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Testing the face shape hypothesis in twins discordant for nonsyndromic orofacial clefting

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Abstract

Nonsyndromic orofacial clefts (OFCs) are complex traits characterized by multifactorial inheritance and wide phenotypic variability. Numerous studies have shown subtle differences in the faces of unaffected relatives from cleft families compared to controls, the implication being that such outward differences are an incomplete expression reflecting an underlying genetic predisposition. Twins discordant for OFCs provide a unique opportunity to further test this idea, as the unaffected co-twin shares on average 50% (for dizygotic twins) and 100% (for monozygotic twins) of the genetic risk factors as the affected twin. We used 3D surface imaging and spatially-dense morphometry to compare facial shape in a sample of 44 unaffected co-twins and age- and sex-matched unaffected controls ($n = 241$). Unaffected co-twins showed statistically significant differences in the midface, lateral upper face, and forehead regions, compared to controls.

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Furthermore, co-twins were characterized by a distinct pattern of midfacial retrusion, broader upper faces, and greater protrusion of the mandible and brow ridges. This same general facial pattern was shown in both unaffected monozygotic and dizygotic co-twin subsets. These results provide additional support that altered facial shape is a phenotypic marker for OFC susceptibility.

Keywords

3D imaging; facial morphology; orofacial clefting; spatially-dense morphometrics; twins

1 | INTRODUCTION

Nonsyndromic orofacial clefts (OFCs) are among the most frequent congenital malformations with an incidence of one in 700 live births that varies considerably by ancestry (Stanier & Moore, 2004). Phenotypically, OFCs can be subdivided in two major classes, cleft lip with/without cleft palate (CL/P) and cleft palate only (CP), based on epidemiological and embryological considerations. The etiology of OFCs is considered multifactorial with both genetic and environmental influences (Dixon, Marazita, Beaty, & Murray, 2011; Leslie & Marazita, 2013), which are only partly understood. One approach to learn more about the etiology of OFCs is to focus on phenotypic features present more frequently in the unaffected relatives from cleft-affected families compared to the general population. These subclinical phenotypes are considered to be an incomplete expression of underlying susceptibility loci for orofacial clefting (Gottesman & Gould, 2003; Weinberg et al., 2006). Several such subclinical phenotypes have now been described (Roosenboom et al., 2015a,b), such as mild disruption of the upper lip musculature (Neiswanger et al., 2007), the appearance of “whorls” on the surface of the lower lip (Neiswanger et al., 2009), dental anomalies (Howe et al., 2015), and altered facial shape (Roosenboom et al., 2015a,b; Weinberg et al., 2008, 2009).

Studies of subclinical phenotypes typically focus on the unaffected parents and siblings of an individual with a cleft. Unaffected co-twins from pairs discordant for OFCs represent another opportunity to explore this concept. The concordance rate for clefting in monozygotic (MZ) twins is between 40–60%, whereas the rate is only 3–5% in dizygotic (DZ) twins, which is similar to the rate seen in non-twin siblings (Grosen et al., 2010, 2012). Discordance in MZ twins may be due to a difference in environmental exposure, epigenetic changes, somatic changes or skewed X-chromosome inactivation (Leslie et al., 2016). However, these hypotheses have not been confirmed in previous studies in MZ twins discordant for OFCs (Jakobsen et al., 2011; Kimani et al., 2007, 2009). However, because MZ twins discordant for OFC effectively share the same DNA profile, they offer an excellent model for testing subclinical OFC phenotypes.

The aim of this study is to compare the facial surface morphology of unaffected co-twins from discordant OFC pairs to demographically matched controls with no history of clefting. Our general hypothesis is that the presence of OFC risk variants, present in the unaffected co-twins, will manifest phenotypically in altered facial morphology. Previous studies of facial morphology in unaffected parents from cleft families have shown a number of subtle

differences compared to the general population (Fraser & Pashayan, 1970; Mossey, McColl, & O'Hara, 1998; Ward, Bixler, & Raywood, 1989). Unaffected parents tend to exhibit increased middle and upper facial widths, midface retrusion, and changes in upper and lower facial height dimensions. Few prior studies have examined the facial morphology in unaffected co-twins using cephalograms (Chatzistavrou, Ross, Tompson, & Johnston, 2004; Laatikainen, Ranta, & Nordström, 1996). In contrast to previous studies of unaffected parents, Chatzistavrou et al. (2004) reported reductions in facial width in their sample of unaffected co-twins discordant for CL/P compared to matched controls. In light of these seemingly contradictory findings, we attempted to reevaluate this question using surface-based morphometric methods designed not to overlook subtle differences in 3D facial shape. Our hypothesis is that our cohort of unaffected co-twins will largely mimic the changes observed in the soft tissue facial morphology of unaffected parents (Weinberg et al., 2009; Roosenboom et al., 2015a,b).

2 | MATERIALS AND METHODS

2.1 | Participants

Forty-five unaffected twins (11 from MZ pairs and 34 from DZ pairs, age 5–69 years) discordant for OFCs (CP and CL/P) and 241 age- and sex-matched controls (age 5–66 years) were recruited from three sources: the Pittsburgh Orofacial Cleft Study, an international research effort that collects extensive phenotypic, and genomic data from families with a history of orofacial clefting (Weinberg et al., 2006); the Danish Facial Cleft Database (Grosen et al., 2010); or were recruited as part of several 3D facial image databases in Belgium or the US (Roosenboom et al., 2015a,b; Weinberg et al., 2016). Due to the limited sample size, it was impossible to stratify for cleft type (CL/P vs. CP) without having a significant loss of power. Three to five matched controls per unaffected co-twin were selected, based on sex and similarity in age (± 1 year). All subjects were of recent European ancestry (self-reported information) in order to mitigate ethnicity effects on facial morphology and were screened for non-genetic factors potentially affecting facial structure, such as surgery or trauma. No group differences in age, sex, and BMI were noted using either a χ^2 test or a two-tailed t -test (Table 1). Statistical analysis were conducted in *R* (version 3.3.2).

The work was conducted in accordance with the declaration of Helsinki. All participants have given written consent. The studies were approved by the ethical committee of the University Hospitals/KU Leuven (ML8636), the University of Pittsburgh IRB (REN16080177 and REN16060170), the Regional Committees on Health Research Ethics for Southern Denmark (S-20080105), and the Danish Data protection Agency (2013-41-2632).

2.2 | 3D imaging and analysis

3D facial surface images were acquired with a 2-pod 3dMDface imaging system (3dMD, Atlanta), using standard protocols (Heike, Upson, Stuhau, & Weinberg, 2010). Subjects were asked to have a neutral facial expression with their eyes open and mouth gently closed while photographed. The pre-processing of images involved removing hair and ears and

mapping the images onto an anthropometric mask in order to represent the 3D image as a configuration of 3D spatially-dense quasi-landmarks (Claes, Walters, & Clement, 2012), and is described in more detail elsewhere (Roosenboom et al., 2015a,b). After removing image acquisition failures (such as facial expression during acquisition) and inaccuracies, the final dataset included 44 unaffected co-twins (10 MZ and 34 DZ) and 241 controls.

The 3D morphometric analysis was performed in MATLAB (version R2012b). Location, scale, and rotational differences among the 3D facial images were removed using a generalized procrustes superimposition (Rohlf & Bookstein, 2003; Shrimpton et al., 2014). Subsequently, facial shapes were symmetrized by taking the average between original and reflected quasi-landmark configurations (Claes et al., 2012). A principal component analysis was performed on these symmetrized quasi-landmarks, and components capturing 98% of the total shape variance were retained. The principal components corresponding to the last two percentages of the variance observed in the complete dataset were dropped, because they typically correspond to insignificant variance due to random errors in the scanning or mapping process. The quasi-landmarks were then reconstructed for each 3D facial surface in the dataset from the set of principal components explaining 98% of the total variance to obtain noise filtered and symmetrical quasi-landmark configurations.

We used partial least square regression (PLSR) as the underlying regression model to investigate effects on facial morphology. Group membership (co-twin or control) was coded as a categorical variable in the regression model and is tested using the permutation framework for partial regression coefficients (Anderson, 2001). Sex, age, and BMI were considered in the model as possible confounding factors, as they are significantly associated with facial morphology. Effect-size was used as test-statistic. Statistical significance was determined using 10,000 permutations under the reduced model. The localized effect, and significance in each quasi-landmark were visualized onto the shape of the overall average face as a heat map using color-coded values. Additionally, shape transformations (morphs) were constructed from the overall average face in opposite directions of the regression path to create hypothetical faces representing the co-twin and control face shapes. To further facilitate visualization group differences in shape, the surface normal 3D displacement of each quasi-landmark from the first shape transformation to the second was visualized using color-coded values. This process highlights facial quasi-landmarks moving relatively inward or outward from one shape transformation to the other and illustrates changes in the prominence of facial features.

3 | RESULTS

When all 44 unaffected co-twins were analyzed together (MZ and DZ combined) there were notable facial differences compared with matched controls. As shown in Figure 1a and 1b, significant differences (overall $p = 0.0387$) were observed at quasi-landmarks grouped around the midface (parts of the face immediately adjacent to the nose, inferior parts of the nose, the philtrum, and lips). Additional clusters of significant quasi-landmarks were found in the forehead, zygomatic arch area, and lateral extent of the mandible. The superimposed facial morph images (Figure 1d) and the normal displacement figure (Figure 1c) show a pattern of midfacial retrusion, broader upper faces (particularly in the zygomatic region),

and greater protrusion in the mandible and brow ridges in unaffected co-twins compared to controls.

Despite the small sample size ($N = 10$), some of the same effects were present in the separate analysis of unaffected MZ co-twins only (Figure 2). Again, a significant cluster of quasi-landmarks can be seen at the midface immediately adjacent to the nose, with less evidence of an effect in other parts of the face. The DZ subset ($N = 34$) largely recapitulated the results for the combined group, except that the significant quasi-landmarks in the midfacial regions were more localized to the philtrum and lips (Figure 3). Looking at the facial displacement figures for both MZ and DZ groups, however, the same general pattern seen in the combined twin group of midface retrusion, increased lateral facial breadth, and mandibular protrusion were apparent (Figures 2c, 2d and 3c, 3d).

When the co-twins discordant for CL/P and the co-twins discordant for CP were compared to a matched control group separately, only the CL/P subset showed the phenotypic characteristics (Supplementary Figures S1 and S2).

4 | DISCUSSION

In this study, we used 3D facial surface images of unaffected co-twins discordant for OFCs in order to compare their facial morphology to age- and sex-matched controls. Our results showed a pattern of midfacial retrusion and lateral facial widening, coupled with mandibular, and forehead protrusion. This pattern was present in both our DZ co-twins and, to a lesser extent, in our small MZ subset. It is tempting to speculate that such midface retrusion is ultimately a reflection of underdevelopment of the maxillary prominences during early facial morphogenesis. Indeed, work from animal models points to hyperplasia of the maxillary prominences as a key feature associated with orofacial clefting (Green et al., 2015). Altered facial widths have long been linked to cleft susceptibility in both animal models and human populations (Vergato, Doeffer, Mooney, & Siegel, 1997), the idea being that the degree of lateral separation of the facial prominences may precipitate a failure of fusion.

These findings are largely consistent with those of previous studies of unaffected parents from OFC families. For example, several early studies noted midface retrusion as a prominent feature of the parental phenotype (Dixon, 1966; Fraser & Pashayan, 1970). More recently, studies using 3D imaging technology have shown that, compared to the general population, unaffected parents are characterized by flattening of the facial profile with midfacial retrusion, mandibular protrusion, decreased middle, and upper facial height, increased lower facial height and increased interorbital width (Roosenboom et al., 2015a,b; Weinberg et al., 2009).

Only a few previous studies have compared craniofacial morphology of unaffected co-twins from pairs discordant for OFCs to controls. Laatikainen and colleagues looked at 11 pairs of MZ twins and 28 pairs of DZ twins, and compared lateral cephalographs of the unaffected co-twins to normative data (Laatikainen et al., 1996). They reported some minor differences, such as slightly smaller SNA/SNB angles, which could indicate midfacial retrusion.

Chatzistavrou et al. (2004) performed a study looking at lateral cephalograms of 33 MZ twins discordant for OFC. Due to their larger sample, they were able to stratify for cleft type (CL/P vs. CP) and found significant differences comparing the unaffected CL/P groups to age- and sex-matched controls (no significant differences were noticed when comparing co-twins discordant for CP to controls). They found a reduced nasal width, longer anterior and posterior cranial base, smaller cranial base width/length ratio, and smaller maxillary width/length ratio in the unaffected co-twins discordant for CL/P. However, these results conflict with the facial differences reported in prior studies of unaffected parents and the results reported here in a similar set of discordant twins. We would expect to see similar facial characteristics in both unaffected parents and unaffected co-twins, as they are expressions of the same underlying susceptibility loci for OFC. It is not clear what accounts for these discrepancies, although the present study does focus exclusively on facial soft-tissues and uses a different morphometric approach.

There are several potential implications arising from this study and others like it. These findings expand the range of what may be considered part of the OFC phenotypic spectrum, further blurring the boundaries between affected and unaffected. While the kinds of subtle surface features observed here have no obvious clinical/functional implications, there are implications for studies of etiology and perhaps for recurrence risk estimation. If the presence of subclinical cleft manifestations is indeed a reflection of underlying susceptibility loci, then the identification of such features within cleft families may be informative for gene mapping studies. Moreover, analysis of the genetic basis of similar facial features in large healthy cohorts may reveal new variants for clefting that perhaps act as modifiers, interacting with known genetic risk factors. For example, several genetic variants for facial width measurements were recently identified in genome-wide association studies of normal-range facial features (Liu et al., 2012; Shaffer et al., 2016).

Our limited sample size prevented us to sub-divide our sample based on multiplex and simplex family definitions. This is unfortunate, as some studies have shown more pronounced facial differences in unaffected family members from multiplex families (Roosenboom et al., 2015a,b; Weinberg et al., 2009). Also, however we analyzed co-twins discordant for CL/P and CP separately (Supplementary Figures S1 and S2), results need to be interpreted with caution because of the very small sample size. The phenotypic effects seen in the overall analysis are still present in the co-twins discordant for CL/P, but not in those discordant for CP. This corresponds to the hypothesis that the genetic etiology of CL/P differs (at least partly) for the genetic etiology of CP. As different susceptibility loci are involved, we also expect different subclinical phenotypes to be expressed in these groups. As twins discordant for OFC are rare, additional sampling efforts will be necessary to increase the sample size, especially for MZ twins. In addition to the small sample size, our results may have been affected by differences in the distribution of cleft types in each of our twin groups. There were more individuals with CP in our MZ group (40%) compared to our DZ group (23%). Because differences in facial shape were not apparent in our CP subset (Supplementary Figure S2), the higher prevalence of CP in the MZ subset could (partially) mask the phenotypic effects seen in the overall (MZ + DZ) analysis and could be one reason why we see a more restricted effect in the MZ group. Despite these limitations, our results show a pattern of facial differences that are both biologically plausible and consistent with

the bulk of prior studies, lending credence to the notion that altered facial shape can indeed be considered a phenotypic marker for cleft risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

Facial morphology in all unaffected co-twins discordant for OFC, compared to age- and sex-matched controls. a, Localized effect. Red: maximal value; blue: value equal to zero. b, Significance level for each quasi-landmark. Yellow: significant effect; green: no significant effect. c, Relative inward/outward displacements of facial features from one shape transformation to the opposite shape transformation. Yellow: outward displacement; blue: inward displacement. d, Shape transformations, ± 3 standard deviations (exaggeration for visualization purposes) from the overall consensus shape in opposite directions of the regression path. Mesh: representation of the control face; solid: representation of the unaffected co-twin face

FIGURE 2.

Facial morphology in unaffected cotwins of MZ twins discordant for OFC, compared to age- and sex-matched controls. a, Localized effect. Red: maximal value; blue: value equal to zero. b, Significance level for each quasi-landmark. Yellow: significant effect; green: no significant effect. c, Relative inward/outward displacements of facial features from one shape transformation to the opposite shape transformation. Yellow: outward displacement; blue: inward displacement. d, Shape transformations, ± 3 standard deviations (exaggeration for visualization purposes) from the overall consensus shape in opposite directions of the regression path. Mesh: representation of the control face; solid: representation of the unaffected co-twin face

FIGURE 3.

Facial morphology in unaffected cotwins of DZ twins discordant for OFC, compared to age- and sex-matched controls. a, Localized effect. Red: maximal value; blue: value equal to zero. b, Significance level for each quasi-landmark. Yellow: significant effect; green: no significant effect. c, Relative inward/outward displacements of facial features from one shape transformation to the opposite shape transformation. Yellow: outward displacement; blue: inward displacement. d, Shape transformations, ± 3 standard deviations (exaggeration for visualization purposes) from the overall consensus shape in opposite directions of the regression path. Mesh: representation of the control face; solid: representation of the unaffected co-twin face

TABLE 1

Demographic information of the study participants

	Mean age	Sex	Mean BMI	Cleft type
Twins (<i>N</i> = 44)	27.7	M:18, F:26	22.8	CP:12, CL/P:32
MZ twins (<i>N</i> = 8)	26.8	M:4, F:6	21	CP:4, CL/P:6
DZ twins (<i>N</i> = 34)	27.9	M:14, F:20	23	CP:8, CL/P:26
Controls (<i>N</i> = 241)	27.1	M:110, F:131	23.3	NA

Age in years.