

# Mapping the brain in autism. A voxel-based MRI study of volumetric differences and intercorrelations in autism

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## Summary

Autism is a disorder of neurodevelopment resulting in pervasive abnormalities in social interaction and communication, repetitive behaviours and restricted interests. There is evidence for functional abnormalities and metabolic dysconnectivity in ‘social brain’ circuitry in this condition, but its structural basis has proved difficult to establish reliably. Explanations for this include replication difficulties inherent in ‘region of interest’ approaches usually adopted, and variable inclusion criteria for subjects across the autism spectrum. Moreover, despite a consensus that autism probably affects widely distributed brain regions, the issue of anatomical connectivity has received little attention. Therefore, we planned a fully automated voxel-based whole brain volumetric analysis in children with autism and normal IQ. We predicted that brain structural changes would be similar to those previously shown in adults with autism spectrum disorder and that a correlation analysis would suggest structural dysconnectivity. We included 17 stringently diagnosed children with autism and 17 age-matched controls. All children had IQ >80. Using Brain Activation and Morphological Mapping (BAMM) software, we measured global brain and tissue class volumes and mapped regional grey and white matter

differences across the whole brain. With the expectation that volumes of interconnected regions correlate positively, we carried out a preliminary exploration of ‘connectivity’ in autism by comparing the nature of inter-regional grey matter volume correlations with control. Children with autism had a significant reduction in total grey matter volume and significant increase in CSF volume. They had significant localized grey matter reductions within fronto-striatal and parietal networks similar to findings in our previous study, and additional decreases in ventral and superior temporal grey matter. White matter was reduced in the cerebellum, left internal capsule and fornices. Correlation analysis revealed significantly more numerous and more positive grey matter volumetric correlations in controls compared with children with autism. Thus, using similar diagnostic criteria and image analysis methods in otherwise healthy populations with an autistic spectrum disorder from different countries, cultures and age groups, we report a number of consistent findings. Taken together, our data suggest abnormalities in the anatomy and connectivity of limbic-striatal ‘social’ brain systems which may contribute to the brain metabolic differences and behavioural phenotype in autism.

**Keywords:** autism; MRI; brain mapping

**Abbreviations:** BA = Brodmann area; ROI = region of interest; STS = superior temporal sulcus; VBM = voxel-based morphometry

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## Introduction

Autism is a highly genetic neurodevelopmental disorder (Folstein and Rutter, 1977; Bailey *et al.*, 1996), characterized by a triad of repetitive and stereotypic behaviour, impaired communication and striking deficits in social reciprocity. Post-mortem studies date the onset of neuropathology to as early as the second half of pregnancy (Kemper and Bauman, 2002), and there is increasing evidence that this results in anatomical abnormalities in fronto-temporo-parietal cortices, the limbic system and cerebellum, but many findings have not been replicated (Bailey *et al.*, 1996; Brambilla *et al.*, 2003). This is probably due in part to differences between studies in diagnostic criteria used and confounders such as the age, IQ and physical health of people studied which impact upon brain structure. These factors hold particular relevance to autism as postnatal brain maturation is altered in autism (Courchesne *et al.*, 2001; Aylward *et al.*, 2002; McAlonan *et al.*, 2002), a majority of people with autism have low IQ and many also have epilepsy. However, failure to replicate may also be a consequence of the methods of data acquisition and analysis and many prior studies have used 'region of interest (ROI)' manual tracing approaches which are not easily reproducible across different laboratories (Brambilla *et al.*, 2003).

It is relatively unlikely that the behavioural phenotype of autism (or any complex neurodevelopmental disorder) can be explained by abnormalities in one brain region. In the general population, studies of social understanding (specifically theory of mind tasks) implicate prefrontal cortex, medial and ventral temporal lobe, superior temporal sulcus (STS), amygdala and cerebellum (Fletcher *et al.*, 1995; Calarge *et al.*, 2003; Schultz *et al.*, 2003) as candidate brain determinants of autism. Theory of mind tasks do not activate all these regions in adults with autism (Happé *et al.*, 1996; Baron-Cohen *et al.*, 1999; Castelli *et al.*, 2001) and, although the early processing in extra-striate visual areas appears normal in individuals with autism, significantly reduced functional connectivity between these areas and STS has been reported (Castelli *et al.*, 2001). Horwitz *et al.* (1988) used PET to address metabolic connectivity in autism and reported significant differences in cortico-subcortical connectivity. Therefore, it is plausible that the anatomy, connectivity and function of brain systems are affected by autism. In a preliminary assessment of the organization of brain systems in autism, Herbert *et al.* (2003) combined structural MRI data from boys with autism and their healthy controls in a factor analysis study (grouping volumes which co-vary or inter-correlate together). They derived three main factor groupings: central white matter; cerebral cortex and hippocampus-amygdala; and other grey matter regions; and found that autism appeared to drive white matter to be larger and cerebral cortex and hippocampus-amygdala to be smaller. However, again this was limited by an ROI approach so that more subtle anatomical differences in distributed neural networks could not be examined *a priori*.

Thus there is a need for detailed anatomical studies of (relatively) homogeneous populations employing newer,

more sensitive techniques such as automated voxel-based morphometry (VBM) of whole brain (Brambilla *et al.*, 2003). In the first study of this type, Abell *et al.* (1999) used statistical parametric mapping to explore grey matter differences in autism. They found grey matter volume reductions in prefrontal lobe and increases in temporal lobe and cerebellum. We subsequently used a non-parametric VBM approach in a study of autistic spectrum adults of normal intelligence in the UK and reported rather more extensive grey matter reductions across frontal, limbic, basal ganglia, parietal and cerebellar regions as well as significant volume changes in related white matter tracts (McAlonan *et al.*, 2002). We concluded that adults with an autistic spectrum disorder have regional differences not only in brain volume, but probably also in anatomical organization of large-scale brain systems. However, we did not know if our findings generalized to other autistic populations (i.e. to children or other cultures) and we were unable to examine the anatomical 'connectivity' of neural systems which putatively underlie the disorder.

Therefore, we examined the brain anatomy of high functioning Chinese children with classical autism at normal schools. We compared these with control children from the same narrow age range and socio-economic class. We used MRI and non-parametric VBM to identify significant between-group differences in the whole brain and regional volumes of grey and white matter and CSF. Interconnecting brain systems have common developmental and maturation influences (Cheverud, 1982, 1984), thus their volumes would be expected to co-vary or positively correlate (Kerwin and Murray, 1992; Bullmore *et al.*, 1998; Zhang and Sejnowski, 2000). We therefore predicted that, compared with controls, children with autism would have disrupted connectivity reflected by fewer and less positive grey matter volume correlation coefficients within the affected grey matter network.

## Methods

### Subjects

Subject characteristics are shown in Table 1. All subjects were ethnic Chinese, right-handed, with IQ >80 (estimated using Raven's progressive matrices). We excluded children with a co-morbid psychiatric or medical condition (e.g. epilepsy), history of head injury

**Table 1** Subject characteristics

	Control	Autism	<i>t</i> test (df = 32)	<i>P</i> value
Age (years)	11 ± 1.2	12 ± 1.8	-1.93	NS
IQ	114 ± 14.1	101 ± 10.0	3.21	0.05
Social class	2.76 ± 1.07	2.88 ± 1.07	-0.30	NS
Education (years)	5.47 ± 1.23	6.12 ± 1.80	-1.22	NS

Social class is based on paternal occupation as defined by the UK Office of Population Censuses and Surveys 1991. Values are given as means ± SD.

or genetic disorder associated with autism (e.g. tuberous sclerosis or fragile X syndrome). Seventeen (16 male, one female) were non-medicated children with autism aged 8–14 years, recruited from a community autism programme. ICD10 diagnosis of autism was confirmed using the 1994 Autism Diagnostic Interview—Revised (Lord *et al.*, 1994) translated into Chinese in-house. Seventeen (16 male, one female) typically developing control children aged 8–14 years old were recruited from local schools and screened for major psychiatric illness using a structured parental interview. We prioritized age matching of groups, as a lower IQ is a recognized complication of autism (Happé and Frith, 1996). Every child's parent gave informed consent for the protocol approved by the University of Hong Kong Faculty of Medicine Research Ethics Committee, and each child gave his/her assent.

### MRI and analysis

Using slices of 3 mm thickness, dual-echo fast spin echo data sets aligned to the anterior commissure–posterior commissure (AC–PC) line were acquired across the whole brain on a GE Sigma 1.5 T system (General Electric, Milwaukee, WI) in Queen Mary Hospital, Hong Kong. A consultant radiologist (K.S.T.), blind to diagnosis, reviewed each MRI scan, and none was found to have any gross anomaly.

Group differences in grey and white matter were mapped using the BAMB software (Brain Analysis Morphological Mapping version 2.5, <http://www-bmu.psychiatry.cam.ac.uk/BAMB/index.html>) on a SPARC workstation (Sun Microsystems Europe Inc., Surrey, UK). This process has been described previously for both adult and child populations (Overmeyer *et al.*, 2001; Sigmundsson *et al.*, 2001; McAlonan *et al.*, 2002). Briefly, images were processed to remove extra-cerebral tissues (Suckling *et al.*, 1999a) and then segmented into grey and white matter, CSF and a fourth class including dura, vessels and other extraneous tissues which subsequently were ignored (Suckling *et al.*, 1999b). Global volumes of grey and white matter, CSF and whole brain were calculated and compared across groups using independent *t* tests. The segmented images were mapped into the standard space of Talairach and Tournoux (1988) by minimizing the sum of square intensity difference of each proton density image to a group-specific template (mean of three children with autism and three controls; Brammer *et al.*, 1997) and smoothed with a 4.4 mm kernel. The effect of diagnostic group was estimated at each voxel by regression of a general linear model. Maps of the appropriate normalized coefficient were subject to an inference procedure in which the significance of three-dimensional cluster statistics was assessed using non-parametric methods (Bullmore *et al.*, 1999). The statistical thresholds were corrected for multiple comparisons by controlling the 'family wise error rate', in this case by setting the *P* value used such that <1 false-positive cluster was expected under the null hypothesis. A cluster of grey or white matter abnormality was defined as 'deficit' or 'excess' depending on whether the volume was reduced or increased in the autism group relative to the control group. To determine whether the IQ difference between groups had an impact on our results, we entered the volumes derived in a multiple analysis of covariance (MANCOVA; SPSS 11.5.1 general linear model, multivariate analysis) with IQ as a covariate.

### Correlation analysis

Volumetric correlations across subjects were explored between each pair of clusters of significant grey matter difference using Pearson's

correlations in both groups separately. The number of intra-regional correlations with  $r \geq 0.4$  found was compared with the  $\chi^2$  square distribution. We also used randomization to assess non-parametrically the significance of between-group differences for each inter-regional correlation. This procedure involved repeated (400) random reassignments of each individual's set of volume measures to diagnostic group to generate correlation matrices which sample the null hypothesis that group differences occurred by chance (Woodruff *et al.*, 1997; Bullmore *et al.*, 1998). To test our prediction that correlation coefficients in the control group would be more positive than the autism group, we used the 99 percentile point as a critical value for a two-tailed test of the null distribution with probability of type 1 error  $\leq 0.01$ .

## Results

### Quantitative structural MRI

The total volume of grey matter in the autism group was significantly reduced [ $t(32) = 3.13$ ,  $P < 0.005$ ] and total volume of CSF significantly increased [ $t(32) = -2.8$ ,  $P < 0.005$ ]. There were no overall group differences in total brain, nor total white matter volumes (see Table 2).

### Regional grey matter differences

Subjects with autism had on average 25% reduction in 13, three-dimensional grey matter clusters. In the right hemisphere, deficit clusters were observed in the orbital, inferior and middle frontal gyri, caudate nucleus, ventral temporal lobe and medial parietal lobe. In the left hemisphere, the deficit clusters were located in orbital, middle and medial frontal gyri, middle and superior temporal gyri, caudate nucleus and medial parietal lobe (cluster threshold  $P = 0.01$ , cluster test significance  $P = 0.002$ ; Fig. 1 and Table 3). Co-varying IQ (MANCOVA) did not alter the significance of group volume differences.

### Regional white matter differences

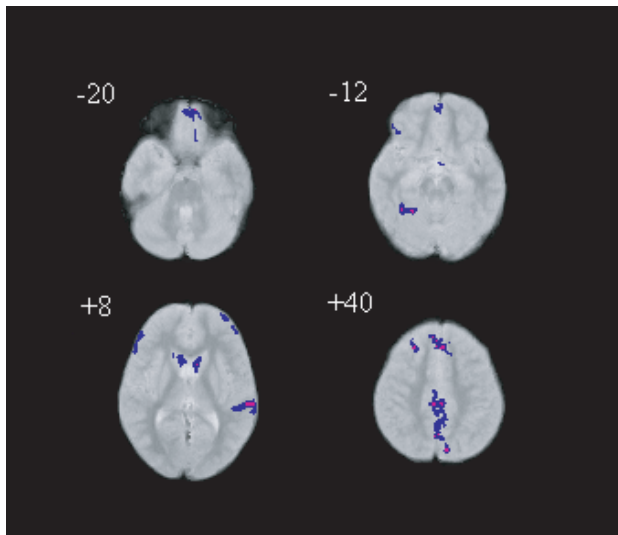
There were two extensive 3D clusters of white matter reduction in autism. White matter was reduced bilaterally in the cerebellum by 19% and in the left internal capsule and bilateral fornices by 21%. At the level of significance selected, no differences in corpus callosum volumes were observed (cluster threshold  $P = 0.05$ , cluster test significance  $P = 0.002$ ; Table 3 and Fig. 2). Co-varying IQ (MANCOVA) did not alter the significance of volume differences.

**Table 2** Global brain volumes

	Control (ml)	Autism (ml)	<i>P</i> value ( <i>t</i> test)
Whole brain volume (SD)	1259.1 (21.3)	1262.7 (26.8)	NS
Total grey matter volume (SD)	626.2 (29.1)	594.1 (31.5)	0.004
Total white matter volume (SD)	468.4 (17.7)	469.1 (5.2)	NS
Total CSF volume (SD)	164.5 (31.2)	199.5 (39.3)	0.008

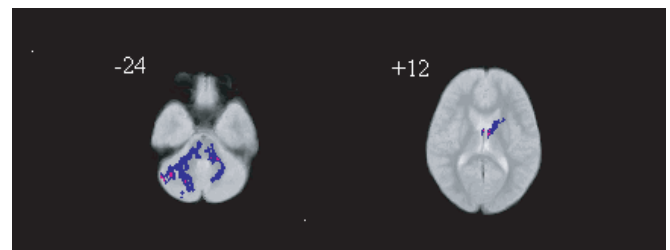
### Correlation analyses

Results are shown in detail in Table 4. Pearson correlation analysis of 3D clusters revealed that, out of a possible 78 correlations in each group, there were large positive correlation coefficients ( $r \geq 0.4$ ) in 24 pairs in the control group and only one sizeable negative coefficient ( $r \leq -0.4$ ).



**Fig. 1** Grey matter deficits in autism ( $P = 0.002$ ). Relative deficit clusters (blue) in grey matter volume in children with autism compared with controls. The maps are orientated with the right side of the brain shown on the left side of each panel. The z-coordinate for each axial slice in the standard space of Talairach and Tournoux is given in millimetres.

In contrast, only eight pairs in the autism group had substantial positive inter-regional volumetric correlations ( $r \geq 0.4$ ), while two pairs were strongly negatively correlated ( $r \leq -0.4$ ). The number of positive correlations in the control group was significantly greater than in the autism group [ $\chi^2(1) = 10.06$ ,  $P < 0.002$ ]. Twenty-three correlation coefficients in the control group, mostly where  $r \geq 0.4$ , were significantly larger than those in the autism group ( $P < 0.01$ ); only five correlation coefficients in the autism group (four, where  $r \geq 0.4$ ) were significantly larger than the corresponding control values. The multiple large positive correlations in the control group were both inter- and intrahemispheric and often involved the caudate nuclei and medial parietal lobe. Positive correlations in the autism group involved the right ventral temporal lobe, left temporal lobe [Brodmann area (BA) 22],



**Fig. 2** White matter deficits in autism ( $P = 0.001$ ). Relative deficit clusters (blue) in white matter volume in children with autism compared with controls. The maps are orientated with the right side of the brain shown on the left side of each panel. The z-coordinate for each axial slice in the standard space of Talairach and Tournoux is given in millimetres.

**Table 3** Relative volume reductions in autism

	x	y	z	Voxels	Control	Autism
Grey matter ( $P = 0.002$ )						
Frontal lobe						
Orbital gyrus BA11 bilateral	-2.6	40.7	-20.0	116	0.64	0.48
Med frontal gyrus BA8 bilateral	-1.2	27.6	39.8	188	0.81	0.62
Mid frontal gyrus BA46 left	-36.0	43.4	17.1	144	0.67	0.49
Inf frontal gyrus BA45 right	45.8	33.5	2.9	170	0.71	0.48
Mid frontal gyrus BA9 right	22.3	33.6	2.5	120	0.63	0.46
Temporal lobe						
Mid temporal gyrus BA22 left	-54.5	-31.3	6.0	151	0.50	0.35
Sup temporal gyrus BA42 left	-49.5	-31.2	21.8	136	0.58	0.42
Parahippocampal BA37/fusiform gyrus BA19 right	21.5	-49.7	-5.7	184	0.82	0.63
Parietal lobe						
Precuneus BA7 bilateral	-5.0	-64.2	33.3	123	0.65	0.51
Cingulate gyrus BA31 bilateral	2.1	-38.5	42.2	310	1.84	1.49
Subcortical						
Caudate nucleus left	-4.9	4.5	1.2	279	1.0	0.77
Caudate nucleus right	8.7	13.1	4.7	129	0.65	0.50
Brainstem	-4.2	-54.7	-38.0	14	0.60	0.43
White matter ( $P = 0.001$ )						
Cerebellum bilateral	13.8	-51.9	-24.3	1362	5.84	4.75
Internal capsule left/fornix bilateral	-6.2	-7.1	2.8	489	1.36	1.18

Suggested anatomical labels and Brodmann areas (BAs) are provided for guidance. The (3D) clusters are not confined to these areas, nor are they all encompassing. A sample Talairach coordinate ( $x$ ,  $y$  and  $z$ ) is given for the approximate centre of each cluster. The volume of grey or white matter within each cluster is shown for control and autism groups in ml.

**Table 4** Correlation matrices of affected grey matter network

Control		Autism												
		Frontal lobe					Temporal lobe			Parietal lobe		Subcortical		
		BA11	BA8	BA46 L	BA45 R	BA9 R	BA22 L	BA42 L	BA37 R	BA7	BA31	Caud L	Caud R	Brainstem
Frontal lobe	BA11		0.37	-0.03	-0.05	0.00	0.17	0.06	0.10	-0.39	0.12	0.01	0.23	0.05
	BA8	0.26		0.34	-0.21	<b>0.43*</b>	0.24	0.03	<b>0.46*</b>	0.05	<b>0.40</b>	0.09	-0.01	-0.08
	BA46 L	<b>0.56*</b>	0.17		0.12	0.05	-0.08	0.02	-0.40	-0.35	0.01	0.08	-0.18	-0.06
	BA45 R	0.35	0.22	<b>0.59*</b>		-0.17	-0.16	-0.25	-0.34	0.21	-0.06	0.28	<b>0.48</b>	0.18
	BA9 R	0.34	0.20	0.03	-0.19		0.00	<b>0.29*</b>	0.19	0.13	0.30	0.27	-0.01	0.11
Temporal lobe	BA22 L	<b>0.48</b>	0.19	<b>0.48</b>	0.34	-0.13		<b>0.44*</b>	<b>0.44</b>	-0.01	0.30	0.05	0.17	-0.56
	BA42 L	0.01	0.31*	0.27	0.26*	0.02	0.12		-0.12	-0.32	0.06	-0.07	0.01	-0.37
	BA37/19 R	-0.01	0.29	0.06	-0.01	-0.01	0.36	0.10		<b>0.48*</b>	0.17	0.20	0.19	-0.38
Parietal lobe	BA7	0.09*	0.36*	-0.14	-0.21	<b>0.57*</b>	-0.40	0.22*	-0.02		0.39	0.29	0.25	-0.22
	BA31	<b>0.47</b>	0.31	<b>0.47*</b>	0.18	<b>0.60*</b>	0.18	<b>0.47*</b>	0.32	<b>0.54</b>		0.06	0.14	-0.13
Subcortical	Caudate L	<b>0.40</b>	<b>0.56*</b>	0.28	0.25	0.69	0.07	0.39*	-0.20	0.46	<b>0.54*</b>		<b>0.77</b>	-0.11
	Caudate R	<b>0.42</b>	0.44	<b>0.43*</b>	0.33	0.36	0.10	0.21	-0.02	0.21	<b>0.58</b>	<b>0.56</b>		-0.13
	Brainstem	0.37	0.27	0.04	-0.11	<b>0.49</b>	-0.07	0.20	-0.05	<b>0.64*</b>	<b>0.71*</b>	<b>0.41</b>	0.34	

Correlation coefficients between brain regions for control (left of diagonal) and autism (right of diagonal) groups. Bold values highlight  $r \geq 0.4$ ; \* indicates a correlation coefficient more positive in one group compared with the other. BA = Brodmann area; L = left; R = right; Caud = caudate nucleus.

prefrontal lobe (BA8 and right BA9), precuneus and the right caudate nucleus. Bonferroni adjustment for multiple comparisons was not applied (Perneger, 1998), as correlation matrices indicated that observations were not independent.

## Discussion

Using a voxel-based analysis in a group of intellectually able children with autism, we found they had reduced global grey matter volumes and increased CSF volumes. Whole brain volume was unchanged, and this agrees with previous reports that megalencephaly is not a feature of autism after age 4 years (Courchesne *et al.*, 2001; Aylward *et al.*, 2002; McAlonan *et al.*, 2002). Our principal findings, however, were that children with autism had significant differences in the regional brain volume of prefrontal and parieto-temporal cortices which are considered to underpin 'social cognition' (Brothers, 1990) and language (Binder, 1997; Binder *et al.*, 1997). In addition, we found marked disruption to cortico-subcortical and cortico-cortical grey matter volumetric relationships which mirror the reduction in cortico-subcortical and cortico-cortical metabolic correlations in autism recorded by Horwitz *et al.* (1988) and add weight to the concept that people with autism spectrum disorders have differences in the anatomical and functional integration of large-scale neural systems.

### Abnormalities in a social brain network

The distribution of grey matter deficits in the autism group is striking in the extent to which it encompasses brain regions with critical socio-emotional function. For example, the right fusiform gyrus, concerned with face recognition (Schultz

*et al.*, 2003), and the STS, with judging changeable aspects of face such as eye gaze, expression and lip movement (Haxby *et al.*, 2002), may be core to face analysis. The orbitofrontal cortex forms an interface between emotion and cognition (Rolls, 2004) and, together with the left middle temporal lobe, precuneus and posterior cingulate, is implicated in making social judgements and empathy (Farrow *et al.*, 2001). Finally, the medial prefrontal lobe (BA8) permits an understanding of the mental state of others, or theory of mind (Fletcher *et al.*, 1995; Frith and Frith, 1999). These results stand in dramatic contrast to a recent VBM study of Williams syndrome which showed that, in this neurodevelopmental disorder of heightened socio-emotional responsiveness, grey matter was increased in a similar circuit through bilateral orbital gyri, left middle temporal gyrus, right fusiform, precuneus and cingulate regions (Reiss *et al.*, 2004). Thus, at the very simplest level of interpretation, there appears to be a dichotomy between developmental pressures which increase grey matter in a social brain network and bias towards socio-emotional behaviour, while grey matter reduction in the same system impairs social interaction.

The network of grey matter change in autism detailed here therefore provides a plausible basis for the differences in brain activation during social and communicative tasks we and others have reported in autism in the medial prefrontal cortex (Happé *et al.*, 1996), superior temporal gyrus (Critchley *et al.*, 2000; Schultz *et al.*, 2000; Just *et al.*, 2004) and ventral temporal lobe (Schultz *et al.*, 2000; Pierce *et al.*, 2001). For instance, the location of the medial prefrontal cortex (BA8) and posterior cingulate (BA31) deficits described here corresponds closely to the areas of activation observed during a 'theory of mind' task in typical adult subjects

(Fletcher *et al.*, 1995) but not in subjects with autism performing the same task (Happé *et al.*, 1996). Similarly, the right fusiform gyrus in the ventral temporal lobe is activated reliably during face recognition, except in individuals with autism (Pierce *et al.*, 2004). Where estimations of functional ‘connectivity’ have been made, these suggest network disintegration in autism (Horwitz *et al.*, 1988; Schultz *et al.*, 2000; Castelli *et al.*, 2001; Just *et al.*, 2004) but the structural basis is unclear. Since interconnecting brain regions exert mutually trophic effects during development, their volumes could be expected to correlate positively (Kerwin and Murray, 1992; Bullmore *et al.*, 1998). Therefore, we suggest that the relative absence of positive inter-regional volumetric correlations in autism points to widespread structural dysconnectivity within the social brain in this condition. We did, however, observe a very few large correlation pairs in autism which were significantly more positive than in the control group. For example, although the volume of the right ventral temporal lobe/fusiform gyrus was reduced in our autism group, it remained strongly correlated with the volume of BA8/medial prefrontal lobe. In typically developing individuals, the fusiform gyrus may be activated preferentially by faces simply because there is strong social motivation to acquire ‘expertise’ in faces (Gauthier *et al.*, 1999; Grelotti *et al.*, 2002). In the absence of this social drive in autism, the fusiform face area may yet be engaged by non-face (expert) objects (Grelotti *et al.*, 2002) and so establish links to components of an otherwise pathological network. Similarly, the volumes of abnormal left middle and superior temporal gyri clusters are strongly correlated in the autism group. The BA22 region is close to an area of unusually increased activity noted by Just *et al.* (2004) in individuals with autism during a sentence completion task. They considered this might reflect hyperlexical skills for single word processing in autism. Thus, against a background of generalized network disintegration in autism, isolated connections of the kind described here might contribute to a neural substrate of aspects of autism which are not ‘deficit’ symptoms.

### **Neuropathological basis of autism**

Exploring inter-regional volume correlations may additionally provide a window onto patterns of very early development (Bullmore *et al.*, 1998). Subcortical afferentation of the cortex (from ~20 weeks gestation) depends upon controlled neuronal migration from periventricular regions across a striatopallidal ‘boundary zone’ (Molnar and Butler, 2002) and is followed by cortico-cortical afferentation. Thus the absence of inter-regional volumetric correlations, together with the particular distribution of cortico-subcortical grey matter anomalies and white matter abnormalities in childhood autism demonstrated here, could reflect afferentation problems during fetal brain development. Consistent with this, post-mortem studies date structural malformations in autism to early in fetal life (Bailey *et al.*, 1998) possibly prior to 28 weeks (Kemper and Bauman, 2002). Of most immediate clinical

interest, this account bolsters the evidence that brain abnormalities in autism are measurable before the possible impact of postulated ‘causal’ postnatal events such as vaccinations (Courchesne *et al.*, 2001). However, we do not intend to suggest that aberrant fetal development can explain all the neuroanatomical changes reported here. Rather we appreciate that brain morphology and organization of its circuitry continues throughout childhood (Munte *et al.*, 2002) by ‘interactive specialization’ (Johnson, 2000, 2003). Brain dysmaturation in autism is therefore likely to be on-going and is reflected by age-related changes in global brain or tissue class volumes (Courchesne *et al.*, 2001; Aylward *et al.*, 2002; McAlonan *et al.*, 2002). Thus our results are therefore most likely to be caused by a combination of factors, including an initial neurobiological ‘insult’ to neural networks which are modified further after birth.

### **Previous VBM studies**

This study of high functioning Chinese children in part replicates earlier work in adults with Asperger’s syndrome in the UK which adopted the same VBM approach (McAlonan *et al.*, 2002). It appears that a core of bilateral grey matter anomalies in prefrontal, caudate and medial parietal areas generalize across different age and ethnic groups who are physically healthy and not learning disabled. However, while the VBM technique provided detailed information about possible abnormalities in this spectrum in our two studies, the results do not overlap fully. In particular, compared with our previous work, here we found grey matter reductions in right ventral temporal lobe and left STS, but no extensive change to putamen or cerebellar grey matter volumes and fewer white matter changes (McAlonan *et al.*, 2002). This is not entirely surprising given a number of important differences between the two studies. The present study addressed classical autism, not Asperger’s. We recruited children in this study, whereas the previous study involved adults. Childhood and adolescence are very dynamic periods of brain modulation; white matter myelination increases linearly by ~12% between the ages of 4 and 22 years (Giedd *et al.*, 1999) while grey matter decreases by ~4–9% during this period (Giedd *et al.*, 1999; Sowell *et al.*, 2002). Given the cross-sectional design of both our studies, the age of participants probably influenced the precise pattern of results. Moreover, participants in our current study were native Chinese speakers and bilingual for Chinese and English, while in the previous study they were native English speakers. Subtle structural anatomical differences in the frontal, temporal and parietal lobes have been reported between Chinese and Caucasians (Kochunov *et al.*, 2003) and learning two languages appears to recruit distinct language modules (Chee *et al.*, 2000). Chinese language experience impacts upon the haemodynamic response in the right ventral temporal lobe (Tan *et al.*, 2000), left superior temporal gyrus (BA22; Tan *et al.*, 2001) and left middle frontal cortex (BA9/46; Tan *et al.*, 2003) and we found group difference in grey matter volume in each of these regions in the current

study but not in our previous study. Children with autism have significant language difficulties, so we postulate that the particular language demands of living in Hong Kong modulate the brain and compound the fronto-temporal abnormalities noted in children with autism in the present study.

The present results were much more at odds with a VBM study which described increased grey matter volumes in the temporal lobe and the cerebellum (Abell *et al.*, 1999). Since the patients in Abell's study were similar to our UK study, methodological differences may be relatively more important. In the study of Abell *et al.*, segmentation of single spectrum images was assisted by determining the probability that voxel values were grey matter (white matter was not addressed). Images were then normalized to the standard MNI (Montreal Neurological Institute) template and smoothed with a 12 mm kernel before parametric testing. Arguably, pre-processing and analysis of our data relied upon fewer assumptions, as it involved bi-modality segmentation, smoothing with a smaller kernel and non-parametric permutation test statistics (Bullmore *et al.*, 1999). In particular, by normalizing images to a group-specific template which incorporated patients, we hoped to introduce less bias (Good *et al.*, 2002). However, type I error control (number of false positives <1) in our study was particularly stringent and, while this lends confidence to the results, it does increase the chance of false-negative results (type II error) which may explain some of the discrepancies between studies.

### Methodological considerations

Other groups have used ROI measurements of structural MRI and sophisticated factor analytical methods to describe the organization of brain systems in neurodevelopmental disorders such as schizophrenia (Wright *et al.*, 1999) and autism (Herbert *et al.*, 2003). This allows grouping of brain regions also according to their volumetric intercorrelations. However, statistical considerations meant that in both examples the authors combined patient and control data sets. We believe that these approaches are not applicable to the present data set since the absolute regional volumes we derived by the VBM analysis are very small. Effectively, as subcomponents of the larger regions measureable using ROI techniques, their impact would probably be diluted. In addition, the decision to combine two groups of modest size to improve statistical power rests upon the assumption that brain regions are organized in the same way. We predicted that this would not be the case in autism, and the marked differences between group correlation matrices agree that this assumption would not have been valid for our data set.

There are a number of limitations to our work. First, we acknowledge that our study only addressed a subset of the autism spectrum and the number of subjects was modest, therefore the results may not apply to the entire population with this condition. The correlation approach we used depends upon the assumption that positive volumetric correlations indicate connectivity. While this seems a logical

interpretation, we cannot say for certain that it is valid. Also, although we found only three sizeable negative correlations in our analysis (two in the autism group and one in the control group), it is difficult to know what they might reflect. Possibly brain maturation leads to a measure of reorganization of neural systems with some connections strengthened at the expense of others. With the advent of diffusion tensor imaging techniques, more direct examination of connectivity is now possible and should help clarify these issues. Finally, a major problem with correlation analysis is that an apparent correlation may exist because both data points in a pair correlate with a third rather than each other. Nevertheless, we present the correlation analysis as a fresh perspective on structural MRI data which may extend our understanding about the development of brain networks in autism and other disorders of neurodevelopment.

### Conclusions

Our main finding is that the anatomy of brain systems implicated in social, emotional and communicative behaviour is disrupted in autism. Within this system, core prefrontal-striato-parietal grey matter abnormalities in autism may be replicable in age-matched and intellectually able groups, using automated voxel-based whole brain analysis methods. We considered correlation analysis as a proxy of network organization and provide preliminary neuroimaging evidence for structural dysconnectivity in the 'social brain' in autism.

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