## *Original Article*

# Morphometric analysis of craniofacial features in mono- and dizygotic twins discordant for unilateral cleft lip and palate

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**ABSTRACT**

**Objective:** To compare craniofacial differences between twins discordant for surgically repaired unilateral cleft lip and palate (CLP) during the developmental ages and to test the effect of zygosity on the shape and size of the craniofacial skeleton of the same twins by means of thin plate spline (TPS) analysis.

**Materials and Methods:** Lateral and posteroanterior (PA) cephalometric films from 19 sets of monozygotic (MZ) twins (15 male and 4 female) and 10 dizygotic (DZ) twins (7 male and 3 female) were analyzed. TPS analysis evaluated statistically significant differences in the craniofacial shape and size between affected and unaffected twins within MZ and DZ twin couples.

**Results:** No statistically significant differences in craniofacial shape or size between CLP and non-CLP MZ or DZ twins were observed. The level of morphological similarity in CLP vs non-CLP MZ twins was statistically greater than in DZ twins.

**Conclusions:** Morphometric analysis showed that surgically repaired CLP does not produce significant shape or size differences in the craniofacial features of MZ or DZ twins discordant for unilateral CLP. (*Angle Orthod.* 2011;81:878–883.)

**KEY WORDS:** Cleft lip and palate; Morphometrics; Monozygotic twins; Dizygotic twins

## INTRODUCTION

Treatment of cleft lip and palate (CLP) involves both a surgical repair and orthodontic treatment at various developmental stages to minimize the impact of the anomaly on the dentofacial structures. It is important, therefore, for orthodontists to be aware of the morphological features in the craniofacial region affected by a surgically repaired CLP in growing patients.[1]

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Several contributions in the literature suggest that the size of the maxilla is reduced in the anteroposterior and vertical dimensions when subjects have had surgical repairs to the cleft palate and/or lip.[2–4] Some investigators have attributed this disharmony to growth restriction induced by the scar tissue following surgical repair.[3,4]

Twins discordant for CLP provide a unique research model also because of the rarity of CLP in twins.[5,6] In this regard, the significance of the literature is limited because of the small sample size, cephalometric analysis that did not differentiate between shape and size differences, or exclusive analysis of either monozygotic (MZ) or dizygotic (DZ) subjects with CLP.[7–10] More recently, Singh et al.[11] analyzed 32 pairs of DZ twins concordant for oral clefting and compared them with 20 sets of noncleft twins with posteroanterior (PA) cephalograms. These authors used traditional cephalometrics, the Euclidean distance matrix analysis, and thin plate spline (TPS) analysis to investigate the maxillary region only. The CLP twins presented with reduced maxillary height, decreased intraorbital width, decreased internasal width, and vertical compression of the midface.

So far, the literature is lacking information about a comprehensive analysis of shape and size differences between both MZ and DZ twins discordant for the

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presence of unilateral CLP by using appropriate methods to visualize morphological differences and appraise them statistically in both the sagittal and frontal planes. Therefore, the aim of this morphometric study was to compare craniofacial differences between twins discordant for surgically repaired unilateral CLP during the developmental ages and to test the effect of zygosity on shape and size of the craniofacial skeleton of the same twins by means of TPS analysis.

## MATERIALS AND METHODS

The original cleft sample consisted of 60 sets of twins, including medical records indicating zygosity, cleft type, and history of surgery (affected sample). The twins were either MZ or DZ, concordant or discordant for CLP, thus resulting in four groups: MZ with one twin affected with a cleft, MZ with both twins affected with cleft, DZ with one twin affected with a cleft, and DZ with both twins affected with cleft. This sample was collected from four studies at various institutions in the United States: The University of Michigan Cleft Palate Study, The University of Illinois Craniofacial Clinic, The University of California San Francisco Cleft Twin Study, and The Lancaster Cleft Palate Clinic at Lancaster General Hospital in Lancaster, Pennsylvania.

The zygosity of the twins was determined by the medical records, including blood types. For those twins without documented medical test diagnosis, zygosity was based on reports of parents and physicians. The National Academy of Sciences–National Research Council twin panel reported that zygosity based on self-reporting was correct at least 95% of the time and therefore is acceptable for the current study.[12]

The original sample of 60 sets of affected twins was subjected to both inclusion and exclusion criteria. Inclusion criteria were represented by the availability of complete records, including paired radiographs taken on the same day and medical records indicating the zygosity, sex, and type of cleft. A nonsyndromic unilateral cleft had to be present in one twin and no cleft in the other twin.

Exclusion criteria were applied when both twins were affected by the cleft and for twin couples affected by a bilateral cleft. Based on the inclusion and exclusion criteria, samples of 19 MZ twins (17 of which with both PA and lateral cephalograms, and 2 with PA films only) and 10 DZ twins (PA films only) were obtained. The MZ twins included 15 boys and 4 girls, and the DZ twins included 7 boys and 3 girls. The mean age for the MZ sample was 8.5 $\pm$ 3.4 years (ranging from 3.5 years through 16 years). The mean age for the DZ sample was 11.2 $\pm$ 4.9 years (ranging from 3.9 years through 17.5 years).

Surgical treatment of the CLP twins consisted of initial lip repair within the first year of life, soft palate repair before 18 months of age, AND secondary cleft lip/cleft palate surgeries such as bone grafting when appropriate.

### TPS Analysis

TPS analysis is a mathematical application that constructs a model of a geometric form to allow analysis of the shape or shape change of a form independent from size.[13] TPS is gaining popularity in CLP research and now is used to determine the effect of a cleft on craniofacial form and the cleft treatment outcome.[14,15] Centroid size can be used in addition to TPS analysis to evaluate size and size changes independent from shape.

Each lateral cephalogram was traced on frosted acetate (0.03-in. thick) by one investigator and checked by another investigator. Landmarks for the description of the craniofacial region were identified (Figures 1 and 2) and digitized by means of appropriate software (Dentofacial Planner version 2.5, Toronto, Ontario, Canada) and a digitizing tablet (Numonics, Lansdale, Penn). The PA cephalograms were orientated for digitization with the cleft of the affected twin always on the right (cleft side). The nonaffected cohort twin then was oriented in the same way.

TPS software (tpsRegr, version 1.31, Ecology & Evolution, SUNY, Stonybrook, NY) computed the orthogonal least-squares Procrustes average configuration of craniofacial landmarks using the generalized orthogonal least-squares procedures as described by Rohlf and Slice.[16]

The average craniofacial configurations on PA and lateral cephalograms were subjected to TPS analysis by means of the following comparisons:

- average configuration on PA cephalograms for the twins with cleft vs average configuration on PA cephalograms for the twins without cleft within the MZ couples,
- average configuration on PA cephalograms for the twins with cleft vs average configuration on PA cephalograms for the twins without cleft within the DZ couples, and
- average configuration on lateral cephalograms for the twins with cleft vs average configuration on lateral cephalograms for the twins without cleft within the MZ couples.

Statistical analysis of shape differences was performed by means of permutation tests with 1000 random permutations on Goodall F statistics (tpsRegr, version 1.31, Ecology & Evolution, SUNY, Stonybrook, NY).

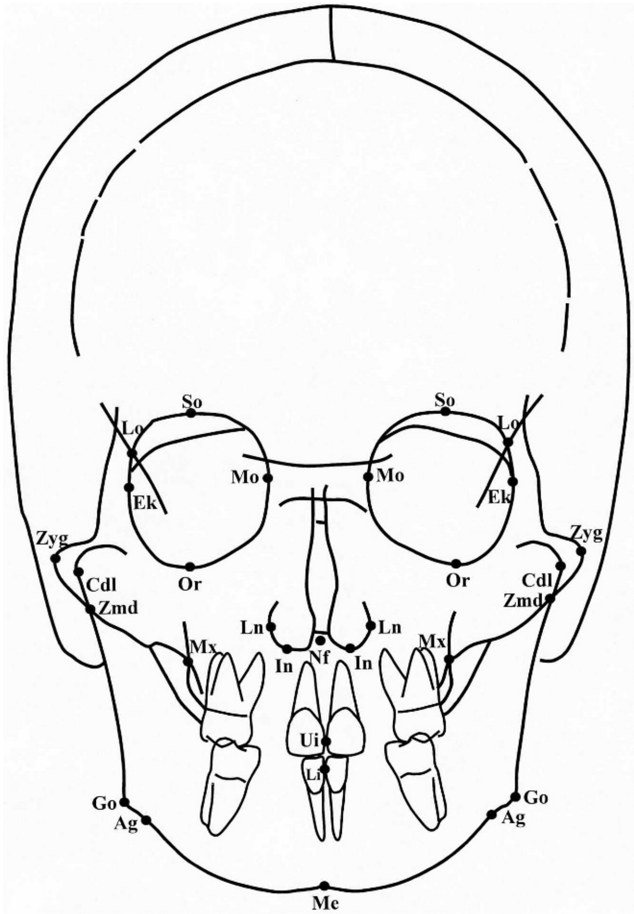


Figure 1. Landmarks for the thin plate spline and centroid analysis of posteroanterior cephalograms. Mo, indicates medio-orbitale, the most medial point of the orbital orifice; Or, orbitale, the lowest point of the orbital orifice; Ek, ectoconchion, the most lateral point of the orbital contour; Lo, latero-orbitale, the intersection of the lateral wall of the orbit and the greater wing of the sphenoid (the oblique line); So, supraorbitale, the highest point of the orbital orifice; Zyg, zygomatic, the most lateral point of the zygomatic arch; Zmd, zygomandibulare, the intersection between the lower margin of the zygomatic bone and the lateral contour of the mandibular ramus; Mx, maxillare a point located at the depth of the concavity of the lateral maxillary contour, at the junction of the maxilla and the zygomatic buttress; Ln, lateronasal, the most lateral point of the nasal cavity; In, inferonasal, the most inferior point of the nasal cavity; Nf, nasal floor, a point located at the intersection of the nasal septum with the floor of the nose; Cdl, condylion lateral, a point located at the lateral pole of the condylar head; Go, gonion; Ag, antegonion; Me, menton; Ui, upper interincisor; Li, lower interincisor.

Centroid size was used as the measure of the geometric size of the craniofacial region in all subjects. It was calculated as the square root of the sum of the squared distances from each landmark to the centroid of each specimen's configuration of landmarks.[13] Differences in size for the twins with vs without cleft within the MZ or DZ couples were tested by means of Mann-Whitney *U*-tests ($P < .05$). Statistical computations for centroid size analysis were performed with

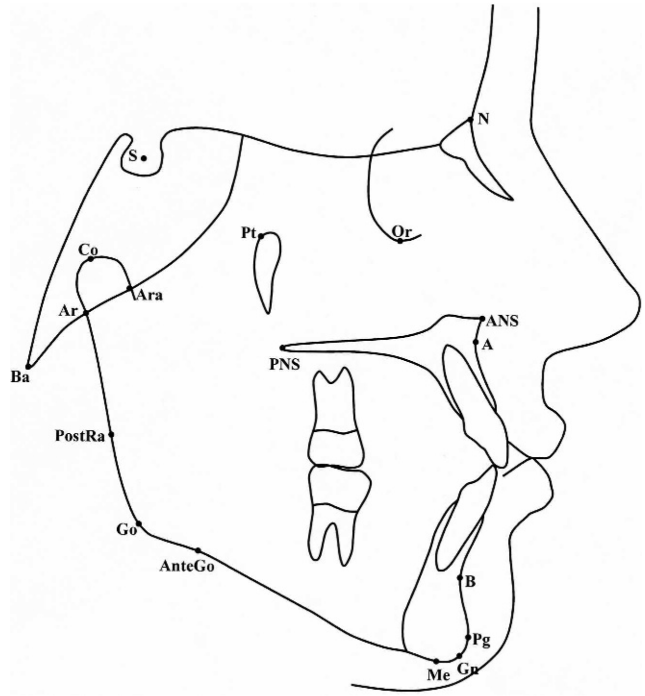


Figure 2. Landmarks used in the thin plate spline and centroid analysis of lateral cephalograms. N, indicates nasion; Or, orbitale; S, sella turcica; Pt, superior Pterygoid point, the most posterosuperior point on the outline of the pterygomaxillary fissure; PNS, posterior nasal spine; ANS, anterior nasal spine; A Point; B Point; Pg, pogonion; Gn, gnathion; Me, menton; AnteGo, antegonial notch, a midplaned point on the inferior border of the mandible at the depth of the inferior concavity; Go, gonion; PostRa, posterior border of the ramus, a midplaned point on the posterior border of the mandibular ramus, approximately halfway between gonion and articulare and at the depth of its anterior curvature; Ar, articulare; Co, condylion; Ara, articulare anterior, the point of intersection of the inferior cranial base surface and the average anterior surface of the mandibular condyles; Ba, basion.

computer software (SPSS, version 12.0, SPSS Inc, Chicago, Ill).

### Method Error

Twenty randomly selected cephalograms were retraced and redigitized to evaluate method error. Dahlberg's formula was used to calculate method error as a combination of location of landmarks, tracing, and digitization. The average method error for landmark identification was $0.7 \pm 0.3$ mm.

## RESULTS

### Comparisons of PA Cephalograms in Monozygotic Twins

The analysis of the transformation grid did not reveal any deformation in the twins with cleft vs the twins without cleft (Figure 3). The results from the permutation test did not show significant shape differences; there was a high level of morphological

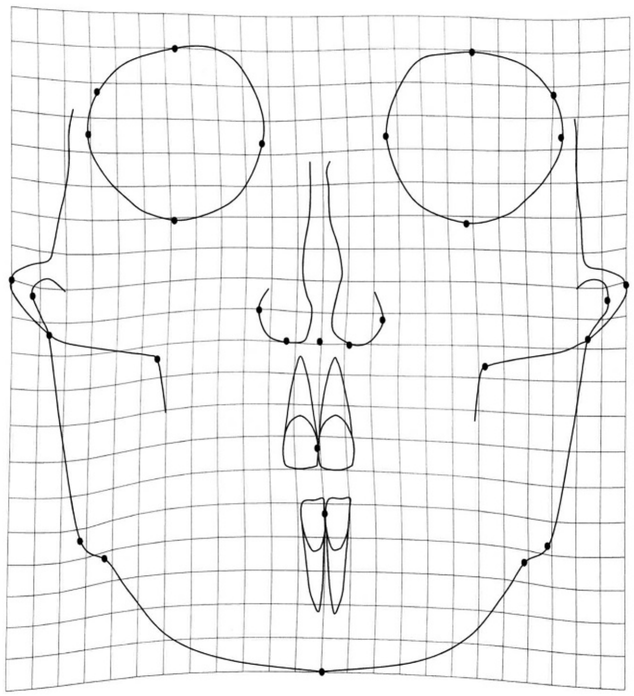

**Figure 3.** Thin plate spline analysis of posteroanterior cephalograms of cleft vs noncleft monozygotic twins.

similarity between twins with and without cleft (96.2%). The results from centroid size analysis also were nonsignificant for any size difference ($z = -0.075$, $P = .956$).

## Comparisons of PA Cephalograms in Dizygotic Twins

The analysis of the transformation grid showed an upward displacement of the orbital floor (landmark Or) and the nasal floor (landmarks Ln and In) on the cleft side (Figure 4). This displacement can be interpreted as a result of the lack of vertical growth/displacement of the hemi-maxilla on the cleft side.

The results from the permutation test, however, did not show significant differences (moderate level of morphological similarity, 56.2%). The results from centroid size analysis also showed nonsignificant size differences ($z = -0.227$, $P = .853$).

## Comparisons of Lateral Cephalograms in Monozygotic Twins

The analysis of the transformation grid did not reveal any noticeable deformation when the average configuration for the twins without cleft was compared with the average configuration for the twins with cleft, with the exception of a slight upward displacement of the palato-maxillary structures on the cleft side (landmarks ANS, PNS, and Point A; Figure 5).

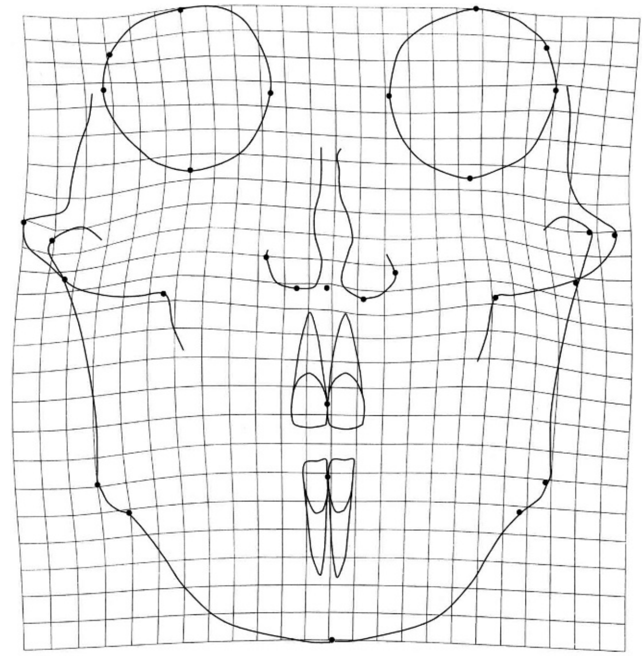

**Figure 4.** Thin plate spline analysis of posteroanterior cephalograms of cleft vs noncleft dizygotic twins.

The results from the permutation test did not show significant shape differences; there was a high level of morphological similarity (85.2%). The results from centroid size analysis also showed nonsignificant differences ($z = -0.038$, $P = .985$).

# DISCUSSION

The aim of the present study was to evaluate shape and size differences between twins affected by unilateral CLP vs unaffected twins in both MZ and DZ twin couples. Morphometric analysis was used for the first time to test these differences as derived from both PA and lateral cephalograms. Also, the current study evaluated morphological characteristics of twins discordant for unilateral CLP during a time period that encompasses most of the developmental ages (from 3 to 16 years of age).

When analyzing the results of this study, three intratwin main factors were considered, as they characterized the subjects within the examined twin couples:

- Physiological factors of concordance, represented by twinning and monozygosity. It is expected that the physical traits displayed by the two siblings are very similar to identical in presence of both these factors.
- Physiological factor of discordance, represented by dizygosity. In the presence of this factor, physiological traits are expected to be shared by the twins to the same extent as would occur with a single birth brother or sister.

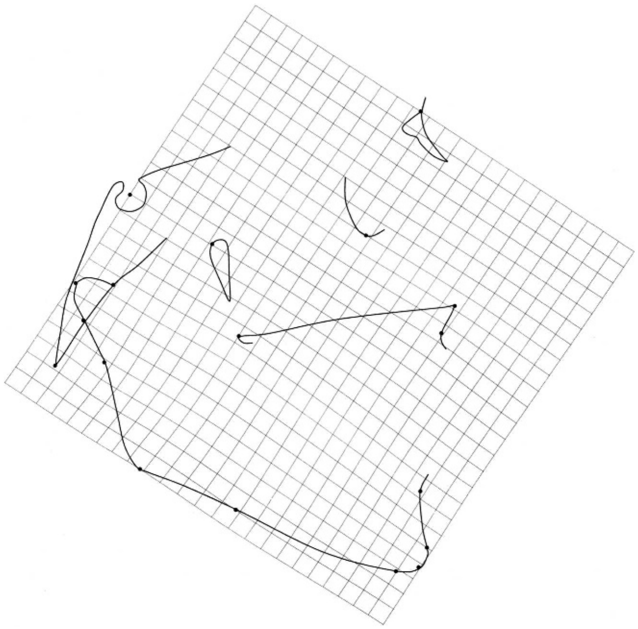

**Figure 5.** Thin plate spline analysis of the lateral cephalograms of cleft vs noncleft monozygotic twins.

- Pathological factor of discordance, represented by the presence of a unilateral cleft, with one twin affected and the other unaffected.

The analysis of the craniofacial form in cleft vs noncleft MZ twins in both the PA and lateral cephalograms showed no significant difference in size and shape. The twins appeared almost identical. This high level of similarity despite the presence of a cleft in one of the twins within the couples presumably was due to the presence of the physiological factors of concordance (twinning and monozygosity). The pathological factor of discordance (cleft vs noncleft) was not able to produce significant differences within the MZ twin couple.

The analysis of the PA cephalograms in DZ twins indicated no significant difference in size and shape. The twins (one affected by cleft and one nonaffected) appeared similar; however, they were less similar than the MZ couples. The level of morphological similarity in the MZ affected twins to unaffected twins on PA cephalograms was 96.2%. This percentage was significantly higher than the 56.2% of morphological similarity of the CLP DZ twins vs their nonaffected co-twins ($z$ test on proportions, $z = 3.38$, $P < .001$). Therefore, in our sample, the presence of DZ vs MZ significantly reduced the degree of similarity in craniofacial shape in twins with vs without cleft.

The reduced level of similarity in DZ twin couples when compared with the MZ twin couples was due to the presence of the physiological factor of discordance (DZ) in association with only one of the two physiological factors of concordance (twinning). Despite the presence of a smaller level of similarity within the DZ twin couples vs the MZ twin couples, the pathological factor of discordance (cleft vs noncleft) was not able to produce significant shape or size differences between cleft and noncleft DZ twins. In these twin couples, the physiological factor of concordance represented by the twinning condition, therefore, appeared to be stronger than the variability produced by the presence of the cleft.

Singh and colleagues[11] analyzed 32 sets of DZ twins using the TPS analysis on PA cephalograms. They compared 20 pairs of nonaffected twins to 12 sets of twins concordant for oral cleft (both twins were affected by a cleft). Their approach examined the effect of only one of the three factors mentioned earlier, that is, the pathological factor of discordance (cleft vs noncleft), and it showed that intraorbital, intranasal, and maxillary base widths were decreased in the twins with cleft.

In the current study, the analysis of both MZ and DZ twins who were discordant for CLP allowed for the evaluation of all three factors of concordance/discordance described previously. Trotman et al.[10] studied both PA and lateral cephalograms of 12 sets of MZ twins discordant for unilateral CLP and CL by means of conventional cephalometrics, and they described a shorter and more posteriorly positioned maxilla in the CLP group. These findings were not confirmed by those of the present study, which used a morphometric approach. Also, the study by Trotman et al.[10] was unable to test for the effect of the physiological factor of discordance (DZ).

CLP is known to have a multifactorial origin due to the interaction of genes and the environment. The existence of twins, and in particular MZ twins, discordant for CLP provides evidence for the nongenetic component in the etiology of this anomaly.[17] The present study offered the opportunity to evaluate the impact of CLP, once treated surgically at an early developmental phase, on the craniofacial phenotype of growing subjects by including ideal controls for affected individuals (their unaffected MZ or DZ twins). The findings appear to indicate that surgically repaired CLP does not lead to a significant dysmorphic facial outcome at the skeletal level.

## CONCLUSIONS

- Morphometric analysis showed that surgically repaired CLP does not produce significant shape or size differences in the craniofacial features of MZ or DZ twins discordant for unilateral CLP.
- Neither the impact of surgically repaired CLP nor differences related to zygosity were able to override

the effect of the twinning condition in terms of morphological similarity in the craniofacial structures.

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