

Investigation of Neuroanatomical Differences Between Autism and Asperger Syndrome

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Background: Autism and Asperger syndrome (ASP) are neurobiological conditions with overlapping behavioral symptoms and of unknown etiologies. Results from previous autism neuroimaging studies have been difficult to replicate, possibly owing to site differences in subject samples, scanning procedures, and image-processing methods. We sought (1) to determine whether low-functioning autism (LFA; IQ < 70), high-functioning autism (HFA; IQ ≥ 70), and ASP constitute distinct biological entities as evidenced by neuroanatomical measures, and (2) to assess for intersite differences.

Methods: Case-control study examining coronally oriented 124-section spoiled gradient echo images acquired on 3 magnetic resonance imaging (MRI) systems, and processed by BrainImage 5.X. Participants were recruited and underwent scanning at 2 academic medicine departments. Participants included 4 age-matched groups of volunteer boys aged 7.8 to 17.9 years (13 patients with LFA, 18 with HFA, 21 with ASP, and 21 control subjects), and 3 volunteer adults for neuroimaging reliability. Main outcome measures included volumetric measures of total, white, and gray matter for cerebral and cerebellar tissues.

Results: Intersite differences were seen for subject age, IQ, and cerebellum measures. Cerebral gray matter volume was enlarged in both HFA and LFA compared with controls ($P = .009$ and $P = .04$, respectively). Cerebral gray matter volume in ASP was intermediate between that of HFA and controls, but nonsignificant. Exploratory analyses revealed a negative correlation between cerebral gray matter volume and performance IQ within HFA but not ASP. A positive correlation between cerebral white matter volume and performance IQ was observed within ASP but not HFA.

Conclusions: Lack of replication between previous autism MRI studies could be due to intersite differences in MRI systems and subjects' age and IQ. Cerebral gray tissue findings suggest that ASP is on the mild end of the autism spectrum. However, exploratory assessments of brain-IQ relationships reveal differences between HFA and ASP, indicating that these conditions may be neurodevelopmentally different when patterns of multiple measures are examined. Further investigations of brain-behavior relationships are indicated to confirm these findings.

Arch Gen Psychiatry. 2004;61:291-298

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AUTISM IS A PERVERSIVE DEVELOPMENTAL DISORDER (PDD) defined by the following triad of behavioral characteristics: social and communication impairments in combination with restricted and repetitive behaviors.^{1,2} Many autistic individuals have cognitive impairments; in the literature, subjects with an IQ of less than 70 are typically designated as having low-functioning autism (LFA), and those with an IQ of at least 70 as having high-functioning autism (HFA).³⁻⁵ Asperger syndrome (ASP), another PDD, is similar to autism, sharing features of social impairment and repetitive behaviors, in the absence of communication and cognitive impairments (ie, phrase language developed before 36 months of age and IQ ≥ 70).^{2,6,7} Because persons with HFA also have IQs of at least

70, and the *DSM-IV* does not require a history of language delay for a diagnosis of autism, this creates a diagnostic overlap between HFA and ASP, resulting in many individuals with ASP who meet *DSM-IV* criteria for autism.^{4,8} As a result, many studies comparing HFA and ASP distinguish these 2 conditions according to history of phrase language development (HFA at 36 months or older, and ASP at younger than 36 months).⁹⁻¹³

Owing to the diagnostic similarities between HFA and ASP, a debate is growing about the validity of ASP as a disorder distinctly different from autism. Several studies have found differences between HFA and ASP on measures of social skills,¹⁰ cognition/executive functioning,¹⁴⁻¹⁶ and motor ability^{17,18}; others reported no differences on similar measures.^{13,19-23} It has been suggested that the spectrum of behavioral

and cognitive patterns seen in individuals with PDD is driven by an underlying severity gradient.^{23,24} Subtypes of PDD align themselves along a severity continuum, beginning with LFA at one end, moving through HFA, and ending with ASP.³ Others argue that these conditions may represent distinct neuropathological disorders with overlapping behavioral and cognitive symptoms.⁵ In either case, the underlying neurodevelopmental mechanisms leading to these conditions are unknown.

A number of structural magnetic resonance imaging (sMRI) studies of the brain in subjects with autistic disorder have revealed neuroanatomical abnormalities of the corpus callosum,²⁵⁻²⁷ cerebellar vermal lobules VI and VII,^{28,29} and amygdala and hippocampus.^{30,31} These findings were not always replicated,³²⁻³⁶ possibly owing to differences between subject populations, scanning procedures, and image processing methods between research sites.

The most consistent sMRI finding is increased brain volume in autistic subjects.³⁷⁻⁴¹ This finding is consistent with reports of increased head circumference⁴²⁻⁴⁴ and brain weight⁴⁵ in autism. Increased brain volume was found regionally in the parietal, temporal, and occipital but not the frontal lobes⁴⁶ and in cortical gray and cerebral white tissue.³⁸ There appear to be age effects on brain volume in autism; children tend to have larger brain volumes than older individuals relative to age-matched controls.^{38,41} Most autism brain volume studies included subjects with LFA and HFA,^{37,38,40,46} but a few studies were restricted to those with HFA.^{39,41} These studies did not specifically include subjects with ASP.

Of the few sMRI studies of subjects with ASP,⁴⁷⁻⁵⁰ only one investigated brain volume, and McAlonan and co-workers⁴⁹ reported no difference in total hemispheric volume between ASP and control subjects. In a related study, Gillberg and de Souza⁵¹ used head circumference data to report macrocephaly in a subgroup of subjects with ASP.

The first goal of the present study is to assess brain volumes in the following 3 PDD groups: LFA, HFA, and ASP. In particular, we sought to determine whether these PDD groups constitute distinct biological entities as evidenced by neuroanatomical measures. This is the first volumetric neuroimaging investigation, to our knowledge, that compares subjects with autism and those with ASP. This study is part of a larger project to investigate intersite differences that might explain the inconsistent replication seen in autism neuroimaging investigations. Thus, our second goal is to examine the differences and similarities in subject populations and neuroimaging data between 2 sites when using the same subject recruitment strategies, scanning protocols, and data measurement procedures.

METHODS

SUBJECT RECRUITMENT

Subjects were boys ranging in age from 7.8 to 17.9 years who met eligibility for 1 of the following 4 subject groups: LFA (Full-Scale IQ [FSIQ], <70), HFA (FSIQ, ≥70), ASP, and age-matched controls. The HFA, ASP, and control groups were recruited jointly by the University of California–Davis (UC Davis) and Stanford University Medical School, Stanford, Calif (Stanford). The LFA group was recruited solely by UC Davis. Both sites re-

cruited through local parent networks and regional professionals who work with the PDD population. All subjects with PDD underwent screening and were excluded if they had any major medical (eg, fragile X syndrome) or psychiatric condition.

Control subjects were recruited at both sites through newspaper advertisements and through friends of the subjects with PDD. The controls were matched by group age with the PDD subjects. All controls were in good physical health and underwent screening to exclude neurological, developmental, or psychiatric disorders. They also underwent screening for any psychiatric symptoms with the Child Behavioral Checklist.⁵² The study was approved by the institutional review boards of Stanford and UC Davis. Written consent was obtained from all subjects and their parents.

COGNITIVE AND BEHAVIORAL ASSESSMENTS

Subjects with PDD first underwent assessment and rating on the *DSM-IV*² criteria for a diagnosis of autism or ASP. They then underwent assessment using the Autism Diagnostic Interview–Revised (ADI-R)⁵³ and the Autism Diagnostic Observation Schedule–Generic (ADOS-G)⁵⁴ by trained examiners (L.J.L. and B.L.G.-J.) who had each established reliability with 1 of the developers of the instrument. Standardized cognitive testing using the Wechsler Abbreviated Scale of Intelligence⁵⁵ was administered to all subjects with the exception of those with LFA, who were administered the Leiter International Performance Scale–Revised.⁵⁶

PDD GROUP ASSIGNMENT

For inclusion in the LFA group, subjects had to have ADI-R and ADOS-G threshold scores for autism and an FSIQ of less than 70. Subjects with HFA had to have ADI-R and ADOS-G threshold scores for autism, an FSIQ of at least 70, and a history of phrase speech development at 36 months or older. The ASP group had to meet *DSM-IV* criteria for ASP or autism, an ADOS-G threshold score for autism or autism spectrum disorder, an FSIQ of at least 70, and a history of phrase speech development at younger than 36 months. Since many persons with ASP also meet ADI-R and *DSM-IV* criteria for autism,⁸⁻¹¹ in this study the primary distinguishing feature between individuals with HFA and ASP was a history of clinically significant language impairment; this strategy has been used in other studies.⁹⁻¹³ In summary, subjects with LFA and HFA were differentiated by FSIQ scores, and subjects with ASP and HFA were differentiated by age of phrase language development.

A total of 73 subjects underwent analysis in this study, including 13 with LFA, 9 with HFA, 11 with ASP, and 11 controls from UC Davis and 9 with HFA, 10 with ASP, and 10 controls from Stanford.

IMAGE ACQUISITION

Subjects at both sites participated in the sMRI protocol described in this study. Subjects at Stanford also participated in a functional MRI protocol and thus had to remain alert throughout scanning. At Stanford, subjects first underwent screening with an MRI simulator. Those subjects with excessive head movement were withdrawn from the study. In contrast, at UC Davis, subjects with PDD who could not remain still underwent scanning under general anesthesia. Images from 29 subjects were acquired on a 3.0-T GE Signa whole-body echospeed MRI system (GE Medical Systems, Milwaukee, Wis) at the Richard M. Lucas Center at Stanford, whereas images from 19 subjects were acquired on a 1.5-T GE Signa Neurovascular-optimized MRI system at UC Davis Imaging Research Center. The remaining 25 subjects with PDD required general anesthesia; accordingly, their

data were acquired on the 1.5-T GE Signa MRI system at the UC Davis Medical Center. A 3-dimensional volumetric radio-frequency spoiled-gradient echo pulse sequence was used to acquire all images in the coronal plane, with the following parameters: repetition time of 35 milliseconds, echo time of 6 milliseconds, flip angle of 45°, number of signals is 1, matrix size of 256 × 192, field of view of 24 cm, full bandwidth of 32 kHz, and slice thickness of 1.5 to 1.7 mm for 124 contiguous sections.

IMAGE PROCESSING AND QUANTIFICATION

At Stanford, all 73 images were imported into the program BrainImage 5.X⁵⁷ for masked semiautomated image-processing analyses and brain volume measurements.⁵⁸ These procedures were previously described and validated.⁵⁹⁻⁶¹ Data processing included removal of nonbrain tissue, correction of image inhomogeneity, data interpolation to cubic dimensions, and segmentation into gray tissue, white tissue, and cerebrospinal fluid (**Figure 1**) for the following structures: cerebral lobes, subcortical nuclei, cerebellum, and lateral ventricles. Specific regions are parcelled and measured using a semiautomated stereotactic method.^{58,62,63}

INTERSITE MRI DATA COMPARABILITY

One of the purposes of using a multisite study design is to elucidate the degree to which differences in methods between research sites might contribute to the variable structural MRI results reported in the autism literature. Although all MRI systems used in this study were GE Signa systems, they differed by magnetic field strength (3 T vs 1.5 T) and software (Stanford, GE Horizon LX Version 8.3; UC Davis Hospital, GE Horizon LX Version 8.3.5; and UC Davis Research Imaging Center, GE Horizon LX Version 8.4M4).

An intersite MRI comparison was conducted using images from 3 normal volunteer adults (1 man and 2 women) who underwent scanning during a 10-month period with the 3 MRI systems described above. Images were acquired and analyzed with the same pulse sequence and BrainImage 5.X⁵⁷ program as that used with the study subjects. Total brain and segmented tissue volumes were compared between the 3 systems; a percentage difference between MRI systems for each subject was averaged across subjects to arrive at a mean percentage difference for each volumetric measure. Only those volumetric values with a mean percentage difference of less than 5% between sites were used in this study. Following are the observed mean percentage differences. For cerebrum measures, these were 1.2% for cerebral total tissue, 1.8% for cerebral gray matter, and 2.3% for cerebral white matter; for cerebellum measures, 6.2% for cerebellar total tissue, 7.0% for cerebellar gray matter, and 17.4% for cerebellar white matter. Cerebral volumes for total, gray, and white tissues had mean intersite differences of 3% or less and thus were used in the analysis of the 3 PDD and control groups.

STATISTICAL ANALYSIS

Age and IQ Measures

We used analysis of variance (ANOVA) followed by Scheffé post hoc testing to assess the 4 subject groups for any differences in age and IQ (performance IQ [PIQ], verbal IQ [VIQ], and FSIQ). The ANOVA followed by Scheffé post hoc testing also was used to examine the 2 sites for any differences in age and IQ for those subject groups who underwent scanning at both sites (HFA, ASP, and control). The subjects with LFA were excluded from the second analysis, because they were recruited only at UC Davis. For these 2 sets of analyses, we used parametric statis-

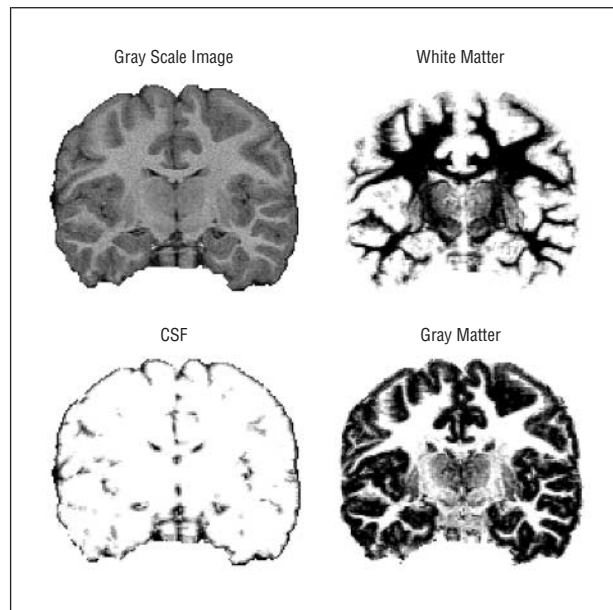


Figure 1. Tissue segmentation. CSF indicates cerebrospinal fluid.

tics, as the distributions of the data did not violate assumptions of normality or homogeneity of variance.

MRI Volumetric Measures

We first applied a parametric method, ANOVA, for MRI volumetric analysis. Because the variance of MRI volumetric findings for the LFA group was larger than for the other subject groups, analysis was repeated using nonparametric methods (Kruskal-Wallis test with post hoc Mann-Whitney test). We assessed interactions between volumetric measures with site, age, and IQ for the 3 subject groups recruited from both sites (ASP, HFA, and control). Interaction terms were excluded from final ANOVA models if they did not approach or reach significance ($P < .10$). Finally, we used the Pearson correlation coefficient to explore the potential effects of age and IQ on MRI volumetric values. We then compared these within-group correlations using the Fisher r -to- z transformation. For all analyses in this report, we used a P value of .05 as a threshold for statistical significance.

RESULTS

AGE AND IQ MEASURES

Among the 73 boys recruited, PIQ ranged from 36 to 142. For the HFA, ASP, and control groups, VIQ scores ranged from 67 to 144 and FSIQ scores ranged from 70 to 140. The VIQ and FSIQ scores were not available for the LFA group, because they were administered the Leiter Scale, which only provides a PIQ.

Means and standard deviations for age and IQ of subject groups are displayed in **Table 1**. The subject groups did not differ significantly in age. There was a significant main effect of subject group on PIQ. Post hoc testing revealed that the LFA group had a lower PIQ compared with the HFA ($P < .001$), ASP ($P < .001$), and control ($P < .001$) groups, whereas the HFA, ASP, and control groups were not significantly different from each other. There also was a main effect of group (ie, HFA, ASP, and control) on VIQ.

Table 1. Age and IQ for Subject Groups*

	Subject Groups				Significance	
	LFA (n = 13)	HFA (n = 18)	ASP (n = 21)	Control (n = 21)	F Value	P Value
Age, y	11.6 (2.1)	12.1 (3.4)	12.7 (2.6)	12.5 (2.8)	0.451	.72
PIQ	46 (10)	105 (19)	104 (20)	113 (12)	52.0	.001
VIQ	†	86 (17)	110 (19)	112 (13)	14.6	<.001
FSIQ	†	94 (17)	108 (20)	114 (10)	7.9	<.001‡

Abbreviations: ASP, Asperger syndrome; FSIQ, Full-Scale IQ; HFA, high-functioning autism; LFA, low-functioning autism; PIQ, performance IQ; VIQ, verbal IQ.

*Data are expressed for combined sites as mean (SD).

†The LFA group was administered the Leiter International Performance Scale-Revised,⁵⁶ which does not measure VIQ or FSIQ.

Table 2. Age and IQ for Sites*

	Sites†		Significance	
	Stanford (n = 29)	UC Davis (n = 31)	F Value	P Value
Age, y	13.7 (2.8)	11.3 (2.5)	12.1	.001
PIQ	117 (11)	99 (19)	19.2	<.001
VIQ	109 (18)	99 (20)	4.1	.048
FSIQ	114 (14)	99 (19)	12.3	<.001

Abbreviations: See Table 1.

*Analyses exclude the low-functioning autism group because they were recruited only at UC Davis. Data are expressed as mean (SD).

†Stanford indicates Stanford University School of Medicine, Stanford, Calif; UC Davis, University of California-Davis.

Post hoc analyses revealed that the HFA group had a significantly lower VIQ compared with the ASP ($P < .001$) and control ($P < .001$) groups. There was no significant difference between the ASP and control groups in VIQ. Groups also differed on FSIQ, which was lower in the HFA group compared with the ASP ($P = .03$) and control ($P = .001$) groups, reflecting the pattern seen in VIQ.

Table 2 shows age and IQ results for both sites across the 3 subjects groups (ie, HFA, ASP, and control); the LFA group was excluded since they underwent imaging only at UC Davis. Stanford subjects were significantly older than UC Davis subjects. This difference in age between sites was seen across all 3 diagnostic groups, but was found to be statistically significant only in the HFA group ($F_{1,16} = 7.9$; $P = .01$). There also was a significant between-site difference in IQ; UC Davis subjects had lower PIQ, VIQ, and FSIQ compared with Stanford subjects. The UC Davis HFA group had lower PIQ ($P < .001$) and lower FSIQ ($P = .01$) compared with the Stanford HFA group, but no differences in VIQ. The UC Davis ASP group had lower PIQ ($P < .001$), VIQ ($P = .01$), and FSIQ ($P = .003$) compared with the Stanford ASP group. Analyses revealed no significant IQ differences between control groups from the 2 sites.

MRI VOLUMETRIC MEASURES

Results of MRI volumetric results for the 4 subject groups are shown in **Table 3**. There was a significant subject group effect for cerebral gray tissue ($F_{3,69} = 4.37$; $P = .007$;

Figure 2) but no significant subject group effect for cerebral total or white tissue. Because of relatively larger variance within the LFA group compared with the other 3 groups, analyses were repeated using nonparametric methods (Table 3). These results were similar to those obtained with ANOVA. Post hoc 2-group analyses using Mann-Whitney indicated that, compared with the control group, the LFA ($P = .04$) and HFA ($P = .009$) groups had enlarged cerebral gray matter volumes, whereas the LFA, HFA, and ASP groups were not significantly different from each other.

Knowing that there were intersite differences for age and IQ, we then looked for effects of 2-way interactions between site and subject group using age and PIQ as covariates and cerebral gray matter volume as the dependent variable. We excluded LFA from this analysis. When all 2-way interactions were included in an initial ANOVA model, the subject group \times site 2-way interaction approached significance ($F_{2,57} = 2.6$; $P = .09$) and was therefore included in the final analysis of covariance model. Consistent with the previous results, a significant main effect of subject group on cerebral gray matter volume was observed ($F_{2,57} = 4.02$; $P = .02$). Age also contributed significantly to the final model ($F_{1,58} = 6.60$; $P = .01$); decreasing cerebral gray matter volumes were correlated with increasing age across the 3 groups.

MRI VOLUMETRIC MEASURES CORRELATED WITH AGE AND IQ

Within-group correlations between cerebral gray matter and the variables of age and IQ are shown in **Table 4**. A negative correlation between cerebral gray matter volume and age was significant in the HFA group ($r_{16} = -0.53$; $P = .02$). A significant negative correlation between cerebral gray matter volume and PIQ was observed for the HFA group ($r_{16} = -0.49$; $P = .04$), and a positive correlation for the same variables approached significance for the ASP group ($r_{19} = 0.42$; $P = .06$). To rule out confounding relationships between age and PIQ in the HFA and ASP groups, these correlations between cerebral gray matter volume and PIQ were repeated using age as a covariate. These partial correlations were of borderline significance for HFA and significant for ASP (Table 4).

Within-group correlations between cerebral white matter and the variables of age and IQ are shown in **Table 5**. The correlation between cerebral white matter and age was significant for the HFA, ASP, and control groups; white matter volume was observed to increase with increasing age across the samples. Only the ASP group had a positive within-group correlation between cerebral white matter volume and PIQ and between cerebral white matter volume and VIQ. Significance was maintained when analyses were repeated as partial correlations using age as a covariate (Table 5).

There were a few significant between-group differences for correlations of volumetric measures with IQ (**Figure 3** and **Figure 4**). The correlations for cerebral gray matter volume and PIQ for the HFA and ASP groups were in opposite directions; this between-group difference was significant (Figure 3). There also were between-group differences for cerebral white matter vol-

Table 3. Neuroanatomical Volumes by Subject Groups*

	Subject Groups				Significance	
	LFA (n = 13)	HFA (n = 18)	ASP (n = 21)	Control (n = 21)	H Value	P Value
Cerebral gray matter	773.2 (104.8)	744.4 (45.5)	719.3 (47.3)	700.0 (48.8)	8.4	.038†
Cerebral white matter	460.6 (73.3)	476.5 (47.2)	470.3 (55.4)	471.4 (36.2)	0.5	.10
Cerebral total tissue	1233.8 (172.5)	1220.8 (70.3)	1189.6 (86.0)	1171.4 (69.6)	3.3	.34

Abbreviations: See Table 1.

*Data are expressed as mean (SD) milliliters.

†The LFA and HFA groups differed significantly from the control group on this measure.

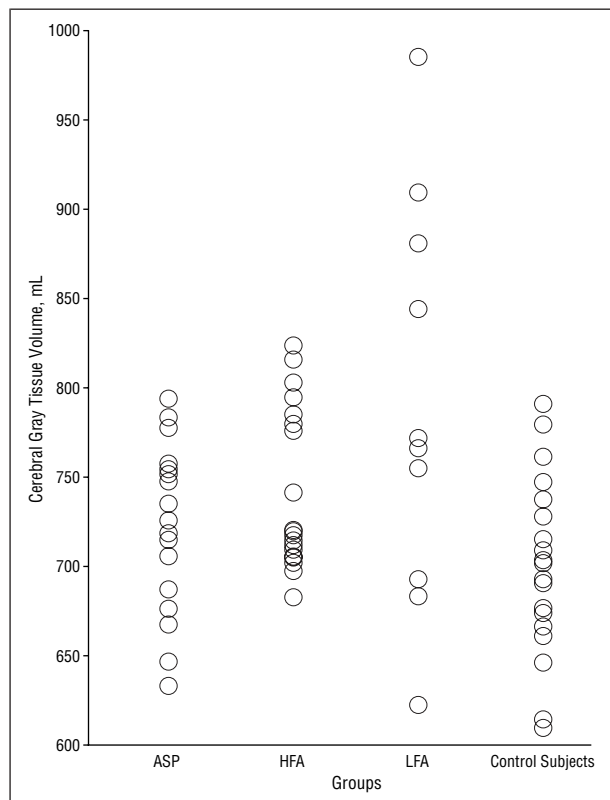


Figure 2. Cerebral gray tissue by groups. Groups included 21 subjects with Asperger syndrome (ASP), 18 with high-functioning autism (HFA), 13 with low-functioning autism (LFA), and 21 control subjects.

ume with PIQ correlations between the ASP and the other 2 groups (HFA and control [Figure 4]).

COMMENT

INTERSITE DIFFERENCES

Despite the use of similar recruitment strategies and scanning protocols, we found site differences in subjects by age and IQ. These differences were not significant in the control group, but were significant in the HFA and ASP groups. Site-specific differences in IQ may be related, in part, to differences in subject enrollment; Stanford's PDD subject recruitment was limited to those with an FSIQ of 70 or above, whereas UC Davis' recruitment included IQs above and below 70. Another reason for site differences in subjects' age and IQ is the subject retention and withdrawal

Table 4. Pearson Correlations Between Age, PIQ, and VIQ and Cerebral Gray Tissue Volume for Subject Groups

	Subject Groups		
	HFA (n = 18)	ASP (n = 21)	Control (n = 21)
Age	-0.53*	-0.11	-0.36
PIQ	-0.49†	0.42‡	0.15
VIQ	-0.09	0.34	0.40

Abbreviations: See Table 1.

*Within group correlation, $P = .02$.

†Within group correlation, $P = .04$; within-group partial correlation covaried for age, $r = -0.39$ ($P = .06$).

‡Within group correlation, $P = .06$; within-group partial correlation covaried for age, $r = 0.51$ ($P = .01$).

Table 5. Pearson Correlations Between Age, PIQ, and VIQ, and Cerebral White Tissue Volume for Subject Groups

	Subject Groups		
	HFA (n = 18)	ASP (n = 21)	Control (n = 21)
Age	0.53*	0.50*	0.44†
PIQ	0.22	0.75‡	0.01
VIQ	0.01	0.52§	0.42

Abbreviations: See Table 1.

*Within group correlation, $P = .02$.

†Within group correlation, $P = .05$.

‡Within group correlation, $P < .001$; within-group partial correlation covaried for age, $r = 0.70$ ($P < .001$).

§Within group correlation, $P = .02$; within-group partial correlation covaried for age, $r = 0.42$ ($P = .03$).

practices dictated by differences in MRI protocols between sites. University of California–Davis used an sMRI protocol only and thus used general anesthesia for those subjects with PDD who could not conform to the motion reduction requirements of MRI, regardless of IQ. In contrast, Stanford used sMRI and functional MRI protocols and withdrew potentially eligible subjects owing to their inability to reduce head movement during the MRI simulation. As a result, Stanford withdrew 12 subjects with PDD, who had an average age of 10.3 years (IQs not measured), and UC Davis anesthetized 12 subjects with HFA and with ASP, who had an average age of 10.7 years and an average VIQ and PIQ of 84 each. As Stanford was withdrawing younger subjects and possibly subjects with lower

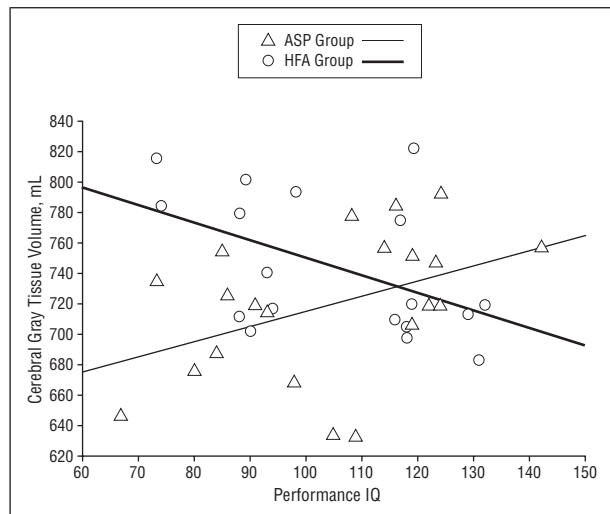


Figure 3. Correlation between cerebral gray tissue volume and performance IQ. Groups included 21 subjects with Asperger syndrome (ASP) and 18 with high-functioning autism (HFA). For the between-group difference, $z = -2.75$ ($P = .005$).

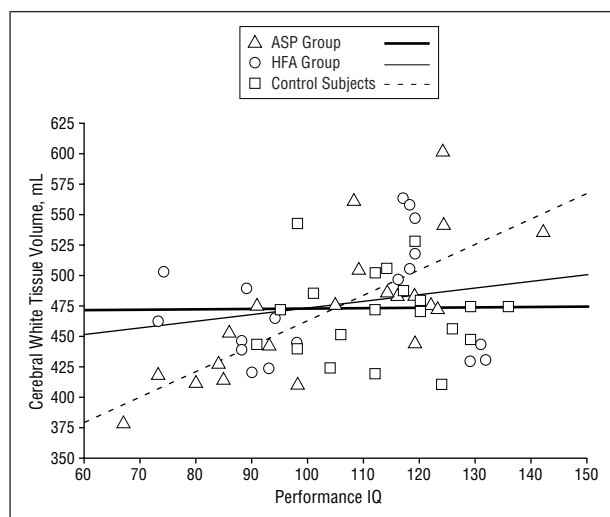


Figure 4. Correlation between cerebral white tissue volume and performance IQ. Groups included 21 subjects with Asperger syndrome (ASP), 18 with high-functioning autism (HFA), and 21 control subjects. For between-group differences, ASP vs HFA, $z = -2.13$ ($P = .03$); ASP vs controls, $z = -2.88$ ($P = .004$).

IQs, UC Davis was retaining similar subjects, resulting in age and IQ differences between sites. Poor replication of autism MRI results may thus be partially explained by differences in eligible subject pools stemming from differences in recruitment and MRI protocols.

Other sources of variation in published PDD neuroimaging results are differences in MRI methods, including field strengths of the MRI system, variations in image acquisition protocols, rater training, methods of image processing, and statistical methods. In this 2-site study, we used uniform imaging acquisition and analysis protocols but different MRI systems with different field strengths (two 1.5T systems at UC Davis and a 3.0-T system at Stanford). Results of the reliability test of 3 volunteer subjects revealed differences of greater than 5% in cerebellar but not cerebral measures. To minimize the effects of using different MRI systems on brain volumet-

rics, we limited our analyses to cerebral volumes. If these MRI reliability results are replicated in a large group of subjects, this may shed light on the lack of agreement between neuroimaging studies, particularly when a cerebellar tissue segmentation procedure is used. Other possible sources of poor replicability between MRI studies are site differences in imaging acquisition and analysis protocols. These could not be addressed in this study, because we used similar protocols. On the basis of these results, MRI reliability analysis should become a standard procedure for multisite neuroimaging studies.

MRI VOLUMETRIC MEASURES

Findings in HFA and LFA Groups

The HFA and LFA groups both had enlarged total cerebral gray matter volumes compared with the control group. In a similar study, Courchesne et al³⁸ reported increased cortical gray matter volume in young autistic children aged 2 to 3 years, but not in older autistic subjects aged 6 to 16 years. Courchesne and colleagues³⁸ studied only a few subjects in the age range reported in the present study, and thus they may not have had the power to detect a difference. In their study and the present one, cerebral gray tissue volume was observed to be reduced with increasing age in autistic subjects. The enlarged cerebral gray matter volume seen in the present study is congruent with findings of enlarged brain volume^{37,41} in autism and a neuropathological study⁴⁵ showing increased cortical volume in autistic adults.

There also appears to be a relationship between cerebral gray matter volume and IQ in autism that is unrelated to mental retardation. That is, within the HFA group, there was a tendency for individuals with large cerebral gray matter volumes to have lower PIQs. This negative correlation stands in contrast to analyses of typically developing children and adolescents, in whom larger brain volumes are associated with higher IQ.⁶⁴ Two previous investigations^{40,41} failed to find a significant correlation between total brain volume and IQ in autistic subjects. This indicates that the brain-IQ relationships in autism may pertain only to cerebral gray matter and not to total brain tissue.

Increased cerebral gray tissue in autism may be due to abnormalities in gray tissue development. Neuropathology studies of autism have revealed cerebral gray matter abnormalities that include an increased number of minicolumns per unit area along with fewer neurons per minicolumn,⁶⁵ smaller and more densely packed neurons in the anterior cingulate gyrus and limbic system,⁶⁶ and an approximately 50% reduction in protein levels of the enzymes that synthesize γ -aminobutyric acid and glutamic acid decarboxylase in parietal and cerebellar cortices.⁶⁷ Overexpression of specific neuropeptides and neurotrophins were reported in neonatal blood of infants who were later diagnosed as having autism.⁶⁸ Overall, a growing body of literature supports the conclusion that abnormalities in gray matter development are a defining feature of autism.

In the present study, we noted that the LFA group had an unusually large variance in cerebral total tissue. This suggests that, neuroanatomically, the LFA sample represents a more heterogeneous population than HFA or ASP samples. Increased heterogeneity implies a greater mixture of dis-

parate etiologies, some of which may be unidentified single-gene disorders. The probability that LFA is more heterogeneous than HFA has previously been discussed.⁶⁹

Autism and ASP Comparisons

To our knowledge, this is the first neuroimaging study to investigate differences in brain volumetric measures between subjects with ASP and those with autism. When ASP and HFA are distinguished by timing of language development, as in this study, there are no differences in cerebral volumetric measures (total, gray, and white tissue) between these 2 PDD subgroups. Also, no differences were observed between ASP and control groups on these same measures, a finding consistent with the report of McAlonan and colleagues,⁴⁹ who found no differences in total cerebral volume in ASP adults compared with controls.

In the current study, the mean cerebral gray matter volume for the ASP group was intermediate between means for the HFA and control groups; this may indicate a continuum in which cerebral gray matter volume increases with the severity of the PDD condition. Using a different MRI technique, voxel-based analysis, 2 investigations^{49,50} reported gray tissue differences in ASP subjects compared with controls. A neuropathology study⁷⁰ reported abnormal minicolumn architecture in ASP subjects similar to that described in autistic subjects, suggesting a common underlying neuropathology.

We also have preliminary evidence that HFA and ASP may differ from each other in specific brain-behavior relationships. First, the HFA group had the atypical pattern of decreasing PIQ associated with increasing gray matter volume, whereas the ASP group had the typical pattern of increasing PIQ associated with increasing gray matter volume.⁶⁴ Second, there was a strong correlation between PIQ and cerebral white tissue volume in the ASP group that differed significantly from the HFA and control groups. Previous studies^{64,71} in typically developing children have suggested that IQ is not related to white tissue volume. This functional white tissue difference between ASP subjects and controls may be congruent with another study,⁴⁹ which used MRI voxel-based analysis and reported white tissue differences between ASP subjects and controls. These suggested brain-behavior differences between HFA and ASP, based on exploratory analyses, are somewhat speculative and require confirmation.

Our attempt to determine whether HFA and ASP disorders are conditions on a continuum or are distinct biological entities was only partially successful. On the single measure of cerebral gray tissue volume, these conditions appear to represent a continuum of severity, with autism exhibiting the greatest aberrant neurodevelopment. However, on multiple measures (ie, brain-behavior correlations of IQ with specific cerebral volumes) there is preliminary evidence of fundamentally different patterns of neurodevelopment between HFA and ASP subjects. These findings are based on differentiating HFA and ASP by history of language development. These dissonant neuroimaging results reflect the present literature on behavioral and cognitive studies of HFA and ASP.^{4,5} Rinehart et al⁵ concluded that results of behavioral and cognitive studies "suggest that it is premature to rule out the possibility

that autism and Asperger disorder may be clinically, and possibly neurobiologically, separate."^{5(p768)} Family studies⁵ indicate that ASP may be genetically different from autism. Our results suggest that when HFA and ASP are differentiated by history of language development, as they are herein, qualitative differences may surface when patterns of multiple measures are examined.

LIMITATIONS OF STUDY

The 2-site design of this study is both a limitation and a strength. Use of different MRI systems and subject groups (ie, differences on age and IQ) introduces confounding variables and is a limitation. However, the 2-site design uncovers those variables that may explain the poor replicability of previous autism MRI investigations and thus is a strength.

We were able to address the known intersite differences in age and IQ by statistically accounting for the effects of age and IQ on the brain volume comparisons. Differences in MRI system field strength were addressed by limiting the analyses to only those volumetric measures with good intersite reliability; this restricted the analyses to measurements focused on the cerebrum. These adjustments may not completely address all intersite differences. Thus, this study needs to be replicated using a intersite design with greater attention to common subject enrollment and withdrawal practices and MRI procedures (ie, sMRI vs functional MRI and the sedation protocol). In an ideal design, traveling subjects should be incorporated for MRI reliability.

Increased sample size would have permitted more robust statistical comparison of the 4 groups. Greater numbers would have given us more power to detect differences where they exist. Since there are age effects on brain development, a prospective study design in which the same subjects undergo scanning every few years into early adulthood should give us the best method to determine differences in gray and white tissue volumes in individuals with PDD.

Submitted for publication February 11, 2003; final revision received August 12, 2003; accepted October 7, 2003.

This study was supported by grants MH01142, MH50047, and HD31715 (Dr Reiss) and MH01832 (Dr Lotspeich) from the National Institutes of Health, Bethesda, Md, and a grant from The MIND Institute, Davis, Calif (Drs Amaral and Reiss).

This study was presented as a poster at the International Meeting for Autism Research; November 1, 2002; Orlando, Fla.

We thank Cindy Johnston, John Ryan, and Meridith Brandt for their contribution to the collection of data, and the study subjects and their families for their participation.

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Correction

Error in Figure. In the Original Article by Lotspeich et al titled "Investigation of Neuroanatomical Differences Between Autism and Asperger Syndrome," published in the March issue of the ARCHIVES (2004;61:291-296), an error occurred in **Figure 4** on page 296. In that figure, the lines representing the Asperger syndrome (ASP) and control groups were reversed. Figure 4 is reprinted correctly here.

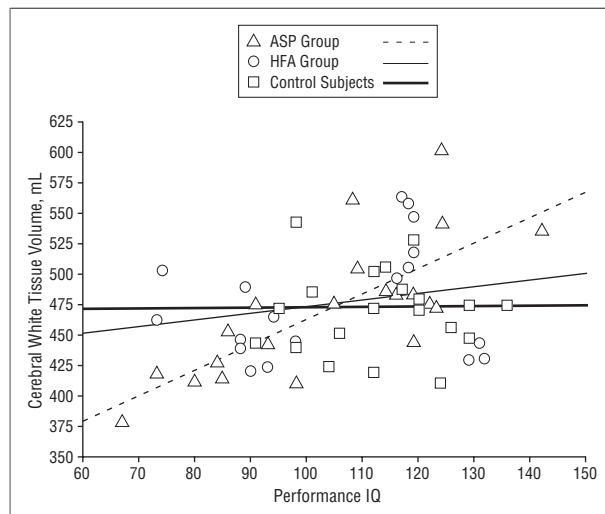


Figure 4. Correlation between cerebral white tissue volume and performance IQ. Groups included 21 subjects with Asperger syndrome (ASP), 18 with high-functioning autism (HFA), and 21 control subjects. For between-group differences, ASP vs HFA, $z = -2.13$ ($P = .03$); ASP vs controls, $z = -2.88$ ($P = .004$).