Published in final edited form as:

Neuroimage. 2012 April 15; 60(3): 1890–1901. doi:10.1016/j.neuroimage.2012.01.120.

Characteristic cortical thickness patterns in adolescents with autism spectrum disorders: Interactions with age and intellectual ability revealed by canonical correlation analysis

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Abstract

To investigate patterns and correlates of cortical thickness in adolescent males with autism spectrum disorders (ASD) versus matched typically developing controls, we applied kernel canonical correlation analysis to whole brain cortical thickness with the explaining variables of diagnosis, age, full-scale IQ, and their interactions. The analysis found that canonical variates (patterns of cortical thickness) correlated with each of these variables. The diagnosis- and age-by-diagnosis-related canonical variates showed thinner cortex for participants with ASD, which is consistent with previous studies using a univariate analysis. In addition, the multivariate statistics found larger affected regions with higher sensitivity than those found using univariate analysis. An IQ-related effect was also found with the multivariate analysis. The effects of IQ and age-by-IQ interaction on cortical thickness differed between the diagnostics groups. For typically developing adolescents, IQ was positively correlated with cortical thickness in orbitofrontal, postcentral and superior temporal regions, and greater thinning with age was seen in dorsal frontal areas in the superior IQ (> 120) group. These associations between IQ and cortical thickness were not seen in the ASD group. Differing relationships between IQ and cortical thickness implies independent associations between measures of intelligence and brain structure in ASD versus typically developing controls. We discuss these findings vis-à-vis prior results obtained utilizing univariate methods.

Keywords

autism spectrum disorders; kernel canonical correlation analysis; varimax rotation; cortical thickness; developmental change

Introduction

Autism spectrum disorders (ASD) are characterized by impairments in social interaction, communication, and repetitive behavior (American Psychiatric Association, 2000). Many studies have shown an atypical developmental trajectory of brain anatomy, particularly gray matter structures, in ASD compared to patterns observed in typical development (Hardan et

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al., 2009; Raznahan et al. 2010; Redcay and Courchesne, 2005; Scheel et al., 2011; Wallace et al., 2010, for review, see Amaral et al., 2008; Courchesne et al., 2007). These studies indicate that age is an important factor and key to understanding neuroanatomical differences in ASD.

In addition to age, intellectual ability (measured via IQ scores) is also related to gray matter structures in typical development (Narr et al., 2007; Shaw et al., 2006; Sowell et al., 2004; Wilke et al., 2003). However, discrepant associations between gray matter structure and IQ in ASD (versus typically developing individuals) have not been shown. Hardan et al. (2006) investigating the cortical thickness differences between typically developing and ASD children, report that no significant correlation between IQ score and cortical thickness was found in either group. Hadjikhani et al. (2006) also did not find any significant correlation between IQ and cortical thinning in ASD adults. These negative results even for typically developing participants could be due to limited sensitivity of their analyses with small sample sizes.

While most of the previous studies use univariate approaches in which the effect of ASD is evaluated at each local region independently, multivariate approaches may more effectively characterize neuroanatomical differences in ASD with higher sensitivity: the effect of ASD is not likely to be restricted to a specific brain region but instead distributed in broad areas; therefore, regional differences can be correlated with one another. In fact, it has been shown that multivariate classification analyses, in which multiple brain regions are used as a multivariate dataset, are more effective in discriminating an ASD group from controls than evaluating their difference at each individual region. Ecker et al. (2010), for example, show high accuracy of classification between ASD and control groups using a whole brain gray matter volume as input for a multivariate classification analysis (support vector machine), while no statistically significant difference between groups was observed with voxel-based analysis. Jiao et al. (2010) also demonstrated that multivariate classification analyses could discriminate ASD children from controls with high accuracy when they used cortical thickness of multiple regions as input for the analysis. They show that cortical thickness is a better classifier of groups than gray matter volume.

While the multivariate classification analysis handles multiple brain regions as a multivariate dataset, its output is still univariate (class label). This analysis, therefore, cannot account for age and IQ effects on multiple brain regions. In the previous studies these effects were excluded beforehand; any possible age confounds were removed at each voxel by a univariate regression in Ecker et al. (2010), or participants' ages were restricted to a small range in Jiao et al. (2010). In the current analysis, however, we do not exclude these factors beforehand because we were interested in evaluating how they and their interactions with group affected multivariate pattern of whole brain cortical thickness.

To investigate cortical thickness in ASD in a multivariate framework with age and IQ interactions taken into consideration, we utilized kernel canonical correlation analysis (KCCA) (Hardoon et al., 2004) in this study. Canonical correlation analysis (CCA) (Hotelling, 1936) evaluates correlations between two multidimensional datasets, so that it can find relationships between cortical thicknesses in multiple brain regions and multiple factors of ASD, age, and IQ. KCCA is an extension of CCA using a kernel method to handle a large dimensional dataset efficiently and robustly.

Even when we have two multidimensional datasets, multiple regression analysis or Pearson's correlation analysis can be utilized if we collapse the dataset into multiple univariate cases. This is called the 'mass-univariate' approach, in which data from each voxel/vertex is used as a univariate value, and univariate correlation analysis or multiple

regression analysis is repeated for each voxel/vertex. However, CCA is disparate from the mass-univariate approach. While the mass-univariate approach evaluates correlation at each brain region independently, CCA can find a pattern correlation; a correlation between linear combinations of multiple variables, so that it can extract more information that can be seen in a pattern of multiple brain regions but cannot be seen at an individual brain region.

Although CCA has been used in analyses of brain imaging data (Correa et al., 2010; Hardoon et al., 2009), it has not, to our knowledge, been applied to evaluations of cortical thickness in ASD. This analysis enables us to find multivariate patterns of cortical thickness correlating with multiple explaining variables. We applied KCCA to the cortical thickness data from groups of ASD and age and IQ matched typically developing control males.

Materials and Methods

The cortical thickness data used in this study was the same as in Wallace et al. (2010). Only the summary of demographics is described here. More details regarding diagnostic procedure, medication history, and criteria for exclusion of participants can be found in Wallace et al. (2010).

Participants

Participants were 40 typically developing (TD) males (12–23 years of age, mean = 17.04, sd = 2.73) and 41 males with an autism spectrum disorder (12–24 years of age, mean = 16.75, sd = 2.84) recruited from the Washington, DC metropolitan area. Informed assent and consent were obtained from all participants and/or their parent/guardian when appropriate in accordance with a National Institutes of Health Institutional Review Board-approved protocol.

Participants with autism spectrum disorders (ASD) included 26 with Asperger's syndrome, 11 with high-functioning autism, three with pervasive developmental disorder not otherwise specified, and one with either Asperger's syndrome or high-functioning autism, which could not be distinguished because of missing early language developmental milestones data. An ASD diagnosis was based on both Diagnostic and Statistical Manual-IV (DSM-IV) criteria as assessed by an experienced clinician and scores from the Autism Diagnostic Interview (ADI or ADI-R; Le Couteur et al., 1989; Lord et al., 1994) and/or the Autism Diagnostic Observation Schedule (Lord et al., 2000), administered by a trained, research-reliable clinician.

Full-scale IQ (FSIQ) was measured for all participants using the Wechsler Abbreviated Scale of Intelligence (34 ASD, 40 TD), the Wechsler Adult Intelligence Scale-III (three ASD), the Wechsler Intelligence Scale for Children-III (two ASD), or the Wechsler Intelligence Scale for Children-IV (two ASD). FSIQ did not differ between groups with scores ranging from 85–143 (mean = 113.27, sd = 15.09) for the ASD group and 97–136 (mean = 114.03, sd = 10.74) for the TD group. Participant groups also did not differ in terms of handedness (ASD: right/left = 37/4, TD: 36/4).

Imaging parameters and cortical thickness calculation

A T1-weighted structural image was obtained from each subject with a magnetization prepared rapid gradient echo sequence on a 3 T General Electric Signa scanner (Milwaukee, Wisconsin) using an 8-channel head coil. Imaging parameters were 124 axial slices, 224 × 224 acquisition matrix, flip angle = 6 degrees, field of view = 24 cm, and voxel size = 1.07 × 1.07 × 1.2 mm.

The FreeSurfer image analysis suite (<http://surfer.nmr.mgh.harvard.edu/>) was used to extract cortical thickness. Cortical surface models were delineated on the gray/white boundary and on the pial surface (Dale et al., 1999; Fischl and Dale, 2000; Fischl et al., 1999). Spatial intensity gradients across tissue classes were used to create surface maps that are capable of detecting sub-millimeter differences between areas. The resulting surface models were reviewed for accuracy and manually edited when needed. Cortical thickness was quantified at each surface location (vertex) as the distance from the gray/white boundary to the pial surface (Fischl and Dale, 2000). This method of cortical thickness measurement has been validated in Rosas et al. (2002), Kuperberg et al. (2003), and Salat et al. (2004), and has shown good reliability across sites and platforms (Han et al., 2006).

Vertex-wise cortical thickness values were mapped onto a normalized cortical surface and smoothed with a 15 mm full width at half maximum kernel. After excluding unreliable areas in the hippocampus and amygdala (cortex label in the FreeSurfer was used as an inclusive mask), 299,881 cortical thickness values on the normalized surface of both hemispheres were obtained for each participant. These values were used in the kernel canonical correlation analysis.

Kernel canonical correlation analysis

The canonical correlation analysis (CCA) finds linear combinations of two multidimensional datasets that have maximum correlation with each other (Hotelling, 1936). Given the two multidimensional datasets, X (each column corresponds to the explaining variables including age, IQ, diagnosis and their interactions, and each row corresponds to one participant) and Y (each column corresponds to cortical thickness at one vertex, and each row corresponds to one participant), canonical correlation ρ is defined as:

$$\rho = \max_{\mathbf{w}_x, \mathbf{w}_y} \frac{\mathbf{w}_x' \mathbf{S}_{XY} \mathbf{w}_y}{\sqrt{\mathbf{w}_x' \mathbf{S}_{XX} \mathbf{w}_x \mathbf{w}_y' \mathbf{S}_{YY} \mathbf{w}_y}} \text{ subjected to } \mathbf{w}_x' \mathbf{S}_{XX} \mathbf{w}_x = 1 \text{ and } \mathbf{w}_y' \mathbf{S}_{YY} \mathbf{w}_y = 1, \quad (1)$$

where $\mathbf{S}_{XX} = X'X$, $\mathbf{S}_{YY} = Y'Y$, and $\mathbf{S}_{XY} = X'Y$. $\mathbf{w}_x$ and $\mathbf{w}_y$ are column vectors. $\mathbf{w}_x$ is given by solving the following eigenproblem:

$$\mathbf{B} \mathbf{w}_x = \rho^2 \mathbf{w}_x, \quad (2)$$

where $\mathbf{B} = \mathbf{S}_{XX}^{-1} \mathbf{S}_{XY} \mathbf{S}_{YY}^{-1} \mathbf{S}_{YX}$. Thus, canonical correlation ρ is given as a square root of eigenvalue of $\mathbf{B}$ and $\mathbf{w}_x$ as an eigenvector of $\mathbf{B}$. $\mathbf{w}_y$ is given by:

$$\mathbf{w}_y = \frac{1}{\rho} \mathbf{S}_{YY}^{-1} \mathbf{S}_{YX} \mathbf{w}_x. \quad (3)$$

In the CCA, multiple canonical correlations can be evaluated with the eigenvalues of $\mathbf{B}$, therefore the CCA can be seen as mapping the original data spaces onto the canonical variate spaces whose dimensions are highly correlated with each other.

When the dimensionality of the data is larger than the number of samples, like in the current case with a dimensionality of nearly 300,000 for 81 samples, CCA is prone to overfit to noise of data, that is, it could find correlations of noise in each dataset. To avoid overfitting (Bishop, 2007), a regularization technique, which restricts the complexity of the model, is used. In this study we used the kernel canonical correlation analysis (KCCA) with regularization.

We used the MATLAB implementation of KCCA by Hadoon et al. (2004), which is available on the website (http://www.davidroi.hadoon.com/Professional/Code_files/kcca_package.tar.gz). Here, we describe only the procedures of the analysis. For proofs, refer to Hadoon et al. (2004).

In this analysis, original data space X and Y are mapped onto kernel spaces K_X and K_Y respectively,

$$\mathbf{K}_X; k_{x_i, x_j} = \langle \phi_X(\mathbf{x}_i), \phi_X(\mathbf{x}_j) \rangle \quad (4)$$

$$\mathbf{K}_Y; k_{y_i, y_j} = \langle \phi_Y(\mathbf{y}_i), \phi_Y(\mathbf{y}_j) \rangle \quad (5)$$

where $\langle, \rangle$ is an inner product operator and ϕ is a kernel function. We used a linear kernel for both X and Y , so that ϕ is identity $k_{x_i, x_j} = \mathbf{x}_i^T \mathbf{x}_j$ and $k_{y_i, y_j} = \mathbf{y}_i^T \mathbf{y}_j$. The kernel matrices, $\mathbf{K}_X$ and $\mathbf{K}_Y$, are further mapped onto a reduced dimensionality space, $\mathbf{R}_X$ and $\mathbf{R}_Y$, using the partial Gram-Schmidt orthogonalization (Hadoon et al., 2004). The canonical correlation analysis was applied to these $\mathbf{R}_X$ and $\mathbf{R}_Y$. The canonical correlation ρ and weight matrix for $\mathbf{R}_X$ are given by solving the following eigenproblem:

$$\mathbf{S}^{-1} \mathbf{Z}_{XY} ((\mathbf{I} - \kappa) \mathbf{Z}_{YY} + \kappa \mathbf{I})^{-1} \mathbf{Z}_{YX} \mathbf{S}^{-1'} \mathbf{W}_{\mathbf{R}_X} = \rho^2 \mathbf{W}_{\mathbf{R}_X}, \quad (6)$$

where $\mathbf{Z}_{YY} = \mathbf{R}_Y' \mathbf{R}_Y$, $\mathbf{Z}_{XY} = \mathbf{R}_X' \mathbf{R}_Y$, $\mathbf{Z}_{YX} = \mathbf{R}_Y' \mathbf{R}_X$, and $\mathbf{S}$ is a Cholesky decomposition of $\mathbf{B} = \kappa \mathbf{Z}_{XX} + (1 - \kappa) \mathbf{I}$. κ is a regularization parameter taking a value between 0 and 1. Weight matrix for $\mathbf{R}_Y$ is given by:

$$\mathbf{W}_{\mathbf{R}_Y} = ((\mathbf{I} - \kappa) \mathbf{Z}_{YY} + \kappa \mathbf{I}) \mathbf{Z}_{YX} \mathbf{W}_{\mathbf{R}_X}. \quad (7)$$

As shown in Hadoon et al. (2004), κ controls the flexibility of the mapping from the input dataset to the canonical variate. This parameter penalizes the norm of the weight values (see Hadoon et al., 2004 for detailed derivation); larger values of κ penalize complex mapping to the canonical variate more, resulting in reducing the flexibility of the analysis. By optimizing κ , we can control the balance of the analysis flexibility and the risk of overfitting. The procedure of κ optimization is described in the next section.

The weight matrices in the reduced kernel space were then transformed to the kernel space:

$$\mathbf{W}_{\mathbf{K}_X} = \mathbf{R}_X (\mathbf{R}_X' \mathbf{R}_X)^{-1} \mathbf{W}_{\mathbf{R}_X} \quad (8)$$

$$\mathbf{W}_{\mathbf{K}_Y} = \mathbf{R}_Y (\mathbf{R}_Y' \mathbf{R}_Y)^{-1} \mathbf{W}_{\mathbf{R}_Y} \quad (9)$$

These weight matrices for the kernel space were further transformed to the original data space:

$$\mathbf{W}_X = \mathbf{X} \mathbf{W}_{\mathbf{K}_X} \quad (10)$$

$$\mathbf{W}_Y = \mathbf{Y} \mathbf{W}_{\mathbf{K}_Y}. \quad (11)$$

With these weight matrices, the canonical variates U and V were defined as:

$$\mathbf{U}=\mathbf{X}\mathbf{W}_x \quad (12)$$

$$\mathbf{V}=\mathbf{Y}\mathbf{W}_y \quad (13)$$

In the current analysis, we used seven explaining variables for $\mathbf{X}$; age, FSIQ, diagnosis (ASD = +1, TD = -1), and their interactions of diagnosis-by-age, diagnosis-by-FSIQ, age-by-FSIQ, and diagnosis-by-age-by-FSIQ. Age and FSIQ variables were normalized to zero mean and unit variance. Interaction terms were made by multiplying the variables. Cortical thickness values were also normalized at each vertex across participants.

While we used a linear kernel for both $\mathbf{X}$ and $\mathbf{Y}$ in equation (4) and (5), KCCA can find nonlinear canonical correlations when a nonlinear kernel is used. We have tested quadratic polynomial kernels for $\mathbf{X}$, but no canonical correlations were statistically significant in the permutation test described in the next section. This suggests that a higher-order kernel was prone to overfit and the sample size was not enough to evaluate higher-order canonical correlations, or a linear kernel was enough for the current dataset. In fact, Shaw et al. (2008) show that most brain regions across the cortex demonstrate linear changes during typical adolescent development.

Optimization of the regularization parameter

The regularization parameter κ was optimized by the permutation test. We randomly permuted sample combinations between $\mathbf{X}$ and $\mathbf{Y}$, then KCCA was applied to the permuted datasets. Repeating this procedure gave us an empirical null distribution of the canonical correlations. The significance of the canonical correlation was evaluated with the Wilks' lambda (Krzanowski, 2000) for taking all orders of canonical correlations into account. Wilks' lambda is defined as:

$$\lambda_i = \sum_{k=i}^D (1 - \rho_k^2), \quad (14)$$

where ρ_k is a k th canonical correlation and D is the number of canonical correlations. For a given κ , false positive rate of λ_1 was estimated by the permutation test with 500 repetitions. The optimal value of κ that returns the most significant λ_1 was searched with the line-search (Nocedal and Wright, 2006) between 0 and 1.

Statistical thresholding the canonical correlation

Seven canonical correlations were evaluated by the KCCA for the current dataset. We used the permutation test to discard non-significant canonical variates. The same procedures of the permutation test described above were repeated 10,000 times with a fixed regularization parameter, κ , optimized in the previous step. The false positive rate (p value) for each λ_i was estimated. We took the canonical correlations until the p value was less than the threshold of $p < 0.05$.

Rotating the canonical variate space

Results of the canonical correlation analysis are usually difficult to interpret since the complex effects of multiple variables are distributed across multiple canonical variates. For clear interpretation of the KCCA results, we rotated the extracted canonical variate space using the varimax rotation (Kaiser, 1958). This method rotates the loading matrix to maximize the sum of variances of squared loading:

$$\sum_{j=1}^m \sum_{i=1}^D (\Lambda \mathbf{R})_{ij}^4 - \frac{1}{D} \sum_{j=1}^m \left(\sum_{i=1}^D (\Lambda \mathbf{R})_{ij}^2 \right)^2, \quad (15)$$

where Λ is a D by m loading matrix (D : dimensionality of original data, m : number of canonical correlations after thresholding). $\mathbf{R}$ is an m by D rotation matrix. We applied the varimax rotation to the correlation matrix between the canonical variates $\mathbf{V}$ in equation (13) and the explaining variables $\mathbf{X}$ to find a multivariate pattern of cortical thickness that is correlated with a specific explaining variable. That is, we used a correlation matrix between $\mathbf{V}$ and $\mathbf{X}$ as the loading matrix Λ in equation (15). This rotation makes each canonical variate related to a specific explaining variable. It should be noted that the varimax rotation is an orthogonal rotation, so that mutual orthogonality across canonical variates is kept after rotation. We used the *rotatefactors* function in the Statistics Toolbox of MATLAB (MathWorks, Natick, MA). The rotation $\mathbf{R}$ evaluated with the varimax was applied for $\mathbf{W}_X$ and $\mathbf{W}_Y$ in equations (10) and (11) to obtain the rotated canonical variates:

$$\mathbf{U}_R = \mathbf{X} \mathbf{W}_X \mathbf{R} \quad (16)$$

$$\mathbf{V}_R = \mathbf{Y} \mathbf{W}_Y \mathbf{R} \quad (17)$$

In the results, we focused on the canonical variates $\mathbf{V}_R$ as a pattern of cortical thickness, each of which is correlated with a specific explaining variable.

Results

The optimal regularization parameter was almost 1.00 ($1.00 - 7.45e^{-9}$). This indicates that we need strong regularization for the best generalized result. In fact, without the regularization parameter all the canonical correlations were 1.0 and none of them were significant with permutation testing.

Table 1 shows the canonical correlations estimated by the KCCA before the varimax rotation. Statistical significance (p values) was evaluated using a permutation test with 10,000 repetitions. Seven canonical correlations were extracted with the seven explaining variables. All the canonical variates were significant ($p < 0.05$). We used these seven variables to make a canonical variate space $\mathbf{V}$ in equation (13), and then rotated it with the varimax to make $\mathbf{V}_R$ in the equation (17).

Table 2 shows percentages of cortical thickness variance explained by the explaining variables via canonical variates. Each percentage (Sx_i) was evaluated with

$Sx_i = \sum_k S v_k \text{corr}(\mathbf{V}_k, \mathbf{x}_i)^2 * 100$, where $\text{corr}(a, b)$ is a correlation between a and b . $S v_k$ is a ratio of cortical thickness variance explained by the canonical variate $\mathbf{V}_k$, which was evaluated

with $S v_k = \sum_j \text{corr}(\mathbf{V}_k, \mathbf{Y}_j)^2 / SST$, where SST is a sum of squared variance of the cortical thickness, which was equal to the number of vertex since all $\mathbf{Y}$ values were normalized. As seen in the table, age had the largest effect on cortical thickness, and the second largest was diagnosis. The main effect of IQ was not as large as those of age and diagnosis; however, its interactions with diagnosis explained more variance than its main effect.

Figures 1 to 7 show the characteristics of each of the rotated canonical variates. In these figures, the relationship between the canonical variate and an explaining variable that was

maximally correlated with the canonical variate are shown in A. Panel B shows the plot of correlations between the canonical variate and all explaining variables. Figures 1B to 7B show that each canonical variate was correlated with each of the explaining variables.

To see which brain regions were related to the canonical variate, correlation values between the canonical variate and cortical thickness were mapped onto an inflated surface model of the template brain in Figures 1C to 7C. While we could use the weight matrix (W_YR in equation 17) as a measure of the relationship, correlation values allowed us to evaluate their relationship with a simple statistical method. This map was thresholded at each vertex ($p < 0.01$) and then thresholded with multiple comparison correction for cluster size ($p < 0.01$). For the cluster size thresholding we used the null distribution of cluster size that was estimated with 10000 iterations of Monte Carlo simulation. The threshold was chosen to be the same as that in the previous study using the same dataset (Wallace et al. 2010), allowing the results to be compared.

To help to interpret the meaning of the canonical variate, actual cortical thickness was plotted against the explaining variable for three representative regions located at a local peak of the map in Figures 1D to 7D. In these graphs, the cortical thickness values were average cortical thicknesses in a circle of 7.5 mm radius centered at the arrowed location in panel C. It should be noted that these plots show the univariate effect in each brain region, so that their results are not necessarily as significant as the multivariate results shown in panel A. While we show the R^2 values to indicate how the data aligned on the fitted line, these figures are shown only for helping interpret the meaning of the canonical variate.

Figure 1 shows the age-related canonical variate. Cortical thinning with age was seen in many areas, including right prefrontal cortex and medial regions of the frontal to occipital cortices. Age-related cortical thickening was seen only in anterior temporal regions.

Figure 2 shows the canonical variates correlated with full-scale IQ (FSIQ) scores. Cortical thickening with FSIQ was seen in the orbitofrontal, postcentral, right precentral, and left medial superior frontal regions. Thinning with higher IQ was seen in the insular and anterior temporal regions.

Figure 3 shows the canonical variate correlated with diagnosis. ASD-related cortical thinning was seen in broad regions specifically in the parietal, superior and ventral temporal, superior frontal, and medial regions extending from the frontal to parietal areas.

Figure 4 shows the canonical variate correlated with the two-way interaction of diagnosis by age. This canonical variate was negatively related with age for typically developing participants, but positively related for participants with ASD. This interaction effect was seen in the left prefrontal, right precentral, and right parietal regions. In these regions, cortical thickness was thinning with age in the TD group, but no change was seen in the ASD group; plots of cortical thickness against age (Fig. 4D-b and 4D-c) revealed thicker cortex for TD than ASD participants at younger ages, but the difference was reduced at older ages due to cortical thinning in the TD group with increasing age. An interaction in the opposite direction was seen in the ventral temporal areas including the fusiform and parahippocampal regions. In these regions, there was cortical thinning with age in the ASD group but there was no age-related change in the TD group. As seen in Figure 4D-a, ASD participants had similar cortical thickness values to those of the TD group at younger ages but there was thinning with age in the ASD participants, resulting in thinner cortex for this group at older ages.

Figure 5 shows the canonical variate correlated with the two-way interaction of diagnosis by FSIQ. This canonical variate was negatively related to FSIQ for TD participants, but

positively related for participants with ASD (Fig. 5A). This interaction was seen in the postcentral, superior temporal, and orbitofrontal regions. As only negative correlations between this canonical variate and cortical thickness were seen on the map (Fig. 5C), the actual pattern of cortical thickness had the opposite direction; positive relationships between FSIQ and cortical thickness were seen for the TD group in these regions, whereas no or negative relationships were found in the ASD group (Fig. 5D).

Figure 6 shows the canonical variate correlated with the two-way interaction of age by FSIQ. To present this interaction in a comprehensive way, we divided the participants into an average/high-IQ group ($FSIQ \leq 120$) and a superior-IQ group ($FSIQ > 120$) in Figure 6A and 6D. This distinction was determined from the results of Shaw et al. (2006), which reports that typically developing individuals with superior-IQ (> 120) had distinct and regionally-specific developmental trajectories of cortical thickness compared to individuals with average or high IQ.

This canonical variate was negatively related with age in the average/high-IQ (A/H IQ) group but positively related with age in the superior-IQ (Sup IQ) group for both TD and ASD participants. Plots of actual cortical thickness (Fig. 6D) show that the left fusiform gyrus is thinning with age in the A/H-IQ group, while it does not change with age in the Sup-IQ group. In the superior paracentral, right pericalcarine, and right lateral occipital regions, cortical thickness in the Sup-IQ group is thinning with increasing age while in the A/H-IQ group it does not change with age.

Figure 7 shows the canonical variate correlated with the three-way interaction of diagnosis by age by FSIQ. This canonical variate was positively related with age for the A/H-IQ group but negatively related for the Sup-IQ group in TD participants (Fig. 7A left). This pattern of interaction was reversed for ASD participants (Fig. 7A right). A wide range of regions contributed to this interaction including frontal, parietal, and occipital areas. In these regions, cortical thickness was negatively related to age for the Sup-IQ group, but no age effect was seen for the A/H-IQ group among TD participants (Fig. 7Db and 7D-c). For ASD participants, the relationship with age was not seen in most of these regions except in the right precuneus, where cortical thickness was negatively related to age for the A/H-IQ group (Fig. 7D-c). The opposite interaction pattern was seen in the anterior cingulate region, in which cortical thickness was positively related to age for the Sup-IQ group of TD individuals and negatively related to age for the Sup-IQ group of ASD participants (Fig. 7D-a).

Discussion

Kernel canonical correlation analysis (KCCA) with varimax rotation revealed patterns of cortical thickness correlating with age, IQ, diagnosis, and their interactions. In contrast to the mass-univariate approach, in which each brain region is analyzed as independent data, and the multivariate classification analysis, in which multiple brain regions are related to a univariate measure (class label), KCCA could find effects of multiple factors on multivariate patterns of cortical thickness.

When examining cortical thickness differences between ASD and TD participants and group by age interactions, KCCA revealed differences of greater magnitude and extent than our earlier study using univariate analyses (Wallace et al., 2010). As seen in Figures 1A to 7A, canonical variables had greater power to detect differences than univariate statistics at any single locations, indicating that this multivariate approach provides higher sensitivity than the more commonly used univariate analyses.

High sensitivity of multivariate analysis, however, also means increased risk of false positive findings. To confirm the robustness of the current results, we used permutation testing. Furthermore in the following sections, to examine the validity of the current results, we compare them with previous studies investigating cortical thickness during adolescence using univariate analyses and consider the meaning of the multivariate analysis results.

Canonical variate as a pattern of cortical thickness

Before we evaluate the current results compared with previous reports, we should note the meaning of the canonical variate as a pattern of cortical thickness. Canonical correlation analysis finds a correlation between linear combinations (patterns) of multivariate datasets. That is, the canonical correlation is not a summary of correlations between each of a set of single variables in the datasets but a correlation between multivariate patterns. In the current analysis, while one explaining variable corresponded to one of the canonical variates because we rotated the weight matrices to do so, one canonical variate represented a pattern of multiple regions' cortical thicknesses. The maps in Figure 1C to 7C do not indicate a direct relationship between cortical thickness and the explaining variable. A combination of cortical thicknesses in these regions was correlated with the explaining variable.

In some regions, raw cortical thickness values (Figs. 1D to 7D) can be seen as inconsistent with a multivariate effect; for example, Figure 2Da indicates an interaction of IQ with diagnosis, while this is shown as a region for the main effect of IQ. These results, however, do not contradict each other considering that the plots of Figures 1D to 7D show a univariate effect in each region. Since one region could be affected by multiple factors, the interaction effect can be seen as mixed with the main effect in the univariate plots. In the canonical variates, in contrast, multiple factors are orthogonalized with each other.

While in the following discussion we evaluate effects of explaining variables on cortical thickness at various brain locations, we do not claim each region in the results is independently related to the explaining variable like in a univariate analysis. All regions in the map have to be combined together to achieve a high canonical correlation. We should remember our results show multivariate patterns of cortical thickness correlated with explaining variables.

Age and IQ effects on the cortical thickness

The age-related canonical variate (Fig. 1) showed cortical thinning with age in many areas of the brain, and thickening in anterior temporal regions. These results are consistent with previous studies investigating human cortical development (Gogtay et al., 2004; Raznahan et al., 2011; Salat et al., 2004; Shaw et al., 2008; Sowell et al., 2003, for review, see Giedd and Rapoport, 2010). Shaw et al., (2008) showed that cortical thickness in most brain regions decreases with age during adolescence, and that some regions of the brain, including anterior portions of the superior temporal gyrus, has a late developmental peak (around 18 years of age), so that cortical thickness in this region continues to thicken in adolescence. The consistency between our results and these earlier studies supports the validity of the current analysis method.

The IQ related cortical thickness pattern, however, was different from the previous report by Shaw et al. (2006). They found positive correlations of cortical thickness with IQ in prefrontal and inferior parietal regions during adolescence. In contrast, our results (Fig. 2) showed that only portions of the previously implicated regions were related to IQ scores. This difference is likely due to the nature of the canonical correlation analysis. The CCA extracts multiple effects simultaneously with orthogonalization across them. Thus, the variance explained by one canonical variate is excluded from the effect of the other

canonical variates. In the current case, the variances in most of the dorsal frontal areas were explained by the three-way interaction of age, IQ, and diagnosis (Fig. 7); therefore, an independent effect of IQ was not seen in these regions.

The mutual orthogonality between the canonical variates also has a preferable effect in evaluating the results; the ASD-related effects can be evaluated free from the general effects of age and IQ.

Characteristic cortical thickness pattern for ASD

For the ASD-related effects, we compared the current results with those of Wallace et al. (2010), in which a univariate analysis was applied to the same dataset. The regions with ASD-related cortical thinning found with the multivariate analysis (Fig. 3) overlapped with the regions found with a univariate analysis in Wallace et al. (2010) (see Fig. 1 in Wallace et al., 2010). While both studies pinpointed overlapping regions of group differences, the multivariate analysis extracted larger regions than those found using the univariate analysis. The regions in the map, however, did not necessarily have a significant correlation in the univariate analysis. All regions on the map worked together as a pattern and this pattern was correlated with diagnosis. Therefore, previous studies reporting localized differences in ASD samples using the mass-univariate approach (e.g. Chung et al. 2005; Scheel et al., 2011) do not necessarily contradict the current results.

The interaction of age and diagnosis was also consistent with the univariate analysis in Wallace et al. (2010). They found greater age-related cortical thinning in the left fusiform area of ASD participants. This thinning was included in the pattern map of the CCA. Wallace et al. (2010) also showed that cortical thickness in the right parietal region is thinner for ASD than for TD participants at younger ages (≤ 17 years) but the difference is diminished at older ages (> 17 years). This interaction effect was also found in the current analysis, which can be seen in the red regions of Figure 4C and in Figure 4D-c. Scheel et al. (2011) also found a consistent age-by-diagnosis interaction in the right parietal and precentral regions as well as in the left parietal region with a univariate analysis, suggesting validity of this interaction effect.

In addition to the aforementioned regions, right ventral temporal/parahippocampal and left prefrontal regions were included in the pattern of cortical thickness. While the effects of ASD on these regions were not significant with a univariate analysis, they contributed significantly to the canonical variate correlating with the age-by-diagnosis interaction. This result indicates that the age-related changes in all of these regions follow a similar pattern, which differentiates the ASD group from the TD group.

Interaction of IQ effect with ASD

The pattern of cortical thickness correlating with the interaction of IQ by diagnosis included the orbitofrontal, postcentral, superior temporal, and ventral occipital regions (Fig. 5C). In these regions, cortical thickness was positively correlated with IQ for the TD participants, whereas no or negative relations were seen for the ASD participants. These regions partly overlap with the results of Shaw et al. (2006) for TD participants in early adulthood (17–29 yr). Considering these regions survived after excluding the interaction effects of age-by-IQ and age-by-IQ-by-diagnosis, this result suggests that the positive IQ effect in these regions did not interact with age and remains until early adulthood in typical development. The current analysis also showed that this IQ effect was not seen within the ASD group.

Shaw et al. (2006) also found an interaction of age and IQ affecting cortical development among TD participants. Their results show that the peak age of cortical thickening is delayed for the Sup-IQ (> 120) group (around 12 years) relative to the A/H-IQ (≤ 120) group (around

9 years) in superior/medial frontal and left middle temporal areas, resulting in thicker cortex in these regions for Sup-IQ in 12 year olds. This difference is transient as the Sup-IQ group displays greater cortical thinning during adolescence.

In our results, the age by IQ interaction was seen only in small regions of superior precentral and right occipital areas (Fig. 6). Most of the regions indicated by Shaw et al. (2006) overlapped with our three-way interaction findings as depicted in Figure 7C. This three-way interaction indicates that the age by IQ interaction effect on cortical thickness was different between TD and ASD participants.

The three-way interaction between diagnosis, age, and IQ (Fig. 7) revealed enhanced thinning for the Sup-IQ group of TD participants, which was consistent with results from Shaw et al. (2006). The ASD group did not display this age by IQ interaction, but an opposite pattern was seen in the right precuneus; the A/H-IQ ASD group had enhanced cortical thinning with age in this region. Also found was cortical thickening for the Sup-IQ group of TD participants and thinning for the Sup-IQ group of ASD individuals in the left anterior cingulate (Fig. 7D-a). These regions comprise the pattern of cortical thickness characterizing the difference of age by IQ interaction effects between TD and ASD participants.

Conclusion

The use of a multivariate approach, kernel canonical correlation analysis, revealed patterns and correlates of cortical thickness in ASD that were not detected as sensitively using univariate methods. The consistency of the current results with prior studies supports the validity of the current analytic method. In addition, our results suggest differing IQ-cortical thickness relationships between ASD and TD groups, which has not been previously reported. This finding is suggestive of independent associations between intelligence and brain structure in ASD versus typically developing controls.

The current results, however, were limited to male adolescents with relatively high IQ scores. We cannot generalize these findings to females, other age ranges, and individuals with both ASD and intellectual disabilities. Although the limitation of sample coverage restricts the generalization of our findings, the KCCA method itself has potential to reveal more complex relationships between wide-ranging variables. With datasets of relatively large size and wide-coverage (e.g., in terms of age and/or IQ), the potential of the multivariate analysis will be demonstrated more prominently.

Acknowledgments

This study was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Mental Health (NIMH).

References

- Amaral DG, Schumann CM, Nordahl CW. Neuroanatomy of autism. *Trends in Neurosciences*. 2008; 31:137–145. [PubMed: 18258309]
- American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders DSM-IV-TR Fourth Edition (Text Revision)*. Washington, DC: American Psychiatric Publishing; 2000.
- Bishop, CM. *Pattern Recognition and Machine Learning*. New York: Springer; 2007.
- Chung MK, Robbins SM, Dalton KM, Davidson RJ, Alexander AL, Evans AC. Cortical thickness analysis in autism with heat kernel smoothing. *NeuroImage*. 2005; 25:1256–1265. [PubMed: 15850743]

- Correa NM, Adali T, Yi-Ou L, Calhoun VD. Canonical Correlation Analysis for Data Fusion and Group Inferences. *Signal Processing Magazine, IEEE*. 2010; 27:39–50.
- Courchesne E, Pierce K, Schumann CM, Redcay E, Buckwalter JA, Kennedy DP, Morgan J. Mapping early brain development in autism. *Neuron*. 2007; 56:399–413. [PubMed: 17964254]
- Dale AM, Fischl B, Sereno MI. Cortical surface-based analysis: I Segmentation and surface reconstruction. *NeuroImage*. 1999; 9:179–194. [PubMed: 9931268]
- Ecker C, Rocha-Rego V, Johnston P, Mourao-Miranda J, Marquand A, Daly EM, Brammer MJ, Murphy C, Murphy DG. Investigating the predictive value of whole-brain structural MR scans in autism: A pattern classification approach. *NeuroImage*. 2010; 49:44–56. [PubMed: 19683584]
- Fischl B, Dale AM. Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc Natl Acad Sci U S A*. 2000; 97:11050–11055. [PubMed: 10984517]
- Fischl B, Sereno MI, Dale AM. Cortical surface-based analysis: II. Inflation, flattening, and a surface-based coordinate system. *NeuroImage*. 1999; 9:195–207. [PubMed: 9931269]
- Giedd JN, Rapoport JL. Structural MRI of Pediatric Brain Development: What Have We Learned and Where Are We Going? *Neuron*. 2010; 67:728–734. [PubMed: 20826305]
- Gogtay N, Giedd JN, Lusk L, Hayashi KM, Greenstein D, Vaituzis AC, Nugent TF, Herman DH, Clasen LS, Toga AW, Rapoport JL, Thompson PM. Dynamic mapping of human cortical development during childhood through early adulthood. *Proceedings of the National Academy of Sciences of the United States of America*. 2004; 101:8174–8179. [PubMed: 15148381]
- Hadjikhani N, Joseph RM, Snyder J, Tager-Flusberg H. Anatomical differences in the mirror neuron system and social cognition network in autism. *Cerebral Cortex*. 2006; 16:1276–1282. [PubMed: 16306324]
- Han X, Jovicich J, Salat D, van der Kouwe A, Quinn B, Czanner S, Busa E, Pacheco J, Albert M, Killiany R, Maguire P, Rosas D, Makris N, Dale A, Dickerson B, Fischl B. Reliability of MRI-derived measurements of human cerebral cortical thickness: the effects of field strength, scanner upgrade and manufacturer. *NeuroImage*. 2006; 32:180–194. [PubMed: 16651008]
- Hardan AY, Libove RA, Keshavan MS, Melhem NM, Minshew NJ. A preliminary longitudinal magnetic resonance imaging study of brain volume and cortical thickness in autism. *Biological Psychiatry*. 2009; 66:320–326. [PubMed: 19520362]
- Hardan AY, Muddasani S, Vemulapalli M, Keshavan MS, Minshew NJ. An MRI study of increased cortical thickness in autism. *American Journal of Psychiatry*. 2006; 163:1290–1292. [PubMed: 16816240]
- Hardoon DR, Ettinger U, Mourao-Miranda J, Antonova E, Collier D, Kumari V, Williams SCR, Brammer M. Correlation-based multivariate analysis of genetic influence on brain volume. *Neuroscience Letters*. 2009; 450:281–286. [PubMed: 19028548]
- Hardoon DR, Szedmak S, Shawe-Taylor J. Canonical correlation analysis: An overview with application to learning methods. *Neural Computation*. 2004; 16:2639–2664. [PubMed: 15516276]
- Hotelling H. Relations between two sets of variants. *Biometrika*. 1936; 28:321–377.
- Jiao Y, Chen R, Ke X, Chu K, Lu Z, Herskovits EH. Predictive models of autism spectrum disorder based on brain regional cortical thickness. *NeuroImage*. 2010
- Kaiser HF. The varimax criterion for analytic rotation in factor analysis. *Psychometrika*. 1958; 23:187–200.
- Krzanowski, WJ. *Principles of multivariate analysis : a user's perspective*. Oxford University Press; 2000.
- Kuperberg GR, Broome MR, McGuire PK, David AS, Eddy M, Ozawa F, Goff D, West WC, Williams SCR, Van der Kouwe AJW, Salat DH, Dale AM, Fischl B. Regionally localized thinning of the cerebral cortex in schizophrenia. *Archives of General Psychiatry*. 2003; 60:878–888. [PubMed: 12963669]
- Le Couteur A, Rutter M, Lord C, Rios P, Robertson S, Holdgrafer M, McLennan J. Autism diagnostic interview: A standardized investigator-based instrument. *Journal of Autism and Developmental Disorders*. 1989; 19:363–387. [PubMed: 2793783]
- Lord C, Rutter M, Couteur AL. Autism diagnostic interview-revised: A revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *Journal of Autism and Developmental Disorders*. 1994; 24:659–685. [PubMed: 7814313]

- Lord C, Risi S, Lambrecht L, Cook EH Jr, Leventhal BL, Dilavore PC, Pickles A, Rutter M. The Autism Diagnostic Observation Schedule-Generic: A standard measure of social and communication deficits associated with the spectrum of autism. *Journal of Autism and Developmental Disorders*. 2000; 30:205–223. [PubMed: 11055457]
- Narr KL, Woods RP, Thompson PM, Szeszkó P, Robinson D, Dimtcheva T, Gurbani M, Toga AW, Bilder RM. Relationships between IQ and regional cortical gray matter thickness in healthy adults. *Cerebral Cortex*. 2007; 17:2163–2171. [PubMed: 17118969]
- Nocedal, J.; Wright, S. Numerical Optimization (Springer Series in Operations Research and Financial Engineering). Springer; 2006.
- Raznahan A, Shaw P, Lalonde F, Stockman M, Wallace GL, Greenstein D, Clasen L, Gogtay N, Giedd JN. How does your cortex grow? *Journal of Neuroscience*. 2011; 31:7174–7177. [PubMed: 21562281]
- Raznahan A, Toro R, Daly E, Robertson D, Murphy C, Deeley Q, Bolton PF, Paus T, Murphy DGM. Cortical anatomy in autism spectrum disorder: An in vivo MRI study on the effect of age. *Cerebral Cortex*. 2010; 20:1332–1340. [PubMed: 19819933]
- Redcay E, Courchesne E. When is the brain enlarged in autism? A meta-analysis of all brain size reports. *Biol Psychiatry*. 2005; 58:1–9. [PubMed: 15935993]
- Rosas HD, Liu AK, Hersch S, Glessner M, Ferrante RJ, Salat DH, Van Der Kouwe A, Jenkins BG, Dale AM, Fischl B. Regional and progressive thinning of the cortical ribbon in Huntington's disease. *Neurology*. 2002; 58:695–701. [PubMed: 11889230]
- Salat DH, Buckner RL, Snyder AZ, Greve DN, Desikan RSR, Busa E, Morris JC, Dale AM, Fischl B. Thinning of the cerebral cortex in aging. *Cerebral Cortex*. 2004; 14:721–730. [PubMed: 15054051]
- Scheel C, Rotarska-Jagiela A, Schilbach L, Lehnhardt FG, Krug B, Vogeley K, Tepest R. Imaging derived cortical thickness reduction in high-functioning autism: Key regions and temporal slope. *NeuroImage*. 2011; 58:391–400. [PubMed: 21749926]
- Shaw P, Greenstein D, Lerch J, Clasen L, Lenroot R, Gogtay N, Evans A, Rapoport J, Giedd J. Intellectual ability and cortical development in children and adolescents. *Nature*. 2006; 440:676–679. [PubMed: 16572172]
- Shaw P, Kabani NJ, Lerch JP, Eckstrand K, Lenroot R, Gogtay N, Greenstein D, Clasen L, Evans A, Rapoport JL, Giedd JN, Wise SP. Neurodevelopmental trajectories of the human cerebral cortex. *Journal of Neuroscience*. 2008; 28:3586–3594. [PubMed: 18385317]
- Sowell ER, Peterson BS, Thompson PM, Welcome SE, Henkenius AL, Toga AW. Mapping cortical change across the human life span. *Nature Neuroscience*. 2003; 6:309–315.
- Sowell ER, Thompson PM, Leonard CM, Welcome SE, Kan E, Toga AW. Longitudinal mapping of cortical thickness and brain growth in normal children. *Journal of Neuroscience*. 2004; 24:8223–8231. [PubMed: 15385605]
- Wallace GL, Dankner N, Kenworthy L, Giedd JN, Martin A. Age-related temporal and parietal cortical thinning in autism spectrum disorders. *Brain*. 2010; 133:3745–3754. [PubMed: 20926367]
- Wilke M, Sohn JH, Byars AW, Holland SK. Bright spots: Correlations of gray matter volume with IQ in a normal pediatric population. *NeuroImage*. 2003; 20:202–215. [PubMed: 14527581]

Figure 1.

Characteristics of the age-related canonical variate. A) Plot of canonical variate against age. Black squares represent typically developing (TD) participants and red circles represent participants with autism spectrum disorders (ASD). Linear regression was performed for each of the TD and ASD groups. The R^2 value indicates a ratio of total variance explained by a fitted line (*: $p < 0.05$, ‡ : $p < 0.01$, ‡ : $p < 0.001$). B) Pearson correlations between the canonical variate and explaining variables. C) Map of Pearson correlations between the canonical variate and cortical thickness at each vertex on an inflated surface model of the template brain. The first two columns present data from the left hemisphere while the latter two columns present data from the right hemisphere. The map was thresholded at each vertex ($p < 0.01$) and then thresholded with multiple comparison correction for cluster size ($p < 0.01$). D) Plots of the cortical thicknesses at three representative regions located at a

peak of the correlation map in C. Average cortical thicknesses in a circle of 7.5-mm radius were plotted against the age. Center of the circle region is indicated by arrow in C.

Figure 2.

Characteristics of the canonical variate correlated with full-scale IQ (FSIQ). Conventions are the same as in Figure 1.

Figure 3.

Characteristics of the canonical variate correlated with diagnosis of autism spectrum disorders (ASD). Conventions are the same as in Figure 1. The results of a two-sample t-test between the typically developing (TD) and ASD groups are shown in A and D.

Figure 4. Characteristics of the canonical variate correlated with the interaction between diagnosis and age. Conventions are the same as in Figure 1.

Figure 5. Characteristics of the canonical variate correlated with the interaction between diagnosis and FSIQ. Conventions are the same as in Figure 1.

Figure 6.

Characteristics of the canonical variate correlated with the interaction between age and FSIQ. The canonical variate and cortical thickness were plotted against age for each of the TD (left panel with black squares) and ASD (right panel with red circles) groups (panels A and D). The filled symbols represent average/high-IQ (≤ 120) participants (A/H IQ) and the blank symbols represent superior IQ (> 120) participants (Sup IQ). Other conventions are the same as in Figure 1.

Figure 7.
 Characteristics of the canonical variate correlated with the interaction between diagnosis, age, and FSIQ. Conventions are the same as in Figure 1 and 6.

Table 1

Canonical correlations between the whole-brain cortical thickness and the explaining variables of age, full-scale IQ (FSIQ), autism diagnosis, and interactions of diagnosis by age, diagnosis by FSIQ, age by FSIQ, and diagnosis by age by FSIQ. p values were estimated with the permutation test.

Order of canonical correlation	Canonical correlation	p value
1	0.8247	0.0124
2	0.8071	0.0072
3	0.8059	0.0056
4	0.7456	0.0103
5	0.7449	0.0026
6	0.7268	0.0011
7	0.5601	0.0059

Table 2

Percentages of cortical thickness variance explained by the explaining variables via canonical variates.

Explaining variable	Explained variance of cortical thickness (%)
Age	5.692
Full-scale IQ (FSIQ)	2.071
Autism diagnosis	4.892
Diagnosis $\times$ Age	1.880
Diagnosis $\times$ FSIQ	3.215
Age $\times$ FSIQ	1.411
Diagnosis $\times$ Age $\times$ FSIQ	3.226
Total	22.389