Neural correlate of autistic-like traits and a common allele in the oxytocin receptor gene

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Sub-clinical autistic-like traits (ALTs) are continuously distributed in the general population and genetically linked to autism. Although identifying the neurogenetic backgrounds of ALTs might enhance our ability to identify those of autism, they are largely unstudied. Here, we have examined the neuroanatomical basis of ALTs and their association with the oxytocin receptor gene (OXTR) rs2254298A, a known risk allele for autism in Asian populations which has also been implicated in limbic-paralimbic brain structures. First, we extracted a four-factor structure of ALTs, as measured using the Autism-Spectrum Quotient, including ‘prosociality’, ‘communication’, ‘details/patterns’ and ‘imagination’ in 135 neurotypical adults (79 men, 56 women) to reduce the genetic heterogeneity of ALTs. Then, in the same population, voxel-based morphometry revealed that lower ‘prosociality’, which indicates strong ALTs, was significantly correlated to smaller regional grey matter volume in the right insula in males. Males with lower ‘prosociality’ also had less interregional structural coupling between the right insula and the ventral anterior cingulate cortex. Furthermore, males with OXTR rs2254298A had significantly smaller grey matter volume in the right insula. These results show that decreased volume of the insula is a neuroanatomical correlate of ALTs and a potential intermediate phenotype linking ALTs with OXTR in male subjects.

Keywords: asperger; endophenotype; imaging genetics; pervasive developmental disorder; sex difference

INTRODUCTION

Autism spectrum disorders (ASDs) are highly heritable neurodevelopmental disorders. Even in sub-clinical populations, autistic-like traits (ALTs) are observed as problems or peculiarities in sociocommunicative behavior, perception of others and self, and adaptations to the environment that do not meet the formal criteria for ASDs, and can be considered as a phenotype resulting from the overlap of genetic factors for ASDs (e.g. Constantino and Todd, 2003; Mosconi et al., 2010; Robinson et al., 2011; Lundström et al., 2012). In addition, contributions of early environmental factors or gene–environment interactions to the pathogenesis of ASDs have recently been emphasized (e.g. Hallmayer et al., 2011). Because it has been proposed that these environment-related effects can differentiate between ALTs and ASDs (Lundström et al., 2012), identifying the genetic correlates of ALTs might enable genetic factors in common with ASDs to be determined. However, to date, the association between ALTs and candidate genetic factors for ASDs is still unclear (Chakrabarti et al., 2009; Kawamura et al., 2011; Chen and Johnson, 2012). Considering the complicated and multi-factorial heritability of ALTs, identifying the neurogenetic basis of ALTs can be done by treating neural correlates of ALTs as an intermediate phenotype, which is more likely to associate with genetic factors for ALTs.

Neural correlates of ALTs might be detectable at brain structural level because previous studies have revealed significant effects of risk alleles for ASDs on regional brain volume [e.g. the oxytocin receptor gene (OXTR)] (Inoue et al., 2010; Tost et al., 2010, 2011; Furman et al., 2011; Yamasue et al., 2011). As grey matter volume (GMV) is a highly heritable trait marker (Baaré et al., 2001), neuroanatomical correlates of ALTs are considered to be good candidate markers for an intermediate phenotype to link ALTs with genetic factors for ASDs. However, to date, only a limited number of studies have examined the neural correlates of ALTs at brain structural level (von dem Hagen et al., 2011; Wallace et al., 2012), and their association with candidate genetic factors for ASDs has not yet been examined. A high total Autism-Spectrum Quotient (AQ) score was found to be associated with decreased white matter volume in the posterior superior temporal sulcus (STS) in healthy adults (von dem Hagen et al., 2011), and high social responsiveness scale scores were associated with thinner temporal and parietal cortices in typically developed children (Wallace et al., 2012).

Notably, a functional-MRI study showed that high ALTs were correlated to less functional connectivity between the insula and the anterior cingulate cortex (ACC) in neurotypical adults (Di Martino et al., 2009b). As interconnecting brain systems have common developmental and maturation influences, their volumes would be expected to covary or positively correlate (Mechelli et al., 2005). A previous study showed a reduction in the degree of correlation among regional GMVs in several regions in people with ASDs (McAlonan et al., 2005). By interconnecting brain systems have common developmental and maturation influences, their volumes would be expected to covary or positively correlate (Mechelli et al., 2005). Therefore, it could be hypothesized that the degree of ALTs would be correlated to regional GMV in certain localized regions as well as to reduced structural coupling between such regions. As genetic heterogeneities of ALTs have also been demonstrated, it has been suggested that the triad of impairment in ASDs and ALTs should be studied separately (Happe et al., 2006). Because previous...
factor analyses have shown three- to four-factor components independently included in ALTs (Austin, 2005; Stewart and Austin, 2009), for the neurobiological basis of ALTs to be examined, the factor structure should be taken into consideration.

Neurobiological substrates for ALTs could also be sexually dimorphic, as ASDs, ALTs and neuroanatomy consistently show significant sex differences (reviewed in Yamasue et al., 2009; Baron-Cohen et al., 2011). The sex ratio of prevalence for ASDs shows a stable male preponderance. Among sub-clinical populations, males show strong ALTs consistently (Baron-Cohen et al., 2001; Constantino and Todd, 2003; Baron-Cohen et al., 2006; Wakabayashi et al., 2006; Auyeung et al., 2008). Significant sex differences in neuroanatomy have also been reported, especially in brain regions involved in social interactions, such as the amygdala, insula, ACC and inferior frontal gyrus (Good et al., 2001; Yamasue et al., 2008; Lombardo et al., 2012).

It could also be hypothesized that sexually dimorphic neural correlates of ALTs would be associated with sexually dimorphic candidate genes for ASDs, such as OXTR rs2254298A. Oxytocin shows sex differences (Carter, 2007), and contributes to social behavior (reviewed in Bartz et al., 2011; Meyer-Lindenberg et al., 2011; van Lijzenoord and Bakermans-Kranenburg, 2011; Chen and Johnson, 2012; Higashida et al., 2012; Yamasue et al., 2012; Yamasue, 2013). OXTR rs2254298A shows associations with ASDs in Asian populations (reviewed in Brune, 2012; Yamasue, 2013) and limbic and paralimbic brain structures (Inoue et al., 2010; Furman et al., 2011; Tost et al., 2011; Yamasue et al., 2011). Thus, we considered OXTR rs2254298A as a candidate allele that could explain individual differences in the neuroanatomical correlates of ALTs, especially their social aspects.

To identify the neurogenetic basis of ALTs using voxel-based morphometry (VBM) of MRI and DNA analysis, the aims of this study are as follows: (i) to examine the correlation between social aspects of ALTs and regional GMV using a whole-brain approach, (ii) to examine structural couplings between the identified neural correlates of social ALTs and other brain regions, (iii) to test the relationship between the identified structural couplings and the social aspects of ALTs and (iv) to test associations between the identified neural correlates of social ALTs and OXTR rs2254298A. To reduce the heterogeneity of ALTs, we employed sub-components of ALTs as behavioural variables by dividing AQ into four factor scores, which fit our data as revealed by factor analysis in the current study subjects. In addition, because ALTs and neuroanatomy are reported to be sexually dimorphic, correlations with neuroanatomy were examined with considering sex differences. Furthermore, on the view of ASD behavior as an extreme on the continuum of ALTs, we examined the associations between regions showing a sexually dimorphic relationship to ALTs and a polymorphism of sexually dimorphic molecules (i.e. OXTR rs2254298A), which have previously been associated with ASDs and limbic and paralimbic brain structure (reviewed in Yamasue, 2013).

**MATERIALS AND METHODS**

**Participants and clinical evaluation**

One hundred and thirty-five right-handed (Oldfield, 1971) ethnically homogeneous Japanese adults (79 male and 56 female) who were mainly college students, hospital staff and their acquaintances participated in the study (Table 1). Of the 135 subjects, 112 whose DNA samples were available also participated in our previous study (Inoue et al., 2010). This study examining neural correlates of ALTs and their association with OXTR rs2254298A was quite distinct from our previous study, in which we examined the association between manually traced amygdala volume and OXTR (Inoue et al., 2010). The magnetic resonance (MR) scanning, collection of peripheral blood and interviews were conducted at The University of Tokyo Hospital. The age of subjects was restricted to the third and fourth decades of life to minimize the effects of aging and menopause on brain morphology [Table 1: mean (range) (s.d.) in male/those in female = 29.4 (21–40) (4.2)/28.1 (22–40) (4.4)]. There were no significant sex differences in age, parental-socio-economic-state (SES) (Hollingshead, 1957) and handedness (Oldfield, 1971), although males had significantly higher self-SES than that of females (P = 0.004). The participants were interviewed by a trained psychiatrist (H.Y. or M.S.) and screened for the presence or absence of neuropsychiatric disorders through the Structured Clinical Interview for DSM-IV Axis I Disorder, Non-patient Edition (First, 1997). Interviews were performed on the same day as MR scanning. Each subject completed a valid Japanese translation (Wakabayashi et al., 2006) of a 50-item AQ (Baron-Cohen et al., 2001). Because the AQ is considered to reflect static trait aspects of behavior, the questionnaire was completed within 1 month before or after the MR scan.

None of the subjects had a known history of neuropsychiatric disorders, serious head trauma with any known cognitive consequences or loss of consciousness for more than 5 min, alcohol/substance abuse or dependence. All participants had to have IQ > 80 (mean (range) (s.d.) = 110.9 (91–122) (8.0)), which was estimated using the Japanese version of the National Adult Reading Test (Nelson, 1982; Matsuoka et al., 2006). The ethical committee of The University of Tokyo Hospital approved this study (No. 397). After a complete explanation of the study, written informed consent was obtained from all participants.

**Factor analysis of the AQ**

A four-point AQ scoring system was used as in previous factor analyses of AQ (Austin, 2005; Hurst et al., 2007; Hoeckastra et al., 2008; Stewart and Austin, 2009; Kloosterman et al., 2011), so that each item was scored on a scale ranging from 0 to 3. Thus, the total score calculated from this system can range from 0 to 150, whereas scores from the original dichotomous version range from 0 to 50. Unlike a dichotomous scale, the four-point Likert scale extracts responses that better

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**Table 1 Subject characteristics**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Male (n = 79)</th>
<th>Female (n = 56)</th>
<th>t-Test</th>
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<tbody>
<tr>
<td>Demographic variables</td>
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</tr>
<tr>
<td>Age (range)</td>
<td>29.4 (21–40)</td>
<td>28.1 (22–40)</td>
<td>4.4</td>
</tr>
<tr>
<td>Handedness (range)</td>
<td>96.3 (25–100)</td>
<td>96.0 (50–100)</td>
<td>10.4</td>
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<tr>
<td>Socioeconomic status (SES)</td>
<td>1.49</td>
<td>0.5</td>
<td>1.8</td>
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<tr>
<td>Parental SES</td>
<td>2.17</td>
<td>0.6</td>
<td>2.07</td>
</tr>
<tr>
<td>Autism-Spectrum Quotient (AQ)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>AQ total</td>
<td>59.4</td>
<td>11.4</td>
<td>57.0</td>
</tr>
<tr>
<td>Prosociality</td>
<td>15.1</td>
<td>6.3</td>
<td>14.3</td>
</tr>
<tr>
<td>Details/Patients</td>
<td>12.4</td>
<td>4.6</td>
<td>11.4</td>
</tr>
<tr>
<td>Communication</td>
<td>10.9</td>
<td>4.3</td>
<td>11.3</td>
</tr>
<tr>
<td>Imagination</td>
<td>6.9</td>
<td>2.8</td>
<td>6.1</td>
</tr>
<tr>
<td>Global brain measures</td>
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<tr>
<td>Total grey matter (cc)</td>
<td>761</td>
<td>51</td>
<td>674</td>
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<tr>
<td>Total white matter (cc)</td>
<td>561</td>
<td>41</td>
<td>491</td>
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<tr>
<td>Total cerebrospinal fluid (cc)</td>
<td>313</td>
<td>28</td>
<td>260</td>
</tr>
<tr>
<td>Intracranial volume (ICV) (cc)</td>
<td>1635</td>
<td>113</td>
<td>1427</td>
</tr>
<tr>
<td>Grey matter/ICV</td>
<td>0.466</td>
<td>0.007</td>
<td>0.473</td>
</tr>
<tr>
<td>White matter/ICV</td>
<td>0.345</td>
<td>0</td>
<td>0.344</td>
</tr>
<tr>
<td>Cerebrospinal fluid/ICV</td>
<td>0.191</td>
<td>0.009</td>
<td>0.183</td>
</tr>
</tbody>
</table>

* Determined using the Edinburgh Inventory (Oldfield, 1971); scores > 0 indicate right-handedness. A score of 100 indicates strong right-handedness. Assessed using the Hollingshead scale (Hollingshead, 1957). Higher scores indicate lower educational and/or occupational status. Higher scores indicate stronger ALTs. A four-point AQ scoring system was used, so that each item was scored on a scale ranging from 0 to 3. Thus, the total score calculated from this system can range from 0 to 150, whereas scores from the original dichotomous version range from 0 to 50.
approximate a continuous distribution, thus providing more information for factor analysis (Kloosterman et al., 2011). After conversion of reversed items, the higher scores indicate strong ALTs. The factor structure of the AQ was examined using SPSS Statistics 18.0 (IBM SPSS, Chicago, IL) with unweighted least squares for extraction and promax rotation.

**OXTR genotyping**

Genomic DNA was extracted from peripheral leukocytes using a standard phenol–chloroform method as described previously (Inoue et al., 2010). DNA was isolated and amplified from blood samples obtained from 112 of the subjects (70 males and 42 females). Blood samples were not obtained from the other 23 subjects because MR data collection time occurred prior to starting blood collection. The single nucleotide polymorphism (SNP) for OXTR rs2254298A was genotyped using the TaqMan® genotyping platform in accordance with the manufacturer’s protocol (www3.appliedbiosystems.com).

**MRI acquisition**

The method of MRI acquisition was the same as that used in our previous studies (e.g. Inoue et al., 2010; Yamasue et al., 2011). Briefly, MRI data were obtained using a 1.5-tesla scanner (General Electric Signa Horizon Lx version 8.2). Three-dimensional Fourier-transformed spoiled-gradient-recalled acquisition with steady state was used. The repetition time was 35 ms, the echo time 7 ms with one repetition, the nutation angle 30°, the field of view 24 cm and the matrix 256 × 256 (192) × 124. Voxel dimensions were 0.9375 × 0.9375 × 1.5 mm in 124 contiguous axial images with no gap. A trained neuroradiologist (O.A.) evaluated the MRI scans and found no gross abnormalities in any of the subjects.

**Image processing for VBM**

Image processing for VBM analysis was performed, as previously described (Yamasue et al., 2011), using SPM8 software (Welcome Department of Imaging Neuroscience) running on Matlab 2008a (Math Works). The non-uniformity of intensity of all acquired images was corrected using non-parametric non-uniform intensity normalization (N3) (available at http://www.bic.mni.mcgill.ca/~jgledslem/thesis/). First, MR images were segmented using the standard unified segmentation model in SPM8. Images were preprocessed using the DARTEL (Ashburner, 2007) SPM8 toolbox. The following steps were used for VBM preprocessing: (i) checking for scanner artefacts and gross anatomical abnormalities for each subject, (ii) setting the image origin to the anterior commissure, (iii) using the DARTEL toolbox to produce a high-dimensional normalization protocol, (iv) checking for homogeneity across the sample and (v) using standard smoothing (i.e. 8 mm). The final voxel resolution after DARTEL was 1.5 × 1.5 × 1.5 mm. A ‘modulation step’ was also included in the normalization to preserve information about the absolute grey matter values.

**Statistical analysis**

Sex differences in demographic information, AQ scores and global brain volumes, calculated from VBM processing, were analysed using two-sampled t-tests. The threshold for statistical significance was set at $P < 0.05$.

To detect neuroanatomical correlates of individual differences in ALTs controlling for sexual dimorphism, an interaction analysis treated sex as a condition and the AQ factor scores as a covariate of interest in the main analysis. As there is a well-established effect of aging on brain volumes and the significant sex differences in the self-SES and intracranial volume (ICV), the interaction analyses included these indices as the nuisance variables. The level of significance was set at peak-level false-discovery rate (FDR)-corrected $P < 0.05$ for testing correlation between social sub-component of AQ factor and regional brain volume, whereas that was defined at uncorrected $P < 0.001$ for testing sex difference in the correlations. Furthermore, interactions without suprathreshold correlation between AQ factor and regional brain volume in one sex were ignored to exclude any false-positive interactions caused by a combination of subthreshold correlation with expected direction in at least one sex and paradoxical direction in the other sex. As it is difficult to predict the location of neural correlates of the newly extracted factor of AQ, this study was designed to search for the neural basis of social aspects of ALTs throughout the entire brain without region-of-interest analysis. However, based on a previous study (von dem Hagen et al., 2011), the correlation between total AQ score and regional brain volume was also examined with STS as an a priori predicted region of interest. For the other three sub-factors and total score of AQ, a Bonferroni correction was employed to correct for multiple comparisons, therefore FDR-corrected $P < 0.0125$ (0.05/4 analyses) was defined as statistically significant.

Then, the structural coupling between regional GMV in the right insula, which showed a significant correlation with ‘prosociality’ specifically in males (see ‘Results’ section), and regional GMV in other regions was examined by structural covariance analysis in males. Structural covariance analysis is a regression analysis (Pezawas et al., 2005), treating voxel intensities extracted from a 10 mm diameter spherical seed centred at the peak voxel in the right insula as covariates of interest and ICV as a nuisance covariate. Voxels identified as significant in this structural covariance approach have regional volumes that are significantly positively or negatively correlated to the right insula across subjects. The significance level was defined as peak-level FDR-corrected $P < 0.05$. Furthermore, Pearson’s correlation coefficients were examined in SPM between ‘prosociality’ and eigenvariates extracted from peak coordinates of brain regions showing significant correlations with the right insula. Statistical significance level was set at $P < 0.05$.

Finally, the relationship of the number of the risk allele for ASDs, OXTR rs2254298A (A/A, A/G and G/G) was obtained and correlated to regional GMV in the brain regions. A significant correlation with ALTs and the right insula was examined using Spearman’s rank-order correlation coefficients in males with SPSS. The correlation was also examined in females to test the specificity of the association in males. To account for the individual differences of ICV in these correlational analyses, the relative regional GMV in the right insula was calculated as regional GMV × 1000/ICV. $P < 0.05$ was defined as the level of statistical significance.

**RESULTS**

**Factor analysis of the AQ**

Using unweighted least squares for extraction, the scree plot for the 50 AQ items suggested extraction of a three-factor structure in the current subjects. First, the three-factor solution was examined using a promax rotation. With eigenvalues of 8.06, 4.84 and 3.70, the three factors explained 33.2% of the variance. Then, 18 items with factor pattern matrix elements smaller than 0.4 were excluded. For the remaining 32 items, the factor internal reliabilities were shown as 0.88, 0.34 and 0.74 (Cronbach’s $\alpha$). Because the second factor was difficult to interpret and had a poor internal reliability, the three-factor solution was deemed to be a poor fit to the data. Then, we examined a four-factor solution. With eigenvalues of 8.06, 4.84, 3.70 and 2.50, the four factors explained 38.2% of the variance. Eleven items with factor pattern matrix elements that were smaller than 0.4 were excluded. The factor internal reliabilities for the remaining 39 items were improved to 0.89, 0.77, 0.76 and 0.54, and the factors were easily interpreted (Table 2).
We decided to adopt this 'four-factor model' in the later analyses. The factors were named 'prosociality', 'communication', 'details/patterns' and 'imagination' in reference to previous literature (Austin, 2005; Hurst et al., 2007; Hoekstra et al., 2008; Stewart and Austin, 2009; Kloosterman et al., 2011). The 12 items included in 'prosociality' significantly overlapped with the homologues factors in previous studies involving larger sample sizes: 12 of 26 in 'social interaction' in Austin, 2005; Hurst et al., 2007; Hoekstra et al., 2008; Stewart and Austin, 2009; Kloosterman et al., 2011; 9 of 12 in 'social skill' in Hurst et al., 2007; 9 of 12 in 'social skill' in Austin, 2005; 9 of 12 in 'social skill' in Stewart and Austin, 2009; and 8 of 8 in 'social skill' in Kloosterman et al., 2011. Thus, 'prosociality' seems to be composed of reasonable items, although the sample size of this study was relatively small compared with previous studies. Of note, a higher score of 'prosociality' indicates highly autistic sociality, whereas the lower scores indicate high levels of prosociality.

Correlation between regional GMV and ALTs, and its relationship to sexual dimorphism

Absolute ICV, total grey matter, white matter, cerebrospinal fluid volume and relative cerebrospinal fluid volume were significantly larger in males (P<0.001), whereas the relative total GMV was significantly larger in females (P<0.001). Although the mean of total AQ score was higher in males than in females, this did not reach statistical significance (Table 1). To control for sex differences, self-SES and ICV were added as confounding covariates in the VBM interaction analysis between sex and AQ factors.

Among the AQ factors, the 'prosociality' score showed a significant negative correlation with regional GMV in the right insula in males (FDR-corrected P<0.05), indicating a relationship between strong social aspects of ALTs and the small right insula. The correlation between 'prosociality' and regional GMV in the right insula was significantly specific to males ([44 0 –11], T[1, 128]=3.98, P<0.001) (Figure 1B). A post hoc additional independent T-test showed that the sex difference in relative regional GMV in the right insula was also significant (T[1, 130]=2.49, P=0.014). Although the level of significance was marginal, the 'prosociality' score also showed a negative correlation with regional GMV in the left insula in the combined sample (FDR-corrected P=0.063) with no significant sexual dimorphism. The regional GMV in other brain regions or white matter volumes throughout the brain showed no significant correlation with the AQ factor scores in the social or in the other domains in males. No significant correlation between the AQ scores and the regional brain volumes was found in females (Figure 1A and Table 3).

We also found a correlation between high total AQ and small regional GMV in several brain regions using a liberal threshold (P<0.001) including the right insula ([36 9 –15], T=3.90 in combined sex; [39 12 –17], T=3.65 in male) by covarying age, self SES and ICV, although the significance disappeared when multiple comparisons were accounted for (FDR-corrected P>0.37). No suprathreshold correlation was detected from the STS.

### Structural coupling of the right insula and its relationship to ALTs

The structural coupling of the right insula to nine different brain regions, which were mainly distributed in the temporal and frontal areas, was found to be statistically significant in males (Table 3, FDR-corrected P<0.05). Furthermore, Pearson's correlation coefficients in SPSS between eigenvariates from correlations of the right insula with these brain regions and 'prosociality' scores revealed that decreased structural coupling of the right insula with the ventral ACC was related to increased social aspects of ALTs (r = -0.23, P<0.05, N = 79) (Figure 2).

### Neural correlates of ALTs and OXTR

A significant association between rs2254298A and the relative regional GMV in the right insula was detected in males (r = 0.270, P = 0.024, n = 70), whereas the corresponding correlation was absent in females (r = 0.091, P = 0.568, n = 42) (Figure 3). Partial correlation analyses with ICV as a regulating variable confirmed a significant association between rs2254298A and regional GMV in the right insula in males (r = 0.268, P = 0.026) but not females (r = 0.033, P = 0.838). These results indicate that having a larger number of risk alleles is associated with a smaller right insula only in males, although the significance of sex difference in the correlation coefficients was marginal (P = 0.067). The insula–ACC coupling or 'prosociality' scores were not significantly associated with rs2254298A (P > 0.216).

When the IQ level was controlled by employing the estimated IQ scores as additional confounding covariates, the statistical conclusions as described above were totally preserved.
DISCUSSION

This study shows that, in adult neurotypical males, strong ALTs in social aspects are associated with smaller regional GMV in the right insula. Furthermore, this correlation was specific to males. Increased social aspects of ALTs were also associated with weak structural coupling of the right insula with the ventral ACC in males. Moreover, the smaller right insula was significantly associated with a larger number of the risk alleles for \( \text{OXTR} \) rs2254298A, in males.

The scoring system for AQ in our study was the same as that of Auyeung et al. (2008), in which the mean total AQ scores were reported as 37.7–45.7 for healthy children. In other studies in which each AQ item was scored on a scale ranging from 1 to 4, the mean total AQ scores were reported as 98.5–111.3 in general populations (Austin, 2005; Hoekstra et al., 2008; Stewart and Austin, 2009). After conversion to the same scoring system as ours in which each item was scored on a scale ranging from 0 to 3, the mean scores in these previous studies were 48.5–61.3. Thus, the mean AQ scores of 57.0–59.4 for the general population in our study were comparable to those in the previous studies.

By extracting the AQ factor structure, this study might reduce the impact of the neurobiological heterogeneity of ALTs. The social domain of ALTs ‘prosociality’ showed a significant correlation with regional GMV, whereas the other domains such as ‘communication’, which mainly include items associated with reading of others’ intention, and ‘details/patterns’ did not. These factors—‘prosociality’, ‘communication’ and ‘details/patterns’—approximately correspond to three core symptoms of ASD: deficits in social, communication and repetitive and restricted behavior, respectively. The core symptoms of ASD have been considered to have different neurobiological origins (Happe et al., 2006). The social domain consists of items relating to prosociality, such as preferences to social situations like parties, chit-chat, social interaction and meeting new people. Recent studies have shown that prosociality (e.g. indexed by group size, social network size or number of Facebook friends) is linked with individual differences at brain structural levels in humans as well as in primates (reviewed in Dunbar, 2011). These findings support the ‘social brain hypothesis’ indicating that prosociality might be a driving force towards development of large brain size in primates and humans.

Our previous studies support the notion that individual differences in prosociality-related personality traits are more likely to be extensively correlated to brain volume (Yamasue et al., 2008) than those in other domains of personality, such as anxiety-related traits (reviewed in Yamasue et al., 2009). Taken together, the current results identifying neuroanatomical correlates of prosociality rather than those of the other factors are consistent with the social brain hypothesis and our own recent findings.

Highly autistic sociality which was indicated by a high score of ‘prosociality’ showed a correlation with smaller GMV in the right insula.

Table 3 Area associated with ‘prosociality’ and its structural coupling with other regions

<table>
<thead>
<tr>
<th>Anatomical location</th>
<th>Peak coordinate</th>
<th>t-Value</th>
<th>FDR-corrected p</th>
<th>Cluster size (k)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>x   y   z</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right insula</td>
<td>44  0  −11</td>
<td>5.04</td>
<td>0.026</td>
<td>531</td>
</tr>
</tbody>
</table>
| Positive correlation of the right insula to other brain regions in males (\( n = 79 \))
| Left insula         | −42  0  −11     | 7.15    | <0.001         | 2164            |
| Left middle temporal gyrus | −65  −16  −2  | 4.58    | 0.001          | 712             |
| Right middle temporal gyrus | 59  −43  −5  | 3.97    | 0.007          | 92              |
| Left superior temporal gyrus | −45  −40  15 | 3.92    | 0.008          | 42              |
| Right middle temporal gyrus | 68  −6   −8  | 3.71    | 0.013          | 47              |
| Right precentral gyrus | 47  −16  40   | 3.59    | 0.017          | 26              |
| Right inferior temporal gyrus | 59  −10  −18 | 3.57    | 0.018          | 22              |
| Left ventral anterior cingulate | −2  30  −3  | 3.36    | 0.028          | 21              |

No suprathreshold cluster

Fig. 1 Male-specific correlation between ‘prosociality’ and GMV in the right insula. (a) The grey matter regions where an interaction between sex and ‘prosociality’ was found are rendered in the MNI space (\( n = 135 \)) (voxel threshold: uncorrected \( p < 0.001 \), L, left; R, right. (b) Scatter plots depicting correlations between regional GMV in the right insula and ‘prosociality’, in which higher score indicates stronger ALTs, in males and females.
insula. Recent evidence highlights a crucial role for the insular cortex in social cognition and behaviours such as sharing other’s sensation and emotions, and processing uncertainty (reviewed in Singer et al., 2009). Although some controversies exist, previous VBM studies have shown smaller than normal GMV in the insula of people with ASDs (reviewed in Duerden et al., 2012). Cortical folding abnormalities (Nordahl et al., 2007) and atypical functional deviations (Ohnishi et al., 2000; Di Martino et al., 2009a; Lai et al., 2010) have also been reported in the insula of ASDs subjects. Thus, individual differences in insular morphology might represent neural correlates of ALTs within the normal range of variation as well as at the extreme.

The sexual dimorphism of the correlation between right insula volume and ALTs in the social domain was found to be statistically significant in this study. Sex differences in activities of the insula have been associated with social cognition and emotional responses in neurotypical adults (Lee et al., 2005; Singer et al., 2006; Kohn et al., 2011). Structural MRI studies have also shown that there are sex differences in GMV (Good et al., 2001; Yamasue et al., 2008) in the insula of healthy adults. The sex difference in relative regional GMV in the right insula was also found to be significant in this study. Although speculative, the high prevalence of ASDs, which can be considered a phenotype of a certain sexually dimorphic pathogenetic factors, can be associated with an enhanced link between ALTs and insula volume in males.

This study revealed that less structural coupling between the insula and the ACC is associated with strong ALTs in the social domain. Although the measures examined here are likely to reflect covariation of regional volumes across subjects and do not directly quantify white matter projections, previous studies have reported structural correlations that correspond well with the known anatomical connectivity of brain regions (Mechelli et al., 2005; Tost et al., 2010). Previous reports of anatomical connections between the insula and the ACC/medial prefrontal cortex are consistent with findings of the current and other previous studies (Augustine, 1996). Reduced functional connectivity between the insula and the ACC appears to be associated with a high level of ALTs (Di Martino et al., 2009b). Further studies have revealed a role of diminished functional (Di Martino et al., 2011) and anatomical (McAlonan et al., 2005; Cheng et al., 2010) connectivity of the insula to other brain regions with social and communicating dysfunction in people with ASDs.

One genetic risk factor for ASDs (i.e. OXTR rs2254298A) was found to be associated with a neural correlate of social domain of ALTs (i.e. smaller right insula volume) in males. Recently, individual differences in trait aspects of social behavior were reported to be associated with OXTR in neurotypical individuals (Tost et al., 2010; Chen et al., 2011). OXTR was also reported to be associated with ASDs that are a quantitative extreme of ALTs (reviewed in Brüne, 2012; Yamasue, 2013). Recent studies have further shown effects of OXTR on brain structure
and function among neurotypical adults (Inoue et al., 2010; Tost et al., 2010, 2011; Furman et al., 2011; Yamasue et al., 2011), although these studies suggest that the effects occur in regions other than the insula, such as the amygdala, hypothalamus and ACC. The difference in location might partially be explained by the fact that in this study, we avoided multiple comparisons of VBM data by first identifying neural correlates of ALTs. Other studies have suggested that there is a relationship between oxytocin and the insula. Oxytocin receptors are densely localized in limbic and paralimbic regions, including the insula, and the distribution of oxytocin receptor appears to be related to social behavior in experimental animals (Febo et al., 2005; Ophir et al., 2012). Moreover, a recent study in humans showed an enhanced insular emotional response induced by intranasal oxytocin (Riem et al., 2011).

The rs2254298A allele has previously been linked to autism in Chinese and Japanese sample populations, but not in Caucasians (reviewed in Brüne, 2012; Yamasue, 2013). It is still not clear whether the OXTR polymorphism (rs2254298) confers a higher vulnerability or differential susceptibility for psychopathology. Furthermore, in sample populations of European ancestry the A allele is uncommon, with the vast majority of individuals being homozygous for the G allele. In Asian populations, the frequency of the A allele is much higher, and 40–50% are GG carriers. This difference in allele frequency also needs to be considered when comparing the current findings with studies in populations of differing ethnicity (Brüne, 2012), although this study revealed that an association between neural correlates of autistic sociability and a risk allele for Asian populations in OXTR was observed in Japanese subjects.

There are several methodological considerations and limitations of our study. First, the limited sample size might contribute to the lack of significant direct relationship of rs2254298A with ALTs and no significant correlation of the allele with the structural coupling. Because an intermediate phenotype should be more likely to associate with genotype as well as with the behavioural phenotype, the combination of current findings seems reasonable. Relatively late white matter development (Lenroot et al., 2007) might make it difficult to detect an association between genotype and interregional connectivity, although a previous large-scale study in 212 Caucasians reported a significant increase in the structural coupling of hypothalamus and ACC in rs2254298A carriers (Tost et al., 2011). Second, there were more male than female subjects in this study. Thus, there remains the possibility that our ability to identify a female-specific correlation was weaker than that to identify a male-specific correlation. Third, this study includes only Japanese subjects. It is possible, however, that the participation of a single ethnic group might have contributed to the clarity of findings. Because previous studies have reported significant ethnic differences in brain morphology (Zilles et al., 2001) and genetics (Brüne, 2012), future replication of our study in other ethnicities is necessary to determine the global significance of our findings. Fourth, this study employed a self-report questionnaire as an index for ALTs. Although the questionnaires used here to study biological aspects of ALTs have been validated (Gomot et al., 2008; von dem Hagen et al., 2011; Kawamura et al., 2011), future studies are needed to confirm the findings using more objective indices of ALTs. Fifth, 112 of 135 subjects and their SNP data overlapped with our previous study (Inoue et al., 2010). However, this study was based on the novel analyses of the novel ALT data in these 135 individuals. Based on these data sets, we first identified the intermediate phenotype (i.e. insular volume) which showed a significant association with social aspects of ALT not investigated in our previous study. Then, we examined association between the identified intermediate phenotype and OXTR rs2254298A. Thus, in addition to the former one, the later association analysis is also considered to be an original one testing association between the originally found intermediate phenotype and rs2254298A. Therefore, this study can significantly contribute to the field, although future studies are expected to replicate the present results in a totally independent population. Finally, although VBM is considered to be better in identifying new potential areas of interest, future studies should replicate the current insular findings by manually tracing volumetry to test the reproducibility of the current results.

In summary, this study shows a neurobiological basis of ALTs in the social domain by considering factor structure and sexual dimorphism of ALTs in neurotypical adults. Smaller GMV in the right insula and its decreased coupling with the ACC were correlated to highly autistic sociality specifically in males. Smaller GMV in the right insula, a sexually dimorphic intermediate phenotype underlying autistic sociality, further showed a significant association with OXTR rs2254298A, which is a candidate genetic factor for ASDs. The present findings suggest that small insula volume can be considered an intermediate phenotype for uncovering the relationship between ALTs and OXTR.

**Conflict of Interest**
None declared.

**REFERENCES**


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