	0.355	1.2487	.089
NSR-NCR	0.7692	1.5555	0.8128	1.4491	.938
MxR-NSR	-0.0283	.7793	0.1128	.9593	.675
MxR-ZyR	-1.87	.8468	-0.47	1.2995	.003
GoR-MxR	0.2225	1.5496	-0.0967	1.1161	.517
Me-GoR	-1.1733	1.0271	-1.3794	1.3208	.652

TABLE VII. Comparisons of findings in Nakasima et al. study and the present study. Variable means found to differ significantly in parents of CP children

NAKASIMA ET AL.	sig.	PRESENT STUDY	sig.
N-ANS (upper facial height)	smaller	N-ANS (upper facial height)	smaller
U-PNS (posterior facial height)	smaller	ANS-Me (lower facial height)	larger
N-ANS/ ANS-Me (facial height proportion	smaller	S-Ba (posterior cranial base)	smaller
MHW/ MHL (cephalic index)	smaller	PNS-ANS (palatal length)	larger
OW/ MHW (orbital width proportion)	larger	PNS-ANS/ N-ANS (upper facial angle)	smaller
FW/ MHW (bizygomaticofrontal suture proportion)	larger	PNS-ANS/ N-Pg (lower facial angle)	smaller
NW/ MHW (nasal width proportion)	larger	S-N-Pg (mandibular position relative to cranial base)	larger
ZW/ MHW (zygomatic width proportion)	larger	N-S-Ba (cranial base flexure)	smaller
AW/ MHW (alveolar width proportion)	larger	Ar-Go-Me (mandibular angle)	larger
MHW (maximum head width)	smaller	MoL-MoR (medial orbital width)	larger
		ZyL-ZyR (zygomatic width)	smaller
		GoL-GoR (gonial width)	smaller
		GoNL-GoNR (gonial notch width)	smaller
		CRO-CNS (upper facial height-frontal)	smaller
		CNS-SD (maxillary height-frontal)	smaller
		CNS-Me (lower facial height-frontal)	smaller
		ID-Me (mandibular height-frontal)	smaller
		NSR-NCR (lateral nasal width)	larger
		MxR-ZyR (maxillary height-frontal)	smaller
		Me-GoR (mandibular body height-frontal)	smaller

DISCUSSION

UNIVARIATE COMPARISON OF PARENTAL SAMPLE TO REFERENCE MEANS AND TO PREVIOUS RESEARCH

The purpose of this study was to assess the hypothesis that differences exist in the cephalometric pattern between first-degree relatives of sporadic cases of isolated CP and reference populations. The demonstration of such differences in facial shape could indicate that such persons have facial morphology that increases the risk for CP. This research on parents of sporadic cases of CP supports the hypothesis that at least some parents have unusual facial features. However, it is also clear that there is heterogeneity among the parents. Our results suggest that at least two different phenotypic patterns can be defined.

Very few studies have been conducted in assessing phenotypic patterns in unaffected relatives of isolated CP. Two studies in the literature have dealt with and separated these individuals. Nakasima et al.[49] conducted a study in 1983 later followed by a study in 1997 from Mossey et al.[51] However, their results are not directly comparable due to significant differences in study designs and assumptions. Nevertheless, Nakasima et al. found that parents of CP children displayed significant differences from controls for upper anterior facial height (N-ANS), upper posterior facial height (U-PNS), and ratio of upper to lower anterior facial height (N-ANS/ANS-Me). All of these lateral measurements were found to be smaller than control data. In regard to PA results Nakasima et al. found that significant differences could be observed in MHW or maximum head width and proportions to MHW; OW/MHW, FW/MHW, NW/MHW,

ZW/MHW, AW/MHW. Specifically, MHW in his parental sample was less than control data and because of this his previous ratios listed were larger.

Taken as a group, the sample of parents in the present study (Table III), while showing several variable means that differed significantly compared to reference norms (Table IV), do not follow the specific findings of Nakasima et al. Significant differences between this study and Nakasima et al. are listed in Table VII. Lateral analysis from our study displayed longer lower faces and shorter upper faces whereas Nakasima et al. found normal lower faces and significantly shorter upper faces. Also longer palatal lengths and a more closed facial angle were observed in our study and not in the study of Nakasima et al. when compared to normal. Finally, retruded mandibles and larger mandibular angles were recorded in our study but not in Naksima et al. The frontal analysis from our study displayed larger orbital width and larger nasal width. Smaller widths were observed in the zygomatic, gonial, and gonial notch areas.

There are several possible explanations for these discrepancies. First, the Nakasima et al. study is based on an Asian population where as the present study concentrates on a largely Caucasian sample. Significant intrinsic differences in facial shape may exist between these two groups. Furthermore, the values of Nakasima et al. were based on generating a "mid-parental average" by combining values of mother and father and then dividing this sum by 2. Because Nakasima et al. did not use z-scores he had to use these averages to control size differences due to gender. However, such averaging will tend to obscure the individual contribution. Finally, the two studies used different landmarks and variables making direct comparison difficult.

Mossey et al.[51] recently studied parents of affected individuals of isolated CP and CL(P). This study used lateral cephalograms only and did not standardize results from normals but compared the two groups. Mossey et al. found parents of individuals with CP to have longer ramus lengths, longer mandibular lengths, larger mandibular area and cranial area than parents of individuals with CL(P). Corresponding measurements of significant data from his study was not found significant in ours. Different study designs were used in their research. Mossey et al. compared CP to CL(P) and did not compare either to reference norms.

CLUSTER ANALYSIS AND EVIDENCE FOR PHENOTYPIC HETEROGENEITY WITHIN THE PARENTAL SAMPLE

Factor analysis reduced our total variables and in conjunction with cluster analysis two clusters were identified. These clustered groups illustrate our non-uniform sample and heterogeneity within our parental group. Relative to reference means and relative to each other Cluster 1 demonstrates a more anterior chin position relative to cranial base (S-N-Pg), smaller facial height (N-Me), smaller posterior facial height (S-Go), smaller cranial base angle (N-S-Ba), larger mandibular angle (Ar-Go-Me), shorter vertical maxillary height (CNS-SD) and shorter lateral mid-facial height (MxR-ZyR). Cluster 2 is distinguished by having a longer lower facial height (ANS-Me), longer mandibular ramus length (Ar-Go), longer palatal length (PNS-ANS), less mandibular breadth (GoNL-GoNR), and a longer mid-face (CRO-CNS). However, both clustered groups are different than reference values and thereby support our hypothesis that there exists differences between reference populations and parents of CP individuals

In Cluster 1, many facial height and cranial base measurements are reduced. The mandibular measurements suggest a down and backward rotation of the lower jaw. This would display the aforementioned large mandibular angle and short ramus length. Functionally, this growth pattern is seen when the tongue is held in an elevated and retruded position in the oral cavity. Developmentally, the tongue needs to fall into the oral cavity to allow the palatal shelves to fuse. An interference prenatally in this fashion often leads to an incidence of CP.[18] Many times a short-face (euryproscopic facial type) individual is seen with a more closed mandibular angle and longer ramus heights. It might be plausible to hypothesize that short facial heights, larger mandibular angles, and decreased ramus heights are a developmental compensation for a CP susceptible individual. Cluster 2 distinguishes itself from Cluster1 by having longer facial heights and smaller widths. It is unclear how these features might relate to predisposition for CP.

Gender difference between clusters was found to be significantly different ($p < 0.025$) and this fact warrants further examination. It should be remembered that z-scores control for gender and age differences, therefore we are not simply observing that females are different than males in general. The fact that there is a preponderance of females in Cluster 1 may relate to differences in embryonic development and observed differences in cleft expression between the sexes. Burdi[60] in 1969 suggested that females close their palates later in development and thus logically are more predisposed to cleft palate. This logic follows the 2:1 female to male ratio of CP incidence between sexes. In this study, our sample consisted of 15 parental pairs or 30 individuals. In analyzing our cluster data, Cluster 1 contained 9/12 (75 percent) female individuals (parental data), while Cluster 2 had 12 of 18 male individuals or 67 percent. The gender differences in

the two clusters suggest that Cluster 1 may be the more predisposing to cleft offspring. Thus, the females in Cluster 1 may carry the predisposing factors and be closer to the threshold of full expression. This could be reflected in their unusual facial shape. It is also possible that some of these individuals are X-linked carriers. One previously published report found X-linked recessive inheritance in two families.[34] In our study, there were four affected individuals from females in Cluster 1 that were males. These females may be carriers who transmit the carrier X chromosome to their sons. The aforementioned females may display phenotypic traits different than reference populations due to lyonization. Alternatively, it could be that females have a lower threshold for expressing the cleft when exposed to causal agents. This might suggest that Cluster 2 with its preponderance of males reflects individuals carrying the risk factors but not expressing the cleft. In other words, males would perhaps show more effect without clefting. The debate is ongoing and can be argued either way.[16] Further analysis of Cluster 1 and 2 reveals other sources of ambiguity. Thus, Cluster 1 is closer to reference means for 15 of 36 variables while Cluster 2 is closer for the other 21 variables.

While the present results are not definitive, the clusters demonstrate that there is phenotypic variability within the parental group and this phenotypic variability may correlate with sex. Thus, it would be unwise to assume that parents always contribute equally to risk. Furthermore, these results suggest that if there are predisposing factors (i.e. major gene or facial shape) in the parents these may be differentially expressed depending on the sex of the parent in which they were present.

Additional or future research with larger sample sizes may help clarify this picture. Such a larger sample size should include a set of matched controls and a set of

unrelated affected individuals to help define the affinities of the phenotypic subgroups among parents. For example, does one of the clusters resemble affected individuals more than the other as was shown for CL(P) parents? In addition, a larger sample including siblings could also allow one to identify a consistent pattern of phenotypic differences that segregate within families with cleft children. Ultimately we could seek to associate particular molecular (genetic) markers with such phenotypes.

SUMMARY AND CONCLUSIONS

In summary, the inheritance for CL(P) has been suspected to be multifactorial in origin. However, CL(P) research has documented consistent cephalometric characteristics for individuals other than the proband in families containing CL(P). Cleft palate research has been less extensive. The few studies that have been conducted contained methodological flaws either with the study population or the way in which data was analyzed.[49, 51]

Evidence from this study, although limited, demonstrates three important points. First, taken as a group, parents of sporadic cases of isolated CP display several distinct differences in mean facial measurements compared to published normal reference values. Second, at least two phenotypic patterns were demonstrated. Third, the phenotypic differences seem to be related to sex of the parent. Thus, the research conducted in this study achieved the stated purpose of finding differences between reference norms and parents of CP individuals.

Parents utilized in this study displayed the following significant findings that were increased relative to reference data: lower facial height (ANS-Me), palatal length (PNS-ANS), mandibular position relative to cranial base (S-N-Pg), mandibular angle (Ar-Go-Me), orbital width (MoL-MoR), lateral nasal width (NSR-NCR). Still other measurements were decreased relative to reference norms; upper facial height (N-ANS), posterior cranial base (S-Ba), upper facial angle (PNS-ANS/ N-ANS), lower facial angle (PNS-ANS/ N-Pg), cranial base flexure (N-S-Ba), zygomatic width (ZyL-ZyR), gonial width (GoL-GoR), gonial-notch width (GoNL-GoNR), PA upper facial height (CRO-

CNS), PA maxillary height (CNS-SD), PA mid-facial height (CNS-Me), PA mandibular height (ID-Me), PA posterior maxillary height (MxR-ZyR), PA mandibular body length (Me-GoR).

Two subgroups were defined via cluster analysis. Significant findings between clusters included the following data that were significantly smaller for Cluster 1 relative to Cluster 2; total facial height (N-Me), lower facial height (ANS-Me), posterior facial height (S-Go), palatal length (PNS-ANS), mandibular ramus length (Ar-Go), PA maxillary height (CNS-SD), PA posterior maxillary height (MxR-ZyR). Variables that were significantly larger for Cluster 1 relative to Cluster 2 were; mandibular position relative to cranial base (S-N-Pg).

Gender difference was also significant between clusters with Cluster 1 containing 75 percent female individuals and Cluster 2 containing 67 percent male individuals. It is suggested that the cephalometric pattern seen in Cluster 1 together with its large concentration of females may reflect a predisposition to CP. However, Cluster 2 with its preponderance of males also might reflect individuals carrying the risk factors but not expressing the cleft. Even though it is unclear which cluster might best be viewed as more predisposing to CP it is clear that there are not equal contributions from both parents in all cases. Thus, all previous research reported on phenotypic patterns in parents of CP children needs to be reevaluated in light of the present findings. Future studies, recognizing the heterogeneity in phenotypic patterns of parents may be able to identify the specific pattern that correlates with an increased risk for having a child with a CP.

REFERENCES

1. Woolf CM. Congenital cleft lip: a genetic study of 496 propositi. J Med Genet 1971; 8:65-83.

2. Carter CO. Genetics of common single malformations. Br Med Bull 1976; 32:21-6.

3. Mendell NR. Multifactorial/ threshold models and their applications to cleft lip and cleft palate. In: Melnick M, Bixler D, Shields E (eds), Alan R. Liss. Etiology of cleft lip and cleft palate. New York, 1980, pp 387-406.

4. Tolarova M. Orofacial clefts in Czechoslovokia. Incidence, genetics and prevention of cleft lip and palate over a 19-year period. Scand J Plast Reconstr Surg 1987; 21:19-25.

5. Fraser FC. The genetics of cleft lip and cleft palate. Am J Hum Genet 1970; 22:336-52.

6. Carter CO. Genetics of common disorders. Br Med Bull 1969; 25:52-7.

7. Melnick M, Marazita ML, Hu DN. Genetic analysis of cleft lip with or without cleft palate in Chinese kindreds. Am J Med Genet Suppl 1986; 2:183-90.

8. Marazita ML, Goldsteing AM, Smalley SL, Spence MA. Cleft lip with or without cleft palate. reanalysis of a three-generation family study from England. Genet Epidemiol 1986; 3:335-42.

9. Chung CS, Bixler D, Watanabe T, Koguchi H, Fogh-Andersen P. Segregation analysis of cleft lip with or without cleft palate: A comparison of Danish and Japanese data. Am J Hum Genet 1986; 39:603-11.

10. Marazita ML, Hu DN, Spence MA, Liu YE, Melnick M. Cleft lip with or without cleft palate in Shanghai, China: evidence for an autosomal major locus. Am J Hum Genet 1992; 51:648-53.

11. Ray AK, Field LL, Marazita ML. Nonsyndromic cleft lip with or without cleft palate in West Bengal, India: evidence for an autosomal major locus. Am J Hum Genet 1993; 52:1006-11.

12. Fitzpatrick D, Farrall M. An estimation of the number of susceptibility loci for isolated cleft palate. J Craniofac Genet Dev Biol 1993; 13:230-5.

13. Czeizel A. Tusnady G. A family study on cleft lip with or without cleft palate and posterior cleft palate in Hungary. Hum Hered 1972; 22:405-16.

14. Ward RE, Bixler D, Jamison PL. Cephalometric evidence for a dominantly inherited predisposition to cleft lip-cleft palate in a single large kindred. Am J Med Genet 1994; 50:57-63.

15. Christensen K, Holm NV, Olsen J, Kock K, Fogh-Andersen P. Selection bias in genetic-epidemiological studies of cleft lip and palate. Am J Hum Genet 1992; 51:645-59.

16. Gorlin RJ, Cohen MM, Levin LS. Syndromes of the head and neck. 4th ed. New York: Oxford University Press, 1990.

17. Ten Cate R. Oral Histology: development, structure, and function. 5th ed. Chicago: Mosby-Year Book, 1998.

18. Fraser FC, Walker BE, Trasler DG. Experimental production of congenital cleft palate: genetic and environmental factors. Pediatrics 1957; 19:1782-7.

19. Gruneberg H. Genetical studies on the skeleton of the mouse. (Pt. 4). Quasi-continuous variations. J Genet 1952; 51:95.

20. Kallen K. Maternal smoking and orofacial clefts. Cleft Palate Craniofac J 1997; 34(1):11-6.

21. Werler MM. Teratogen update: smoking and reproductive outcomes. Teratology 1997; 55(6): 382-8.

22. McDevitt JM, Gautieri RF, Mann DE Jr. Comparative teratogenicity of cortisone and phenytoin in mice. J Pharm Sci 1981; 70(6):631-4.

23. Sphrintzen RJ, Siegel-Sadewitz VL, Amata J, Goldberg RB. Anomalies associated with cleft lip, cleft palate or both. Am J Med Genet 1985; 20:585-95.

24. Rank BK, Thomson JA. Cleft lip and palate in Tasmania. Med J Aust 1960; 2:681-9.

25. Eiberg H, Bixler D, Nielsen LS, Conneally PM, Mohr J. Suggestion of linkage of a major locus for nonsyndromic orofacial cleft with F13A and tentative assignment to chromosome 6. Clin Genet 1987; 32:129-32.

26. Beiraghi S, Foroud T, Diouhy S, et al. Possible localization of a major gene for cleft lip and palate to 4q. Clin Genet 1994; 46:255-6.

27. Ardinger HH, Buetow KH, Bell GI, Bardach J, Van Demark DR, Murray JC. Association of genetic variation of the transforming growth factor-alpha gene with cleft lip and palate. Am J Hum Genet 1989; 45:348-53.

28. Chenevix-Trench G, Jones K, Green AC, Duffy DL, Martin NG. Cleft lip with or without cleft palate: associations with transforming growth factor-alpha and retinoic acid receptor loci. Am J Hum Genet 1992; 51:1377-85.

29. Sassani R, Bartlett SP, Feng H, Goldner-Sauve A, Haq AK. Association between alleles of the transforming growth factor-alpha locus and the occurrence of cleft lip. Am J Med Genet 1993; 45:565-9.

30. Hecht JT, Wang YP, Blanton SH, Michels VV, Daiger SP. Cleft lip and palate: no evidence of linkage to transforming growth factor-alpha. Am J Hum Genet 1991; 49:682-6.

31. Vintiner GM, Holder SE, Winter RM, Malcolm S. No evidence of linkage between the transforming growth factor-alpha gene in families with apparently autosomal dominant inheritance of cleft lip and palate. J Med Genet 1992; 29:393-7.

32. Fogh-Anderson P. Inheritance of harelip and cleft palate. Copenhagen: Munksgaard, 1942.

33. Marazita ML, Spence MA, Melnick M. Genetic analysis of cleft lip with or without cleft palate in Danish kindreds. Am J Med Genet 1984; 19:9-18.

34. Rollnick BR, Kaye CI. Mendelian inheritance of isolated nonsyndromic cleft palate. Am J Med Genet 1986; 24:465-73.

35. Christensen K, Mitchell L. Familial recurrence pattern analysis of nonsyndromic isolated cleft paalte- a Danish registry study. Am J Hum Genet 1996; 58:182-90.

36. Trasler DG. Pathogenesis of cleft lip and its relation to embryonic face shape in A/J and C57BL mice. Teratology 1968; 1:33-50.

37. Trasler DG, Fraser FC. Role of the tongue in producing cleft palate in mice with spontaneous cleft lip. Dev Biol 1963; 6:45-60.

38. Streeter GL. Developmental horizons in human embryos. Carnegie Institution of Washington Publication 575. Contr Embryol 1948; 32:133-203.

39. Gruneberg H. The development of some external features in mouse embryos. Heredity 1943; 34:89-92.

40. Kraus BS, Kitamura H, Latham RA. Atlas of developmental anatomy of the face (with special reference to normal and cleft lip and palate). Hoeber Medical Division: Harper and Row, 1966.

41. Stark RB. The pathogenesis of harelip and cleft palate. Plast Reconst Surg 1954; 13:20-39.

42. Carter CO. The genetics of congenital malformations. Second international conference on congenital malformations, July 14, 1963. International Medical Congress Ltd.

43. Burston WR. The development of cleft lip and palate. Ann R Coll Surg Eng 1959; 25:225-33.

44. Juriloff DM, Trasler DG. Test of the hypothesis that embryonic face shape is a causal factor in genetic predisposition to cleft lip in mice. Teratology 1976; 14:35-41.

45. Fraser FC, Pashayan H. Relation of face shape to susceptibility to congenital cleft lip. A preliminary report. J Med Genet 1970; 7:112-7.

46. Kurisu K, Niswander JK, Johnston MC, Mazaheri M. Facial morphology as an indicator of genetic predisposition to cleft lip and palate. Am J Hum Genet 1974; 26:702-14.

47. Erickson JD. Facial and oral form in sibs of children with cleft lip with or without cleft palate. Ann Hum Genet 1974; 38:77-88.

48. Trasler DG, Machado M. Newborn and adult face shapes related to mouse cleft lip predisposition. Teratology 1979; 19:197-206.

49. Nakasima A, Ichinose M. Characteristics of craniofacial structures of parents of children with cleft lip and/ or palate. Am J Orthod 1983; 84:140-6.

50. Ward RE, Bixler D, Raywood ER. A study of cephalometric features in cleft lip-cleft palate families. (Pt 1). Phenotypic heterogeneity and genetic predisposition in parents of sporadic cases. Cleft Palate J 1989; 26:318-26.

51. Mossey PA, McColl JH, Stirrups DR. Differentiation between cleft lip with or without cleft palate and isolated cleft palate using parental cephalometric parameters. Cleft Palate Craniofac J 1997; 34:27-35.

52. Crawford F, Sofaer J. Cleft lip with or without cleft palate: identification of sporadic cases with a high level of genetic predisposition. J Med Genet 1987; 24:163-9.

53. Fraser FC. The multifactorial threshold concept – uses and misuses. Teratology 1976; 14:267-80.

54. Saksena SS, Walker GF, Bixler D, Yu P. A clinical atlas of roentgencephalometry in norma lateralis. New York: Alan R. Liss, 1987.

55. Saksena SS, Bixler D, Yu P. A clinical atlas of roentgencephalometry in norma frontalis. NewYork: Alan R. Liss, 1990.

56. Dentofacial planner- Version 6.2. Dentofacial Software; Toronto, Canada, 1993.

57. Nie NH, Hull CH, Jenkins JG, Steinbrenner K, Bent DH. Statistical package for the social sciences. St. Louis: McGraw-Hill, 1975.

58. Sohal R, Rohlf FJ. Biometry. 2nd ed. San Francisco: WH Freeman and Co., 1981.

59. Ward JH, Jr. Hierarchical grouping to optimise an objective function. J Am Stat Assoc 1963; 58(301):236-44.

60. Garn SM, Smith BH, LaVelle M. Applications of pattern profile analysis to malformations of the head and face. Radiology 1984; 150:683-90.

61. Burdi AR, Silvey RG. Sexual differences in closure of the human palatal shelves. Cleft Palate J 1969; 6:1-7.

APPENDIX

A more extensive review of the multivariate methodologies used in this research may be of use to those unfamiliar with these techniques. Two types of multivariate analysis were used to reduce the number of variables so that subsequent procedures could be conducted more efficiently. Factor analysis refers to a category of related techniques that utilize correlation statistics in order to determine if some underlying "common factors" can be identified that would allow the data to be grouped or rearranged to create a smaller set of variables. This procedure is valuable in situations in which there may be many highly interrelated variables (as is likely to be the case in cephalometrics of the head and face). In this study, factor analysis was used because of the small sample size of subjects. It is generally the case that multivariate techniques are sensitive to discrepancies between number of variables and sample size.

Factor analysis, thus was used to identify a subset of components that consisted of a series of interrelated variables. This reduction is accomplished in several steps. First, a correlation matrix is calculated for all pairs of variables, next a set of factors is extracted by the construction of a new set of variables in which combinations of variables are produced that, together, account for the greatest amount of variability in the data. Thus, variables may be added to the linear combination until such additions actually start to decrease the effectiveness of the combination. The first such constructed variable is sometimes referred to as the "first principle component". Subsequent iterations of the process attempt to define the best combination of variables in terms of accounting for the greatest proportion of the remaining variation (after that accounted for by the first

principle component has been removed). Thus, subsequent factors account for less and less variation until all the variation in the sample has been exhausted. Generally there can be as many factors as there are variables. Clearly with this last provision in mind data reduction requires another step. In the present analysis, the SPSS procedure generates a plot of the percentage of variation explained by each factor. It is often the case that the first few factors account for the majority of the variation and subsequent factors explain fractional amounts. This plot is referred to as a "scree plot" because it frequently resembles a mountain, with the first few variables forming a "peak" and subsequent variable leveling off as does the "scree" slope on the shoulder of the mountain. Thus, the number of factors can be defined by examining the plot and determining when a certain preset level of explanatory power has been reached. In the case of the present study, we used the criteria of 90 percent of the total variance explained by the accumulated factors. This resulted in the reduction of the original 36 variables into a set of 12 factors. More importantly for subsequent analysis, these factors can be treated as numerical variables and "scores" can be calculated for each subject on each factor based on their original values on those variables included in the factor.

Finally, additional information can be obtained from considering the combination of variables that comprise each factor. Often, the underlying commonality will be obvious. For example, several variables around the orbits may be combined in a single factor, or, alternatively variables relevant to the facial profile may be discerned in a factor. This information can be important in subsequent attempts to interpret results of multivariate analyses.

The second multivariate procedure utilized in this study was cluster analysis. Like factor analysis, clustering involves a series of steps that begin with a correlation matrix. However, instead of looking for the correlation between variables, clustering looks at the correlation between individual subjects. Thus it examines pair-wise groupings of individuals to identify those who have the most similarity in their variable scores. From this matrix a second procedure is performed in which, using any of a variety of grouping algorithms, an attempt is made to sort the correlated individuals into groupings that either maximize the distance between it and other groups or minimize this distance. The result of either procedure is produced graphically, as a dendogram or "tree diagram" in which the history of the joining or splitting is represented hierarchically. Thus, at one point all (n) individuals are represented and then progressively, these are joined in larger and more inclusive groupings or clusters.

Interpretation of the results of cluster analysis has an obvious subjective element, since there theoretically are from n to 1 possible clusters to interpret. However, in situations where meaningful differences between groups of individuals do exist, this will be evident on the dendogram as distinct bifurcations, such that groups of individuals cluster together with high levels of similarity (low distance scores) and remain separate from other such groupings until low levels of similarity (large distance scores) are reached.

In the present study, factor scores were used instead of the original variables to get around the limitation imposed by having more of these original variables than subjects (see above). The resulting dendogram was then reviewed and the most distinct clusters identified, using the criteria described above. These clusters were then

characterized by descriptive statistics within the clusters. Pattern profiles were constructed from the mean variable scores (original variables) for each cluster.

ABSTRACT

CEPHALOMETRIC SIMILARITY AMONG PARENTS OF INDIVIDUALS WITH SPORADIC ISOLATED CLEFT PALATE: IS THERE EVIDENCE FOR AN INHERITED PREDISPOSITION?

by

Edward M. Sammons

Indiana University School of Dentistry
Indianapolis, Indiana

Isolated cleft palate is one of the most frequent congenital conditions that affect the oral and facial structures, yet its etiology remains obscure. Previous studies of both cleft palate (CP) and cleft lip and/ or palate [CL(P)] have shown that there may be unusual facial characteristics among the parents of such sporadic cases. Such findings have been used to support the possibility that there are predisposing familial (genetic) factors for both conditions. However, previous studies have generally not controlled for the possibility of genetic heterogeneity or for different contributions from each of the parents. The objective of this study is to examine parents of individuals with CP in order to test the hypothesis that these "non cleft" individuals have abnormal facial structures. Lateral (LA) and Posterior-Anterior (PA) cephalograms were examined from thirty parents of fifteen individuals with sporadic CP. Seventeen LA and twenty-five PA variables were obtained on each subject and converted to standardized "z-scores" through comparison to published age and sex matched reference data. Multivariate cluster

analysis was used to define groupings of individuals who shared similar patterns of facial features. Results demonstrate that as a group, relatives of CP individuals show significantly different patterns of facial measurements compared to reference norms. Values significantly larger ($p < 0.05$) from parental data included: ANS-Me, PNS-ANS, S-N-Pg, Ar-Go-Me, MoL-MoR, NSR-NCR. Values significantly smaller ($p < 0.05$) from parents included: N-ANS, S-Ba, PNS-ANS/ N-ANS, PNS-ANS/ N-Pg, N-S-Ba, ZyL-ZyR, GoL-GoR, GoNL-GoNR, CRO-CNS, CNS-SD, CNS-Me, ID-Me, MxR-ZyR, Me-GoR. These findings were not entirely consistent with those few previously reported findings. Additional analysis of the present data demonstrated that such inconsistencies may be due in part to the presence of distinct phenotypic subgroupings within the parental sample. Cluster analysis identified two such subgroups. Significant findings ($p < 0.05$) that were smaller for Cluster 1 relative to Cluster 2 included: N-Me, ANS-Me, S-Go, PNS-ANS, Ar-Go, CNS-SD, MxR-ZyR. Significant variables that were larger for Cluster 1 included: S-N-Pg. In addition, gender was significantly different across clusters with Cluster 1 containing 75 percent female individuals and Cluster 2 containing 67 percent male individuals. These results extend those reported in other studies by demonstrating that unusual facial patterns, when present are not uniformly distributed in parents of sporadic cases of CP. Phenotypic assessment in conjunction with multivariate analysis may help to identify families in which there is a significant heritable component for CP.

CURRICULUM VITAE

Edward M. Sammons

April 23, 1969	Born in Brook, Indiana
May 1991	BA, Indiana University Bloomington, Indiana
May 1995	DDS, Indiana University School of Dentistry Indianapolis, Indiana
June 17, 1995	Married to Christine L. Wattles Bloomington, Indiana
July 1997	MSD Joint Program- Pedo/ Ortho Pediatric Dentistry Certificate James Whitcomb Riley Hospital for Children Indiana University School of Dentistry, Indianapolis, Indiana
July 1997 to July 1999	MSD Joint Program- Pedo/ Ortho Orthodontic Progam Indiana University School of Dentistry, Indianapolis, Indiana

Professional Organizations

American Dental Association
American Student Dental Association
American Association of Pediatric Dentistry
Pediatric Dentistry Alumni Association
American Society of Dentistry for Children
American Association of Orthodontics
Omicron Kappa Upsilon, Theta Theta Chapter