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ORIGINAL ARTICLE

Brain morphometry volume in autistic spectrum disorder: a magnetic resonance imaging study of adults

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Background. Several prior reports have found that some young children with autism spectrum disorder [ASD; including autism and Asperger's syndrome and pervasive developmental disorder – not otherwise specified (PDD-NOS)] have a significant increase in head size and brain weight. However, the findings from older children and adults with ASD are inconsistent. This may reflect the relatively small sample sizes that were studied, clinical heterogeneity, or age-related brain differences.

Method. Hence, we measured head size (intracranial volume), and the bulk volume of ventricular and peripheral cerebrospinal fluid (CSF), lobar brain, and cerebellum in 114 people with ASD and 60 controls aged between 18 and 58 years. The ASD sample included 80 people with Asperger's syndrome, 28 with autism and six with PDD-NOS.

Results. There was no significant between-group difference in head and/or lobar brain matter volume. However, compared with controls, each ASD subgroup had a significantly smaller cerebellar volume, and a significantly larger volume of peripheral CSF.

Conclusions. Within ASD adults, the bulk volume of cerebellum is reduced irrespective of diagnostic subcategory. Also the significant increase in peripheral CSF may reflect differences in cortical maturation and/or ageing.

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Key words: Autism spectrum disorder, Asperger's syndrome, magnetic resonance imaging, brain.

Introduction

Autism spectrum disorder [ASD; comprising autism, Asperger's syndrome and pervasive developmental disorder – not otherwise specified (PDD-NOS)] is being increasingly diagnosed. For example, the prevalence of ASD in South London was recently reported as 116.1/100 000 (Baird *et al*. 2006). ASD is characterized by stereotyped and obsessional behaviours, and pervasive abnormalities in socio-emotional and communicative behaviour (Wing, 2004). There is, however, variation in the clinical phenotype. Individuals with autism also have delayed language development, and are frequently intellectually disabled (mentally retarded). In contrast, people with Asperger's syndrome have no history of language delay and have normal or superior intellectual abilities. The biological basis of ASD is poorly understood and it is unknown if classical differences in the clinical phenotype are associated with brain differences.

There is converging evidence from both post-mortem and *in vivo* neuroimaging studies that some people with ASD have a significant increase in head size, and in brain weight and volume (e.g. see Bailey *et al*. 1998; Kemper & Bauman, 1998; Courchesne *et al*. 1999; Carper *et al*. 2002; Herbert *et al*. 2003). This is frequently reported in young children (Sparks *et al*. 2002; Courchesne *et al*. 2003; Hazlett *et al*. 2005). However, the results from studies of adolescents and adults with ASD are inconsistent, i.e. some have reported increased brain volume (Piven *et al*. 1995; Hardan *et al*. 2001) but others found no difference (Courchesne *et al*. 2001; McAlonan *et al*. 2002). One possible explanation for these discrepant findings is that different investigations have included different subtypes of people with ASD. However, to date only one study (Lotspeich *et al*. 2004) has examined brain volume in subtypes of ASD. This study assessed total

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cerebral grey and white matter volume in adolescents and found an increased volume for individuals with autism but not with Asperger's syndrome compared with controls.

Alternatively, people with ASD may have differences in post-natal brain maturation, which only occur (and/or are only detectable) at certain ages. For example, there is preliminary evidence that people with ASD may be born with a smaller brain (Courchesne *et al*. 2003) but then have a period of pathologically rapid growth detectable as a larger head size in early childhood, and a subsequent 'plateauing' of brain growth in ASD, so that differences in head size are not detectable in late childhood (Redcay & Courchesne, 2005).

This suggestion is indirectly supported by other work. For example, a recent *in vivo* magnetic resonance imaging (MRI) study demonstrated an increase in total brain volume, and cortical grey and white matter in 2-year-old children with autism compared with controls (Hazlett *et al*. 2005). Also, while head size was similar in both groups at birth, after 12 months of age those with autism had a larger head size (Hazlett *et al*. 2005) – suggesting that they have a larger brain. However these studies require replication.

The hypothesis that people with ASD have age-restricted differences in brain size is further supported by other studies which reported that children, but not adolescents and adults, with ASD have a significantly larger brain volume than healthy controls (Aylward *et al*. 2002). Also, in the same cohort, all three age groups had a significantly increased head circumference compared with controls. The larger head size (i.e. intracranial volume) in adults with ASD may have reflected an earlier larger brain volume, because head size is proportional to maximal brain volume (Wickett *et al*. 2000). This suggests that once maximal brain size (and consequently head size) is achieved in young people with ASD, there may be continuing subtle differences in brain maturation which continue to remodel the brain into adulthood. In support of this we previously reported no significant difference in bulk brain volume between adults with Asperger's syndrome and controls, but did report significant differences compared with controls in age-related loss of total brain grey and white matter (McAlonan *et al*. 2005).

In addition to differences in the developmental trajectory of whole brain, it has been suggested that there are regionally specific abnormalities in the morphometry of lobar brain and cerebellum, and in the anterior–posterior gradient and right–left asymmetry. For example, differences have been described in the anatomy of the cerebellum (Courchesne *et al*. 1988), fronto-temporal regions (Abell *et al*. 1999; McAlonan *et al*. 2005) and ventricular system (Howard *et al*. 2000; Hardan *et al*. 2001). Most (Courchesne *et al*. 2001; Sparks *et al*. 2002), but not all (Piven *et al*. 1995), studies of young children reported increased cerebellar volume in those with ASD. In contrast, within adolescents and adults, cerebellar volume has been reported as increased (Piven *et al*. 1997), no different (Piven *et al*. 1992) and decreased (McAlonan *et al*. 2002). Also, an anterior–posterior gradient has been suggested in brain volume for both ASD children (Carper *et al*. 2002; Hazlett *et al*. 2005) and adolescents/adults (Hazlett *et al*. 2006), with the frontal and temporal lobes demonstrating greater enlargement than the parietal and occipital lobes. Both increased leftward asymmetry (Courchesne *et al*. 1998) and a reversal of the normal leftward asymmetry (Herbert *et al*. 2005) have also been reported in ASD. It is unknown why such disparate findings have been reported in cerebellar volume, but it may reflect the relatively small sample sizes, together with differences between studies in the inclusion criteria of subjects [e.g. in age, intelligence quotient (IQ), presence of epilepsy, and subcategory of ASD].

Increased cortical thickness (Hardan *et al*. 2006) and differences in cortical gyrification (Hardan *et al*. 2004) have been demonstrated in children with autism, suggesting an increased cortical surface area and thus increased grey matter in children with autism. Further, a differential decrease in cortical gyrification with age in ASD has also been reported (Hardan *et al*. 2004). Hence, we would expect to find differences in the volume of peripheral cerebrospinal fluid (CSF) (as cortical gyrification and grey matter is lost/modified) in people with ASD compared with control subjects. However, no one has examined peripheral CSF in ASD.

In summary, it has been suggested that people with ASD have age-restricted differences in brain size and perhaps also in cortical gyral maturation. However, to date no one has examined both total and regional brain and CSF volume in a relatively large sample of ASD adults. We therefore used quantitative MRI to measure head size (intracranial volume), and the volume of ventricular and peripheral CSF, lobar brain and cerebellum in 114 individuals with ASD and 60 controls between 18 and 58 years. We tested the main hypothesis that ASD adults would not have any difference from controls in bulk brain volume, but would have a significantly larger intracranial volume (i.e. an increased head size reflecting prior brain overgrowth) and volume of peripheral CSF. We also tested the subsidiary hypothesis that this would be true in all the different subtypes of ASD we studied.

Method

Subjects

People with ASD were recruited from our clinical research programme [sponsored by the Medical Research Council (MRC) UK A.I.M.S. network and the South London and Maudsley NHS Foundation Trust]. Controls were recruited locally through advertisement. We excluded subjects with a co-morbid medical condition (e.g. epilepsy), history of head injury, psychosis, cardiovascular disease, a genetic disorder associated with ASD (e.g. tuberous sclerosis or fragile X syndrome), or clinically abnormal findings on routine MRI.

The ASD sample comprised 114 ASD adults over the age of 18 years (80 with Asperger's syndrome, 28 with typical autism and six with PDD-NOS) and 60 healthy controls. Of these, 17 controls and 10 Asperger's subjects were participants in a previous study (McAlonan *et al*. 2002). Subjects with Asperger's syndrome and autism were differentiated by the presence of abnormal phrase language development.

People with ASD were clinically diagnosed using International Classification of Diseases (ICD)-10 research criteria (WHO, 1992). This was confirmed in 69 cases where parental informants were available with the Autism Diagnostic Interview (ADI; Lord *et al*. 1994) or the Autism Diagnostic Observation Schedule (ADOS) in 18 cases (Lord *et al*. 2000) if parents were unavailable and the subject was willing to undergo further interviewing.

We measured overall intelligence using the Wechsler Adult Intelligence Scale – Revised (Wechsler, 1981). Research was approved by the respective local research ethics committees, and each participant (and/or their carer) provided written consent/assent as appropriate. The subjects were familiarized with the MRI scanner before imaging. No sedation was used during the scanning process.

MRI scanning protocol

All subjects and controls underwent MRI scanning on the same GE Signa 1.5-T MR system (General Electric, Milwaukee, WI, USA) at the Maudsley Hospital, London. A coronal three-dimensional spoiled grass (SPGR) dataset covering the whole head was acquired (124 slices, 1.5 mm slice thickness) from all subjects. Manual tracing of brains was performed on the reformatted SPGR dataset using MEASURE software and using previously published anatomical definitions (Barta *et al*. 1997). All image analysis was carried out blind to subject status.

Manual tracing of brain structures was performed on SPGR datasets, using MEASURE software (Johns Hopkins University, Baltimore, MD, USA). As described previously (Murphy *et al*. 1992, 1996), we traced the total intracranial volume and total brain matter volume of cerebral hemispheres, cerebellum and brainstem, and individual brain lobes. We also measured the volume of ventricular CSF (the lateral and third ventricles) and peripheral CSF [total intracranial volume – (cerebral hemispheres + cerebellum + brainstem + ventricles)]. The volume of each region was calculated by multiplying the summed pixel cross-sectional areas by slice thickness. Inter-rater reliabilities were determined for all brain regions traced by the operators and were highly significant ($r > 0.90$) (Bartko & Carpenter, 1976). Ten scans were used to determine inter-rater reliability. One author reviewed all scans (B.H.). Rater drift was assessed; 10 scans that were traced at the beginning of the analysis were retraced at the end of the study and intra-rater reliabilities was attained ($r = 0.999$ for all regions except for the brainstem, $r = 0.94$ and cerebellum, $r = 0.98$).

Statistical analysis

MRI volumetric measurements

The analysis of manually traced volumes was carried out using SPSS (version 14.0 for Windows; SPSS, Inc., Chicago, IL, USA). Between-group differences in total and regional brain volumes were calculated using analysis of covariance with group (ASD or controls) as the between-subject variable and total intracranial volume as covariate. We firstly compared all people with ASD (combined as one group) with controls, and then compared each subtype. As IQ is known to modulate cortical growth (Shaw *et al*. 2006), and was also (as expected) significantly different between the groups (Table 1), we repeated our analysis adding IQ as a covariate. *Post-hoc* testing (Scheffé test) was used to assess for any between-group differences in brain volume between the different ASD subgroups.

To measure right–left asymmetry of brain regions, we employed a symmetry index (SI) (Galaburda *et al*. 1987), using the formula $SI = 2(L - R)/(L + R) \times 100$. Positive values indicate left-sided preponderance. To classify each structure as being significantly left- or right-asymmetrical, one-sample Student's t tests were used to assess the probability that the mean SI for each structure was non-zero (significantly asymmetrical) for both ASD individuals and controls. We then compared ASD subjects and controls using unpaired Student's t tests.

Also we carried out a *post hoc*, preliminary, exploratory analysis on the effect of ageing. We correlated (within each group) brain and CSF volumes

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Table 1. *Demographics*

	ASD ($n=114$)	Asperger's syndrome ($n=80$)	Autism ($n=28$)	PDD-NOS ($n=6$)	Controls ($n=60$)
Age (years)	32 (11)	33 (11)	29 (7)	30 (9)	32 (9)
Full-scale IQ[a]	98 (18)	102 (15)	85 (19)	83 (10)	114 (12)
Verbal IQ[a]	97 (19)	102 (16)	83 (18)	86 (13)	114 (13)
Performance IQ[a]	97 (19)	101 (17)	87 (21)	81 (7)	114 (14)
Sex, male/female	96/18	71/9	21/7	4/2	53/7

ASD, Autistic spectrum disorder; PDD-NOS, pervasive developmental disorder – not otherwise specified; IQ, intelligence quotient.

Values are given as mean (standard deviation).

[a] All IQ measurements are significantly lower in all groups compared with controls ($p<0.001$; analysis of covariance).

with age using Pearson's correlation coefficient. We then investigated group differences in brain ageing by transforming Pearson's r coefficient into Fisher's Z score to test the significance of the difference between correlations, where a $Z_{\text{observed}} \geqslant 1.96$ or $Z_{\text{observed}} \leqslant 1.96$ is significant (Pallant, 2001).

Results

Demographic profile

There was no between-group difference in age at the time of MRI acquisition. There was no difference in the male:female ratios between the different groups. However, as expected, there was a significant group effect for IQ in the adult group. The Asperger's subgroup had a higher IQ than all the other ASD subgroups [$F(2,113)=17.60$, $p<0.001$] (Table 1).

Volumetric measurements

Cerebral hemispheres and lobar brain

There was no significant difference between the whole ASD group and controls in head size, or volume of whole brain or bulk lobar brain. Similarly there was no difference in whole brain or bulk lobar brain between each ASD subgroup and controls. There were no significant differences in intracranial volume or any bulk lobar volume between the ASD subgroups. Correcting for IQ did not change the results.

Cerebellum

The combined group of subjects with ASD had a significantly smaller cerebellar volume than controls, [$F(1,173)=6.137$, $p=0.014$]. This was present before and after correcting for both intracranial volume and IQ. Similarly, each ASD subgroup (except for those with PDD-NOS) had a significantly smaller cerebellar volume than controls. However, there was no significant difference in cerebellar volume between the ASD subgroups (Table 2, Fig. 1).

CSF

Total peripheral CSF was significantly increased in the whole group of people with ASD compared with controls [$F(1,173)=6.940$, $p=0.009$] but there were no differences in lateral ventricular volume. This was also true when we corrected for IQ, and before and after we removed five outliers. Removing these outliers reduced the lateral ventricular mean value to 16.4 cm^3 and 16.3 cm^3 in the ASD and Asperger's groups respectively. Also each ASD subgroup (except for those with PDD-NOS) had a significantly larger volume of peripheral CSF than controls, both before and after correcting for IQ. Lateral ventricular volume was significantly [$F(1,87)=4.807$, $p=0.031$] larger than controls in people with autism, but this also became non-significant when we corrected for IQ. There was no significant difference between the ASD subgroups in peripheral CSF or lateral ventricular volume (Fig. 2).

Ageing

There was no significant age-related difference between the combined ASD group and controls in volume of whole brain or any bulk lobar region. A significant increase in peripheral CSF with ageing was found in subjects with ASD ($r=0.237$, $p=0.015$) but not in control subjects ($r=0.177$, $p=0.257$). However, there was no significant differential increase in peripheral CSF volume with ageing in ASD individuals compared with controls ($Z=0.38$). We found a similar pattern in each subgroup (Fig. 2).

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Table 2. *Bulk brain (grey + white matter) and CSF volumes*[a]

	ASD ($n=114$)	Asperger's syndrome ($n=80$)	Autism ($n=28$)[b]	PDD-NOS ($n=6$)	Controls ($n=60$)
Intracranial volume					
Total	1422.40 (149.21)	1436.43 (139.87)	1397.50 (169.49)	1375.82 (176.00)	1429.70 (128.88)
Right	706.73 (82.01)	715.72 (75.13)	691.55 (94.58)	672.63 (92.38)	708.98 (61.76)
Left	715.67 (78.01)	720.72 (76.53)	705.95 (81.72)	703.19 (86.19)	720.72 (82.01)
Cerebral hemispheres					
Total	1072.77 (126.66)	1083.58 (118.71)	1055.06 (136.22)	1012.82 (178.19)	1098.22 (108.69)
Right	536.49 (64.23)	542.22 (60.40)	526.98 (69.22)	504.57 (86.36)	548.51 (54.02)
Left	536.27 (63.54)	541.35 (59.73)	528.08 (67.63)	508.25 (92.03)	549.71 (64.23)
Frontal lobes					
Total	498.05 (70.51)	503.88 (68.03)	485.60 (70.92)	488.06 (99.68)	509.91 (47.69)
Right	249.53 (35.98)	252.81 (34.44)	241.94 (37.75)	246.78 (46.15)	255.36 (23.47)
Left	248.51 (35.46)	251.06 (34.53)	243.66 (34.03)	241.28 (53.96)	254.55 (25.57)
Temporal lobes					
Total	117.16 (20.97)	119.42 (19.99)	113.36 (22.80)	111.02 (19.28)	120.52 (16.61)
Right	59.67 (11.01)	60.55 (10.68)	57.73 (12.22)	58.48 (9.25)	61.20 (9.00)
Left	57.50 (10.69)	58.86 (10.28)	55.63 (11.19)	52.53 (11.93)	59.31 (8.46)
Parietal lobes					
Total	331.81 (52.27)	338.55 (55.49)	332.33 (55.54)	293.61 (48.01)	336.52 (55.82)
Right	165.78 (26.10)	169.20 (27.65)	166.22 (28.11)	145.98 (24.01)	167.88 (27.74)
Left	166.03 (26.71)	169.35 (28.24)	166.12 (28.33)	147.63 (24.06)	168.64 (28.33)
Occipital lobes					
Total	126.43 (27.09)	127.36 (26.72)	125.37 (25.82)	120.55 (29.71)	136.68 (23.64)
Right	61.09 (13.78)	61.85 (12.90)	60.84 (14.64)	53.51 (19.54)	65.66 (12.09)
Left	65.34 (14.29)	65.51 (15.10)	64.53 (13.03)	67.04 (11.81)	71.01 (14.31)
Cerebellum					
Total	129.37 (19.74)*	130.80 (17.98)*	125.56 (24.81)*	130.34 (14.63)	139.50 (16.69)
Right	64.87 (9.84)**	65.48 (8.92)**	63.43 (12.31)**	64.39 (7.84)	69.43 (8.67)
Left	64.49 (10.00)**	65.32 (9.02)**	62.14 (12.52)**	65.95 (6.94)	70.08 (8.66)
Brainstem					
Total	27.04 (4.85)	26.87 (4.88)	27.05 (5.14)	29.08 (2.60)	27.77 (5.31)
Right	13.05 (2.74)	12.96 (2.65)	13.12 (3.11)	13.73 (2.06)	13.62 (2.94)
Left	13.99 (2.89)	13.90 (3.04)	13.93 (2.73)	15.35 (1.60)	14.15 (3.13)
Lateral ventricles					
Total	22.57 (37.15)	20.04 (24.58)	31.74 (62.67)	14.92 (6.54)	14.65 (9.93)
Right	9.91 (12.50)	9.73 (12.04)	11.08 (14.84)	6.56 (2.74)	6.93 (4.90)
Left	13.45 (27.61)	10.99 (14.23)	20.66 (48.07)	8.36 (4.25)	7.72 (6.64)
Third ventricle	0.51 (0.60)	0.52 (0.68)	0.51 (0.44)	0.40 (0.21)	0.40 (0.32)
Peripheral CSF					
Total	168.67 (56.42)*	171.42 (58.35)*	157.58 (53.24)*	188.25 (45.05)	145.95 (41.60)
Right	81.90 (33.87)*	83.87 (34.89)	76.69 (32.93)**	83.18 (27.64)	70.91 (21.73)
Left	86.77 (32.32)	87.55 (33.69)	80.89 (28.29)	105.07 (30.43)	75.04 (23.51)
Hemispheric	155.61 (51.46)*	159.10 (53.01)	144.08 (49.08)*	168.69 (40.45)	136.93 (37.08)
Cerebellar	13.06 (9.03)	12.32 (9.28)*	13.50 (8.60)	19.56 (5.78)*	9.02 (7.55)

CSF, Cerebrospinal fluid; ASD, autistic spectrum disorder; PDD-NOS, pervasive developmental disorder – not otherwise specified.

Values are given as mean (standard deviation).

[a] Analysis of covariance with intracranial volume and intelligence quotient as covariates in all analyses of brain volume (cm^3).

[b] The autism group includes 20 subjects with high-functioning autism and eight with low-functioning autism.

Significantly different from the control sample: * $p<0.05$, ** $p<0.01$.

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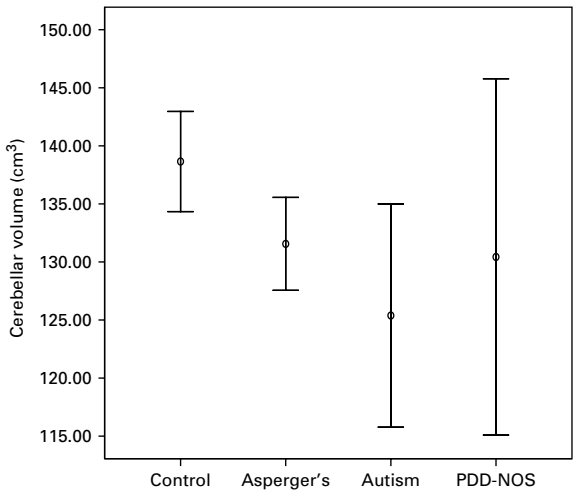

Fig. 1. Cerebellar volume in adults with autistic spectrum disorder and controls. Values are means and 95% confidence intervals. PDD-NOS, Pervasive developmental disorder – not otherwise specified.

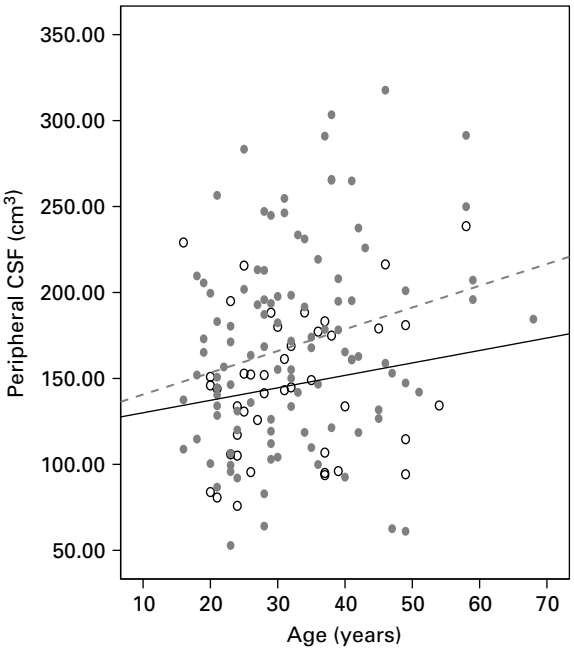

Fig. 2. Peripheral cerebrospinal fluid (CSF) in adults with autistic spectrum disorder (●, – – –, $r=0.237$, $p=0.015$) and controls (○, —, $r=0.177$, $p=0.257$). $Z=0.38$.

Right–left asymmetry

There was no significant difference in asymmetry between the combined ASD group or any subgroup and controls.

Matched-IQ Asperger's group

As the control subjects had a higher mean IQ than the Asperger's subjects, we repeated our analysis using a subgroup of controls ($n=51$, mean full-scale IQ = 111, S.D. = 12) and Asperger's subjects ($n=69$, mean full-scale IQ = 108, S.D. = 11). In the Asperger's subgroup, cerebellar volume was significantly reduced in volume [$F(1,119)=5.338$, $p=0.023$] and total peripheral CSF was significantly increased in volume [$F(1,119)=5.768$, $p=0.018$].

Autism subgroups

We repeated our analysis comparing individuals with high-functioning autism (HFA) ($n=20$) with controls and low-functioning autism (LFA) ($n=8$) with controls. Cerebellar volume was reduced [$F(1,79)=8.092$, $p=0.004$] and peripheral CSF was increased [$F(1,79)=4.916$, $p=0.03$] in the HFA group compared with controls. In the LFA group, left cerebellar volume was reduced [$F(1,67)=4.848$, $p=0.03$] and lateral ventricular volume was increased [$F(1,67)=4.842$, $p=0.030$] compared with controls.

Discussion

We examined the bulk volume of brain and CSF in adults with ASD using *in vivo* MRI and hand tracing. We found no significant between-group difference in whole-brain, lobar, or intracranial volume. However people with ASD had a significantly smaller volume of cerebellum and a significantly larger volume of peripheral CSF.

Macrocephaly

Our failure to find an increased total brain volume in ASD adults supports a number of previous reports in older age groups (McAlonan *et al*. 2002; Redcay & Courchesne, 2005). Also our finding of no differences in right–left asymmetry is consistent with some other studies in both children (Hazlett *et al*. 2005) and adults (Courchesne *et al*. 1988).

However, Redcay & Courchesne (2005) suggested that the developmental trajectory of brain growth in ASD is significantly different – with maximal brain volume being achieved at a much younger age in individuals with ASD compared with healthy controls. Thus our sample may have had transient differences in brain volume compared with controls when they were much younger, but these were not detectable by us at a later age. Nevertheless, our findings do suggest that, in our sample of people with ASD, maximal brain size (as reflected by current intracranial volume) was never significantly greater than controls.

Cerebellum

All the subgroups of ASD (except for PDD-NOS) had a significantly smaller bulk volume of cerebellum.

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This is consistent with a number of previous MRI studies (Murakami *et al*. 1989; Courchesne *et al*. 2001). Our tracing methodology did not allow us to determine which particular subregions, or tissue class, were most affected. However, we previously reported a significant reduction in both grey and white matter in the cerebellum of adults with Asperger's syndrome using voxel-based morphometry (McAlonan *et al*. 2002). It is thus likely that our findings indicate a reduction in both of these tissue compartments in ASD adults.

Our findings are at odds with a number of previous studies that have found increased cerebellar volume in ASD (Piven *et al*. 1997; Hardan *et al*. 2001; Sparks *et al*. 2002). However, these studies examined a different patient group compared with our study. The studies carried out by Piven *et al*. (1997) and Hardan *et al*. (2001) used different MRI statistical software and examined a younger cohort, including both adolescents and adults with mean ages of 18 and 22 years respectively, both significantly younger than our study; whilst Sparks *et al*. (2002) examined only young children.

We found evidence that all subgroups of ASD (except for PDD-NOS, where we only had modest numbers and thus a large variance in volume) have a significant reduction in volume of cerebellum. Hence this may be a common feature across the disorder in adults, although there is no direct evidence linking cerebellar function and higher-order functions. This suggestion is supported by evidence that the cerebellum is important in higher-order functions frequently impaired in people across the spectrum – e.g. attention (Allen *et al*. 1997), social interaction (Townsend *et al*. 1999) and executive functioning (Ronning *et al*. 2005). In addition, autistic-like symptoms (such as social and communicative deficits) have been demonstrated in previously normal-functioning individuals with acquired cerebellar lesions (Schmahann & Sherman, 1998; Levisohn *et al*. 2000). Moreover, functional MRI studies in ASD individuals have demonstrated differences in cerebellar activation as compared with controls during both motor (Allen *et al*. 2004) and social tasks (processing facial expressions) (Critchley *et al*. 2000).

CSF

We found no significant difference in volume of ventricular CSF in people with ASD at any age, and this is consistent with some (but not all) previous reports (Hardan *et al*. 2001). A difference in ventricular CSF is normally assumed to be a proxy measure for variation in development (or loss) of brain matter, and is significantly increased in people with other neurodevelopmental pathologies including epilepsy and some types of intellectual disability (mental retardation) and neuropsychiatric disorders. We deliberately only included very healthy people with ASD and we found that ventricular CSF was only increased in autistic people before we corrected for IQ. Hence prior reports that people with ASD have differences in ventricular volume may be explained by variation in the intellectual level and physical and mental health characteristics of the participants.

This is the first study to examine peripheral CSF in people with ASD. All ASD groups (except for PDD-NOS) had significantly larger volumes of peripheral CSF compared with controls, without an associated increase in (current) bulk brain size or total intra-cranial volume. Also we found preliminary evidence for a differential increase in peripheral CSF with age in ASD compared with controls – although this difference did not reach statistical significance.

Peripheral CSF includes all non-ventricular subarachnoid CSF surrounding the brain. This is generally accepted as a proxy measure for the mismatch between current head size and maximal brain size attained during development [because intracranial volume (i.e. head size) is proportional to brain size (Wickett *et al*. 2000)].

The cause of this increase in peripheral CSF in ASD is unknown but may include differences in the developmental trajectory (or ageing) of the two groups. For example, differences in cortical thickness have been reported in ASD which contribute to increased grey matter volume (Hardan *et al*. 2006). Normally, loss of bulk lobar brain volume affecting both grey and white matter is associated with expansion in ventricular CSF – whereas we found differences only in peripheral CSF. Hence our findings may be partially explained by differences in neurodevelopmental processes affecting predominantly cortical grey matter, with loss of cortical grey matter, and/or differences in cortical gyrification being associated with expansion of peripheral CSF in ASD. This hypothesis, however, is speculative – and we need further studies which directly measure cortical thickness and gyrification in ASD.

Nevertheless, if we are correct, there are several (testable) potential explanations for any putative abnormalities in the developmental trajectory of cortex. Among these are differences in neurotrophins and apoptotic proteins. For example, some have reported that newborns later diagnosed with autism (Nelson *et al*. 2001) have a significantly increased plasma concentration of brain-derived neurotrophic factor (BDNF), neurotrophin-4 (NT-4), vasoactive intestinal peptide and calcitonin-related gene peptide. Furthermore, increased BDNF levels have been demonstrated

in post-mortem autistic brains (Nelson *et al*. 2001) and both BDNF and NT-4 can decrease Purkinje cell survival by excitotoxic methods (Morrison & Masone, 1998). Abnormalities in apoptotic proteins in ASD include decreased levels of reelin (Fatemi *et al*. 2001) and Bcl-2 (Morrison & Masone, 1998) and increased levels of p53 (Araghi-Niknam & Fatemi, 2003). Both reelin and Bcl-2 are anti-apoptotic proteins, while p53 is a key regulator of normal apoptosis (Araghi-Niknam & Fatemi, 2003). Thus abnormalities in neurotrophins, and/or apoptotic proteins, may partially explain differences in brain maturation, and hence expansion of peripheral CSF in people with ASD.

Study limitations

There are a number of limitations with our study. First, although we had a relatively large sample size, we only had very modest numbers of individuals with PDD-NOS. Also, our study was cross-sectional and therefore we can only report age-related differences as opposed to differences in ageing of individuals. Further, we did not relate our volumetric MRI findings to ADI scores (as these were not available in all subjects), and so we cannot definitively conclude that the anatomical differences we identified are related to the behavioural phenotype of ASD. We did not have an ADI or ADOS available on all individuals in this study; however, all subjects were diagnosed using ICD-10 research criteria and our clinical interview, screening and investigations for each individual (excluding carrying out the ADI or ADOS) takes an entire day to complete. We repeated our analysis including only individuals who had an ADI or ADOS and our volumetric findings were unchanged. Finally, we used hand-tracing methods of large brain regions, and not voxel-based morphometry, and we did not have measures of cortical thickness/gyrification. Hence we were unable to measure subtle differences in grey and white matter of whole brain and other brain regions implicated in ASD. This is of importance because we have previously demonstrated that brain regions which do not differ in bulk volume do have relative differences in grey and white matter (McAlonan *et al*. 2005). Nevertheless this study design did allow us to rapidly examine a large number of adults from across a relatively wide age range, which would almost be impossible in a longitudinal study. Also our simple tracing approach gives volumes in millilitres that can be easily, and rapidly, replicated by other laboratories without sophisticated resources. Due to multiple testing there was an increased risk of type 1 errors. We carried out multiple-comparison procedures (Bonferroni testing) on all parametric analysis to control for this.

Conclusions

To our knowledge this is the largest quantitative MRI study of bulk brain volume and CSF in people with ASD. We found no significant between-group difference in current brain or head size, or volume of ventricular CSF. However, the adults with ASD had a significant reduction in cerebellar volume and an increase in peripheral CSF. Hence, we suggest that the ASD adults we studied never had differences from controls in their maximal brain size, but did have differences in cerebellar development. Our findings of increased peripheral CSF in those with ASD may be due to subtle differences in the ageing of cortical grey gyrification.

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Declaration of Interest

None.

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