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Prenatal alcohol exposure alters the patterns of facial asymmetry

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Abstract

Directional asymmetry, the systematic differences between the left and right body sides, is widespread in human populations. Changes in directional asymmetry are associated with various disorders that affect craniofacial development. Because facial dysmorphology is a key criterion for diagnosing fetal alcohol syndrome (FAS), the question arises whether in utero alcohol exposure alters directional asymmetry in the face. Data on the relative position of 17 morphologic landmarks were obtained from facial scans of children who were classified as either FAS or control. Shape data obtained from the landmarks were analyzed with the methods of geometric morphometrics. Our analyses showed significant directional asymmetry of facial shape, consisting primarily of a shift of midline landmarks to the right and a displacement of the landmarks around the eyes to the left. The asymmetry of FAS and control groups differed significantly and average directional asymmetry was increased in those individuals exposed to alcohol in utero. These results suggest that the developmental consequences of fetal alcohol exposure affect a wide range of craniofacial features in addition to those generally recognized and used for diagnosis of FAS.

Keywords: Directional asymmetry; Discriminant function; Facial dysmorphology; Fetal alcohol syndrome; Procrustes superimposition

Introduction

Directional asymmetry is defined as systematic differences between the left and right sides of the body. Examples are the conspicuous asymmetry of internal organs and the more subtle asymmetry of the human brain (e.g., Toga and Thompson, 2003). With the advent of powerful methods of geometric morphometrics, it has become apparent that subtle, but significant directional asymmetry is nearly ubiquitous even for apparently symmetric features, such as craniofacial features or limbs and is found in a wide range of organisms (Auffray et al., 1996; Klingenberg, 2002; Klingenberg et al., 1998, 2002), including humans (e.g., DeLeon, 2007; Ercan et al., 2008; Schaefer et al., 2006).

Although subtle facial directional asymmetry is present in healthy individuals (DeLeon, 2007; Ercan et al., 2008; Schaefer et al., 2006), stronger directional asymmetry is often associated with conditions that disrupt normal craniofacial development, such as cleft lip and palate (Bock and

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Bowman, 2006), deformational plagiocephaly, or craniosynostosis (Netherway et al., 2006). Changed patterns of directional asymmetry have been reported among individuals diagnosed with disorders in which facial changes may be a secondary consequence of abnormal brain development, such as schizophrenia (Hennessy et al., 2004) and autism spectrum disorder (Hammond et al., 2008).

Specific facial dysmorphology, resulting from prenatal exposure to alcohol, remains the key diagnostic feature of fetal alcohol syndrome (FAS) (Astley and Clarren, 2000; Hoyme et al., 2005). Anthropometric measurements of facial changes have been shown to correctly distinguish FAS from non-FAS in different ethnic populations (Moore et al., 2007). In addition, modern techniques of geometric morphometrics have confirmed that prenatal alcohol exposure has significant effects on facial shape (Mutsvangwa and Douglas, 2007). Thus far, little attention has been paid to the effects of prenatal alcohol exposure on facial asymmetry, although Kieser (1992) reported that maternal alcohol consumption correlates with fluctuating asymmetry in the teeth of children. Although asymmetry is normal in most populations (Ercan et al., 2008; Kimmerle and Jantz, 2005; McIntyre and Mossey, 2002; Shaner et al., 2000), no study has investigated the effects of prenatal exposure to alcohol on directional asymmetry.

Here, we report changes in the pattern of directional asymmetry among individuals prenatally exposed to alcohol and those who were not exposed. We have included ethnically distinct samples (Moore et al., 2007). We used sensitive morphometric methods specifically developed for measuring asymmetry of shape (Bock and Bowman, 2006; Klingenberg and McIntyre, 1998; Klingenberg et al., 2002). These methods detected subtle, but statistically significant, directional asymmetry in the face and revealed that asymmetry is not the same for individuals who were prenatally exposed to alcohol as compared with those without prenatal alcohol exposure.

Material and methods

Study design

Participants were assessed as part of an ongoing international consortium, the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD). Participants were from two sites: Cape Town, South Africa and Helsinki, Finland. This study was approved by the Institutional Review Board at each site and at the grantee institutions (Indiana University School of Medicine, Wayne State University School of Medicine, and Biomedical Sciences, San Diego State University). All participants and/or their parent(s)/legal guardian(s) provided written informed consent and assent.

As part of the study visit, each participant was examined by members of the CIFASD Dysmorphology Core, who completed a standardized, uniform assessment as described by Jones et al. (2006). Details of the study visit are provided by Moore et al. (2007). Briefly, a standard classification system, based solely on structural features and growth deficiency, was used to determine a preliminary classification of FAS, not FAS, or deferred (Hoyme et al., 2005; Jones et al., 2006). Analyses were limited to participants with either a diagnosis of FAS from the Dysmorphology Core, or to participants with a diagnosis of ‘‘control’’ from the Dysmorphology Core who were not exposed to alcohol during pregnancy, according to maternal interview data. Individuals who were known to have been exposed to alcohol in utero but did not receive a diagnosis of FAS were labeled ‘‘deferred.’’ Because the focus of this article was to determine whether asymmetry differs between individuals with FAS and controls that were not exposed to alcohol, we excluded these deferred individuals from the current analyses. Due to potential differences in morphometric facial structure between ethnicities and sample size considerations, only participants reported to be Finnish Caucasian (FC: 40 FAS, 50 control) or Cape Coloured (CC: 49 FAS, 29 control) were included in the analysis. Demographic data are provided in Table 1.

Collection of 3-dimensional (3D) images

Facial images were captured using a commercially available laser scanner, the Minolta Vivid 910fw (Konica Minolta Sensing Americas, Inc., Boulder, CO). The scanner shines a low-intensity ‘‘eye safe’’ laser on the participant. Details describing calibration assessment of the scanners are provided by Moore et al. (2007). Participants were seated approximately 660 mm from the scanner and a trained operator located seven soft-tissue landmarks (bilateral: frontotemporale, tragion, gonion; unilateral: menton) by inspection and/or palpation, and marked them on the skin using an eye-liner pencil. Two frontal and two lateral left and right scans were obtained for each subject. For the lateral scans, the participant faced at near right angles to the scanner. Collected images were processed using a commercially available software package, Rapidform™ 2006 (INUS Technology Incorporated, Seoul, Korea).

Image processing and measurement

Rapidform™, a reverse modeling software package that scans physical objects and creates a digital version of the object, was used to merge the best lateral and frontal scans into a single, 3D model of the participant’s face. For each subject, the better of the two scans of each of the three positions was determined such that neutral expressions were present in all scans, lighting was optimal, and the number of visible landmarks was maximized. Each 3D facial image was analyzed using a customized software plug-in, written by one of the authors (J.R.) using Visual C++ and the Rapidform™ application programming interface. An

Table 1
Subject demographics

Ethnic group	Diagnosis	Number of subjects (%)	Number of males (%)	Mean age (S.D.)	Mean IQ (S.D.)
FC	FAS	40 (41)	17 (42)	13.2 (3.6)	90.7 (16.0)
	Control	50 (59)	20 (40)	13.7 (3.6)	99.9 (17.7)
CC	FAS	49 (60)	22 (48)	5.2 (1.3)	84.1 (11.5)
	Control	29 (39)	15 (52)	4.4 (1.0)	86.9 (13.3)

FC = Finnish Caucasian; CC = Cape Colored; FAS = fetal alcohol syndrome; S.D. = standard deviation. *Note:* The tabled information is for those subjects included in the analyses.

anthropologist (E.M., R.W.) identified a series of landmarks on the 3D model, of which 17 are included in this study (Fig. 1). The software required double redundant measurement accuracy. This redundant approach required the user to identify at least two sets of facial landmarks resulting in less than 2 mm difference per linear measurement. If the user failed to identify redundant landmarks, she or he was forced to pick a third set or re-pick the existing landmarks until accuracy met the 2-mm specification. Analyses were based on two configurations of landmarks per subject, excluding any divergent measurements. Because of problems with hair around the face and ears, some individuals did not have complete landmark data. These individuals were omitted from all analyses.

Morphometric analysis

Geometric morphometrics is based on a definition of shape as the complete geometric information about an object, except its size, position, and orientation (e.g., Dryden and Mardia, 1998). The variation among shapes can be extracted from information on the landmark positions by a Procrustes fit, which removes variation of size, position, and orientation (Dryden and Mardia, 1998). The criterion for an optimal fit is the sum of squared distances between corresponding landmarks. This measure, which is minimized in the Procrustes fit, can also be used as a measure of the absolute magnitude of the difference between two shapes and is known as Procrustes distance (Dryden and Mardia, 1998). The coordinates of the landmarks after the Procrustes fit contain only shape variation and can be used for further analyses with the methods of multivariate statistics. These coordinates define a multidimensional shape space; because some information was removed by eliminating variation in size, position, and orientation, this space has seven dimensions fewer than the number of landmark coordinates that were used (in our example, $44 = (17 \times 3) - 7$).

For objects that are symmetric, such as the human face, a special version of this approach is required, which can characterize components of symmetric shape variation and asymmetry (Bock and Bowman, 2006; Klingenberg et al., 2002; Mardia et al., 2000). For each landmark configuration, this method used the original configuration and a copy that was reflected to its mirror image; the labels of the paired landmarks of the reflected copy were then exchanged; and finally, all original configurations and their reflected and relabeled copies were then included in a joint Procrustes fit (for details, see Klingenberg et al., 2002). After this Procrustes fit, the asymmetry of shape was

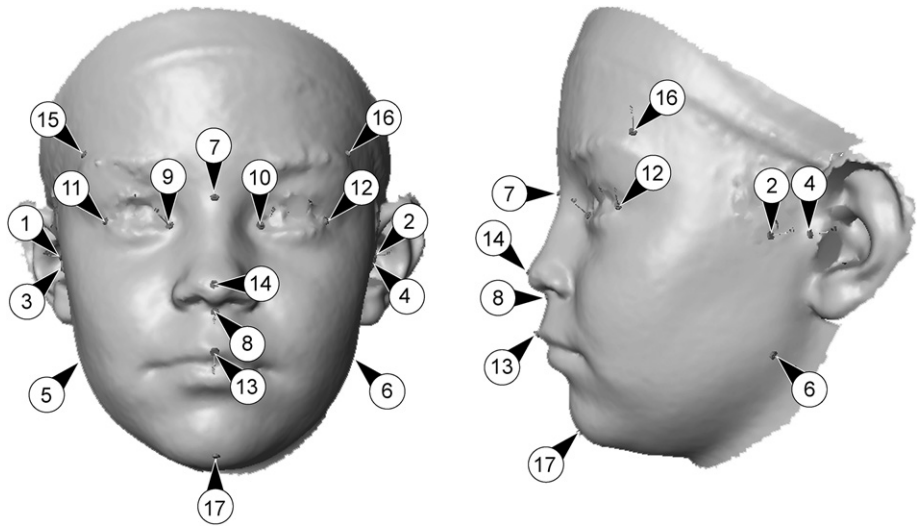

Fig. 1. The set of landmarks used in this study. Five of the 17 landmarks are in the median plane, whereas the other 12 occur in six pairs on both sides of the face. Landmarks: 1, right zygonion; 2, left zygonion; 3, right tragion; 4, left tragion; 5, right gonion; 6, left gonion; 7, nasion; 8, subnasale; 9, right endocanthion; 10, left endocanthion; 11, right exocanthion; 12, left exocanthion; 13, labiale superior; 14, pronasale; 15, right frontotemporale; 16, left frontotemporale; and 17, menton.

computed for each individual as the deviation in the landmark coordinates of the original configuration from the symmetric shape obtained by averaging the original and reflected copies for that individual. These computations were carried out automatically in the MorphoJ software package (Klingenberg, 2008). This method uses information from all landmarks equally to determine the plane of symmetry and makes no assumptions regarding the symmetry or asymmetry of any landmarks. In the shape space defined by the Procrustes fit, the components of symmetric variation and asymmetry occupy mutually orthogonal subspaces (for our data, the symmetric component has 24 dimensions and the asymmetry component has 20 dimensions; Klingenberg et al., 2002).

Three subjects had measurements with large Mahalanobis distances from the average shape (a multivariate measure of distance relative to within-sample variation; Mardia et al., 1979). This set of landmarks for the three subjects were excluded from the study, and only a single set was included in the analysis for the respective subjects. Individuals with known craniofacial disorders (e.g., Williams Syndrome) were also removed from the study.

Measurement error has been previously identified as an important consideration when subtle biological effects are the focus of interest, such as in studies of asymmetry (Palmer and Strobeck, 1986; Robinson et al., 2002; Ward and Jamison, 1991). To quantify measurement error and the amount of variation due to the various factors in the study design, we used Procrustes ANOVA (Klingenberg and McIntyre, 1998; Klingenberg et al., 2002). This method is an extension of the one-way ANOVA proposed by Goodall (1991) for landmark data. It adds up sums of squares and mean squares over the coordinates of the landmarks after the Procrustes fit and can therefore quantify the amounts of shape variation in units of squared Procrustes distance as an easily interpretable measure of the magnitude of the effects in the ANOVA. The method has been generalized to more complex designs, including asymmetry (Klingenberg and McIntyre, 1998; Klingenberg et al., 2002). For this Procrustes ANOVA, the degrees of freedom from the ANOVA design are multiplied with the dimensionality of the shape space. In the context of analyses of asymmetry for symmetric objects, the multiplier for the degrees of freedom depends on whether an effect is part of the symmetric or asymmetric component of variation (Klingenberg et al., 2002). A fully multivariate ANOVA (MANOVA) approach has been developed, which provides additional information for interpreting the results (Klingenberg et al., 2002).

Asymmetry in a configuration of landmarks is computed from the difference between the original configuration and a copy that has been reflected and relabeled (swapping the labels of paired landmarks; e.g. Klingenberg et al., 2002). Thus, the overall directional asymmetry was computed as the average asymmetry across all individuals included in the analysis. Directional asymmetry was also estimated separately for each ethnic group (CC and FC).

Statistical comparisons of the mean asymmetries of the two ethnic groups (CC, FC) were done with permutation tests (Good, 2000; Manly, 2007) using both Procrustes distance and the T^2 statistic (equivalent to using Mahalanobis distance) as test statistics. Procrustes distance is a measure of the absolute magnitude of shape differences that does not take into account the direction of the mean differences. In contrast, the T^2 statistic does take into account the extent of variation in different directions of shape space (Klingenberg and Monteiro, 2005).

To assess whether differences in directional asymmetry were sufficient to distinguish children in the alcohol exposure groups, we used linear discriminant functions (separately for the CC and FC samples). The reliability of the classification was assessed with cross-validation that left out each individual in turn and evaluated whether the discriminant function computed with the remaining sample obtained a correct classification for the subject that was "left out." From cross-validation results, sensitivity (number of correctly classified FAS cases/total number of FAS cases) and specificity (number of correctly classified controls/total number controls) were computed for each ethnic group.

Morphometric analyses were conducted with the MorphoJ software (Klingenberg, 2008). To visualize the shape changes identified by the analyses, we morphed the surface from a single facial scan to fit the required landmark positions (using the Landmark software; Wiley et al., 2005). This morphing used the thin-plate spline method (Bookstein, 1989) to interpolate shape changes at the landmark positions to every point on the facial surface between the landmarks.

Results

The overall Procrustes ANOVA (Table 2) shows highly significant effects on shape of the sample (CC vs. FC), of prenatal alcohol exposure, and a significant effect of sex. The Procrustes ANOVA also indicated that the main effect of side (original vs. reflected copies) was highly significant ($P < .0001$), revealing the presence of directional asymmetry. The analysis also indicated that measurement error is small relative to the individual-by-side interaction and was therefore not a confounding factor in this study. Because we focused our analyses on directional asymmetry, which is the vector of average asymmetry in a sample, the effect of measurement error is further reduced by the large sample size. The MANOVA results for the analysis were similar, except for the fact that the effect of sex was only significant for the symmetric component of shape variation but not for the asymmetry component ($P = .48$). This indicates that sex dimorphism of facial shape does not interfere with the analyses of asymmetry. Separate analyses for the CC and FC samples produced similar results.

Table 2
Procrustes ANOVA for facial shape (Klingenberg et al., 2002)

Effect	Sums of squares	Mean squares	Degrees of freedom	*F*	*P*
Country	0.2231	0.009296	24	32.74	<.0001
FAS vs. control	0.2117	0.008819	24	31.06	<.0001
Sex	0.0115	0.000481	24	1.69	.0185
Individual	1.1176	0.000284	3,936	5.15	<.0001
Side	0.0354	0.001770	20	32.10	<.0001
Individual × side	0.11842	0.000055	3,340	5.08	<.0001
Residual	0.0764	0.000011	7,040		

The effect of individuals represents the variation among individuals in the symmetric component of shape. The main effect of side is directional asymmetry (the systematic difference between the original and reflected copies of each individual). The individual × side effect quantifies fluctuating asymmetry; both of the latter effects concern the asymmetry component of shape. The residual variation is due to measurement error and includes both symmetric and asymmetric components of shape variation.

Analyses of asymmetry were then performed in each ethnic group separately. The CC FAS and control individuals had directional asymmetries that were significantly different for both test statistics (Procrustes distance 0.0091, $P = .029$; $T^2 = 58.41$, $P = .017$). The principal shape features associated with the directional asymmetry were a shift of the midline landmarks to the right and a shift of the eyes to the left (Fig. 2). Moreover, the eye and the frontotemporale were displaced anteriorly on the right side and posteriorly on the left side. Smaller asymmetries were observed for the zygonion (higher and slightly more anterior on the left than on the right side), the tragion (more

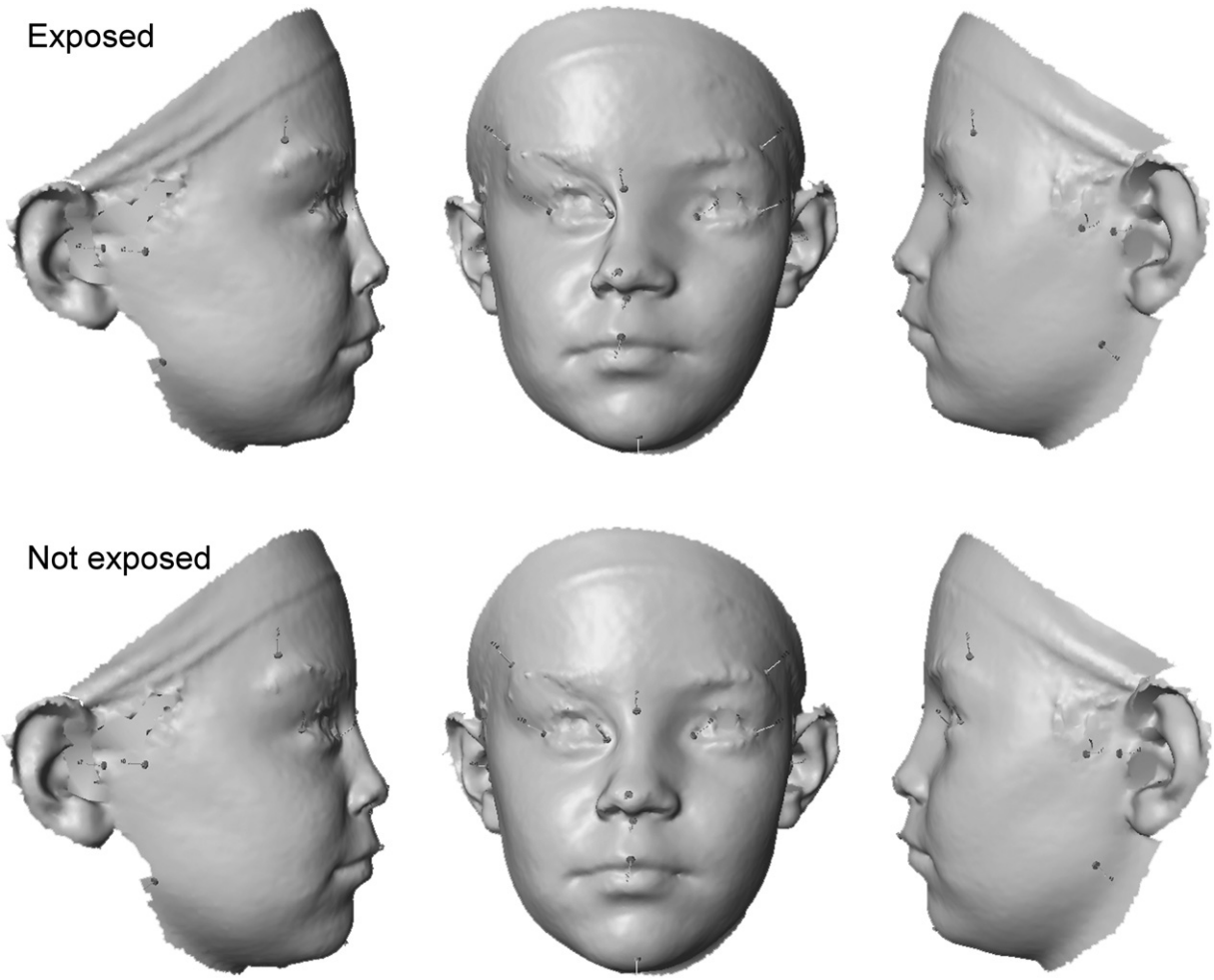

Fig. 2. Directional asymmetry in the fetal alcohol syndrome and control subjects in the Cape Coloured sample. For better visibility, the observed asymmetry has been exaggerated 5-fold. The facial surfaces were generated by morphing a single facial scan according to the landmark positions for the mean asymmetries (Wiley et al., 2005).

anterior and slightly higher on the left than on the right side) and for the gonion (more posterior and slightly higher on the left than on the right side). Comparison according to alcohol exposure indicated that these features were much more accentuated for the FAS group exposed to alcohol than for the nonexposed control group (Fig. 2). The cross-validation of the linear discriminant classification rule using the asymmetries resulted in a sensitivity of 69% and specificity of 52%.

The directional asymmetries in the FC sample also differed significantly between the FAS and control groups for both test statistics (Procrustes distance 0.0066, $P = .043$; $T^2 = 55.59$, $P = .008$). Similar to the results in the CC sample, the main features of directional asymmetry were a shift of the midline landmarks (except menton) to the right and a shift of the eyes to the left. In addition, the FC showed substantial directional asymmetry of the frontotemporale, which is in a more posterior and somewhat lower position on the left than on the right side (particularly noticeable in relation to exocanthion; Fig. 3). Differences in directional asymmetry between the control and FAS groups, as in the CC sample, included a stronger rightward shift of the midline landmarks for the exposed group. Unlike the CC population, however, there was relatively little change in the directional asymmetry of the eyes or of frontotemporale. In contrast, there was a marked difference in directional asymmetry at gonion (more posterior and lateral position on the left than on the right side in the exposed group; in addition, substantially higher on the left than on the right side in the control group). Moreover, the control group also showed a parallel shift of zygonion and tragion (more anterior and medial on the left than on the right side), whereas the exposed group showed opposite anterior–posterior shifts (zygonion more posterior and tragion more anterior on the left than on the right side). The cross-validation of the discriminant function in the FC population resulted in a sensitivity of 60% and specificity of 66%.

Discussion

The finding of significant directional asymmetry in all groups agrees with most published analyses (Ercan et al., 2008; Kimmerle and Jantz, 2005; McIntyre and Mossey,

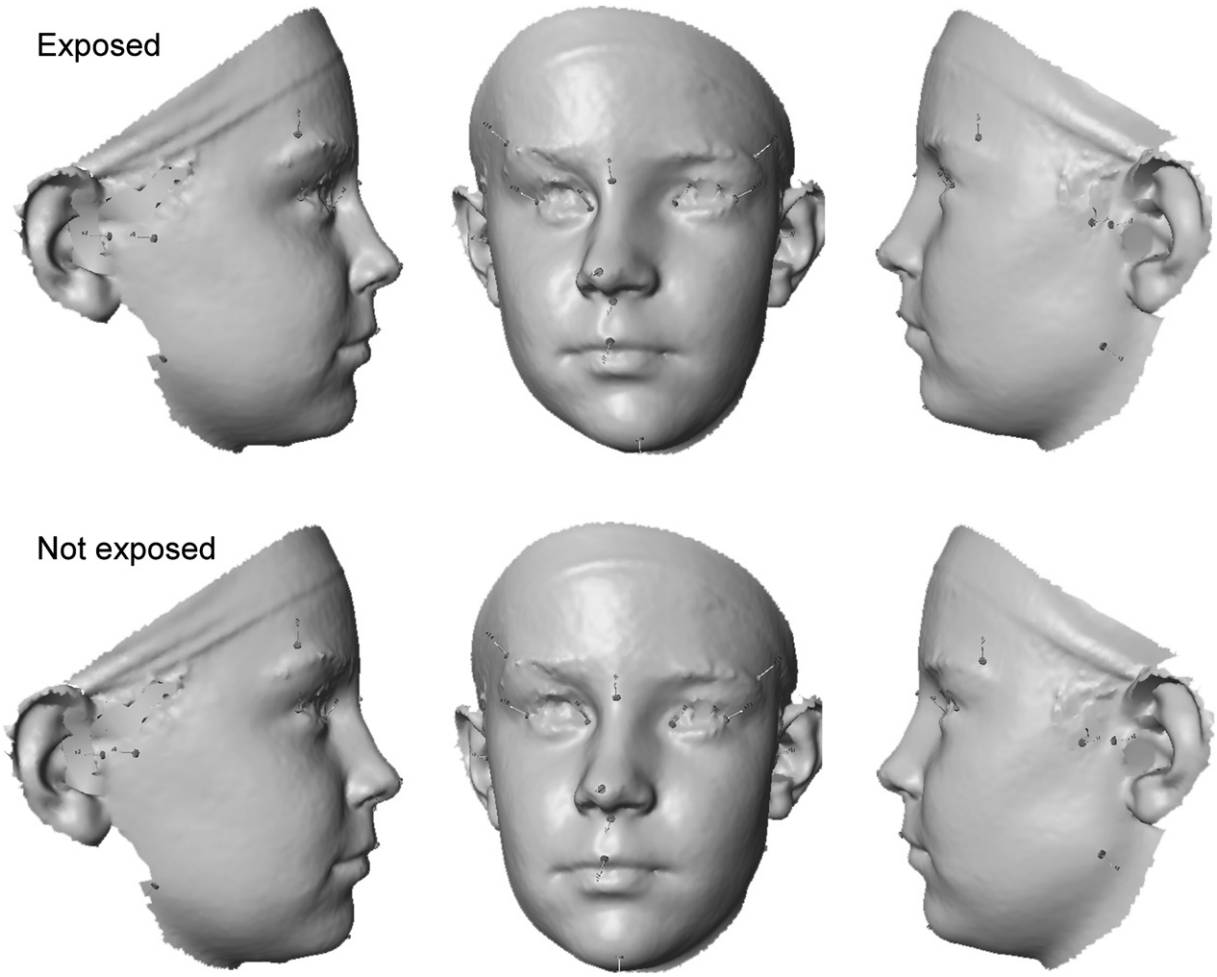

Fig. 3. Directional asymmetry in the fetal alcohol syndrome and control subjects in the Finnish Caucasian sample. For better visibility, the observed asymmetry has been exaggerated 10-fold.

2002; Shaner et al., 2000). The average directional asymmetry was dominated by a shift of the midline landmarks to the right and of the eyes to the left (Figs. 2 and 3). This is consistent with the strong tendency for a left dominance in facial distance measurements found in some published studies (Ercan et al., 2008; McIntyre and Mossey, 2002; Ras et al., 1994) but contrasts with the right dominance reported by other investigators (Ferrario et al., 1995; Shaner et al., 2000). It is unclear to what extent the inconsistency of published results corresponds to true differences between the populations under study or to differences in the methods used for measurements and analyses. For this study, the crucial results concern the changes of the directional asymmetry between groups with or without alcohol exposure in each of the two ethnic samples (i.e., the bottom vs. top row in Figs. 2 and 3).

Our analyses demonstrated significant differences in facial asymmetry between individuals with a diagnosis of FAS and individuals not exposed to alcohol prenatally (controls) in both populations. Differences in directional asymmetry were large enough to classify subjects into the correct group. The cross-validation results indicated that facial asymmetry could classify individuals with a fair degree of accuracy. Although this performance approaches the accuracy of diagnoses by pediatricians after a 2-day training program (Jones et al., 2006), it is unlikely that facial asymmetry is practical as a diagnostic tool. The effects of alcohol exposure on facial asymmetry are very subtle, and it is impossible with the present data to quantify the degree of asymmetry due to amount or timing of alcohol exposure in utero. It is possible, however, that facial asymmetry discreetly provides complementary information to dysmorphologists and may provide further insight into the pathophysiology of alcohol on the developing fetus. The CIFASD project is now collecting data on brain structure and function, which will allow for exploratory analysis to identify principal features of covariation between brain and the dysmorphology of the face.

Sample sizes were sufficiently large for detailed analyses of the shape features that differ between the directional asymmetry of FAS and control groups (Fig. 3). In both samples, changes of directional asymmetry were associated with a strengthening of features that were already present in the overall average directional asymmetry: the shift of midline landmarks to the right and, less strongly so, the opposite displacement of both eyes. Overall, children prenatally exposed to alcohol with a FAS diagnosis tend to have stronger directional asymmetry than the children in the respective nonexposed control groups. This strengthening of overall directional asymmetry for children with alcohol exposure is not the only change, however, because other aspects of directional asymmetry are affected as well.

Prenatal alcohol-induced alterations in directional asymmetry are similar in the two study sites overall and differ only in relatively minor ways (Fig. 3). It is not possible to assign these differences to a particular cause because the two samples differ in several factors, including age and ethnicity. Although there is a substantial age difference between the samples: the Finnish sample consists of older individuals and has a greater age range than the Cape Town sample, a regression analysis of facial asymmetry on age did not indicate a systematic trend of directional asymmetry to change with age (results not shown). Accordingly, it is not possible with the present data to disentangle completely the effects of ethnic composition, age, and other possible factors.

Because craniofacial development of the face is intimately tied to the development of the brain, the question arises whether the increase of facial directional asymmetry associated with alcohol exposure correlates with a change in the asymmetry of the brain. Sowell et al. (2002) reported that individuals with severe prenatal alcohol exposure have reduced asymmetry of the cortical surface and gray matter density. Normal asymmetry is right-biased and particularly accentuated in the posterior inferior temporal lobes (Sowell et al., 2002). This region is not directly adjacent to the region of the face considered in this study; thus, it is unlikely that there is a direct developmental link as has been suggested for the facial and brain asymmetry in autism spectrum disorder (Hammond et al., 2008). On the other hand, Sulik and Johnston (1983) have demonstrated direct links between damage to the frontal lobes and craniofacial dysmorphology in mice prenatally exposed to ethanol and Johnson et al. (1996) observed a similar association in human subjects. It is likely that a range of developmental processes, and their disruption by various disorders, can produce associations between the structure or function of the brain and facial shape and asymmetry. Examples include altered facial shape and asymmetry in schizophrenia (Hennessy et al., 2004) and correlations between facial features and cognitive performance in healthy subjects (Hennessy et al., 2006).

This study has demonstrated a clear difference in facial asymmetry between individuals with prenatal exposure to alcohol and those with no prenatal exposure. The powerful methods of geometric morphometric analysis will allow us to study the association of facial asymmetry with other brain-related functions as well as with neuropsychological and behavioral deficits that have been shown to exist in children with prenatal alcohol exposure (Mattson et al., 1998; McGee et al., 2008). Identification of patterns of asymmetry with brain morphology, function, or neuropsychological deficits will present health care providers with expanded opportunities for effective intervention.

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