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ORIGINAL ARTICLE

Face–brain asymmetry in autism spectrum disorders

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The heterogeneity of autism spectrum disorders (ASDs) confounds attempts to identify causes and pathogenesis. Identifiable endophenotypes and reliable biomarkers within ASDs would help to focus molecular research and uncover genetic causes and developmental mechanisms. We used dense surface-modelling techniques to compare the facial morphology of 72 boys with ASD and 128 first-degree relatives to that of 254 unrelated controls. Pattern-matching algorithms were able to discriminate between the faces of ASD boys and those of matched controls (AUC = 0.82) and also discriminate between the faces of unaffected mothers of ASD children and matched female controls (AUC = 0.76). We detected significant facial asymmetry in boys with ASD ($P < 0.01$), notably depth-wise in the supra- and periorbital regions anterior to the frontal pole of the right hemisphere of the brain. Unaffected mothers of children with ASD display similar significant facial asymmetry, more exaggerated than that in matched controls ($P < 0.03$) and, in particular, show vertical asymmetry of the periorbital region. Unaffected fathers of children with ASD did not show facial asymmetry to a significant degree compared to controls. Two thirds of unaffected male siblings tested were classified unseen as more facially similar to unrelated boys with ASD than to unrelated controls. These unaffected male siblings and two small groups of girls with ASD and female siblings, all show overall directional asymmetry, but without achieving statistical significance in two-tailed *t*-tests of individual asymmetry of ASD family and matched control groups. We conclude that previously identified right dominant asymmetry of the frontal poles of boys with ASD could explain their facial asymmetry through the direct effect of brain growth. The atypical facial asymmetry of unaffected mothers of children with ASD requires further brain studies before the same explanation can be proposed. An alternative explanation, not mutually exclusive, is a simultaneous and parallel action on face and brain growth by genetic factors. Both possibilities suggest the need for coordinated face and brain studies on ASD probands and their first-degree relatives, especially on unaffected mothers, given that their unusual facial asymmetry suggests an ASD susceptibility arising from maternal genes.
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Introduction

Autism spectrum disorders (ASDs) include autistic disorder, Asperger's syndrome and pervasive development disorder (not otherwise specified), which are characterized by significant impairments in social interaction and communication, as well as inappropriately focused behaviour and restricted interests. ASDs affect as many as 1 in 166 individuals[1] and have a major impact on families, requiring extensive support from the education, social welfare and health care systems. ASDs are considered complex polygenic disorders, resulting from interactions of several genes with one another and with the environment.[2] Heterogeneity in phenotype and aetiology has confounded attempts to identify causes and develop effective treatments. Detailed analyses of the ASD phenotype beyond behavioural indices alone could improve the identification of homogeneous subgroups, informing more incisive molecular analyses of prospective genes involved in its pathogenesis.

It has been known for over 40 years that the face and the brain develop in exquisite coordination and that abnormalities or differences in facial morphology

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can be indicative of underlying brain pathology.[3] Until recently, the analysis of facial morphology has employed visual inspection, manual anthropometry, 2D photogrammetry, laser-based 3D image capture and conventional statistical analysis. Rapid 3D photogrammetric devices and pioneering developments in morphometric analysis techniques[4] now enable more sensitive surface shape analyses of facial morphology.[5,6] Studies using 3D dense surface models (DSMs) of face shape[7] have proven accurate at recognizing the characteristic facial phenotype of a variety of neurodevelopmental syndromes and have delineated the 3D facial gestalt of conditions, such as Fabry's disease.[8–12] Dense-surface-modeling techniques have not previously been used to evaluate facial morphology of homogeneously selected subjects with ASD, despite their clear potential to define biological markers of atypical or disrupted neurodevelopment. Here, we demonstrate significant facial asymmetry in multiplex families with children affected by ASD. Notably, affected boys and unaffected mothers of children with ASD have discriminating and statistically significant facial asymmetry compared to age/sex/racially matched controls.

Materials and methods

We recruited subjects with idiopathic ASD through the ASD-Canadian American Research Consortium (ASD-CARC; www.AutismResearch.com). Because of our interest in heritable genetic factors contributing to autism, each family had at least two affected members. However, not all affected individuals were available for imaging and occasionally an image proved later to be in some way inadequate for analysis. The 72 boys and 12 girls with ASD, 41 unaffected mothers and 27 unaffected fathers of children with ASD, and the 20 male and 28 female unaffected siblings were all of Caucasian ethnic origin and were age/sex/ethnicity matched with 254 unrelated controls. Of the boys with ASD, there were 19 pairs of siblings and 34 singletons. Diagnoses were established using psychometric standards compatible with DSM-IV TR criteria, such as the autism diagnostic interview-revised (ADI-R) and the autism diagnostic observation schedule-generic (ADOS-G).[13,14] The handedness of the boys with ASD did not differ significantly from that of the general population. Individuals with identifiable syndromes were excluded from the study. Written informed consent was obtained from affected individuals and/or their parents, depending on age and level of functioning. The study received ethical approval both in the UK (JREC 00/E042, UCLH Trust) and in Canada (CREB C01-0507; UBC).

In the study, the 3D face images were captured using commercial photogrammetric devices (www.canfieldscientific.com; www.3dMD.com). The number of surface points captured by the most recent models of these devices is of the order of 25 000 on an adult face. Both systems transform four different views/images of a face, overlaid with a random pattern, into a 3D point cloud to represent the surface of the subject's face. Two additional three-quarter colour portraits are mapped onto the mesh formed by the point cloud to reproduce facial appearance.

One individual (PH) manually annotated each face surface with 18 anatomical landmarks, adopted because of their known reproducibility.[15] Twelve of the landmarks (inner and outer canthi, alare, cheilion, crista major and otobasion inferius) were paired and labelled in a left–right manner. Six other landmarks (nasion, pronasale, subnasale, labiale superius, labiale inferius and gnathion) were not paired. To avoid the inaccuracies associated with incompletely scanned regions near the ears and hairline, we excluded these from analyses. Using the procrustes algorithm to compute mean landmarks and thin-plate splines to warp face surfaces to the mean landmarks, a set of face surfaces can be closely aligned. The points on a selected face can then be mapped to the closest points on each face to produce a dense correspondence of tens of thousands of points across all images. An average face surface of the set is then readily computed. The differences between the positions of the densely corresponded points on each face surface and those on the overall average face are subjected to a principal component analysis (PCA). From the resulting set of principal components (PCs) or PCA modes, those accounting for 99% of all shape variation are selected and we refer to them as a DSM.[5,7] In the present study, for example, the full-face DSM computed from the 177 images of both controls and affected boys contained 40 PCs. Each face surface can be resynthesized as a weighted linear sum of the PCs.

The averages of the corresponding DSM weightings of any subset of the face surfaces used to build the DSM synthesize the average face of that subset. Similarity between two individual face surfaces, or between a face surface and the average face of a subset, is computed as the square root of the sum of squares of differences of DSM weightings. Thus, we can calculate shape similarity or proximity of an individual's face, or the mean face of one subset, to another individual, or to the mean of another subset. This provides a simple categorization of a face with respect to two disjoint subsets of faces in terms of the subset whose average face is closest or most similar. The normalized position of a face relative to affected and unaffected means is defined as follows:

Let U and A, respectively, denote the DSM representations of the subsets of 3D face images for the unaffected and affected individuals. Let u and a respectively denote the DSM representations of the mean faces of U and A. Then,

$$\underline{u} = \{\Sigma \underline{x}\}/|U| \quad \text{and } \underline{a} = \{\Sigma \underline{x}\}/|A| \qquad \underline{x} \in U \qquad \underline{x} \in A$$

Let n represent the number of modes in the DSM covering 99% of shape variation and let R denote the set of real numbers. Define the mapping $\text{norm}_{\underline{u},\underline{a}}: R^n \rightarrow R$ as follows: for $\underline{x} \in \text{U} \bigcup \text{A}$

$$\mathrm{norm}_{\underline{u},\underline{a}}(\underline{x}) = (\underline{u} - \underline{a}).(\underline{u} + \underline{a} - 2\underline{x})/(\underline{u} - \underline{a}).(\underline{u} - \underline{a})$$

using vector inner product.

Then

$$\mathrm{norm}_{\underline{u},\underline{a}}(\underline{u}) = -1 \text{ and } \mathrm{norm}_{\underline{u},\underline{a}}(\underline{a}) = +1$$

and thus $\mathrm{norm}_{\underline{u},\underline{a}}$ maps the average control and affected faces to the positions -1 and $+1$, respectively.

With the closest mean discrimination testing, the average faces are computed for the control and family member (for example, affected males, unaffected brothers and mothers) subgroups in the training set, and each unseen test face is classified according to which average it is nearest using its DSM representation. For linear discriminant analysis, the goal is a linear combination of PC modes that exhibits the largest difference in the subgroup means relative to the within-group variance. Support vector machines, or large margin classifiers, focus on individual cases in the overlap of the subgroups to be classified that help to define a separating surface with largest margin between the subgroups.

The validity of our discrimination results is determined using receiver-operating characteristic analysis and, in particular, the area under the curve (AUC) arising from a plot of pairs of false positive rate and true positive rate, when a classification parameter is varied through its full range. The intuitive interpretation of AUC is the probability of correctly classifying a randomly selected pair of subjects, one negative and one positive, or in this case, a control and an individual with a confirmed ASD.

Biological asymmetry of a physical object is usually studied by comparing original and mirrored (reflected) forms.[16,17] In anticipation of our analysis of asymmetry, we generated a mirrored form of each face surface in the study and swapped over the manually placed and left–right-paired landmarks before resampling the surface, effectively interpolating a dense set of landmarks on both sides of the face. Thus, the interpolated landmarks are matched-paired across the face; to study face asymmetry, a DSM was constructed from the combined set of densely corresponded face surfaces and their mirrored forms. The Euclidean distance between the DSM-based representations of the surface of a face (or a face patch) and its mirrored form was used as a measure or index of asymmetry.

Results

Visualization of mean face shape differences in boys with ASD

First, we analysed the 3D face scans of 72 boys (2.2–18.2 years; mean 9.2 years) with a definitive ASD diagnosis, and 105 unrelated, unaffected boys (2.0–18.0 years; mean 9.5 years) from which we derived the mean affected and mean control faces (Figure 1a). These mean faces are indistinguishable using just the unaided eye. Therefore, we colour-distance coded points on the mean ASD face to highlight their distance along the surface normal from corresponding points on the mean control face (Figure 1b). Shape difference is then visibly detectable in the supraorbital, nasal, zygomatic and perioral regions, especially on the right side. Along the normal to the face surfaces, the accuracy of the 3D scanning devices we used is of the order of 0.1 mm. We generated an alternative comparison in the form of a dynamic morph or rapid interpolation from one mean face to the other (Supplementary Animation 1). This confirmed the right supraorbital and zygomatic dominance and also demonstrated an increased width of the nose in the ASD boys that is statistically significant ($P < 0.05$). Colour–distance-coded comparisons of the two means, parallel to three orthogonal axes, provided additional evidence of right dominant asymmetry for the ASD boys of the supraorbital region and zygoma that is more lateral and depth-wise than vertical (Figure 1c). Tangential to the face surface, the scanner accuracy is about 1.4 mm. But, the depth asymmetry comparison of the regions of particular interest (supraorbital and periorbital) will largely reflect difference along the surface normal.

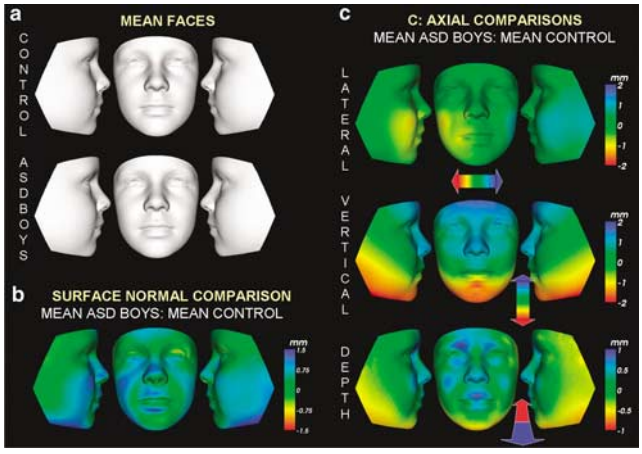

Figure 1 Comparison of mean face of boys with ASD ($n = 72$) to that of control boys ($n = 105$). (**a**) Top row: mean face of control boys; bottom row: mean face of ASD boys; (**b**) Colour–distance coding of points on mean ASD boys' face to show distance along the surface normal from corresponding points on mean control boys' face. The colour scale uses blue (or red) to indicate regions where the mean ASD boys' face is at least 1.5 mm outside (or at least 1.5 mm inside) corresponding regions of the mean control boys' face. Parallel to surface normal, scanner accuracy is 0.1 mm. (**c**) Colour–distance comparisons of mean ASD boys' face to show displacement relative to corresponding points on mean control face along three orthogonal axes. First row: lateral axis with scale (−2,2); second row: vertical axis with scale (−2,2); third row: depth axis with scale (−1,1). Parallel to the surface, scanner accuracy is about 1.4 mm. For depth accuracy, where interest is in forward-facing regions, the accuracy along the normal, which is of the order of 0.1 mm, is most relevant.

Table 1 Mean AUC for ROC curves for 20-folded unseen classification of face regions for affected boys and unaffected mothers versus age and sex-matched controls

	ASD boys (n = *72*) *vs male controls* (n = *105*)			*Unaffected mothers* (n = *41*) *vs female controls* (n = *52*)		
	CM	*LDA*	*SVM*	*CM*	*LDA*	*SVM*
Face	0.77	0.77	**0.82**	**0.76**	0.75	0.75
Supra-orbit	0.73	0.75	**0.82**	0.68	**0.71**	0.68
Periorbit	0.77	0.79	**0.79**	**0.69**	0.65	0.67
Perinasal	0.76	**0.81**	0.79	0.60	**0.65**	0.61
Perioral	0.59	0.66	**0.69**	0.63	**0.66**	0.63

Abbreviations: AUC, area under curve; ASD, autism spectrum disorders; CM, closest mean; LDA, linear discriminant analysis; ROC, receiver operating characteristic; SVM, support vector machines.
Values in bold indicate best or statistically significant results.

Discriminating face shape differences in boys with ASD
To determine if the face shape differences in ASD boys described above were discriminating in an unbiased fashion, receiver-operating characteristic curves were generated for each of 20 DSMs constructed from 90% training sets and evaluated against unseen 10% test sets, randomly sampled in a stratified manner from the ASD and control subgroups. Using closest mean as the classification criterion, the mean AUC of the 20 receiver-operating characteristic curves was 0.77 and the mean value of the point on the receiver-operating characteristic curve where sensitivity and specificity coincide was 70%. Linear discriminant analysis achieved an identical mean AUC and the support vector machines algorithm scored a mean AUC of 0.82, and mean equal sensitivity and specificity of 79%. To determine discrimination accuracy for individual regions of the face, we completed similar 20-fold testing of four regions (supraorbital, periorbital, perinasal and perioral). This showed the supraorbital and perinasal regions to be more discriminating than other parts of the face (Table 1).

Because the colour-coded comparisons of the mean faces of the affected and unaffected boys suggested this, we next considered facial asymmetry.

Significant facial asymmetry in boys with ASD
To investigate asymmetry, previous studies have typically compared the positions of landmarks and/or measurements derived from landmarks on the original and mirrored objects. Above, we showed that the ASD boys and controls have discriminating differences in face shape. The colour–distance-coded comparison of the mean face of the boys with ASD to that of the matched controls emphasized that the face shape differences are most evident in the orbit, zygomatic arch and forehead, where anatomical landmarks are extremely difficult to place. So, an analysis of regions where landmarks could be placed, and/or where landmark measurements derived, was unlikely to detect the subtle face shape differences we had identified. Therefore, we generated a DSM combining the original face surfaces (or face patches) and their mirrored forms and employed the Euclidean distance between the DSM-based representations of an individual face (or a face patch) and its mirrored form as a measure or index of asymmetry. We found this asymmetry measure to be significantly greater for the ASD boys when compared to the controls (Figure 2) for this combined DSM of the original and reflected faces ($P = 0.009$), and also for the periorbital ($P = 0.009$) and perioral (0.002) original and mirrored patches for similar combined DSMs (Table 2).

Table 2 Significance testing of asymmetry index for face regions

	Male controls (n = *105*)		*ASD boys* (n = *72*)		t-*test*
	Mean	*Var*	*Mean*	*Var*	P
Face	6.10	2.66	6.77	2.84	**0.009**
Supra-orbit	6.88	9.07	7.46	8.64	0.202
Periorbit	7.68	2.91	8.41	3.54	**0.009**
Perinasal	6.32	3.71	6.68	5.05	0.251
Perioral	6.84	3.81	8.06	6.84	**0.002**

Abbreviation: ASD, autism spectrum disorder.
Values in bold indicate best or statistically significant results.

Visual confirmation of the localized nature of this asymmetry is seen in Figure 3, where the mean original faces for the ASD and control boys are colour-coded in terms of difference from the mean mirrored faces using surface normal and axial comparisons. Even though there is a mild signal showing depth asymmetry in the control boys, the most notable signal is along the depth axis for the ASD boys, emphasizing the supraorbital and periorbital regions. The distance between the mean of a set of faces and that of their mirrored forms is a measure of the overall directional asymmetry (DA) of the original faces. Using a bootstrap analysis of 1000 iterations, estimates were computed for the mean DA and a 95% confidence interval for the ASD boys (2.150, (2.139, 2.161)) and control boys (1.777, (1.770, 1.784)) adding further evidence for greater facial asymmetry in the ASD boys (Table 3). Supplementary Animation 2 shows dynamic morphs between the mean original and mean mirrored face surfaces for the affected and

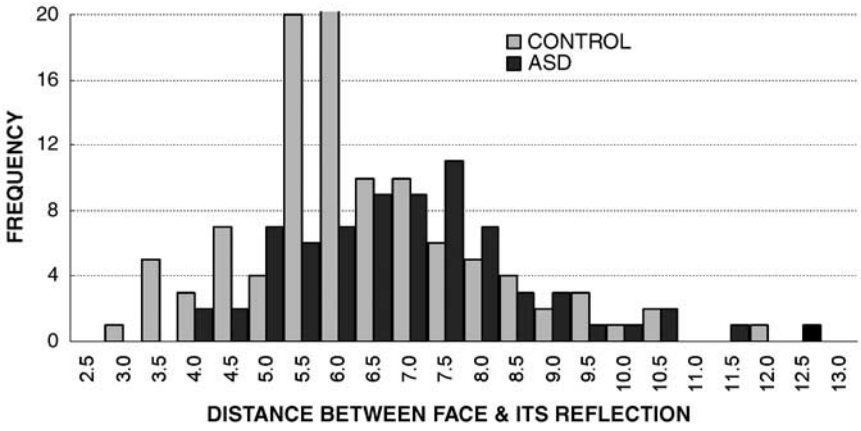

Figure 2 Asymmetry index for ASD boys and related controls. Histogram of distance between faces and their reflected or mirrored forms (a measure of asymmetry) for ASD boys ($n = 72$) and control boys ($n = 105$). The index is significantly greater for the ASD boys than for the control group ($P = 0.009$).

unaffected boys. Both morphs have been exaggerated to demonstrate the subtle differences in asymmetry. The morph for the control boys shows a twist to the upper face with orbits coordinated as opposed to an anterior–posterior change in orbit position for the ASD boys, once again emphasizing much greater right dominance in the supra- and periorbital regions.

In summary, then, we have established discriminating and statistically significant facial asymmetry in the ASD boys, especially in the supraorbital region, in comparison with appropriate controls. Because, preliminary data have suggested that unaffected first-degree relatives of individuals with ASD share some of the same brain characteristics,[18] we next undertook similar analyses of affected and unaffected first-degree relatives.

Significant discriminating facial asymmetry in unaffected mothers of children with ASD

We compared 3D face images of 41 unaffected mothers of children with ASD (32.4–58.3 years; mean 42.2 years) to those of 52 age/sex/ethnicity matched and unrelated controls (30.4–59.6 years; mean 42.1 years). As with the ASD boys, the difference between the mean faces of the unaffected mothers and matched controls is too subtle to be detected by the unaided eye (Figure 4a). The colour–distance comparison along the surface normal of the mean face of the unaffected mothers to the mean control identifies greater width of the mean face of the unaffected mothers (Figure 4b), which is statistically significant ($P < 0.05$) for the distance between the lower ear attachment points. The axial colour–distance comparisons show asymmetric differences in both the vertical position of the orbit and depth of the supraorbit, the latter being similar to that of the ASD boys (Figure 4c).

Next, it was natural to carry out discrimination testing of these face shape differences. A 20-fold unseen comparison of the unaffected mothers and age-matched female controls produced a mean AUC of 0.76 for closest mean discrimination using the full face. Linear discriminant and support vector machines analyses both scored an AUC of 0.75. For a DSM for the full face, constructed from both original faces and mirrored forms, the asymmetry measure (distance between a face and its mirrored form) was significantly greater ($P < 0.03$) for the mothers compared to adult female controls (Figure 5).

A bootstrap analysis of 1000 iterations produced DA estimates and 95% confidence intervals for the mothers of the ASD boys (2.863, (2.851, 2.875)) and age-matched control women (2.381, (2.371, 2.391)), adding further quantitative evidence for greater facial asymmetry in the mothers. As with the ASD boys–control comparison, visual confirmation of regions of asymmetry difference were demonstrated by colour-coded comparisons of mean original to mean mirrored faces (Figure 6). The depth axial comparison demonstrates greater right-sided dominance for the unaffected mothers of the supraorbital, periorbital and zygomatic regions. In addition, there is a stronger signal for the vertical axial comparison in the orbital region. The latter, in particular, is confirmed by the dynamic morphs in Supplementary Animation 3.

Thus, as with boys with ASD, we established discriminating and statistically significant facial asymmetry in unaffected mothers of children with ASD compared to controls. Moreover, besides right-sided dominance of the supraorbital region, these unaffected mothers additionally showed vertical asymmetry of the orbital region.

Face shape difference in unaffected male siblings

We scanned the faces of 20 unaffected male siblings. Such a small number was inadequate for meaningful multi-folded discrimination testing. Instead, we computed separate DSMs using the faces of the 72 ASD boys and 105 controls, omitting on each occasion affected brothers of the unaffected sibling being tested unseen. Figure 7 shows a background closest mean classification scatter of 172 ASD boys and controls for a single DSM. It is overlaid by the unseen classification position of the unaffected siblings also using the

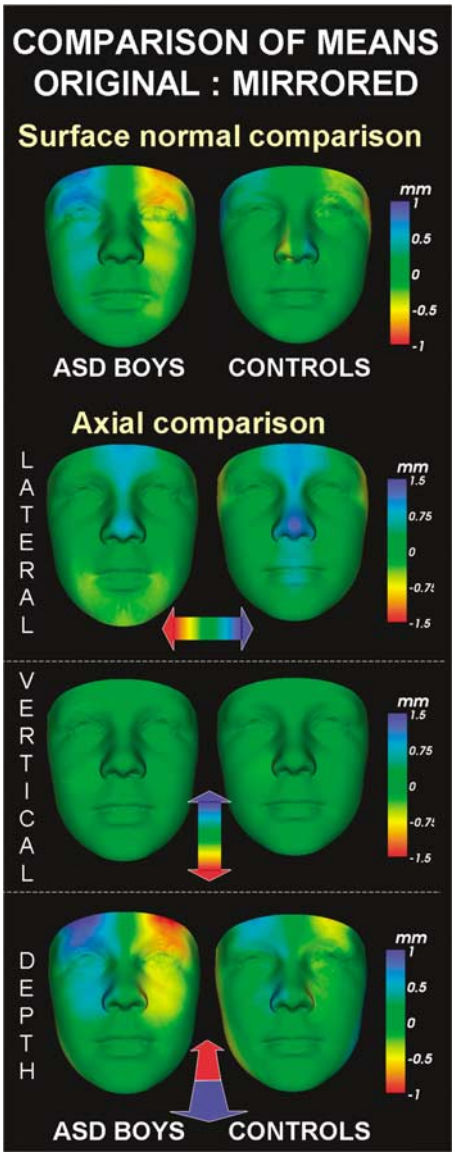

Figure 3 Comparisons of mean original to mean mirrored form for ASD ($n = 72$) and controls boys ($n = 105$). Separate colour–distance codings of points on mean original faces to show distance from corresponding points on mean mirrored faces for ASD and control boys using the surface normal and three orthogonal axes. The interpretation of the scales is as in Figure 1. The ASD boys show most difference in the form of right-dominance of the supraorbital region.

closest mean algorithm for the DSM computed from the 177 ASD and control boys less any affected brothers of the sibling being tested. About two thirds of the unaffected male siblings are classified as more like the average of the (unrelated) ASD boys than the average control. This suggests that a majority of these unaffected male siblings have facial features more like those of unrelated ASD boys than age-matched controls.

For the visualizations of face shape differences, the 20 male siblings (2.1–21.0 years; mean 8.7 years) were compared with 89 male controls (2.0–18.0 years; mean 8.7 years). Supplementary Figure S1 illustrates

Table 3 Mean directional asymmetry and 95% confidence error arising from a 1000 iteration bootstrap for subgroups of the ASD families and matched controls

	ASD families			*Controls*		
1.96 s.e.*	*Subgroup*	n	*DA*	*DA*	n	*Subgroup*	*1.96* s.e.*
0.011	ASD boys	72	2.150	1.777	105	Boys	0.007
0.012	Mothers	41	2.863	2.381	52	Women	0.010
0.016	Fathers	27	3.243	2.356	47	Men	0.010
0.020	Male siblings	20	2.817	1.763	89	Boys	0.008
0.027	ASD girls	12	2.680	1.969	46	Girls	0.012
0.016	Female siblings	28	2.286	1.906	42	Girls	0.012

Abbreviations: ASD, autism spectrum disorder; DA, directional asymmetry, s.e., standard error.

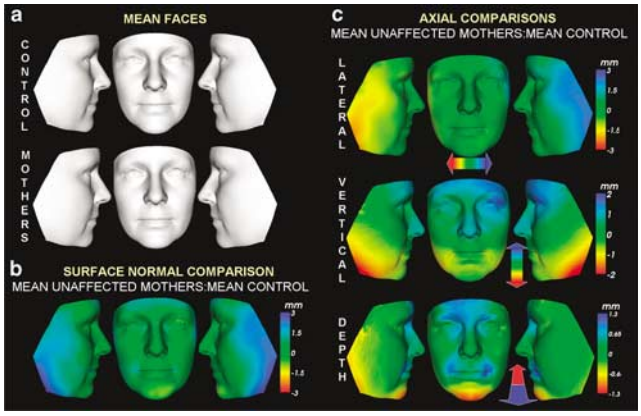

Figure 4 Comparison of mean face of unaffected mothers of children with ASD ($n = 41$) to that of female adult controls ($n = 52$). (**a**) Top row: mean face of adult female controls ($n = 52$); bottom row: mean face of unaffected mothers of children with ASD ($n = 41$). (**b**) Colour–distance coding of points on mean unaffected mothers' face to show distance along surface normal to corresponding points on mean control face. The colour scale uses blue (or red) to indicate regions where the mean unaffected mothers' face is at least 3 mm outside (or at least 3 mm inside) corresponding regions of the mean control face. Parallel to the surface normal, scanner accuracy is about 0.1 mm. (**c**) Colour–distance comparisons of mean unaffected mothers' face to show displacement relative to corresponding points on mean control face along orthogonal axes. First row: lateral with scale (−3,3); second row: vertical with scale (−2,2); third row: depth with scale (−1.3,1.3). The vertical comparison shows upward displacement of the left orbit in the unaffected mothers compared to the controls. The depth comparison shows right-dominance of the supraorbital region in the unaffected mothers compared to the controls.

the face shape difference of the average unaffected male sibling compared to the average control. Colour-coded comparisons of mirrored mean to original mean for the unaffected siblings and associated controls are

shown in Supplementary Figure S2. Both figures are consistent with greater right-sided dominance in the periorbital region and zygomatic arch, as found in the ASD boys. A bootstrap analysis of 1000 iterations produced estimates for the mean overall DA and a 95% confidence interval for the male siblings (2.817, (2.787, 2.837)) and matched control boys (1.763, (1.755, 1.771)). However, the individual asymmetry index for the full face for the male siblings did not show significant difference from the controls.

Face shape difference in unaffected fathers of boys with ASD

A group of 27 unaffected fathers (32.8–59.2 years; mean 43.3) were compared with 47 unaffected male controls (30.4–60.0 years; mean 42.5 years). Once again, the small numbers precluded meaningful multi-folded discrimination analysis. The axial depth comparison of the mean face of the unaffected fathers compared to that of the matched controls (Supplementary Figure S3) suggests there could be minor mid-facial hypoplasia in the unaffected fathers, but it was not shown to be statistically significant. The asymmetry comparisons (Supplementary Figure S4) along the surface normal suggested greater right-sided dominance for the fathers, as does the axial depth comparison, but it was far less convincing than for the ASD boys, unaffected mothers and male siblings. Although a bootstrap analysis produced estimates of mean DA for the fathers (3.243, (3.227, 3.259)) and matched male controls (2.356, (2.346, 2.366)) that indicate greater paternal group directional asymmetry, a *t*-test of the individual asymmetry index of fathers compared to male controls did not achieve statistical significance.

Face shape differences in girls with ASD and unaffected female siblings

Finally, we compared 12 girls with ASD (2.2–15.1 years; mean 8.9 years) with 46 unaffected female controls (1.9–16.1 years; mean 8.8 years) and also 28 unaffected female siblings (4.4–18.2 years; mean 9.9 years) with 42 unaffected female controls (4.0–16.4 years; mean 10.0 years). Because of the small numbers involved, no multi-folded discrimination testing was carried out. The comparisons in Supplementary Figures S5 and S6 of mean faces of the ASD girls and unaffected sisters to their respective control group means were not very informative, probably because of the small ASD family group sizes. In contrast, the comparisons of mean original face to mean mirrored face (Supplementary Figure S7) did suggest greater right dominance in the supraorbital and periorbital regions and zygomatic arch in both the ASD girls and female siblings, when compared to their respective control group. In agreement with the visualizations of overall DA, the bootstrapping analysis established greater group DA for both the ASD girls (2.680, (2.653,2.707)) and unaffected female siblings (2.286, (2.270,2.302)), compared to their matched control groups (1.969, (1.958,1.981)) and (1.906,(1.894,1.918)), respectively. However, as for the unaffected fathers and brothers, it was not possible to establish statistical significance for the individual asymmetry index for either group compared to their respective matched controls.

Discussion

Variation in facial morphology results from variation in embryonic and fetal development and is likely due to small environmental changes, genetic constitution and natural selection. Facially symmetric individuals have been found to be in better physiological, psychological and affective health.[19] The intimate embryological relationship between the face and brain also serves as an informative index of brain dysmorphogenesis in neurologic and psychiatric disorders of early developmental origin.[6] The facial

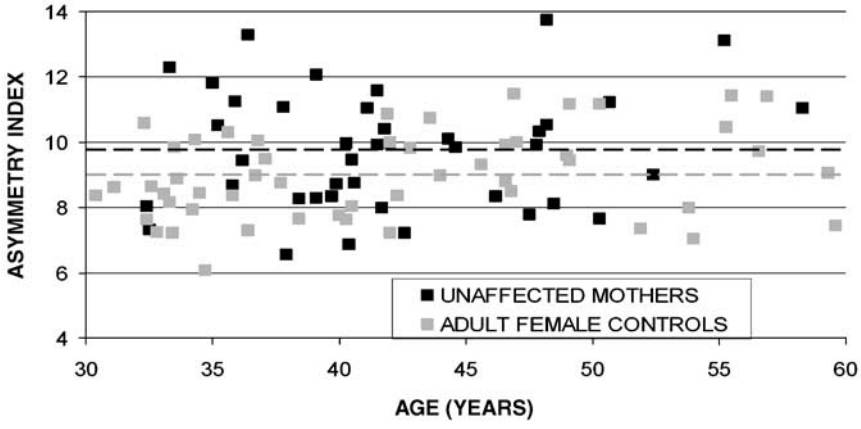

Figure 5 Asymmetry index of unaffected mothers and female controls. The scatter plot shows asymmetry index (distance between a face and its reflection) against age for the full-face-combined dense surface model of original and mirrored faces for the unaffected mothers of ASD children and matched adult female controls. The overlaid broken horizontal lines indicate mean asymmetry indices for the two groups The mean index (9.75) for the unaffected mothers is significantly larger than that (8.99) of the matched control group using a two-tailed *t*-test ($P<0.03$).

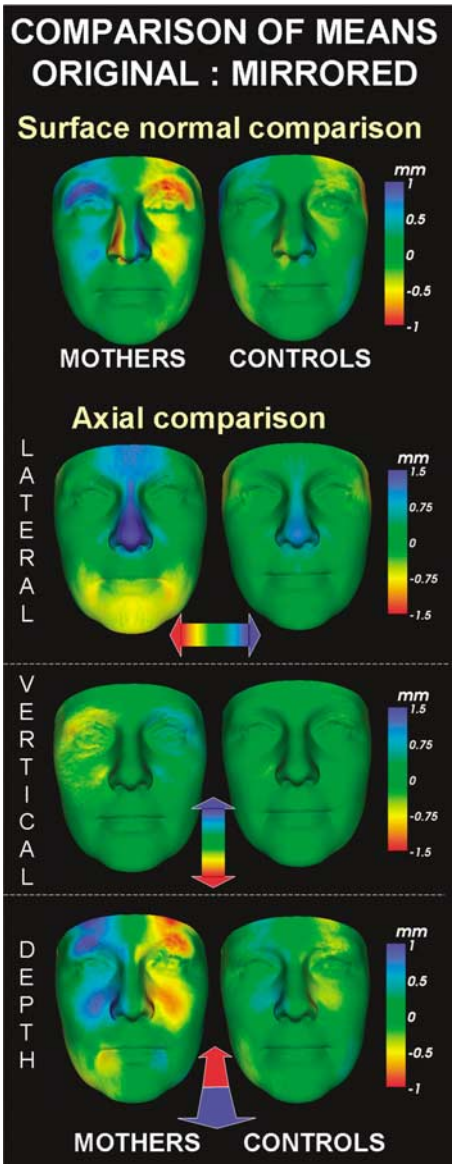

Figure 6 Comparisons of mean original to mean mirrored form for female controls ($n=52$) and mothers of ASD children ($n=41$). Separate colour–distance codings of points on mean original faces to show distance from corresponding points on mean mirrored faces for unaffected mothers and female controls using the surface normal and three orthogonal axes. The interpretation of the scales is as in Figure 1. The unaffected mothers show slightly less supraorbital difference as well as considerable difference in the periorbital, zygomatic and nasal regions. The vertical difference suggests a rotation about an axis approximately normal to the plane of the face.

asymmetry that we have established in both affected and unaffected members of ASD families inevitably encourages a comparison with known asymmetric brain morphology. We begin the discussion by focusing on the control population of our study.

Figures 3 and 6 show colour–distance-coded comparisons of mean original to mean mirrored face for four subgroups in our study: ASD boys, control boys, unaffected mothers of ASD children and control women. For each control group, these comparisons and the upper face twisting shown in the corresponding control morphs of Supplementary Animations 2 and 3 suggest minor right dominance in the supraorbital region. A similar mild right dominance can be seen in the corresponding colour-coded comparisons for the controls in Supplementary Figures S2, S4 and S7. This is consistent with so-called Yakovlevian torque found in the general population in which the right hemisphere protrudes anteriorly beyond the left, and the left hemisphere extends posteriorly beyond the right, reflecting volume differences. Additionally, the left occipital lobe extends across the midline, bending the interhemispheric fissure towards the right.[20] We hypothesise, therefore, that for the general population, such a torque in brain growth is closely related to the tendency for the upper face to have a similar leftward twist.

For the boys with ASD, the colour–distance comparisons of Figure 3 show their mean face to have negligible vertical asymmetry and, compared to the control boys, less lateral asymmetry but much greater depth asymmetry. For the control boys, Supplementary Animation 2 shows the upper part of the face rotating in a twist-like motion about a vertical axis while the left and right palpebral fissures maintain their position relative to one another. By comparison, the related morph for the ASD boys emphasizes an alternating, forward and backward motion of the left and right supraorbital and periorbital regions. This results in the right and left palpebral fissures moving in an inferior–posterior fashion relative to each other and indicates much stronger right depth dominance of the supraorbital and periorbital regions. How might this additional depth asymmetry arise? Could it be related to brain asymmetry in boys with ASD?

In the past, MRI studies have identified a variety of global and localized brain size anomalies in ASD, which were not always concordant.[21,22] A recent meta-analysis showed that head size is normal at birth and that atypical, accelerated enlargement is restricted to the first 2–4 years of life with subsequent deceleration of brain growth.[23] For 45 of the 72 boys with ASD that we studied, head circumference was available for analysis. There was no statistically interesting relationship between our computed asymmetry index and head circumference. A strong correlation (Pearson product moment of 0.764) was established between head circumference and mode 1 of the DSM for the full face, but this is to be expected since mode 1 typically reflects overall face size and is itself even more strongly correlated with age.

The dorsolateral and medial frontal regions of the brain have been found to be most abnormal in 2- to 4-year-old children with ASD.[21] The white matter underlying the frontal pole was found to be 1.2 s.d., larger than normal in one study of autism in boys.[24] When the frontal cortex was divided into four subregions, the dorsolateral and medial frontal re-

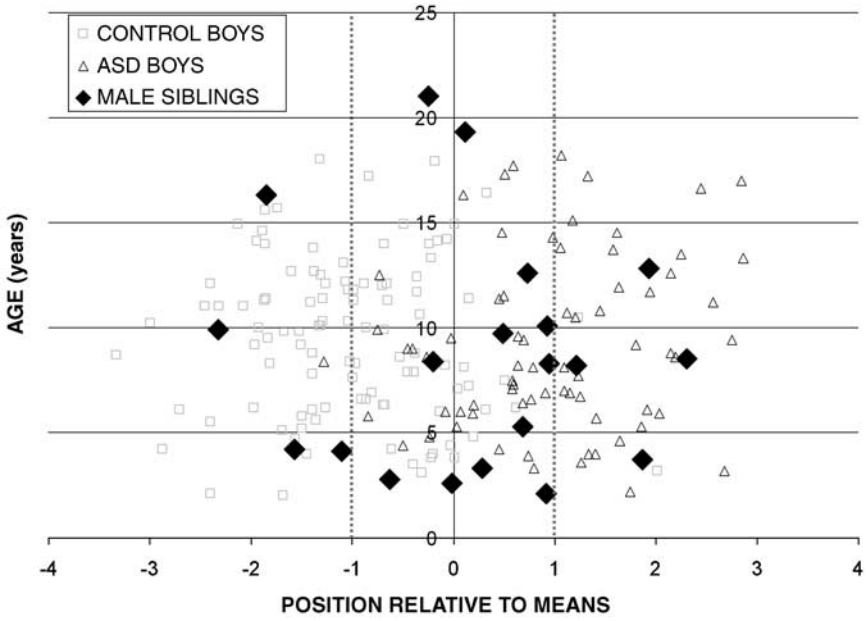

Figure 7 Unseen categorization of unaffected male siblings using the closest mean classification. The background scatter is provided by an unblinded classification of the faces of the control and ASD boys normalized to the interval (−1,1) using the closest mean for a single dense surface model (DSM) for the combined groups. Overlaid are the unseen classification positions of each of the 20 unaffected male siblings with respect to individual DSMs computed for all ASD and control boys but omitting affected individuals related to the unaffected sibling being tested.

gions were enlarged in 2–5 year-old boys with autism, but thereafter, growth of the dorsolateral region was decreased compared to controls.[25] In particular, the frontopolar cortex has been shown to be significantly right-dominant asymmetric (right volume greater than left) in affected boys aged 5–11 years than in controls.[26] Thus, if the atypical facial asymmetry in ASD boys found in the present study is secondary to asymmetry of the frontal poles, this provides strong evidence of a correlation between brain asymmetry and ASD.

Conclusion

We conclude that the faces of boys with ASD have atypical right dominant asymmetry of the supraorbital and periorbital regions anterior to the frontal cerebral pole, and that it is significantly discriminating from age- and ethnicity-matched male controls. Previously identified right dominant brain asymmetry of the frontal pole in boys with ASD could explain their atypical facial asymmetry through an indirect effect of asymmetric brain development on facial development. An alternative explanation, not necessarily mutually exclusive, is that genetic factors are acting in parallel on the development of the face and the brain in ASD. Unaffected mothers of children with ASD also show significantly different facial asymmetry from matched controls, not only right-dominant depth asymmetry supraorbitally but also orbital asymmetry vertically. If it could be proven that there is no right frontal pole dominance in unaffected mothers of children with ASD, then this would strongly favour the second explanation. Both explanations suggest the need for future coordinated studies of face and brain development in appropriately selected ASD families and controls. This is particularly the case for unaffected mothers, given that their depth and vertical facial asymmetry, and the apparent lack of a similar degree of paternal asymmetry, suggest an ASD susceptibility arising from maternal genes.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)