Original Article

Phenotypic Characterization of Class III Patients

A Necessary Background for Genetic Analysis

Chi Bui[a]; Terri King[b]; William Proffit[c]; Sylvia Frazier-Bowers[d]

ABSTRACT

Objective: The objective of this study is to characterize the convergences of dentofacial form of skeletal Class III malocclusion in individuals to test the fundamental hypothesis that there are distinct subtypes of Class III malocclusion.

Materials and Methods: A detailed phenotypic characterization was performed on a retrospective cohort of 309 subjects using cluster and principal component analyses on 67 cephalometric variables.

Results: The results indicated that there are five clusters representing distinct subphenotypes. The principal component analysis suggested that the groupings of variables reflect anteroposterior and vertical dimensions rather than specific craniofacial structures. This may ultimately suggest that different genes are involved in controlling dimension vs structures.

Conclusions: Our phenotypic dissection of Class III malocclusion established distinct subtypes in a large sample of patients and will ultimately provide the basis for future familial studies to identify a causative gene. (*Angle Orthod* 2006;76:564–569.)

KEY WORDS: Class III; Prognathism; Multivariate; Analysis

INTRODUCTION

Skeletal Class III malocclusion clearly has a significant genetic component. It has been observed for many years that mandibular prognathism, and, perhaps to a lesser extent, maxillary deficiency run in families. It also is apparent that multiple patterns of Class III malocclusion exist. Even a diagnosis that is extended to identify the jaw most at fault is not adequate in distinguishing different phenotypes.

A necessary first step in establishing the genetic contribution to these problems is to distinguish phenotypes that can be related to different expressions of that patient's genotype. Once the Class III phenotypes are fully characterized by establishing distinct subtypes in a large sample of patients, the resultant subtypes can be compared to familial cases and provide the basis for linkage studies to identify a causative gene. Moreover, clues to identifying genetic factors influencing skeletal Class III malocclusion may be found in detailed analyses of the facial characteristics from an extensive family study.

Cephalometric analysis probably is still the best way to approach the definition of phenotypes within the Class III population, although three-dimensional analysis via computed tomography undoubtedly will play a larger role in the future. A great advantage of cephalometrics at present is that these radiographs exist for a large population of Class III individuals who have been evaluated for possible treatment. Newer methods of analysis to evaluate the shape and form of the craniofacial complex, such as elliptical Fourier analysis, thin-plate spline analysis, and finite-element morphometry, can be applied to the definition of phenotypes.[1–3] In addition, multivariate analyses (discriminant, cluster, and principal component analyses) have been used to distinguish between Class I and Class III subjects and potentially are quite useful in distinguishing phenotypic variations.[4–7]

The goal of this study is to characterize the conver-

[a] Former Graduate orthodontic resident, Department of Orthodontics, The University of North Carolina at Chapel Hill, Chapel Hill, NC; currently in private practice, Orange County, Calif.

[b] Assistant Professor, Department of Pediatrics, The University of Texas Health Sciences Center at Houston, Houston, Tex.

[c] Professor, Department of Orthodontics, The University of North Carolina at Chapel Hill, Chapel Hill, NC.

[d] Assistant Professor, Department of Orthodontics, The University of North Carolina at Chapel Hill, Chapel Hill, NC.

Corresponding author: Dr. Sylvia Frazier-Bowers, Department of Orthodontics, School of Dentistry, The University of North Carolina at Chapel Hill, 7450 Brauer Hall, Chapel Hill, NC 27599 (e-mail: sylvia_frazier@dentistry.unc.edu)

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Table 1. Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
ANB < 1	Previous orthodontic treatment
Overjet ≤ 0	Congenital abnormalities
Anterior crossbite	Trauma
Concave profile	Missing or undiagnostic cephalogram

The inclusion and exclusion criteria for study group indicate inclusion criteria summarized above where two out of three attributes were required for inclusion and lists exclusion criteria.

Table 2. Descriptive Statistics for Study Group

Variables	
Sample size	309
Race[a]	
Caucasian	225 (72.82)
African-American	52 (16.83)
Hispanic	9 (2.91)
Asian	16 (5.18)
Other	7 (2.27)
Sex[a]	
Male	131 (42.39)
Female	178 (57.61)
Age[b]	19.10 $\pm$ 9.89
Male	19.43 $\pm$ 10.16
Female	18.86 $\pm$ 9.71
Range	5.92–56.25

[a] n (%).
[b] Mean (years) $\pm$ SD and range.

gences of dentofacial form into subtypes of skeletal Class III malocclusion in individuals in a retrospective cohort study as a step toward future studies to determine whether certain subtypes of Class III are more likely to be genetically determined. The findings from this population study would be applicable to familial cases to aid in future genetic analyses, such as genotyping and linkage studies.

MATERIALS AND METHODS

Subjects

The study sample was derived from the cohort of 356 patients with a clinical diagnosis of skeletal Class III malocclusion who presented to the University of North Carolina (UNC) between 1995 and 2004 for treatment in the graduate orthodontic clinic or evaluation for orthognathic surgery. Of this group, 47 were excluded because of the inability to find their pretreatment lateral cephalometric radiographs or failure to meet the radiographic diagnostic criteria noted in Table 1. Table 2 summarizes the demographic characteristics of the final sample.

Cephalometric analysis

The lateral cephalometric radiographs were taken in natural head position with posterior teeth in maximum intercuspation, except when an anteroposterior shift was detected. In those cases, the radiographs were taken in centric relation. The radiographs were digitized using a 67-point model for anteroposterior and vertical structures in Dolphin Imaging 9.0 (Dolphin Imaging Systems, Chatsworth, Calif), and 38 linear, 25 angular, and 4 proportional measurements were calculated.

Method error

Ten randomly chosen radiographs were retraced three times at 2-week intervals. The error method between the replicate tracings was calculated using the intraclass correlation coefficient, *R*, which ranged from 85.7% to 99.8%. However, the intraclass correlation coefficient was greater than 97% for most variables. In fact, only two variables (% nasal height and posterior : anterior face height) were less than 90%.

Data normalization

For the statistical analysis in this study, all measured values were adjusted with linear regression with age, race, and sex as covariates and the residual values were used. This method was chosen for normalization of the data to provide a variance structure appropriate for our sample. Although *z* scores would also adjust for age and sex, the variance would be greater when compared with our Class III population because the *z* scores are based on norms of Class I individuals.

Multivariate analysis

A cluster analysis (SAS version 7.1, Cary, NC) using the normalized cephalometric values was performed. The upper limits of the cluster were determined by evaluating the percentage of variances explained as a function of the number of clusters. We sought to minimize the number of clusters while explaining the most variance. The maximum iteration was set at 50, and the convergence criterion was 0.02. To obtain a derived phenotype, a pattern analysis was performed. One representative case that was closest to the mean value of the variables of the cluster was chosen as the prototype of that cluster. In addition, a principal component analysis was performed. Again, we maximized the variance explained with the principal components.

RESULTS

The preliminary cluster analysis revealed 10 groups of Class III subphenotypes based on the cephalomet-

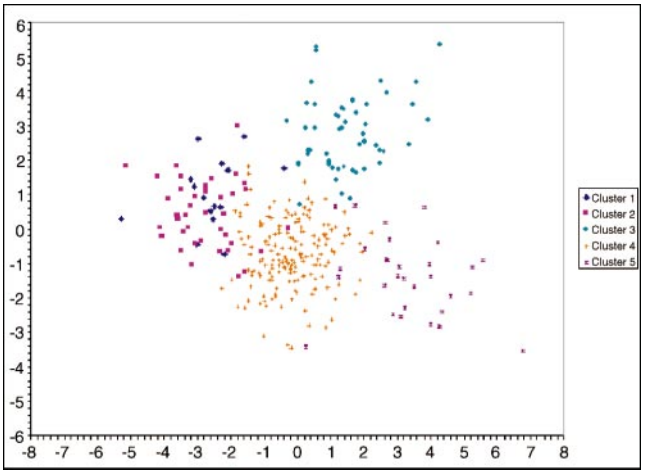

FIGURE 1. Diagrammatic representation of the five clusters identified, each represented by a unique symbol and color as indicated on the legend at right.

Table 3. Summary of Cluster Analysis[a]

Cluster	No. Members	Nearest Cluster	Description
1	31	4	Mandibular prognathic, long face
2	55	4	Maxillary deficient, low angle
3	96	4	Maxillary deficient, high angle
4	77	3	Mildly mandibular prognathic, normal
5	50	4	Combination, normal

[a] Summary of cluster analysis shown by cluster number (1–5); number of participants in each cluster; cluster with the most overlap; and description of the facial type represented by each cluster.

ric measurements. However, we limited the analysis to five clusters because they yielded the most distinct subphenotypes (Figure 1; Table 3). Cluster 4 was centrally located, and members of this cluster overlapped with other clusters. Cluster 3 had the largest number of members, whereas cluster 1 the fewest.

The prototypes of each cluster are illustrated in Figure 2. Cluster 1 is characterized by very extreme mean values of the variables and corresponds with mandibular prognathic and long-face subjects. Cluster 4 represents the mildly mandibular prognathic subphenotype. Subjects in cluster 2, on the other hand, have maxillary deficiency and decreased vertical dimensions. Individuals in cluster 3 also exhibit maxillary deficiency but are high angle. Except for decreased maxillary unit length, most of the mean variables in clusters 2 and 3 deviate in opposite directions. Cluster 5 is defined by borderline Class III individuals, and the mean for most of the variables in cluster 5 are increased.

The results of the principal components analysis (PCA) revealed five principal components, which explained 67% of the variance (Figure 3). Although there is a 17% change in the variance explained between the third and fifth PC, only the first three principal components were used because the first three PCs were more direct in their anatomic explanation. The variables in each principal component are summarized in Table 4. The first principal component consisted of sagittal parameters. The variables, such as ANB and facial taper, primarily describe the anterior part of the face. The second principal component was significant for vertical measurements, such as lower face height and SN to mandibular plane angle. It also consists of variables to describe lower incisor position. The third principal component consists of variables related in both anteroposterior and vertical dimensions. The remaining principal components consisted of multiple variables, including saddle angle.

DISCUSSION

The cluster analysis enabled us to systematically determine the classification of the subjects to describe skeletal Class III malocclusion. Although cluster analysis is based on advanced statistical theory, there is variability in determining the number of clusters. This depends in part on the number of variables and their intercorrelation and on the investigators' decision as to the clinical relevance of the clusters that are differentiated statistically.

The cephalometric variables used in our study were standard angular, linear, and proportional relationships that are included in well-established analyses. Some studies used esoteric analyses and less clinically useful landmarks. Mackay et al[8] used a centroid method and Hong and Yi[9] relied on the Delaire analysis to evaluate craniofacial morphology. We chose five clusters because this placed subjects into clinically useful subphenotypes. This number of clusters is comparable to previous studies involving cluster analysis of Class III subjects. Mackay et al[8] also found five clusters, whereas Abu Alhaija and Richardson[10] described three clusters and Hong and Yi[9] had seven clusters.

Although there is a certain amount of subjectivity in the cluster analysis, we believe that our results can be generalized to the population in the United States. The cohort at UNC consisted predominantly of Caucasians, with less than 20% African-Americans. These racial proportions are representative of most areas of the country. Many of the previous studies that attempted to categorize Class III facial forms were based on homogeneous populations with limited subjects, and thus, the results are less likely to be relevant to the American population. Mackay et al[8] based their studies on a group of British schoolchildren from Manchester. The study of Mouakeh[11] was based solely on Syrian children and that of Lu et al[12] was based on a Japanese population. Another aspect in which our

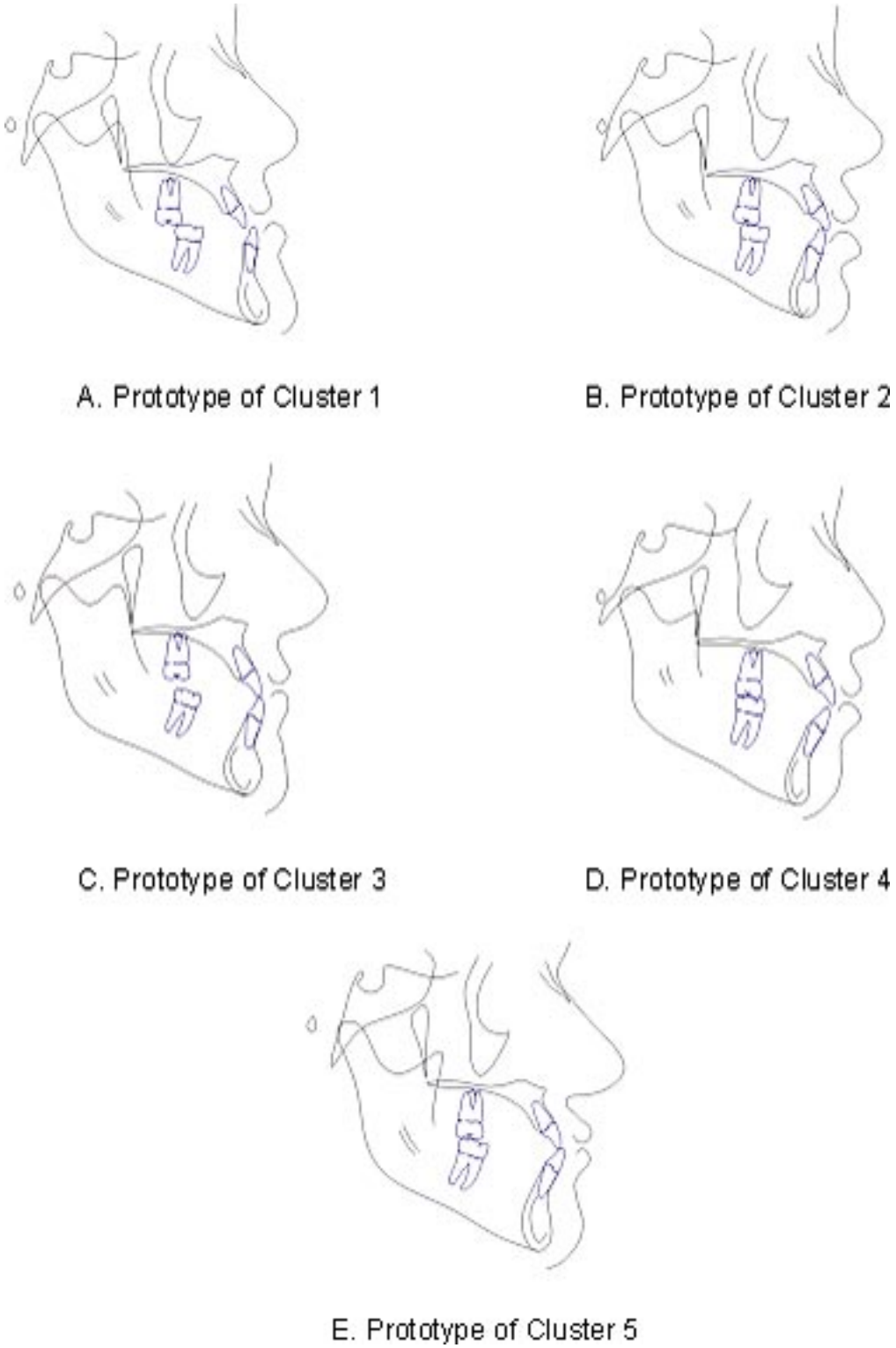

FIGURE 2. Representative cephalometric tracing from each cluster as described in Results.

sample is more generalized is that it contains subjects with mild to severe cases because our goal was to characterize the spectrum of variation in the Class III subphenotypes. The level of severity of the Class III patients in other studies was usually more severe than our cohort. Many of the previous studies used patients who required orthognathic surgery to correct their facial deformity.[7,8,13]

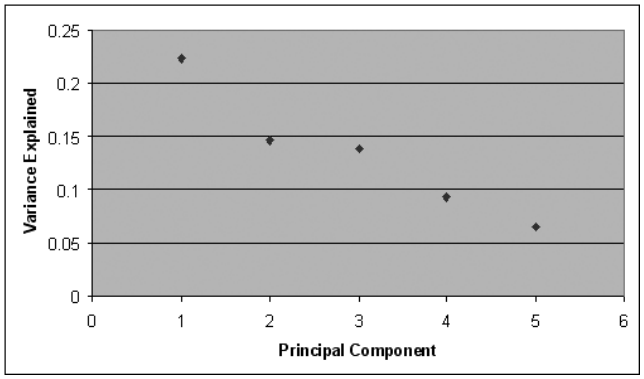

FIGURE 3. Scree plot of principal components illustrating the variance explained by each component in percentage. The total variance explained can be attained by adding each value plotted on the y-axis for a total variance explained of 67%.

The principal component analysis was performed to obtain information that could serve as a precursor for genetic analyses. The PCA identified the most highly correlated variables in the data set. Although these principal components cannot be directly related to clinical subphenotypes, they are useful in highlighting some cephalometric parameters that may often be overlooked in our UNC analysis, such as the saddle angle. The principal component analysis also raised some questions on the validity of the conventional Class III parameters. Variables such as overjet and Wits analysis, traditional measures of Class III, were not as highly correlated as were other variables in the PCA. The groupings of variables in the principal components reflect anteroposterior and vertical dimensions rather than specific craniofacial structures. This may suggest that different genes are involved in controlling dimension, not structures. If this is true, it may cause a paradigm shift in our current clinical categorization of skeletal Class III malocclusion, which is largely based on maxillary deficiency and mandibular excess. Other studies that have explored this topic agree that Class III malocclusion contains a genetic component but also highlight the importance of the gene-environmental interaction.[13]

This study is a part of a larger study to determine the phenotype-genotype correlation of skeletal Class III malocclusion. The phenotype dissection will be applied to families to determine whether there is a convergence of subphenotypes within families. The multivariate analyses used in this study used values that were adjusted for race and sex, and hence, we could not report the effects of those factors. However, because previous studies have reported that there is sexual dimorphism of the Class III trait, future studies should investigate this aspect further.[14–16] Also, the possibility that individuals of the same racial group show a convergence of the Class III subphenotype would further support the role of genetics in this skeletal disharmony. Furthermore, newer technologies in both clinical and molecular diagnosis are developing and will be incorporated into the ongoing study.

Although determining the genetic basis of skeletal Class III malocclusion may not have a direct clinical application in the immediate future, detection of the gene(s) involved holds promise for vast improvements in the management of such patients. Such knowledge may be used to accurately predict long-term growth changes and therefore treatment modalities. In turn, the genetic and molecular discoveries can direct pharmacological interventions. In any case, as the field of orthodontics continues to develop technologically and philosophically, we can expect that advances in diag-

Table 4. Summary of Principal Component Analysis[a,b]

	1	2	3	4	5
(a) Principal component					
(b) Variance explained	0.2226	0.1466	0.1386	0.0933	0.0647
(c) Cumulative variance		0.3693	0.5079	0.6012	0.6659
(d) Variables	Facial plane-SN	SN-GoGn	L1-FH	S-Articulare	Saddle°
	ANB	L1-N B point	Mandibular unit length	SN	FH-SN
	Facial angle	L1 protrusion	Unit length difference	Ramus height	ANB
	B point-N⊥	L1-N B point	Interincisal	Midface	Wits appraisal
	Pogonion-N⊥	Total face height	Upper face height	Maxillary unit length	Overjet
	STN⊥-Lower lip	Lower face height		Posterior face height	A-N vert
	STN⊥-Pogonion	L1-GoGn		U6-Mandibular plane	ST N vert-Upper lip

[a] ST indicates soft tissue; L1, lower incisor; SN, saddle-nasion; FH, Frankfort Horizontal plane; U6, upper first molar.

[b] A summary of principal components (a) shows the variance (of the Class III trait) explained by each principal component (b); the cumulative variance explained by sequentially adding each component (c); and the variables contained in each component (d).

nosis and treatment planning are imminent and inevitable.

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