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# The macrostructural and microstructural abnormalities of corpus callosum in children with attention deficit/hyperactivity disorder: A combined morphometric and diffusion tensor MRI study

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ABSTRACT

The corpus callosum (CC) is one of focused target areas which may play an important role in the pathophysiology of attention deficit hyperactivity disorder (ADHD). Conventional structural magnetic resonance imaging (MRI) studies have revealed the macrostructural abnormalities of CC and its subdivisions in ADHD compared with controls. However, no study has examined the macrostructural and microstructural characteristics of the CC in the same ADHD group. In this study, MRI morphometric and diffusion tensor imaging (DTI) techniques were combined to explore the area and measure fractional anisotropy (FA) abnormality of CC and its seven subdivisions in children with ADHD. Twenty-eight boys with ADHD (13.3±1.5 years) and 27 age- and gender- matched controls (13.2±0.9 years) were included. We co-registered individual structural MRI and DTI images manually and subdivided the midsagittal CC into seven subdivisions. The area and FA of the CC and its subdivisions were then compared between the patients and the matched controls. Results showed that ADHD had decreased area of entire CC, anterior middle-body, and isthmus. Meanwhile, reduced FA value of the isthmus was found in the ADHD group compared with the controls. Our study indicated that not only macrostructural abnormalities but also microstructural alterations in CC, especially in isthmus occurred in ADHD. The abnormality of the isthmus, the subdivision that contains the fibers connecting posterior regions of brain, may play an important role in the pathophysiology of ADHD and may be implicated in the disorders of attention.



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## 1. Introduction

Attention deficit/hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders of children, which affected approximately 5% school-age population and characterized by age inappropriate symptoms of inattention, hyperactivity, impulsivity and motor restlessness (American Psychiatric Association, 1994). Although the etiology of this disorder is unclear, converging evidence from structural neuroimaging studies demonstrated that abnormalities throughout the brain may result in ADHD symptomatology (Seidman et al., 2005). Among these aberrant brain regions, the corpus callosum (CC) is one of the focused target areas (Roessner et al., 2004).

As the main white matter fiber tract in the brain, the CC consists of approximately 200–350 million fibers in humans and is responsible for conduction of signals between homologous and heterotopic cortical regions. So, this structure is an essential component for brain lateralization and interhemispheric communication (Bellis et al., 2008; Gazzaniga, 2000). Surgical transection of CC in humans provides evidence that function of the corpus callosum is to communicate perceptual, cognitive, mnemonic, learned and volitional information between the two brain hemispheres (Bogen et al., 1965). One of the main components of these functions is the allocation of attention and relative levels of arousal in the two hemispheres (Rueckert and Levy, 1996). Psychological studies from Rueckert and Levy (1996) showed that the efficiency of the transfer of CC is related to the ability of sustained attention. In a case report of Roessner et al. (2004), a 6.5-year-old boy with the total agenesis of CC had symptoms of inattention, hyperactivity, impulsivity and aggressiveness since early childhood and met the criteria of ADHD combined type and oppositional defiant disorder (ODD) based on *DSM-IV* (Van der Oord et al., 2008). This case indicated that the abnormality of CC may play an important role in the pathophysiology of ADHD.

The results of several structural MRI researches concerning the abnormalities of area of CC and its subdivisions in ADHD were inconsistent. Hynd et al. (1991) reported smaller overall CC area, especially in the region of the genu, splenium and in the area just anterior to the splenium in ADHD. The reduced size of splenium in ADHD was further supported by the findings from Semrud-Clikeman et al. (1994) and Hill et al. (2003). Smaller splenium and isthmus areas were indicated in ADHD adolescents compared to adolescents with other disruptive behaviour diagnosis (Lyoo et al., 1996) and the decreased thickness of isthmus was also found in ADHD in a recently study (Luders et al., 2008). But Giedd et al. (1994) and Baumgardner et al. (1996) found smaller rostrum of CC in ADHD compared to controls. In another two studies, however, no differences of the area of CC and its subdivisions were found between ADHD and controls (Castellanos et al., 1996; Overmeyer et al., 2000). Recent meta-analyses of morphometric MRI studies for CC in ADHD indicated that the splenium of the CC was smaller in ADHD than in controls, which added further evidence that CC is involved in the pathophysiology of ADHD (Hutchinson et al., 2008; Valera et al., 2007). The smaller area of the CC and its subdivisions in ADHD may suggest that the quantity of the interhemispheric connective fiber tracts across this certain area is fewer (Lyoo et al., 1996; Semrud-Clikeman et al., 1994). However, the macrostructural abnormalities of CC measured by conventional MRI may not reflect the underlying quality of tissue in its microstructure. Another type of measure, diffusion tensor imaging (DTI), is a promising technique for depicting microstructural abnormalities of fiber tracts.

In contrast to conventional MRI technique, DTI provides a tool to examine microscopic characteristics of white matter in vivo (Basser et al., 1994). The values most often used to characterize the integrity of white matter tracts are fractional anisotropy (FA). FA values estimate the presence and coherence of oriented structures, e.g. myelinated axons, and range between 0 and 1, where 0 corresponds to isotropic diffusion and 1 to fully anisotropic diffusion (Filippi et al., 2003).

Up to now, several DTI studies have been conducted to examine the structural integrity of white-matter in ADHD. In a voxel-based DTI study, Ashtari et al. (2005) did not find differences in the FA value of CC between ADHD group and normal controls. Another regions-of-interest (ROI) based analysis did not treat the CC as the ROI (Makris et al., 2008). Hamilton et al. (2008) selected the CC as the ROI, they did not find differences in average FA of the entire CC between ADHD and controls. Silk et al. (2008) examined FA and mean diffusivity within major white-matter pathways throughout the whole-brain of ADHD and also did not find abnormality in the CC. The latter two studies, however, did not explore the FA abnormality in subdivisions of CC. Pavuluri et al. (2008) investigated the splenium among paediatric bipolar disorder, ADHD and normal controls with three DTI parameters, including FA, apparent diffusion coefficient (ADC), and regional fiber coherence index (r-FCI), and no differences were found. No study has explored the abnormality of DTI parameters in subdivisions other than splenium of CC in ADHD yet. Since specific regions of CC (i.e., rostrum, genu, rostral body, anterior midbody, posterior midbody, isthmus and splenium) comprise fibers connecting heterotopic and unimodally associated cortical regions and the FA value are linked to the associated cortical regions (Hasan et al., 2005), and previous neuroimaging studies have found structural and functional abnormalities throughout the brain in ADHD (Bush et al., 2005; Seidman et al., 2005), the abnormalities of the FA value in subdivisions of CC in ADHD patients need further investigation.

To our knowledge, combining MRI morphometric and DTI techniques have not been applied to the target brain area in the same subjects of ADHD yet. Such studies could reflect not only macrostructural abnormality but also microstructural alteration in the interested region. A combined MRI volumetric and DTI study investigating the entorhinal region of schizophrenia suggested that combinations of different MRI modalities are a promising approach for the detection and characterization of subtle brain tissue abnormality (Kalus et al., 2005). Another study, measuring the volume, FA and mean diffusivity (MD) in the CC and its subdivisions in the same group of schizophrenia patients, found that not all regions were equally affected by anatomical changes, which suggests that using different methods in evaluation of white matter in patient group could reduce false negative findings (Rotarska-Jagiela et al., 2008). So the purpose of the current study is to investigate the macrostructural and microstructural differences of CC and its subdivisions between patients with ADHD and controls combining conventional MRI morphometric and

DTI techniques. Based on the results of previous studies (Ashtari et al., 2005; Bush et al., 2005; Hill et al., 2003; Hynd et al., 1991; Lyoo et al., 1996; Seidman et al., 2005; Semrud-Clikeman et al., 1994; Silk et al., 2008), we hypothesize that the area of entire CC as well as its subdivisions, especially the posterior regions, would be smaller in ADHD compared with controls, and that the FA value of CC subdivisions maybe decreased in ADHD group.

## 2. Results

### 2.1. Participants

Table 1 shows that the two groups have no significant difference in mean age. The controls had higher IQ than ADHD ($t=3.542$, $p=0.001$). As expected, the ADHD group scored significantly higher than the controls on the inattention, hyperactivity/impulsivity symptoms and total of the ADHD RS-IV and total of CRPS ($p\leq 0.001$).

**Table 1 – Demographic and psychometric variables in ADHD and controls.**

| Variables/group | ADHD ($n=28$) | Controls ($n=27$) | $p$ |
|---|---|---|---|
| Age | 13.3 (1.5) | 13.2 (0.9) | 0.605 [a] |
| Full scale IQ | 102.8 (12.9) | 115.1 (12.7) | 0.001 [a] |
| ADHD RS-IV | | | |
| Total scores | 44.9 (11.6) | 25.5 (6.8) | <0.001 [b] |
| Inattention | 24.4 (4.8) | 14.0 (5.4) | <0.001 [a] |
| Hyperactivity/ impulsivity | 20.5 (7.9) | 11.5 (2.1) | 0.001 [b] |
| CPRS | | | |
| Total scores | 36.2 (15.6) | 6.5 (7.3) | <0.001 [b] |

Data are presented as mean (SD).
ADHD RS-IV, ADHD Rating Scale-IV; CPRS, Conners' Parents Rating Scales.
[a] $p$ values are for 2-tailed $t$ tests.
[b] $p$ values are for $\chi^2$ tests.

### 2.2. Corpus callosum measures

Although the controls showed numerically larger intracranial area, which represents the brain size than the children with ADHD, there was no significant difference between groups on this variable ($F=1.553$, $p=0.218$). The area and mean FA value for entire CC and its seven subdivisions of the two groups are shown in Table 2. Using the age, IQ and intracranial area as covariates, an ANCOVA revealed that there was a significant difference for the total CC area between the two groups ($F=7.529$, $p=0.008$). We set the $p$ value at the 0.007 level[2] and revealed that the areas of isthmus ($F=14.157$, $p<0.001$) and anterior middle-body ($F=9.226$, $p=0.004$) were significantly different between two groups. As indicated in Table 2, the areas of isthmus and anterior middle-body in ADHD were smaller than that in controls, while the areas of posterior middle-body and splenium had a trend level of being smaller in ADHD ($F=4.776$, $p=0.034$ and $F=4.844$, $p=0.031$, respectively).

The two groups showed no difference in the mean FA value of entire CC indicated by the ANCOVA using age and IQ as covariates ($F=2.897$, $p=0.096$). MANCOVA tests at the 0.007 level indicated significant difference of the mean FA value in isthmus between two groups ($F=7.854$, $p=0.007$). Table 2 showed that the mean FA value in isthmus was lower in ADHD group.

### 2.3. Correlations between area and FA

Spearman's rank correlation analysis revealed no significant relationship between the value of area and FA in CC and its subdivisions in all participants or in two groups (ADHD and normal control), respectively. In isthmus, the area value correlated positively with the FA value at a trend level ($r=0.265$, $p=0.051$) in all participants.

## 3. Discussion

In the current study, we combined the volumetric and DTI parameters to explore the macrostructural and microstructural changes of the CC and its subdivisions in children with ADHD. Consistent with previous MRI morphometric studies (Hill et al., 2003; Hynd et al., 1991; Luders et al., 2009; Lyoo et al., 1996), we found that the area of the entire CC and the isthmus in the midsagittal slice were smaller in ADHD compared with controls. We also found that the area of the anterior midbody was reduced in ADHD group. As for the DTI parameters, we did not find difference of the mean FA value in the entire CC between ADHD and controls, which was in line with the results of previous studies (Ashtari et al., 2005; Hamilton et al., 2008; Silk et al., 2008). Significant reduction of the mean FA value was only seen in the isthmus in ADHD after correction for multiple comparisons.

Smaller area of the overall CC and its certain region may suggest the quantity of the interhemispheric connective fiber tracts across two hemispheres and certain homologous cortical regions are fewer (Lyoo et al., 1996; Semrud-Clikeman et al., 1994) and that the abnormality in a given area of the corpus callosum may reflect abnormality in the specific corresponding region of the brain from where those fibers originate. So the reduction of the overall area of CC in ADHD may suggest the abnormalities of brain structures and functions in ADHD. Several structural MRI studies have shown that the total cerebrum volume is 3%–5% smaller in ADHD than that in normal control (Castellanos et al., 1996, 2001, 2002; Hill et al., 2003; Mostofsky et al., 2002). Previous studies have also reported smaller total gray and white matter in ADHD (Castellanos et al., 2002; Mostofsky et al., 2002). A recent study found that patients with ADHD not only had decreases in total cerebral volume and total cortical volume of over 7% and 8%, respectively, but also showed a decrease in surface area of over 7% and a significant decrease in cortical folding bilaterally (Wolosin et al., 2007). A positron emission

[2] *Note*: To avoid type I errors in the multiple comparisons, the p value was set at 0.007 (0.05/7, Bonferonni correction) for area and FA comparisons in seven subdivisions of CC between the two groups.

**Table 2 – The area and average FA value in CC and its seven subdivisions in two groups.**

| Measure | Area ($mm^2$) | | | | $p$ Value[a] | FA | | | | $p$ Value[b] |
|---|---|---|---|---|---|---|---|---|---|---|
| | Controls ($n=27$) | | ADHD ($n=28$) | | | Controls ($n=27$) | | ADHD ($n=28$) | | |
| | Mean | SD | Mean | SD | | Mean | SD | Mean | SD | |
| Rostrum | 18 | 8.6 | 17.9 | 7.8 | 0.607 | 0.235 | 0.09 | 0.296 | 0.141 | 0.234 |
| Genu | 127.8 | 26.6 | 120.4 | 27.3 | 0.922 | 0.438 | 0.151 | 0.446 | 0.133 | 0.841 |
| Rostral body | 76.9 | 15 | 76.8 | 14.1 | 0.676 | 0.365 | 0.091 | 0.375 | 0.082 | 0.630 |
| Anteriormid-body | 72.4 | 11.1 | 64 | 9.8 | 0.004 | 0.467 | 0.136 | 0.425 | 0.115 | 0.043 |
| Posterior mid-body | 65.6 | 10.6 | 60.1 | 9.8 | 0.034 | 0.481 | 0.139 | 0.406 | 0.132 | 0.029 |
| Isthmus | 61.3 | 13.9 | 51.1 | 8.2 | <0.001 | 0.48 | 0.127 | 0.406 | 0.126 | 0.007 |
| Splenium | 174.6 | 21.6 | 159.4 | 21 | 0.031 | 0.585 | 0.085 | 0.574 | 0.101 | 0.452 |
| Total CC | 596.6 | 63 | 549.7 | 54.5 | 0.008 | 0.479 | 0.081 | 0.457 | 0.074 | 0.096 |
| intracranial area | 14724 | 881.7 | 14460 | 676.6 | 0.218 | | | | | |

[a] Treated the age and IQ as covariates for intracranial area comparison and age, IQ and intracranial area as covariates for total CC and seven subregions comparisons.
[b] Treated the age and IQ as covariates.

tomography (PET) study reported that the global cerebral glucose metabolism was 8.1% lower in ADHD (Zametkin et al. 1990). These structural and functional abnormalities in cerebrum may correlate with the smaller overall CC area in ADHD.

According to the Witelson's scheme, the anterior midbody of CC contains the fibers originating from motor cortical regions, but recent studies using fiber tractography showed that these fibers originate from premotor and supplementary motor cortical areas (Hofer and Frahm, 2006; Huang et al., 2005; Park et al., 2006). A previous study found that the volume of premotor and deep white matter was decreased in ADHD (Mostofsky et al., 2002). A voxel-based morphometric MRI study also indicated volume reduction in the premotor, motor and somatosensory cortex in ADHD (Carmona et al., 2005). A DTI study by Ashtari et al. (2005) showed that the children with ADHD had lower FA in right premotor region. Furthermore, fMRI studies also found hypoactivation in the same area during response inhibition and attentive tasks (Cao et al., 2008; Tamm et al., 2004).

As indicated by recent tracing studies (Park et al., 2006), isthmus, a subdivision of CC, contains fibers originating from the sensory-motor cortical areas, such as precentral gyrus, paracentral gyrus and postcentral gyrus. In our study, both the volumetric and DTI parameters demonstrated abnormality in isthmus in ADHD subjects. The involvement of this region in transcallosal inhibition has also been proved (Karbe et al., 1998) and the value of its area has a significant negative correlation with externalizing symptoms score in subjects at high risk for alcohol dependence (Venkatasubramanian et al., 2007). The structural and functional abnormalities of the sensory-motor cortex have been demonstrated by recent studies. A large-scale, longitudinal MRI study including 163 children with ADHD and 166 controls indicated that the cortical thickness of the left precentral gyrus was thinning in ADHD (Shaw et al., 2006). The increased perfusion of sensory-motor regions were found both in children with ADHD by single photon emission computerized tomography (SPECT) study (Kim et al., 2002) and in adults with ADHD by PET study (Schweitzer et al., 2003). In resting state, the amplitude of low-frequency (0.01–0.08 Hz) fluctuation (ALFF) in the sensory-motor cortex was abnormal in ADHD in a fMRI study (Zang et al., 2007). A recent meta-analysis also indicated the aberrant activations of sensory-motor cortex during cognitive tasks (Dickstein et al., 2006). These studies suggested that the structural and functional abnormalities of corresponding brain regions, which sent fibers through the isthmus, may play an important role in the pathology of ADHD.

In addition to reflecting abnormalities in the cortical sources of the fibers traversing through it, the abnormality of the CC in ADHD may reflect problems in interhemispheric connectivity itself. The macrostructural and microstructural abnormalities in isthmus of CC in ADHD could be related to number, density and diameter of axons, integrity and thickness of the myelin sheath, as well as number and size of nonaxonal components, such as glia, neurons and blood vessels in the certain region (Innocenti et al., 2003). Our correlation analysis, although at a trend level, showed the area value was correlated positively with FA value in isthmus in all participants. In other words, for the children and adolescents who participated in this study, the larger the area of the isthmus, the higher the value of FA. As the increase in midsagittal area of the CC was related more to increased myelination rather than increased axonal density (Aboitiz et al., 1992) and increased myelination was related to increased FA in DTI studies (Muetzel et al., 2008), we postulated that the abnormality of macrostructure and microstructure in isthmus in ADHD may result from poor myelination in this region.

In typically developing individuals, with the maturing of the CC, the area and FA keep increasing during childhood and adolescence (Giedd et al., 1999; Keshavan et al., 2002; Muetzel et al., 2008; Thompson et al., 2000). However, the growth within the CC shows a region specific pattern. The greatest increase occurs in posterior regions of the CC during childhood (Giedd et al., 1999; Keshavan et al., 2002; Thompson et al., 2000). In this study, deficits in CC, particularly in its posterior regions in adolescents with ADHD could result from relative slow development of the brain in early years. These abnormal developments of CC in adolescents with ADHD could result from many different developmental perturbations in early years for the disorder. According to the theories of excitation for CC, the corpus callosum enforces the integration of

cerebral processing between the two hemispheres (Lezak, 1995) and activates the unstimulated hemisphere (Yazgan et al., 1995). Therefore, the macrostructural and microstructural abnormalities of CC, especially in its posterior regions, may weaken the efficiency of information conduction between the posterior brain regions of the two hemispheres, which implicated in the disorders of attention.

The present study has a few limitations. Firstly, several individuals in the ADHD group met diagnostic criteria for oppositional defiant disorder (ODD) and conduct disorder (CD). Although the results of Luders et al's study (2009) indicated that the thickness of posterior callosal regions (mainly isthmus) were independent on comorbid ODD, on what extent ODD/CD comorbidity influencing the macrostructural and microstructural characters needs further investigated. Secondly, although previous study had suggested the gender effects on the area of CC (Hutchinson et al., 2008), our study did not include the females with ADHD. A larger sample including males and females with ADHD are optimal. Thirdly, we did not restrict the subtypes and lacked sufficient power to analyze subgroup differences. Further analyses of subtype differences may be particularly informative. Finally, the influences of different scanning parameters on the area measurements of entire CC and its subdivisions should be considered. Totally, there were four kinds of parameters. Based on these high-resolution structural data according to four different scanning parameters, the outline of CC was easy to determine. Meanwhile, two kinds of scanning parameters were used in most (52 of 55) of participants. These two main scanning sequences have similar distribution across the two groups ($\chi^2=2.226$, $p=0.136$) and they may have similar effect on the two groups.

In summary, by using combined MRI morphometric and DTI techniques, we found that children with ADHD have not only macrostructural abnormality but also microstructural alteration in CC, especially in isthmus. The abnormality of the isthmus may play an important role in the pathophysiology of ADHD.

## 4. Experimental procedures

### 4.1. *Participants*

Twenty-eight boys with ADHD and 27 age- and gender-matched controls took part in this study. They were all aged between 11 and 16 years. All the participants met the following criteria: (1) right-handedness, (2) no lifetime history of head trauma with loss of consciousness, (3) no history of neurological illness or other serious physical disease, (4) the full scores of Wechsler Intelligence Scale for Chinese Children-Revised (WISCC-R, Gong and Cai, 1993) higher than 85, (5) born after 33 weeks of gestation. This study was approved by the Research Ethics Review Board of Institute of Mental Health, Peking University. After complete description of the study procedures, written informed consent was obtained from parents or guardians of all participants. All children agreed to take part in the experiment.

Children with ADHD were recruited from the outpatients of Institute of Mental Health, Peking University. A structured diagnostic interview, the Clinical Diagnostic Interviewing Scales (CDIS) (Yang et al., 2001), which is based on DSM-IV criteria, was administered to diagnose ADHD. The inclusion criteria for ADHD were: (1) predominantly inattention subtype (ADHD-I) or combined subtype (ADHD-C), (2) no history of emotional disorders, affective disorders, Tourette disorder and other Axis I psychiatric disorder, (3) no evidence of severe language development delay and communication problems as determined through clinical history, parents interview, and observation of the children. Boys with ADHD comorbiding conduct disorder (CD) or oppositional defiant disorder (ODD) were included. Twelve patients met the criteria for ADHD-C and 16 for ADHD-I. Twenty-three of the 28 patients were stimulants naïve and the other 5 patients were withheld from stimulants at least 2 weeks before the MRI scanning. Six had comorbid ODD and 3 had comorbid CD. Controls were recruited from a local middle school. The inclusion criteria for them were same as the ADHD group except that they were not diagnosed as ADHD according to CDIS. The Conners' Parents Rating Scales (CPRS) and the ADHD Rating Scale-IV (ADHD RS-IV) reported by parents were administered for both ADHD and control groups. The ADHD RS-IV contains all the inattention and hyperactivity/impulsivity symptoms of ADHD according to DSM-IV. Each symptom is scored based on how often it occurred, rating from 1 to 4 (i.e. "never" is rated as 1, "occasionally" is 2; "often" is 3; and "always" is 4). Thus, the total possible score on this scale was from 18 to 72. Table 1 summarizes the major clinical and demographic data.

### 4.2. *MRI scans*

MRI data were acquired using a SIEMENS TRIO 3-Tesla scanner (Siemens, Erlangen, Germany) at the Institute of Biophysics, Chinese Academy of Sciences. All participants have normal structural MRI by macroscopic observation. Then, $T_1$-weighted, sagittal three-dimensional (3D) images were acquired with spoiled gradient recalled (SPGR) sequence. Because the data collection continued about 2 years, some modifications were made in the sequence of the structural images, and participants were scanned with one of the following four kinds of parameters: (1) TR=1700 ms, TE=3.92 ms, slice thickness=1.0 mm, skip=0 mm, flip angle=12°, inversion time=1100 ms, FOV=256×256 mm, matrix=512×512, 176 slices, used in 7 patients and 13 controls; (2) TR=2000 ms, TE=3.67 ms, slice thickness=0.96 mm, skip=0 mm, flip angle=12°, inversion time=1100 ms, FOV=240×240 mm, matrix=256×256, 192 slices, used in 18 patients and 14 controls; (3) TR=1950 ms, TE=2.60 ms, slice thickness=1.30 mm, skip=0 mm, flip angle=10°, inversion time=900 ms, FOV=240×256 mm, matrix=240×256, 128 slices, used in 2 patients; (4) TR=2530 ms, TE=3.37 ms, slice thickness=1.33 mm, skip=0 mm, flip angle=7°, inversion time=1100 ms, FOV=256×256 mm, matrix=256×256, 128 slices, used in 1 patients. It should be noted that the boundary of the CC is very easy to be defined in the sagittal plane of the high-resolution 3D images, although the scanning parameters of the 3D images varied across subjects. In addition, the two main sets of scanning parameters were similarly distributed across the two groups of subjects ($\chi^2=2.226$, $p=0.136$), the effects of the scanning

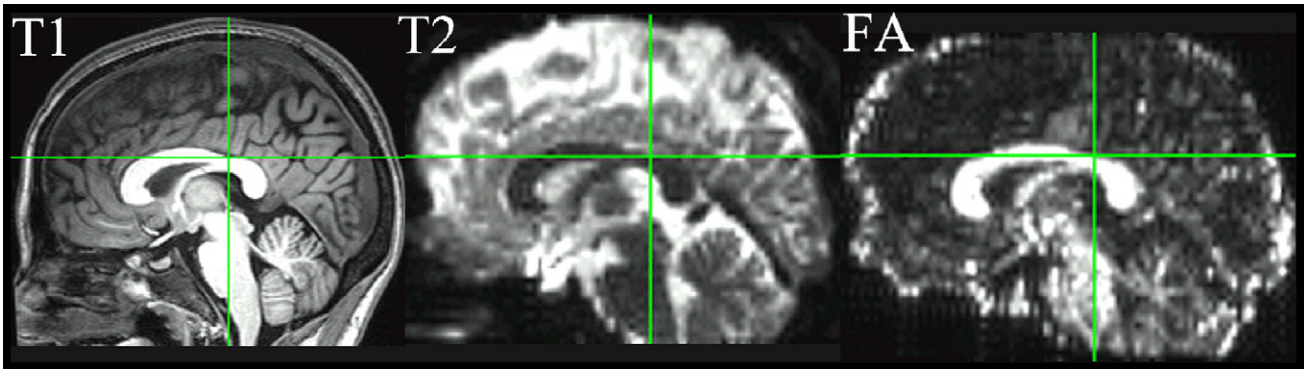


**Fig. 1 – Registration of individual $T_1$-weighted, sagittal three-dimensional (3D) image and FA map through $T_2$ ($b_0$) image on midsagittal slice.**

parameters on the manual lineation of the CC could be, if any, very small. DTI were acquired parallel to the AC-PC based on a spin echo echo-plannar imaging (EPI) sequence with the following parameters: TR=8600 ms, TE=130 ms, slice thickness=4 mm, skip=1 mm, flip angle=90° flip, FOV=22×22 cm, matrix=128×128, 30 axial-oblique slices, diffusion was measured along 13 noncolinear directions, diffusion weighting was $b$=1000 s/mm$^2$, number of excitations (NEX)=3, and a T2 volume without diffusion weighting ($b$=0; NEX=3). The total DTI scanning time was 340 s.

### 4.3. *Imaging analysis*

#### 4.3.1. *Reconstruction of 3D T1-weighted MR images*

The 3D T1-weighted images were resampled to a voxel resolution of 1×1×1 mm$^3$ by using AFNI (http://afni.nimh.nih.gov/afni). The motion artifacts of the scans for all the participants were acceptable by visual inspection.

#### 4.3.2. *DTI processing*

A in-house software was used to estimate and diagonalize the diffusion tensor in each voxel and FA maps were calculated for all participants (Basser and Pierpaoli, 1998).

#### 4.3.3. *Registration of the 3D T1-weighted MR image and DTI*

Before the combined analysis, the registration of the 3D T1-weighted MR images and DTI was done on midsagittal slice. This step was done using AFNI. $T_2$ ($b_0$) images were manually registered to 3D T1-weighted MR images by shifting and rotating, and then the same spatial transformation was done to the FA map. Fig. 1 shows the degree of registration of the 3D T1-weighted MR images and DTI.

#### 4.3.4. *Segmentation of the corpus callosum in the midsagittal plane*

The midsagittal slice was designated as the slice that contained the easily identified cerebral aqueduct, and then the CC was manually outlined. In order to segment the CC, three points, the anterior point of the CC, posterior point of the CC and the anteriormost point of the inner convexity of the CC, were selected manually on the CC. According to these three points, a procedure was used to divide the CC into seven segments based on previous work by Witelson (1989), as shown in Fig. 2. The area and mean FA of the entire CC as well as its seven subdivisions were calculated.

#### 4.3.5. *Intracranial area*

The midsagittal intracranial area was manually outlined and was used as a covariate. The boundary of the intracranial area was determined as described by Baumgardner et al. (1996).

All the manual delineation procedures were performed by two raters (QJ Cao and XH Cao) who were blind to diagnosis. The average intraclass correlations for the inter-rater and intra-rater reliability were 0.912 and 0.930, respectively, for

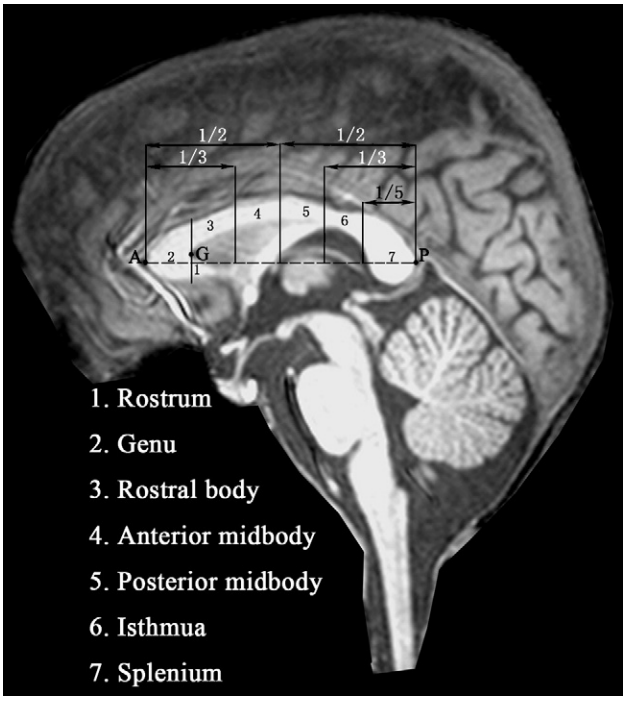


**Fig. 2 – Sub-divisions of corpus callosum according to the work by Witelson (1989). A, the anterior point of the corpus callosum; P, the posterior point of the corpus callosum; G, the anteriormost point of the inner convexity of the corpus callosum.**

area measurements, 0.956 and 0.980, respectively, for intracranial area measurements and 0.976 and 0.981, respectively, for FA measurements ($n$=55).

### 4.4. *Statistical analysis*

SPSS was used for all statistical analyses. Student's $t$-tests and chi-square tests were used to examine group differences on continuous and categorical demographic variables, respectively. All tests were two-tailed. We used univariate analysis of covariance (ANCOVA) to compare the differences in the intracranial area, area and mean FA value of entire CC and multivariate analysis of covariance (MANCOVA) to compare the area value and mean FA value in seven subdivisions of CC between ADHD and controls. For the area analysis, the age, IQ and intracranial area were treated as covariates controlling for their effects on measurement. As for comparison of the FA, the age and IQ were treated as covariates. We did not treat the intracranial area as covariate in FA analysis because it has been shown that FA values are not influenced by brain volume (Schulte et al., 2005). A $p$ value less than 0.007 (0.05/7, Bonferonni correction) was considered significant for area and FA comparisons in seven subdivisions of CC between the two groups to avoid type I errors in the multiple comparisons. Spearman's rank correlation analysis was used to examine the relationship between the area and FA of the CC and its subdivisions in all participants.

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