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Evaluation of Corpus Callosum Anisotropy in Young Adults With Fetal Alcohol Syndrome According to Diffusion Tensor Imaging

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Background: Fetal alcohol syndrome (FAS) and associated disorders resulting from maternal alcohol use during gestation are among the most common developmental disorders. However, they are rarely diagnosed and not fully understood in terms of their behavioral and neurocognitive phenotype. Prenatal exposure leads to alterations in facial morphology, growth, and neurocognition. The nature and extent of teratogenic effects on the brain and the relationship between such effects and observed behaviors remain in debate because there are no established markers for the neurological effects of exposure. In this study, we examined the impact of prenatal alcohol exposure on white-matter integrity in the corpus callosum by using diffusion tensor imaging (DTI) and herein describe the relationship between such effects and observed physical and behavioral outcomes.

Methods: DTI was used to evaluate diffusion anisotropy in the genu and splenium of corpus callosum in 16 low-income, primarily African-American volunteers. Volunteers were recruited from a cohort of young adults who had received neuropsychological evaluations during adolescence. Nine had been prenatally exposed to alcohol and had characteristics of FAS, and seven were nonexposed controls.

Results: Significant difference in the means for diffusion fractional anisotropy ($t = 2.26$, $df = 9$, $p < 0.002$) and apparent diffusion coefficient ($t = 2.14$, $df = 14$, $p < 0.008$) were observed in the corpus callosum of alcohol-exposed youth compared with nonexposed youth. No significant differences were found in intracranial volume between these groups.

Conclusions: Our results illustrate that DTI can be used in evaluating the integrity of corpus callosum in alcohol-exposed individuals. If future studies support these findings, diffusion anisotropy, represented by fractional anisotropy, has the potential to be used as a clinical marker in the diagnosis of FAS.

Key Words: Fetal Alcohol Syndrome, Diffusion Tensor Imaging, Fractional Anisotropy.

FETAL ALCOHOL SYNDROME (FAS) and the spectrum of associated disorders that result from maternal alcohol use during gestation (e.g., partial FAS, alcohol-related neurodevelopmental disorders) (Stratton et al., 1996) are among the most common developmental disorders (CDC, 1993). Prenatal exposure leads to alterations in facial morphology, growth, and neurocognition (Jones and Smith, 1973), presumably due to teratogenic effects on the nervous system. However, there is an incomplete understanding of the brain-behavior relationship in this disorder. On the behavioral side, a broad range of outcomes has been documented in exposed and affected individuals. Because of inconsistencies from one study to another and across developmental stages, there have not, as yet, been specific and invariant behavioral or neurological markers identified. This situation results from the real-world methodological constraints intrinsic to research on such a topic, including difficulties inherent in characterizing a study sample that is not associated with a variety of social confounders as well as problems in specifying neurological or neurocognitive sequelae that can be attributed to prenatal exposure rather than to associated factors.

Both human studies of behavior and animal models leave no doubt that alcohol is a teratogen that has toxic effects on the brain. In the last decade, it has become possible to evaluate directly the effect on human brain structure and functioning. Imaging studies of alcohol-affected patients have been carried out and have shown effects on the brain in older children and adults. Effects are reported both in clinical samples of patients with FAS and in exposed individuals who do not meet the criteria for the full syndrome.

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The majority of published studies have used structural neuromagnetic imaging (sMRI) and focused on morphology. Several studies (Bookstein et al., 2001; Bookstein et al., 2002; Sowell et al., 2001b) have correlated structural outcomes with neurobehavioral data. In this research, microcephaly has been the most consistent finding, with individuals with FAS showing a general reduction in brain volume relative to contrast groups (Archibald et al., 2001; Bhatara et al., 2002; Riley et al., 1995; Sowell et al., 2001a; Sowell et al., 2001b) that is usually controlled for in subsequent analyses. More specifically, relative reductions in white matter have been noted in the corpus callosum and in the parietal lobe (Archibald et al., 2001; Riikonen et al., 1999; Sowell et al., 2001b), particularly in patients with full FAS.

The corpus callosum has been a focus of interest because it is a midline structure and particularly vulnerable to disruption in neural migration. Agenesis and hypoplasia of this structure have been reported repeatedly, particularly in dysmorphic individuals (Bhatara et al., 2002; Johnson et al., 1996; Riikonen et al., 1999; Riley et al., 1995; Sowell et al., 2001a; Swayze et al., 1997). Sowell et al. (2001a) reported reductions and displacements in the corpus callosum in both the anterior region and the splenium. These differences were associated with deficits in verbal learning and memory. Bookstein et al. (2001) reported that there is greater variability in corpus callosum shape among alcohol-affected individuals and that thicker or thinner structures are related to specific patterns of neurodevelopmental deficit. The cerebellum also has been found to be altered. Riikonen et al. (1999) reported atrophy in this area, whereas Sowell et al. (1996) noted size reductions, particularly in the anterior vermis (lobules I–V), a finding supported by earlier animal studies (Goodlett et al., 1991). Autti-Ramo et al. (2002) reported hypoplasia in cerebellar hemispheres in 10 of 17 individuals. Other specific deficits in morphology have been seen also, although not reported as consistently.

These studies of the structural impact of prenatal alcohol exposure on the brain indicate that there are often significant morphological differences in alcohol-affected individuals. However, it is not clear from this research that a specific area is always indicated, suggesting a more general process at work Several "candidates" for further research do emerge from these data, but even the corpus callosum, which has been implicated frequently, does not show specific macrostructural patterns related to alcohol exposure across all of these studies. For instance, although callosal agenesis is reported (Bhatara et al., 2002; Johnson et al., 1996; Riley et al., 1995), other studies found thinning of these areas or posterior displacement (Sowell et al., 2001b), whereas many studies have not reported macrostructural anomalies among all of the subjects studied (Autti-Ramo et al., 2002). These outcomes suggest that in some exposed individuals, gross anatomical studies may not be adequate to delineate effects of prenatal exposure, and evaluation of microstructural alterations may be warranted.

In research on other disease conditions, diffusion tensor imaging (DTI) (Basser et al., 1994a; Basser et al., 1994b; Lim and Helpern, 2002) has refined understanding of the relationship between pathological conditions and such outcomes. By measuring the diffusion characteristics of water in tissue along at least six noncolinear directions in space, DTI maps the directional dependence of water diffusion and is useful for visualizing microstructural tissue organization in the human brain. The advent of DTI in the last decade has made it possible for noninvasive visualization of neural fiber tracts *in vivo* (Basser and Pierpaoli, 1996; Pierpaoli and Basser, 1996), which enables the study of brain development and pathology of the diseases associated with white-matter damage and disruption. DTI appears to be a promising methodology for use with alcohol-exposed individuals for several reasons. In individuals with FAS, structural studies frequently reported effects on integrity of white-matter tracts (Archibald et al., 2001; Bhatara et al., 2002; Clark et al., 2000; Johnson et al., 1996; Sowell et al., 2001a; Swayze et al., 1997). The corpus callosum, the major white-matter tract crossing the interhemispheric fissure in the human brain, has often been implicated. In studies of other pathologies, white-matter integrity has been associated with many of the behavioral outcomes and neuropsychological tasks found to be affected as a result of alcohol exposure (Kable and Coles, 2003). For instance, Pfefferbaum et al. (2000) have explored the impact of chronic alcoholism on white-matter microstructural disruption, finding that DTI discriminated alcoholics from controls and correlated with behavioral outcomes even in the absence of macrostructural evidence of damage. Pfefferbaum and Sullivan (2002) investigated the extent of white-matter damage in chronic alcoholic women by using conventional sMRI and DTI and found that, although there were no significant group differences in regional sMRI outcomes, DTI revealed differences in fractional anisotropy (FA) and intervoxel coherence (C) in white matter in the genu (anterior) and splenium (posterior) of the corpus callosum. Similar findings were also reported in other patient populations who had white-matter lesions or who demonstrate evidence of white-matter loss (Bozzali et al., 2002; Foong et al., 2000; O'Sullivan et al., 2001; Rao et al., 1989). These studies suggest that investigation of callosal integrity in alcohol-exposed individuals with DTI is warranted.

As a result of these previous findings, in the current study, we examined the impact of prenatal alcohol exposure on the brain in young adults who have been followed up since the prenatal period with the use of 1) MRI for the morphometric measurement to obtain intracranial volume (ICV) and 2) DTI for the measurement of diffusion anisotropy. We hypothesized that prenatal alcohol exposure would be associated with smaller ICV, less FA, and higher diffusion coefficients (ADCs). Furthermore, we investigated the relationship between various regions of the cor-

Table 1. Demographic Characteristics of Alcohol Exposed and Contrast Groups

Group	Age	Gender	Ethnicity	Self Report of Ounces Absolute Alcohol Weekly (Oz AA/wk) during Pregnancy	Highest dysmorphia Score*	Full Scale IQ**	Processing Speed***
Alcohol Affected							
1	18	F	African-American	10	20	48	54
2	20	F	African-American	2	9	52	64
3	22	M	African-American	10.4	27	44	50
4	25	F	White	40	15	74	uncollected
5	18	M	African-American	4	14	60	67
6	20	M	African-American	18	8	65	72
7	18	F	African-American	2	10	71	96
8	20	M	African-American	34	25	62	86
9	22	M	African-American	6	27	53	67
Mean (Std)	20 (2)	n/a	n/a	14.10 (14.027)	17.22 (7.74)	58.33 (9.6)	69.50 (15.33)
SES Controls							
1	21	M	African-American	0	2	80	101
2	22	F	African-American	0	2	83	106
3	20	F	African-American	0	uncollected	75	88
4	21	F	African-American	0	3	86	96
5	18	M	African-American	0	5	86	99
6	22	F	African-American	0	4	87	122
7	22	F	African-American	0	8	82	99
Mean (Std)	21 (1)	n/a	n/a	0	4.0 (2.28)	82.7 (4.23)	101.57 (10.53)

* $F = 16.18$, $p < 0.001$; ** $F = 38.76$, $p < 0.000$; *** $F = 21.61$, $p < 0.000$.

pus callosum to determine whether certain areas (i.e., genu or splenium) are differentially affected in alcohol-affected individuals. Finally, in exploratory descriptive analyses, we graphed the relationships between MRI outcomes and physical (i.e., alcohol-related dysmorphia) and cognitive (i.e., full-scale IQ, [FSIQ] and processing speed [PS], as measured by standardized tests of ability) characteristics associated with prenatal alcohol exposure.

METHODS

Participants

Participants were 16 young adults recruited from a cohort enrolled in a longitudinal study of effects of prenatal alcohol exposure on subsequent development. The original sample was recruited prenatally from an Atlanta urban hospital between 1978 and 1986. The sample is predominantly of low socioeconomic status and African-American. Both women who reported ingesting at least two drinks per week during pregnancy and women who did not use alcohol were asked to participate. Self-reported alcohol use by women during pregnancy is reported in Table 1. Information on reported alcohol use is presented as ounces of absolute alcohol per week, which were calculated on the basis of the quantity and frequency of use and type of alcohol consumed (Coles et al., 1985). Members of the cohort have been evaluated periodically since birth (Brown et al., 1991; Coles et al., 2002; Coles et al., 1985). All participants in the current study were seen as part of a Teen Assessment Study between 1996 and 2000, and information was available concerning maternal characteristics, prenatal exposure to alcohol and other drugs, physical characteristics associated with alcohol exposure (dysmorphia scores), and results of neurocognitive testing during adolescence. This information was used to classify participants as to alcohol exposure and effects and provide the information in Table 1.

For the present study, nine young adults who had been exposed to alcohol were selected primarily on the basis of scores of physical dysmorphia. Our goal was to identify individuals who demonstrated alcohol effects consistent with a diagnosis of FAS. In some cases, we also considered evidence of cognitive deficits (FSIQ on the Wechsler Intelligence Scale for Children, third edition [WISC-III IQ]; Wechsler, 1991) associated with prenatal exposure, because the diagnosis of FAS and partial FAS include such evidence. The third criterion for diagnosis, physical growth, was not used in selection because there is evidence (Streissguth, 1997) that growth deficits may not persist in adolescence and adulthood. In this cohort, physical dysmorphia was assessed at evaluations during infancy, childhood, and adolescence, and the measure used in this study was the highest dysmorphia score (Coles et al., 1985) ever assigned to the individual over that period. Alcohol-exposed individuals whose dysmorphia scores were less than 10 were considered eligible when their IQ score was less than 70, thereby classifying them as mentally retarded (and presumably, alcohol affected). Seven normal controls were recruited from the nonexposed contrast group without consideration of dysmorphia score or IQ. At recruitment, potential participants were contacted by study outreach workers and told about the study. If they were interested in participating, a short screening interview was completed to determine whether they were eligible to have an MRI. Exclusion criteria included having implanted medical devices or metal in the body (e.g., pacemaker, aneurysm clips, hearing aids or cochlear implants, pins, screws, braces on teeth, plates, shrapnel, etc.), pregnancy, nonremovable jewelry or body piercing, claustrophobia, extreme obesity, and vision problems not correctable with contact lenses. During recruitment, three individuals were found to be ineligible according to these criteria and were not included in this study. If participants were interested and eligible, outreach workers completed the consent procedure and asked participants to sign the consent forms approved by the Emory University School of Medicine Institutional Review Board. Parents or guardians were asked to consent for minors or participants not cognitively competent to give consent.

Procedures

On the day of evaluation, participants were transported to the Emory University Child Development Study Laboratory to complete training to prepare them for the imaging session. After training, they were transported to the Imaging Laboratory at Emory University Hospital. They were accompanied by study staff and family members, if they wished. The imaging session lasted ~45 min. After the session, the participants were transported home. The full session, including training and imaging, lasted ~2 hr. The participants received $100 to compensate them for their effort and inconvenience.

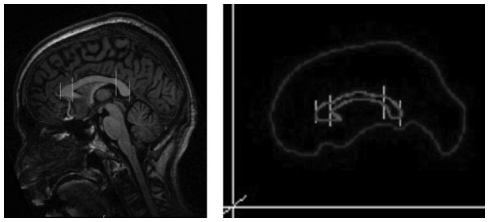

Fig. 1. Midsagittal slice of 3D T1-weighted images, demonstrating the scheme of corpus callosum as divided for morphometric measurement.

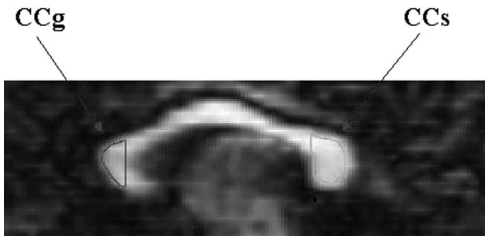

Fig. 2. Midsagittal slice of FA map, corresponding to the same slice for morphometric measurement, demonstrating the ROIs of the CCg and CCs of corpus callosum for FA and ADC measurement.

MRI: Imaging Procedures

All imaging experiments were performed on a 3-T Siemens Magnetom Trio scanner (Siemens, Erlangen, Germany).

Morphometric Measurement of Corpus Callosum

High-resolution, T1-weighted, three-dimensional (3D) sagittal images were acquired with a 3D MPRAGE (magnetization prepared rapid gradient echo) sequence for all participants. The scan protocol, optimized at 3 T, used an repetition time/inversion time/echo time of 2600/900/3.93 msec, a flip angle of 8°, a field of view of 256 × 224 × 176 mm^3, a matrix of 256 × 224 × 176, corresponding to an isotropic resolution of 1 mm. Scan time was 7:18 min. A T1-weighted 3D data set was discarded because one subject among nine young adults who had been exposed to alcohol did not complete the scan. The morphometric measurement was performed according to the methods used by Geidd and Sowell (Giedd et al., 1994; Sowell et al., 2001b). The 3D image data set was first realigned and registered in reference to AC-PC (anterior commissure to posterior commissure) line. A midsagittal slice was selected and used to identify the corpus callosum, to define the subregion of genu and splenium, and to define the ICV. The maximum length of corpus callosum was obtained by measuring the distance between the anteriormost and posteriormost points. The genu (CCg) and the splenium (CCs) are defined as illustrated in Fig. 1. Then, the areas of genu, splenium, and total CC were determined with BRAINS2 (University of Iowa, Iowa City). The ICV of each subject, taken as the intracranial area within the slice, was determined. For consistency, all areas were manually delineated by one of the authors who was blinded to the subject's age, gender, and diagnosis. As detailed below, group comparisons of the genu, splenium, and total CC were performed with and without normalization by ICV. For the normalized comparison, the areas of genu, splenium, and total CC of each subject were divided by his/her ICV.

Measurement of Diffusion Anisotropy

DTI data were acquired with a diffusion-weighted, single-shot, spin-echo echoplanar imaging sequence. A dual spin-echo technique combined with bipolar gradients (Alexander et al., 1997) was used to minimize the geometric distortion induced by eddy currents. Diffusion weighting gradients were applied in 12 directions with a b value of 1000 sec/mm^2. The following parameters were used: repetition time = 2660 msec, echo time = 86 msec, field of view = 22cm × 22 cm, slice thickness = 2.5 mm, slice gap = 0 mm, number of slices = 19 (to cover the whole range of corpus callosum), b = 0, 1000 s/mm^2, and 12 averages. Scan time was 7:27 min. Images were originally acquired in the axial orientation with a matrix size of 128 × 96; they were then interpolated into a matrix of 256 × 256 with the use of an online sinc interpolation algorithm, corresponding to a pixel size of 0.86 × 0.86 mm^2.

Before the calculations for DTI on a voxel basis, the linear interpolation, implemented in IDL (RSI, Boulder, CO), was applied to the multislice DTI images (acquired without gapping) in the through-plane direction to generate the high-resolution images in the sagittal plane. Interpolations performed on the raw diffusion images, provided that the volume data with a voxel size was 0.86 × 0.86 × 0.5 mm^3. Then the diffusion tensor was calculated on the basis of the interpolated data set for each voxel, and ADC, FA maps, and color-coded maps were generated with DtiStudio (Johns Hopkins University, Baltimore, MD; (Mori et al., 1999).

As demonstrated in Fig. 2, regions of interest (ROIs) were manually defined in the areas of the genu and splenium of the corpus callosum (i.e., CCg and CCs) in the midsagittal plane for each individual. Care was taken to make sure that the boundary of the ROI was definitely within the corpus callosum to avoid measurement errors due to a partial-volume effect. For consistency, data were manually digitized by one of the authors who was blinded to the subject's age, gender, and diagnosis. The ROI calculations were performed to obtain the mean value and standard deviation for the FA and ADC. Between-group comparisons were made to ascertain differences in callosal diffusion anisotropy. Regression analyses were performed on data sets of FA and ADC in alcohol-affected individuals to examine the correlation between these measures and dysmorphia scores. Finally, in exploratory analyses, the relationships between FA and characteristics of prenatal alcohol exposure (i.e., dysmorphia, FSIQ, and PS, a measure of cognitive efficiency that is part of the WISC-III [Wechsler 1991], obtained during the adolescent follow-up) were graphed. PS was selected for analysis because it was expected to be sensitive to the effects of alcohol exposure (Kable and Coles, 2004). The PS summary score is composed of two speeded subtests, coding and symbol search, which require efficient visual/motor processing and as shown in Table 1, appears to be less sensitive to the effects of environmental deprivation than does FSIQ in this low-income sample.

RESULTS

Several different analyses were done to evaluate the status of alcohol-affected young adults in comparison with nonexposed controls. The results of the morphometric measurement are shown in Table 2 and Fig. 3. The comparison between exposure groups was done with two-way ANOVAs with both the raw values and the ratio of genu and splenium to the ICV area on the midsagittal slice. Because previous studies (e.g., Sowell et al., 2001a) found differences in brain volume between alcohol-affected and contrast groups, the mean volumes of CCg ($df = 7$, $F = 0.55$, $p < 0.23$), CCs ($df = 7$, $F = 1.65$, $p < 0.28$), total CC ($df = 7$, $F = 0.98$, $p < 0.49$), and ICV ($df = 7$, $F = 0.87$, $p < 0.43$) were compared between groups. For this small sample, the results for the unexposed control subjects were similar to those of the alcohol-affected group, and statistical testing indicated that differences were not significant, although there was a trend consistent with previously published results. In a second comparison with CCg and CCs normalized to the ICV, the differences between groups also

Table 2. MRI Measurement of Genu and Splenium of Corpus Callosum in Alcohol-Affected Young Adults and Controls

	Area (mm^2)				Normalization	
	CCg	CCs	Total CC	Total brain ICV	CCg/total brain ICV	CCs/total brain ICV
Normal ($n = 7$)	132.90 ± 28.86	168.96 ± 30.19	631.11 ± 89.28	10984.95 ± 873.75	0.0120 ± 0.0020	0.0155 ± 0.0028
Alcohol-affected	108.79 ± 22.17	131.35 ± 37.33	521.64 ± 85.03	9459.92 ± 931.31	0.0118 ± 0.0031	0.0138 ± 0.0037
Young adults ($n = 8$)	df = 7, F = 0.55, $p < 0.23$	df = 7, F = 1.65, $p < 0.28$	df = 7, F = 0.98, $p < 0.49$	df = 7, F = 0.87, $p < 0.43$	df = 7, F = 1.94, $p < 0.22$	df = 7, F = 1.31, $p < 0.38$

were not statistically significant, suggesting that area reduction is not disproportional to overall brain volume reduction in these individuals.

DTI MEASUREMENT

The DTI data were used to examine both callosal diffusion anisotropy (FA) and mean diffusivity (ADC). For both of these response variables, a 2 × 2 two-way ANOVA was performed using cohort (control/AAYA) and anatomical region of the CC (CCg/CCs) as factors. The results of the comparisons are shown in Fig. 4 and Table 3.

ROI measurements in the genu and splenium of the corpus callosum (CCg and CCs) in exposed individuals showed a lower FA value ($t = 2.26$, $df = 9$, $p < 0.002$) and a higher ADC value ($t = 2.14$, $df = 14$, $p < 0.01$) (Table 3) compared with those of normal volunteers, and these group comparisons were statistically significant. It was noticed also that the FA value of CCs was consistently slightly higher than that of CCg, whereas the ADC of CCs was lower than that of CCg, in both the alcohol-exposed volunteers and controls. However, no significant difference was observed in FA and ADC between CCg and CCs areas in these groups.

EXPLORATORY ANALYSES

To investigate whether FA and ADC may correlate with dysmorphia score, linear regressions (two-tailed) of FA and ADC in CCgs and CCs of alcohol-affected individuals as a function of dysmorphia score were also performed (Fig. 5). The results showed no indication that FA and ADC were correlated with dysmorphia score in this sample. Finally, fractional anisotropy outcomes were correlated with the WISC-III FSIQ and PS standard scores (see Table 1), obtained previously. These scores had been collected a mean of 5.5 years previously. Ability scores of this kind are stable over adolescence and young adulthood, and we anticipated that PS would be related to the efficiency of neural transmission, as indexed by FA outcomes. When correlations were done within groups, significant outcomes were not found for most of the analyses. There was a significant linear correlation between PS standard scores and one DTI outcome (FA in the genu) for those in the control group, despite the small sample size ($r_{(7)} = 0.714$, $p < 0.04$) but not for the alcohol-affected group. Figure 5 includes scattergrams showing the distribution of these scores.

DISCUSSION

The goal of the present work was to explore the usefulness of DTI as a tool for understanding the impact of prenatal alcohol exposure on neurodevelopment. DTI was used because it has proved useful in the understanding of neuropathology in other clinical conditions (Lim and Help-

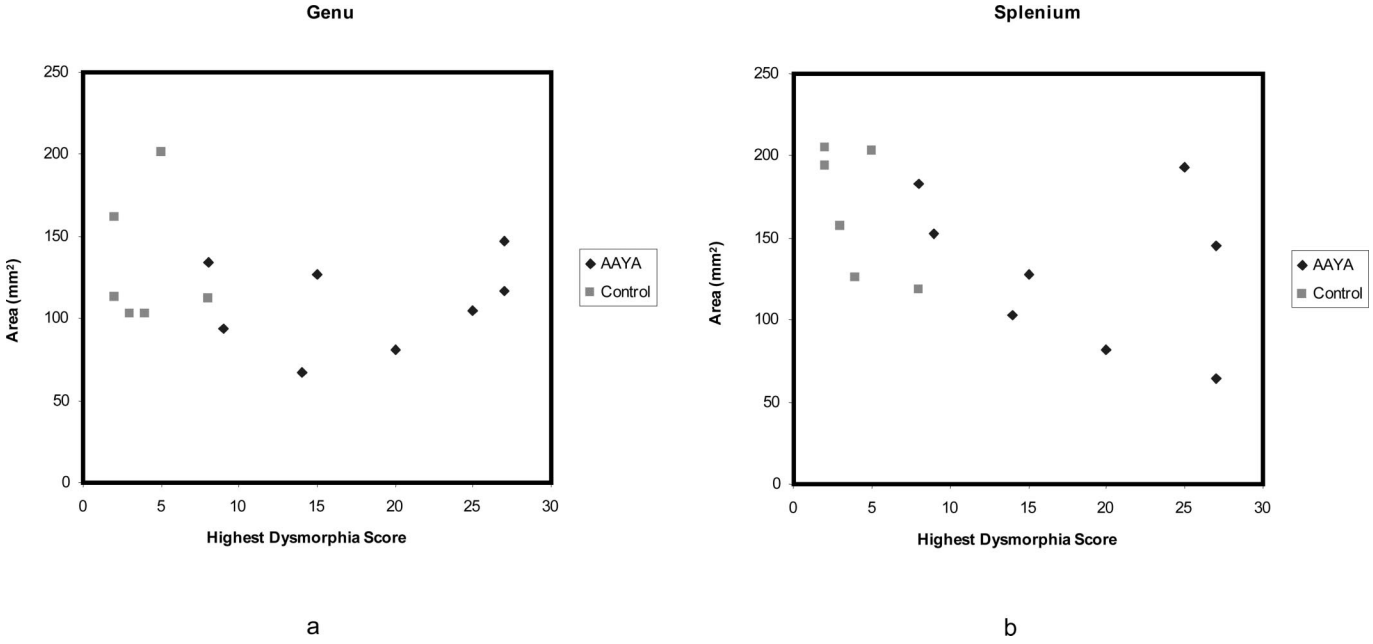

Fig. 3. Scatterplot of morphometric measurement versus highest dysmorphia score in (1) genu and (2) splenium of corpus callosum between alcohol-affected young adults (AAYA) and controls.

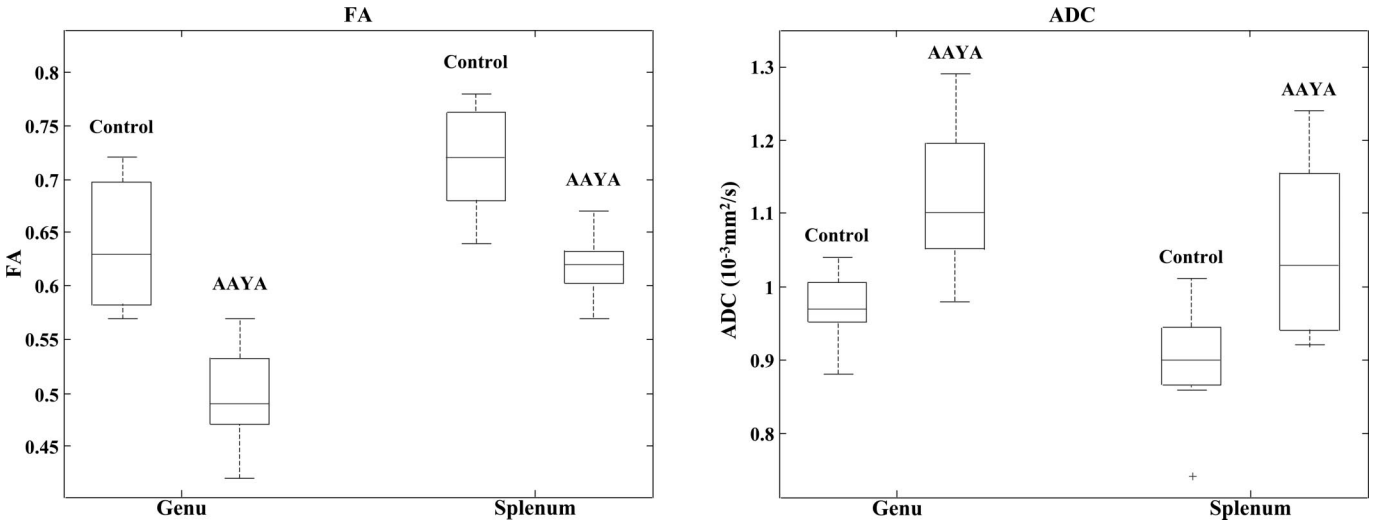

Fig. 4. Group comparison of (1) FA and (2) ADC in genu and splenium of corpus callosum between alcohol-affected young adults (AAYA) and controls.

Table 3. Comparison of ADC and FA Values Between Alcohol-Affected Young Adults and Controls

	CCg		CCs	
	ADC (10^{-5} cm^2/sec)	FA	ADC (10^{-5} cm^2/sec)	FA
Normal (7)	0.971 ± 0.036	0.640 ± 0.054	0.896 ± 0.059	0.716 ± 0.042
FAS Patients (9)	1.121 ± 0.081	0.498 ± 0.038	1.058 ± 0.104	0.617 ± 0.021
	$t = 2.18$, $df = 12$, $p < 0.003$	$t = 2.20$, $df = 11$, $p < 0.001$	$t = 2.14$, $df = 14$, $p < 0.008$	$t = 2.26$, $df = 9$, $p < 0.002$

ern, 2002). Particularly relevant to the current investigation are those studies that have examined the effects of alcohol abuse on white-matter conductivity in adults (Pfefferbaum et al., 2000; Pfefferbaum and Sullivan, 2002) and in brain development associated with developmental disabilities (Arfanakis et al., 2002; Barnea-Goraly et al., 2003). The results of this study demonstrate that DTI also can be used to study the effects of prenatal alcohol exposure.

Significant differences were observed in FA and ADC in both genu and splenium of corpus callosum, with the young adults who showed physical and cognitive effects associated with FAS having less optimal findings than did members of

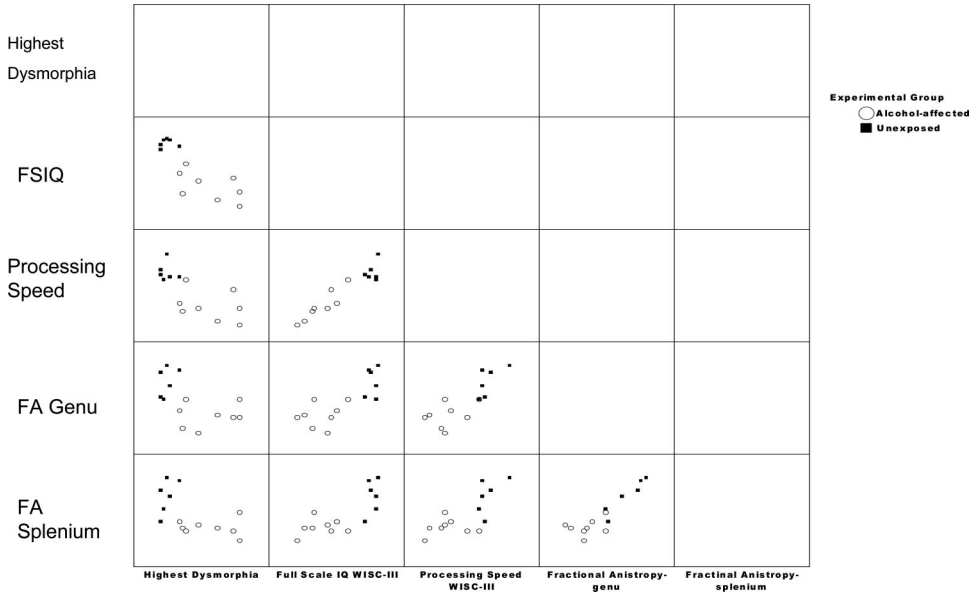

Fig. 5. Scattergrams showing relationships among subject variables.

a control group matched for ethnicity and socioeconomic status. Quantitative measurements in the genu and splenium of the corpus callosum (CCg and CCs) in alcohol-affected individuals showed a lower FA and a higher ADC compared with those values in normal controls. Because brain regions with dense and highly oriented fiber bundles have a high anisotropy, the lower FA suggests that there is abnormal development of white matter in the CC in this group, such that fibers may be less dense or less coherent, causing diffusion to be more isotropic. In other clinical populations (Lim and Helpern, 2002), such patterns are associated with degradation of white matter as well as functional deficits. Thus, the differences observed in diffusion anisotropy and fiber coherence in the alcohol-affected individuals may reveal a disruption of connectivity within the corpus callosum that may be associated with impairments in neurocognition that characterize this group.

In contrast, we did not observe significant morphometric differences in CCg and CCs between alcohol-exposed individuals and unexposed socioeconomic status-matched controls. Thus, the results do not confirm the difference in ICV reduction in alcohol-affected individuals reported by other investigators (Archibald et al., 2001; Bhatara et al., 2002; Riley et al., 1995; Sowell et al., 2001a; Sowell et al., 2001b). This finding could be interpreted to suggest either that alcohol exposure is not associated with microcephaly or reduced ICV, conclusions that are not reasonable given the definition of the disorder (Stratton et al., 1996), as well as the previous studies cited in the Introduction, or that the morphometric measurement is less sensitive than the DTI procedures to the teratogenic effects of alcohol. If so, macrostructure may be a less efficient indicator for differentiating effects of alcohol exposure than functional status or DTI. Such an outcome would be consistent with the findings of Pfefferbaum and Sullivan (2002) in their work with alcohol-abusing women and suggests that DTI is a more sensitive measure than morphology. However, it is important to remember that the sample size used in this study was very small, and power may not have been sufficient to detect real differences, given that there was high variability in macrostructural outcomes within clinical groups. Therefore, these results may not necessarily contradict the previously published results (Sowell et al., 2001a). We believe our future study with a larger sample size will provide the information necessary to discriminate these possibilities.

A second issue involving the morphometric measurement is that of "normalization" of results to make comparisons among different brain regions between the two groups. When this procedure was used, no relative differences in size were noted. That is, group mean measurements of regions were in the same proportion in the alcohol-affected group as in the control group. However, according to Mathalon et al. (1993), "the effect of head-size correction appears to depend on a trade-off between the introduction of additional measurement error and the elimination of irrelevant true score variance (p 136)," and its effect on the ability to differentiate two groups could be mixed, even though such correction appears to be most appropriate for measurement of white matter. Nonetheless, given that we did not see any significant difference in the genu and splenium volumes with or without normalization, it is fair to say that for this study, no significant volume differences were found.

In addition, these initial results in a small sample of affected individuals suggest that FA results are not strongly correlated with physical dysmorphia in the alcohol-affected group. Certainly, this finding may reflect limitations in the measurement of dysmorphia as well as other factors. In functional terms, although there is an implication of a relationship between DTI findings and neurocognitive deficits seen in alcohol-affected individuals, such a relation-

ship cannot be established by this study due to the subject selection procedures. Because DTI had not been studied in FAS previously, we deliberately selected participants who showed physical effects of their prenatal exposure to maximize the possibility of finding differences from those in the control group, and there was a strong correlation between dysmorphia and ability level (presumably subsumed by neurodevelopmental integrity). However, because such a relationship exists, it will be difficult to identify measurement tools that are not influenced by general ability level. This exploratory analysis of DTI outcomes and available cognitive measures do suggest some direction for future exploration. Previous studies with other populations have found measures of PS to be related to degradation of white matter (Pfefferbaum et al., 2000). These patterns suggest that further evaluation of these relationships is warranted, both in clinically-referred patients and in alcohol-exposed individuals without dysmorphia. With more comprehensive research into the integrity of white matter in the brain in alcohol-exposed individuals, patterns of functional deficits may become apparent that will improve understanding of these relationships.

These results should be interpreted cautiously given the small sample size (discussed above) and the preliminary nature of these investigations. In addition, in selecting the sample for study, we identified individuals who showed physical and cognitive signs of damage associated with prenatal alcohol exposure, and these results may not be generalizable to alcohol-exposed individuals who do not have dysmorphia. Nevertheless, this study provides *in vivo* evidence for disruption of white-matter microstructure in alcohol-affected individuals and indicates that anisotropy measurement with DTI can be very sensitive in examining the neurodevelopmental effects of teratogens. Our results illustrate that the integrity of the corpus callosum in alcohol-exposed individuals may be compromised and suggest the potentially unique usefulness of diffusion anisotropy measurement for group discrimination between alcohol-affected young adults and normal controls. This work also indicates that the potential of FA as a clinical marker in the diagnosis of FAS should be explored more fully.

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