Supplementary Online Content


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This supplementary material has been provided by the authors to give readers additional information about their work.
eAppendix

MRI Acquisition. Spoiled gradient recalled (SPGR) was acquired at two flip angles (\(\theta\)) with the sequence parameters given in Table 1 from which an estimate of T1 was derived at each cerebral voxel. These quantitative T\(_1\) maps were then used to create simulated structural T\(_1\)-weighted inversion recovery (IR) images, with 176 contiguous slices (1 mm x 1 mm x 1 mm resolution), a field-of-view of 25.6 cm, a simulated repetition time/inversion time (TR/TI) of 1800/850 ms, and flip angle of 20°. This combination of parameters gave optimal deep and cortical grey / white-matter contrast in the subsequent tissue segmentation without modulation by B\(_0\) and B\(_1\) field in-homogeneities

VBM Permutation Testing. Permutation testing was used to assess statistical significance at the level of voxel clusters. Here, group membership was randomly reassigned and the general linear model refitted to generate 100 permuted maps sampling the statistics under the null-hypothesis of no group differences. Three-dimensional clusters in both observed and permuted maps were obtained by initial thresholding at \(p<0.05\) and the sum of voxel statistics for each resulting cluster taken. The statistical threshold of cluster mass was then calculated, controlling for multiple comparisons by adjusting the two-tailed probability threshold such that less than one false positive cluster is expected per map under the null-hypothesis. A significant cluster was defined as a deficit or excess depending on a respective reduction or increase in the ASD group relative to the control group.

Tract-specific labels for white-matter clusters. A diffusion tensor imaging (DTI)-derived atlas from 40 healthy subjects was used to optimally localize the white-matter abnormalities detected in the VBM analysis. This atlas provides maps of the major long-range white-matter tracts normalized in a common space of reference (MNI). Briefly the method consists in performing virtual dissections of the tracts in the native space and creating binary visitation maps for each tract by assigning each voxel a value of 1 or 0 depending on whether the voxel is intersected by the streamlines of the tract. The binary visitation maps of each subject are normalized to the MNI space and percentage overlap maps created by
summing at each point in the MNI space the normalized visitation maps of each subject. In this case the visitation maps are binary and unsmoothed, hence the overlap of the visitation maps varies according to inter-subject variability \(^6\). Only maps with an overlap above 50\% were used for comparisons. Full details of the method have been recently published in \(^43\). The results from the voxel-based analyses of white-matter volume were therefore overlapped on the digital masks of each tract provided in the atlas. This approach enabled the localization of tracts with the highest degree of confidence (i.e. only those clusters intersecting regions with > 90\% of tract overlap).

**Partial Least Square analysis (PLS).** PLS estimates the correlations between group membership (ASD: 0; Controls: 1) and normalized grey-matter volume at each voxel where the probability of grey-matter P(GM) was greater than 0.05 (thus excluding all voxels representing predominantly white-matter or CSF from the analysis). The same threshold was applied to white-matter probabilities. The overall strength of the correlations between grey/white-matter volume and group were then summarized by a scalar \(d = \sqrt{\sum (r_i)^2}\), where \(r_i\) is the correlation at the \(i\)th voxel and the sum is over all voxels. The association between grey/white-matter volume and group membership was tested for statistical significance by means of a permutation test of \(d\) using 500 permutations \(^7\). Brain systems strongly correlated with group membership were visualized by thresholding the correlations at each voxel with an arbitrary, \(|r_i| > 0.15\) and a minimum cluster size of 75 voxels. The choice of visualization thresholds makes no difference to the statistical significance of \(d\) (the overall correlation between anatomical images and group membership) and was chosen for illustration purposes.
eReferences


**eTable 1.** Summary of SPGR image acquisition parameters used at each centre

<table>
<thead>
<tr>
<th>Centre</th>
<th>Scanner Manufacturer</th>
<th>Field of view</th>
<th>Image matrix</th>
<th>T&lt;sub&gt;E&lt;/sub&gt; (ms)</th>
<th>T&lt;sub&gt;R&lt;/sub&gt; (ms)</th>
<th>FA (deg)</th>
<th>Bandwidth (Hz/pixel)</th>
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<td>3.74</td>
<td>8.01</td>
<td>18.4</td>
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<tr>
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<td>4.80</td>
<td>9.10</td>
<td>20.4</td>
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</tr>
</tbody>
</table>

*Note.* All images were acquired at a field strength of 3Tesla; GE: General Electric
eFigure 1. Significant grey-matter differences (VBM) in ASD ($p = 0.0049$) using linear registration (FLIRT). Relative excesses in gray matter volume in adults with ASD compared with controls are displayed in orange/red for (A) temporal cluster and (B) frontal cluster, while deficits are displayed in blue for (C) occipital cluster. The maps are orientated with the left side of the brain shown on the right side of each panel. The $z$-coordinates for each axial slice in the standard space of MNI are given in millimeters.
eFigure 2. Significant white-matter differences (VBM) in ASD (p = 0.0076) using linear registration (FLIRT). Relative excesses in white-matter volume in adults with ASD compared with controls are displayed in orange/red, while deficits are displayed in blue. The maps are orientated with the left side of the brain shown on the right side of each panel. The z-coordinates for each axial slice in the standard space of MNI are given in millimeters.
eFigure 3. Brain map (PLS) illustrating regions where white-matter volume was associated with group membership. Orange/red regions indicate areas with a positive association between grey-matter volume and ASD (i.e. ASD > control); blue regions indicate brain systems of decreased grey-matter volume in the ASD group. The maps are orientated with the right side of the brain shown on the left side of each panel. The z-coordinates for each axial slice in the standard space of MNI are given in millimeters.
**eFigure 4.** Relationship between group membership and associated brain scores (summary measures of grey- and white-matter correlation with grouping membership over the whole brain). (A) Boxplots of brain score by group membership for gray matter showing significantly larger scores for individuals with ASD compared to controls ($r = -0.46$, $N = 178$, $p < 0.01$). (B) Boxplots of brain score by group membership for white matter showing significantly smaller scores for individuals with ASD compared to controls ($r = 0.52$, $N = 178$, $p < 0.01$)