A Comprehensive Volumetric Analysis of the Cerebellum in Children and Adolescents with Autism Spectrum Disorder

Julia A. Scott, Cynthia Mills Schumann, Beth L. Goodlin-Jones, and David G. Amaral

Magnetic resonance imaging (MRI) and postmortem neuropathological studies have implicated the cerebellum in the pathophysiology of autism. Controversy remains, however, concerning the nature and the consistency of cerebellar alterations. MRI studies of the cross-sectional area of the vermis have found both decreases and no difference in autism groups. Volumetric analysis of the vermis, which is less prone to ''plane of section artifacts'' may provide a more reliable assessment of size differences but few such studies exist in the literature. Here we present the results of a volumetric analysis of the structure of the whole cerebellum and its components in children and adolescents with autism spectrum disorders. Structural MRI's were acquired from 62 male participants (7.5 to 18.5 years-old) who met criteria for the following age-matched diagnostic groups: low functioning autism, high functioning autism (HFA), Asperger syndrome, and typically developing children. When compared to controls, the midsagittal area of the vermis, or of subgroups of lobules, was not reduced in any of the autism groups. However, we did find that total vermis volume was decreased in the combined autism group. When examined separately, the vermis of only the HFA group was significantly reduced compared to typically developing controls. Neither IQ nor age predicted the size of the vermis within the autism groups. There were no differences in the volume of individual vermal lobules or cerebellar hemispheres. These findings are discussed in relation to the pathology of autism and to the fairly common alterations of vermal morphology in various neurodevelopmental disorders.

Keywords: Asperger; MRI; developmental delays; vermis; neurodevelopmental disorder

Introduction

Autism is a neurodevelopmental disorder characterized by impaired social interaction, deficits in communication, and restricted activities or interests [American Psychiatric Association, 1994]. The potential role of the cerebellum in the pathophysiology of autism spectrum disorders has been explored by many groups (Table I). Subject ages and clinical characteristics in these studies vary widely and conclusions concerning the type and consistency of cerebellar pathology remain controversial. Some studies suggest that cognitive functions that are mediated, in part, by the cerebellum, such as imitation, are impaired in autism [Rogers, Hepburn, Stackhouse, & Wehner, 2003; Williams, Whiten, Suddendorf, & Perrett, 2001].

Previous MRI studies have found that the midsagittal area of the vermis, particularly lobules VI–VII, is reduced in idiopathic autism and autism with co-morbid conditions, such as Down syndrome [Courchesne et al., 1988, 1994; Hashimoto et al., 1995; Kaufmann et al., 2003; Schaefer et al., 1996]. Other studies, however, have found no evidence of vermal hypoplasia in autism [Holttum et al., 1992; Kleiman, Neff, & Rosman, 1992; Manes et al., 1999]. A recent meta-analysis of the issue of vermal hypoplasia [Stanfield et al., 2008] concluded that the area of lobules I–V and VI–VII of the vermis are reduced in individuals with autism compared to controls [Stanfield et al., 2008]. Moreover, the authors suggested that the vermal reduction was negatively associated with age and IQ.

Postmortem neuropathological studies also suggest that the cerebellum may be abnormal in autism; lower Purkinje cell density has been reported for both the vermis and hemispheres [Bailey et al., 1998; Kemper & Bauman, 2002]. A caveat to these studies is that Purkinje cell loss is associated with seizure history [Crooks, Mitchell, & Thom, 2000], which was present in some cases. However, Whitney, Kemper, Bauman, Rosene, and Blatt [2008] recently suggested that only about half of postmortem autism cases show a lower density of Purkinje cells [Whitney et al., 2008].

Part of the ambiguity related to cerebellar pathology in autism may also be due to limitations in the strategy employed in early MRI analyses. For example, most studies measured the midsagittal area of the vermis as

Additional Supporting Information may be found in the online version of this article.

From the Department of Psychiatry and Behavioral Sciences, University of California, Davis, M.I.N.D. Institute, Sacramento, California (J.A.S., C.M.S., B.L.G.-J., D.G.A.)

Received February 10, 2009; revised August 31, 2009; accepted for publication September 3, 2009

Address for correspondence and reprints: David G. Amaral, University of California, Davis, The M.I.N.D. Institute, 2825 50th Street, Sacramento, CA 95817. E-mail: dgamaral@ucdavis.edu

Grant sponsor: NIH; Grant numbers: MH41479; MH01832; MH01142; MH50047; NS16980; HD31715; Grant sponsor: UC Davis M.I.N.D. Institute.
Published online 2 November 2009 in Wiley InterScience (www. interscience.wiley.com)
DOI: 10.1002/aur.97

Table I. Main Findings on Cerebellar Structure in Autism Spectrum Disorder

First author, year	Age range	ASD group	Comparison group(s)	Exclusion criteria	Measurement	Primary finding(s)	Statistical significance
Courchesne, Yeung-Courchesne, Press, and Hesselink [1988]	6–30 years	18 ASD 16 Male 2 Female	12 Typically–developing (TD) 9 Male 3 Female	Mental retardation Comorbid neurological conditions	Area of vermal lobules I–V, V–VII, VIII ROI	Reduced area of VI–VII	I–V, $P > 0.55$ VI–VII, $P = 0.003$ VIII, $P > 0.55$
Piven et al. [1992]	18–53 years	15 ASD 1 subject, 8 years	15 matched for IQ 15 matched for age and SES	Seizure history Female	Area of vermal lobules I–V, VI–VII, fourth ventricle, midsagittal brain area ROI	Reduced area of VI–VII when compared to age-matched but not IQ-matched group No differences in the area of fourth ventricle Larger brain area in HFA	Used ratio measures No difference after a multivariance anaylsis was adjusted for brain area, age and IQ
Holttum, Minshew, Sanders, and Phillips [1992]	11–42 years	18 HFA	18 TD matched for age, gender, SES, IQ, race	Mental retardation Comorbid neurological conditions Female	Area of vermal lobules I–V, VI–VII, VIII, fourth ventricle ROI	No differences found	I–V, $P = 0.34$ VI–VII, $P = 0.31$ VIII–X, $P = 0.15$
Kleiman et al. [1992]	2.7–16.8 years	13 ASD 3 Seizure history 10 Seizure history absence	28 controls 11 Seizure history 17 Seizure history absence	History of ataxia, tremor or clumsiness	Area of vermal lobules I–V, VI–VII Fourth ventricle height Computer-assisted planimetry	No differences found in comparison of seizure history groups No differences found in comparison of absence of seizure history groups	I–V, $P = 0.99$ VI–VII, $P = 0.45$ Fourth ventricle, $P = 0.17$
Courchesne, Townsend, and Saitoh [1994]	2–40 years	32 ASD 25 Male 7 Female	41 TD 34 Male 7 Female	Fragile X syndrome	Area of vermal lobules I–V, V–VII ROI	Reduced area of VI–VII	I–V, $P = 0.71$ VI–VII, $P = 0.031$
Hashimoto et al. [1995]	3 months–20 years	102 ASD 76 Male 26 Females	122 TD	Hyperactivity disorder	Area of vermal lobules I–V, VI–VII, VIII–X Area of brainstem: midbrain, pons, medulla oblongata ROI	Accelerated growth of I–V, VI–VII, and pons Reduced area of vermis and brainstem structures	Vermis, $P = 0.001$ in ASD and TD Brainstem, $P = 0.001$ in ASD and TD
Schaefer et al. [1996]	0–90 years	13 ASD	125 TD 89 Neurogenetic Disorders	None	Area of vermal lobules I–V, VI–VII, VIII–X	Reduced area of VI–VII in some Neurogenetic Disorder groups with and without autistic traits	VI–VII, $P < 0.05$ in Retts syndrome and Chiari malformations
Piven et al. [1997]	12–29 years	35 ASD 26 Male 9 Female	36 TD 20 Male 16 Female	Comorbid neurological conditions	Area of lobules I–V, VI–VII Volume of total cerebellum ROI	No area difference in I–V, VI–VII Larger cerebellar volume without correction for TBV	I–V, $P = 0.59$ VI–VII, $P = 0.89$ Uncorrected cerebellar volume, $P = 0.0002$ Corrected cerebellar volume for TBV and IQ, $P > 0.05$

Table I. Continued

First author, year	Age range	ASD group	Comparison group(s)	Exclusion criteria	Measurement	Primary finding(s)	Statistical significance
Manes et al. [1999]	6–21 years	27 LFA	17 matched for mental age	CARS Score > 30 in comparison group IQ tested in only 4 of the comparison group and 1 LFA	Area of I–V, VI–VII, VIII–X as a proportion of intracranial area ROI	No difference in area	I–V, $P = 0.87$ VI–VII, $P = 0.68$ VIII–X, $P = 0.92$
Hardan, Minshew, Harenski, and Keshavan [2001]	12–52 years	22 ASD	22 TD matched for IQ, age, race, SES, gender	Comorbid developmental or neurological conditions Seizure history Female	Volume of cerebellum and vermis Area of vermal lobules I–V, VI–VII, VIII–X ROI	Larger cerebellar volume with correction for TBV No differences in vermal area	Cerebellar volume, $P = 0.03$ Cerebellar hemispheres, $P = 0.05$ Vermis, $P = 0.23$
Courchesne et al. [2001]	2–16	60 ASD	52 TD	Female Comorbid conditions PDD	Gray and white matter volumes Area of vermal lobules I–V, VI–VII, VIII–X	From 2–3 years, greater white matter in ASD Less gray matter in ASD at all ages Reduced VI–VII at all ages	White matter volume, $P < 0.001$ Gray matter volume, $P < 0.01$ VI–VII area, $P < 0.05$ one-tailed
Sparks et al. [2002]	3–4.5 years	45 ASD 38 Male 7 Female	26 TD 14 DD 16 PDD-NOS	Comorbid neurological conditions	Volume of cerebellum Cavalieri grid	Larger cerebellar volume was proportional to TBV	Uncorrected cerebellar volume for ASD v. TD, $P = 0.03$ Corrected for ASD v. TD, $P > 0.05$
Herbert et al. [2003]	7–11 years	17 ASD	15 TD	Mental retardation Seizure history History of head injury Sensorimotor deficits Encephalopathy Female	Volume of gray and white matter in total brain and cerebellum Voxel-based morphometry	Larger cerebellar volume was proportional to TBV	Uncorrected cerebellar volume, $P = 0.009$ Corrected cerebellar volume for TBV, $P > 0.05$
Kaufmann et al. [2003]	3–9 years	39 ASD 10 Idiopathic autism 16 Down Syndrome+ autism 13 Fragile X+ autism	22 TD 11 Down Syndrome only 9 Fragile X only Matched for age	History of mental health issues Female	Area of vermal lobules I–V, VI–VII, VIII–X Vermal measures are expressed as ratios of intracranial area ROI	Reduced area of VI–VII and VIII–X in FX+autism and DS+autism Reduced area of VI–VII in idiopathic autism VI–VII:intracranial area dependent on autism status only in FX	DS:VI–VII, $P = 0.010$ VIII–X, $P = 0.003$ FX:VI–VII, $P = 0.003$ VIII–X, $P = 0.086$ ASD: VI–VII, $P = 0.052$ Intracranial area in DS and DS+autism compared to TD, $P = 0.0001$

McAlonan et al. [2005]	8–14 years	17 HFA 16 Male 1 Female	17 TD 16 Male 1 Female	Co-morbid psychiatric or medical conditions Mental retardation History of head injury Fragile X syndrome	Volume of gray and white matter and CSF in total brain and cerebellum Voxel-based morphometry	Reduced total gray matter in ASD Increased CSF volume in ASD Decreased white matter in cerebellum in ASD	Total Gray Matter, $P = 0.004$ CSF, $P = 0.008$ Total White Matter, NS Whole Brain Volume, NS
Palmen et al. [2005]	7–15 years	21 HFA, medication-naive	21 TD	Epilepsy, head trauma, and other neurological illness Mental retardation	Volume of gray and white matter and CSF in total brain and cerebellum Semi-automated method	Larger cerebellar volume was proportional to TBV Larger CSF volume disproportionate to TBV	Uncorrected cerebellar volume, $P = 0.032$ Uncorrected lateral ventricle $P = 0.032$ Uncorrected third ventricle $P = 0.001$
Hazlett et al. [2005]	1.5–3 years	51 ASD	14 TD 11 DD	Epilepsy, Fragile X syndrome, head trauma, and other neurological illness	Volume of gray and white matter and CSF in total brain and cerebellum Estimation Maximizati on Software	Cerebellar volume was not enlarged compared to TD and DD	NS differences in total volume, GM, or WM
Rojas et al. [2006]	7–44 years	24 ASD	23 TD	Female Fragile X	Voxel-based morphometry	Reduced left and right hemispheric lobule VII Reduced lobules VIII–IX along midline	Reduced regions, $P < 0.05$ with small volume corrections
Hallahan et al. [2009]	18–58 years	80 ASP (71 Male) 28 ASD (21 Male) 6 PDD-NOS (4 Male)	60 TD (53 Male)	Comorbid medical condition, head injury, psychosis, genetic disorder associated with autism	Volume of intracrainal space, cortical lobes, cerebellum, ventricular CSF, and peripheral CSF ROI	Reduced cerebellum volume in all groups Increased peripheral CSF in all groups	Reduced cerebellum and increased peripheral CSF, $P < 0.05$ with intracranial volume as a covariate
Webb et al. [2009]	36–58 months	45 ASD (38 Male)	26 TD (18 Male) 14 DD (6 Male)	Comorbid medical condition, perinatal trauma, genetic disorder	Volume of the cerebrum and cerebellum; area of vermis lobules I–V, VI–VII, VIII–X	Reduced area of lobules I–V and VI–VII compared to TD DD reduced areas and volumes compared to TD	Reduced regions, $P < 0.001$ with age, gender and cerebellar volume as covariates

ASD, autism spectrum disorder; DD, developmental delay; DS, Down syndrome; FX, Fragile X syndrome; ROI, region of interest; TBV, total brain volume; TD, typically developing.

well as defined lobule groups [Courchesne et al., 1988; Gaffney, Tsai, Kuperman, & Minchin, 1987; Holttum et al., 1992]. The cross-sectional area measurement is dependent on only a single slice through the vermis. Since slight variations in the orientation of the head can profoundly affect the size of the vermal region, so-called ''plane of section'' artifacts may be easily introduced. The midsaggital area measurement also does not assess more laterally placed portions of the vermis. Recent volumetric studies of the cerebellum have not addressed the question of vermal abnormalities [Hazlett et al., 2005; McAlonan et al., 2005; Palmen et al., 2005] but examined total volume and tissue class volume differences.

The goal of this study was to carry out a comprehensive MRI volumetric analysis of the cerebellum in male children and adolescents with low and high functioning autism (HFA), Asperger syndrome and typically developing controls. Traditional midsaggital measurements were carried out with careful alignment of the brain. In addition, the volume of the vermis, vermal lobule groups, and cerebellar hemispheres were measured.

Methods

Participants

A parent or guardian for each study participant gave informed consent, and children with typical cognitive development gave their assent, to participate in these studies as approved by the Institutional Review Board of the University of California, Davis. Study participants were recruited through local advocacy groups and the M.I.N.D. Institute clinic. Seventy-two male volunteers between the ages of 7.5 and 18.5 years participated in the study. All participants were healthy volunteers who met criteria in one of four diagnostic groups: low functioning autism (LFA, $n = 19$), HFA ($n = 19$), Asperger syndrome (ASP, $n = 16$), and typically developing controls (CON, $n = 18$).

Diagnostic assessments were conducted at the M.I.N.D. Institute clinic. The Autism Diagnostic Interview (ADI-R) [Lord, Rutter, & Le Couteur, 1994] and Autism Diagnostic Observation Schedule (ADOS-G) [DiLavore, Lord, & Rutter, 1995; Lord et al., 2000] were administered by a clinician (B.L.G.J.), who had previously obtained reliability with an author of these measures (C. Lord). An IQ exam was administered to all participants. Depending on verbal ability, the appropriate test was used from the following: the Wechsler Intelligence Scale for Children, the Wechsler Abbreviated Scale of Intelligence [Wechsler, 1999] or the nonverbal Leiter International Performance Scale-Revised [Roid & Miller, 1997]. A full scale IQ of 70 divided the high and low functioning autistic groups. Exclusionary criteria included diagnosis of Fragile X, seizure disorder, tuberous sclerosis, a primary diagnosis of obsessive–compulsive disorder, bipolar disorder or any other major neurological illness. Details of the diagnostic assessments are available in a previous publication that included this cohort of subjects [Schumann et al., 2004].

Neuroimaging

A parent or guardian for each participant was present throughout the duration of the scan in an adjacent waiting room. Those study participants requiring anesthesia to undergo MRI were imaged at the UC Davis Hospital on a 1.5T GE Signa NV/I system (LFA, $n = 19$; HFA, $n = 13$; ASP = 7). All remaining participants were scanned at the UC Davis Research Imaging Center on a 1.5T GE Signa NV/I system (HFA, $n = 6$; ASP, $n = 9$; CON, $n = 18$). These systems were calibrated prior to scan acquisition and similar image acquisition on both scanners was experimentally validated [Lotspeich et al., 2004].

The protocol for scanning each participant included a three-dimensional coronal SPGR series (TR: 35 ms; TE: 6 ms; FOV: 24 cm; matrix: 256×256; section thickness: 1.5 mm; number of slices: 124; total scan time: 14:24 min), which was used for the volumetric assessment of the cerebellum. In addition, a two-dimensional sagittal T1 spin echo, two-dimensional PD/T2 interleaved double echo, and a diffusion tensor sequence were collected on all participants for other analyses.

Upon review of the images, ten participants were excluded from the study due to excessive movement, distorted images resulting from orthodontics, or additional diagnostic information that precluded the series from being used (LFA, $n = 1$; HFA, $n = 4$; ASP = 1; CON, $n = 4$). Within each diagnostic group, excluded participants did not differ from included participants with respect to age, IQ, or symptom severity.

Structural Analysis

Each coronal SPGR series was imported into ANALYZE 6.0 [Robb et al., 1989] and converted to cubic voxel dimensions of 0.9375 mm using a cubic spline interpolation algorithm. Images were reoriented along an axis through the anterior and posterior commissures. Measurements of total cerebral volume used in the current study were described in a previous report [Schumann et al., 2004]. Briefly, each series of images was edited manually to remove nonbrain structures, the brainstem, and the cerebellum. Using a Gaussian cluster multispectral thresholding tool, the ventricles were defined and excluded. Total cerebral volume was calculated from a mask of the remaining brain tissue.

Prior to volumetric analyses, the midsagittal area of the cerebellar vermis was measured (Fig. 1A). The vermis was outlined on a single section that approximated as closely as possible the midline of the brain. The vermis was subdivided into lobule groups including lobules I–V, VI–VII, and VIII–X along the primary and prepyramidal fissures.

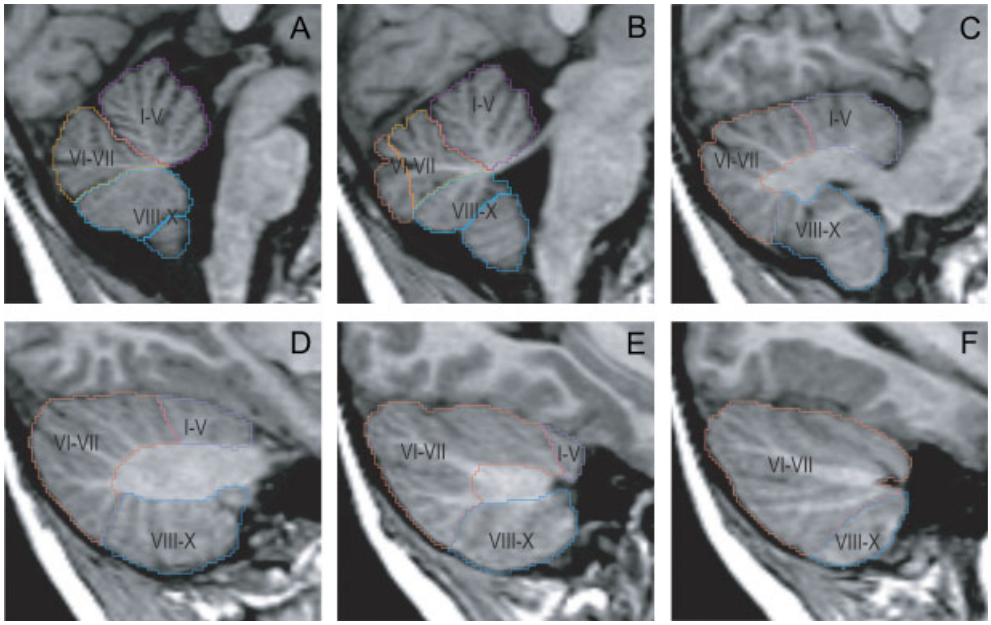

Figure 1. Sagittal series of MRI sections illustrating lobar segmentation of the cerebellum. Panels are arranged from midsagittal (top left) to lateral (bottom right). Lobule groups: I–V, lobules one through five; VI–II, lobules six through seven; VIII–X, lobules eight through ten. Light colored profiles are of vermal lobule groups; darker colors indicate hemispheric lobule groups.

The whole cerebellar volume was also measured. The total volume was segmented into a medullary core (the central white matter and deep nuclei of the cerebellum), the hemispheres (cortex and white matter), and the vermis (midline region of the cerebellum) (Fig. 2A). The vermis and hemispheres were separately subdivided into lobules I–V, VI–VII, and VIII–X (Figs. 1 and 2B). All structures were manually defined by a set of raters who achieved greater than 0.96 inter- and intrarater reliability on each of the structures. *The MRI Atlas of the Cerebellum* [Schmahmann, Doyon, Toga, Petrides, & Evans, 2000] and *The Human Cerebellum* [Angevine, Mancall, & Yakovlev, 1961] were closely consulted in the development of the region of interest tracing protocols. Detailed protocols for analysis of all of the cerebellar structures are provided in the Supplemental Materials.

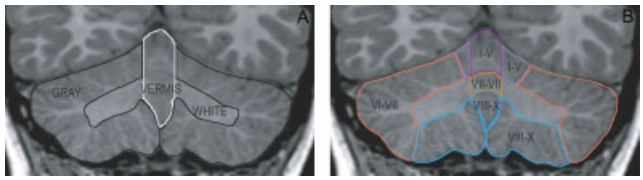

Figure 2. Coronal sections illustrating segmentation into whole cerebellar structures (A) and lobar structures (B). GRAY includes the cortex of the hemispheres; WHITE includes the medullary core and deep nuclei.

Statistical Analyses

All statistical analyses were conducted with SPSS 16.0 (SPSS Inc., Chicago, Illinois). Prior to analysis of the anatomical data, age and IQ were compared between groups by an analysis of variance (ANOVA) to detect any group differences. Tukey's post hoc test followed up on any main effects.

Due to the small and uneven sample size of the groups, there was a potential for the data to be distributed nonparametrically or adversely influenced by outliers. Tests of kurtosis and skewness were conducted on each anatomical measure to determine which type of analysis of variance should be used. The data were sufficiently normally distributed and only one measure, medullary core, was skewed with a right-handed tail.

Since all of the data were normally distributed, univariate and multivariate general linear models (GLM) were used to compare anatomical structures between groups. Age and total cerebral volume were entered as covariates in each analysis. Simple contrasts were made with the typically developing group as the reference category. Tests were conducted at each anatomical level. Univariate GLM was applied to vermis area and a multivariate GLM was applied to the analysis of the area of vermal lobule groups. A univariate GLM was used for the cerebellum volume. Separate multivariate GLM were conducted for major parts of the cerebellum, and the lobule groups on the left and right hemispheres and vermis. A significance level of a two-tailed α of 0.05 was selected a priori. These analyses were repeated for comparison of the collective autism spectrum group to the typically developing group without specific contrasts.

Subregions of the cerebellum were also analyzed as a ratio to the total cerebellar volume (i.e. normalized). Multivariate GLM, with simple contrasts, were repeated for the normalized volumes (the major parts of the cerebellum, and the lobule groups of the left hemisphere, right hemisphere, and the vermis).

The potential relationships between age and IQ and the anatomical measures within each group were evaluated by linear regression. A regression analysis was also performed for vermis volume in which vermis area was a regressor. This regression tested the degree to which the midsagittal area measurement predicted the volume measurement. A Pearson's correlation analysis between total cerebellar volume and total cerebral volume determined whether the cerebellum was proportional to the cerebrum.

Results

Age and IQ Measures

Group demographics are summarized in Table II. There was no difference in the mean age of the groups at the time of MRI acquisition. There was a significant group effect for full scale IQ ($P<0.001$). As expected, post hoc tests indicated that full scale IQ for the LFA group was lower than all other groups ($P<0.01$). The full scale IQ for HFA and Asperger syndrome were also lower than the group of typically developing controls ($P<0.01$ and $P<0.05$, respectively). Both verbal and performance IQ were compared between the HFA, Asperger syndrome and control groups. The verbal IQ for HFA was lower than Asperger syndrome ($P<0.05$) and controls ($P<0.05$). The performance IQ for the high functioning group was lower than for the control group ($P<0.01$).

Midsagittal Area of the Vermis

Uncorrected areal measurements are summarized in Table III. When all autism groups were analyzed together, total vermis area was not reduced relative to controls ($P=0.37$) (Fig. 3A). Neither were there significant differences when the vermis was broken down into lobule groups (I–V, VI–VII, VIII–X) ($P>0.067$). When autism groups were separated into LFA, HFA, and Asperger syndrome, similar findings were obtained. The vermis area was not reduced in any of the autism groups ($P=0.38$) (Fig. 4A) and the areas of lobule groups were not significantly different between groups ($P>0.11$) (Fig. 4C–E). Age and IQ did not predict the area of the vermis in any of the groups (for all groups, $\beta<0.416$, $P>0.15$).

Table II. Participant Demographics

	LFA ($n=18$)	HFA ($n=15$)	ASP ($n=15$)	CON ($n=14$)
Age in years	13.1 (3.0)	11.7 (3.2)	12.3 (3.2)	12.5 (3.1)
Full scale IQ	56 (10)**	88 (16)**	97 (17)*	113 (12)
Verbal IQ	n/a	86 (21)**	105 (23)	110 (14)
Performance IQ	n/a	92 (13)*	98 (32)	115 (14)

*$P<0.05$; **$P<0.01$ when compared to CON.

Cerebellum and Major Subregions

Volume measurements are summarized in Table IV. Total cerebellar volume did not differ between the diagnostic groups ($P=0.509$) (Fig. 5A). In an analysis of the major subregions of the cerebellum (right and left hemispheres, medullary core and vermis) across each diagnostic group, only the vermis showed a main effect of group (raw: $P=0.016$, $pEta^2=0.166$, normalized: $P=0.143$) (Figs. 4B and 5B–C). The HFA group in particular had a smaller vermis volume than the control group (raw: $P=0.002$; normalized: $P=0.022$) (Fig. 4B). When data for the collective autism group was compared to the typically developing group, the vermis volume was significantly smaller (raw: $P=0.019$, $pEta^2=0.091$; normalized: $P=0.039$, $pEta^2=0.071$) (Fig. 3B). Raw and normalized volumes were compared for the lobule groups (I–V, VI–VII, and VIII–X) of the vermis (Fig. 4F–H). None of the lobule groups were reduced in LFA, HFA, or Asperger

Table III. Midsagittal Area Data

	LFA ($n=18$)	HFA ($n=15$)	ASP ($n=15$)	ASD ($n=48$)	CON ($n=14$)
Vermis	11.59 (1.3)	11.81 (1.0)	12.19 (1.0)	11.8 (1.1)	11.54 (1.2)
I–V	4.92 (0.47)	4.79 (0.58)	5.10 (0.54)	4.9 (0.50)	4.89 (0.54)
VI–VII	3.02 (0.55)	3.28 (0.54)	3.17 (0.41)	3.2 (0.50)	3.14 (0.53)
VIII–X	3.66 (0.53)	3.73 (0.36)	3.91 (0.50)	3.8 (0.50)	3.50 (0.32)

Mean (standard deviation) of volumes measured in square centimeters.

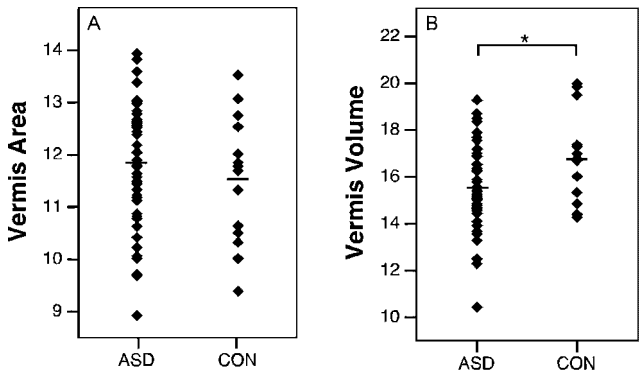

Figure 3. Scatter plots with means of volumes (cm^3) and areas (cm^2) of vermis structures collapsed across autism spectrum disorder groups. ASD, autism spectrum disorder; CON, typically developing (*$P<0.05$).

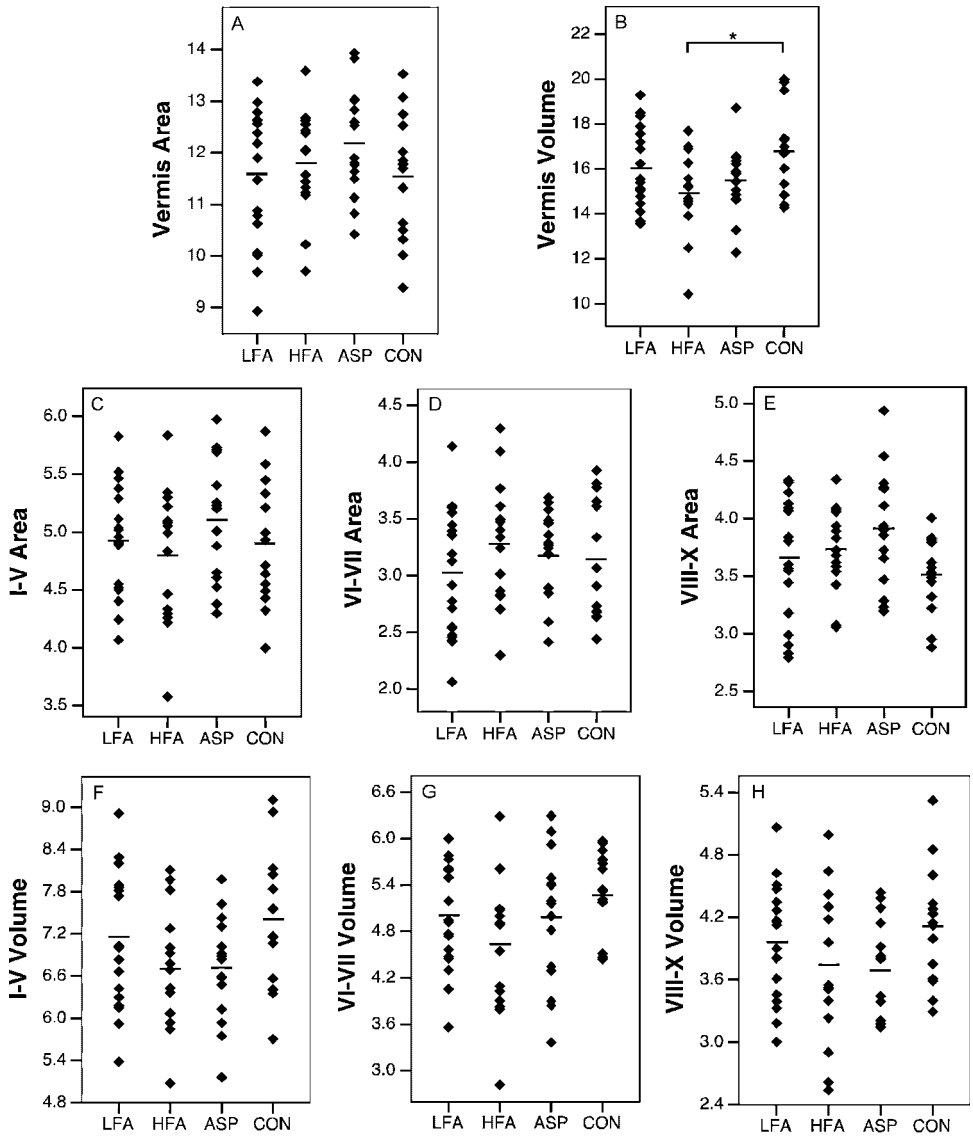

Figure 4. Scatter plots with means (indicated by horizontal lines) of volumes (cm^3) and areas (cm^2) of vermal structures: total vermis area and volume (top panel), vermis area of lobule groups (middle panel), vermis volume of lobules groups (bottom panel). ASP, Asperger syndrome; HFA, high functioning autism; LFA, low functioning autism ($^*P<0.05$).

syndrome (for all comparisons, $P>0.097$). When diagnostic groups were collapsed, no differences were found in the major subregions of the cerebellum (for all comparisons, $P>0.062$). The effects of age and IQ on the volume measurements were tested (Fig. 6). There were no significant regressors for cerebellar volume in any of the groups (for all groups, $\beta<0.315$, $P>0.22$). No significant relationships between age and IQ and vermis volume were found (for all groups, $\beta<0.361$, $P>0.137$).

Correlation analyses indicated that cerebellar and cerebral volumes were highly associated (Pearson = 0.295, $P = 0.02$). Across all subjects, vermis volume was not predicted by the midsagittal area of the vermis ($\beta = 0.086$, $P = 0.509$).

Discussion

We have carried out a comprehensive MRI analysis of the whole cerebellum and its subregions in children 7.5–18.5 years of age with autism spectrum disorder. Carefully conducted midsagittal areal measurement of the vermis did not reveal any differences between the autism groups and controls. We also found that the total cerebellar volume did not differ between those with autism and typically developing controls. However, the volume of the vermis, but not any particular lobule group, was reduced in the autism spectrum group. Somewhat surprisingly, the reduction in vermal volume was most prominent in the HFA group. This difference was not due

Table IV. Volumetric Data

	LFA ($n = 18$)	HFA ($n = 15$)	ASP ($n = 15$)	ASD ($n = 48$)	CON ($n = 14$)
Total cerebral volume	1,237 (160)	1,219 (107)	1,181 (85)	1,195 (201)	1,190 (78)
Total cerebellar volume	154.4 (16.7)	148.8 (15.8)	149.8 (11.6)	151.0 (14.9)	152.4 (11.8)
Cerebellar subregions					
Vermis	16.0 (1.8)	14.9 (1.8)*	15.5 (1.5)	15.5 (1.7)*	16.8 (1.9)
Right hemisphere	62.3 (6.9)	60.0 (6.8)	60.6 (4.9)	61.0 (6.2)	61.6 (5.2)
Left hemisphere	62.1 (7.1)	60.8 (6.7)	61.4 (4.9)	61.5 (6.2)	61.8 (5.0)
Core	14.0 (2.5)	12.6 (1.8)	12.2 (1.9)	13.0 (1.7)	12.9 (2.1)
Vermal lobules					
I–V	7.16 (.99)	6.70 (.86)	6.71 (.75)	6.87 (.89)	7.4 (.97)
VI–VII	5.01 (.71)	4.63 (.89)	4.97 (.86)	4.88 (.82)	5.27 (.58)
VIII–X	3.96 (.56)	3.74 (.74)	3.69 (.48)	3.81 (.60)	4.11 (.57)
Right hemisphere lobules					
I–V	5.30 (1.6)	5.03 (1.2)	5.57 (1.1)	5.30 (1.34)	5.33 (1.5)
VI–VII	41.2 (4.7)	40.1 (5.3)	39.7 (3.1)	40.39 (4.45)	41.5 (2.8)
VIII–X	15.7 (2.5)	14.8 (1.7)	15.4 (2.1)	15.26 (2.10)	14.9 (2.3)
Left hemisphere lobules					
I–V	5.31 (1.1)	5.01 (1.2)	5.17 (1.3)	5.17 (1.17)	4.91 (1.3)
VI–VII	40.4 (5.6)	39.0 (5.2)	40.2 (2.7)	39.89 (4.68)	40.7 (2.4)
VIII–X	16.4 (1.7)	16.7 (1.9)	15.8 (2.9)	16.29 (2.17)	15.9 (2.6)

Mean (standard deviation) of areas measured in cubic centimeters. $^*P < 0.05$, when compared to CON.

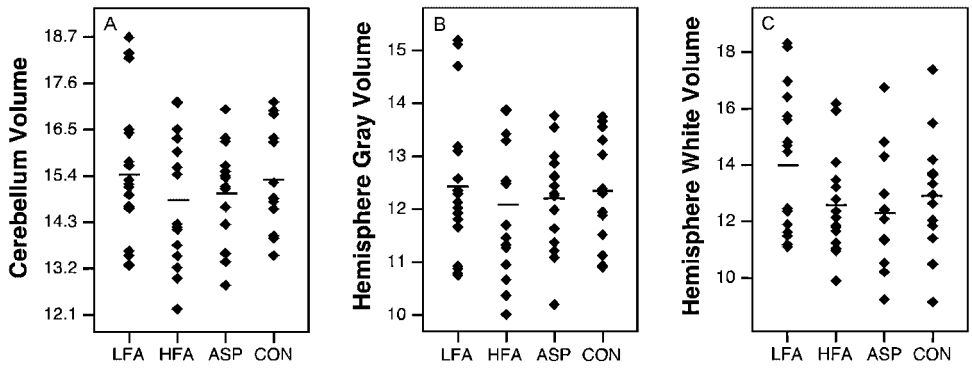

Figure 5. Scatter plots with means of volumes (cm^3) of cerebellum and gray and white matter of the hemispheres ($^*P < 0.05$).

to differences in IQ, as IQ was not a significant predictor of vermal volume.

Comparison with Previous Findings

Hypoplasia of the vermis, especially of lobules VI–VII, has been highlighted as a prominent component of the neuropathology of autism [Courchesne et al., 1988]. However, as indicated in Table I, this finding is inconsistently observed across studies. In fact, even the Courchesne group has suggested that subgroups of individuals with autism demonstrate either hypoplasia or hyperplasia of the vermis [Courchesne et al., 1994]. Our area measurements of the vermis in a sample of 48 children and adolescents with autism did not detect any reliable differences in comparison to typically developing controls. Given the substantial heterogeneity in reports related to vermal area in autism [Stanfield et al., 2008; Table I], the conclusion that vermal hypoplasia is not consistently seen across all individuals with autism appears to be warranted. It will be interesting to determine what phenotypes of autism may be more consistently associated with vermal hypoplasia.

Although we did not find a difference in the mid-sagittal area of the vermis, we did find evidence for a decreased volume of the vermis in the autism spectrum group. Individual comparisons of the diagnostic groups indicated that this difference was driven primarily by a smaller vermis in the individuals with autism and IQ greater than 70 (HFA group). In a previous study of cerebellar volumes carried out in autistic males with a broader age range (12–52), Hardan et al. [2001] found that hemispheric and total cerebellar volumes were enlarged, but vermal volume was not different from controls. Cross-sectional area of the vermis was not

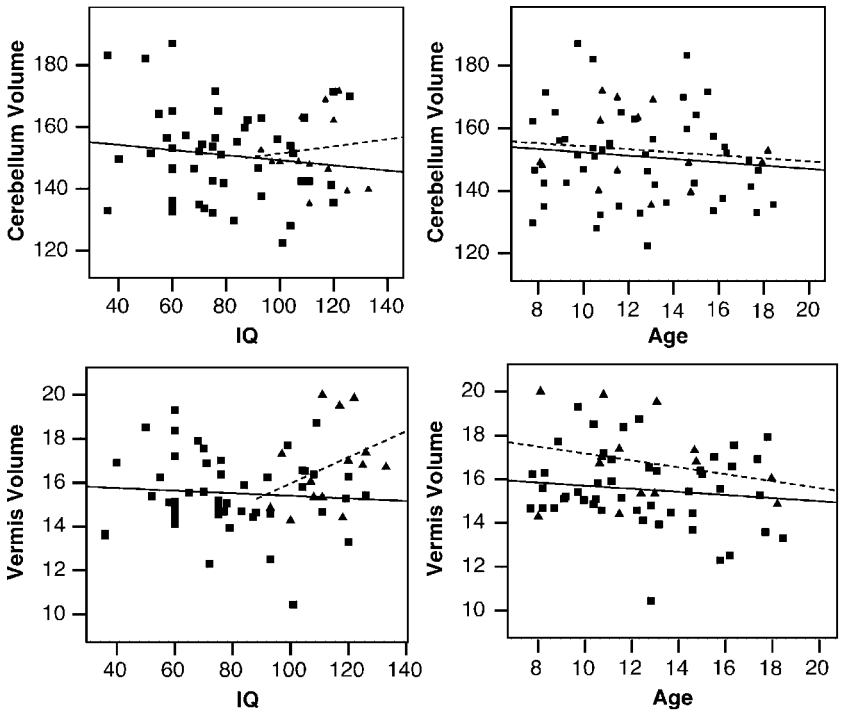

Figure 6. Linear regression of total cerebellum and vermis volumes for IQ and age variables. Solid line, ASD; Dashed line, CON.

different in this study either. The authors concluded that the enlargement of the cerebellum was in line with a more general increase in brain size that they and others had observed. Our cohort of males (aged 7.5–18.5) did not demonstrate an overall increased brain volume, though the volume of the cerebellum was highly correlated with the volume of the cerebrum.

The surprising finding in our study was that the HFA group was the group that had a reduction in vermal volume. Even in this group, however, there was no difference in the midline area measurement. This suggests that the more laterally situated portions of the vermis were smaller in this group. We have no good explanation for why a vermal volume reduction was observed in the high functioning autistic group and not in either those individuals with LFA or Asperger syndrome, with the caveat that the LFA group also exhibited varying levels of mental retardation, which may confound their neuropathological profile. Additionally, the sample size in this study is insufficient to handle the inherent heterogeneity of the autism cohort to parcellate subphenotypes of autism or isolate incidental findings.

Interestingly, vermal hypoplasia in general as well as reductions specifically in lobules VI–VII has been found in other neurodevelopmental disorders [Ciesielski, Harris, Hart, & Pabst, 1997; Soto-Ares, Joyes, Lemaitre, Vallee, & Pruvo, 2003]. Kaufmann and colleagues, for example, examined vermis area in autism with and without the presence of Down syndrome or fragile X syndrome [Kaufmann et al., 2003]. Reductions in lobules VI–VII were found in groups with single diagnoses and in the dual diagnosis of autism and Down syndrome. Similar outcomes were observed in another study in which vermal hypoplasia was found in both neurogenetic disorders with and without autistic traits [Schaefer et al., 1996]. Other studies looking at developmental disabilities, such as nonspecific mental retardation and juveniles treated with radiation and chemotherapy, also report vermis size reduction when compared to controls, particularly in lobules VI–VII [Ciesielski et al., 1997; Soto-Ares et al., 2003]. In fact, several studies of nonautism neurodevelopmental disorders, including Dandy–Walker syndrome [Aldinger et al., 2009], attention deficit/hyperactivity disorder [Curatolo, Paloscia, D'Agati, Moavero, & Pasini, 2009], fetal alcohol syndrome [Astley et al., 2009] and chromosome 22q deletion syndrome [Bish et al., 2006] find vermal hypoplasia, which indicates that this form of neuropathology is not specific to autism but a common feature of atypical development.

IQ and Age Correlates

Previous studies have suggested that reductions in the volume of the vermis in individuals with autism may be related to IQ or age. Early on, IQ was suggested to be a major factor in vermis reductions [Piven et al., 1992]. A recent meta-analysis found that lower IQ does appear to be associated with greater reduction in the area of

vermal lobules VI–VII in autistic groups [Stanfield et al., 2008]. In this study, which included individuals with a broad range of IQ scores, regression analyses indicated that IQ was not significantly related to the vermal area or volume. We also found that none of the cerebellar measures were correlated with age in either the control or autism groups. Other studies of cerebellar volume in the age range we measured have also not reported a correlation with age [Herbert et al., 2003; Palmen et al., 2005].

Conclusion

In this study of children and adolescents with autism spectrum disorders, we did not replicate the finding of a reduced area of vermal lobules VI–VII. We did, however, observe a decrease of overall vermal volume in this population; this finding was driven primarily by observations from the HFA group. This came in the context of no global cerebellar volume changes in the autism spectrum group. Since the vermis appears to be vulnerable to a variety of neurodevelopmental disorders and insults, it is not surprising that it is also pathological in some individuals with autism. However, given the current finding in the context of previous studies, this form of neuropathology is neither a specific nor a sensitive biological signature of autism.

Acknowledgments

We thank Meridith Brandt for participant recruitment and scheduling at UC Davis, John Ryan for his assistance in carrying out MRI acquisition, and Kristine Strohbin, Carolynn Nolte and Jonathan Lee for assisting in anatomical segmentation. We also thank the study participants and their families for their contribution.

References

Aldinger, K.A., Lehmann, O.J., Hudgins, L., Chizhikov, V.V., Bassuk, A.G., et al. (2009). FoxC1 is required for normal cerebellar development and is a major contributor to chromosome 6p25.3 Dandy-Walker malformation. Nature Genetics, 41, 1037–1042.

Angevine, J.B., Mancall, E.L., & Yakovlev, P. (1961). The human cerebellum: an atlas of gross topography in serial sections. Great Britain: J&A Churchill Ltd.

Astley, S.J., Aylward, E.H., Olson, H.C., Kerns, K., Brooks, A., et al. (2009). Magnetic resonance imaging outcomes from a comprehensive magnetic resonance study of children with fetal alcohol spectrum disorders. Alcoholism Clinical and Experimental Research, 33, 1671–1689.

Bailey, A., Luthert, P., Dean, A., Harding, B., Janota, I., et al. (1998). A clinicalpathological study of autism. Brain, 121, 889–905.

Bish, J.P., Pendyal, A., Ding, L., Ferrante, H., Nguyen, V., et al. (2006). Specific cerebellar reductions in children with chromosome 22q11.2 deletion syndrome. Neuroscience Letters, 22, 245–248.

Ciesielski, K., Harris, R., Hart, B., & Pabst, H. (1997). Cerebellar hypoplasia and frontal lobe cognitive deficits in disorders of early childhood. Neuropsychologia, 35, 643–655.

Courchesne, E., Karns, C.M., Davis, H.R., Ziccardi, R., Carper, R.A., et al. (2001). Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. Neurology, 57, 245–254.

Courchesne, E., Townsend, J., & Saitoh, O. (1994). The brain in infantile autism: posterior fossa structures are abnormal. Neurology, 44, 214–223.

Courchesne, E., Yeung-Courchesne, R., Press, G., Hesselink, J., & Jernigan, T. (1988). Hypoplasia of cerebellar vermal lobules VI and VII in autism. The New England Journal of Medicine, 318, 1349–1354.

Crooks, R., Mitchell, T., & Thom, M. (2000). Patterns of cerebellar atrophy in patients with chronic epilepsy: a quantitative neuropathological study. Epilepsy Res, 41, 63–73.

Curatolo, P., Paloscia, C., D'Agati, E., Moavero, R., & Pasini, A. (2009). The neurobiology of attention deficit/hyperactivity disorder. European Journal of Paediatric Neurology, 13, 299–304.

DiLavore, P., Lord, C., & Rutter, M. (1995). The pre-linguistic autism diagnostic observation schedule. Journal of Autism and Developmental Disorder, 25, 355–379.

Gaffney, G.R., Tsai, L.Y., Kuperman, S., & Minchin, S. (1987). Cerebellar structure in autism. American Journal of Diseases of Children, 141, 1330–1332.

Hallahan, B., Daly, E.M., McAlonan, G., Loth, E., Toal, F., et al. (2009). Brain morphometry volume in autistic spectrum disorder: a magnetic resonance imaging study of adults. Psychol Med, 39, 337–346.

Hardan, A., Minshew, N., Harenski, K., & Keshavan, M. (2001). Posterior fossa magnetic resonance imaging in autism. Journal of American Academy of Child and Adolescent Psychiatry, 40, 666–672.

Hashimoto, T., Tayama, M., Murakawa, K., Yoshimoto, T., Miyazaki, M., et al. (1995). Development of the brainstem and cerebellum in autistic patients. Journal of Autism and Developmental Disorders, 25, 1–18.

Hazlett, H.C., Poe, M., Gerig, G., Smith, R.G., Provenzale, J., et al. (2005). Magnetic resonance imaging and head circumference study of brain size in autism: birth through age 2 years. Archives of General Psychiatry, 62, 1366–1376.

Herbert, M.R., Ziegler, D.A., Deutsch, C.K., O'Brien, L.M., Lange, N., et al. (2003). Dissociations of cerebral cortex, subcortical and cerebral white matter volumes in autistic boys. Brain, 126, 1182–1192.

Holttum, J., Minshew, N., Sanders, R., & Phillips, N. (1992). Magnetic resonance imaging of the posterior fossa in autism. Biological Psychiatry, 32, 1091–1101.

Kaufmann, W.E., Cooper, K.L., Mostofsky, S.H., Capone, G.T., Kates, W.R., et al. (2003). Specificity of cerebellar vermian abnormalities in autism: a quantitative magnetic resonance imaging study. Journal of Child Neurology, 18, 463–470.

Kemper, T.L., & Bauman, M.L. (2002). Neuropathology of infantile autism. Molecular Psychiatry, 7, S12–S13.

Kleiman, M., Neff, S., & Rosman, N. (1992). The brain in infantile autism: are posterior fossa structures abnormal? Neurology, 42, 753–760.

Lord, C., Rutter, M., & Le Couteur, A. (1994). Autism diagnostic interview-revised: a revised version of a diagnostic for caregivers of individuals with possible pervasive developmental disorders. Journal of Autism and Developmental Disorder, 24, 659–689.

Lord, C., Risi, S., Lambrecht, L., Cook, E., Leventhal, B., et al. (2000). The autism diagnostic observation schedule-generic: a standard measure of social and communication deficits associated with the spectrum of autism. Journal of Autism and Developmental Disorder, 30, 205–223.

Lotspeich, L., Kwon, H., Schumann, C., Fryer, S., Goodlin-Jones, B., et al. (2004). Investigation of neuroanatomical differences between autism and Asperger syndrome. Archives and General Psychiatry, 61, 291–298.

Manes, F., Piven, J., Vrancic, D., Nanclares, V., Plest, C., & Starkstein, S. (1999). An MRI study of the corpus callosum and cerebellum in mentally retarded autistic individuals. Journal of Neuropsychiatry and Clinical Neuroscience, 11, 470–474.

McAlonan, G.M., Cheung, V., Cheung, C., Suckling, J., Lam, G.Y., et al. (2005). Mapping the brain in autism. A voxel-based MRI study of volumetric differences and intercorrelations in autism. Brain, 128, 268–276.

Palmen, S.J., Hulshoff Pol, H.E., Kemner, C., Schnack, H.G., Durston, S., et al. (2005). Increased gray-matter volume in medication-naive high-functioning children with autism spectrum disorder. Psychological Medicine, 35, 561–570.

Piven, J., Nehme, E., Simon, J., Barta, P., Pearlson, G., & Folstein, S. (1992). Magnetic resonance imaging in autism: measurement of the cerebellum, pons, and fourth ventricle. Biological Psychiatry, 31, 491–504.

Rojas, D.C., Peterson, E., Winterrowd, E., Reite, M.L., Rogers, S.J., & Tregellas, J.R. (2006). Regional gray matter volumetric changes in autism associated with social and repetitive behavior symptoms. BMC Psychiatry, 6, 56–74.

Robb, R., Hanson, D., Karwoski, R., Larson, A., Workman, E., & Stacy, M. (1989). Analyze: a comprehensive, operator-interactive software package for multidimensional medical image display and anaylsis. Computerized Medical Imaging and Graphics, 13, 433–454.

Rogers, S.J., Hepburn, S.L., Stackhouse, T., & Wehner, E. (2003). Imitation performance in toddlers with autism and those with other developmental disorders. Journal of Child Psychology and Psychiatry, 44, 763–781.

Roid, G., & Miller, L. (1997). Leiter international performance scale-revised. Illinois: Stoelting.

Schaefer, G.B., Thompson, J.N., Bodensteiner, J.B., McConnell, J.M., Kimberling, W.J., et al. (1996). Hypoplasia of the cerebellar vermis in neurogenetic syndromes. Annals of Neurology, 39, 382–385.

Schmahmann, J., Doyon, J., Toga, A., Petrides, M., & Evans, A. (2000). MRI atlas of the human cerebellum. San Diego, CA: Academic Press.

Schumann, C.M., Hamstra, J., Goodlin-Jones, B.L., Lotspeich, L.J., Kwon, H., et al. (2004). The amygdala is enlarged in children but not adolescents with autism; the hippocampus is enlarged at all ages. Journal of Neuroscience, 24, 6392–6401.

Soto-Ares, G., Joyes, B., Lemaitre, M.-P., Vallee, L., & Pruvo, J.-P. (2003). MRI in children with mental retardation. Pediatric Radiology, 33, 334–345.

Stanfield, A.C., McIntosh, A.M., Spencer, M.D., Philip, R., Gaur, S., & Lawrie, S.M. (2008). Towards a neuroanatomy of autism: a systematic review and meta-analysis of structural magnetic resonance imaging studies. Eur Psychiatry, 23, 289–299.

Webb, S.J., Sparks, B.F., Friedman, S.D., Shaw, D.W., Giedd, J., et al. (2009). Cerebellar vermal volumes and behavioral correlates in children with autism spectrum disorder. Psychiatry Research, 172, 61–67.

Wechsler, D. (1999). Wechsler abbreviated scale of intelligence. San Antonio, TX: The Psychological Corporation.

Whitney, E.R., Kemper, T.L., Bauman, M.L., Rosene, D.L., & Blatt, G.J. (2008). Cerebellar Purkinje cells are reduced in a subpopulation of autistic brains: a stereological experiment using calbindin-D28k. Cerebellum, 7, 406–416.

Williams, J.H., Whiten, A., Suddendorf, T., & Perrett, D.I. (2001). Imitation, mirror neurons and autism. Neuroscience and Biobehavioural Reviews, 25, 287–295.