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Soft-Tissue Nasal Asymmetry as an Indicator of Orofacial Cleft Predisposition

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Abstract

The biological relatives of offspring with nonsyndromic orofacial clefts have been shown to exhibit distinctive facial features, including excess asymmetry, which are hypothesized to indicate the presence of genetic risk factors. The significance of excess soft-tissue nasal asymmetry in at-risk relatives is unclear and was examined in the present study. Our sample included 164 unaffected parents from families with a history of orofacial clefting and 243 adult controls. Geometric morphometric methods were used to analyze the coordinates of fifteen nasal landmarks collected from 3D facial surface images. Following generalized Procrustes analysis, Procrustes ANOVA and MANOVA tests were applied to determine the type and magnitude of nasal asymmetry present in each group. Group differences in mean nasal asymmetry were also assessed via permutation testing. We found that nasal asymmetry in both parents and controls was directional in nature, although the magnitude of the asymmetry was greater in parents. This was confirmed with permutation testing, where the mean nasal asymmetry was significantly different ($p < 0.0001$) between parents and controls. The asymmetry was greatest for midline structures and the nostrils. When subsets of parents were subsequently analyzed and compared (parents with bilateral vs unilateral offspring; parents with left vs right unilateral offspring), each group showed a similar pattern of asymmetry and could not be distinguished statistically. Thus, the side of the

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unilateral cleft (right vs left) in offspring was not associated with the direction of the nasal asymmetry in parents.

Keywords

geometric morphometrics; cleft lip and palate; stereophotogrammetry; shape variation

Introduction

Facial asymmetry is present to some degree in every human face (Ercan et al., 2008; Claes et al., 2012). In some genetic conditions, such as hemifacial microsomia, this facial asymmetry is exacerbated. Orofacial clefts are among the most common congenital anomalies affecting humans, with estimates ranging from 1:500 to 1:2500 depending on regional and ethnic variation (Dixon et al., 2011). Often isolated, when clefts affect the primary palate (lip and alveolus), they can occur either unilaterally (~75%) or bilaterally (25%). Unilateral isolated clefts occur most frequently on the left side (Paulozzi and Lary, 1999), although the biological mechanism responsible for this phenomenon is unknown. Whether right or left sided, unilateral clefts are associated with excess facial asymmetry, due to the loss of tissue integrity on one side of the midface and the subsequently altered growth patterns (Ferrario et al., 2003; Stauber et al., 2008; Starbuck et al., 2014; 2015). Excess facial asymmetry may be present even at the mild end of the phenotypic spectrum of orofacial clefting, e.g., the “cleft nasal deformity” microform (Sigler and Ontiveros, 1999; Fisher et al., 2014).

A number of studies have also documented subtle facial differences (compared to the general population) in the biological relatives of individuals affected with clefts (Fraser and Pashayan, 1970; Ward et al., 1989; Mossey et al., 1998; Weinberg et al., 2009, Miller et al., 2014). The general hypothesis is that such facial differences are indicative of an underlying genetic risk for clefting, which failed to manifest as an overt defect in these relatives. Such studies typically focus on changes in overall facial shape; only a few have focused on asymmetry. Several early case series described excess nostril asymmetry and/or nasal cavity deformities in the unaffected parents of cleft-affected offspring (Fukuhara and Saito, 1962; Fukuhara, 1965; Niswander, 1968). This tendency was not observed by Mills et al. (1968) and, in contrast to these qualitative studies, Pashayan and Fraser (1971) measured the nostrils in unaffected parents and failed to find any evidence of increased asymmetry compared to controls. Fukuhara (1987) subsequently criticized the methods Pashayan and Fraser used in their study to measure nostril form as inadequate. Moreover, nostril asymmetry has been shown to be relatively common in the general population (Farkas and Cheung, 1979), suggesting that large samples may be needed to statistically discriminate between unaffected parents and controls.

Several studies have also considered overall facial asymmetry, including the nasal region. Cephalometric studies, for example, have shown increased asymmetry in the nasomaxillary complex of unaffected parents (McIntyre and Mossey, 2010). In one such study, the side of the nasal cavity asymmetry in parents corresponded to the side of the cleft in offspring (Yoon et al., 2003). This finding has not been replicated and it is not clear if such

correspondence is evident in the overlying soft-tissues. Soft-tissue facial shape asymmetry in unaffected parents and siblings was also recently evaluated using 3D surface imaging (Miller et al., 2014). While nostril shape was not the focus of this study, evidence of increased asymmetry was observed at the root of the nose in addition to other regions of the face (e.g., eyes and chin). Thus, while several prior studies have shown increased asymmetry in the nasal region of unaffected parents from families with a history of orofacial clefting, the nature and location of this asymmetry and its relationship to the type of cleft present in the child remains unclear.

In the current study, we quantified nasal asymmetry from 3D facial surface images in the parents of children with nonsyndromic forms of clefting that affect the primary palate. We then tested whether nasal asymmetry was capable of distinguishing between our sample of unaffected parents and controls. Finally, we examined the relationship between cleft laterality in affected offspring and the pattern of nasal asymmetry observed in their unaffected parents. Our predictions were that (1) unaffected parents from cleft families will demonstrate greater nasal asymmetry than controls; (2) unaffected parents of children with unilateral clefts will display more nasal asymmetry than parents of children with bilateral clefts; and (3) unaffected parents of children with left and right unilateral clefts will display distinctive patterns of nasal asymmetry.

Materials and Methods

The unaffected parental sample (N=164) was recruited as part of a large international family-based study of the genetics of nonsyndromic orofacial clefting (Weinberg et al, 2006). Families were recruited at several sites (Pittsburgh, Iowa City, Houston, St. Louis, and Denmark) through either cleft registries or craniofacial centers providing treatment. A total of 92 unaffected mothers and 72 unaffected fathers were included in this study. The parental sample was limited to families where the affected child had either nonsyndromic cleft lip (CL) or nonsyndromic cleft lip and palate (CLP). The unaffected status of each parent was based on both self-report and direct visual examination during the study visit. Unaffected controls (N=243), with no family or personal history of clefting or other craniofacial condition, were recruited from four US sites: Pittsburgh, Houston, Seattle, and Iowa City. All participants were adults between the age of 21 and 59; the mean age of parents was 40 years, while the mean age of controls was 30.5 years. Both unaffected parents and controls were excluded if they had a personal history of craniofacial surgery or significant facial trauma. To mitigate potential confounding effects of ethnicity of facial shape, all participants were limited to self-identified whites of European descent. Institutional ethical approval was obtained at each recruitment site and all participants provided written informed consent.

3D facial surface images were obtained on all participants using a 3dMDface camera (Atlanta, GA) following established protocols (Heike et al., 2010). A set of 15 anatomical landmarks (Figure 1) corresponding to morphological aspects of the external nose and nostrils were identified on each 3D facial surface and the associated 3D coordinates were saved for later analysis. Standard anthropometric definitions apply to most of these landmarks (Kolar and Salter, 1997). The three bilateral landmarks relating to the nostrils

were defined as follows: nostril superior (nos) marked the upper-most point along the long axis of the nostril; nostril inferior (noi) marked the lowest point along the long axis of the nostril; columella (col) marked the natural inflection point along the medial margin of the nostril where it meets the columella or, if no inflection point is present, simply the medial-most point along the nostril. As described previously (Weinberg et al., 2016) all landmarking personnel were first calibrated against a single expert rater (SMW) and then tested for landmark location reliability on a set of training images. All landmarks showed high intraobserver reliability, with intraclass correlation coefficients exceeding 0.90. After screening the landmark configurations for outliers, the configurations were superimposed using generalized Procrustes analysis (Rohlf, 1999), which scales, rotates, and centers the landmark configurations via an iterative least-squares process. The resulting transformed 3D coordinates (Procrustes coordinates) reflect shape variation, which can then be subjected to traditional multivariate statistics.

Because orofacial clefts are known to display a left-right bias, this analysis focused on directional asymmetry, which measures the systematic (non-random) difference between left and right anatomical structures. Specific methods have been developed for the analysis of shape asymmetry using geometric morphometrics (Klingenberg et al., 2002). During the Procrustes fit, each landmark configuration is reflected in order to capture symmetric shape variation. Simultaneously, asymmetric shape is quantified as the difference between the original landmark configuration and the reflected symmetrical landmark configuration for each individual in the dataset. In this manner, the total shape variation is broken down into symmetric and asymmetric components, each of which can be analyzed separately. Because we are focused on asymmetry for the current analysis, only the asymmetric component of shape variation was taken into account.

Preliminary investigations showed no sex differences in mean nasal asymmetry (Procrustes distance = 0.0045, $p = 0.12$; $T^2 = 0.0044$, $p = 0.24$) and no meaningful relationship between age and asymmetry in our sample. Both sexes and all ages were therefore combined for our analyses. Following established analysis protocols (Klingenberg et al., 2002; 2010), Procrustes ANOVA was used to determine whether there was evidence of directional asymmetry in each of the parental and controls groups. In addition, MANOVA was applied as a confirmatory test of the directional asymmetry effect, as this test does not assume that the variation at each landmark is isotropic – an assumption that is often violated in biological datasets (Klingenberg et al., 2002). Mean nasal shape asymmetry was then compared between groups (all parents versus all controls; parents of unilateral cleft children versus parents of bilateral cleft children; parents of left unilateral cleft children versus parents of right unilateral cleft children) via discriminant function analysis (DFA). Within the DFA framework, differences in mean shape asymmetry were quantified using both the Procrustes distance and T^2 statistic, with statistical significance determined through permutation testing (5000 resamples). The threshold for statistical significance was set at $p < 0.05$. All morphometric analyses were performed in MorphoJ (Klingenberg, 2011). Wireframe and surface deformations were generated to assists with visualizing the asymmetric shape variation. Surface deformations were created in Landmark v3.0 (Wiley et al., 2005).

Results

Procrustes ANOVA revealed that the predominant type of nasal shape asymmetry in each of the six groups/subgroups was directional in nature. The directional asymmetry effect (side) was significant in both ANOVA and MANOVA tests in each group ($p \leq 0.003$). These results are presented in Table 1.

For the first group comparison, mean nasal shape asymmetry was significantly different between all unaffected parents and controls ($p < 0.0001$; Table 2). Wireframe and surface warps showing the pattern of mean nasal asymmetry for each group are shown in Figure 2. It is clear that the unaffected parent group exhibited greater nasal asymmetry than controls. Both differences and similarities were apparent in the nasal asymmetry patterns. A clear left shift in the three midline landmarks (nasion, pronasale, subnasale) was apparent in the unaffected parents, while in controls only nasion showed a slight shift to the right. The alare and subalare landmarks were shifted counterclockwise (right landmarks inferior, left landmarks superior) in the unaffected parents. The opposite pattern was observed in controls, but to a lesser degree. Further, the left alare and subalare landmarks were shifted posteriorly and right landmarks anteriorly in unaffected parents, while in controls there was little change in these landmarks in the anterior-posterior direction. In both groups, the left alar curvature point was shifted posteriorly and the right shifted anteriorly. The pattern of nostril asymmetry was also similar in parents and controls. In both groups, but more extreme in the unaffected parents, the left nostril landmarks were displaced inferiorly and the right nostril landmarks were displaced superiorly. From the inferior view, it is clear that the left and right anterior nostril landmarks were shifted to the right (more extreme in parents) while the posterior landmark was shifted left (more extreme in controls). This resulted in a counter-clockwise rotation of the nostril.

Additional group comparisons focused on subsets of unaffected parents. Mean nasal asymmetry did not differ significantly ($p > 0.05$) between parents of children with unilateral clefts and parents of children with bilateral clefts. While some differences were observed at a few midline landmarks (nasion, subnasale), both sets of parents exhibited shape asymmetry that was very similar in terms of overall pattern and magnitude (Figure 3, top row). Likewise, no significant differences ($p > 0.05$) in mean nasal shape asymmetry were observed between parents of children with left and right unilateral clefts, and both sets of parents exhibited nearly identical patterns of asymmetry (Figure 3, bottom row).

Discussion

Our results indicate that both unaffected parents and controls exhibited significant directional asymmetry in soft-tissue nasal shape. There were some similarities and differences in the overall pattern of nasal asymmetry observed in parents and controls, but the asymmetry tended to be more extreme in the unaffected parents. This finding is supported by several prior studies, which have documented excess nasal or nasomaxillary asymmetry in the unaffected parents and/or siblings of cleft-affected individuals as part of a broader pattern of facial asymmetry (McIntyre and Mossey, 2010; Yoon et al., 2003; Miller

et al., 2014). Our findings are also in agreement with early descriptive studies reporting greater nostril asymmetry in unaffected parents (Fukuhara and Saito, 1962; Fukuhara, 1965).

Although multiplex families frequently have both bilateral and unilateral clefts present in the pedigree, there is some evidence from population-based epidemiological studies that affected parents tend to have affected offspring with the same pattern of laterality (Grosen et al., 2010). Parents of unilaterally affected children might be expected, therefore, to exhibit greater nasal asymmetry compared with parents of children with bilateral clefts. We found weak evidence to support this claim. While parents of unilaterally affected children did exhibit slightly greater nasal asymmetry, this difference was not statistically significant ($p=0.069$). Miller et al. (2014) reported similar results when looking at asymmetry involving the whole face. Likewise, we failed to find evidence that either the pattern or magnitude of nasal asymmetry differed between parents of children with unilateral clefts, according to whether the cleft occurred on the right or left side. In contrast, Yoon et al. (2003) reported that the side of the cleft in offspring corresponded to the side of the nasal cavity that was larger in parents (based on cephalometry). In our sample, we found no such correspondence for nasal soft tissue morphology. It appears that in our dataset, the same general pattern of nasal asymmetry is present in all parental subgroups, regardless of the type of cleft present in their offspring. This disagreement may potentially indicate that asymmetry of the internal and external nose are not necessarily tightly connected, which has been previously suggested in the literature (Bastir and Rosas, 2013; Maddux et al., 2017).

It is also notable that, while the magnitude clearly differed, the direction and location of the nasal asymmetry was similar in several respects between unaffected parents and controls. This was particularly true for the nostrils. This suggests that whatever etiological factors are predisposing parents are likely also present to some degree in the general population. It is an open question whether such a ubiquitous pattern of directional asymmetry might help explain the left-right bias observed in clefts of the primary palate, as this epidemiological feature of orofacial clefting has never been explained adequately. Perhaps in the presence of additional genetic risk factors for clefting, this general tendency for facial asymmetry in one direction favors fusion on one side of the developing lip. Looking at facial asymmetry in embryonic mouse strains with high rates of spontaneous clefting may provide some clues. Another important question is how to explain rarer, right-sided clefts. It must be remembered that, while the average tendency shows nasal asymmetry biased in one direction, individuals may also display the opposite pattern. By grouping parents together into a single cohort, less common patterns of asymmetry may be statistically diluted by the more dominant pattern.

It is unclear what the excess directional asymmetry represents in this sample of unaffected relatives. It is possible that excess nasal asymmetry observed in parents is part of a broader pattern of somatic asymmetry, perhaps indicating a general developmental disturbance. Additional morphological traits, including dental and dermatoglyphic features, have also been shown to exhibit elevated asymmetry in both cleft affected individuals and their unaffected family members (Sofaer, 1979; Neiswanger et al., 2002). Alternatively, the presence of excess nasal asymmetry in the unaffected parents of children with orofacial clefts may represent a specific subclinical manifestation of orofacial clefting. If this is the

case, then both traits should have some genetic risk factors in common. Focusing on a small set of cleft candidate genes, Miller et al. (2014) showed that facial asymmetry in a sample of unaffected relatives was associated with SNPs in *SNAI1*, a gene known to be involved in left-right patterning (Murray and Gridley, 2006) and to be expressed in the developing murine palate (Murray et al., 2007). Uncovering the genetic architecture of the facial asymmetry patterns observed in the present study (both in at-risk parents and in healthy controls) may reveal additional insights into the genetic etiology of clefting. The availability of large-scale 3D facial imaging datasets with associated genomic markers (Hochheiser et al., 2011) can facilitate novel discoveries in this area.

Conclusion

We observed that nasal asymmetry in both unaffected parents of offspring with orofacial clefts and controls was directional in nature. The magnitude of the asymmetry was greater in parents and tended to be most pronounced in midline structures and the nostrils. When subsets of parents were subsequently analyzed and compared (e.g., parents with bilateral versus unilateral offspring; parents with left versus right unilateral offspring), each group showed a similar pattern of asymmetry and could not be distinguished statistically. These results may provide insights into the genetic basis of clefting and may help explain the well-documented left-right bias observed in clefts affecting the primary palate.

Acknowledgments

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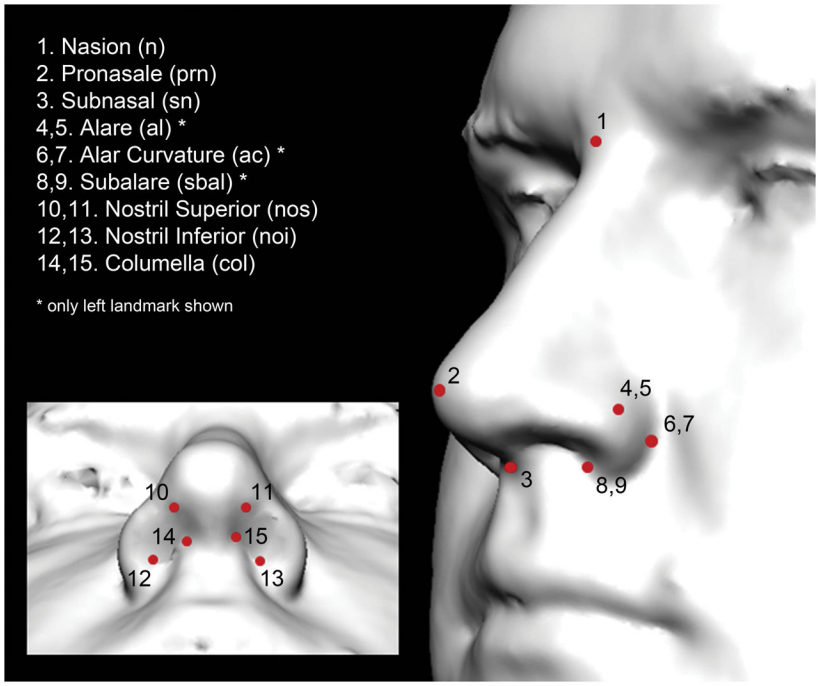

Figure 1.
Example of the 3D facial surface (skin texture and color map removed) showing the location and names of the 15 landmarks used in the present study.

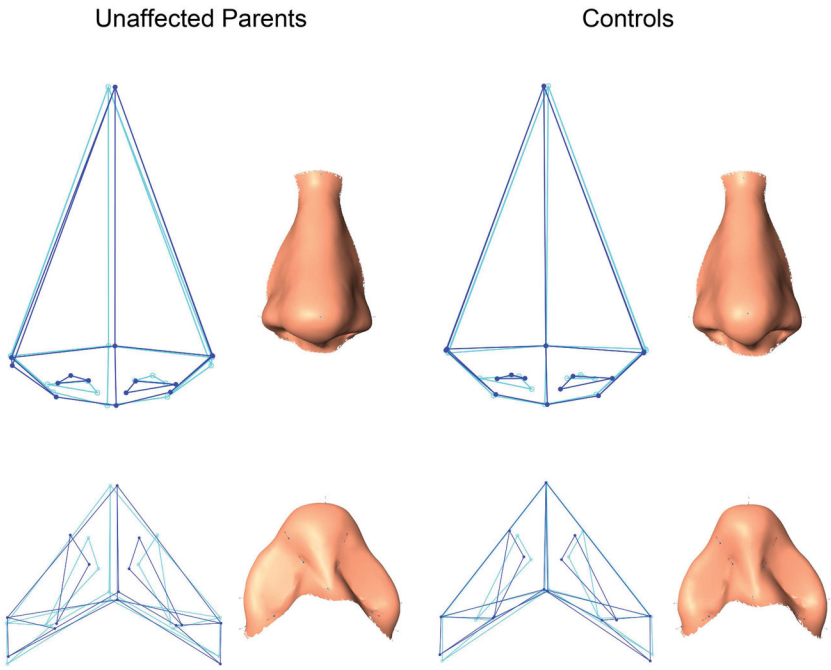

Figure 2.

Wireframe and surface warps showing the patterns of nasal shape asymmetry present in the combined unaffected parents group (left column) and control group (right column). The patterns are based on Procrustes ANOVA results. The frontal view is represented in the top row. The subnasal view is represented in bottom row. For the wireframes, the dark blue lines illustrate the directional asymmetry effect. Shape variation is magnified 10× for visualization.

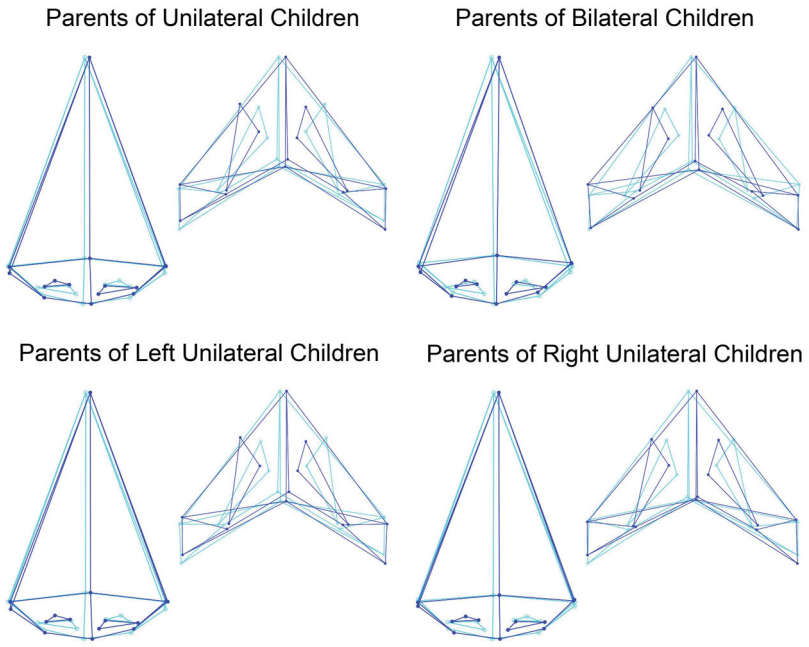

Figure 3.

Wireframe warps showing the patterns of nasal shape asymmetry present in the four unaffected parent groups. The top row corresponds to the comparison between parents of unilateral and bilateral affected children. The bottom row corresponds to the comparison between the parents of left and right unilateral children. The patterns are based on Procrustes ANOVA results. For each group a frontal and subnasal view is presented. For the wireframes, the dark blue lines illustrate the directional asymmetry effect. Shape variation is magnified 10× for visualization.

Table 1

Procrustes ANOVA and MANOVA results for nasal shape asymmetry among the parental groups and controls.

		ANOVA					MANOVA	
Group	**Effect**	**SS**	**MS**	**DF**	**F**	**P**	**Pillai Tr**	**P**
All parents	Individual	1.6258736	0.0004987	3260	6.37	<0.0001		
	Side	**0.0192256**	**0.0010681**	**18**	**13.64**	**<0.0001**	**0.63**	**<0.0001**
	Individual*Side	0.2297667	0.0000783	2934				
All controls	Individual	2.0303732	0.0004195	4840	6.60	<0.0001		
	Side	**0.0188920**	**0.0010496**	**18**	**16.50**	**<0.0001**	**0.63**	**<0.0001**
	Individual*Side	0.2770176	0.0000636	4356				
Parents of unilateral children	Individual	1.2288074	0.0004763	2580	6.22	<0.0001		
	Side	**0.0163818**	**0.0009101**	**18**	**11.89**	**<0.0001**	**0.68**	**<0.0001**
	Individual*Side	0.1776909	0.0000765	2322				
Parent of bilateral children	Individual	0.3886490	0.0005889	660	6.89	<0.0001		
	Side	**0.0042063**	**0.0002337**	**18**	**2.73**	**0.0002**	**0.86**	**0.0007**
	Individual*Side	0.0507752	0.0000855	594				
Parents of left unilateral children	Individual	0.8701721	0.0004834	1800	6.23	<0.0001		
	Side	**0.0129803**	**0.0007211**	**18**	**9.29**	**<0.0001**	**0.72**	**<0.0001**
	Individual*Side	0.1257547	0.0000776	1620				
Parents of right unilateral children	Individual	0.3503878	0.0004610	760	6.16	<0.0001		
	Side	**0.0042253**	**0.0002347**	**18**	**3.14**	**<0.0001**	**0.75**	**0.0037**
	Individual*Side	0.0511832	0.0000748	684				

Individual = Among-individual variation in shape; Side = Directional (systematic) asymmetry effect; Individual*Side = Fluctuating (random) asymmetry effect.

Table 2

Results of permutation testing for group differences in mean nasal shape asymmetry

Group Comparison	Procrustes distance (p-value)	T^2 (p-value)
All parents vs. controls	0.00816 (< 0.0001)	118.2395 (< 0.0001)
Parents of unilateral vs. parents of bilateral	0.00702 (0.4702)	31.7984 (0.0692)
Parents of left unilateral vs parents of right unilateral	0.00542 (0.8392)	10.3554 (0.9622)

* p-values based on permutation with 5000 resamples