

Outcome Classification of Preschool Children With Autism Spectrum Disorders Using MRI Brain Measures

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ABSTRACT

Objective: To test the hypothesis that a combination of magnetic resonance imaging (MRI) brain measures obtained during early childhood distinguish children with autism spectrum disorders (ASD) from typically developing children and is associated with functional outcome. **Method:** Quantitative MRI technology was used to measure gray and white matter volumes (cerebrum and cerebellum), total brain volume, and the area of the cerebellar vermis in 52 boys with a provisional diagnosis of autism (aged 1.9–5.2 years) and 15 typically developing young children (aged 1.7–5.2 years). Diagnostic confirmation and cognitive outcome data were obtained after the children reached 5 years of age. **Results:** A discriminant function analysis of the MRI brain measures correctly classified 95.8% of the ASD cases and 92.3% of the control cases. This set of variables also correctly classified 85% of the ASD cases as lower functioning and 68% of the ASD cases as higher functioning. **Conclusions:** These results indicate that variability in cerebellar and cerebral size is correlated with diagnostic and functional outcome in very young children with ASD. *J. Am. Acad. Child Adolesc. Psychiatry*, 2004;43(3):349–357. **Key Words:** autism spectrum disorders, magnetic resonance imaging, neuroanatomy.

Autism is a neurodevelopmental disorder in which behavioral impairments and anomalies form the sole foundation for clinical diagnosis. Subject characterization for biological investigations is made difficult by the heterogeneity and overlap of the behavioral phenotype associated with autism and related disorders (Lord et al., 2001). While a number of brain abnormalities have been identified in postmortem and magnetic reso-

nance imaging (MRI) studies of individuals with autism, commonalities have been difficult to find across the clinical population (Akshoomoff, 2000; Akshoomoff et al., 2002).

In a recently published study, we found that whole brain volume, cerebral and cerebellar white matter volume, and cerebral gray matter were significantly larger in young children with autism (aged 2–5 years) compared to age-matched typically developing children (Courchesne et al., 2001). This pattern of significant overgrowth was not observed in the older children with autism who were studied, suggesting age-related changes in the neural abnormalities associated with the disorder. Across studies of older adolescents and adults with autism, megalencephaly appears to be the exception, not the rule (Akshoomoff et al., 2002). A recent paper also reported abnormally large whole brain volume in 3- to 5 year-old children with a diagnosis of autism spectrum disorder (ASD) (Sparks et al., 2002). No differences in whole brain volume were found between the children who appeared to have less severe autistic symptoms (pervasive developmental disorder-not otherwise specified [PDD-NOS]) and those who met the full criteria for autistic disorder.

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Although a clinical diagnosis of autism is often sensitive and stable over time in children younger than age 3 years, stability increases when an autism spectrum approach is used (Cox et al., 1999; Lord, 1995; Stone et al., 1999); that is, a proportion of the children identified with possible autism before age 3 years do not meet criteria for autism at later follow-up but are highly likely to meet criteria for PDD-NOS. Children younger than age 3 years suspected of having autism but who do not meet criteria for autism on a standardized parent report measure, such as the Autism Diagnostic Interview-Revised, may nonetheless receive a clinical diagnosis of autism at follow-up (Cox et al., 1999; Lord, 1995). The use of a standardized observation of social and communicative behavior and play, such as the Autism Diagnostic Observation Schedule-Generic, may be more sensitive and stable over time than a standardized parent report measure. In one study, use of the Pre-Linguistic Autism Diagnostic Observation Scale, Autism Diagnostic Observation Schedule-Generic, or Childhood Autism Rating Scale (instead of the Autism Diagnostic Interview-Revised alone) allowed inclusion of children who appeared to meet the social and communication criteria for autism but did not yet show significant evidence of restricted or repetitive behaviors as required to meet the cutoff for autism on the Autism Diagnostic Interview-Revised or according to *DSM-IV* criteria due to their young age (Lord and Risi, 2000).

In the current study, we compared neuroanatomical data from young children with autism, young children with PDD-NOS, and typically developing young children. MRI scanning was conducted when the children were aged 1.9 to 5.2 years. Diagnostic and psychological testing was repeated when the children were approximately 5 years of age. We sought to test the hypothesis that neuroanatomical characteristics observed early in life are strongly associated with diagnostic and functional outcome ascertained after age 5, when behavior-based diagnostic distinctions and long-term outcome are more reliable and predictable (Lord and Risi, 2000; Venter et al., 1992). Within the diagnosis of autism, there is a great deal of variability in level of function, with approximately 75% of children being mentally retarded and most children having limited functional language. We therefore also tested the hypothesis that differences in functional level at the time of outcome diagnosis (e.g., language skills, IQ level) are related to earlier differences in neural growth patterns. We hypothesized that while whole

brain volume may be abnormally large across young children with a diagnosis of ASD, children with less severe symptoms (and correspondingly better functional outcome) would perhaps have less severe overgrowth on more specific neuroanatomical measures or perhaps when a combination of variables was used. Our prospective diagnosis approach provided an avenue for examination of these hypotheses. Discriminant function analysis was used to determine how children who were separated into groups based on their behavioral profiles would be classified based on MRI data.

METHOD

The Institutional Review Board of Children's Hospital San Diego approved the study. Informed consent was obtained from each participant's parents after the study procedures were explained. All subjects were paid for their participation.

ASD Group

Autism is more common in boys than girls, and therefore fewer girls (15%) than boys (85%) were originally identified and recruited for this study. Significant morphometric differences between male and female neuroanatomy are discernible in children even as young as age 2 years (Courchesne et al., 2001; Durston et al., 2001). This presents difficulties in attempting to compare neuroanatomical abnormalities in boys and girls with ASD. We therefore chose to compare the behavioral and MRI results of only the boys who received an outcome diagnosis of autism with the boys who received an outcome diagnosis of PDD-NOS.

Prospective Diagnosis Procedure. Children were initially recruited for the ASD group if they had a clinical presentation consistent with autism or PDD-NOS, as determined by at least one experienced clinician. At referral, the parent of each potential participant answered a series of questions in a phone screen and completed a medical and family history questionnaire. Any child who had a diagnosable medical condition that might affect brain development, such as fragile X, a known brain lesion, history of exposure to teratogens, an infectious or metabolic disorder, epileptic aphasia, or tuberous sclerosis, was not included in the study.

Potential participants were next given a set of tests to determine an initial diagnosis. Initial diagnostic testing was completed when these 52 children were aged 1.9 to 4.8 years (mean 3.4 ± 0.7 years). In terms of maternal level of education, 48% had at least a degree from a 4-year college, 35% had some college education, 15% were high school graduates, and 2% did not have a high school diploma. Overall, 86% were white. Evaluation was similar across all participants; slight differences in the diagnostic measures used were contingent on the availability of test editions at the time of data collection. Each child was administered at least one diagnostic test—the Childhood Autism Rating Scale (CARS) (Schopler et al., 1988), the Pre-Linguistic Autism Diagnostic Observation Scale (PL-ADOS) (DiLavore et al., 1995), or the Autism Diagnostic Observation Schedule-Generic (ADOS) (Lord et al., 2000)—and a parent interview—the Autism Diagnostic Interview (ADI) (Le Couteur et al., 1989) or the Autism Diagnostic Interview-Revised (ADI-R) (Lord et al., 1994), or a developmental history. Examiner reliability in administration and scoring of the ADI, ADI-R,

PL-ADOS, and ADOS was established through examiner training and coding of the standard international pack of videotapes with Dr. Catherine Lord, one of the developers of these instruments.

Following initial diagnostic testing, each child was given a comprehensive medical and neurological examination by a pediatric neurologist to describe the clinical neurological characteristics of the young participants and to ensure they did not have any diagnosable medical condition that would exclude them from the study. DNA fragile X analysis, chromosome analysis, and in most cases a clinical EEG were performed. MRI scanning was conducted when the children were aged 1.9 to 5.2 years (mean = 3.8 ± 0.8 years).

Final Diagnosis Procedure. While clinically significant signs of autism are present by the second or third year of life, a diagnosis of autism is considered most reliable when a child is at least 4 years old (Lord and Risi, 2000). Therefore, all the participants were reevaluated as close to age 5 as possible. The reevaluation battery consisted of the ADI-R, ADOS, CARS, and the appropriate standardized psychological tests for each child's functional level. The ADI-R and the CARS were completed for each child by at least one clinical psychologist from the laboratory. If a child had achieved at least three-word phrase speech at the time of the final ADI-R, algorithm items for both nonverbal and verbal communication were used to compute the communication domain score (Lord et al., 1994). Level of functioning was evaluated using one or more standardized tests including the Leiter International Performance Scale (Arthur, 1980), the Stanford-Binet IV (Thorndike et al., 1986), the Wechsler Intelligence Scale for Children III (Wechsler, 1991), the Mullen Scales of Early Learning (Mullen, 1995), the Peabody Picture Vocabulary Test-Revised (Dunn and Dunn, 1981), and the Vineland Scales of Adaptive Behavior (Sparrow et al., 1984). The specific tests administered were selected on a case-by-case basis depending on the child's age and level of ability. Fourteen of the lower-functioning participants were not able to achieve above basal level on the verbal tests. One examiner (C.L.), who was blind to all previous information other than the child's expressive language level, administered the appropriate module of the ADOS to each child. The appropriate ADOS module is selected based on the child's age and/or expressive language level (preverbal or single words, phrase speech, fluent speech/adolescent, fluent speech/adult). The CARS was also completed at the conclusion of the testing.

Based on these test data, the clinical psychologists reached a final best estimate diagnostic decision. To be diagnosed with autism, a child had to meet ADI-R, ADOS, and *DSM-IV* criteria for autistic disorder. Children identified as having intellectual and adaptive abilities in the severe to profound level of mental retardation (IQ < 35 or mental age < 18 months) were not included in the analysis. Of the original sample of boys in the study, seven boys who were reevaluated after age 5 were found to be severely/profoundly mentally retarded and thus were excluded from the present study.

Children who did not meet both ADOS and ADI-R cutoffs for autism but had enough positive symptoms to meet the *DSM-IV* criteria for PDD-NOS received a diagnosis of PDD-NOS. If ADI-R criteria for autism were not met in all three domains, a diagnosis of PDD-NOS was considered if a child obtained a score within 2 points of the cutoff on the ADI-R reciprocal social interaction domain and within 1 point of the cutoff on either the communication or repetitive behaviors and stereotyped patterns domain. If ADOS criteria for autism were not met, a diagnosis of PDD-NOS was considered if a child at least met ASD criteria in the social domain on the ADOS. If a child obtained scores on the ADI-I and ADOS in the autism range, the clinicians used a final best estimate diagnostic decision to determine whether other factors

may have inflated the scores and a diagnosis of PDD-NOS was more appropriate.

Typical Development Control Group

This group consisted of 15 typically developing, healthy boys aged 1.7 to 5.2 years (mean = 3.6 ± 1.1 years) at the time of MRI scanning. In terms of maternal level of education, 20% had at least a degree from a 4-year college, 13% had some college education, and 40% were high school graduates. Overall, 60% were white. Participants were recruited through advertisements in the community. Based on information obtained through interview and responses to the medical, family, and educational history questionnaire, none of these children showed evidence of developmental, educational, medical, psychological, or psychiatric abnormalities. Depending on the child's age at the time of testing, verbal and nonverbal abilities were evaluated with one of the following tests: the Stanford-Binet IV (Thorndike et al., 1986), the Wechsler Intelligence Scale for Children III (Wechsler, 1991), the Mullen Scales of Early Learning (Mullen, 1995), the Leiter International Performance Scale (Arthur, 1980), the Peabody Picture Vocabulary Test-Revised (Dunn and Dunn, 1981), or the Differential Abilities Scales (Elliott, 1990). Standard scores obtained ranged from 86 to 132 for Verbal IQ (mean = 111.9) and 90 to 140 for nonverbal IQ (mean = 108.1). Data from 13 of these 15 children (87%) were included in the control group in our previous paper on cerebral and cerebellar volume in autism (Courchesne et al., 2001).

MRI Procedures

Imaging and Image Processing Procedures. These procedures were described previously (Courchesne et al., 2000, 2001). All participants in the ASD group were scanned while under anesthesia (propofol). Participants in the typical development control group (CON) were scanned during natural sleep, although some remained awake during scanning. Scans were collected between 1992 and 1999 as part of a continuing project on brain growth in autism. Scans for both patients and controls were collected throughout that period of time, rather than in any circumscribed blocks of time, on the same scanner.

To obtain images for calculating whole brain, cerebral, and cerebellar volumes, a dual-echo intermediate- and T2-weighted sequence was performed in the axial plane (repetition time [TR] = 3,000 ms, echo time [TE] = 30 and 80 ms, 1 NEX, field of view [FOV] = 20 cm, matrix = 256×256 , 3 mm interleaved [no gaps]) using a 1.5-Tesla GE Signa (Milwaukee, WI) MR scanner. Image data were coded so that operators were blind to each subject's identity, sex, age, and study group membership. For each brain dataset, a semiautomated three-dimensional local contrast algorithm, SEGMENT, was used to classify all pixels as gray matter, white matter, CSF, CSF partially volumed with skull, or other (Courchesne et al., 2000). Validation of SEGMENT in normal children and adults has been previously detailed (Courchesne et al., 2000). The smallest volume examined in the 3-mm axial scans was the cerebellum. In nearly all cases, the cerebellum was evident on at least 18 slices (typically 18–23 slices). Based on Cavalieri principles, this sample is more than adequate for a reliable estimate of volume for this structure. With a minimum of seven slices, a coefficient of error of <5% can be obtained. This error will be substantially less with 18 or more slices.

The midsagittal cerebellar vermis was imaged with a high in-plane resolution two-dimensional spin-echo T1 sagittal sequence (in-plane pixel area = $0.625 \text{ mm} \times 0.625 \text{ mm}$) (TR = 600 ms, TE = 25 ms, 2 NEX, FOV = 16 cm, matrix = 256×256 , 4 mm inter-

leaved [no gaps]). Localizer images were used during image acquisition to orient the sagittal T1 scan in the plane of the cerebellar vermis. When the T1 scan was complete, the images were examined immediately to ensure that a mid-vermis image had been acquired and that the lobules of the vermis were clear. If this was not the case, the orientation was further adjusted and the scan was repeated (see Courchesne et al., 1994a, and Courchesne and Plante, 1996, for a discussion of this subject).

Anatomical Parcellation

Brain volume was the sum of all gray and white matter voxel volumes, according to procedures previously described (Courchesne et al., 2000, 2001). Cerebral cortical boundaries were demarcated by raters experienced in neuroanatomy using manual tracing and explicitly defined anatomic landmarks and boundaries with the aid of neuroanatomical atlases (Duvernoy, 1991). Excluded from all cerebral measures were the corpus callosum, caudate nucleus, putamen, globus pallidus, diencephalon, ventricular CSF, brain stem, and cerebellum. Experienced raters, using appropriate neuroanatomical rules and reference to neuroanatomical atlases (Press and Courchesne, 1992; Schmahmann, 2000), demarcated cerebellar boundaries. Vermis lobules I–V and VI and VII were measured following procedures detailed in previous publications (Courchesne et al., 1994a, 1988; Saitoh et al., 1995).

Reliability. Rater reliability was established according to procedures used in our previous study (Courchesne et al., 2001). To establish interrater reliability, the raters traced eight cerebrums and nine cerebellums; statistical analyses revealed intraclass correlation coefficients of 0.99. To ensure the maintenance of intrarater reliability (i.e., no measurement drift), a series of reference cerebrums and cerebellums that the raters had previously measured were randomly distributed throughout the dataset (intraclass correlation coefficient = 0.99); raters were blind to which were reference and which were study brains.

RESULTS

Diagnostic and Behavioral Results

At the completion of the final diagnosis procedure for the 52 participants with ASD, 42 children (81%) were diagnosed with autism and 10 children (19%) were diagnosed with PDD-NOS. Data from 28 of the 42 children (67%) with a final diagnosis of autism were included in our previous paper on cerebral and cerebellar volume in autism (Courchesne et al., 2001). None

of the data from the children with a final diagnosis of PDD-NOS appeared in that paper.

Functional Outcome. The 10 children in the PDD-NOS group had Performance (nonverbal) IQ scores that ranged from 75 to 120 and Verbal IQ scores that ranged from 68 to 103. Data from the children in the PDD-NOS group were compared with children in the autism group who were similar in terms of (higher) intellectual ability. The autism group was therefore divided into two groups (Table 1). The 12 children in the higher-functioning autism (HFA) group were those who obtained a nonverbal IQ of 70 or above and a Verbal IQ of 70 or above; nonverbal IQs ranged from 72 to 122 and Verbal IQs ranged from 73 to 90. They ranged in age from 5.1 to 8.2 years at reevaluation (mean = 6.1 years). The 30 children in the lower-functioning autism (LFA) group had nonverbal IQs that ranged from 35 to 128 and verbal skills that ranged from nonverbal to a Verbal IQ of 78. They ranged in age from 4.0 to 10.0 years at reevaluation (mean = 6.2 years). Only one child was younger than age 5 at reevaluation (4.0 years) due to time constraints. This child was the last child scanned in the study (age 1.9). The 10 children in the PDD-NOS group ranged in age from 5.2 to 8.3 years at reevaluation (mean = 6.3 years).

Group data were compared using analysis of variance followed by Tukey honestly significant difference post hoc tests. There were no significant differences in age at the time of the MRI scan between the children in the four groups ($F_{3,63} = 1.50, p > .2$). The HFA, LFA, and PDD-NOS groups differed significantly in terms of nonverbal IQ ($F_{2,49} = 9.34, p < .001$) and Verbal IQ ($F_{2,35} = 33.53, p < .001$). The children in the LFA group had significantly lower nonverbal IQ and Verbal IQ scores than those in the HFA and PDD-NOS groups ($p < .05$), but the children in the HFA and PDD-NOS groups did not differ significantly from each other in nonverbal or Verbal IQ scores ($p > .05$).

TABLE 1
Group Characteristics at Time of Final Diagnosis

Group	<i>n</i>	Age	CARS	Nonverbal IQ	Verbal IQ
LFA	30 males	6.2 ± 1.1 (5.1–8.2)	39.0 ± 4.0	71.4 ± 24.0	44.4 ^a ± 18.4
HFA	12 males	6.1 ± 1.0 (4.0–10.0)	31.7 ± 4.9	97.9 ± 15.8	79.2 ± 5.94
PDD-NOS	10 males	6.3 ± .8 (5.2–8.3)	26.0 ± 4.8	95.4 ± 15.2	86.1 ± 13.5

Note: Data are presented as mean ± SD (range). CARS = Childhood Autism Rating Scale; LFA = lower-functioning autism; HFA = higher-functioning autism; PDD-NOS = pervasive developmental disorder-not otherwise specified.

^a Based on 16 of the subjects; the remaining 14 subjects were nonverbal.

The HFA, LFA, and PDD-NOS groups differed significantly in scores on the CARS (Table 1; $F_{2,49} = 38.11$, $p < .001$). The children in the LFA group obtained significantly higher scores on the CARS (i.e., more severe autism symptoms) than those in the HFA and PDD-NOS groups, and the children in the HFA group obtained significantly higher scores on the CARS than those in the PDD-NOS group ($p < .05$).

At age 5, 9 of the 10 children in the PDD-NOS group obtained scores above the autism cutoff in all three ADI-R domains, while one child did not receive a score above the cutoff in the repetitive domain. As expected, the ADI-R scores in the social and communication domains were significantly lower (i.e., less impaired) for the PDD-NOS group than for the LFA and HFA groups ($p < .05$; Table 2). The LFA and HFA groups did not differ significantly in ADI-R social and communication domain scores. There were no significant group differences in the repetitive behavior domain scores.

As expected, the distribution of ADOS modules administered differed across the three groups. Distributions of Module 1, Module 2, and Module 3 were as follows: in the LFA group, 25 (83%), 5 (17%) and 0 (0%); in the HFA group, 2 (17%), 7 (58%), and 3 (25%); and in the PDD-NOS group, 0 (0%), 3 (30%), and 7 (70%). Using the cutoff scores for each module, two (20%) of the children in the PDD-NOS group met ADOS threshold scores for autism. However, the clinicians believed that other factors appeared to have inflated the scores, and therefore the final best estimate diagnostic decision in these cases was a diagnosis of PDD-NOS. Three of the 10 children in the PDD-NOS group (30%) met the broader threshold scores for ASD on the ADOS and the 3 remaining children (30%) did not meet threshold scores for ASD in the communication plus social domain but met ASD criteria in the social domain.

While clearly meeting criteria for autism, the children in the HFA group exhibited less severe symptoms than the LFA group, as reflected by their lower scores on the CARS, better functional language, and higher nonverbal abilities. In turn, the children in the PDD-NOS group exhibited less severe symptoms than the children in the HFA group, as reflected by their lower scores on the CARS, ADI-R, and ADOS.

MRI Results

Group Measurement Comparisons. The group means and standard deviations are shown in Table 3. The HFA, LFA, PDD-NOS, and CON groups differed significantly in terms of whole brain volume ($F_{3,63} = 3.20$, $p < .05$). The LFA group had significantly larger whole brain volume than the CON group ($p < .05$). Although the HFA group mean was also greater than the CON group mean, the difference did not reach significance ($p = .08$). The groups differed in overall cerebral volume ($F_{3,63} = 2.82$, $p < .05$). Cerebral volume was significantly larger in the LFA group than the CON group ($p < .05$). Group differences in overall cerebellar volume approached significance ($F_{3,57} = 2.5$, $p = .07$), with the greatest difference between the HFA group and the CON group.

The groups did not differ significantly with respect to cerebral white matter volume ($F_{3,63} = 1.49$). There were significant group differences in cerebral gray matter volume ($F_{3,63} = 3.50$, $p < .05$). Cerebral gray matter volume was significantly larger ($p < .05$) for the LFA group than the CON group. Although there was a tendency for greater than normal cerebral gray matter volume in the HFA and PDD-NOS groups, these differences did not reach significance.

There were significant group differences in cerebellar white matter volume ($F_{3,57} = 15.32$, $p < .001$). As shown in Figure 1, the LFA, HFA, and PDD-NOS

TABLE 2
ADI-R Domain Scores for Each Group at Final Diagnosis

	LFA ($n = 30$)	HFA ($n = 12$)	PDD-NOS ($n = 10$)	ANOVA Results
Social interaction (cutoff = 10; max. = 30)	23.5 (4.1)	23.9 (4.6)	18.9 (6.5)	$F_{2,49} = 3.96$ $p < .05$
Communication: nonverbal (cutoff = 7; max. = 17)	10.9 (2.4)	11.0 (3.5)	6.0 (3.2)	$F_{2,49} = 12.0$ $p < .001$
Communication: nonverbal + verbal (cutoff = 8; max. = 26)	18.2 (3.3) ^a	18.9 (4.1)	14.6 (4.6)	$F_{2,32} = 3.7$ $p < .05$
Repetitive behavior (cutoff = 3; max. = 12)	6.6 (2.2)	5.9 (2.1)	7.4 (3.0)	$F_{2,49} = 1.11$ NS

Note: Data are presented as mean (SD). ADI-R = Autism Diagnostic Interview-Revised; LFA = lower-functioning autism; HFA = higher-functioning autism; PDD-NOS = pervasive developmental disorder-not otherwise specified; ANOVA = analysis of variance; NS = not significant.

^a Only 13 (43%) of the children in the LFA group had at least three-word phrase speech at the time of the final ADI-R.

TABLE 3
Group Means of the Neuroanatomical Measurements of Interest

	LFA (<i>n</i> = 30)	HFA (<i>n</i> = 12)	PDD-NOS (<i>n</i> = 10)	CON (<i>n</i> = 15)
Whole brain volume (mL)	1,280.49 ^a (100.17)	1,283.56 (125.31)	1,272.47 (79.6)	1,188.49 (92.77)
Overall cerebral volume (mL)	1,042.08 ^a (85.01)	1,043.51 (105.68)	1,033.02 (71.91)	967.43 (80.85)
Cerebral white matter volume (mL)	275.2 (31.9)	285.7 (32.7)	277.6 (14.0)	261.4 (34.0)
Cerebral gray matter volume (mL)	766.9 ^a (57.0)	757.8 (75.2)	755.45 (62.2)	706.0 (53.5)
Overall cerebellar volume (mL)	134.27 (12.87)	137.05 (14.0)	130.59 (12.91)	124.85 (8.43)
Cerebellar white matter volume (mL) ^c	32.1 ^a (3.6)	31.0 ^a (3.6)	31.9 ^a (4.5)	23.6 (4.3)
Cerebellar gray matter volume (mL) ^c	102.1 (10.3)	106.0 (12.6)	98.7 (11.5)	101.3 (5.1)
Anterior cerebellar vermis area (mm ²)	445.6 ^a (46.5)	478.8 ^a (42.5)	458.1 ^a (44.0)	406.8 (33.0)
Posterior cerebellar vermis area (mm ²) ^b	261.5 (36.2)	305.1 (46.9)	276.8 (43.0)	287.3 (44.8)

Note: Standard deviations are indicated in parentheses. LFA = lower-functioning autism; HFA = higher-functioning autism; PDD-NOS = pervasive developmental disorder-not otherwise specified; CON = control.

^a Group mean significantly different from control group mean.

^b LFA group mean significantly lower than HFA group mean.

^c LFA: *n* = 26 and CON: *n* = 13.

groups all had significantly larger than normal cerebellar white matter volume than the CON group ($p < .001$). The groups did not differ with respect to cerebellar gray matter volume ($F_{3,57} = 1.0$). As can be seen in Table 3, the range of measurements was larger in the patient groups than the CON group.

There were significant group differences in the mid-sagittal area of cerebellar vermis lobules I–V across the groups ($F_{3,63} = 6.80$, $p < .001$; Table 3). The

area of lobules I–V (anterior vermis) was significantly larger than normal in the HFA, PDD-NOS, and LFA groups. There was also a significant group difference with respect to area of cerebellar vermis lobules VI–VII ($F_{3,63} = 3.59$, $p < .05$; Table 3). The area of lobules VI and VII (posterior vermis) was significantly smaller in the LFA group compared to the HFA group, with a trend toward smaller than normal area of the posterior vermis in the children in the LFA group ($p = .07$).

Discriminant Function Analysis. Six predictor variables (cerebellar white and gray matter volumes, area of the anterior and posterior cerebellar vermis, and cerebral white and gray matter volumes) were entered into a discriminant function analysis as predictors of group membership. Six cases were dropped from the analysis due to missing cerebellar volume data (four in the LFA group and two in the CON group). The overall multivariate test of the analysis was significant (Wilks $\lambda = 0.32$, $\chi^2_{18} = 62.29$, $p < .0001$). The first discriminant function had an eigenvalue of 1.18. As shown in Table 4, cerebellar white matter volume made a significant contribution to the first discriminant function. The second discriminant function had an eigenvalue of 0.38. Three additional variables made a significant contribution to the second discriminant function (area of the anterior cerebellar vermis, area of the posterior cerebellar vermis, and cerebral white matter volume).

As shown in Figure 2, the first discriminant function maximally separated the LFA group from the CON group, with the PDD-NOS and HFA groups falling between these two groups. The second discriminant

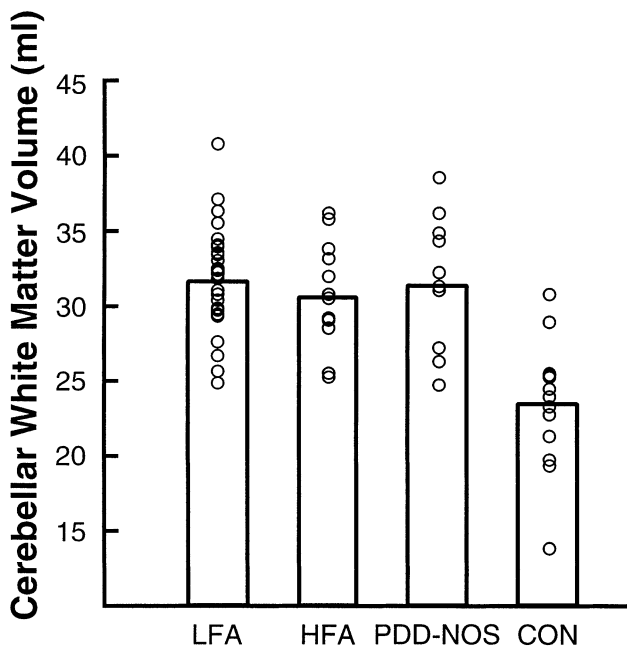


Fig. 1 Group mean volume of cerebellar white matter volume with individual values superimposed. LFA = lower-functioning autism; HFA = higher-functioning autism; PDD-NOS = pervasive developmental disorder-not otherwise specified; CON = control.

TABLE 4

Pooled Within-Groups Correlations Between Discriminating Variables and Standardized Canonical Discriminant Functions

	Function	
	1	2
Cerebellar white matter volume	0.794 ^a	0.395
Area of anterior vermis	0.238	0.792 ^a
Area of posterior vermis	-0.231	0.667 ^a
Cerebral white matter volume	0.223	0.365 ^a
Cerebellar gray matter volume	-0.023	0.218
Cerebral gray matter volume	0.326	0.164

^a Largest absolute correlation between each variable and any discriminant function.

function separated the HFA group from the CON and LFA groups, with the PDD-NOS group falling between the groups.

The subjects in the LFA, HFA, and CON groups were very well classified by the discriminant functions (Table 5). All of the children in the LFA group were classified with “autism” (22 as LFA and 4 as HFA). Classification for the HFA group was good, with 92% of the children classified as “ASD” (8 as HFA, 2 as LFA, and 1 as PDD-NOS). Similarly, 75% of these

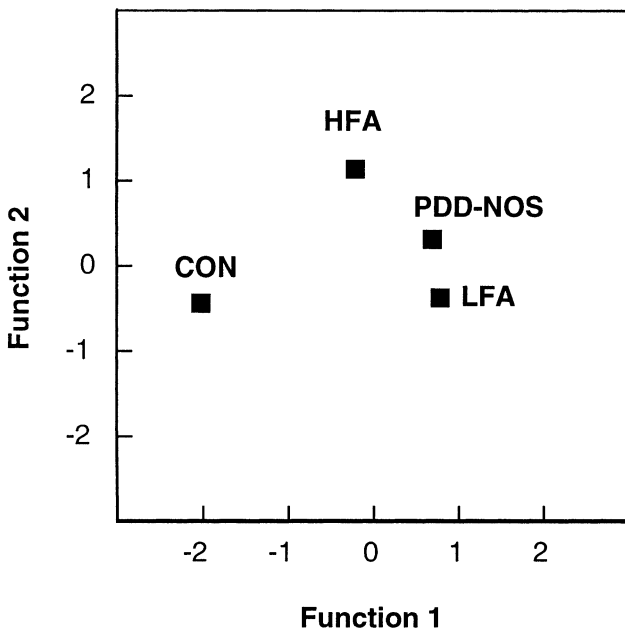


Fig. 2 Plot of the four group centroids on two discriminant functions derived from two cerebral and four cerebellar variables. Cerebellar white matter volume made the largest contribution to the first discriminant function. Area of the anterior vermis, area of the posterior vermis, and cerebral white matter volume made significant contributions to the second discriminant function. LFA = lower-functioning autism; HFA = higher-functioning autism; PDD-NOS = pervasive developmental disorder-not otherwise specified; CON = control.

TABLE 5

Classification Results From Discriminant Function Analysis

Actual Group	n	Predicted Group							
		LFA		HFA		PDD-NOS		CON	
	n	%	n	%	n	%	n	%	
LFA	26	22	84.6	4	15.4	0	0	0	0
HFA	12	2	16.7	8	66.7	1	8.3	1	8.3
PDD-NOS	10	5	50.0	3	30.0	1	10.0	1	10.0
CON	13	1	7.7	0	0	0	0	12	92.3

Note: LFA = lower-functioning autism; HFA = higher-functioning autism; PDD-NOS = pervasive developmental disorder-not otherwise specified; CON = control.

children were classified as “high functioning” (HFA or PDD-NOS). Classification was poorer for the PDD-NOS group, with 80% classified with “autism” (three as HFA and five as LFA) and only 10% classified as PDD-NOS. All but one of the control subjects was correctly classified. It appears that this one exception occurred due to his very large cerebellar white matter volume (see outlier on Fig. 1).

DISCUSSION

In this study, young children who met the criteria after age 5 for PDD-NOS as well as those who met the criteria for autistic disorder were successfully discriminated from typically developing children on the basis of a combination of neuroanatomical measures obtained from MRI. Despite the relatively small control group and the degree of overlap between the patient and control groups for some MRI measures, there was excellent separation between control and patient groups when all of the MRI variables were combined in a discriminant function analysis. The results also demonstrate that there are neuroanatomical differences between young children with autism who are higher functioning compared to those who are lower functioning.

The children in the PDD-NOS group were successfully discriminated from the typically developing children on the basis of morphometric variations. However, they were not as distinctly classified as the other three groups. In fact, they were more likely to be classified as LFA or HFA than as PDD-NOS or control cases. Additional studies are needed to determine whether other biological factors distinguish PDD-NOS, the milder variant, from autistic disorder. It is important to note that the children in the PDD-NOS group were initially recruited for the study because it

appeared likely that they would also continue to meet criteria for autism after age 5. Further study is needed to determine whether there were behavioral characteristics early on that were predictive of their later outcome. It is therefore possible that neuroanatomical measures obtained earlier in development are useful to distinguish these groups.

The neuroanatomical similarities and differences between the higher-functioning and lower-functioning children with autism are intriguing. Since cerebellar white matter volume was significantly larger in all three patient groups compared to the control group, these young children with ASD may all share a common pathological finding. On the other hand, MRI studies of high-functioning older children and adults with autism have concluded that the size of the anterior and posterior cerebellar vermis does not differ from normal (Hardan et al., 2001; Piven et al., 1997), while studies that include both low- and high-functioning individuals consistently report decreased size of the posterior vermis (Courchesne et al., 1994b, 1988; Hashimoto et al., 1995). It may be that less loss in the posterior cerebellar vermis early in life is associated with better functional outcome, while abnormalities in other parts of the cerebellum and in the cerebrum are associated with the persistent autistic symptoms observed in these children. The cerebellum has been shown to be important for and functionally active in a variety of cognitive tasks (Allen et al., 1997; Gao et al., 1996; Kim et al., 1994; Schmahmann, 1997). Abnormal development of cerebrocerebellar connections would therefore be expected to interfere with the development of a variety of functions. If this includes both the cerebellar white matter and the vermis, this may lead to poorer outcome.

Increased brain volume, particularly white matter volume, would be expected to have a significant impact on early neural and behavioral development. At this point, the etiology of what appears to be increased brain volume in autism is not known, but some recent pieces of evidence are consistent with this finding. Abnormally high levels of certain neonatal brain growth factors have been reported for children later diagnosed with autism (Nelson et al., 2001). A recent postmortem study reported minicolumnar abnormalities in the brains of patients with autism, suggestive of an abnormal increase in the number of processing units in the autistic brain (Casanova et al., 2002). Increased brain volume could also indicate a delay in the timing of the normal course of neuronal pruning and synapse elimination in autism, resulting in a period of apparent

“overgrowth” followed by possible excessive elimination or reduction in some regions (Akshoomoff et al., 2002; Carper et al., 2002). Each of these possibilities would potentially result in poorly controlled functioning at a neural and behavioral level, consistent with many of the symptoms and delays commonly observed in young children with autism.

Limitations

There are limitations to be noted. The sample sizes are relatively small, primarily due to the difficulties inherent in a study of this type with young children. Mean whole brain volume and cerebral gray matter volume was abnormally large in the LFA group, and although the means for the HFA and PDD-NOS group were larger than the control group, the differences did not reach significance. This may be due to the disparity in group size, such that if there had been more subjects in the HFA and PDD-NOS groups, these differences would have also reached significance. A replication study with a new sample of subjects is necessary to test the results of the discriminant function analysis. We are currently conducting such a study. The combination of neuroanatomical measures in the current study led to near-perfect separation of patients from controls but provided less specificity with regard to autism versus PDD-NOS. Although it is difficult to interpret negative findings, it is possible that there is little difference from a neuroanatomical perspective between relatively high-functioning young children who do not meet full criteria for autistic disorder and those who do. Alternatively, MRI data obtained earlier in life may give insight into the earliest manifestations of pathology and thus lead to better classification of children according to functional outcome. Finally, these MRI results do not address the issue of specificity relative to other developmental disorders. The results of these analyses suggest that lower intellectual functioning in ASD is associated with overall larger total brain volume during early childhood. We are unaware of any other developmental disorder where this type of association has been observed. This finding may indicate that if excessive brain growth associated with autism is more pervasive, this leads to poorer outcome.

Clinical Implications

Implementation of a statistically based “neuroanatomical profile” for classification of individual cases would require normative data based on age and sex and common methods of quantitative analysis of data ob-

tained from MRI scans. In the present study, some of the children were close to 5 years of age at their initial MRI scan, calling into question the added benefit of MRI data over careful psychological examination. Diagnostic testing relies on experienced behavioral clinical procedures. Further study is needed to determine whether in the future MRI data of this type would provide a significant added benefit in diagnosis and treatment recommendations for very young children or children at risk for developing ASD. It may also serve as a component of subject characterization in clinical treatment trials with young children to assist in determining how a child's level of functioning and biological characteristics are predictive of treatment response. Identification of reliable, common, and potentially fundamental biological defects also has significant implications for quantitative trait genetic linkage analyses and animal models of the neurodevelopmental abnormalities in autism.

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