CRANIOFACIAL MORPHOLOGY IN FAMILIAL CASES OF CLEFT LIP/PALATE: PHENOTYPIC HETEROGENEITY AND GENETIC PREDISPOSITION IN UNAFFECTED FAMILY MEMBERS

by

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INTRODUCTION

The hypothesis of this research can be stated as follows: In families that have multiple cases of primary palate clefts (CL/P) the genetic risk for cleft offspring should be increased over the general population risk. Since these persons by definition have an increased risk of recurrence, the research question is--do these persons show any abnormality of facial morphology that is characteristic of that group? Hence, the research hypothesis is:

Unaffected family members of CL(P) multiplex families can be identified and defined by the demonstration of specific abnormal facial features. These features will be defined by tracing boney landmarks recorded on cephalometric x-ray headplates. Thus, this research proposed that unique facial features can be used to predict genetic liability for cleft offspring.

Normal, for the purpose of this study, is defined as those family members who are related to others only by marriage. This obligate normal population is so defined because their chance of having genetic liability for cleft offspring is similar to that of the general population risk, a figure much smaller than what must be the risk for blood relatives of the multiple clefts--assuming a genetic basis for clefting.

The pedigree defined group of suspected gene carriers (as shown by pedigree analysis) will be compared to these family obligate normals for definition of any phenotypic differences. Finally, a group of individuals designated as the unknown population consists of blood relatives of the cleft family who are not cleft themselves. These individuals will be analyzed to determine which group (normal or carrier) they best duplicate phenotypically.

Previous studies have shown that three major subgroups for both cleft lip with or without cleft palate, CL(P), and isolated cleft palate, CP, are recognizable. The three are: (1) syndromes with clefts, (2) sporadic (single) occurance of the non-syndromic type of cleft, and (3) familial (multiple) cases of non-syndromic clefts in one family. It is often stated that this subgroup has the true genetically caused cleft. Furthermore, careful inspection of pedigrees will often show individuals in a genetic line of descent who are not cleft themselves and who subsequently prove to be at increased risk to have cleft children. These persons can be designated as "gene carriers" and they are the focus of this research, which attempts to identify their facial characteristics that relate to an increased liability for clefting.

The ability to predict the risk for reproducing a child with a cleft would improve our ability to provide accurate genetic counselling. Furthermore, such findings support the concept of a major gene

responsible for the transmission of CL(P) and would be extremely helpful in carrying out the molecular DNA linkage studies necessary for identifying and defining a "cleft" gene.

REVIEW OF LITERATURE

For many years researchers have studied the inheritance patterns of various congenital malformations. Of these, probably because it is so common. the most often studied deformity over the past 50 years has been cleft lip and palate. Before discussing the proposed etiologies of clefting, a review of the embryogenesis of the primary and secondary palates is in order. The primary palate develops into the upper lip, alveolar ridge and the anterior one-third of the hard palate, while the secondary palate consists of the remainder of the hard palate and the soft palate.

In normal development of the oro-facial complex, the margins of the olfactory placodes curl up as the first stage in the formation of the lateral and medial nasal processes. As the lateral margins of the developing face move toward the midline, the two medial nasal processes make contact with each other and fuse. Subsequently, the lateral-inferior margin of the MNP (globular process) makes contact with the maxillary process which is rapidly growing forward. Thus, the position of the placodes may be important for the clefting process. Since nasal placode position is established by the 17th day of gestation, mapping of the clefting site must occur within the first three weeks of gestation. The upper lip and alveolar bone develop from the three-way fusion of the

medial nasal process (MNP), lateral nasal process (LNP) and maxillary process (MxP). Fusion occurs when the epithelium first adheres and then breaks down to allow the internal mesenchyme to make contact.[1] In other words, final upper lip development is dependent upon the outward growth and fusion of all three of these development processes. Johnston believes that the MxP and MNP contact is first and probably most superficial in promoting the subsequent fusion of the MNP and LNP.[1] At any rate, all three processes are primarily involved in the formation of a normal upper lip by six weeks of embryonic age.

For development of the secondary palate, a different series of events must occur. The tongue, which is normally elevated in the roof of the mouth, must drop into a space between the two halves of the mandible. This then allows the palatal shelves to elevate to a horizontal position, move toward each other, make contact, and eventually fuse together in three-way contact with the nasal septum. These events begin at about eight weeks of development and are completed by the ninth week.

The embryogenesis of cleft lip with or without cleft palate occurs as the result of a failure of the processes to fuse. It is believed that the MNP and MxP initially fuse normally, and the proposed cause of clefting is a failure of the LNP to meet and fuse with the MNP. This initial contact and fusion of the MNP and MxP eventually breaks down in 90 percent of the cases. In studies of monozygotic twins, two-thirds of

the clefts were caused by the underdevelopment of the MNP resulting in contact failure. The other one-third of cases were caused by the underdevelopment of the MxP.[1]

Either genetic or environmental factors cause the shelves not to fuse, resulting in a cleft palate. Facial morphology, as an example of the genetic factor, differs between races. Asians, for example, are twice as likely to have primary clefts as Caucasians. Blacks, on the other hand, have only half this incidence of clefting.

The multifactorial/threshold theory, in contrast, exemplifies the environmental etiologies of clefting. This will be discussed at length later in the literature review.

Cleft microforms (the minor forms of expression of a cleft) are expressed in various ways. The aberrant features include: (1) asymmetrical nares, (2) high palatal vault, (3) a fibrous band of tissue extending from the base of the nose to the vermillon border, (4) a "notched" vermillon border, (5) supernumerary, congenitally missing, or deformed teeth, and/or (6) bifid uvula.

Whether microforms are aesthetically significant is a moot question. Thomson evaluated patients for improvement in appearance after plastic surgery. The evaluators consisted of two panels, one made up of lay persons and the other of professionals. The professionals were more critical preoperatively and, therefore, ultimately saw more improvements after surgery. In contrast, the panel of lay persons was

twice as likely to feel that the child's appearance did not improve.[2] This suggests that for one to better determine the significance of microform clefting, the views of both patients and parents need to be considered.

Some of these microforms have been reported to occur more frequently in the unaffected (not cleft) members of a family. To examine this hypothesis, Erickson[3] studied three aspects of the face: (1) facial profile, (2) dental arch shape, and (3) palatal form. Siblings of the affected group had a tendency toward maxillary hypoplasia but this was not significant. Differences in arch shape and palatal form, however, were significant. The arch shape of the affected siblings was tapered versus square, and the palatal vault was high versus flat. Though these differences existed, they were not of such magnitude that the siblings could be classified into specific populations.

Schubert et al.[4] concluded that the overall incidence of microforms of CL/P in relatives of cleft patients was greater than the normal population. The incidence of microforms was higher in parents and siblings of the proband (86.2 percent) and also in the familial group (57.9 percent) than in sporadic cases of clefting (45.7 percent). These studies support the conclusion that the presence of microforms cannot be liked to an increased recurrence risk for clefts, but may help

divide the sporadic cases into another category with recurrence risks similar to that of the familial group.

An anthropometrical study of nasal asymmetry resulted in several conclusions, probably the most important being that further research is needed in this area. Some variations of the nostril area, including narrower nasal floor or deviation of the nasal bridge away from the asymmetrical side, apparently do not coincide with the cleft lip/palate microforms. Most of the nostril asymmetries (88.6 percent) were considered as normal deviations. Severe asymmetries, including the alar base location, nasal floor, columella and nasal bridge, were seen 1.6 percent of the time. These are the most serious variations and are suggestive of microforms of CL/P anomaly.[5]

McCarthy et al.[6] presented an overview of clefting, and concluded that the incidence of CL/P is increasing, with a number of possible explanations including: a decrease in post-natal and operative mortality as well as an increase in the number of affected persons reproducing. With an increase in the affected population that is reproducing, there is an accompanying elevated risk factor to siblings/parents.

The etiology of cleft lip either with or without a cleft palate has had detailed population studies over the past 20 years that have revealed CL(P) to be a triad of very complex etiology. These results necessitated a re-evaluation of the nature of the genetic contribution

of CL(P). In 1941 Fogh-Andersen[7] showed that cleft lip with or without an accompanying cleft palate, and cleft palate alone, were two different entities.[8] The explanation for their uniqueness lies in the genetic regulation of differing developmental patterns and their timing.

For example, there has never been a reported case of monozygotic twins where one twin had a cleft lip with or without a cleft palate and the other had an isolated cleft palate. Since CL(P) monozygotic twins have a concordance of less than 100 percent, other factors contributing to clefting must be involved. In support of a strong genetic influence, though, is the fact that the monozygotic twins concordance even though less than 100 percent, is roughly 10 times the concordance of dizygotic twins.[9,10]

Juriloff[11] studied the hereditary control of clefting utilizing inbred mice. After four generations of inbreeding (brother/sister matings), the number of cleft lip producers in the strain remained constant. Apparently, homozygosity for the primary genetic factors controlling clefting was achieved. Thus, cleft liability in the mouse was due to a mutant gene brought to the homozygous state. This transmission pattern suggests a recessive mode of inheritance for the gene itself. Determining the reason for this transmission pattern could help in the identification of modifiers of facial development.

In the 1970s and '80s, most researchers supported one or the other of two mathematical concepts purporting to describe the inheritance of

CL(P). These two model systems were: (1) the polygenic model and (2) the multifactorial/threshold model (MF/T). Polygenic describes an inheritance pattern in which many gene loci each contribute a small but additive effect to the cleft phenotype. Multifactorial, on the other hand, refers to the presence of multiple, differing but interrelated factors causing clefting. These factors may or may not be additive, but include a mix of both genetic and environmental influences. When summed, these factors describe one's predisposition for developing a cleft. If this possibility is above a defined threshold, the person is affected; if it is below, he is not.[12]

The development of an isolated cleft palate (CP) depends on an interference with any one of several normal patterns. For instance, if the palatine shelves of the maxilla remain in their normal vertical position too long (timing), when the shelves do elevate, fusion may not be possible.[13] Other potential impediments to proper palatal (and hence facial) development include the position of the tongue and its alteration as a result of mandibular growth, head width (and hence palatal shelf width), forces affecting shelf movement, and overall growth of the mandible. All these factors are considered to play a part in the MF/T model. Fraser proposed that once the developmental threshold of any of these interacting factors is exceeded, a cleft occurs.

The current MF/T model has four mathematical tests that were derived from both the Edward's polygenic model and Falconer's MF/T model. Researchers have used these maxims to test their applicability from an unknown population to a cleft population. The following are the accepted tests:

(1) the incidence of clefts in near relatives of probands compared to that of clefts in the general population decreases as one goes from first to second to third degree relatives.[14]

(2) As the number of cleft persons increases in a single family, the risk for recurrence also increases. In a situation where a parent and one child are affected, the risk to subsequent children increases in a single case family from 3-4 percent (sporadic) to greater than 10 percent for the multiplex family.[7]

As the severity of the cleft increases in a given family or its members, the risk to future children also increases. The lowest risk is approximately 2.5 percent to a sibling of a child with a unilateral cleft lip, while the risk increases to approximately 6 percent if the affected sibling has a bilateral cleft lip and palate.[14]

(3) The least affected sex (i.e., females for CL(P) and males for CP) have a higher risk for passing the malformation on to relatives,[14] presumably because anyone who can transmit the cleft trait to offspring

and yet is not cleft himself (or herself) must have a higher threshold for liability, and therefore carries a higher genetic liability for clefting without expressing it.

(4) Compared to the general population, there is an increase in the frequency of clefting in intra-family or consanguineous (i.e., between first cousins) marriages.

While the MF/T model provides an explanation for major common birth defects (such a CL(P) and CP), it has been repeatedly challenged. By using soft tissue and bony x-ray examinations, Fukuhara and Saito reviewed 12 cases involving a history of cleft lip and palate and found defects (microforms) in siblings or parents of the affected person. This pattern of penetrance is suggestive of a dominant gene.[15]

Crawford and Sofaer[16] divided their subjects into five groups: controls, familial affected (probands and affected parents), non-cleft relatives of the familial cases, sporadic cases, and relatives of sporadic cases. Stepwise logistic regression selected bilateral asymmetrical variables that discriminated between groups. By using these variables, 85 percent of familial cleft patients and controls were classified correctly. Twenty-six percent of sporadic cases were similar to the majority of the familial patients supporting a high genetic predisposition even in the sporadic (non-familial) situation.

Researchers have also examined the incidence of clefting in different racial groups. A larger study of Danish families with clefts

used both the classical and a more complex type of segregation analysis (COMSEG). The conclusion was reached that there was at least one major gene responsible for producing the cleft phenotype, a finding which does not support the MF/T model.[17,18]

A smaller study using COMSEG was conducted with 12 Caucasian families in the United States involving parent to child transmission. Five families demonstrated either a dominant or codominant appearing major gene model. However, four families did not support this, but still rejected the autosomal recessive model. The remaining three families were non-contributory to conclusions drawn about any model.[19]

Chung and associates[20] compared data from the Danish families noted above to that of families in the Japanese population. Although the Japanese have a higher incidence of cleft lip and palate than do Caucasians, the empiric liability for relative of clefts is lower. Furthermore, the Danish data when analyzed by COMSEG showed support for a combination of the two etiologic theories. Thus, there was evidence for a major gene acting in the Danish population while the MF/T model best filled the Japanese families.

The major gene influence, at least for some populations, appears to be recessive. Approximately one-third of the Danish cleft population were due to this major gene trait. The other two-thirds favored the MF/T model. By contrast, the Japanese population data best fit the MF/T model exclusively with no evidence for major gene effects. This support

also takes into account the fact that the facial morphology of the Japanese is unique. As an illustration, the overall distance between their palatal shelves is greater than it is in Caucasians. This factor alone could represent the mechanism for failure of the palatal shelves to fuse, thereby resulting in a cleft palate.

The Chinese may be different genetically, however, since a COMSEG analysis of the cleft population of Beijing supported the monogenic theory. Melnick et al. addressed each of the four tests used to test for the presence of a MF/T in a given population. They reported a decreasing cleft incidence from first to second to third degree relatives of cleft probands, as would be expected in any familial pattern, and this rate was not statistically different from the expected. This was supported by the fact that the incidence of clefting in these relatives was higher than predicted by the MF/T model. Furthermore, women, the least frequently affected sex for CL(P), did not show a higher incidence in their first degree relatives than males. Consanguinity rates were found to be higher as predicted in the MF/T model, but not significantly so. Thus, three out of the four mathematical predictions of the MF/T model were rebutted. Interestingly, the data showed a marked decrease in the total number of females in the proband families. This resulted in a significantly altered sex ratio, both for the normal and the cleft females, which may be evidence for an x-linked recessive gene.[21,22]

Marazita et al.[23] compared results published from three different populations (Danish, English and Chinese). All were evaluated by (1) the MF/T predication tests, (2) a goodness-of-fit Chi-square test, and (3) both the standard segregration analysis and complex segregation (COMSEG) analysis. The MF/T prediction tests consistently failed to support a multifactorial mode of inheritance for this data in the Danish and Chinese, but they were only partially rejected by the English data set.

For purposes of performing a classical segregation analysis, the Danish population was divided into two family subgroups: Denmark-MG (multigenerational) and Denmark-N (nuclear). Data analyzed by this method was compatible with an autosomal recessive trait for all three populations, but for the Denmark-N subgroup no major gene was shown. The results obtained using the complex segregation analysis, then, could not be clearly interpreted.

Temple et al.[24] examined a family with four generations of clefts. This type of multiplex pedigree itself supports the major gene locus concept. However, the linear family pattern present showed an autosomal dominant transmission pattern instead of the possible recessive one suggested by the Chinese data and the CL Fraser mouse data.

Eiberg et al.[25] also reported that Danish pedigrees suggested dominant inheritance. Subjects were typed for various gene markers to see if a genetic linkage could be shown. The blood clotting factor

XIIIa located on chromosome #6 had the highest lod score (i.e., Logarithm of the odds of the two loci being linked). The greater the lod score the more likely the loci are linked. A score of 3, for example, represents a one in a thousand chance that the loci are not linked. Thus, a single gene locus for clefting may be linked to the F-XIIIa locus on chromosome #6.

Several studies have now presented data that question the applicability of the MF/T model to clefting. It appears that, as more data is reported, increasing support for a major gene locus regulating clefting is found. Fraser[26] reviewed the literature by comparing these two concepts and their supporting research, concluding that proponents of the MF/T model need to find a biological attribute that supports their theory, while major gene concept supporters will need to identify the gene locus itself.

If a single gene can control the clefting process, then perhaps it may be possible to identify gene "carriers" by their facial morphology. Before discussing specific research and facial morphology, addressing the tool that helps assess the ora-facial complex is important. Cephalometry was initially used to assess the face, allowing for a valid study of the morphology of the face and its components.[27] The lateral (LA) and posterior-anterior (PA) cephlographs are used to examine different aspects of the face. PA radiograph analyze height, width and depth in a two-dimensional plane. LA cephalographs, on the other hand,

allow evaluation of facial depth and height but provide little information on facial width. By using both PA and LA cephalographs, the intricate parts of the face can be examined, allowing division of the face into the upper, middle and lower as well as anterior and posterior dimensions.

An important aspect of using any diagnostic tool is the ability to reproduce the data accurately. A study by Midtgard et al.[28] determined that there were no significant differences between two different observers on the same film, rendering this type of diagnostic aide very useful.

Vincent and West's[29] study in this area is important. They determined that each landmark has a pattern of error that is characteristic of that landmark. The amount of error varies from study to study. Even with this knowledge, it is generally agreed that cephalometrics is a valid method to measure facial morphology and that the results can be reproduced accurately.

Many investigators, including Fraser and Pashayan,[30] used cephalometric analysis for their research. They hypothesized that if the embryonic face at the time of clefting is related to configuration of the postnatal face, and further, if face shape is genetically determined and related to the clefting process, then parents of children with clefts would have unique facial features not common in the general population of non-cleft persons.

Abnormally high levels of developmental facial asymmetry were found in the non-affected, near relative of cases of clefting, although many of the sporadic cases showed the same range of asymmetry as was seen in the familial cases. This result lends itself to support of a genetic predisposition regulating facial symmetry.[16]

Several studies have compared the facial features (both soft and bony tissues) of parents with affected children to parents of children without clefts (control group). The advantage of this type of anthropometric study is in its simplicity of design, in the availability of data bases for producing the norms, and the production of specific quantitative data for analysis.[31] Differences were reported in the facial features of parents at risk but exactly which facial features correlated best was found to be different between investigators.

Coccaro et al.[32] found the cranial base angle (Ba-S-N) of the experimental group to be significantly more acute. Measurements evaluating the upper face (N-ANS) and maxillary length (ANS-PNS) were different in the cleft lip and palate group--they showed a short vertical facial height dimension and shorter palate length (anterior two-thirds of hard palate). In contrast, the lower face showed an increased mandibular body length (Go-Gn) in the cleft lip and palate group, which tended to support the presence of a prognathic skeletal relationship. When the embryo has parents with these craniofacial

features, there appears to be a significant interference with proper formation of the facial processes.

In contrast with Coccaro's results, Nakasima and Ichinose[33] found that the cranial base was more obtuse in the experimental group. The frontal view showed wider facial features such as interorbital width, bizygomatic-frontal suture width, nasal width and bigonial width. Parents with upper face dimensions showing increased horizontal and decreased vertical face dimension values appeared to be more susceptible to producing cleft offspring. Kurisu et al.[34] made similar observations of decreased facial height and protrusive mandibles in at-risk parents but they came to a different conclusion. They compared their findings to the MF/T hypothesis, and postulated that if the MF/T model was correct, parents showing the largest deviations in facial dimensions should produce children with the most severe defects. When their data did not support this idea, they concluded there was no support for the concept of altered craniofacial features predisposing to clefting.

Ward et al.[35] divided parents of sporadic cleft cases into three groups by the use of a cluster analysis of the sporadic data. Two of these three major clusters showed significant deviation from the controls with an increased lower facial height and concave facial profile. One of these two groups also had a significantly larger mandible. These deviant features were also those reported to be present in children with cleft lip and palate.

There are many advantages to employing this familial, cephalometric research technique. Researching familial cases will explore new territory and concentrate on the genetic liability. Indiana families are readily available through the Indiana University Craniofacial Clinic, and the cephalometric method study of the face is well established. As noted, the significance of being able to identify such facial features is that it may allow geneticists to recognize individuals predisposed to having a child with a cleft, thereby allowing for more accurate counseling.

METHODS AND MATERIALS

Experimental and Control Populations

Families included in this study were obtained from the Craniofacial Clinic at the Indiana University Medical Center. They were chosen based on the presence of at least two persons affected with either a cleft lip or a cleft lip and palate in each kindred (first, second, and third degree relationship). A total of 50 families received a letter of invitation (appendix A) informing them of the study. Fourteen of these families expressed an interest in participating. An abbreviated family pedigree was made from the family history taken during the telephone call. As each family member was individually contacted, complete background information and medical history were obtained, and a detailed pedigree was constructed. After reviewing the familial relationships shown in these pedigrees, certain families were eliminated from further study because of failure to meet the above stated multiplex family history of cleft relatives. From the remaining participating families, a clinical/genetic designation was given to each family member as follows:

Each family member was placed in one of the following four categories: (1) Affected (cleft)--those persons with cleft lip or cleft lip and palate*. This group consisted of 20 subjects.

*Note that isolated cleft palate families were not chosen for the study since the studies of Fogh-Andersen[7] clearly show CP to be a different entity from CL(P). (2) Normal (non-cleft)--for this study, "normals" were defined as those persons who marry into the family. This group totaled 22 subjects. (3) Obligate genetic carriers--this definition comes exclusively from the pedigree and was given as follows: persons in the pedigrees located in a direct line for transmission of the cleft trait, but who are themselves not cleft. Such a situation in genetic studies is called non-penetrance and means a failure to express the phenotype (cleft) when the genotype for it is present. Thus, in Figure 1, individual II-2 has an affected mother and two affected children but she herself shows no clinical sign of a cleft. A total of 12 such subjects were identified from the pedigrees. (4) Unknown--these are non-cleft persons who are blood relatives of a cleft person but by chance could be either gene carriers or non-carriers. The unknown individuals in Figure 1 for example are II-3, II-4, II-5, III-3 or III-4. This group totaled 26 subjects.

Family members age 6 and older participated in this study. The total number of subjects was 79, and their ages ranged from 6 to 89 years. Of all participants, 76 percent were adults and the M/F sex ratio was 34/45 or 57 percent, with a preponderance of females in the total sample. All of these 79 subjects were assigned to one of the four groups described above. The unknown population was the largest of

the four groups (32 percent) while, the gene carrier group was the smallest (15 percent). By definition, there could be no children in either the family normal or carrier groups, so if the children were not cleft they were automatically assigned to the unknown group.

Cephalometric Techniques

Utilizing a standard lead apron for radiation protection from ionization, standard radiographic methods were used to make the lateral (LA) and posterior-anterior (PA) cephalographs. In order to minimize variation only one investigator made these records. Each study patient was first examined clinically for an overt cleft of the lip and palate, a submucosal cleft, or any microform of the cleft process. Also noted were any other physical abnormalities especially of the face, which including a scarring raphe of the upper lip, and all irregularities of the nasal cavity and orofacial region (Figure 2). This was necessary to rule out the presence of a cleft syndrome such as the van der Woude syndrome. All such syndromes were to be excluded from the study because of their unusual range of heritability.

To establish reliability in identification of radiographic anatomic landmarks, the investigator reviewed these landmark points to be recorded with another researcher experienced in their identification. Using a viewbox with high intensity light, the investigator first traced each study cephalograph by hand. The points to be recorded for both the

LA and PA films are listed in Tables I and II,[36] respectively, and are then illustrated in Figures 3 and 4, respectively. If the radiograph was not in focus with a sharp contrast, it was retaken. Even so, some landmarks occasionally proved to be difficult to visualize (ANS, BA, etc.). In this instance the point was estimated. Therefore, to minimize such variation, which could be due to subjective decision making as well as to inherent examiner variability, outlying points with a measurements greater than 3 standard deviations were routinely remeasured. To determine this investigator's reproducibility of measurement, 10 LA and 10 PA cephalographs were measured three times--once directly from the radiograph using computerized digitizer hardware with a software program,[37] and twice from the tracing on cellulose acetate that was made from the same radiograph. For the tracing data, the points were both measured by hand (this investigator) and digitized by another investigator (Dr. David Bixler) thereby providing an estimate of inter-examiner variability. The inter-examiner error will be discussed later in the paper.

Data Preparation

Various landmarks, 16 on the LA and 13 on the PA cephalographs, outlined different areas of the face. They all met the following criteria: (1) These landmarks are descriptive of the specific anatomic region in question. In other words they are anatomic, not constructed

(with the notable exception of GO in the LA radiograph), and (2) They are points descriptive of different regions, and hence, those representing duplication were omitted. The landmarks then incorporated into 28 linear measurements, and the measurements defined the face in various dimensions: facial height, width and depth in each third of the face (upper, middle and lower). The linear measurements and their definitions are listed in Table III.

All landmarks were digitized using a program that converted the X/Y coordinates of each variable into a series of linear measurements. To minimize differences in sex and age within the sample, the data were compared to those of previously published age and sex matched normal values.[38,39] This comparison of variables in the two populations resulted in a Z score (i.e., variation in standard deviation units), which is calculated by finding the difference between the individual measurement and the mean of the control or normal population divided by the variable's standard deviation in that same population. All subsequent analyses and comparisons were made with Z score data.

Statistical Analysis

Both univariate and multivariate statistical techniques were used to analyze the data. The programs used were from the computerized SPSS package[40] and included the following:

a. Univariate Analysis

Analysis of variance, ANOVA, was used to determine the differences in the means for each variable in each of the four groups (univariate F-ratio). Twenty-eight variables were individually compared as indicated in Table IV.

b. Multivariate Analysis

This research effort was specifically focused on identifying facial differences between the family normals and obligate trait carriers. Therefore, the population of clefts was eliminated from the discriminate function analysis.

Multivariate stepwise discriminate function analysis was the methodologic approach to defining the best combination of discriminating variables that will separate the two groups and hence identify them.

Once the discriminant function was computed, it was tested for its ability to predict group membership. The percentage of correctly classified individuals then is an indication of the effectiveness of the function. The discriminant function and those additional variables that correlate highly with it, but are not a part of it, provide information on the nature of those factors that best separate the two groups. The ultimate test is to apply this function to the members of the unknown group to see if this mixture of cleft family members can be individually identified.

RESULTS

Univariate Analysis Results

Table IV presents the mean Z score and the standard deviation for each variable in the normal and carrier groups. It also presents results of the univariate analysis on the individual mean Z scores, their F ratios, and the probabiltiy (significance) for each F-value. Univariate analysis of the 28 variables showed that only one variable had a significant difference ($p < .01$) between the two groups. This variable was NCR-MO, and its F-value was 11.42. The means of the other 27 variables were not significantly different between the obligate carriers and the normal population.

By converting the mean values for each variable in the two groups into Z scores,[41] they can then be directly compared to the same or similar mean values from a normal population, as has been illustrated by Saksena et al.[37] However, differences can be seen in the "mean pattern profiles" of these two groups (Figures 5-7).

In this study, "normal" refers to persons who were not cleft and who were related to the cleft persons only by marriage. Figures 5-7 show the pattern profiles for variables of these normals. This population deviates somewhat from published normal values, but this deviation is not unusual due to the small sample of normals used. A few

variables were +/- 2 standard deviations (or very close to it), and several others showed differences of more than 1 standard deviation. Thus, the control in this family is somewhat more variable than the normal data used here for the baseline of this study. Nevertheless, the important aspect to observe are any differences between the two study groups, the family normals and carriers.

When comparing the study normals and obligate carriers (Figure 5), the largest differences in Z scores were seen for the following variables: NCR-MO, CNS-SD and NSR-NC. Anatomically, these variables represent landmarks for upper and middle facial height.

Figure 6 illustrates the differences in facial width between the family normals and carriers. The Z scores for variables NS-NS and MX-MX show the largest differences between the two groups, and these two variables are descriptive of the middle of the face.

Concerning the variables for facial depth (Figure 7), GO-PG, N-Ba, ME-GOR and PNS-ANS showed the greatest differences, while the variables with the next highest F-value were MX-MX (3.04), CNS-SD (2.58), S-PNS (2.49), and NSR-NC (2.40). All these variables are related to mid-face development, some more significantly than others. Three of the four variables represent facial height while the other single variable relates to facial width.

Multivariate Analysis Results

Stepwise discriminant function analysis was used to select a set of variables that effectively separated and distinguished between the two groups of non-cleft persons in these families. The function as seen in Table V was comprised of the following variables: NCR-MO, LO-LO, GOR-MX from the PA radiograph and N-BA, S-PNS and PNS-ANS from the LA radiograph.

Table VI lists the standardized and unstandardized canonical function coefficients. The standardized coefficients illustrate the relative contribution of each variable to the overall discriminant function using the unstandardized coefficients. The efficiency of this function is measured by the accuracy with which it predicts actual group membership. Discriminant scores for each member of the two groups were calculated.

Table VII demonstrates the structure matrix. A structure matrix shows the correlation between the initial variables and the discriminant function. This information is valuable because, in constructing the discriminant function, the statistical procedure will exclude variables that correlate highly with those in the function. Thus, the nature of the morphological differences between the two groups can be better understood by studying this matrix. The variables with the highest correlation include NSR-NC, CNS-SD, CRO-CNS, CNS-ME and MXR-ZY. Four of these variables define facial height while the fifth (MXR-ZY) relates

to facial depth. The first two of these variables were among those with the highest F-value.

Figure 8 shows a histogram of predicted membership for both the normal and carrier individuals. The normal individuals tend to fall below a score of 1.0, while the carrier population tends to score above 1.0. When this predicted group membership is compared to actual group membership, it was found that these six variables could correctly classify 94 percent of the normal population (16/17) and 89 percent of the carriers (8/9).

Discriminant scores for the family group designated as unknown were also calculated. The histogram in Figure 9 illustrates the distribution of members of the unknown group between the normal and carrier populations, with 38 percent of the unknown group being classified as normal.

The family pedigrees in Appendix B illustrate the prediction of the classification of these unknown individuals according to their discriminant score. Initially, each individual's risk of an increased liability for cleft offspring was uncertain. A discriminant score was calculated for each individual, and a prediction of their group placement was made. Therefore, depending upon their phenotype, each individual was assigned to either the normal or carrier population. Many of the unknowns were either children or adults who had no offspring. Eight out of 12 in this subgroup were classified as normal.

Three of the families (IV, XI, XIII) were not sure on which side of the pedigree the clefting trait was being transmitted. Interestingly, in all of these cases both parents were classified as carriers.

FIGURES AND TABLES

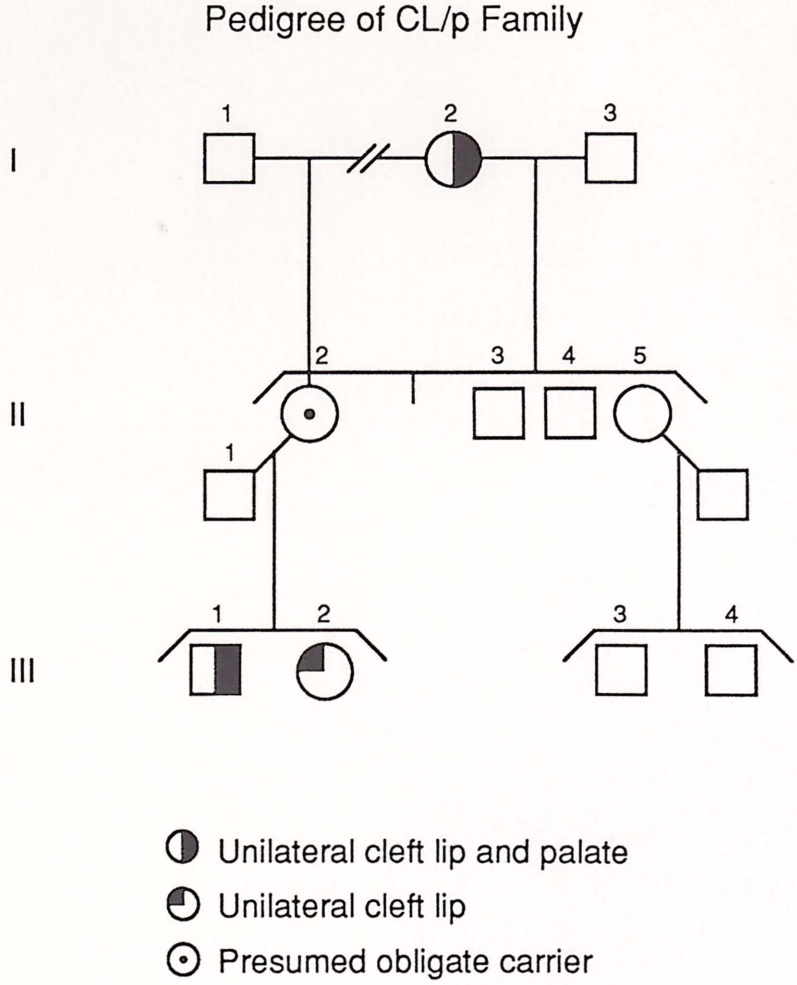

FIGURE 1. Pedigree of cleft lip/palate family.

ORAL-FACIAL VARIATION EXAMINATION

Name_____ Date_____

Birth Date_____ Sex_____

	Present	Absent	Comments
Telecanthus			
Bifid Uvula			
Submucous Cleft			
Notching of Hard Palate			
Palatal Transillumination			
Raphe of Upper Lip			
Notching of Alveolus			
Mandibular Lip Pits			
Commissural Lip Pits			
Asymmetry of Nose			
Missing/Malformed Incisors			
Other Anomalies			

FIGURE 2. Oral-facial variation examination sheet for cleft lip and palate families

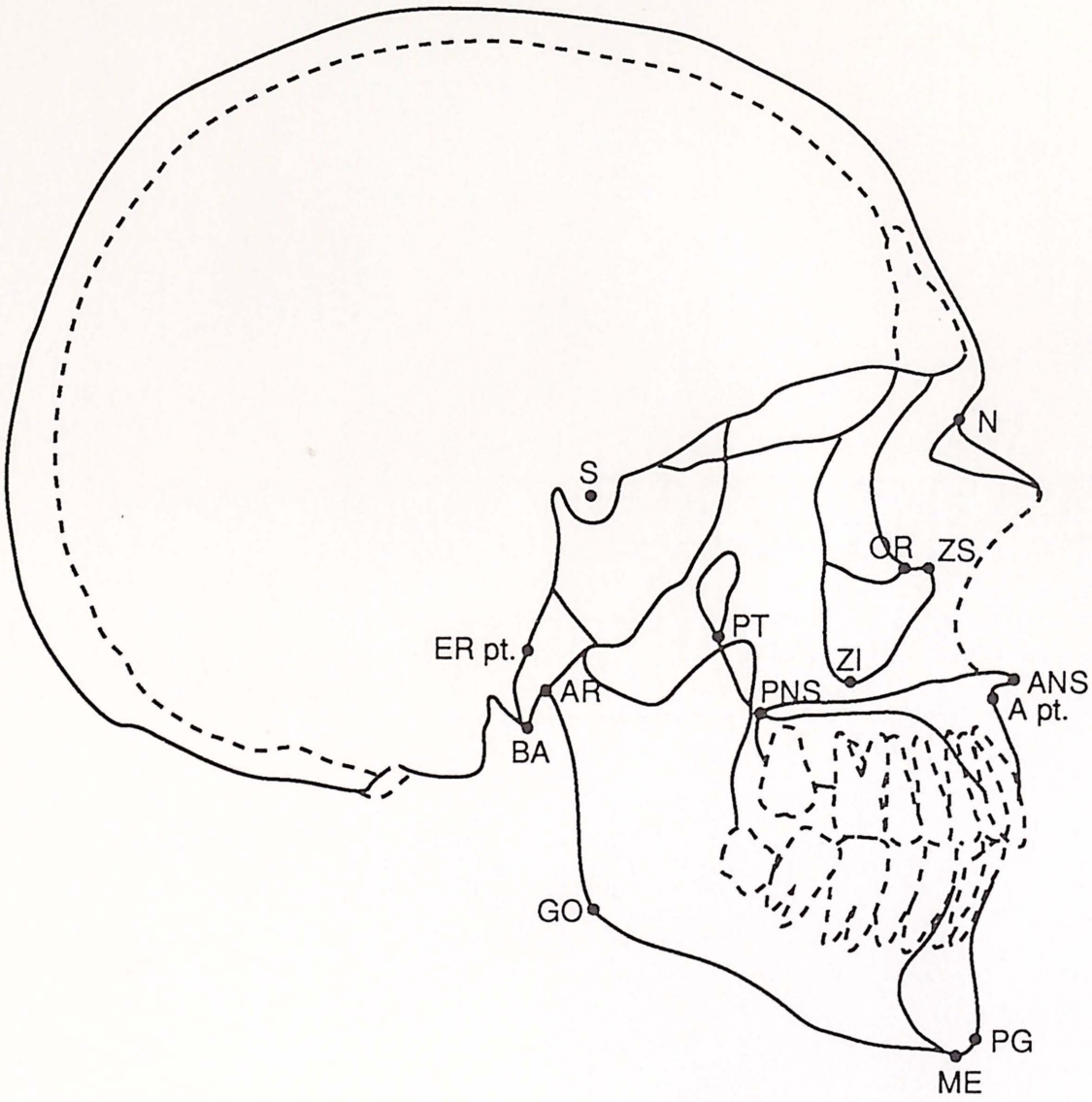

FIGURE 3. LA cephalometric landmarks utilized for measurements.

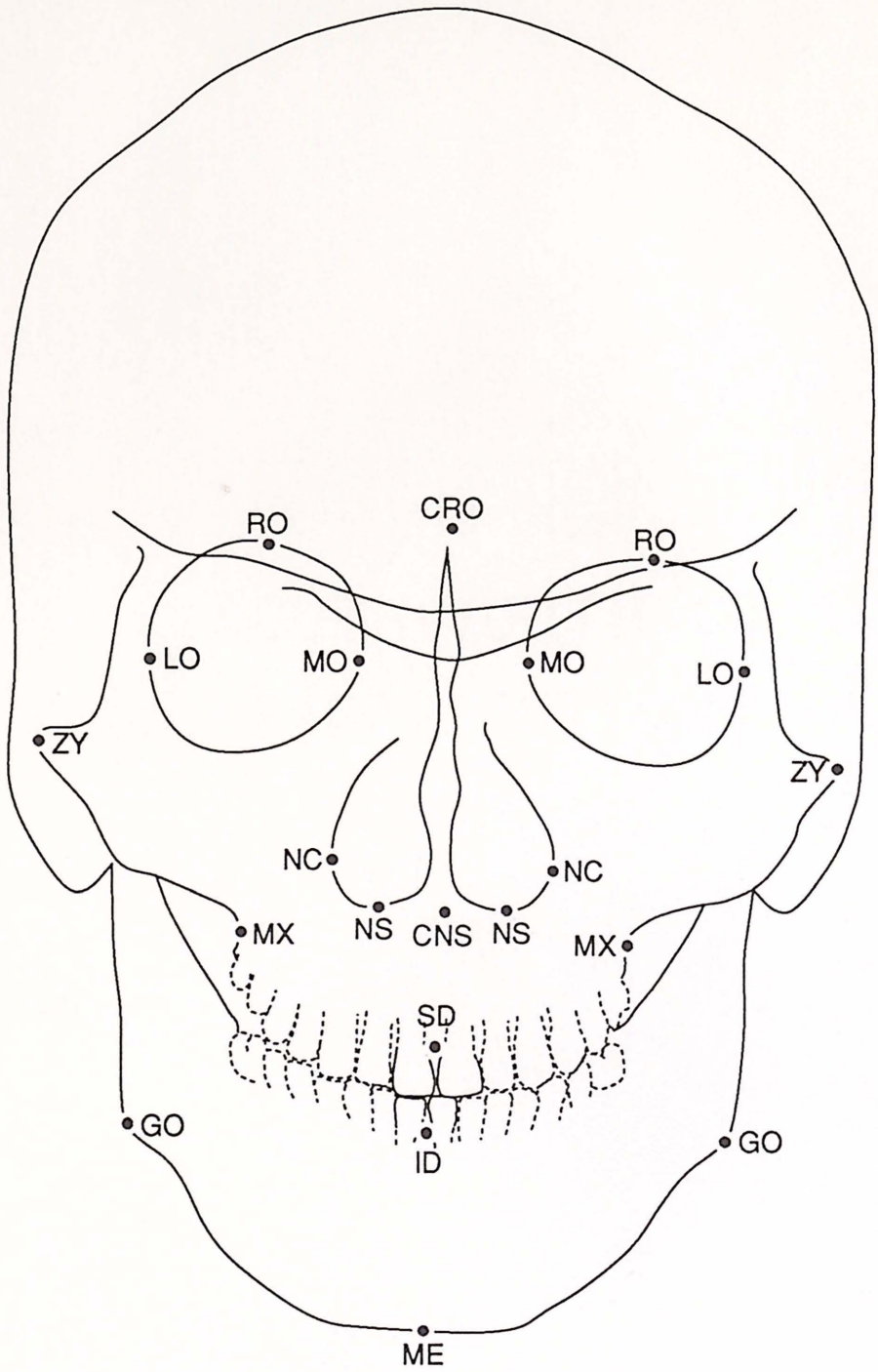

FIGURE 4. PA cephalometric landmarks utilized for measurements.

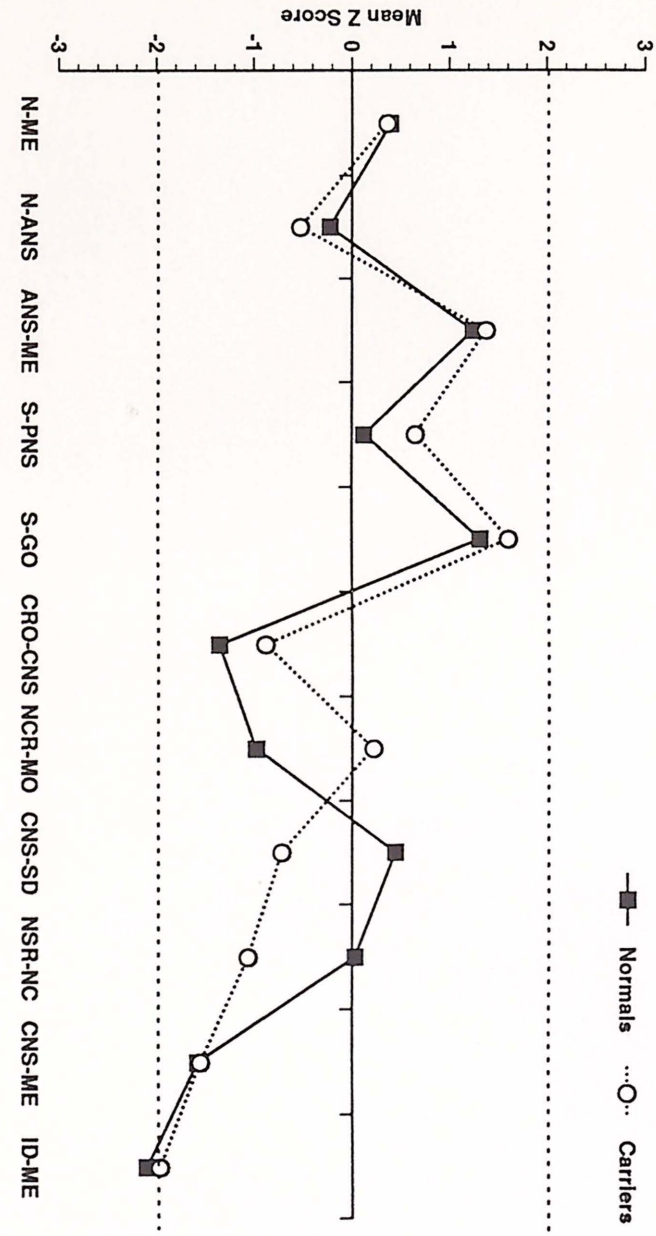

FIGURE 5. Mean Z score pattern profile for facial height.

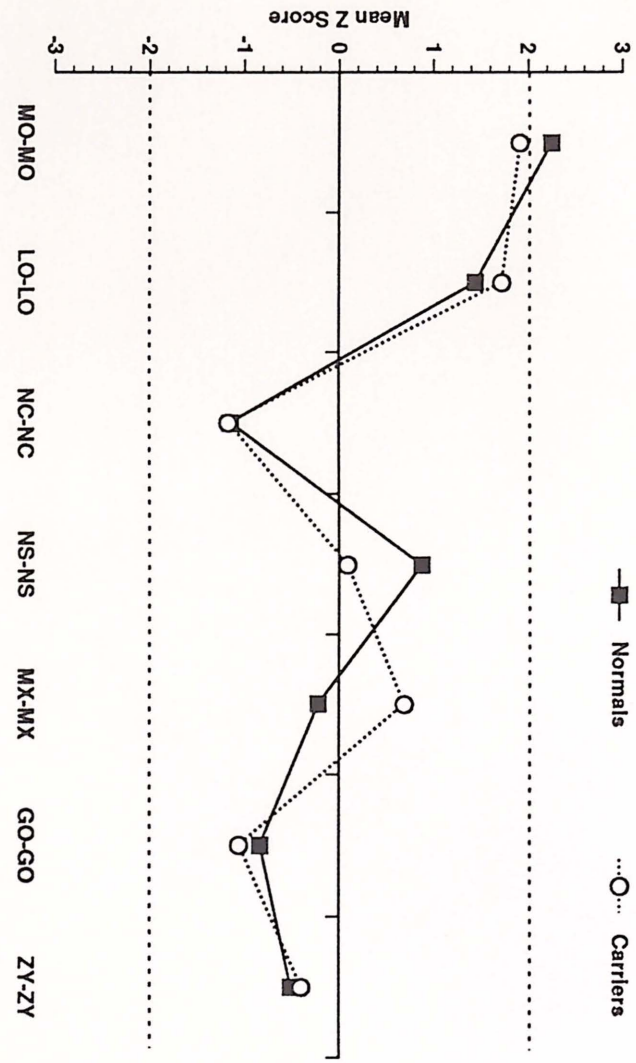

FIGURE 6. Mean Z score pattern profile for facial width.

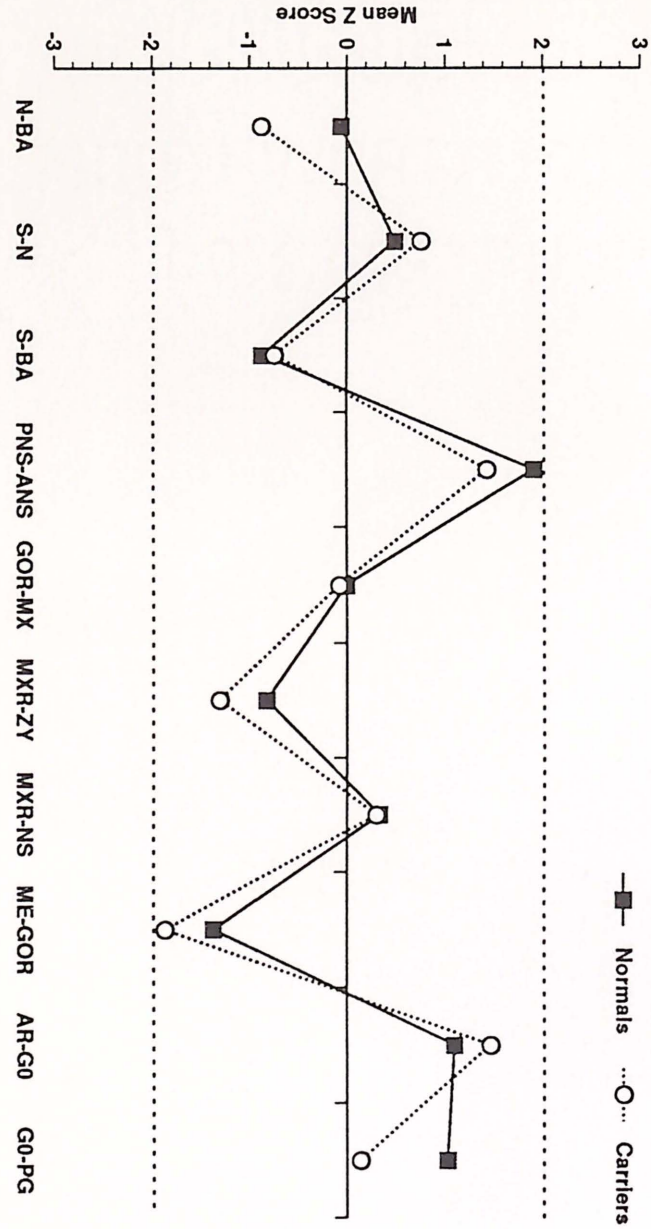

FIGURE 7. Mean Z score pattern profile for facial depth.

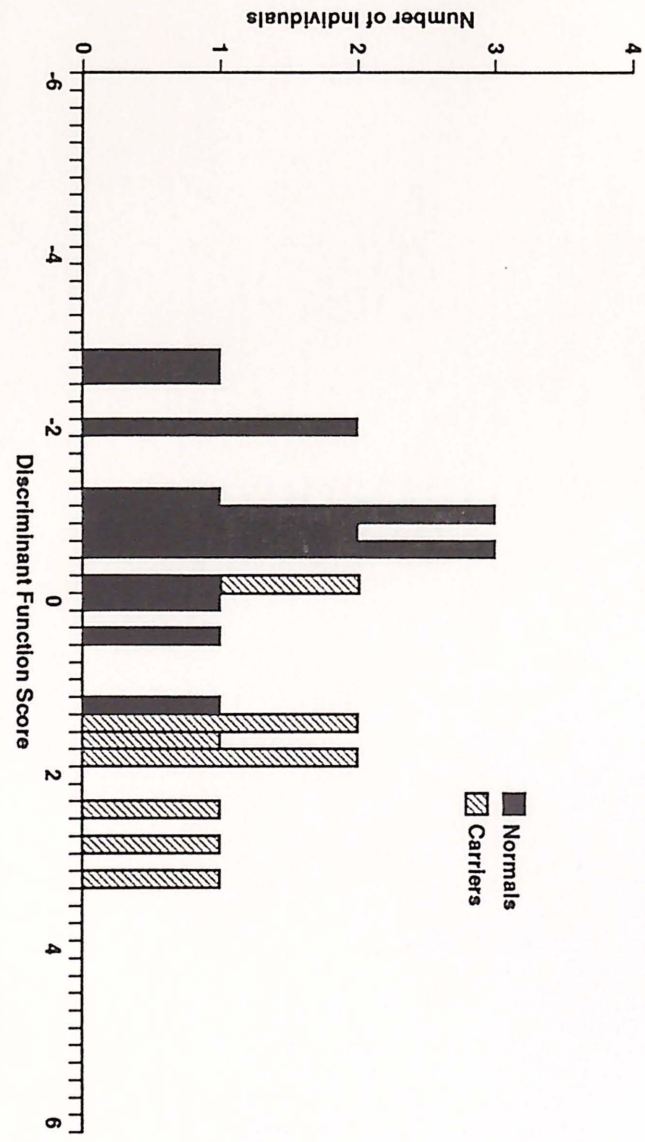

FIGURE 8. Histogram of the discriminant score for the normal and carrier population.

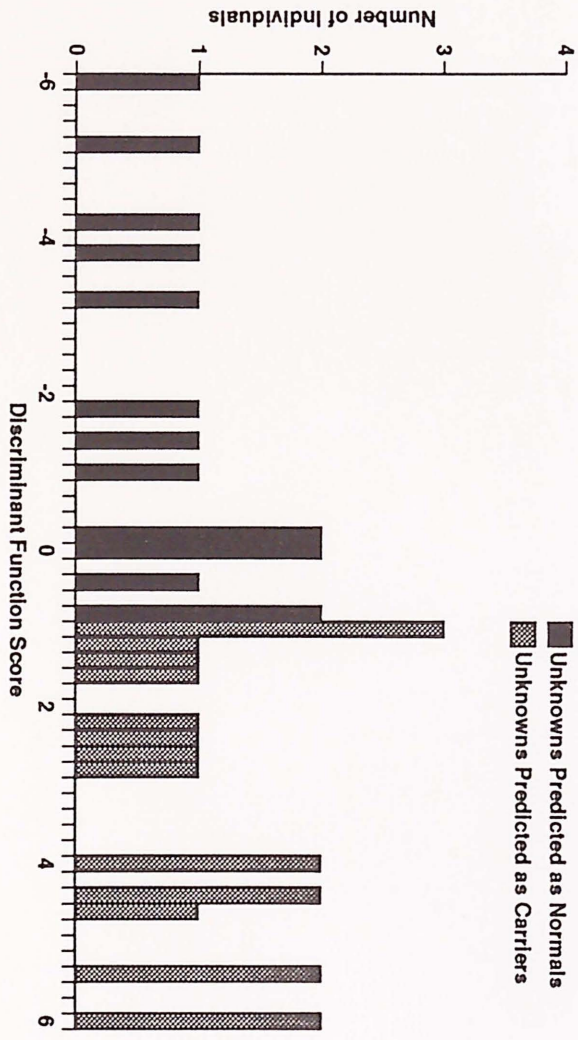

FIGURE 9. Histogram of the discriminant score for the unknown population.

TABLE I

LA cephalometric points used to evaluate the head of persons in the cleft lip and palate families

1.	Nasion	N
2.	Sella	S
3.	Orbitale	OR
4.	Zygoma Superior	ZS
5.	Zygoma Inferior	ZI
6.	Anterior Nasal Spine	ANS
7.	Posterior Nasal Spine	PNS
8.	A point	A pt.
9.	Pogonion	PG
10.	Menton	ME
11.	Gonion	GO
12.	Basion	BA
13.	Articulare	AR
14.	Pterygo-Mandibular Fissure	PT
15.	Ear Rod point	ER

TABLE II

PA cephalometric landmarks used to evaluate the head of persons in the cleft lip and palate families

	Bilateral Features	
1.	Roof of Orbit	RO'-RO
2.	Lateral Orbital Wall	LO'-LO
3.	Medial Orbital Wall	MO'-MO
4.	Zygoma	ZY'-ZY
5.	Nasal cavity	NC'-NC
6.	Nasal shelf	NS'-NS
7.	Maxilla	MX'-MX
8.	Gonion	GO'-GO
	Midline Features	
1.	Center of roof of orbit	CRO
2.	Center of nasal shelf	CNS
3.	Supra-dentale	SD
4.	Infra-dentale	ID
5.	Menton	ME

TABLE III

LA and PA cephalometric linear measurements
used to evaluate cleft lip and palate families

LA LANDMARKS/DEFINITIONS

Cranial Base	Maxilla	Mandible
N-BA	PNS-ANS	AR-GO
S-N		GO-PG
S-BA		

Facial Height-Anterior	Facial Height-Posterior
N-ME	S-PNS
N-ANS	S-GO
ANS-ME	

PA LANDMARKS/DEFINITIONS

Facial Width-Orbit	Facial Width-Nasal	Facial Width
MO-MO	NC-NC	ZY-ZY
LO-LO	NS-NS	MX-MX
		GO-GO

Facial Height-Upper	Facial Height-Middle	Facial Height-Lower
CRO-CNS	CNS-SD	CNS-ME
NCR-MO	NSR-NC	ID-ME

Facial Dept-Middle	Facial Depth-Lower
GOR-MX	ME-GOR
MXR-ZY	
MXR-NS	

TABLE IV

Variable mean Z score, standard deviation, mean Z score differences, univariate F ratio, and probability for carrier and normal family members

	Normal (N=17)		Carriers (N=9)				
	XZN	SDN	XZC	SDC	XZDiff	F	P
MOZ	2.244	1.243	1.910	2.456	0.334	0.215	0.647
LOZ	1.443	1.296	1.722	1.565	0.279	0.237	0.631
NCZ	-1.145	1.255	-1.176	1.453	0.031	0.003	0.955
NSZ	0.877	2.258	0.093	1.987	0.784	0.767	0.390
ZYZ	-0.511	1.708	-0.404	1.524	0.107	0.025	0.877
MXZ	-0.222	1.206	0.697	1.407	0.919	3.044	0.094
GOZ	-0.836	2.155	-1.067	1.463	0.230	0.082	0.777
CROCNSZ	-1.359	1.203	-0.886	1.211	0.473	0.906	0.351
CNSDZ	0.442	1.692	-0.721	1.878	1.163	2.583	0.121
CNSMEZ	-1.581	1.766	-1.551	1.315	0.029	0.002	0.965
IDMEZ	-2.101	1.488	-1.966	1.904	0.136	0.040	0.843
NCRMOZ	-0.981	0.933	0.219	0.696	1.200	11.42	0.003*
NSRNCZ	0.028	1.944	-1.063	1.087	1.080	2.404	0.134
MXRNSZ	0.329	1.682	0.299	2.148	0.031	0.002	0.968
MXRZYZ	-0.826	1.506	-1.300	1.161	0.474	0.673	0.420
GORMXZ	-0.009	1.097	-0.080	0.937	0.071	0.027	0.870
MEGORZ	-1.371	2.088	-1.863	1.323	0.492	0.408	0.529
NBAZ	-0.061	1.980	-0.880	1.263	0.819	1.256	0.274
SNZ	0.484	1.189	0.754	1.201	0.271	0.304	0.587
SBAZ	-0.870	2.185	-0.744	1.688	0.126	0.022	0.882
PNSANSZ	1.906	1.383	1.430	1.438	0.476	0.678	0.418
ARGOZ	1.094	1.505	1.467	1.319	0.373	0.391	0.538
GOPGZ	1.029	1.600	0.138	0.869	0.891	2.385	0.136
NMEZ	0.394	1.844	0.363	1.192	0.031	0.002	0.964
NANSZ	-0.225	1.100	-0.530	1.722	0.305	0.306	0.586
ANSMEZ	1.244	2.387	1.377	2.130	0.133	0.019	0.890
SPNSZ	0.126	0.928	0.652	0.490	0.526	2.485	0.128
SGOZ	1.313	0.991	1.606	1.070	0.293	0.486	0.492

* indicates significantly different Z score values from the normal population $p < .01$

TABLE V

Multivariate analysis discriminators for the normal and carrier populations

Variable	Description
NCR-MO	Middle Facial Height
S-PNS	Posterior Facial Height
GOR-MX	Lower Facial Height
N-BA	Upper Facial Depth
PNS-ANS	Palatal Length
LO-LO	Orbital Width

TABLE VI

Standardized and unstandardized canonical discriminant function coefficients

Variable	Standardized coefficients	Unstandardized coefficients
LOZ	1.205	0.866
NCRMOZ	1.262	1.465
GORMXZ	-0.857	-0.819
NBAZ	-0.644	-0.363
PNSANSZ	0.675	0.482
SPNSZ	0.935	1.156
(CONSTANT)		-1.852

TABLE VII

Structure matrix of correlations between discriminating variables and canonical Discriminant Functions

Variable	Function 1
NSR-NC	-0.43
CNS-SD	-0.37
CRO-CNS	0.30
CNS-ME	-0.29
MXR-ZY	-0.29

DISCUSSION

The results of this research support the original hypothesis that family members identified by pedigree analysis as obligate carriers of an increased genetic liability for clefting can also be phenotypically differentiated from the normal family members (non-cleft persons who are not related by blood). By definition, they do not carry such an increased liability. Since normals in this study were those persons who married into the family, and assuming even a modestly high population gene frequency for clefting, they should be at a very much lower risk to carry this trait than will those blood relatives of the clefts. Thus, "normals" are referred to hereinafter as obligate normals, a description that highlights their lack of "genes in common" with blood relatives of clefts. Obligate carriers in the study received a careful clinical examination, which showed no signs of any of the more commonly proposed microforms of clefting. However, their sub-clinical phenotype, as defined by a roentgen-cephalometric analysis appeared to confirm the idea that such individuals are at an increased risk for producing affected offspring. Indirectly, this data also supports the contention that there is a major gene that regulates the formation of cleft lip with or without cleft palate.

The univariate analysis data summarized in Table IV showed only one variable to be significantly different between the normal and carrier populations. This variable, NCR-MO, reflects facial height. It was found to be significantly smaller in the carrier population. Other investigators' research [32-35] has corroborated this finding. However, this variable taken alone is not as effective a discriminator for clefting as is the combination of variables defined in the subsequent analyses.

Examination of the mean pattern profiles provides a graphic overview of the relationship of all the variables between the two groups (Figures 5-7). Facial height, as presented by the variables NCR-MO, CNS-SD and NSR-NC, showed the greatest separation between the groups.

Figure 6 illustrates the pattern profile for facial width. The largest separation on this profile was nasal shelf widths and maxillary width, both structures of the mid-face directly related to those structures affected by palatal closure. Nasal width for carrier population was smaller than that in the normal population. Smaller nasal widths were also reported in the twin study by Johnston et al.[1] These investigators found that the non-cleft twin (of a pair of discordant, monozygotic (MZ) twins) demonstrated nasal width reduction just as the cleft lip and palate twin did. Note that from a genetic viewpoint, the non-cleft MZ twin is actually the same as an obligate gene carrier defined in this study.

The observed finding (Figure 6) of increased maxillary width (MX-MX) in the carrier population is also supported by the literature.[30,33,34]

Facial depth (Figure 7) correlates with the widest separation among the variables GO-PG, N-BA, ME-GOR and PNS-ANS. ME-GOR tends to be increased in the carrier population. This tends to support the presence of a longer mandibular body and a tendency toward a Class III occlusion, but only in relation to the maxilla. Asians show a natural predilection for a Class III occlusion, and most importantly, also show a much higher incidence of clefts than do Caucasians.[42]

While the pattern profiles suggest differences between the two groups, the wide variation in the expression of these variables makes a univariate discrimination between carriers and normal individuals difficult if not impossible.

By employment of multivariate discriminant analysis, six variables were defined, which when taken together and considered collectively readily separated the two groups. These variables are listed in Table V. When the carriers were compared to the normals, lower facial height was increased while the middle facial height was reduced. Palatal length was decreased and upper facial depth was increased in the carriers. Collectively, these results suggest a concave facial profile for carriers a tendency for a Class III occlusion. Orbital width was also increased in the carrier population. Altogether, the

discriminators proved to be effective in classifying carrier individuals 89 percent of the time and correctly classified normal individuals 94 percent of the time.

These findings can be related to the embryology of the oro-facial complex. Johnston et al.[1] have produced a lucid and detailed description of lip and palate embryogenesis based upon their observations on the formation of clefts of the lip and palate in experimental animals. According to their research, the basic etiopathogenesis of cleft lip lies in the failure of the medial nasal prominence (MNP) to fuse with the lateral nasal prominence (LNP) and the maxillary prominence (MxP). By contrast, the etiology of cleft palate is primarily related to a failure of the palatal shelves to fuse. In the 1990 study by Johnston et al.[1] of monozygotic twins, they found that in two-thirds of the cases, the primary palate (lip) cleft was caused by an underdevelopment of the MxP. This implies that there may well be two or more major mechanisms producing cleft lip--a failure of fusion (1) between the MNP and LNP and (2) between the MNP and MxP.

The set of discriminators which best differentiates carriers from obligate normals is generally compatible with those events in embryogenesis, which are most important to the clefting process as described by Johnston et al. The non-cleft trait carriers were found to have shorter palatal length, the logical result of a deficient MNP.

Other discriminators included an increased upper facial depth and lower facial height.

Palatal clefting is most often a failure of the palatal shelves to fuse. This could be due to either an abnormal tongue position or an increased facial width. Data from this study showing an increase in facial width (LO-LO) was compatible with a failure of fusion between MNP and LNP. However, this fusion failure itself can be the result of several other earlier errors such as: (1) increased head width or decreased palatal width--in both cases the palatal shelves may not make contact and fail to fuse; (2) shelves fail to elevate to the midline--due to their being blocked by an abnormal tongue position or perhaps even a biochemical failure to generate shelf force; (3) shelves contact but do not fuse--failure of epithelium to break down; or (4) shelves contact and fuse but the fusion breaks down.

An article recently submitted for publication by Ward and associates[43] describes a family study of clefts using techniques and methods similar to this one. The discriminators reported in the Ward et al. study, though not identical to those here, did define similar aspects of the face. They found not only an increase in facial width and lower facial height, but also a decrease in upper facial height in the carrier population. His study included a mandibular angle measurement where this study did not. He also found the facial profiles of the carrier group to be flatter (more concave). The conclusion was

that individuals predicted by pedigree analysis to be gene carriers of clefting have phenotypic findings which differentiate this group from normal individuals.

By examining the structure matrix (Table VII), the variables that are most highly correlated to this six element derived function but not in the function itself, can be reviewed. The variables describing mid-facial height (NSR-NC and CNS-SD), are the most highly correlated to the function. The normal group had a greater mid-facial height compared to the carriers. This coincides with the study done by Ward et al.[43] in which the carriers had a mid-facial insufficiency similar to that of clefts. This finding supports the theory proposed by Johnston et al.[1] that the etiology of clefting is associated with an underdevelopment of the median nasal prominence.

The next two variables, the first with upper (CRO-CNS) and the second with lower (CNS-ME) facial height are highly correlated, but are not part of the function itself. Both of these were found to be greater in the carrier population.

The last variable in the structure matrix representing facial depth, (MXR-ZY), is greater in the normal population. This makes sense because the carrier group would be expected to have a less convex profile again resulting from an insufficient mid-face. This time it would support the theory of a deficient lateral maxillary prominence.[1]

This study has produced a discriminant function that divides the individuals in cleft families into various subgroups. These subgroups are compared to that of the initial subgrouping which was defined and determined by pedigree analysis. By using the discriminant function to verify the initial groupings, the percent classified correctly is calculated. When utilizing this same function on the normal population, those persons who married into the proband's family but who are without a cleft themselves, all but one (94 percent) was classified correctly.

The family members who are identified as carriers of a genetic trait for liability to clefting were defined by the family pedigree as those non-cleft family members who passed on a major gene associated with clefting to their offspring (Figure 4, II-2). Again, all but one (89 percent) were classified correctly by use of the discriminant function.

A comparison was made between family normals and obligate carriers to determine the efficacy of their separation using the discriminant function. The histogram (Figure 8) helps one to visualize the actual separation of the normal and carrier populations. One of the most valuable aspects of this research lies in the attempt to classify the unknown members of the family. The members of the unknown group were defined as those people who were blood relatives of the clefts but whose risk for an increased liability for cleft offspring was uncertain. This

group's Z score data was evaluated at the group centroids by using the discriminant function. The centroids are also the group means for a particular population.

In the case of the normal population, the centroid was -0.95, and for the carrier population it was 1.79. The data obtained from the unknowns were compared to the centroids of the two groups. The assignment of phenotype to the unknowns as either normal or carrier is shown in Figure 9.

Note: When the individual's score was below 1.0 he or she was classified as normal. If it was above 1.0, he or she was classified as a carrier.

Of the 34 people designated as unknown, 38 percent were subsequently reclassified as normals and 62 percent as carriers. Although these percentages appear to be reversed since it would seem more likely to have the higher percentage of normals come out of the unknown group, they are probably correct, which suggests that the gene for this liability trait may be quite common.

An important aspect of any study employing humans is to examine the potential error involved in attempting to make objective decisions about the research data. To be able to reproduce the results as accurately as possible is essential to determining the power of the test method and hence the validity of the research results. In this study the intra-observer error was calculated. For the author, the error

figure was quite small when landmarks were attempted to be reproduced from the tracing. It was 1.3 percent for the LA and 1.8 percent for the PA cephalographs. However, when the landmark was not reproducible froma tracing but was determined from the headplate itself, the error rate was as high as 10.2 percent and overall gave a mean value of 6.8 percent. This result illustrates the problems involving objective diagnosis from un-fixed radiographic landmarks. Such an increase makes it clear why established analyses require large sample size to achieve significance. Sources of error in this study include: Locating the same point repeatedly. This can be done only if the point in question is always available for identification. On the radiographs, various points are constructed, GO and S on the LA and CRO and CNS on the PA. Identification of landmarks may be difficult lending itself to error. For the LA radiograph, these points include ANS, N, and BA and for the PA radiograph they are SD, ID and ME. In each case (LA, PA), there are landmarks which cannot be identified if the quality of the radiograph is not at least good to excellent.

In reviewing this study, various aspects could be changed to improve further research on familial clefting. Initially, better scrutiny of the family selection would be helpful. By minimizing the number of families to only five, a more thorough collection of data could be obtained by reaching more family members within that family.

More emphasis should be made on members in direct line of genetic transmission. It would also be helpful to study all first degree relatives of clefted family members if possible.

Concerning the data collection, more care could be taken while taking the radiographs so that the film's contrast is optimal. This would allow for better detection of landmarks. A review of the criteria for landmarks selection could be useful because some may be able to be added or deleted so that a better assessment of the face could be made.

In this study only a relatively small number of linear measurements were made and no angular measurements. The latter would have been most helpful in defining the facial profile, thereby allowing these results to be compared to those of Ward et al.[43]

In summary, the finding of this research appear to support the hypothesis that unaffected individuals labeled as genetic carriers by means of pedigree analysis have phenotypic features that distinguish them from normal individual and their risk for cleft offspring. This phenotype in carriers can be best discriminated by the use of just six variables describing facial characteristics.

Additional research findings could be of significance. Through genetic counselling which would include pedigree analysis and cephalometric analysis, there is the potential to identify people at risk for clefting without performing a DNA analysis. Once this group has been identified however, a blood sample for DNA molecular testing

could lead to the identification of the gene responsible for clefting. Ultimately, an individual may be able to discover the risk of producing a child with a cleft lip with or without cleft palate by either a blood test or a radiograph of the head.

SUMMARY AND CONCLUSIONS

This study investigated the craniofacial morphology in familial cases of cleft lip with or without cleft palate. The purpose was to determine if unaffected family members differed phenotypically from the so-called normal individuals in these families. This information could be very helpful in predicting and counselling unaffected persons about their genetic liability for cleft offspring.

Fourteen families with a total of 79 individuals participated. After pedigree analysis, each person was placed into one of four groups: (1) Affected--persons manifesting some form of clefting, (2) Normal--persons who married into the family but who are without a positive history of clefting in their own family, (3) Carriers--persons believed to carry the trait who may or may not show a cleft type of deformity, and (4) Unknown--unaffected blood relatives of cleft persons who must be either carriers or non-carriers for a clefting gene. LA and PA cephalographs were taken on each individual. The radiographs were digitized and multiple linear measurements of craniofacial structures were made. By converting the raw data to Z scores, the non-linear effects of growth manifested by the age and sex differences were essentially eliminated.

Univariate analysis compared the mean Z scores for 28 variables and only on variable, NCR-MO, proved to be significant in the group comparisons. This variable alone could probably not be a sufficiently good discriminator between the groups to be useful.

The mean pattern profiles illustrate the basic differences in facial morphology between the family normals (which were different than the published normals) and the gene carrier group. That is, mid-facial height was decreased and upper facial height was increased in the carrier group. Differences in facial width are seen as a narrower nasal shelf and a wider maxilla in the carrier group. The profile for facial depth once again showed variables defining the mid-face as having the largest differences between the groups.

Through multivariate, stepwise analysis, a set of six variables were found to be able to significantly distinguish between the normal individuals and the carriers. This set of variables were NCR-MO, LO-LO and GOR-MX from the PA cephalograph and N-BA, S-PNS and PNS-ANS from the LA. What these results signify is that gene carriers have: (1) an increased lower facial height and facial width, and (2) a decreased mid-facial height and palatal length. These variables, when used collectively, proved effective 89 percent of the time for the carrier population and 94 percent of the time for the obligate normal population.

The structure matrix consisted of variables which are highly correlated to the function but are not a part of that function. The first four variables represent facial height (NSR-NC, CNS-SD, CRO-CNS, and CNS-ME) and the last variable defines facial depth, MXR-ZY.

The discriminant function was utilized to calculate a discriminant score for the unknown individuals. This score allows a prediction to be made for these persons to be placed into either the carrier or normal group depending upon their phenotype. Thirty-eight percent of the unknowns were reclassified as normals.

In conclusion, the hypothesis that unaffected (non-cleft) individuals that are labeled as carriers through pedigree analysis have phenotypic features which distinguish them from the normal individual is supported by this research. These phenotype differences can be uncovered by only six variables. Employing simple cephalometric analysis, individuals could be identified at risk for clefting. These findings are not only helpful in genetic counselling but, in addition, also by taking blood samples of these high risk individuals, the gene responsible for clefting may be obtained through DNA molecular testing.

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APPENDICES

APPENDIX A

Dear Parent:

We are excited by the current research studies that are occurring around the country attempting to identify the genes that regulate specific diseases. We are initiating such a study at the Indiana University Medical Center. This study aims at the identification of the specific persons who have a gene or genetic factors involved in the production of cleft lip and palate. We plan to examine the hereditary pattern for cleft lip (with or without a cleft palate) in those families which have at least two affected members (for example: child, uncle, and grandparent; a mother and two of her children). By comparing all the facial features found in cleft persons (not counting the cleft deformity itself) to other family members who are unaffected by clefting, itself) to other family members who are unaffected by clefting, we plan to identify those features that specifically go with the clefting process. Several Indiana families meet the requirements for this study, (enough cleft and normal children to make a comparison) and yours is one of those. Therefore, we are asking you to participate in the study. If you and your family can help us, you will be asked to fill out a confidential family background questionnaire and to have two x-rays taken just as the orthodontist does.

If your child is currently being seen in the Oral Facial Clinic before the end of 1991, I will plan to adjust to your schedule and meet with you during that time. If you do not plan or need to return to the clinic in the next few months, I can set up a special time to meet with you at your convenience.

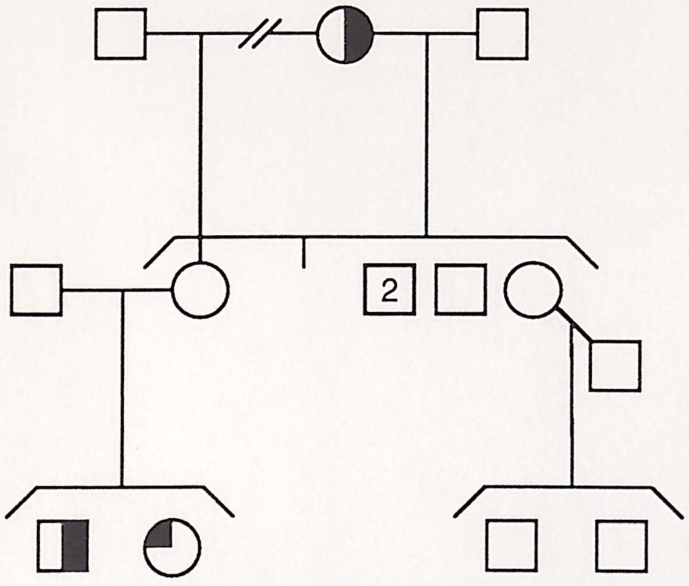
Thank you for your time. I will be in touch with you soon to discuss the details of this project.

Sincerely yours,

Stephanie M. Litz, D.D.S.
Asst. Professor of Pediatric Dentistry

Patricia Severns, M.A.
Craniofacial Program Coordinator

APPENDIX B: I



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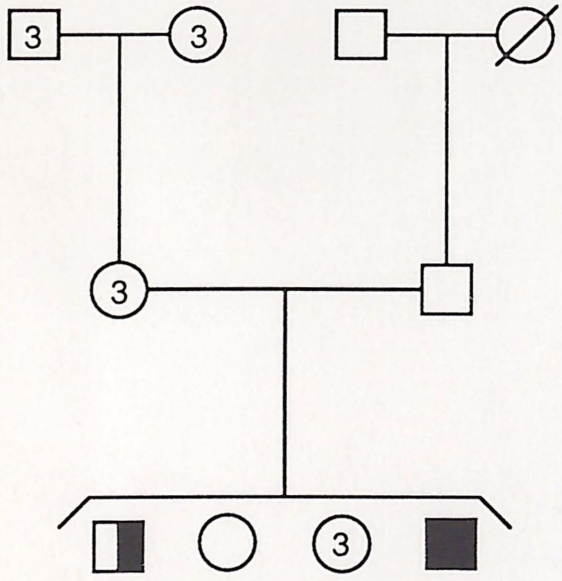

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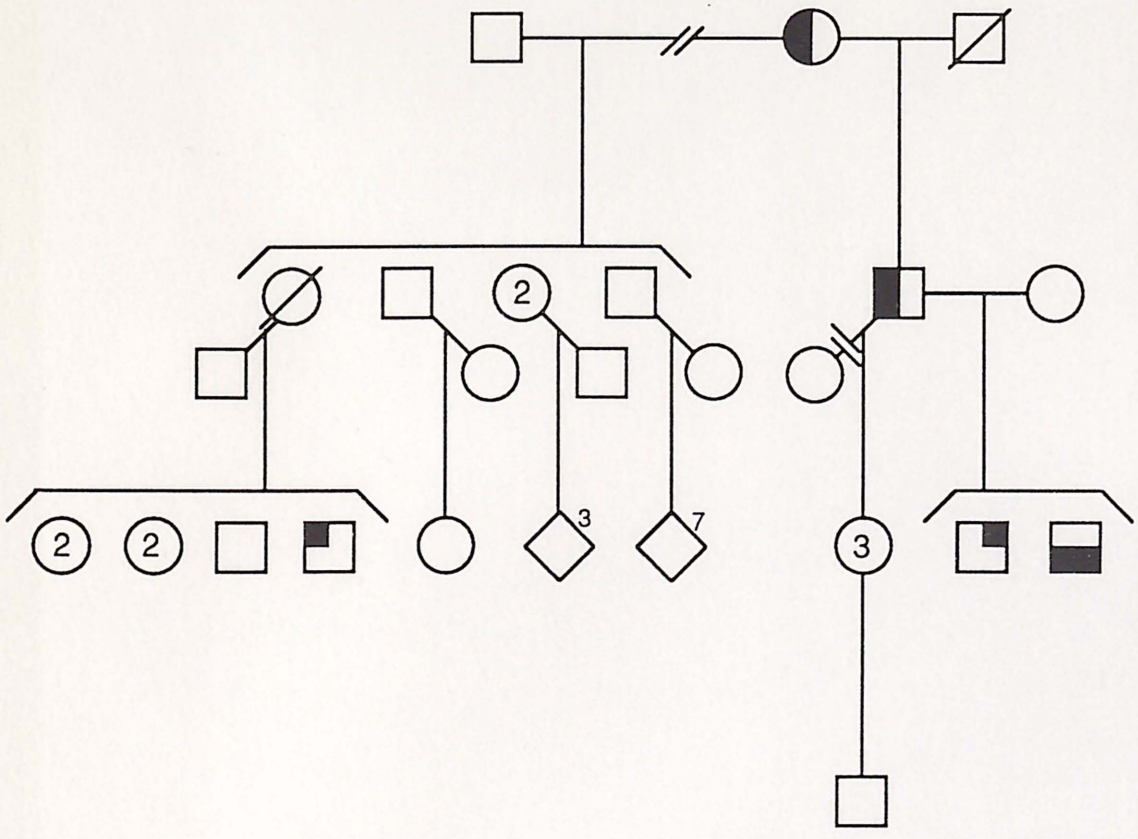

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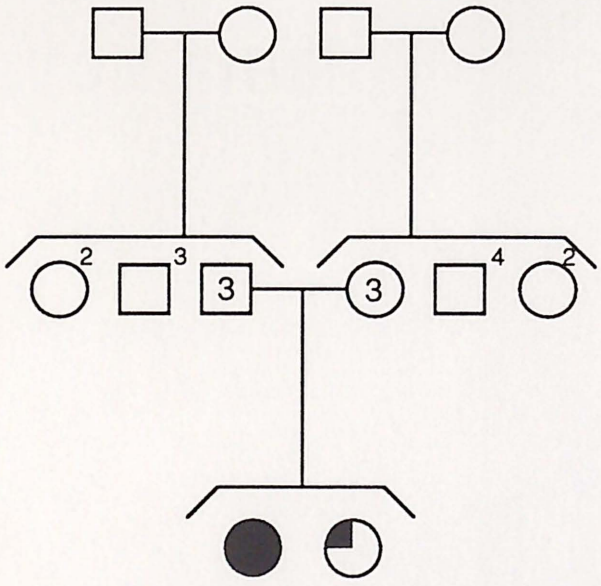

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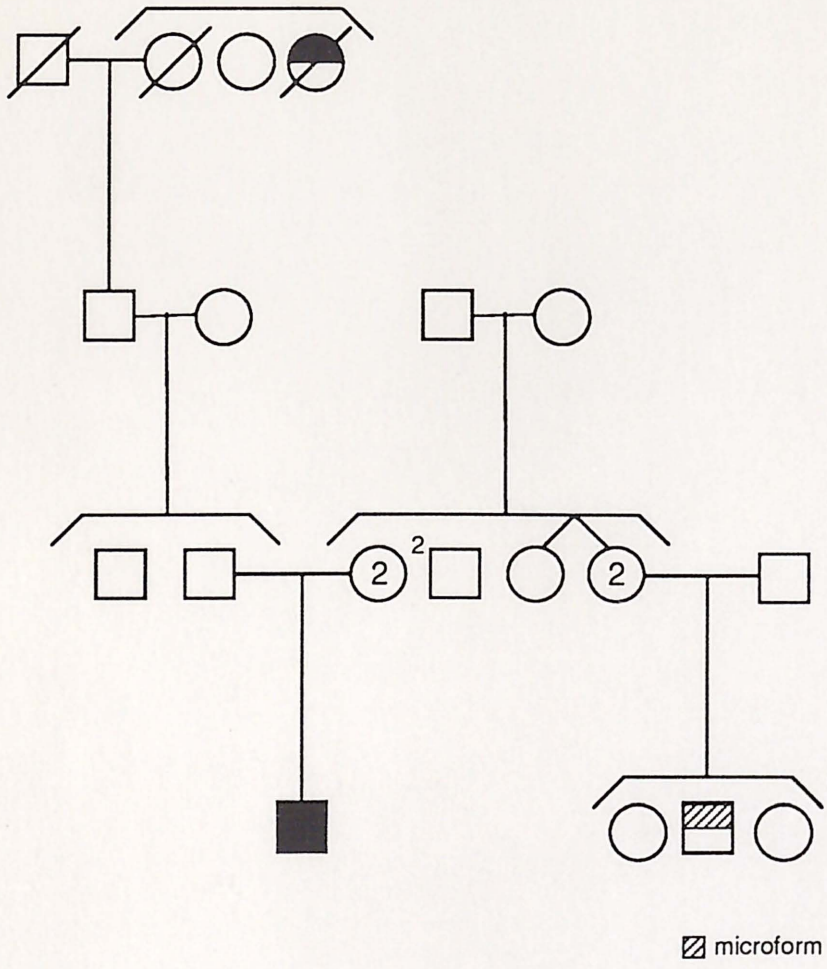

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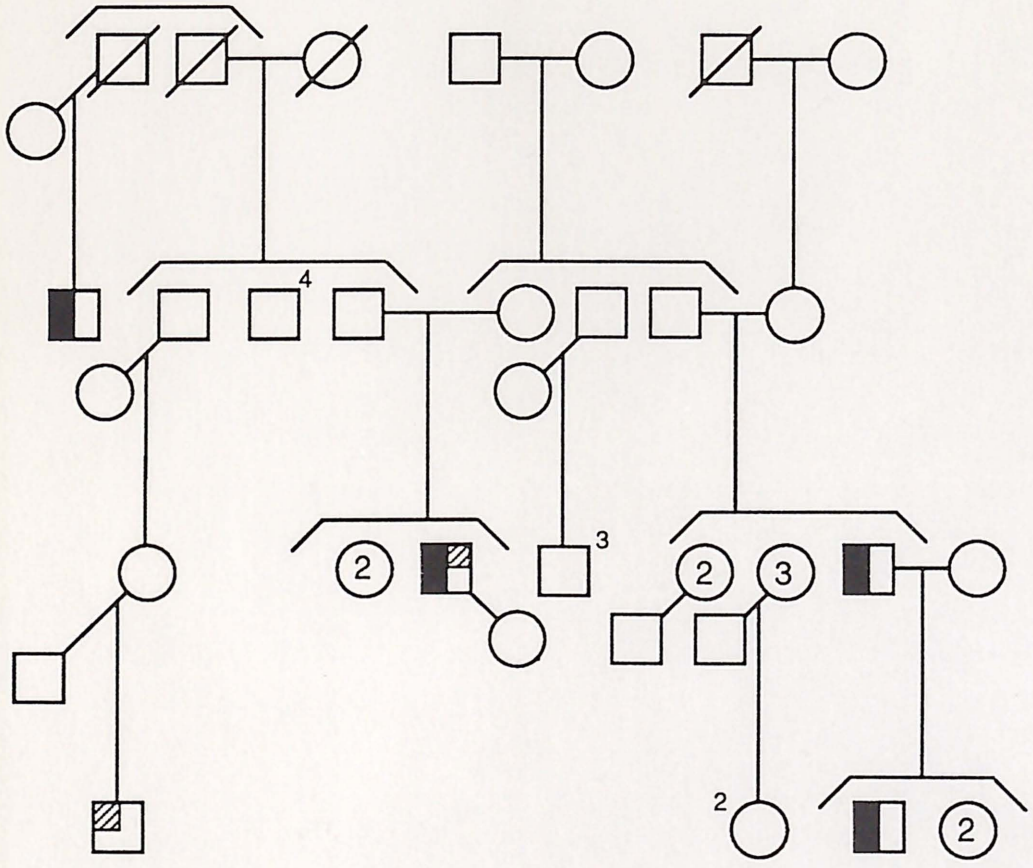

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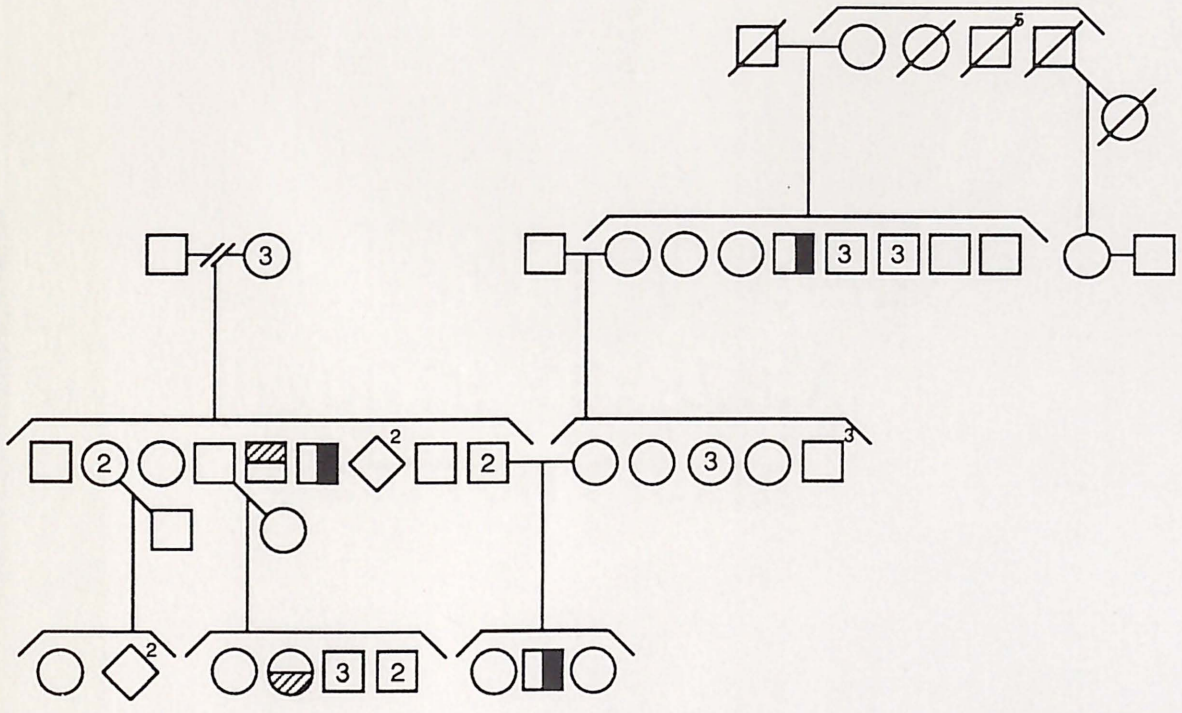

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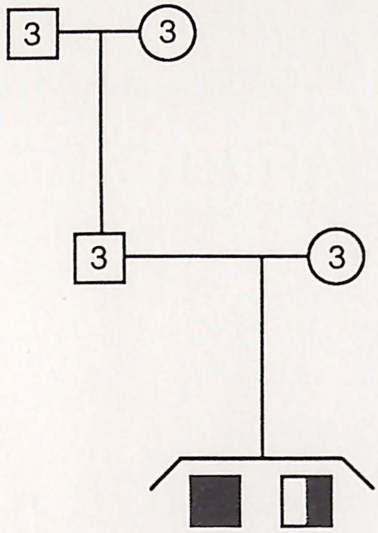

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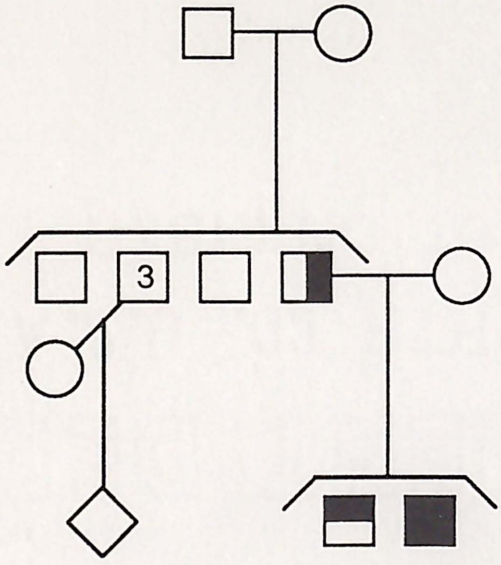

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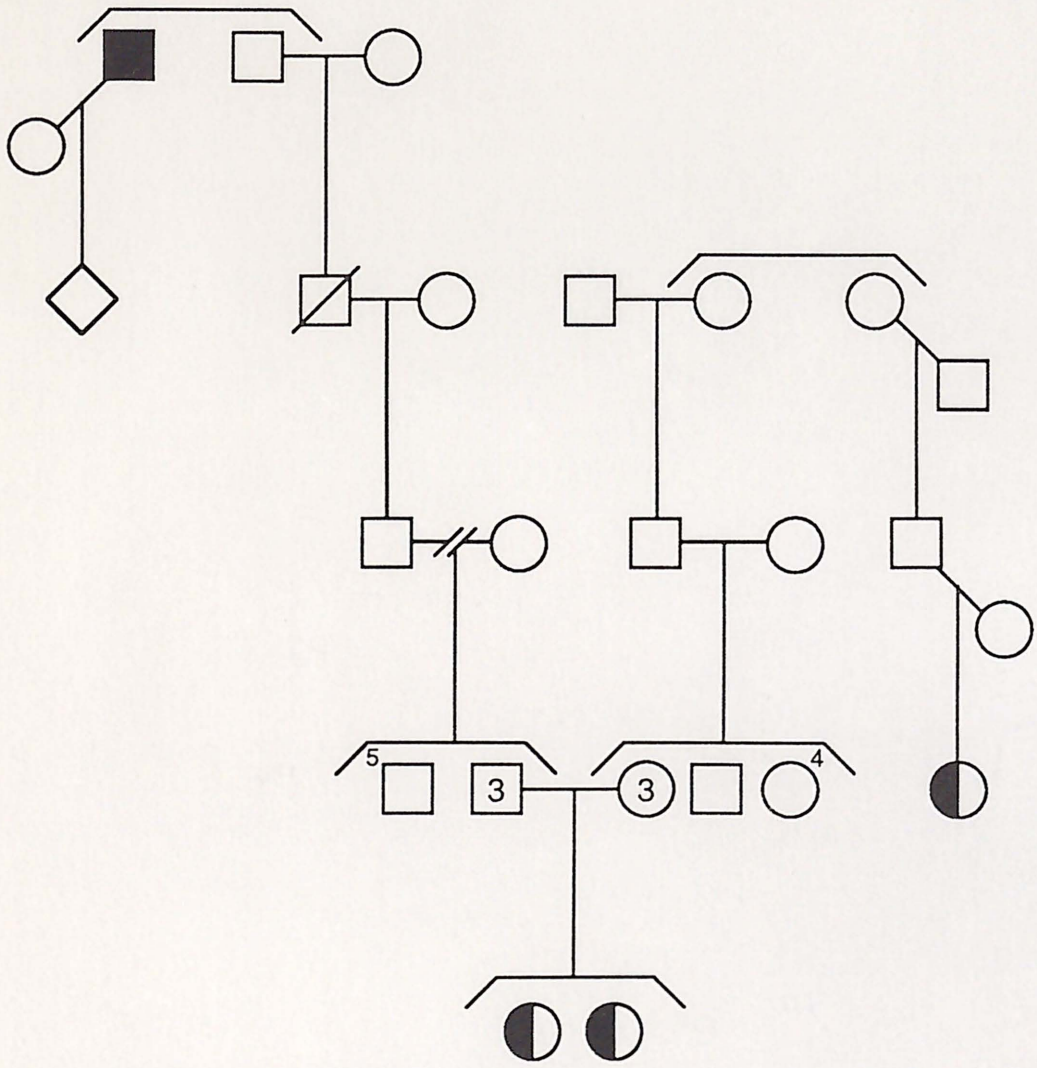

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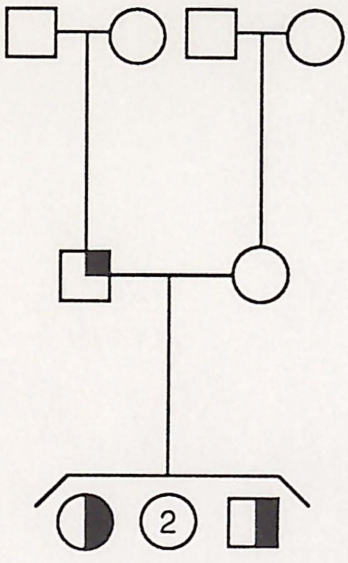

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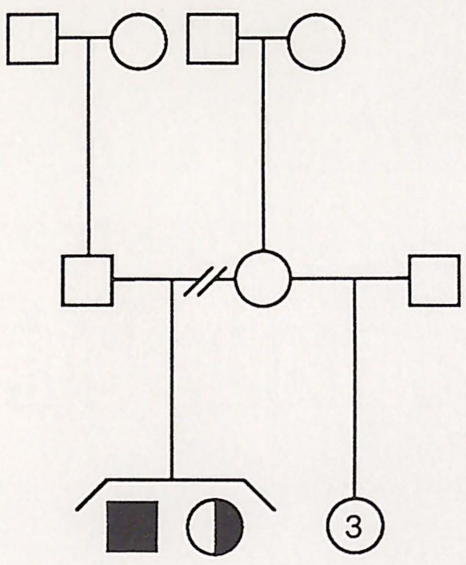

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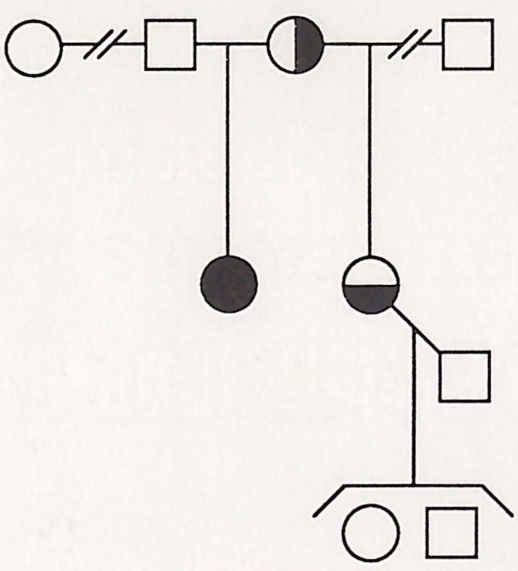

ABSTRACT

CRANIOFACIAL MORPHOLOGY IN FAMILIAL CASES OF CLEFT LIP/PALATE: PHENOTYPIC HETEROGENEITY AND GENETIC PREDISPOSITION IN UNAFFECTED FAMILY MEMBERS

by

Stephanie M. Litz

Indiana University School of Dentistry
Indianapolis, Indiana

This study investigated familial cases of cleft lip with or without cleft palate to determine whether the unaffected members of each family can be identified as gene carriers for the cleft trait. This research presumes that such carriers will have henotypic features identifiable by cephalometric analysis that are associated with an increased risk to cleft offspring. Using population genetics methodology, a pedigree analysis was made for each family member was assigned to one of four groups: (1) obligate normal, (2) affected, (3) carrier, and (4) unknown. LA and PA cephalographs were taken on each

subject and a clinical oral-facial examination carried out on participating family members. Various anatomic landmarks located on the LA and PA films were digitized and from them, a total of 28 linear measurements were made. To eliminate the effect of sex and differential age responses, Z scores were calculated.

Through univariate analysis, only one variable, NCR-MO, was shown to be significantly different between the two groups. This variable difference by itself is not adequate to differentiate those in the normal group from the carrier group. Even though only one variable was significant, other differences in the variables between theses groups become obvious when the group variables were plotted as Z scores. Since Z scores are pure values with no limits (2--the number of standard deviations in a given variable differs from normal). Thereby, age-related growth differences were minimized. Further information is gained when these Z scores are plotted as pattern profiles, Figures 5-7.

These profiles of mean Z scores for each variable pointed out areas of the face in which the differences were so great that specific anatomic areas appeared to be associated with one of the four groups. For example, gene carriers demonstrated specific alterations in facial height that might conceivably be used to discriminate that group from the other three groups.

The family normals and carriers were then analyzed by using a stepwise multivariate analysis. By this approach, a discriminant

function was generated consisting of six variables (three each from the lateral and frontal headplates), which proved to be significant in distinguishing an individual's phenotype. These variables define facial height, width and depth. The specific findings included a decrease in mid-facial height and depth along with an increased lower facial height and width in the gene carrier population as compared to the normals.

The function then was used to predict group membership of the same two groups. Comparing this analytical prediction to that of the grouping system that resulted from the pedigree analysis, all but one individual was classified correctly in both the normal and carrier population.

A discriminant score was also determined for the unknown population of family members which were defined as non-cleft blood relatives of cleft probands. Thus, they were a mixture of two types--those unaffected who carried a genetic liability for producing a cleft child and those unaffected who did not. A prediction of their placement into either the normal or carrier group was made with the discriminate function. One-third were classed in the normal group and two-thirds as gene carriers.

The results of this study confirm that the phenotype of these unaffected family members designated as obligate gene carriers differs significantly from that of the family normals. This information is not

only quite useful for genetic counselling but gives both a better understanding or the genetic control of clefting and can lead to molecular research to identify the specific gene in question.

CURRICULUM VITAE

Stephanie Marie Litz

August 2, 1961	Born in Gary, Indiana
May 1984	Associates Degree, Dental Hygiene, Indiana University School of Dentistry, Indianapolis, Indiana
September 1984	Married Steven Craig Litz, Merrillville, Indiana
November 1988	Delivered twins: Brandon William Litz and Marika Danielle Litz
May 1989	DDS, Indiana University School of Dentistry, Indianapolis, Indiana
June 1991	Pediatric Dental Residency, Indiana University School of Dentistry, James Whitcomb Riley Hospital for Children, Indianapolis, Indiana
June 1991	Recipient of the Ralph McDonald Outstanding Pediatric Dentist Award
July 1991	Appointed Assistant Professor at James Whitcomb Riley Hospital for Children, Indianapolis, Indiana
January 1993	Appointed Faculty at Methodist Children's Hospital, Indianapolis, Indiana

January 1993

Appointed Pediatric Dentist on the Craniofacial Team at Methodist Children's Hospital, Indianapolis, Indiana

Professional Organizations

American Dental Association
Indiana Dental Society
American Academy of Pediatric Dentistry