Deformations of the Midfacial Complex in Twins With Orofacial Clefts

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Objective: **To investigate the craniofacial morphology in twins with cleft lip and/or palate (OFC) and localize differences, compared with noncleft (NC) twins.**

Design: **Retrospective study.**

Setting: **School of Dentistry, University of Michigan.**

Sample: **Posteroanterior cephalographs of 32 pairs of dizygotic, concordant, like-sexed twins. The NC group consisted of 20 pairs of noncleft twins. The cleft twin (CT) group consisted of 12 pairs of concordant twins (both exhibited OFC).**

Main Outcome Measures: **Changes in linear distances, differences in form difference matrices, and visualization of deformations of thin-plate spline (TPS) transformation grids.**

Results: **Linear analysis indicated significant reductions in interorbital distance (≈12%; $p < .01$) and reduced maxillary heights (≈27%; $p < .001$) in CTs. Euclidean distance matrix analysis strongly supported these findings, confirmed that the form matrices were significantly different ($p < .05$), and indicated relative decreases in internasal width (≈12%) and maxillary base width (≈10%). The TPS analysis produced a transformation grid that showed superoinferior compression, suggesting that OFC is associated with a downward displacement of the nasomaxillary complex as well as distortion in the region of the maxillary base.**

Conclusions: **Twins with orofacial clefts differ from their unaffected counterparts by a midfacial skeletal morphology characterized by decreases in interorbital and internasal widths and relatively shorter maxillary basal heights and widths. Although most of these differences appear to be due to compression and regionalized deformation, the resultant inferior displacement of the medial region of the midface concomitant with horizontal widening in the presumptive palatal region may be a development model associated with OFC.**

KEY WORDS: *cleft lip palate, geometric morphometrics, twins*

Craniofacial morphology is heritable (Stein et al., 1956; Suzuki and Takahama, 1991; Manfredi et al., 1997), and attempts have been made to demonstrate that some features are under genetic control. For example, Ayra et al. (1973) suggested that mandibular phenotypes have a greater genetic component, whereas Lobb (1987) suggested that the maxilla is influenced to a greater extent than the mandible. Nevertheless, craniofacial shape heritability also contains an environmental component (Saunders et al., 1980). For example, orofacial clefts (OFCs), which are produced as a result of incomplete union of embryonic processes that normally form the upper lip and palate, can occur as a characteristic feature in certain syndromes. However, most patients with OFC are nonsyndromic and have no association with any other structural abnormality. Thus, environmentally induced changes in early morphologic relationships may precipitate a departure from the developmental program. Thus, the etiologies of OFC are complex and heterogeneous (Wyszynski and Beaty, 1996).

The multifactorial model (Fraser, 1970) assumes that transmission of OFC will be due to both parents contributing predisposing factors to an affected child, including both multiple genes and environmental components. Therefore, because environmental factors also contribute, the risk of OFC in close relatives usually is lower than that observed with single gene disorders. The recurrence risk of unaffected parents who already have a child with OFC is about 4%. If they have two children affected by OFC, the risk increases to about 9% (Fras-

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er, 1970). Thus, OFC is a so-called multifactorial disorder because the presence of OFC is determined by the interaction of a number of genes at different loci, each with a small but additive effect, together with environmental factors. When these genetic and environmental influences reach a threshold, malformation will occur.

The above hypothesis, however, has been superseded by the theory of etiologic heterogeneity, which suggests that genetic influences on OFC will be minimal in some cases, heavily weighted toward one parent in others, and approximately equal when each parent happens to possess the same degree of predisposing factors. Indeed, much attention has focused on the transforming growth factor (TGF) α gene locus and the possibility of polymorphisms within this gene being of importance in OFC (Chenevix-Trench et al., 1991; Sassani et al., 1993; Romitti et al., 1999). On the other hand, areas of interest for environmental factors include maternal smoking and alcohol consumption during pregnancy. Shaw and Lammer (1999) reported that increased risk of OFC does not occur with moderate alcohol consumption but increases with increasing intake. Similarly, Romitti et al. (1999) reported that smoking significantly elevates the risk of OFC, but the risk is increased if the child also has a polymorphism at the TGFβ3 locus.

Twin pairs can provide a source of environmentally or genetically matched controls and test cases. Dizygotic (DZ) twins are no more alike genetically than any other pair of siblings. But during intrauterine life, a pair of DZ twins shares the same environmental factors. Thus, any environmentally induced changes in the early, critical morphologic relationships during palatogenesis may precipitate OFC, even though nonrelated DZ twin pairs do not share the same environments postnatally. However, only 1 in 40,000 live births results in a twin with OFC. In a large study, twinning was increased for all types of OFCs (Robert et al., 1996). In contrast, Nordstrom et al. (1996) reported that twinning was not associated with OFCs. In a study in which the sample was composed of discordant twins, the maxilla was found to be shorter in cleft twins (Hunter, 1981), even though the environmental factors to which each twin was presumably exposed during intrauterine life did not differ markedly. More recently, however, Laatikainen et al. (1996) reported that twinning does not have an effect on maxillofacial morphology. This current study attempts to address the environmental/genetic impact by using DZ twins, one set being concordant for OFCs and the other set being concordant for noncleft (normal, controls). Thus, the impact of intrauterine environmental differences for each pair in the two groups will be reduced, and any differences found will be attributable mostly to the genome of the twin pair.

Most previous studies investigating the morphology in orofacial clefts have assessed the maxillozygomatic complex quantitatively by using conventional cephalometry on posteroanterior cephalometric radiographs. On this basis, Maue-Dickson (1979) proposed that maldevelopment occurs in the midface and cranial base. Similarly, Horswell and Gallup (1992) concluded that during embryonic development, basicranial abnormalities might result in OFCs. However, Bishara et al. (1976) compared unoperated children with OFC with a matched sample of normal control individuals and found the cranial base and skeletal face were not extensively malrelated. This finding was supported further by Mars and Houston (1990), but it is important to remember that conventional methods of cephalometric analysis, although widely recognized, do not consider size differences. This problem can be overcome to some extent by using geometric morphometric techniques (Bookstein, 1991; Singh et al., 1997, 1998). These techniques take into account the geometrical relationships of biologic landmarks, rather than measured distances or angles, and have been used successfully in several biological and clinical studies (Lele and Richtsmeier, 1991; Hay and Singh, 2000; Hay et al., 2000; Singh, 2002).

Therefore, the aim of this study was to investigate the phenotype of unoperated twins with or without OFCs to identify differences in craniofacial morphology between DZ twins concordant for OFCs and noncleft (unaffected, normal, control) twins. The null hypothesis to be tested was that there are no detectable differences in the maxillozygomatic complex between nonsyndromic twins with OFCs and noncleft twins of the same ethnicity. Rejection of the null hypothesis would indicate the genetic impact in OFCs when intrauterine environmental factors are reduced and provide a developmental hypothesis that could be tested experimentally.

Materials and Methods

After gaining appropriate consent, posteroanterior cephalographs were retrieved for like-sexed groups of twins from the Department of Orthodontics and Pediatric Dentistry at the University of Michigan. The total sample consisted of 32 pairs of DZ, concordant twins. The twins were divided into two groups on the basis of OFCs. The noncleft (NC) group consisted of 20 pairs of NC twins (i.e., individuals who formed pairs of NC, normal, control twins, ascertained from medical records). The cleft twin (CT) group consisted of 12 pairs of concordant twins (i.e., in each pair, both twins exhibited OFC). In all cases, the OFCs were nonsyndromic and untreated. Exclusion criteria included a history of previous orthodontic treatment, plastic or maxillofacial surgery, and facial trauma that required hospital attention.

To determine whether twins with OFCs showed a difference in morphology of the midface, compared with NC twins, 11 homologous landmarks, representing the midface (which were distributed relatively evenly) were identified and digitized to obtain their x, y coordinates (Fig. 1). (The degree of digitizing error was found to be $< 1\%$ with $p > .05$ on duplicate digitization.) Therefore, cephalometry was undertaken by calculating 11 mean linear distances between the landmarks for each group. All lengths were compared between groups using a two-tailed Student's *t* test.

Next, a perturbation model was used to compare the mean cleft and mean NC group morphologies. A Procrustes superimposition routine was implemented to obtain a generalized rotational fit (Rohlf and Slice, 1990). This technique normal-

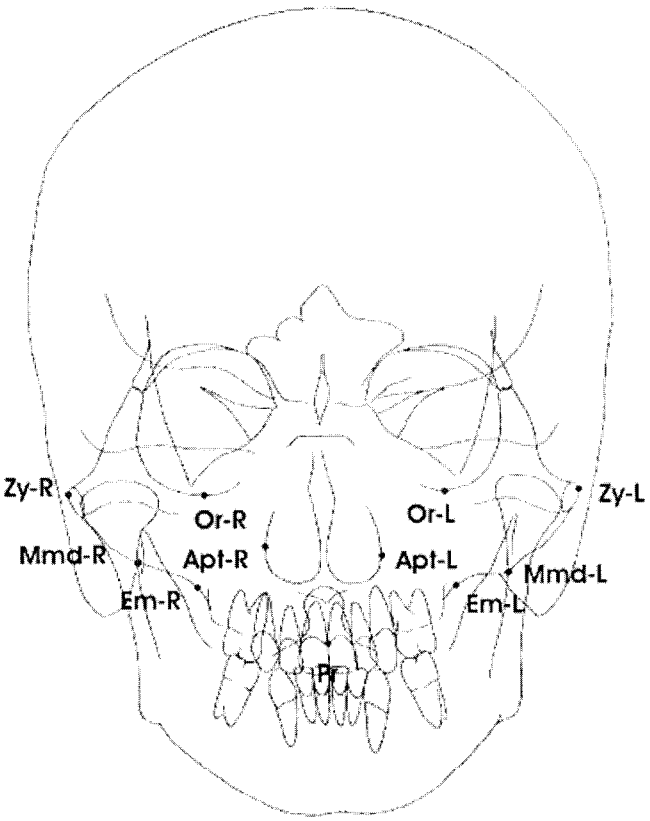

FIGURE 1 Definitions of midfacial landmarks employed. Or-R = Orbitale: the most inferior point of the orbital cavity (right side). Zy-R = Zygion: the most lateral point on the zygomatic bone (right side). Mmd-R = Maxillomandibulare: the intersection between the lower margin of the maxilla and the medial contour of the ramus (right side). Em-R = Ectomaxillare: the intersection of the lateral contour of the upper alveolar process and the lower contour of the zygomatic process of the maxilla (right side). Apt-R = Apertion: the most lateral point of the nasal cavity (right side). Pr = Prosthion: the tip of the alveolar crest between the maxillary incisors. Apt-L = Apertion: the most lateral point of the nasal cavity (left side). Em-L = Ectomaxillare: the intersection of the lateral contour of the upper alveolar process and the lower contour of the zygomatic process of the maxilla (left side). Mmd-L = Maxillomandibulare: the intersection between the lower margin of the maxilla and the medial contour of the ramus (left side). Zy-L = Zygion: the most lateral point on the zygomatic bone (left side). Or-L = Orbitale: the most inferior point of the orbital cavity (left side).

ized the geometric areas, i.e., all configurations were scaled to an equivalent size and registered with respect to one another (Singh et al., 1997). Thus, the mean midfacial morphologies were determined for the NC and CT groups.

To compare cleft and NC group morphologies further, WinEDMA (Zumpano et al., 1999; Cole, 2002) was used. This program is a coordinate-free statistical procedure for the comparison of two forms, using all possible linear distances between homologous landmarks. Form matrices were constructed for NC and CT configurations (Richtsmeier et al., 1992). The NC group configuration was used as the denominator, whereas the cleft morphology was the numerator. To compare these matrices, WinEDMA generated form difference matrices that allowed determination of the way two groups' shapes differed. It identified those linear distances that are the most and least different among the forms being compared (Lele and Richtsmeier, 1991). Therefore, distances between each of the homologous landmarks were calculated and form matrices determined for the cleft and NC configurations. For example, if no difference exists between two landmarks, the relevant form difference matrix value would be 1.00. Consequently, values of greater than or less than 1 indicate that distances between landmarks differ in size. Thus, a value of 1.15 indicates that the numerator configuration distance is 15% longer than that of the denominator configuration. Therefore, corresponding linear distances were compared, and the statistical significance of form difference was tested by the nonparametric bootstrap method (Lele and Richtsmeier, 1991).

TABLE 1 Results of Conventional Linear Analysis.

	Noncleft Twins		*Cleft Twins*		
Linear Distance†	*Distance (mm)*	*SD*	*Distance (mm)*	*SD*	*Significance*
ZyR-MmdR	30.98	4.06	32.95	5.47	NS
ZyR-OrR	32.76	2.38	31.71	4.73	NS
MmdR-EmR	12.29*	3.09*	9.02*	2.60*	$p < .001$*
EmR-AptR	19.34	1.91	19.88	2.52	NS
AptR-Pr	30.51	3.58	29.66	4.20	NS
ZyL-MmdL	21.92	3.86	20.52	3.93	NS
ZyL-OrL	30.92	3.00	32.24	4.57	NS
MmdL-EmL	12.45*	2.22*	8.97*	2.40*	$p < .001$*
EmL-AptL	24.80	2.45	24.53	3.02	NS
AptL-Pr	30.93	4.66	31.62	5.32	NS
OrR-OrL	69.76*	3.74*	61.64*	6.69*	$p < .01$*

† See Figure 1 for explanation of terms.
* Statistically significant differences.

Finally, thin-plate spline (TPS) analysis also was undertaken because results are presented in a graphical form, allowing visualization of form changes as transformation grids (Hay and Singh, 2000). The TPS can be thought of as a thin sheet of steel extending infinitely in all directions (Bookstein, 1991); deformations represented by it describe the transformation from one form to the other. The displacements required for the (x, y) landmark data are represented in the z plane (Zelditch et al., 1992). The mathematical function describing these changes is depicted by explicitly minimizing the net bending energy required to match the mean homologous landmarks for the chosen comparison. The superimposed grid allows visualization of the transformation in the areas around the landmarks (Bookstein, 1991). In this way, TPS transformations are geometric descriptions of shape change, ranging from the entire form to specific localized changes. The mean cleft and NC configurations were subjected to TPS analysis executed on a PC (Rohlf, 1996). Thus, a transformation grid depicting deformation of the midfacial region, transforming the NC group morphology into a cleft configuration was produced.

RESULTS

The results of conventional cephalometry, EDMA, and TPS complemented each other strongly. Conventional cephalometry demonstrated that most of the differences in linear, intertwin distances were insignificant (Table 1). There were, however, a

TABLE 2 Results from EDMA Showing the Sorted Form-Difference Matrix

*Parameter**	*Form Difference*
Em-L-Mmd-L	0.716†
Mmd-R-Em-R	0.730†
Em-L-Or-L	0.764
Or-R-Em-R	0.790
Apt-L-Or-L	0.825
Or-R-Apt-R	0.830
Apt-R-Or-L	0.831
Or-R-Mmd-R	0.847
Pr-Or-L	0.859
Mmd-L-Or-L	0.863
Mmd-R-Or-L	0.870
Em-R-Or-L	0.874
Apt-R-Apt-L	0.877†
Mmd-R-Pr	0.878
Or-R-Apt-L	0.878
Or-R-Pr	0.879
Mmd-R-Apt-L	0.880
Or-R-Or-L	0.881†
Apt-R-Mmd-L	0.892
Mmd-R-Mmd-L	0.895†
Zy-R-Or-L	0.899
Or-R-Mmd-L	0.902
Em-R-Apt-L	0.908
Pr-Mmd-L	0.908
Em-R-Mmd-L	0.910
Apt-R-Zy-L	0.912
Apt-L-Mmd-L	0.913
Mmd-R-Em-L	0.914
Or-R-Em-L	0.914
Mmd-R-Zy-L	0.918
Zy-R-Mmd-L	0.920
Zy-R-Zy-L	0.923
Or-R-Zy-L	0.923
Apt-R-Em-L	0.924
Em-R-Pr	0.924
Mmed-R-Apt-R	0.924
Em-L-Zy-L	0.925
Zy-R-Apt-L	0.925
Em-R-Zy-L	0.928
Pr-Apt-L	0.935
Zy-R-Em-L	0.935
Em-R-Em-L	0.938
Zy-R-Pr	0.940
Zy-R-Apt-R	0.944
Pr-Zy-L	0.945
Apt-L-Zy-L	0.947
Or-R-Zy-R	0.953
Zy-R-Em-R	0.955
Apt-R-Pr	0.965
Pr-Em-L	0.972
Apt-L-Em-L	0.983
Em-R-Apt-R	1.009
Mmd-L-Zy-L	1.013
Zy-L-Or-L	1.031
Zy-R-Mmd-R	1.054

* See Figure 1 for explanation of terms.

† Appear to contribute to the deformation of the midfacial complex depicted in Figure 2. For the overall comparison of cleft and noncleft twin form matrices, $p < .05$.

few notable exceptions. Cephalometry showed significant decreases in oblique maxillary lengths of the midface between Em-L-Mmd-L and Mmd-R-Em-R (≈27%; $p < .001$), and a significant decrease in interorbital width (Or-R-Or-L) in group CT (≈12%; $p < .01$), compared with the NC group (Table 1).

The EDMA strongly supported these findings and confirmed that the form matrices were significantly different ($p < .05$).

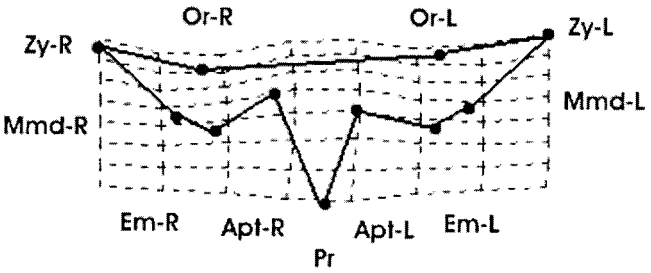

FIGURE 2 Total nonaffine spline transforming the intertwin noncleft (NC) configuration into the cleft twin (CT) morphology. The deformations indicate a superior-inferior compression in the vertical Or-Em axes particularly. Thus, the midfacial heights Em-Mmd, nasal width (Apt-R-AptL), maxillary width (MmdR-Mmd-L), and interorbital width (OrR-OrL) are seen to decrease in the CT configuration.

The results from EDMA for the comparison are shown in Table 2, which indicates that, in general, the majority of differences were relative decreases in CT parameters of the midface. The most significant changes in the midface were relative decreases in oblique maxillary lengths on both sides of the midface between Em-L-Mmd-L and Mmd-R-Em-R (≈27%) and a decrease in interorbital width (Or-R-Or-L) in group CT (≈12%). These results match the cephalometric results above closely. As well, in the nasomaxillary regions, horizontal distances are relatively shorter in the CT group, compared with the NC group, indicated by relative decreases in internasal width (AptR-AptL, ≈12%) and maxillary base width (MmdR-MmdL, ≈10%). If these findings are true, deformations illustrating these changes should be demonstrable.

Using TPS analysis, when transforming the NC configuration into the CT morphology, the deformation grid (Fig. 2) shows evidence of superior-inferior compression, most striking along the vertical Or-Em axes. The changes associated with this compression in the Or-Em axes are a decrease in the interorbital width (OrR-OrL) and a decrease in the nasal width (AptR-AptL). As well, the configuration indicates an oblique compression between Em and Mmd bilaterally and a decrease in maxillary base width (Mmd-R and Mmd-L). Importantly, although most of the differences appear to be due primarily to compression in Or-Em plane, the deformation grid shows regionalized vertical displacement of the transformation grid between Pr-Apt (bilaterally) with concomitant horizontal stretching in the presumptive palatal region.

DISCUSSION

Phenotypic variability can be investigated using geometric morphometrics. These techniques, however, can be used only to hypothesize developmental mechanisms that might account for the OFC phenotype under consideration because natural selection often acts on heritable variation in morphology. To attempt to understand biologic processes responsible for phenotypic variation, quantification is necessary. Procrustes superimposition was used in the current study to generate two-dimensional mean configuration for the two groups. The disadvantages of this method are its reliance on the assumptions of equality of vari-

ance and normality of distribution. The inequality of the variance-covariance matrix is fundamentally important within a biological population (Lele and Cole, 1996). However, WinEDMA takes consideration of these assumptions and takes into account the limited size of the cross-sectional samples but does not provide a graphical display. Thus, TPS was used in conjunction with WinEDMA to obtain a graphical output. The TPS allowed the depiction of differences in CT and NC midfacial forms. Nevertheless, the Procrustes and TPS methods used in this study were applied to two-dimensional data, and it could be argued that the landmarks should have been located in three-dimensional space. Although three-dimensional data were not used in this present study, the use of posteroanterior cephalograms restricts the landmarks to a single plane (the frontal facial plane). Moreover, to prevent artifacts of orientation, the landmarks were distributed relatively evenly around the configuration so that portions of the configuration were not over- or underrepresented.

Most previous studies in OFC have been intratwin and have studied twins discordant for OFC mainly. This current study investigated intertwin differences. However, the likelihood of giving birth to twins is low, and the chance of a child being born with OFCs is also low (~1:750 births; Berkowitz, 1994). Thus, it is difficult to obtain a large sample of concordant twins. Therefore, to gain a reasonable sample, unilateral and bilateral clefts were grouped together in this study despite the fact that cleft lip, with or without cleft palate, and cleft palate are distinct etiological events (Fraser, 1970). Nevertheless, Bishara et al. (1986) noted that individuals with similar types of unoperated OFCs have similar dentofacial characteristics overall. Earlier, Bishara et al. (1976) suggested that there is little scientific information on the morphology of the face of individuals with unrepaired clefts, particularly among twins, on whom there seems to be very little information. To investigate the impact of genetic factors on the morphology of OFCs, it is necessary to have a group of discordant monozygotic twins. However, DZ twins were used in this study, and, although not all genes are identical for both twins, they will share many genes, being siblings, as well as the same intrauterine environment during the critical period of palatogenesis.

Indeed, facial development is influenced by environmental factors (Saunders et al., 1980), and because DZ twins share very similar environmental conditions during development, their craniofacial morphology may be more highly correlated than other siblings, negating the identification of abnormal maxillary microforms associated with OFCs. Although it has been shown that correlation of craniofacial morphology between siblings generally is high (Stein et al., 1956; Suzuki and Takahama, 1991; Manfredi et al., 1997), it is suggested that some parameters are influenced genetically to a greater degree than others. The maxilla is such a structure (Lobb, 1987), but sibling craniofacial morphology, as determined by their genomes, will be similar, and shape differences may be subtle and difficult to detect. The extent to which OFC is hereditary remains an enigma, however, and assuming that there are differences in form, Laatikainen et al. (1996) suggested that twinning itself does not seem to influence craniofacial morphology, compared with nontwins. But it has been shown from twin studies that heredity is still an important factor for OFCs (King et al., 1993).

Although it is acknowledged that OFC may be an epigenetic, not a genetic, condition, it has been reported that interocular distance is increased in OFCs (Nakasima and Ichinose, 1983; Suzuki et al., 1999). This notion contrasts with the finding of this present study in which interorbital width was found to decrease. However, linear analysis fails to correct for size differences, and the shortcomings of cephalometry have been well documented (Moyers and Bookstein, 1979). Nevertheless, an array of linear distances was measured in this study to highlight morphological differences and specify features associated with OFCs. However, few of the parameters selected in the study were found to be statistically different, and many showed a relative decrease when subjected to EDMA. These findings concur with previous findings showing few differences in midfacial profile (Bishara et al., 1976; Mars and Houston, 1990). Midfacial profiles similar to those reported in this study were found in operated patients with both unilateral and bilateral clefts (Johnson, 1980). Moreover, Hunter and Dijkman (1977) reported decreased height and weight in DZ twins, reflecting our geometric morphometric results. These findings suggest that maxillary hypoplasia may be one feature that may increase the risk of OFCs occurring in a child genetically predisposed to OFCs.

In addition, the current study identified statistically significant decreases of the oblique lengths of the midface between Em and Mmd. These results support the above contention and confirm the report of Maue-Dickson (1979), based on conventional cephalometry, that in OFCs, maldevelopment occurs in the midface, which may be due to intrinsic tissue abnormalities. It is thought that in normal growth the sutural complex behind the maxillary tuberosity involving the pyramidal process of the palatine bone is involved in growth in height of the midface. However in OFCs, the palatine bone does not form correctly. This abnormality may suppress the growth in height of the midface and account for the findings of this study, showing a decrease in vertical height of the midface. In contrast, using conventional cephalometry, twins with OFCs have been shown in previous studies to have increased anterior face heights (Cronin and Hunter, 1980; Hunter, 1981). The results from the geometric morphometric part of this study seem to suggest otherwise, providing a developmental hypothesis that could be tested experimentally in animal models; localized suppression of midfacial growth in OFC, which could be induced by subjecting pregnant dams at known gestational ages with known teratogens (Ferguson, 1981; Singh and Moxham, 1996). Consequently, regionalized deformation and the resultant inferior displacement of the medial region of the midface may induce concomitant horizontal widening in the presumptive palatal region, which may represent a development model associated with OFC.

The fact that genetic and environmental influences combine to affect structures creating randomly perturbed forms, however, is supported by the present study that found a significant

difference in midfacial phenotype between twins who had OFCs, compared with NC twins. Moreover, in this study only the morphology of the craniofacial skeleton was examined, but OFC is not confined to hard tissues alone. In particular, cleft lip is predominantly a soft tissue malformation. Examination of the hard tissues alone, especially when the OFC group contains subjects with both cleft lip and cleft palate, does not cover the possibility of microforms existing in the soft tissues. Evaluation of whether DZ cleft twins also display soft tissue microforms requires further study in which soft tissues can be examined, using three-dimensional digital surface stereophotogrammetry.

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