Association Between the Probability of Autism Spectrum Disorder and Normative Sex-Related Phenotypic Diversity in Brain Structure

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**IMPORTANCE** Autism spectrum disorder (ASD) is 2 to 5 times more common in male individuals than in female individuals. While the male preponderant prevalence of ASD might partially be explained by sex differences in clinical symptoms, etiological models suggest that the biological male phenotype carries a higher intrinsic risk for ASD than the female phenotype. To our knowledge, this hypothesis has never been tested directly, and the neurobiological mechanisms that modulate ASD risk in male individuals and female individuals remain elusive.

**OBJECTIVES** To examine the probability of ASD as a function of normative sex-related phenotypic diversity in brain structure and to identify the patterns of sex-related neuroanatomical variability associated with low or high probability of ASD.

**DESIGN, SETTING, AND PARTICIPANTS** This study examined a cross-sectional sample of 98 right-handed, high-functioning adults with ASD and 98 matched neurotypical control individuals aged 18 to 42 years. A multivariate probabilistic classification approach was used to develop a predictive model of biological sex based on cortical thickness measures assessed via magnetic resonance imaging in neurotypical controls. This normative model was subsequently applied to individuals with ASD. The study dates were June 2005 to October 2009, and this analysis was conducted between June 2015 and July 2016.

**MAIN OUTCOMES AND MEASURES** Sample and population ASD probability estimates as a function of normative sex-related diversity in brain structure, as well as neuroanatomical patterns associated with low or high ASD probability in male individuals and female individuals.

**RESULTS** Among the 98 individuals with ASD, 49 were male and 49 female, with a mean (SD) age of 26.88 (7.18) years. Among the 98 controls, 51 were male and 47 female, with a mean (SD) age of 27.39 (6.44) years. The sample probability of ASD increased significantly with predictive probabilities for the male neuroanatomical brain phenotype. For example, biological female individuals with a more male-typic pattern of brain anatomy were significantly (ie, 3 times) more likely to have ASD than biological female individuals with a characteristically female brain phenotype ($P = .72$ vs .24, respectively; $\chi^2 = 20.26; P < .001$; difference in $P$ values, 0.48; 95% CI, 0.29-0.68). This finding translates to an estimated variability in population prevalence from 0.2% to 1.3%, respectively. Moreover, the patterns of neuroanatomical variability carrying low or high ASD probability were sex specific (eg, in inferior temporal regions, where ASD has different neurobiological underpinnings in male individuals and female individuals).

**CONCLUSIONS AND RELEVANCE** These findings highlight the need for considering normative sex-related phenotypic diversity when determining an individual’s risk for ASD and provide important novel insights into the neurobiological mechanisms mediating sex differences in ASD prevalence.

Published online February 8, 2017.

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Editorial
Supplemental content
Autism spectrum disorder (ASD) is a complex neurodevelopmental condition that is 2 to 5 times more common in male individuals than in female individuals.\(^1,2\) While the male preponderant prevalence of ASD might partially be explained by sex differences in clinical symptoms,\(^3,4\) etiological models suggest that the biological male phenotype itself (ie, in general) carries a higher risk for ASD than the female phenotype.\(^5\) However, despite the growing number of studies examining sex differences in the brain in ASD (eg, those by Lai et al\(^6\) and by Schaer et al\(^7\)), this hypothesis has never been tested directly, to our knowledge, and the neurobiological mechanisms that underpin the male preponderant prevalence of ASD remain elusive.\(^8\)

This study examined the probability of ASD as a function of normative sex-related phenotypic diversity in brain structure. To do so, we initially developed a predictive model of biological sex based on multivariate differences in brain structure in a sample of typically developing (TD) male and female control individuals. This normative model was subsequently applied to male individuals and female individuals with ASD. Unlike recent studies examining sexual dimorphism of the brain (reviewed by Rujgrok et al\(^8\)), we used a probabilistic pattern classification approach,\(^9\) which allowed us to accommodate interindividual neuroanatomical diversity within and across the binary categories dictated by biological sex.\(^10\) As a result, we were able (1) to examine the probability of ASD along a normative phenotypic axis ranging from the characteristic female to male brain phenotype and (2) to identify the patterns of sex-related neuroanatomical variability associated with low or high probability of ASD.

We based our analysis on measures of cortical thickness (CT) because these measurements have previously been shown to be highly variable between neurotypical male individuals and female individuals\(^12,13\) and tend to be significantly altered in individuals with ASD (eg, as shown by Ecker et al\(^14\) and by Wallace et al\(^11\)). Moreover, measures of CT are less sensitive to global confounders associated with biological sex (eg, differences in total brain volume) than other morphometric features (eg, surface area).\(^16-19\) Last, we investigated whether the cortical underpinnings of ASD are significantly modulated by biological sex and whether ASD has common or distinct neuroanatomical underpinnings in male individuals and female individuals.

**Methods**

**Participants**

Ninety-eight right-handed adults with ASD (49 male and 49 female; mean [SD] age, 26.88 [7.18] years) and 98 matched neurotypical controls (51 male and 47 female; mean [SD] age, 27.39 [7.18] years) aged 18 to 42 years were recruited locally by advertisement and assessed at the Institute of Psychiatry, Psychology and Neuroscience (IoPPN), London, England, and the Autism Research Centre, Cambridge, England (Table). Approximately equal ratios of cases to controls and male individuals to female individuals were recruited within sites (eTable 1 in the Supplement). Exclusion criteria included a history of major psychiatric disorder (eg, psychosis), head injury, ASD-associated genetic disorders (eg, fragile X syndrome and tuberous sclerosis), or any other medical condition affecting brain function (eg, epilepsy). We also excluded individuals taking antipsychotic medication, mood stabilizers, or benzodiazepines.

All participants with ASD were diagnosed according to the International Statistical Classification of Diseases, Tenth Revision research criteria. The clinical diagnosis was confirmed using the Autism Diagnostic Interview–Revised (ADI-R)\(^20\) or the Autism Diagnostic Observation Schedule (ADOS).\(^21\) All participants diagnosed with the ADI-R reached algorithm cutoffs in the 3 domains of (1) communication (cutoff of 8), (2) reciprocal social interaction (cutoff of 10), and (3) repetitive behaviors and stereotyped patterns of interest (cutoff of 3), although failure to reach the cutoff in the repetitive domain by maximally 2 points was permitted. Because reliable informants were unavailable, we were unable to obtain ADI-R diagnostic data from 5 female individuals with ASD and confirmed diagnostic status using ADOS cutoffs. In all other participants, the ADOS scores were used to assess the severity of current symptoms. One female individual with ASD fell short by 1 point on the ADI-R communication and repetitive behavior domains but met ADOS criteria. All participants had a full-scale IQ greater than 70 assessed using the Wechsler Abbreviated Scale of Intelligence.\(^22\) Participants gave informed written consent in accord with ethics approval by the National Research Ethics Committee, Suffolk, England. The study dates were June 2005 to October 2009, and this analysis was conducted between June 2015 and July 2016.

**Magnetic Resonance Imaging Data Acquisition**

Imaging took place at the IoPPN and the Addenbrooke’s Hospital, Cambridge, using a 3-T system (Sigma; GE Medical Systems). A specialized acquisition protocol using quantitative, T1-weighted mapping was used to ensure standardization of structural magnetic resonance imaging scans across sites,\(^23,24\) which resulted in high-resolution structural T1-weighted inversion recovery images, with a \(1 \times 1 \times 1\)-mm resolution, a \(256 \times 256 \times 176\)-pixel matrix, repetition time of 1800 milliseconds, inversion time of 50 milliseconds, flip angle of \(20^\circ\), and field of view of 25 cm.
Table. Participant Demographics and Global Brain Measures

<table>
<thead>
<tr>
<th>Variable</th>
<th>ASD (n = 98)</th>
<th>TD Control (n = 98)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Individuals (n = 100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>49</td>
<td>51</td>
</tr>
<tr>
<td>Age, y</td>
<td>26.16 (7.20) [18-41]</td>
<td>27.22 (5.58) [18-42]</td>
</tr>
<tr>
<td>IQ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full-scale WASI</td>
<td>112.61 (12.27) [89-135]</td>
<td>114.67 (10.88) [93-137]</td>
</tr>
<tr>
<td>Verbal</td>
<td>110.51 (13.01) [83-137]</td>
<td>109.86 (11.03) [88-137]</td>
</tr>
<tr>
<td>Performance</td>
<td>112.00 (13.65) [85-138]</td>
<td>116.74 (10.90) [93-133]</td>
</tr>
<tr>
<td>ADI-R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social</td>
<td>17.57 (5.50) [10-28]</td>
<td>NA</td>
</tr>
<tr>
<td>Communication</td>
<td>13.86 (4.16) [8-24]</td>
<td>NA</td>
</tr>
<tr>
<td>Repetitive behavior</td>
<td>4.96 (2.37) [1-10]</td>
<td>NA</td>
</tr>
<tr>
<td>ADOS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social plus communication</td>
<td>9.43 (4.39) [1-21]</td>
<td>NA</td>
</tr>
<tr>
<td>Stereotypic behavior</td>
<td>1.27 (1.27) [0-5]*</td>
<td>NA</td>
</tr>
<tr>
<td>Cortical thickness, mm</td>
<td>2.35 (0.10)</td>
<td>2.33 (0.10)</td>
</tr>
<tr>
<td>Volume, L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total gray matter</td>
<td>0.76 (0.08)</td>
<td>0.76 (0.05)</td>
</tr>
<tr>
<td>Total intracranial</td>
<td>1.59 (0.21)</td>
<td>1.58 (0.17)</td>
</tr>
<tr>
<td>Female Individuals (n = 96)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>49</td>
<td>47</td>
</tr>
<tr>
<td>Age, y</td>
<td>27.60 (7.12) [18-48]</td>
<td>27.60 (7.31) [19-52]</td>
</tr>
<tr>
<td>IQ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full-scale WASI</td>
<td>114.88 (12.36) [84-136]</td>
<td>118.38 (7.32) [99-129]</td>
</tr>
<tr>
<td>Verbal</td>
<td>116.60 (12.15) [76-144]</td>
<td>117.83 (8.81) [96-135]</td>
</tr>
<tr>
<td>Performance</td>
<td>110.56 (14.50) [67-138]</td>
<td>114.38 (8.26) [96-128]</td>
</tr>
<tr>
<td>ADI-R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social</td>
<td>16.32 (4.24) [10-26]</td>
<td>NA</td>
</tr>
<tr>
<td>Communication</td>
<td>12.43 (3.96) [7-22]</td>
<td>NA</td>
</tr>
<tr>
<td>Repetitive behavior</td>
<td>4.36 (1.98) [1-9]</td>
<td>NA</td>
</tr>
<tr>
<td>ADOS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social plus communication</td>
<td>7.71 (5.28) [0-19]</td>
<td>NA</td>
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<tr>
<td>Stereotypic behavior</td>
<td>0.75 (0.97) [0-3]*</td>
<td>NA</td>
</tr>
<tr>
<td>Cortical thickness, mm</td>
<td>2.32 (0.11)</td>
<td>2.34 (0.10)</td>
</tr>
<tr>
<td>Volume, L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total gray matter</td>
<td>0.67 (0.06)</td>
<td>0.68 (0.06)</td>
</tr>
<tr>
<td>Total intracranial</td>
<td>1.32 (0.18)</td>
<td>1.32 (0.17)</td>
</tr>
</tbody>
</table>

Abbreviations: ADI-R, Autism Diagnostic Interview-Revised; ADOS, Autism Diagnostic Observation Schedule; ASD, autism spectrum disorder; NA, not applicable; TD, typically developing; WASI, Wechsler Abbreviated Scale of Intelligence.

* There were no significant between-group differences in age, full-scale IQ, mean cortical thickness, total gray matter volume, or total intracranial volume (2-tailed P < .05). The ADI-R values were based on 49 male individuals and 44 female individuals with ASD. The ADOS values were based on 49 male individuals and 48 female individuals with ASD.

Statistically significant between male individuals and female individuals based on 2-tailed P < .05 (t_{male} = 2.23, P = .03).

Verbal and performance IQ data were not available for 1 female individual with ASD.

Cortical Reconstruction Using FreeSurfer
A software program (FreeSurfer, version 5.3.0; http://surfer.nmr.mgh.harvard.edu) was used to derive cortical surface models for each T1-weighted image. These well-validated and fully automated procedures have been extensively described elsewhere.25-27 All surface models were visually inspected for errors, and scans with visible reconstruction inaccuracies were excluded from the statistical analysis (dropout <10%). We based our analysis on measures of CT (ie, the closest distance from the gray matter (GM) or white matter boundary to the GM or cerebrospinal fluid boundary at each cerebral vertex).28 Cortical thickness maps were smoothed with a 15-mm surface-based gaussian kernel.

Gaussian Process Classification
Gaussian process classification (GPC)10,29 was used for the probabilistic prediction of biological sex based on neuro-anatomical variability in CT (details are available in the eMethods in the Supplement). In brief, we initially developed a normative model of biological sex by predicting the binary class labels y ∈ {−1, +1} for female and male TD controls, respectively, using CT measures estimated at approximately 320 000 vertices on the cortical surface (Gaussian Processes for Machine Learning Matlab toolbox; http://www.gaussianprocess.org/gpml/code/matlab/doc). In addition to the overall model accuracy (ie, proportion of biological male individuals or female individuals correctly classified as phenotypic male individuals or female individuals), GPC returned a set of predictive class probabilities indicating the probability of an individual belonging to the male or female category. Class probabilities ranged from 0 to 1 for the characteristic female to male brain phenotype, respectively, so that a class probability of 0.5 represents a binary cutoff separating both classes. Classifier performance was validated by cross-validation and tested for statistical significance via 1000 permutations of class labels. The normative model was subsequently applied to male individuals and female individuals with ASD.

A predictive mapping approach30 was used to identify (1) the spatially distributed patterns of neuroanatomical variability in CT characteristics for the neurotypical female or male brain phenotype and (2) the set of brain regions associated with low or high probability of ASD. Normative or risk (ie, low or high probability) patterns were derived by quantifying the extent to which the individual’s neuroanatomy interacts with the spatial representation of the decision function (ie, the weight vector w) separating neurotypical male individuals from female individuals phenotypically (ie, the product of w and CT). The high ASD probability pattern included all male individuals (or female individuals) with a male neuroanatomical phenotype (ie, class probability >0.5). The low ASD probability pattern included all male individuals (or female individuals) with a female neuroanatomical phenotype (ie, class probability <0.5). We also identified these patterns across all individuals along the normative axis of predictive class probabilities. At each vertex, the resulting maps were summarized by the mean value across all individuals within examined groups.
Estimation of ASD Probability
To examine the probability of ASD as a function of normative phenotypic variability in brain structure, we converted the continuous axis of class probabilities into a set of discrete bins (eg, from 0 to 1 in steps of 0.125). Within each bin, the sample probability of ASD was determined as the ratio of individuals with ASD relative to the total number of individuals per bin. Moreover, these data allowed us to estimate the population prevalence of ASD given that an individual exhibits a male or female neuroanatomical phenotype in addition to being biologically male or female (details are available in the eMethods in the Supplement). In brief, using Bayes theorem, we combined our sample prevalence estimates with previously published population prevalence rates of ASD for biological male individuals and female individuals (ie, 1:42 for male individuals and 1:89 for female individuals). In this way, we were able to estimate the population prevalence of ASD (D = 1) given a male (or female) neuroanatomical brain phenotype (M) for biological male individuals (or female individuals) (S), that is P (D = 1 | M, S). Confidence intervals were determined using an exact binomial test implemented in a software package (R Project for Statistical Computing; https://www.r-project.org).

Vertexwise Between-Group Comparison of CT

Vertexwise statistical analysis of CT measures was conducted using the R2014a Matlab toolbox (SurfStat; http://www.math.mcgill.ca/keith/surfstat). For the comparison between male and female controls, parameter estimates were obtained by regression of a general linear model at each vertex i, with biological sex and center as categorical fixed-effects factors and total GM volume as a continuous covariate. To examine whether the neuroanatomy of ASD is significantly modulated by biological sex, we also included a main effect of diagnostic group and biological sex and a group × sex interaction as follows:

\[ y_i = \beta_0 + \beta_1 \text{Group} + \beta_2 \text{Sex} + \beta_3 (\text{Group} \times \text{Sex}) + \beta_4 \text{GM}_{\text{total}} + \beta_5 \text{Center} + \epsilon_i, \]

where \( y \) refers to the variable that is predicted by the model (ie, the CT at voxel \( i \)) and \( \epsilon_i \) is the residual error. Effects were estimated from the coefficients \( \beta_{1-4} \) normalized by the corresponding standard error. Corrections for multiple comparisons were performed using a random field theory-based cluster analysis for nonisotropic images at a cluster threshold of 2-tailed \( P < .05 \). We also confirmed our findings using a non-parametric clustering approach (2-tailed \( P < .05 \)) using 10,000 permutations of the original data.

Results

Prediction of Biological Sex Based on Normative Variability in CT

Overall, our CT-driven probabilistic classifier predicted biological sex at an accuracy of 71.4% under cross-validation in neurotypical controls, which was significantly higher than would be expected by chance (\( P < .001 \) obtained via permutation testing). In total, 68.1% (32 of 47) of all biological female individuals were correctly allocated to the category of phenotypic female individuals and 74.5% (38 of 51) of all biological male individuals to the category of phenotypic male individuals (Figure 1A, lower panel). Across individuals, class probabilities ranged continuously from 0 to 1 for the characteristic female to male brain phenotype, respectively (Figure 1A, upper panel). Moreover, the patterns of CT variability associated with the phenotypic shift of the brain from a female to male presentation resembled the set of brain regions where female individuals significantly differed from male individuals (eFigure 1 and eFigure 2 in the Supplement). Therefore, while a perfect phenotypic differentiation between biological sexes could not be achieved, we were able to separate male individuals from female individuals in most cases based on multivariate differences in brain structure.

Phenotypic Prediction of Biological Sex for Individuals With ASD

When applying our normative predictive model of biological sex to the independent sample of individuals with ASD, we found that, in female individuals with ASD, predictive class probabilities for the male neuroanatomical brain phenotype were significantly increased relative to female controls (\( t_{\text{ASD}} = 6.13, P < .001 \)) (Figure 1B). Overall, 39 of 49 female individuals (79.6%) with ASD were allocated to the category of phenotypic male individuals, which was significantly more frequent than expected based on the typical rate of misclassification for female controls (31.9% [15 of 47], respectively; \( \chi^2 = 20.26, P < .001 \)). No such differences were observed in male individuals with ASD, who were correctly allocated to the male category in 81.6% (40 of 49) of all cases and also did not differ significantly from male controls in predictive class probabilities (\( t_{\text{ASD}} = 1.35, 2\text{-tailled} P > .17 \)). Therefore, when representing individuals along a single axis of normative sex-related phenotypic diversity in brain structure, female individuals with ASD displayed a CT pattern that resembled more closely the neurotypical male rather than female neurophenotype. This phenotypic shift in female individuals is likely to influence the probability of ASD.

ASD Probability as a Function of Normative Sex-Related Phenotypic Variability in Brain Structure

In female individuals with ASD, the sample probability of ASD increased with increasing predictive probabilities for the male neuroanatomical brain phenotype (eFigure 3A in the Supplement). More specifically, female individuals with class probabilities exceeding the binary sex cutoff of 0.5 (ie, biological female individuals falling into the category of phenotypic male individuals) were significantly (ie, 3 times) more likely to have a diagnosis of ASD than biological female individuals with a class probability lower than 0.5 (\( P = .72 \) vs .24, respectively; \( \chi^2 = 20.26, P < .001 \)). In contrast, male individuals with class probabilities lower than 0.5 (ie, biological male individuals falling into the category of phenotypic female individuals) were 1.2 times less likely to have ASD than male individuals allocated to the category of phenotypic male individuals (\( P = .41 \) vs .51, respectively).
Figure 1. Gaussian Process Classification of Biological Sex

A. Gaussian process classification between male and female typically developing (TD) control individuals based on normative (ie, neurotypical) variability in cortical thickness. The y-axis indicates predictive class probabilities. Therefore, a class probability of 0.5 served as a binary cutoff separating male individuals from female individuals. The y-axis indicates the position of each individual on the normative axis of sex-related phenotypic diversity in brain structure (lower panel). The upper panel shows the density (ie, frequency) of male individuals and female individuals along the normative axis of class probabilities. B. Probabilistic predictions for male individuals and female individuals with autism spectrum disorder (ASD) using the normative model for biological sex. The density functions for male individuals and female individuals with ASD (upper panel) show the phenotypic shift of the brain in female individuals with ASD toward a more male phenotypic presentation. Of 49 female individuals with ASD, 39 (79.6%) fell within the category of phenotypic male individuals (lower panel).

respectively; $\chi^2 = 0.38; P > .53$; difference in $P$ values, $-0.10$; 95% CI, $-0.36$ to $0.15$) (eFigure 3B in the Supplement). If one combines these sample probabilities with previously published prevalence rates of ASD in the general population (eg, 1:42 for male individuals and 1:189 for female individuals31), our study shows that biological female individuals with male neuroanatomical features were 6.5 times more likely to have ASD than biological female individuals with a characteristically female neuroanatomy, which translates to an estimated variability in population prevalence between 0.2% to 1.3%, respectively (eTable 2 in the Supplement). Therefore, normative sex-related phenotypic diversity in brain structure significantly affected the probability of ASD in addition to biological sex alone, with male neuroanatomical characteristics carrying a higher intrinsic risk for ASD than female characteristics.

The particular patterns of neuroanatomical variability associated with low or high ASD probability differed between men and women in sign and regional composition (Figure 2 and eFigure 2 in the Supplement). For example, CT variability in the left and right inferior temporal lobe differentiated between low or high risk in women but not in men ($t_{max} = 3.78, cluster P = 6.29 \times 10^{-4}$ for the left; $t_{max} = 3.47, cluster P = 1.09 \times 10^{-2}$ for the right). Therefore, while it is possible to link the probability of ASD to particular patterns of neuroanatomical variability in CT, our findings suggest that these patterns are sex specific.

Biological Sex Significantly Modulates the Cortical Anatomy of ASD

Last, using a conventional general linear model, we tested for significant CT differences between men and women with ASD and group × sex interactions. We found that the cortical neuroanatomy of ASD was significantly modulated by biological sex, particularly in the bilateral parahippocampal and entorhinal cortex (Brodmann area [BA] 28/34), the fusiform and lingual gyrus (BA 20/37/19), and the inferior or middle temporal lobe (BA 21/22) (Figure 3 and eTable 4 and eFigure 5 in the Supplement). In these regions, the degree of cortical abnormality in female individuals significantly exceeded the degree of abnormality observed in male individuals compared with their respective normative populations ($t = 3.29$, cluster $P = 3.5 \times 10^{-4}$ for the left; $t = 3.42$, cluster $P = 5.49 \times 10^{-4}$ for the right) (Figure 3B and C and eFigure 6 in the Supplement). However, female individuals with ASD were not more significantly impaired than male individuals in clinical symptom severity (eTable 5 in the Supplement).
The clusters with significant group × sex interactions also remained significant when controlling for variability in verbal and performance IQ (eTable 5 and eFigure 7 in the Supplement) and thus do not seem to reflect a simple functional difference in these clinical or neurocognitive measures (further details are available in eFigure 8 and the eAppendix in the Supplement).

**Discussion**

Our study demonstrates that normative sex-related phenotypic diversity in brain structure affects the prevalence of ASD in addition to biological sex alone, with male neuroanatomical characteristics carrying a higher intrinsic risk for ASD than female characteristics. This increase in risk was predominantly driven by female individuals with ASD, who displayed a pattern of neuroanatomical variability in CT that resembled more closely the neurotypical male rather than female neurophenotype overall. Moreover, our study links low and high risk (ie, probability) of ASD to particular patterns of sex-related neuroanatomical variability, thus providing important novel insights into the neurobiological mechanisms underpinning the male preponderant prevalence of ASD.

An important feature of GPC is that it is possible to summarize the highly complex and multivariate pattern of normative sex-related phenotypic diversity in brain structure to
Figure 3. Significant Group × Sex Interactions

A. Clusters with significant group × sex interactions in cortical thickness (CT) as examined by a conventional general linear model-type approach. In these regions, the difference in CT between female individuals with autism spectrum disorder (ASD) and female control individuals significantly exceeded the difference between male individuals with ASD and male controls (statistical details are available in eTable 4 in the Supplement). The group × sex interactions were driven by reduced CT in female individuals with ASD relative to female controls and increased CT in male individuals with ASD relative to male controls (eFigure 6 in the Supplement).

B. Clusters with significantly increased CT in male individuals with ASD relative to male controls (random field theory–based, cluster-corrected P < .05).

C. Clusters with significantly reduced CT in female individuals with ASD relative to female controls (random field theory-based, cluster-corrected P < .05). eFigure 5 in the Supplement shows results of the permutation-based cluster thresholding of the same contrasts. TD indicates typically developing.

A single measure (ie, class probability) indicative of the individual's neurophenotypic sex. This ability sets our approach apart from existing studies (eg, those by Ruigrok et al9 and by Escorial et al34) examining sexual dimorphism of the brain based on univariate group differences between male individuals and female individuals, which precludes the
investigation of interindividual phenotypic diversity across the binary categories dictated by biological sex. This study is of importance because it has recently been suggested that, on a phenotypic scale, the brain should be considered a “mosaic” of regions, each of relative maleness or femaleness, resulting in significant interindividual variability both within and between sexes.\textsuperscript{11,35} Therefore, the multivariate patterns derived from the classification between neurotypical male and female controls may be interpreted as being representative of such a mosaic, which drives the brain toward a female or male phenotypic end point overall (see also the study by Chekroud et al\textsuperscript{36}).

Furthermore, we demonstrated that the normative pattern of sex-related neuroanatomical variability is predictive of ASD probability, particularly in female individuals, and that low and high ASD probability can be linked to regional differences in CT. These patterns not only included many of the brain regions where women typically differ from men in CT\textsuperscript{12,13} but also highlighted brain areas that have previously been linked to the core behavioral deficits characteristic of ASD (reviewed by Amaral et al\textsuperscript{10} and by Ecker et al\textsuperscript{38}). Notably, some of these brain regions (eg, inferior temporal lobes) carried low or high probability of ASD in women but not in men. Herein, we found that the degree of cortical abnormality in female individuals with ASD significantly exceeded the degree of abnormality in male individuals compared with their respective normative population. Yet, female individuals with ASD were not more severely impaired than male individuals on the level of autistic symptoms. Therefore, our results with ASD were not more severely impaired than male individuals (reviewed by Werling and Geschwind\textsuperscript{5}) and may need to deviate more neurobiologically from their normative population to demonstrate a clinical ASD phenotype.

Limitations
While our approach holds promise for future investigations into the neurobiological mechanisms that underpin risk and resilience for conditions with a sex difference in prevalence, this study has several limitations. First, our study was designed to establish the statistical association between normative sex-related phenotypic diversity and ASD probability. Therefore, future studies are needed to examine the causal mechanisms for this association (eg, as shown by Lai et al\textsuperscript{8} and Baron-Cohen et al\textsuperscript{39}). Second, because of reasons outlined above, we based our study on measures of CT in a sample of high-functioning adults with ASD. While this scope is sufficient to provide proof of concept, it will be necessary in the future to extend our approach to other neurobiological features that have previously been linked to ASD (eg, cortical surface area and regional volumes\textsuperscript{14}) and to replicate our findings in other subgroups on the autism spectrum (eg, different age groups and individuals with learning disabilities). Third, in future research, it will be crucial to further explore the functional relevance of our findings and to examine how normative sex-related phenotypic diversity in brain structure relates to sex differences in general cognitive or behavioral profiles (eg, as shown by Miller and Halpern\textsuperscript{40} and by Hyde\textsuperscript{41}) and to different clinical ASD phenotypes.\textsuperscript{3,42-44}

Conclusions
Our findings suggest that the neurobiological male phenotype carries a higher intrinsic risk for ASD than the female phenotype across the binary categories dictated by biological sex. In addition to genetic and environmental factors, normative sex-related phenotypic diversity should thus be taken into account when determining an individual’s probability of ASD. Therefore, our approach to modeling normative sex-related phenotypic diversity may be more widely used in the future to elucidate the neurobiological mechanisms that underpin risk and resilience for mental health disorders.
Association of Autism Spectrum Disorder With Sex-Related Phenotypic Diversity in Brain Structure

Original Investigation Research

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Conflict of Interest Disclosures: Dr Bullmore reported being employed half-time by GlaxoSmithKline and reporting being a stockholder of GlaxoSmithKline shares. No other disclosures were reported.

Funding/Support: This work was supported by the Autism Imaging Multicentre Study Consortium, funded by Medical Research Council United Kingdom grant G0400061, and by the European Union–Autism Imaging Multicentre Study Consortium, supported by the Innovative Medicines Initiative Joint Undertaking under grant 115300, which includes financial contributions from the European Union Seventh Framework Programme (FP7/2007-2013), the European Federation of Pharmaceutical Industries and Associations companies in kind, and Autism Speaks.

Dr Ecker acknowledges support by grants EC480/1-1 and EC480/2-1 from the German Research Foundation under the Heisenberg Programme. Dr Marquand acknowledges support by grant 024.001.006 from the Netherlands Institute for Scientific Research under the Gravitation Programme. Dr Lai acknowledges support from the Wellcome Trust Autism Neuroscience Fellowship, Cambridge, England, and the O'Brien Scholars Program within the Child and Youth Mental Health Collaborative at the Centre for Addiction and Mental Health and the Hospital for Sick Children, Toronto, Ontario, Canada. Drs Ruigrok and Baron-Cohen acknowledge support from the Autism Research Trust.

Role of the Funder/Sponsor: The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

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Additional Contributions: We thank the National Institute for Health Research Biomedical Research Centre for Mental Health and The Dr Mortimer and Theresa Sackler Foundation. The MRC AIMS Consortium is a United Kingdom collaboration between the Institute of Psychiatry, Psychology and Neuroscience at King’s College London, the Autism Research Centre at the University of Cambridge, and the Autism Research Group at the University of Oxford.

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