Inverse correlation between frontal lobe and cerebellum sizes in children with autism

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Summary
Certain cognitive and behavioural deficits suggest that the frontal lobe functions abnormally in patients with autism, but little anatomical research is available to either verify or refute this. In contrast, several neuropathological and neuroimaging studies have demonstrated anatomical abnormalities in the cerebellum in autistic patients. The current study shows that frontal lobe cortex volume is increased in a subset of patients with autism and that this increase correlates with the degree of cerebellar abnormality. This evidence of concurrent structural abnormalities in both the frontal lobe and the cerebellum has important implications for understanding the development and persistence of the autistic disorder.

Keywords: magnetic resonance imaging; cerebrum; vermis; neuroanatomy; autism

Abbreviations: ADI = Autism Diagnostic Interview; CARS = Childhood Autism Rating Scale; ERP = event-related potential; LIPS = Leiter International Performance Scale; PD = proton density; PPVT = Peabody Picture Vocabulary Test—Revised; SBIS = Stanford Binet Intelligence Scale; WISC-III = Wechsler Intelligence Scale for Children, Third Edition

Introduction
Autism is a pervasive neurodevelopmental disorder characterized by impairments in social interaction and communication, behavioural stereotypes and a range of cognitive deficits. Although a consensus has not been reached regarding its aetiology or its brain substrates, a number of hypotheses have been put forward. One early and influential speculation suggested that dysfunction of the frontal lobe might underlie some of the characteristic behavioural abnormalities (Damasio and Maurer, 1978). Subsequent studies of autistic patients, which demonstrated commonly accepted indicators of frontal damage including abnormalities in cognition, e.g. deficits in attention, set-shifting, cognitive planning and problem-solving (Rumsey and Hamburger, 1990; Hughes et al., 1994; Pennington and Ozonoff, 1996; Townsend et al., 1996), cerebral blood flow (e.g. decreased blood flow; George et al., 1992; Zilbovicius et al., 1995) and neurophysiology (e.g. decreased amplitude of event-related potentials; Courchesne et al., 1984; Ciesielski et al., 1990; Dawson et al., 1995), helped to preserve a place for frontal dysfunction in more recent theoretical views. However, none of these reports documented an anatomical abnormality of the frontal cortex, and the findings could easily have been the consequence or by-product of maldevelopment in other neural systems that interact with the frontal lobe. As an example, autistic patients show an almost total absence of a frontally localized neurophysiological response in relation to attention, but the same effect is seen in non-autistic adults who have no evidence of structural damage of the frontal lobe but have acquired cerebellar lesions from strokes or tumours (Westerfield et al., 1998; see also Courchesne et al., 1984; Ciesielski et al., 1990). Thus, a finding which would seem to indicate abnormalities in the frontal lobe might also be explained by non-frontal lobe damage. In addition, if a frontal lobe abnormality is to explain the characteristic behaviours of autism, it must precede the onset of these behaviours chronologically. The question therefore remains whether the autistic phenotype includes frontal lobe abnormalities of a structural rather than simply a functional nature, and whether these abnormalities have an onset in early development.

Although the issue of neuroanatomical involvement of the frontal cortex is clearly important, the majority of the evidence for developmental neuropathology in autism has been localized to the cerebellar cortex. This region has been examined in a total of 20 post-mortem autism cases, 19 of whom showed evidence consistent with developmental abnormalities. In all but two of these cases, the cerebellar pathology consisted of a substantial reduction in the number
of Purkinje neurons, the amount of the decrease varying across cases (Guerin et al., 1996; Courchesne, 1997; Bailey et al., 1998). The frontal lobe, on the other hand, has been examined in only a handful of post-mortem cases. In three cases examined by three different laboratories, no abnormalities related to autism were reported (Williams et al., 1980; Bauman and Kemper, 1985; Guerin et al., 1996; note that only case 3 in the study of Williams et al. fits current diagnostic criteria for autism). But in a new post-mortem study of six adults and one child with autism (Bailey et al., 1998), two adult cases appeared to have thickened cortices in the frontal lobe and other regions, and one other adult case and the single child were reported to have irregular cortical laminar patterns in the frontal lobe. It is important to note that, among these four cases with frontal lobe abnormalities, three also had a decrease in Purkinje neuron numbers in the cerebellar vermis and hemispheres and the fourth had aberrant Purkinje neurons, most prominently seen in the cerebellar vermis. Therefore, this new post-mortem study not only provides the first anatomical evidence of frontal lobe abnormalities in autism but also raises the possibility that such abnormalities may occur in conjunction with established cerebellar abnormalities. Another important question, therefore, is whether cerebellar and frontal lobe abnormalities correlate with each other in autism, i.e. whether the degree of anatomical abnormality in one site is related to the degree of abnormality in the other. If the two abnormalities were to correlate, this would suggest that they are developmentally linked. This could result from a common aetiological event such as a genetic defect, or from abnormal interactions between the two regions, such as abnormal neural signals affecting the anatomical development of the regions to which they are transmitted.

To determine whether neuroanatomical abnormalities in the frontal lobe are typically seen in early autism, we studied a large sample of autistic children (n = 42) and healthy normal children (n = 29) using quantitative MRI to measure the volume of the frontal cortex. In order to examine whether frontal lobe abnormalities might be developmentally related to established cerebellar abnormalities, we also measured the superior posterior cerebellar vermis and performed correlation analyses on the two structures.

Methods

Patients and control subjects

The parents of all subjects gave informed consent for their child’s participation. The experimental procedures were approved by the Institutional Review Board of San Diego Children’s Hospital Research Center. All patients and control subjects were paid for their participation.

Patients with autism

Forty-two male patients with autism were examined; their ages ranged from 3.1 to 9.1 years (mean ± SD, 5.4 ± 1.7 years). Neuroanatomical measures for 11 of these subjects have been reported previously as part of a report on possible neuroanatomical contributions to orienting deficits in children with autism (Harris et al., 1999).

Diagnosis procedures. All subjects were assessed by a trained psychologist and met criteria for the diagnosis of autism according to all of the following (Table 1): DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, American Psychiatric Association, 1994); CARS (Childhood Autism Rating Scale, Schopler et al., 1988); ADI (Autism Diagnostic Interview, Lord et al., 1994); and ADOS (Autism Diagnostic Observation Schedule, Lord et al., 1989). All subjects who were scanned prior to the age of 5 years met clinical criteria at that time, and were also given a second diagnostic evaluation by Dr Cathy Lord (an expert in the diagnosis of autism, who was blind to the MRI measures) when they reached 5 years of age or older. These patients were included only if they met all of the above criteria after the age of 5 years. Patients diagnosed with pervasive developmental disorders other than autistic disorder, or with fragile-X syndrome, were excluded. Subjects were given a complete neurological examination, including EEG and brainstem auditory evoked response testing. Six of the patients had a history of seizures or evidence of seizure disorder on EEG.

Intelligence estimates. Subjects were given one or more standardized tests of intelligence, depending on the child’s level of cognitive functioning and co-operation. These included the Arthur adaptation of LIPS (Leiter International

### Table 1 Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Autistic subjects (n = 42)</th>
<th>Controls (n = 29)</th>
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<tbody>
<tr>
<td>Age at MR scan (years)</td>
<td>5.4 ± 1.7</td>
<td>6.0 ± 1.8</td>
</tr>
<tr>
<td>Seizures (n)</td>
<td>6</td>
<td>–</td>
</tr>
<tr>
<td>CARS</td>
<td>40.0 ± 4.9</td>
<td>–</td>
</tr>
<tr>
<td>ADI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social</td>
<td>25.6 ± 4.9</td>
<td>–</td>
</tr>
<tr>
<td>Verbal communication</td>
<td>17.0 ± 3.4</td>
<td>–</td>
</tr>
<tr>
<td>Non-verbal communication</td>
<td>11.6 ± 2.9</td>
<td>–</td>
</tr>
<tr>
<td>Restricted/repetitive</td>
<td>7.3 ± 2.4</td>
<td>–</td>
</tr>
<tr>
<td>IQ†</td>
<td>79.5 ± 22.3</td>
<td>114.0 ± 12.0</td>
</tr>
<tr>
<td>PPVT†</td>
<td>55.9 ± 13.9</td>
<td>102.7 ± 11.8</td>
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</table>

*Mean IQ for autism patients is based on performance IQ tasks only. Scores are from LIPS, WISC-PIQ or SBIS-Abstract Reasoning. Five patients did not complete any of these tests. Mean IQ for control subjects is based on composite IQ scores and is taken from WISC-FSIQ or SBIS-Composite. †Mean PPVT (Peabody Picture Vocabulary Test—Revised) score for autism patients is for 15 patients. An additional 10 patients took the test but achieved raw scores too low to determine an IQ equivalent (i.e. IQs below ~45); 17 patients did not complete the test. PPVT scores were not available for two control subjects. ADI = Autism Diagnostic Interview; CARS = Childhood Autism Rating Scale.
Performance Scale, Arthur, 1980), SBIS (Stanford Binet Intelligence Scale, Thorndike et al., 1986) and WISC-III (Wechsler Intelligence Scale for Children, Third Edition, Wechsler, 1991). Subjects were also administered the PPVT (Peabody Picture Vocabulary Test—Revised, Dunn and Dunn, 1981), a measure of receptive language ability. Nearly all of the subjects performed better on non-verbal portions of the tests than on the verbal portions, which is typical of patients with autism (Lincoln et al., 1994). Because of this, the child’s highest score from among the LIPS, WISC-III performance IQ and SBIS Abstract Reasoning test was used for the intelligence estimates.

Normal control subjects
Twenty-nine normal healthy male control subjects were examined (age 6.0 ± 1.8 years, range 3.4–9.0 years). They were recruited through advertisements in the community, and showed no evidence of developmental, educational, medical or psychiatric abnormalities on pre-MRI screening.

Intelligence estimates. Control subjects were given the PPVT and either the SBIS or the WISC-III depending on their age at the time of testing. Composite IQ scores and PPVT scores are shown in Table 1.

Imaging and image processing
Autistic patients were anaesthetized prior to scanning. Control subjects were typically scanned during normal sleep, although some remained awake during scanning. All subjects were scanned between 1992 and 1997 on the same 1.5 T magnet (Signa, General Electric, Milwaukee, Wis., USA) using two imaging protocols: (i) a T1-weighted sagittal protocol [TR (repetition time) = 600 ms, TE (echo time) = 25 ms, 2 NEX (number of excitations), FOV (field of view) = 16 cm, matrix = 256 × 256, 4 mm slices, no gaps]; and (ii) a double-echo, T2- and PD-weighted (PD = proton density) axial protocol (TR = 3000 ms, TE = 30 and 80 ms, 1 NEX, FOV = 20 cm, matrix = 256 × 256, 3 mm slices, no gaps). Data were transferred to Silicon Graphics (Mountain View, Calif., USA) workstations for analysis. Image sets from both subject groups were coded with random numbers and intermixed to ensure blindness of the experimenter to groups.

The axial image sets were processed using an automated tissue classification program (SEGMENT) that was designed in our laboratory. The techniques used in this program were similar to those described by other researchers in the semiautomated segmentation of nearly identical PD/T2 imaging protocols (Jackson et al., 1994; Matsumae et al., 1996). SEGMENT used a maximum likelihood criterion (Vannier et al., 1985) applied to the signal intensities on the PD and T2 images to classify pixels as parenchyma, CSF or non-brain tissue. Further discrimination of parenchyma into grey and white matter was based on a local threshold computed from pixel statistics within a three-dimensional space of 29 pixels × 29 pixels × 3 slices surrounding the pixel being classified. Skull and extracranial structures were removed from the T2-weighted images using a combination of thresholding and manual tracing. These images were then used as a mask on the tissue-classified images to create a data set containing tissue-classified intracranial structures only. Additional details regarding these algorithms and their validation are available upon request.

Measurement of frontal lobe volume
The classical anatomical boundaries of the frontal lobe (for review, see Zilles, 1990) were traced on the axial images at each slice level for every subject. The majority of the tracing was performed on the T2 images, but frequent reference was also made to the segmented images. In the more superior slices (Fig. 1A and D), a line was drawn through the centre of the central sulcus to mark the posterior limit of the frontal lobe. A line oriented perpendicular to the midline was then drawn from the cortical ribbon to the interhemispheric fissure to fully separate the frontal lobe from the rest of the hemisphere. In slices below the level of the central sulcus (Fig. 1B and E), a line was drawn through the centre of the sylvian fissure and then anteriorly along the surface of the insula, thereby excluding the insular cortex from the measurements. The frontal lobe was traced at the most ventral levels (Fig. 1C and F) by using the basal part of the lateral fissure. On all slices, the left and right frontal lobes were separated from each other by the interhemispheric fissure. After completion, the full set of boundary tracings was applied to the tissue-classified images to determine the number of pixels of each tissue type that fell within the frontal lobe.

Measurement of cerebellar vermis area
In a separate process, the cross-sectional area of cerebellar vermis lobules VI–VII was measured on the sagittal images (Fig. 2). A straight line from the anterior limit of the primary fissure to the apex of the fourth ventricle formed the boundary between lobules I–V and VI–VII. The border between lobules VI–VII and lobule VIII was defined by a straight line from the anterior limit of the prepyramidal fissure to the apex of the fourth ventricle. This cross-sectional area was used because: (i) the area of lobules VI–VII was found to be reduced in large studies of autism (Courchesne et al., 1994; Hashimoto et al., 1995); (ii) hypoplasia (reduced growth) of the cerebellar vermis is highly correlated with hypoplasia of the cerebellar hemispheres in patients with autism (Murakami et al., 1989); (iii) a variable degree of Purkinje cell reduction or abnormality was demonstrated in the vermis in 13 of 19 post-mortem cases (Guerin et al., 1996; Courchesne, 1997; Bailey et al., 1998); (iv) in autism patients, vermis hypoplasia is associated with deficits in shifting of attention, automatic orienting of attention and exploratory behaviour (Harris et al., 1999; K. Pierce and E. Courchesne, submitted for
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Fig. 1 Anatomical boundaries of frontal lobe. (A–C) T₂-weighted axial images at three representative slice levels, illustrating the location of major neuroanatomical landmarks. (D–F) Segmented images at the same slice levels as in A–C, illustrating the anatomical boundaries used for measurement of frontal lobe volumes. A detailed description of the method is included in the text. 1 = central sulcus; 2 = interhemispheric fissure; 3 = superior frontal gyrus; 4 = postcentral sulcus; 5 = lateral fissure; 6 = insula; 7 = cingulate gyrus; 8 = insular sulcus (circular sulcus); 9 = basal part of lateral fissure; 10 = middle cerebral artery.

publication); and (v) this cross-sectional area can be measured quickly and accurately, making it a convenient index of cerebellar hypoplasia.

Data processing and analysis
All statistical analyses were performed using SPSS 6.1.1 software (Chicago, Ill., USA). Independent sample t-tests were used to compare structure sizes between the autistic and control subjects as well as for post hoc analysis. Either separate or pooled variance analyses were used, as indicated by Levene’s test for equality of variances. One-tailed tests were used in the initial between-group comparisons based on the hypothesis that frontal lobe tissue volume would be larger in autistic patients, while lobule VI–VII area would be smaller. Linear regression analysis was used to test for possible relationships between the size of lobules VI–VII and the volume of the frontal lobe cortex in each subject group. Since autism, and perhaps even normal development, may involve some significant degree of biological heterogeneity, these analyses did not include statistically identified outliers. In order to identify such subjects, linear regression analyses were performed first, with all subjects from the group included. The standardized residuals were then used to identify outliers and the analyses were repeated with outliers removed on an analysis-by-analysis basis. That is, for any given comparison any subject with a standardized residual more than 2 standard deviations from the mean on the initial comparison was removed and the analysis was
repeated. Three autistic patients and one normal control were identified and excluded by this process.

Results

Results of the group-wise t-tests showed that, as expected, the area of vermis lobules VI–VII was significantly smaller in patients with autism than in normal controls \[t(69) = -2.37, P = 0.01; \text{Table 2}\]. In contrast, comparisons of frontal lobe tissue volumes (grey, white, CSF) did not show significant differences \[\text{all } |t(69)| \leq 0.56; \text{all } P \geq 0.29\]. In order to control for individual variation in overall brain size, we also performed the \(t\)-test on the ratio of each structure to total brain volume (e.g. frontal grey ratio = frontal grey volume/total brain volume), and ANCOVA (analysis of covariance) using total brain volume as a covariate. The results of these analyses were similar to those of the initial comparisons: in both analyses lobules VI–VII were significantly smaller in the autism group \[t(69) = -2.30; F(1,68) = 5.74; \text{both } P \leq 0.01\]; and none of the frontal tissue types showed significant differences between groups \[\text{all } F(1,68) \leq 2.11; \text{all } |t(69)| \leq 1.17; \text{all } P \geq 0.08\].

Linear regression analysis of the autistic group indicated that the volume of the frontal cortex was inversely correlated with the size of cerebellar vermis lobules VI–VII \[r = -0.37; F(1,37) = 5.73; P = 0.01\]. In contrast, in the normal control subjects there was no significant correlation between frontal cortex volume and the size of vermis lobules VI–VII \[r = 0.07; F(1,26) = 0.12; P = 0.73\]. The calculated regression lines for autistic and normal subjects are shown in Fig. 3. Since the primary finding here is an inverse correlation between structures (i.e. the increased size of one structure is associated with the decreased size of another), the correlation is clearly not an artefact of variance in overall brain size.

Comparison of the two lines shown in Fig. 3 suggests that, while the autistic patients with almost normal vermis measurements had almost normal frontal cortex volumes, the autistic patients with the greatest degree of hypoplasia had frontal cortex volumes larger than normal. To test this in our data, we performed a median split on the autistic sample based on the size of vermis lobules VI–VII (median = 255.1 mm\(^2\)). We then compared the frontal cortex volumes of these two groups of autistic patients with those of the normal control subjects. The \(t\)-tests (one-tailed) showed that the patients with more hypoplastic vermis sizes had frontal lobe grey matter volumes that were significantly larger than the normal controls \[t(45) = 1.75, P = 0.04\], while the patients with more normal vermis sizes had frontal volumes that did not differ from normal \[t(46) = -0.56, P = 0.29\] (Fig. 4). In a separate post hoc analysis, we examined frontal lobe volume in normal subjects with particularly small vermis

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**Table 2 Volume and area comparisons**

<table>
<thead>
<tr>
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<th>Autism ((n = 42))</th>
<th>Control ((n = 29))</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal lobe volume (cm(^3))</td>
<td>(292.85 \pm 30.67)</td>
<td>(288.67 \pm 31.36)</td>
<td>0.29</td>
</tr>
<tr>
<td>Grey</td>
<td>(111.75 \pm 16.05)</td>
<td>(113.07 \pm 17.79)</td>
<td>0.37</td>
</tr>
<tr>
<td>White</td>
<td>(37.51 \pm 18.47)</td>
<td>(37.58 \pm 20.43)</td>
<td>0.49</td>
</tr>
<tr>
<td>CSF</td>
<td>(258.36 \pm 34.58)</td>
<td>(279.84 \pm 41.64)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

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Fig. 2 Mid-sagittal, T\(_1\)-weighted image illustrating the anatomical boundaries used for measurement of cerebellar vermis lobules VI–VII.
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Fig. 3 Area of cerebellar vermis lobules VI–VII versus frontal lobe grey matter volume. (A) Patients with autism showed a significant inverse correlation between lobules VI–VII and the frontal grey matter \( r = -0.37, F(1,37) = 5.73, P = 0.01 \). (B) Control subjects did not show a significant relationship between the two regions \( r = 0.07, F(1,26) = 0.12, P = 0.73 \). consistent with the finding of Bailey and colleagues of cortical abnormalities in four of seven post-mortem cases (Bailey et al., 1998), and with reports of abnormal frontal lobe metabolism (George et al., 1992; Zilbovicius et al., 1995) and reduced or absent attention-related event-related potential responses over the frontal lobe in autism (Courchesne et al., 1984; Ciesielski et al., 1990; Dawson et al., 1995; Westerfield et al., 1998). Structural abnormalities in the frontal lobe would be expected to affect attention, working memory and problem-solving, cognitive functions which are deficient in autism. The present study also shows that the degree of frontal lobe abnormality correlates with the degree of cerebellar abnormality, so that the frontal lobe fig. 4 Mean frontal grey matter volumes for control subjects and subgroups of autism patients. Autism patients with lobule VI–VII size below the group median (VI–VII < med; \( n = 19 \)) had a significantly larger frontal lobe grey matter volume than control subjects [Control; \( n = 28, t(45) = 1.75, P = 0.04 \)]. Autism patients with lobule VI–VII size above the group median (VI–VII > med; \( n = 20 \)) had a grey matter volume that did not differ significantly from that of controls \( t(46) = -0.56, P = 0.29 \). Error bars represent ±1 SE. sizes. Since the control group had a more restricted range of vermis lobule VI–VII sizes, it is possible that the same frontal lobe enlargement might exist in these controls but remain undetected by the regression analysis. We therefore compared the frontal grey matter volume of control subjects whose vermis size fell below the autism median (\( n = 6 \)) with that of controls whose vermis was greater than the autism median vermis size (\( n = 22 \)). Rather than having an enlarged frontal cortex, the control subjects with small vermis sizes had frontal volumes that tended to be smaller than those of other control subjects [median = 272.4 versus 284.8 cm\(^3\); \( t \)-test (two-tailed): \( t(18.53) = -1.73, P = 0.10 \)].

Discussion
These results indicate that anatomical abnormalities of the frontal lobe occur in autism in at least some cases. This is
and any other brain regions receiving this input. Regardless of whether the abnormalities in the frontal lobe and cerebellum have their origins in a common aetiology or result from the influence of one region upon the other, the reciprocal neural connections between these two misconstructed regions would have a continued detrimental influence on development. This bidirectional maldevelopment would probably exacerbate the concurrent anatomical abnormality not just in the frontal lobe, but also in the cerebellum and possibly also in other brain regions. It remains to be determined whether a similar increase in volume exists elsewhere in the brain (particularly the cerebrum) and whether such abnormalities also correlate with the cerebellar abnormalities.

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