ORIGINAL ARTICLE

Reduced Acetylcholinesterase Activity in the Fusiform Gyrus in Adults With Autism Spectrum Disorders

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Context: Both neuropsychological and functional magnetic resonance imaging studies have shown deficiencies in face perception in subjects with autism spectrum disorders (ASD). The fusiform gyrus has been regarded as the key structure in face perception. The cholinergic system is known to regulate the function of the visual pathway, including the fusiform gyrus.

Objectives: To determine whether central acetylcholinesterase activity, a marker for the cholinergic system, is altered in ASD and whether the alteration in acetylcholinesterase activity, if any, is correlated with their social functioning.

Design: Using positron emission tomography and a radiotracer, *N*-[^{11}C]methyl-4-piperidyl acetate ([^{11}C]MP4A), regional cerebrocortical acetylcholinesterase activities were estimated by reference tissue–based linear least-squares analysis and expressed in terms of the rate constant k_3. Current and childhood autism symptoms in the adult subjects with ASD were assessed by the Autism Diagnostic Observation Schedule and the Autism Diagnostic Interview–Revised, respectively. Voxel-based analyses as well as region of interest–based methods were used for between-subject analysis and within-subject correlation analysis with respect to clinical variables.

Setting: Participants recruited from the community.

Participants: Twenty adult subjects with ASD (14 male and 6 female; age range, 18-33 years; mean [SD] intelligence quotient, 91.6 [4.3]) and 20 age-, sex-, and intelligence quotient–matched healthy controls.

Results: Both voxel- and region of interest–based analyses revealed significantly lower [^{11}C]MP4A k_3 values in the bilateral fusiform gyri of subjects with ASD than in those of controls ($P < .05$, corrected). The fusiform k_3 values in subjects with ASD were negatively correlated with their social disabilities as assessed by Autism Diagnostic Observation Schedule as well as Autism Diagnostic Interview–Revised.

Conclusions: The results suggest that a deficit in cholinergic innervations of the fusiform gyrus, which can be observed in adults with ASD, may be related to not only current but also childhood impairment of social functioning.

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AUTISM SPECTRUM DISORDERS (ASD), comprising autistic disorder, Asperger disorder, and pervasive developmental disorder not otherwise specified, are characterized by impairment in both social interaction and communication as well as by restricted or repetitive behaviors and interests.[1] Although individuals with ASD also have significant deficits in face perception,[2,3] this aspect is not included in the current diagnostic criteria.[1] Because of the fundamental role of face processing in social interactions, it has been hypothesized that abnormalities in the neural circuitry involved in face processing contribute to social disabilities in ASD.

It is well recognized from functional magnetic resonance imaging (fMRI) studies that a specific region of the fusiform gyrus called the fusiform face area (FFA) is consistently active during face viewing in typically developing individuals.[4,5] The FFA activity in face processing is known to be dominated by the right hemisphere,[6] and the functional volume of the right FFA, defined by fMRI during face viewing, may increase with age.[7] Most previous fMRI studies have found that subjects with ASD lack FFA activation in response to strangers' faces,[8-10] although subsequent studies have shown that differences in FFA activation between typically developing and autistic people may

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be mediated by task demand,[11,12] familiarity,[13,14] or the amount of time spent fixating on the eyes.[15] The FFA hypofunction, especially in the right hemisphere, that occurs when children and adults with ASD view strangers' faces, is the best-replicated fMRI abnormality.[14,16] This phenomenon may arise from neuropathological abnormalities in the fusiform gyrus in ASD; in a recent postmortem study by van Kooten et al,[17] compared with controls, patients with autism showed significantly lower neuron densities in layer III, total neuron numbers in layers III, V, and VI, and mean perikaryal volumes of neurons in layers V and VI in the fusiform gyrus.

Several neurotransmitters including acetylcholine, dopamine, noradrenaline, and serotonin have been found to play important roles in cortical activity.[18,19] In the visual cortex, acetylcholine makes the greatest contribution to the biophysical properties of the neurons and synaptic efficacy, although the involvement of noradrenaline and serotonin is also implicated.[19] Evidence of the critical effect of acetylcholine on fusiform activity has been derived from the results of fMRI and positron emission tomography (PET) studies, which have demonstrated that pharmacological manipulation of cholinergic activity can alter the function of the fusiform gyrus; scopolamine reduced fusiform activity in individuals who performed a long-term encoding task,[20-23] while cholinergic enhancement by the cholinesterase inhibitor physostigmine augmented the relative neuronal response in the middle fusiform gyrus during emotional processing.[24] These findings suggest that abnormalities in cholinergic function could occur in the fusiform gyrus in individuals with ASD and that such abnormalities would be associated with social disability. To test this hypothesis, we assessed acetylcholinesterase (AChE) activity, a marker for the central cholinergic system,[25] in adult individuals with ASD and age- and sex-matched controls by PET and the radioactive tracer *N*-[^{11}C]methylpiperidin-4-yl acetate ([^{11}C]MP4A), which is an analog of acetylcholine and is selectively hydrolyzed by AChE.[26,27] Furthermore, we examined the clinico-biomarker relationship by comparing clinical variables with regional [^{11}C]MP4A PET data in subjects with ASD. Because previous studies describe right-hemisphere dominance in fusiform hypofunction during face processing[14,16] and unaltered choline acetyltransferase activities in the postmortem parietal and frontal cortices[28] in autism, we predicted that abnormalities in AChE activity measured by [^{11}C]MP4A PET would be less prominent or not altered in the cerebral cortex, other than the right-hemisphere fusiform gyrus, in subjects with ASD.

METHODS

SUBJECTS

Twenty subjects with ASD (14 male and 6 female; mean [SD] age, 23.5 [4.3] years; age range, 18-33 years) and 20 age- and sex-matched control subjects (14 male and 6 female; mean [SD] age, 23.1 [4.2] years; age range, 19-34 years) participated in this study. All of the participants were right-handed and had an intelligence quotient greater than 70. None of the participants were tobacco smokers or taking any medication, including psychotropic drugs. All of the subjects with ASD were diagnosed by 2 trained child psychiatrists (K.N. and T.S.) according to the *DSM-IV*.[1] The subjects with ASD did not have any other psychiatric comorbidity disorders, as confirmed by applying the Structured Clinical Interview for *DSM-IV* axis I disorders.[29] In addition, they had no notable dysmorphology, neurocutaneous abnormalities, significant neurologic deficits, history of epileptic seizures, or disorders known to be associated with autism such as fragile X syndrome, neurofibromatosis, or tuberous sclerosis. The Autism Diagnostic Observation Schedule (ADOS)[30] module 4 and Autism Diagnostic Interview-Revised (ADI-R)[31] were used to evaluate current and childhood autism symptoms, respectively, by trained clinicians (K.J.T. and K.M., respectively). Fifteen of 20 subjects with ASD were diagnosed with autistic disorder and remaining 5 were considered to have pervasive developmental disorder not otherwise specified according to the ADOS scores, although all 20 subjects met ADI-R criteria of autism disorder. All control subjects were found to be mentally and physically healthy according to comprehensive assessments of their medical histories and neuropsychiatric examinations. The study was approved by the local ethics committees. Written informed consent was obtained from each of the participants.

MRI AND PET PROCEDURES

As described elsewhere,[32,33] we performed 3-dimensional MRI scans using a 0.3-T MRI unit (model MRP7000AD; Hitachi Medical, Tokyo, Japan) and PET scans with a high-resolution brain PET scanner with the ability to yield 47 PET images simultaneously (model SHR-12000; Hamamatsu Photonics, Shizuoka, Japan). Details in image acquisition and preprocessing procedures are described in the online-only material (eAppendix 1; http://www.archgenpsychiatry.com). The MRI measurements and a mobile PET gantry allowed us to reconstruct PET images parallel to the anterior-posterior intercommissural line without resectioning. Using this approach, we were able to allocate a region of interest (ROI) to the target area of the PET image.[33] Before dynamic PET scanning, a 20-minute transmission scan was performed for attenuation correction using a ^{68}Ge/^{68}Ga source with the participant's head fixed by means of a radiosurgery-purpose thermoplastic face mask. Then, after a bolus intravenous injection of a 380-MBq dose of [^{11}C]MP4A, 32 serial PET scans (time frames, 4×30 seconds, 20×60 seconds, and 8×300 seconds) were performed for 62 minutes. No sedatives were administered during either the MRI or the PET scan.

IMAGING DATA ANALYSIS

Regional cerebrocortical AChE activities were estimated using PMOD 2.95 software (PMOD Technologies, Zurich, Switzerland). Production of parametric k_3 images was based on the reference tissue model designated for [^{11}C]MP4A k_3 value quantification,[34] and the ROI analysis was based on the reference tissue–based linear least-squares method[35] (eAppendix). In brief, a target cortical region and the cerebellum as a reference region were delineated on MRIs from each participant and transferred onto PET images. The regional k_3 value, representing the rate of tracer hydrolysis by AChE, and the R_1 value, which is the delivery of the tracer in the target region relative to the reference and reflects regional cerebral blood flow, were calculated using multilinear regression from time-activity curves from the target and reference regions.[35] The R_1 value is important for ruling out the effect of regional cerebral blood flow on the regional k_3 value. Using the PMOD, whole-brain parametric maps of k_3 and R_1 were generated. We masked extracerebral structures by demarcating cerebral regions on MRIs for further analysis of the k_3 and R_1 parametric maps.

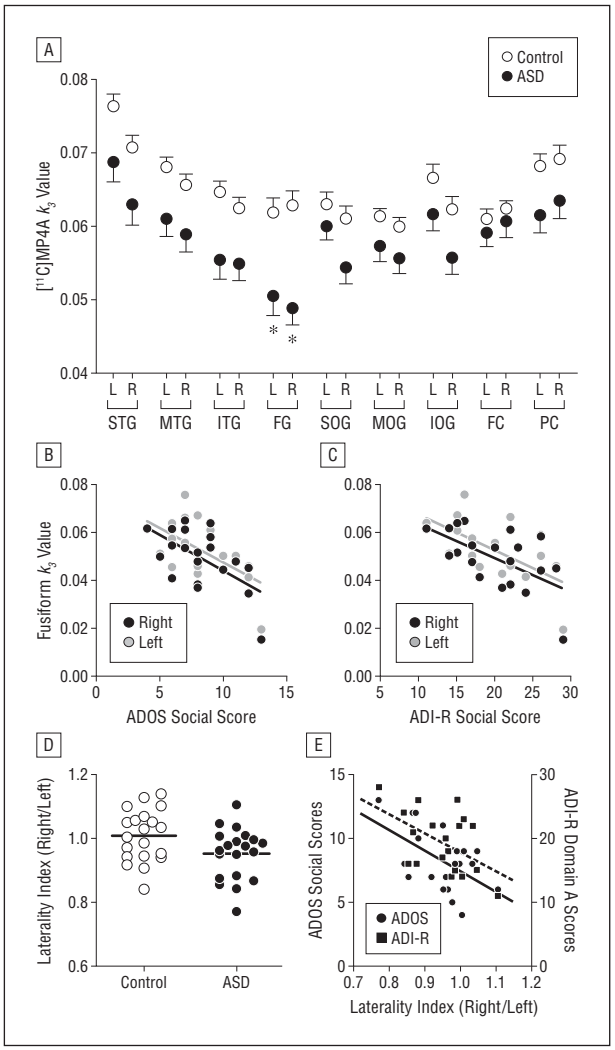

Figure 1. Results of region of interest–based analysis. A, Mean regional brain *N*-[^{11}C]methylpiperidin-4-yl acetate ([^{11}C]MP4A) k_3 values in controls and subjects with autism spectrum disorder (ASD). Subjects with ASD had significantly lower k_3 values in the bilateral fusiform gyri compared with controls. Error bars represent the standard error of the mean. Note the significance ($P<.01$ by post hoc Bonferroni test following 2-way analysis of variance). Correlation between [^{11}C]MP4A k_3 values in the fusiform gyrus and social scores of the ADOS (B) and ADI-R (C) is shown. Values of [^{11}C]MP4A k_3 in both the right and left fusiform gyrus were significantly and negatively correlated with the Autism Diagnostic Observation Schedule (ADOS) social scores (A) as well as the Autism Diagnostic Interview–Revised (ADI-R) domain A scores (B). D, The mean laterality index (right to left ratio) of the fusiform k_3 values in controls and subjects with ASD is shown. E, Correlation between laterality index of fusiform k_3 and social scores of ADOS or ADI-R in subjects with ASD is also shown. ADI-R indicates Autism Diagnostic Interview–Revised; ADOS, Autism Diagnostic Observation Schedule; FC, dorsolateral prefrontal cortex; FG, fusiform gyrus; IOG, inferior occipital gyrus; ITG, inferior temporal gyrus; MOG, middle occipital gyrus; MTG, middle temporal gyrus; PC, parietal cortex (angular gyrus); SOG, superior occipital gyrus; and STG, superior temporal gyrus.

VOXEL-BASED IMAGE ANALYSIS

We performed voxel-based whole-brain analyses using statistical parametric mapping software (SPM5; Wellcome Department of Cognitive Neurology, Institute of Neurology, London, England). In the SPM analysis of [^{11}C]MP4A k_3 and R_1 parametric maps, between-group comparisons were performed to investigate regional differences in each value, using the *t* test for each voxel. The SPM analyses were performed without proportional scaling of k_3 and R_1 values. Correlations between k_3 and R_1 values were examined on a voxel-by-voxel basis using the Biological Parametric Mapping toolbox.[36] The Biological Parametric Mapping toolbox examines any correlation voxelwise between multimodal images that are coregistered and aligned within the same space (ie, Montréal Neurological Institute space). To test the effect of tracer delivery (R_1) on the metabolic rate (k_3), an analysis of covariance was performed using the k_3 map as the primary modality and the corresponding R_1 map as the regressor using the Biological Parametric Mapping toolbox. In addition, we performed exploratory correlation analyses between the regional changes in [^{11}C]MP4A k_3 values and the severity of social disabilities in subjects with ASD. Age and sex were treated as covariates, and the scores on the ADOS and ADIR were considered to be variables of interest. To test hypotheses about the regional specific effects of these variables, the estimates were compared using 2 linear contrasts (positive or negative correlation).

ROI-BASED ANALYSIS

In addition to the voxel-based analysis that is suitable for an exploratory examination of tracer distribution altered in the brain, we performed ROI-based analysis because it enabled us to generate quantitative differences in [^{11}C]MP4A k_3 and R_1 values in specific regions. Manual delineation on individual MRI scans in ROI-based approaches is often biased by the variability between raters and side differences in ROI size, whereby direct case-control comparability is compromised. Therefore, we chose to delineate ROIs by application of a standardized ROI template based on the Anatomical Automated Labeling atlas[37] fitting the Montréal Neurological Institute standard brain. Both the k_3 and R_1 parametric maps were normalized to the Montréal Neurological Institute space by applying a nonlinear iterative algorithm using PMOD software. Then we chose ROIs of 9 brain areas bilaterally including visual processing pathways (the fusiform gyrus, superior, middle, and inferior temporal gyri, and the superior, middle, and inferior occipital gyri), dorsolateral prefrontal cortex (Brodmann area 9), and parietal cortex (angular gyrus, Brodmann area 39). Averaged k_3 and R_1 values for each ROI were obtained. To determine whether there is laterality in the regional k_3 values, we calculated a laterality index (right k_3/left k_3) in bilateral fusiform ROIs in the 2 groups.

STATISTICAL ANALYSIS

Demographic and clinical variables were compared between the ASD and control groups using the unpaired *t* test using statistical software (SPSS version 17J; SPSS Japan Inc, Tokyo, Japan). In the voxel-based analyses, the results were corrected for multiple comparisons of whole-brain analysis at a significance level of $P<.05$ (false discovery rate). The significance level was determined using a voxel-level threshold of $P<.001$. In ROI-based analyses, we tested the main effect of the diagnosis of ASD on [^{11}C]MP4A k_3 or R_1 values derived from 9 brain regions using 2-way analysis of variance followed by post hoc Bonferroni test. We further conducted an analysis of covariance using the k_3 value as the independent variable and the corresponding R_1 value as the covariate in ROIs on the fusiform gyrus, based on the results of the 2-way analysis of variance (**Figure 1**A). In the laterality analysis, an unpaired *t* test was used for the comparison between the 2 groups. Evaluation of relationships between the regional k_3 values from each ROI and ADI-R or ADOS scores among subjects with ASD was performed with the Pearson *r* correlation coefficient. Statistical significance was set at $P<.05$.

RESULTS

The characteristics of all the participants are summarized in **Table 1**. There was no significant difference in the distribution of age or the sex ratio. The difference in intelligence quotient between the 2 groups did not reach statistical significance ($t=1.43$; $P=.16$). In quantitative PET brain imaging, the motion artifact is the important degrading factor. Therefore, we fixed the head of each participant using a thermoplastic face mask, observed participants carefully during each scan, and confirmed that all of the participants had remained immobilized. Another major confounding factor in PET image analysis is the partial volume effect (PVE) that can be observed in measuring small brain structures and lead to an underestimation of tracer activity. The present results were generated without PVE correction. To minimize PVE, we used a high-resolution brain-purpose PET scanner for data acquisition and MRI data for image analysis, the latter of which allowed us to select the brain loci with no extraparenchymal spaces to estimate k_3 value, an index of AChE activity, and R_1, an index of tracer delivery, with reference tissue–based linear least-squares analysis[34] of dynamic [^{11}C]MP4A PET images. When we conducted an additional volumetric brain morphometry study using a 3-T scanner on the participants of the MP4A PET study, there was no significant difference in whole-brain or regional gray matter volumes between subjects with ASD and controls (eAppendix 2, eTable, eFigure 1 and eFigure 2).

VOXEL-BASED WHOLE-BRAIN ANALYSIS

We first obtained parametric maps of k_3 and R_1 values of ASD and control subjects. **Figure 2**A illustrates normalized and averaged [^{11}C]MP4A k_3 parametric maps from control subjects and subjects with ASD. The ASD group showed significant reductions in [^{11}C]MP4A k_3 values in ventral portion of the bilateral temporal lobes compared with the control group (Figure 1B). There was no voxel where [^{11}C]MP4A k_3 values were greater in subjects with ASD than in controls. In contrast, there was no significant difference in R_1 values in the whole brain between groups (eFigure 3 and eFigure 4). Although it was found that R_1 values did not differ significantly between the ASD and control groups, to exclude further a possible adverse effect of the [^{11}C]MP4A delivery on its retention, we conducted an analysis of covariance using the k_3 map as the primary modality and the corresponding R_1 map as the regressor. After controlling the effect of R_1 value, the reduction in [^{11}C]MP4A k_3 values in the ASD group was still significant within the fusiform gyrus bilaterally (Figure 1C; $P<.05$, corrected).

We further examined the possible relationships between [^{11}C]MP4A k_3 values and clinical features in subjects with ASD (**Table 2**). Figure 1D shows a cluster on the fusiform gyrus in which the [^{11}C]MP4A k_3 values were significantly negatively correlated with the ADOS social score ($P<.05$, corrected). Figure 1E indicates a cluster on the fusiform gyrus in which a significantly negative correlation between the [^{11}C]MP4A k_3 values and the ADI-R domain A (social) score was noted ($P<.05$, corrected). Clusters associated with the ADOS social score (Figure 1D) and the ADI-R social score (Figure 1E) were located within the clusters shown in Figure 1C. The other scores in the ADOS and ADI-R did not correlate significantly with [^{11}C]MP4A k_3 values (data not shown).

Table 1. Demographic Characteristics of the Participants

Characteristic	Mean (SD) [Range]	
	Control (n = 20)	ASD (n = 20)
Male:female, No.	14:6	14:6
Age, y	23.1 (4.2) [19-32]	23.5 (4.3) [18-33]
WAIS-III, full IQ	100.5 (19.4) [70-136]	91.6 (19.7) [70-140]
ADOS score		
Social	NA	8.6 (2.3) [5-13]
Communication	NA	4.3 (1.9) [2-8]
Stereotype	NA	0.8 (0.9) [0-3]
ADI-R score, Domain		
A (social)	NA	20.0 (5.2) [11-29]
B (communication)	NA	14.8 (5.0) [9-23]
C (stereotype)	NA	5.2 (2.3) [4-13]

Abbreviations: ADI-R, Autism Diagnostic Interview–Revised; ADOS, Autism Diagnostic Observation Schedule; ASD, autism spectrum disorder; NA, not applicable; WAIS-III, Wechsler Adult Intelligence Scale, 3rd edition.

ROI ANALYSIS

The results of analyses of multiple ROIs are shown in Figure 2. Consistent with the findings derived from the voxel-based analysis, [^{11}C]MP4A k_3 values in the bilateral fusiform gyri in subjects with ASD were significantly lower than the corresponding values in control subjects (Figure 2A; $t=4.91$, $P<.001$ for the right; $t=3.98$, $P=.002$ for the left). There was no difference in [^{11}C]MP4A R_1 values between subjects with ASD and controls in either side of the fusiform gyrus (eFigure 5; $t=1.47$, $P=.15$ for the right; $t=1.66$, $P=.10$ for the left). Analysis of covariance showed that differences in k_3 values between the 2 groups were significant in bilateral fusiform ROIs after controlling the effect of R_1 value ($F_{1,37}=12.51$, $P=.001$ for the right; $F_{1,37}=6.78$, $P=.01$ for the left).

Examination of the correlation between [^{11}C]MP4A k_3 values in the bilateral fusiform gyri and the clinical characteristics revealed that the [^{11}C]MP4A k_3 values were significantly negatively correlated with social scores of both the ADOS (Pearson $r=-0.559$, $P=.009$ for the right; $r=-0.512$, $P=.02$ for the left) and ADI-R ($r=-0.594$, $P=.007$ for the right; $r=-0.572$, $P=.008$ for the left) (Figure 2B for ADOS and Figure 2C for ADI-R). No correlation was found between [^{11}C]MP4A *k3* values in the fusiform gyrus and other scores of the ADOS or the ADI-R (data not shown). Values of [^{11}C]MP4A k_3 in ROIs other than the fusiform gyrus did not correlate significantly with any ADOS or ADIR scores (data not shown).

Results from the laterality analysis of [^{11}C]MP4A k_3 values in the fusiform gyrus are shown in Figure 2, D and E. The group mean of the laterality index, a right to left ratio of the k_3 value, in subjects with ASD was significantly lower than that of controls ($t=2.21$; $P=.03$). The laterality index was weakly but significantly and negatively correlated with ADOS social scores (Pearson

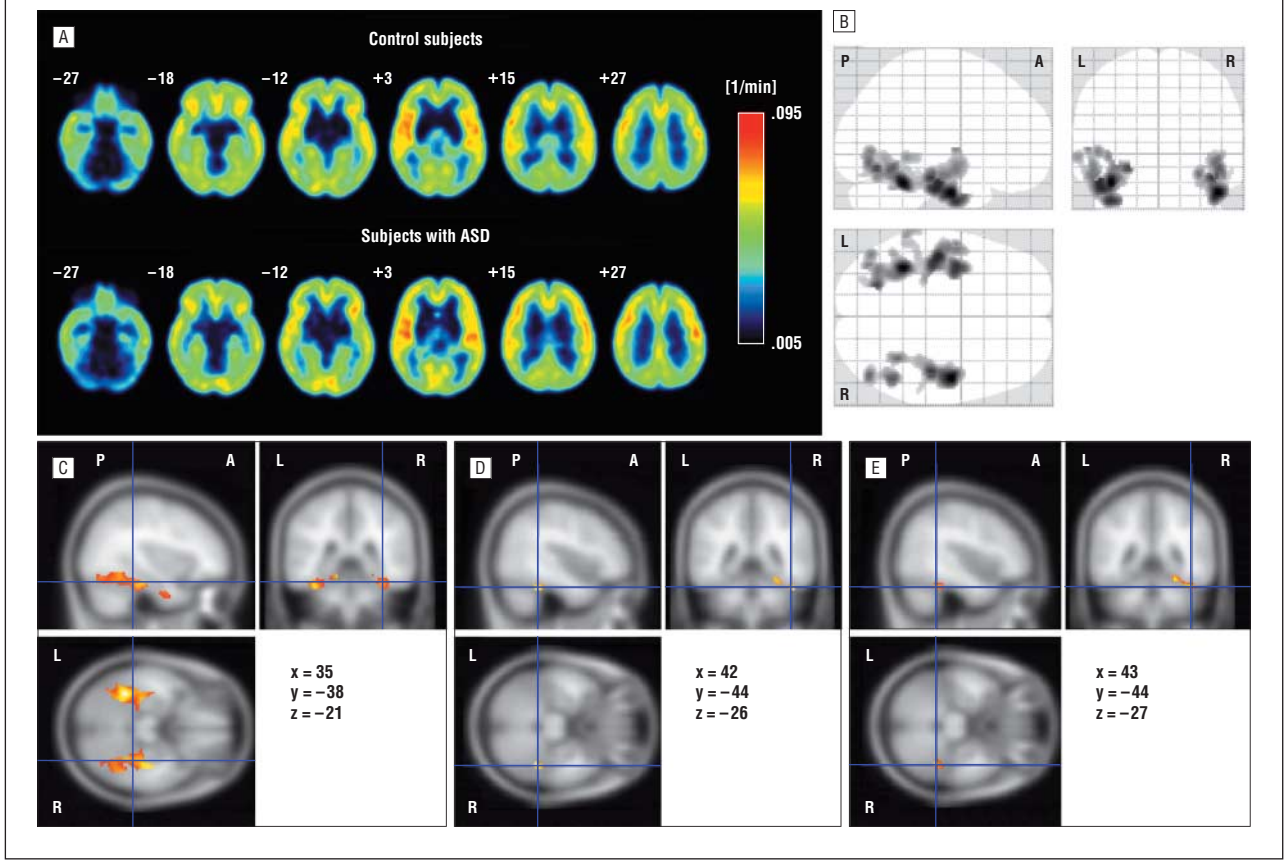

Figure 2. Results of the whole-brain voxel-based statistical parametric mapping analysis of the *N*-[^{11}C]methylpiperidin-4-yl acetate ([^{11}C]MP4A) k_3 value distribution maps. A, Normalized and averaged [^{11}C]MP4A k_3 parametric maps from control subjects and subjects with autism spectrum disorders (ASD) are shown. B, Areas with significantly reduced [^{11}C]MP4A k_3 values in subjects with ASD compared with those in controls ($P<.05$, corrected) are rendered on glass brains. C, Results from analyses of covariance are shown. Areas with significantly lower [^{11}C]MP4A k_3 values in the ASD group than in the control group ($P<.05$, corrected) are indicated. The location of a cluster with significant negative correlations between [^{11}C]MP4A k_3 values and Autism Diagnostic Observation Schedule social scores (D) or Autism Diagnostic Interview–Revised social scores (E) in subjects with ASD ($P<.05$, corrected) is shown. The locations are rendered on the standard-brain T1 template. A indicates anterior; P, posterior;

$r=-0.508$; $P=.02$) as well as ADI-R domain A scores ($r=-0.505$; $P=.02$) (Figure 2E).

COMMENTS

Adults with ASD had significantly and locally reduced [^{11}C]MP4A k_3 values, a representative measure of the hydrolytic activity of AChE in the bilateral fusiform gyri, with no significant change in [^{11}C]MP4A k_3 values in the other cortical areas. As mentioned previously, motion during PET and PVE are potential confounding factors that influence the results of PET analysis. In this study, however, we confirmed that all of the participants had remained immobilized during each PET scan by fixing the head of each participant. An additional volumetric brain morphometry study showed no significant difference in whole-brain or regional gray matter volumes between subjects with ASD and controls (eAppendix 2, eTable, eFigure 1 and eFigure 2). Therefore, the obtained PET data may correctly represent the condition in the brain. Acetylcholinesterase is most abundant along cholinergic pathways, where it terminates neurotransmission through the rapid hydrolysis of acetylcholine. Although AChE has a very good correspondence with choline acetyltransferase, the enzyme that synthesizes acetylcholine, several other cortical AChE-rich neurons have no choline acetyltransferase activity and are classified as noncholinergic but cholinoceptive.[38,39] However, the AChE-rich cortical axons in the adult brain are almost exclusively cholinergic, arise mostly from the basal forebrain, and contain AChE that is transported anterogradely from cholinergic perikarya in the basal forebrain.[40-42] Therefore, the present result of reduction in the [^{11}C]MP4A k_3 values localized in the bilateral fusiform gyri suggests that presynaptic cholinergic innervation of a specific cortical region is selectively impaired in adult individuals with ASD. Previously, Perry et al[28] measured cholinergic enzyme activity as well as the levels of muscarinic and nicotinic receptors in the frontal and parietal cortices in deceased adults with autism and found no change in the activities of AChE and choline acetyltransferase, although there were decreases in some types of muscarinic and nicotinic receptors. The results of Perry et al[28] may support our contention that the presynaptic cholinergic innervations of the cortex, other than the restricted region of the fusiform gyrus, are intact in ASD. Serotonergic and dopaminergic, as well as cholinergic, innervations may play important roles in the regulation

Table 2. Clusters Where [^{11}C]MP4A κ_3 Values Significantly Correlated With Social Scores From ADOS and ADI-R in Subjects With ASD in the Fusiform Gyrus[a]

Hemisphere	Cluster Size	*F*	x	y	z
Negatively Correlated With ADOS Social Scores					
R	58	29.92	42	–44	–26
L	16	24.92	–46	–52	–20
R	20	23.78	24	–60	–20
R	38	19.65	24	–46	–16
L	14	16.89	–28	–74	–16
Negatively Correlated With ADI-R A (Social) Scores					
R	267	32.78	38	–46	–24
L	36	28.70	–46	–52	–20
L	59	16.53	–28	–76	–16
R	39	15.55	24	–80	–16

Abbreviations: [^{11}C]MP4A, *N*-[^{11}C]methylpiperidin-4-yl acetate; ADI-R, Autism Diagnostic Interview–Revised; ADOS, Autism Diagnostic Observation Schedule; ASD, autism spectrum disorder; NA, not applicable.
[a] $P<.05$, corrected; cluster extent threshold, 10 voxels.

of cortical activity in the visual area.[19] However, our recent PET study in which brain serotonin and dopamine transporter bindings were evaluated in adults with high-functioning autism showed no changes in the serotonergic or dopaminergic terminals in the fusiform gyrus.[32] Therefore, the deficit in the fusiform gyri of individuals with ASD may be relatively specific to the cholinergic neurotransmission, although more study of the influences of other neurotransmitters, such as noradrenaline, is necessary. In our ROI-based analysis, AChE activities tended to be lower in the ASD groups than in the controls across all of the ROIs tested, although it reached significance only in the bilateral fusiform gyri after correction for multiple comparisons. It may be possible that the cholinergic transmission is globally impaired in ASD. Further study is therefore required on the subject.

When the relationship between the [^{11}C]MP4A k_3 value and the diagnostic algorithm scores from the ADOS and ADI-R was examined in each side of the fusiform gyrus, lower levels of the k_3 value in both fusiform gyri were found to be associated with more severe social reciprocity, as evaluated by the ADOS and ADI-R. The ADOS social score reflects the current social function, while the ADI-R social (domain A) scoring is based on early social development. Therefore, a deficit in cholinergic innervation of the fusiform gyri, which can be observed in adults with ASD, may be related not only to the current but also the childhood impairment of social functioning. The participation of the fusiform gyrus in this regard may be more predominant in the right than the left hemisphere, since our laterality analysis showed that the individual laterality indices were negatively correlated with social scores from the ADOS and ADI-R. It is currently unknown whether children with ASD have abnormalities in cholinergic innervations of the fusiform gyri. However, a lack of interest in the human face is a major symptom of autism and is evident as early as the first year of life,[43] suggesting the emergence of a functional impairment of the face-processing system, including the FFA within the fusiform gyrus, in the early development of ASD. Although speculative, the association of the current deficit in cholinergic innervations of the fusiform gyri with the present and early impairment of social functioning may reflect the existence of the cholinergic insult in the early development of ASD, persisting into adulthood. Recently, Nacewicz et al[44] demonstrated that a smaller amygdala exhibits more significant impairment in social reciprocity as determined by the ADI-R. When Kleinhans et al,[45] using the fMRI technique, investigated functional connectivity within the limbic system during face identification in high-functioning adults with ASD, abnormal functional connectivity between the right fusiform gyrus and the left amygdala was associated with ADI-R social scores in childhood. At this time, it is unclear whether cholinergic transmission impairment in the right-hemispheric fusiform gyrus is involved in the time-independent association described by Nacewicz et al[44] and Kleinhans et al.[45]

A previous neuropathological study of autism described significant reductions in neuron density in layer III, total neuron numbers in layers III, V, and VI, and mean perikaryal volumes of neurons in layers V and VI in the fusiform gyrus.[17] The neuropathological changes may be specific to the fusiform gyrus because none of these alterations were found in the primary visual cortex or in the whole cerebral cortex.[17] The pyramidal cells in layers III and V have been suggested to be cholinoceptive.[40,46-51] Because acetylcholine is known to play an important role in the regulation of both structural and functional maturation of cortical circuits,[18,52,53] and because the modulatory effect of acetylcholine seems to depend on the level of AChE activity,[54] we suppose that the reduced AChE activity in the fusiform gyrus observed here may partly contribute to the reduction in the number of cholinoceptive neurons in layers III and V.

Our study has some limitations. The small sample size renders the data presented here preliminary, and a larger study with more ASD subjects will be necessary. However, recruitment for the current study was limited to a group of high-functioning subjects with ASD, none of whom were given psychotropic drugs, and all were able to complete PET examination without sedation. Therefore, our data are free from possible confounding factors and thus reflect a certain common pathology among

people with ASD. Another methodological issue is that the absence of PVE correction using a low-resolution PET camera would affect quantitative values such as k_3.[55] One solution is the use of a higher-resolution PET camera. Compatible with the reported high-resolution human PET scanner,[55] our PET camera has an intrinsic 2.9-mm resolution, which previously allowed us to evaluate the change in tracer accumulation in a small region such as the midbrain.[56] The fusiform is actually larger than the midbrain, and it was reported that the fusiform cortex is thicker in ASD than in controls.[57] Thus, the use of a high-resolution brain-purpose PET camera and MRI-guided ROI determination on the thicker cortical region could minimize the PVE issue in the present study. It was reported that hypometabolism exceeded atrophy in most altered structures in Alzheimer disease.[58] Although the disease is different, that observation suggests that the present [^{11}C]MP4A k_3 reduction reflects the pathophysiology of ASD rather than the atrophy.

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Correction

Error in Figure Citations and Legend. In the Original Article "Reduced Acetylcholinesterase Activity in the Fusiform Gyrus in Adults With Autism Spectrum Disorders" by Suzuki et al, published in the March 2011 issue of the *Archives* (2011;68(3):306-313), some figure citations and some parts of the legend are incorrect. Though the first figure citations (those in boldface) are correct, all subsequent ones are incorrect. Thus, Figure 1 should be Figure 2 and Figure 2 should be Figure 1 in these citations. Also, in the legend of Figure 1, lines 11 and 12, (A) and (B) should be (B) and (C), respectively. The Suzuki et al article was corrected online.