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White matter microstructure, alcohol exposure, and familial risk for alcohol dependence

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ABSTRACT

Offspring from families with alcohol dependence (AD) have been shown to exhibit brain morphological alterations that appear to be related to their familial/genetic risk for AD. Greater susceptibility for developing AD may be related to structural underpinnings of behavioral traits that predispose to AD. We examined white matter (WM) integrity in 81 individuals with either a high density of AD in their families ($N=44$) or without a family history for either alcohol or drug dependence ($N=37$). Magnetic resonance images were acquired on a Siemens 3 T scanner with fractional anistropy (FA) and the apparent diffusion coefficient (ADC), along with radial diffusivity (RD) and longitudinal (axial) diffusivity calculated for major white matter tracts in both hemispheres. Extensive personal histories of alcohol and drug use were available from longitudinal collection of data allowing for reliable estimates of alcohol and drug exposure. We found that the interaction of personal exposure to alcohol and familial risk for AD predicts reduction in WM integrity for the inferior longitudinal fasciculus (ILF) and the superior longitudinal fasciculus (SLF) in the left hemisphere and the forceps major tract. Only one tract showed a significant difference for exposure alone, the anterior thalamic radiation.

1. Introduction

The neurotoxic effects of alcohol consumption have been evident since the time that Victor et al. (1971) published their classic work. Using autopsy data, it was shown that the debilitating behavioral and neurocognitive effects of long-term alcohol use seen in Wernicke–Korsakoff patients, who have among other deficits a profound short-term memory loss, were associated with significant neuropathological changes in a number of subcortical regions.

It is now clear from numerous reports focused on individuals with chronic alcohol dependence that long-term consumption of alcohol is associated with brain morphological changes (Chanraud et al., 2010), as well as neurocognitive changes (Sullivan and Pfefferbaum, 2005; Oscar-Berman and Marinkovic, 2007). Although alcohol diffuses throughout the brain, there is evidence that not all regions are equally affected by alcohol. Regional changes have been suggested by work showing impairment in frontal and parietal networks in long-term alcohol dependence (Pfefferbaum et al., 2010) and a tendency for lateralized effects to be seen, with the right hemisphere showing greater impairment (Oscar-Berman and Marinkovic, 2007; Pfefferbaum et al., 2009) in association with the neurotoxic effects of alcohol.

There is an intriguing possibility that some of the observed variation in structural and functional characteristics of individuals who are heavy consumers of alcohol may have existed prior to the initiation of drinking and that some of these characteristics may actually be markers of vulnerability. Structural differences have been observed family-history-positive youth selected for a high density of familial alcohol dependence. These include reduced volume of the right amygdala (Hill et al., 2001), reduced volume of the right orbitofrontal cortex (Hill et al., 2009a), and greater volume of cerebellum for age (Hill et al., 2007a; Hill et al., 2011a) suggesting a slower rate of neural pruning of grey matter in those with a family history of alcohol dependence. Also, youth with parental alcoholism have been reported to have smaller total brain volume (Gilman et al., 2007). Electrophysiological differences between family-history-positive and-negative youth were first identified using event-related potential (ERP) recordings in boys without any significant drinking history (Begleiter et al., 1984). Reduction in the P300 component of the ERP was seen in those with a family history. Several other studies have found similar effects (for reviews, see Polich et al., 1994; Hill, 2010). These deficits may reflect an inability to reach age-appropriate levels of P300 in high-risk youth (Hill et al., 1999) and, importantly, slower trajectories of P300 change are related to childhood psychopathology (Hill and Shen, 2002).

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A variety of functional imaging paradigms have been used to address possible differences between high- and low-risk youth. Although the paradigms and functions have varied across studies (for review see Tessner and Hill, 2010), the general tendency has been to see reduced activation in frontal regions associated with attentional tasks (Spadoni et al., 2008) and with tasks requiring inhibition of a prepotent response (Schweinberg et al., 2004). Functional imaging studies that have focused on brain connectivity using functional magnetic resonance imaging (fMRI) in family-history-positive and -negative youth have reported weaker fronto-parietal connectivity (Wetherill et al., 2012) and weaker fronto-cerebellar connectivity (Herting et al., 2011) in family-history-positive youth.

Attempts to differentiate what is cause and what is consequence are complicated by the fact that youth from families with a history of alcohol dependence have a higher risk for developing an alcohol use disorder (AUD) or related substance use disorder (SUD) than do youth without such a history (Milberger et al., 1999; Merikangas et al., 2009). Follow-up of youth from multiplex, multigenerational families with multiple cases of alcohol dependence is associated with a four-fold increase in risk for substance use disorder (SUD) (Hill et al., 2008, 2011b). In addition, youth with a positive family history of alcohol dependence tend to start using alcohol at an earlier age than do those without a history (McGue et al., 2001; Hill and Yuan, 1999; Hill et al., 2000). Because of this earlier onset of substance use, those with a family history may be at greater risk for incurring alcohol- and drug-related exposures that could potentially affect brain morphology.

Magnetic resonance (MR) diffusion tensor imaging (DTI) provides an opportunity to investigate white matter microstructure often revealing disruption that is not apparent upon macrostructural inspection (Pfefferbaum and Sullivan, 2002). DTI studies are possible because of characteristics of water diffusion in the brain. Diffusion that is unconstrained is isotropic as is seen in the cerebrospinal fluid whereas anisotropy results from constraints imposed by fiber tracts. The metrics of DTI include fractional anistropy (FA) and the apparent diffusion coefficient (ADC), which can be decomposed into two components, the transverse (radial) diffusivity (λT), and the longitudinal (axial) diffusivity (λL). (Beaulieu, 2002). Specifically, FA reflects the tendency for water to diffuse along an axis parallel to the fiber tract such that higher values of FA are associated with more intact white matter microstructure. Radial diffusivity (RD), which measures water diffusion perpendicular to the tract, is associated with disruption of white matter integrity so that higher values are indicative of greater disruption. These DTI metrics provide a method for assessing the likelihood that white matter integrity has been affected. Radial diffusivity (RD) increases with loss of myelin integrity. Longitudinal diffusivity can be altered where disruption of axonal integrity or axonal deletion is present. Typically, disruption of white matter through breakdown of the myelin sheath is associated with decreased FA and increased radial diffusivity RD (Song et al., 2002).

Among the applications of DTI methodology are assessments of whether white matter integrity has been compromised by exposure to substances, whether neurodegenerative conditions may be taking place, or whether white matter variation might be the result of a familial/genetic characteristic of the individual. Evidence that FA may also vary due to inborn characteristics comes from recent studies showing a substantial heritability of FA in related healthy individuals (Jahanshad et al., 2010). FA values change during development, typically with changes in mean diffusivity. In their review of 30 studies that have addressed DTI in child, adolescent, young adult, and older adult samples, Schmithorst and Yuan (2010) conclude that there is an overall tendency for FA values to rise during childhood, adolescence and young adulthood, peaking at about 40 years of age and then declining. The rise in FA seen during this period is mirrored by a decrease in mean diffusivity, declining until age 40 and then rising again.

DTI studies have provided evidence that white matter microstructural disruption occurs in alcohol-dependent men and women in the corpus callosum and spenium and in multiple white matter tracts (Pfefferbaum et al., 2006, 2009). DTI can reveal regional white matter disruption and assess white matter tracts (Lehericy et al., 2004). Applying quantified fiber tracking, Pfefferbaum et al. (2009) showed that alcoholism affected FA and diffusivity of several fiber bundles with frontal and superior regions most affected (frontal forceps, longitudinal fasciculi, internal and external capsules, fornix and superior cingulate). Curiously, more posterior and inferior structures were relatively spared.

Although there is substantial evidence that exposure to alcohol and drugs is associated with white matter microstructural changes (Sullivan and Pfefferbaum, 2005; Pfefferbaum et al., 2009), it is unclear if some of these microstructural changes may have been present in those with a family history of alcohol dependence before they started drinking. Only a few studies have examined white matter microstructure in youth with and without a family history of alcohol dependence (Medina et al., 2008; Bava et al. 2009, 2010; De Bellis et al., 2008; Herting et al., 2010; Wetherill et al., 2012). One study reported reduced FA for the inferior longitudinal fasciculus and right optic radiation in family-history-positive youth (Herting et al., 2010), while another (Wetherill et al., 2012) did not find altered white matter microstructure in tracts connecting the frontal and parietal regions, though they did find evidence for reduced frontoparietal connectivity using fMRI. Bava et al. (2009) reported a family history effect for the right crus cerebri, though no other regions showed an association. Exposure to marihuana and alcohol predicted FA above and beyond parental history, though no interaction between family history and exposure was seen. However, in a later report, Bava et al. (2010) found FA in inferior longitudinal fasciculus (right) to be significantly correlated with parental SUD.

Alcohol or marihuana exposure during adolescence and young adulthood appears to have an adverse effect on white matter microstructure (Medina et al., 2008; De Bellis et al., 2008; McQueeny et al., 2009; Bava et al., 2009; Thatcher et al., 2010). Three of the studies that evaluated exposure effects also evaluated family history (Bava et al., 2009; Medina et al., 2008; De Bellis et al., 2008) with one reporting no effect of familial risk for alcohol dependence background (Medina et al., 2008), the other showing minimal effect (Bava et al., 2009), while the third found an unexpected increase in FA (De Bellis et al., 2008).

Because of the possibility that both familial loading for alcohol dependence and exposure to alcohol or drugs of abuse might affect white matter tracts, this study was designed to address these issues in a sample that varied in familial loading for alcohol dependence and for whom extensive data were available for personal exposure to alcohol, marihuana, and cigarettes. Based on results from previous studies that have assessed alcohol exposure, we predicted that subjects with heavier alcohol exposure would have reduced FA and increased ADC (either axial or transverse diffusivity) reflecting loss of white matter integrity. With previous reports of reduced FA in those with a family history of alcohol dependence, we predicted that individuals with multiplex family history would have reduced FA. It was also hypothesized that those with a multiplex family history who were among the heavier consumers of alcohol might have the greatest decline in FA and the largest increases in radial or longitudinal diffusivity. Our first goal was hypothesis-driven and was designed to assess whether or not previously identified changes in specific tracts (inferior longitudinal fasciculi and superior longitudinal fasciculi) would be seen in association with multiplex

Table 1
Demographic characteristics of high-and low—risk adolescent and young adults.

	High-risk ($N=44$)		Low–risk ($N=37$)		F	df	p
	Mean	SD	Mean	SD			
Age males ($N=44$) [a,b]	25.33	5.05	21.45	5.24	6.23	1/42	0.02
Age females ($N=37$)[c]	24.65	5.10	24.0	5.70	0.13	1/35	NS
SES[d]	37.84	11.41	47.91	8.61	19.46	1/79	0.001
Number of years drinking (males)	10.0	5.42	3.3	3.81	21.6	1/42	0.001
Average drinking density (males)	364.75	610.78	154.05	292.35	1.99	1/42	NS
Number of years drinking (females)	10.5	6.13	7.82	5.64	1.88	1/35	NS
Average drinking density (females)	99.18	92.41	99.57	145.36	0.00	1/35	NS
Number in top tercile > 140 drinks/years.	19		8				
Number in top tercile for marijuana (15.3 occasions/years)	23		4				
Overlap between heavy drinking and marihuana use	13		2				
Right handed (%)	93.2		94.6				
Alcohol or drug abuse/dependence[f]	26		6		10.18[e]	1	0.001
Age of onset of SUD	18.58	3.21	20.33	5.43	1.11	1/30	NS

[a] Age range for high-risk subjects was 16–34 years and 13–33 years for low-risk controls.
[b] There were 24 high-risk males. Of these, 15 met criteria for SUD before the scan. There were 20 low-risk males. Of these, 3 met criteria for SUD before the scan.
[c] There were 20 high-risk females. Of these, 11 met criteria for SUD before the scan. There were 17 low-risk females. Of these, 3 met criteria for SUD before the scan.
[d] Hollingshead (1975) Four-Factor Index.
[e] Chi square value.
[f] Number of cases meeting criteria for alcohol or drug abuse or dependence before their scan. Diagnoses were made using the KSADS for those under age 19, and the CIDI for those 19 or greater.

familial risk for alcohol dependence and determine if this would be modified by personal exposure to alcohol. Our second goal was to perform exploratory analyses with the remaining Johns Hopkins University Atlas tracts to determine if other tracts might reveal an association with these variables.

2. Methods

2.1. Participants

The sample included 44 subjects (24 male and 20 female) from multiplex for alcohol dependence families and 37 (20 male and 17 female) low-risk control subjects (Table 1). Overall the groups did not differ in mean age, though the high-risk males were on average somewhat older than the low-risk males. A similar proportion was left handed. There was a statistically significant difference in the socioeconomic status (SES) of the two groups. However, the mean Hollingshead Four-Factor Index scores (Hollingshead, 1975) for the high- and low-risk groups are in adjacent SES classes suggesting greater similarity in SES for the two groups than the mean differences indicate.

2.2. Risk group status

2.2.1. High-risk group

A three-generation study of multiplex alcohol dependence (AD) alcohol dependence families initiated in 1985 provided the participants for the present study. These multiplex AD families were originally identified through the presence of two adult brothers (proband pair) with AD. Extensive in person and family history information are available for these targeted families, including the probands' parents (first generation) and the second generation siblings of the identified probands. The multiplex sampling strategy used in this study resulted in a high density of AD in the targeted pedigrees (an average of 4.41 first and second degree relatives for the present sub-sample) as previously described (Hill et al., 2008). Third generation offspring of the brother pairs and offspring of their siblings in these multiplex families provided the high-risk offspring for the present report. These offspring have a greater familial loading for alcohol dependence than is commonly seen in studies designed to identify offspring of AD parents (Table 2).

Psychiatric status of the "marrying in" side of the offspring's family was also obtained. Among the high-risk offspring, 76% of the fathers and 33% of the mothers had AD by lifetime history. A total of eight offspring (18.2%) were from families where both parents were alcohol dependent. Although six high-risk offspring did not have a father or mother with a lifetime diagnosis of AD, the sampling design insured that each individual designated as high risk came from multiplex pedigrees. As a result, the average number of second degree relatives (aunts and uncles), even for the six high-risk offspring without AD parents, averaged 4.33 relatives who were alcohol dependent.

Table 2
Family history of alcohol dependence.

	Average number of first degree[a] relatives	Average number of second[b] degree relatives	Average number of first and second degree relatives	Average family density[c]
High risk	0.95	3.45	4.41	0.55
Low risk	0.11	0.05	0.16	0.02

[a] First degree relatives included parents only. The subjects' brothers and sisters were not included in the calculation because most were too young to have passed through the age of risk for AD.
[b] Second degree relatives included aunts, uncles and grandparents.
[c] Density was calculated using the number of alcohol-dependent relatives divided by the number of known relatives.

2.2.2. Low-risk group

Low-risk control families were identified through two adult brothers without a lifetime history of alcohol or drug dependence. Parental AD among low-risk offspring was infrequent but did occur where "marrying in" spouses had AD. For fathers, 8.1% were alcohol dependent and 2.7% were dependent on drugs. For control mothers, 5.4% had a lifetime diagnosis of drug dependence. Two second degree relatives of the control offspring were alcohol or drug dependent.

2.2.3. Recruitment by risk group

All third generation offspring who were enrolled in a longitudinal follow-up initiated in 1989 were eligible to participate in the MRI portion of the study. Letters were sent to all offspring and appointments set up for MRI scans for those whose schedules would allow it.

2.3. Contaminating variables

In order to control for variables other than familial/genetic risk that might influence brain morphology, careful attention was paid to personal characteristics of the subjects including their personal history of prenatal use. Similarly, prenatal use of substances by mothers was obtained.

2.3.1. Mothers' prenatal use of substances

Mothers of both high- and low-risk offspring were administered a structured interview designed to assess quantity and frequency of use of alcohol, drugs and cigarettes during pregnancy. The assessment was retrospective, being obtained at the child's first longitudinal assessment (typically before the age of 12 years).

Comparison of prospective and retrospective data for drinking during pregnancy has shown retrospective data to be valid (Griesler and Kandel, 1998). Moreover, follow-up of women for 4 and 5 years following their pregnancies has shown substantial reliability ($r=0.53$ and 0.67, respectively) between reports obtained during pregnancy and those obtained following the pregnancy (Ernhart et al., 1988; Jacobson et al., 1991).

2.3.2. Personal history of psychiatric disorders

All participants are currently enrolled in the ongoing longitudinal study that has followed youngsters from childhood through young adulthood. Extensive clinical information was available for determining if any psychiatric disorder including substance use disorder was present by the time the MRI assessment was performed. Children/adolescents were assessed yearly with the Kiddie Schedule for Affective Disorders and Schizophrenia for School-Aged Children (K-SADS) (Chambers et al., 1985) using separate interviews of parent and offspring to determine the presence or absence of Axis I DSM-III diagnoses. (DSM-III was the current methodology at the initiation of the study in 1989.) Young adults were assessed using the Composite International Diagnostic Interview (CIDI) diagnostic instrument (Janca et al., 1992) to obtain DSM-IV Axis I diagnoses. The CIDI-Substance Abuse Module (CIDI-SAM) was used to supplement the CIDI to obtain detailed substance use history.

2.3.3. Personal history of substance use

Using quantity and frequency information available from the K-SADS, CIDI and CIDI-SAM, it was possible to determine the average yearly density of each substance and the number of years of use. This measure was used as an index of exposure to take into account the number of years the subject had been drinking and the number of visits that occurred between the age of onset to drink and the time the scan was performed. The total number of drinks reported collectively from all visits was divided by the number of years drinking. If the number of visits was less than the number of years, the estimate was adjusted accordingly.

2.4. Informed consent and safety monitoring

All participants signed informed consent documents after having the study explained to them. All were screened to insure absence of ferromagnetic metal in or on their body. All female subjects were screened for absence of pregnancy using Icon® 25hCG pregnancy kits. A neuroradiologist reviewed any scan considered suspicious for abnormality.

2.5. Image acquisition

All subjects were scanned on a Siemens 3T scanner located in the Department of Radiology MR Research Center. To verify subject position, cooperation, and image quality, a set of coronal and sagittal scout images (TE=2.5 ms, TR=250 ms, slice thickness=4 mm, FOV=256 × 256 4 mm, 24 slices) were obtained. The axial sections are obtained parallel to the anterior commissure/posterior commissure line (AC/PC) which provides a reproducible guide for image orientation.

2.5.1. T1 weighted images

The T1 weighted images were acquired using the MPRAGE sequence (TR=2300 ms, TI=1000 ms, TE=2.98, flip angle=9 degrees, NEX=1, FOV=256 × 256) with full coverage of the whole head for all subjects.

2.5.2. FSE PD-T2

Using the Siemens technology, the proton density and T2 weighted images are acquired in a single scan, as a dual echo acquisition turbo spin echo. The parameters for this sequence are TR=3000 ms, flip angle =150 degrees, echo times TE1=11 ms and TE2=101 ms, NEX=1, FOV=256 × 256. The geometry of the acquisition is 48 slices (3 mm thickness) with zero gap.

2.5.3. Axial DTI

A single shot echo planar imaging (EPI) dual spin echo sequence modified to include bipolar diffusion-sensitizing gradients in arbitrary directions was used to acquire the DTI raw data. The parameters for the sequence were TR=5300, TE=88, NEX=4, FOV=256 × 256. Twelve diffusion gradient orientations were used and a total of four signal averages were performed for each orientation with a b-value of 1000 s/mm^2. A total of 40 slices with a thickness of 3 mm and an interslice gap of 0 mm were acquired for each subject.

2.6. DTI processing

The FMRIB Software Library (FSL; University of Oxford) linear image registration tool (FLIRT) was used for preprocessing, including eddy current correction for the diffusion-weighted images, correction for spatial distortions including head motion, and for transformation of each diffusion image. The Brain Extraction Tool (BET) was used to mask out all structures other than brain tissue. The eddy corrected images were used to calculate the diffusion tensor components. The program MedINRIA (Asclepios Research Project; INRIA Sophia Antipolis, Cedex France), which provides an initial estimate of DTI parameters using a method described by Basser and Pierpaoli (1996), was used to obtain three eigenvalues ($\lambda1$, $\lambda2$, and $\lambda3$,) and FA. An average of the $\lambda2$ and $\lambda3$ images provided a measure of radial diffusivity (RD). The method proposed by Basser and Pierapoli in which a family of tensor-derived scalar anistropy measures is used to characterize the diffusion in DTI remains a well recognized approach (Basser and Pierpaoli, 2011).

Using TBSS, images from all study scans were combined to provide a representative template for further analysis. Although a template from the low-risk control sample could have been used, use of all study scans provided a larger sample for constructing a representative template. We used an FA threshold of 0.2 for determining the FA skeleton for the first stage where registration is done. The 3-D FA images were nonlinearly registered and aligned to FMRIB58_FA_1mm MNI 152 standard-space image. Images were registered using a two-step process. First images were registered linearly using FLIRT. This normalized the brain volume, shape, and center of mass. Images were further registered locally using FNIRT to nonlinearly align local structures. Every image was reviewed for registration misalignment. If the registration resulted in a failure, we rejected the subject's scan. Using fslmerge, these images were then merged into a single 4-D image. Next, fslmaths was used to mask out voxels not belonging to all subjects. The remaining voxels were averaged over all 81 subjects to obtain the mean FA. The mean FA was then skeletonized and used as the basis for skeletonizing each individual subject.

Our goal was to map our findings to the Johns Hopkins University Atlas (JHUA), which describes nine bilateral major fiber tracts along with the forceps major and minor (Wakana et al., 2004). Included were four bilateral association tracts: the superior longitudinal fasciculus (SLF), inferior longitudinal fasciculus (ILF), inferior frontal-occipital fasciculus (IFOF) and uncinate fasciculus (UF). Additionally, bilateral tracts of the anterior thalamic radiation (ATR) and corticospinal tracts (CST), cingulum (CG) and cingulum-hippocampus (CH) were assessed. Also, the portion of the superior longitudinal fasciculus that courses through the temporal lobe was assessed bilaterally.

The JHUA atlas was used to mask the skeletonized voxels of interest with all voxels within the mask being included. The JHUA mask for each major tract was applied to all 81 subjects using fslmaths. Fslstats was then applied to all of the resulting images to calculate the mean, median, and center of gravity for that tract for each subject. Our method is most similar to voxel-based statistical techniques but uses a standardized region of interest (ROI), an atlas-defined tract. By registering all of the subjects' data into a common space and common skeleton, our method makes it possible to apply the same structural mask to the entire set of subjects which leads to less bias in the outcome.

The mean FA, and components of the apparent diffusion coefficient (ADC) components, the transverse (radial) diffusivity (λT) or RD values, and the longitudinal (axial) diffusivity (λL) values were then analyzed using SPSS (Version 20) using mixed model analyses that included fixed effects (familial risk status, alcohol exposure, and their interaction) with mixed model covariates included for sex, age, SES and mother's lifetime drinking status. Although the self-reported drinking and drug use during pregnancy was minimal in all mothers, the most conservative method for correcting for any possible underreporting would be to presume that those with a lifetime history might have used more substances during pregnancy than was reported. A random effect was included in the model to account for the presence of multiple siblings in some of the families. Tracts showing significant effects of alcohol exposure and risk were further tested to determine if exposure to marihuana or cigarettes would change the results obtained.

3. Results

3.1. JHUA tracts

The close correspondence between the JHUA SLF tract (illustrated in blue) and the clusters of voxels for our subjects (red) may be seen in Fig. 1.

3.2. Alcohol exposure by risk group and gender

The adjusted yearly drinking variable was used to group individuals into high- and low-consumption groups. Terciles were constructed based on the reported yearly quantity consumed, with individuals in the bottom two terciles contrasted with those falling in the top tercile. Also, a total lifetime exposure variable was calculated representing total drinks consumed which was then converted to kg. Results may be seen in Table 3.

3.3. Risk, alcohol exposure and fractional anistropy (FA)

Fractional anistropy varied with risk group membership and alcohol exposure for three tracts: inferior longitudinal fasciculus (ILF) (left), superior longitudinal fasciculus (SLF) (left), and the portion of the superior longitudinal fasciculus that courses through the left temporal lobe (Table 4A). To examine the effect of alcohol exposure, correlations were performed between FA and lifetime number of drinks first for the full sample and then confined to the high-risk sample. Correlations for the full sample were all nonsignificant. However, analysis confined to the high-risk sample showed significant results. For ILF left $r=-0.313$, $p=0.038$, for SLF left, $r=0.297$, $p=0.05$, and for the temporal portion of SLF left, $r=0.301$, $p=0.047$.

3.3.1. Gender and FA

Gender was a significant covariate for ILF left, and for the SLF left (temporal lobe portion) for FA (Table 4A), but was not significant for any of the tracts showing significant changes in transverse (radial) diffusivity.

3.3.2. Age effects and FA

Age was entered into the analyses both as a linear and a quadratic term. The tracts that were found to differ in association with the main effects of risk and exposure (ILF left, SLF left, SLF left [temporal lobe]) did not appear to be influenced by age.

To further explore the impact of age, all tracts were analyzed to determine if significant main effects of age would be apparent by regressing age on FA, testing for linear and quadratic components for the 81 subjects studied. Over the age range studied, significant age effects were not found with the exception of the corticospinal tract (CST) (left) ($F=3.95$, $df=1,79$, $p=0.050$). An analysis was then performed to address the age-related changes within each familial risk group. To control for gender, analyses were performed within a sub-sample of male low-risk controls ($N=20$) and a sub-sample of high-risk males ($N=24$). As may be seen in Fig. 2, a significant quadratic relationship ($F=4.70$, $df=2,17$, $p=0.024$) between age and FA was seen in the low-risk control males. In contrast, high-risk males did not show an age-related change in FA for any fit. For the quadratic fit that was significant in controls, high-risk males appeared to show minimal change with age ($F=0.11$, $df=2,21$, $p>0.05$) (Fig. 3). Neither the high- nor the low-risk female groups displayed significant age-related changes.

3.4. Risk, alcohol exposure and diffusion metrics ADC, radial diffusivity(λT), and longitudinal diffusivity (λ L)

Analysis to determine the extent of radial diffusivity across the nine bilateral tracts and the forceps major and minor (Table 4B) in association with familial risk, alcohol exposure and their interaction revealed marginally significant results for the inferior lon-gi-tu-di-nal fasciculus ... the forceps major (left) and significant results for the forceps major ($p=0.046$). Also, the anterior thalamic radiation showed increased radial diffusivity in association with alcohol exposure alone ($p=0.025$). Additionally, ADC for the anterior thalamic radiation (ATR) right tract differed significantly for those in the lower terciles of alcohol exposure versus those in the top tercile ($p=0.014$). Increased longitudinal (axial) diffusivity was also seen for this tract ($p=0.023$). To further examine factors associated with increased diffusivity, correlations were performed between lifetime drinking, ADC, RD, and λL for ATR right within the male only and female only sub-samples. Within the male sample (high and low risk) two significant correlations and one nonsignificant correlation were observed. For ADC $r=0.355$, $p=0.018$, for RD $r=0.355$, $p=0.018$, and for λL $r=0.287$, $p>0.05$. Results for the ATR right may be seen in Fig. 4.

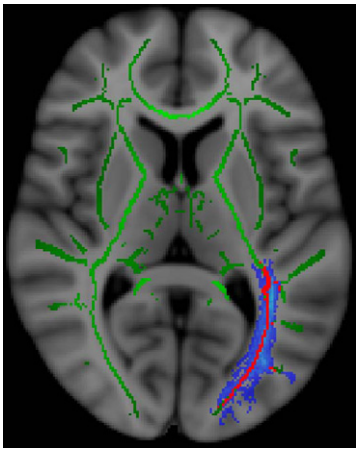

Fig. 1. The Johns Hopkins University Atlas was used to identify major white matter tracts. Note the close correspondence between the JHUA SLF tract (illustrated in blue) and clusters of voxels for our subjects (red).

Table 3
Lifetime alcohol exposure (kg) by risk group and gender.

	Mean kg		Median kg	Lower terciles kg		Upper tercile kg	
	Mean	SE		Mean	SE	Mean	SE
High-risk males	120.2	±33.9	57.9	10.7	2.8	107.9	26.9
High-risk females	37.9	±12.0	17.0	9.2	1.7	46.5	17.1
Low-risk males	27.3	±14.2	2.3	2.7	1.1	48.3	24.3
Low-risk females	41.8	±19.9	8.1	4.9	1.1	74.6	38.2

3.5. Effects of marihuana and cigarette use on FA and RD

Although the goal of the study was to evaluate the effects of alcohol exposure in offspring with and without a family history of alcohol dependence, the overlap with marihuana exposure and cigarette smoking suggested the need to analyze the results to account for possible effects of these substances. Models were run for the ILF left, SLF left and the SLF in the left temporal lobe to test for the effects of risk and alcohol exposure, covarying gender and age, and additionally evaluating first marihuana exposure and then cigarette smoking. As may be seen in Tables 4A and 4B, yearly adjusted use of marihuana and cigarettes when entered as covariates in the full model did not show an effect of these potentially contaminating variables.

3.5.1. Effects of marihuana exposure on FA

Marihuana exposure was analyzed as a main effect without consideration of familial risk or alcohol exposure to assess the extent of its impact on white matter integrity. It should be noted that 42 subjects reported using marihuana with 39 reporting none. Of the 42 users 20 reported a 1–9 year history and 22 a history of 10–20 years. Lifetime number of marihuana smoking occasions was analyzed using a group analysis with (1) no previous use; (2) use between 2.5 and 263 occasions (lower exposure) and 365 or more occasions. The tracts showing significant exposure effects in association with alcohol use (Table 4A) were tested for main effects of marihuana. Although there was a trend for FA to be reduced in association with group

Table 4a
Effects of familial risk and exposure to alcohol in late adolescent/young adults on fractional anisotropy.

	Risk	Exposure	Risk × exposure	Age linear	Age quad	Gender	SES	Marihuana[b]	Cigarettes[b]
Ant. Thal. Rad L. and R	–	–	–						
CST L. and R.	–	–	–						
Cingulum Gyrus L. and R.	–	–	–						
Cingulum Hippoc. L. and R	–	–	–						
Forceps major	–	–	$F=3.39$[c] $p=0.070$						
Forceps minor	–	–	–						
Inf. Fronto-Occip. Fas L. and R	–	–	–						
Inf. Long. Fas. L.	–	–	$F=5.91$ $p=0.018$	–	–	$F=5.18$ $p=0.026$	–	–	–
Inf. Long. Fas. R.	–	–							
Sup Long. Fas L.	–	–	$F=4.71$ $p=0.033$	–	–	NS[a]	–	–	–
Sup. Long. Fas. R.	–	–	–						
Uncinate Fas. L.and R	–	–	–						
Sup. Long. Fas. (Temp) L	–	–	$F=4.47$ $p=0.038$	–	–	$F=3.99$ 0.050	–	–	–
Sup. Long. Fas (Temp)R.	–	–	–						

[a] $p=0.062$.
[b] Yearly adjusted marihuana density and smoking density were included in the full model analysis that also included covariates for gender, age, SES. A separate analysis was conducted to assess the effects of possible prenatal exposure in mothers reporting a lifetime history of alcohol or drug dependence by entering this information as a covariate in an analysis that included testing for main effects of risk, exposure, risk × exposure with any significant covariates from the full analysis (seen above) and a covariate for mothers' lifetime history. This covariate did not affect the results. Similarly, removal of these 14 cases in a separate analysis did not alter the significance of the reported findings.
[c] Although this tract showed marginally significant results for FA, it is included because of the significant RD effect seen (see Table 4B).

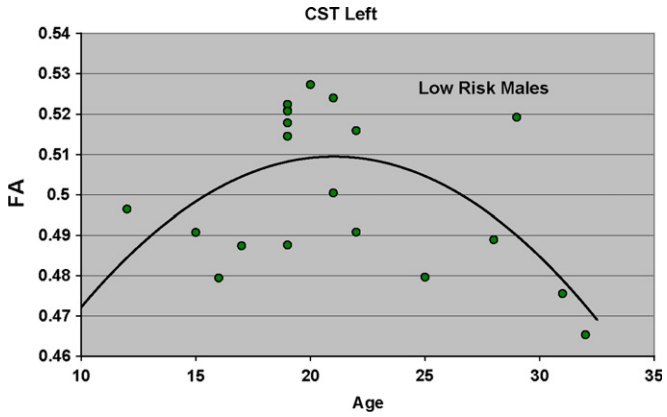

Fig. 2. Age-related changes in FA are illustrated for the low-risk control males ($N=20$). A quadratic fit of the data showed statistical significance ($p=0.024$).

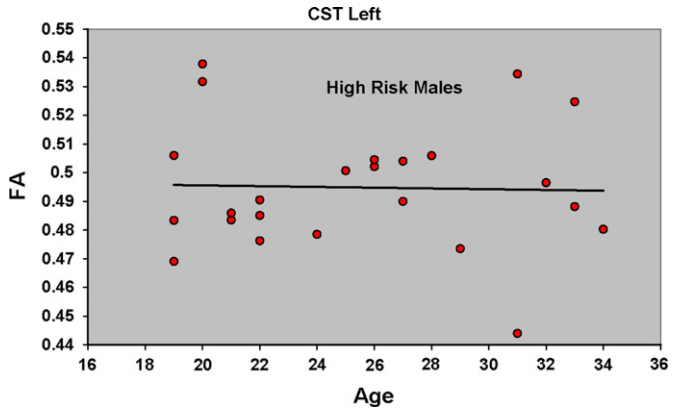

Fig. 3. Age-related changes in FA are illustrated for the high-risk males ($N=24$). A significant effect of age was not seen indicating an absence of increased FA with age. Note the high degree of variability among subjects in this group.

membership, marihuana exposure did not prove to be statistically significant for these tracts. Correlation analysis for total lifetime marihuana use and FA for ILF left, SLF left and SLF left (temporal) were not significant with r values ranging between -0.01 and -0.07.

3.5.2. *Effect of cigarette smoking on FA*

When cigarette smoking was analyzed as a main effect without consideration of alcohol consumption, no effect was seen using in a three group analysis in which lifetime number of cigarettes smoked was considered. Correlation analysis was performed for ILF left, SLF left and SLF left (temporal) and total lifetime exposure (number of cigarettes). All correlations ranged between $r=-0.09$ and -0.11 and were not significant. It should be noted that our results for the effects of smoking are based on a small sample of smokers ($N=30$) from the total group of 81 subjects. This may have limited the statistical power to uncover such effects.

3.6. *Prenatal exposure to alcohol, cigarettes, and other drugs*

Mothers of both high- and low-risk offspring were interviewed concerning their use of alcohol or drugs during pregnancy and found to be free of heavy use during pregnancy. Drinking among mothers in both groups was quite low. Of the 81 subjects studied, prenatal exposure data for drinking were available for 74, with data for drug use and cigarette smoking available for 70. A total of 81.1% of the mothers of the offspring studied reported no drinking with an additional 18.9% drinking less than one drink per day. A total of 1.4% reported using any drugs during pregnancy. Absence of cigarette use was reported by 77.1% of mothers. However, of those who smoked, a greater percentage were from high-risk families (30.7%) versus low-risk families (12.9%).

Although the reported level of drinking, drug use and cigarette smoking was low in this sample, poor recall or distortion of self-report can occur. Therefore, we performed two additional analyses to insure that our results were not influenced by prenatal exposure but rather reflected personal exposures and familial risk. First, a dummy variable was constructed in which any mother with a lifetime history of alcohol or drug dependence was coded as "1" and those without such a diagnosis coded as "0". These values were entered into the full analysis shown in Tables 4A and 4B as a covariate. This analysis showed that the mothers' potential use during pregnancy did not alter our results. Next, we removed the data for 14 offspring of these mothers. In spite of reduced sample

Table 4b
Effects of familial risk and exposure to alcohol in late adolescent/young adults on Radial Diffusivity (λT).

	Risk	Exposure	Risk × exposure	Age linear	Age quad	Gender	SES	Marihuana	Cigarettes
Ant. Thal. Rad L.	–	–	–						
Ant Thal. Rad R.[a]	–	$F=5.27$ $p=0.025$	–						
CST L. and R	–	–	–						
Cingulum Gyrus L. and R	–	–	–						
Cintulum Hippoc. L. and R	–	–	–						
Forceps major	–	–	$F=4.12$ $p=0.046$						
Forceps minor	–	–	–						
Inf. Fronto-Occip. Fas L. and R	–	–	–						
Inf. Long. Fas. L.	–	–	$F=3.43$ $p=0.068$						
Inf. Long. Fas. R.	–	–	–						
Sup Long. Fas L. and R.	–	–	–						
Uncinate Fas. L. and R.	–	–	–						
Sup. Long. Fas. (Temp) L and R	–	–	–						

[a] Significant effects were seen for ADC and for longitudinal diffusivity (λL) as well as RD (λT) for the ATR right. For ADC $F=6.32$, $p=0.014$ and for (λL) $F=5.37$, $p=0.023$. Separate analyses by gender revealed the effect was confined to male subjects. [b]Although only marginally significant, this value is included to illustrate that ILF (left) shows an increase that accompanies the decline in FA for ILF (left) reported in Table 4A.

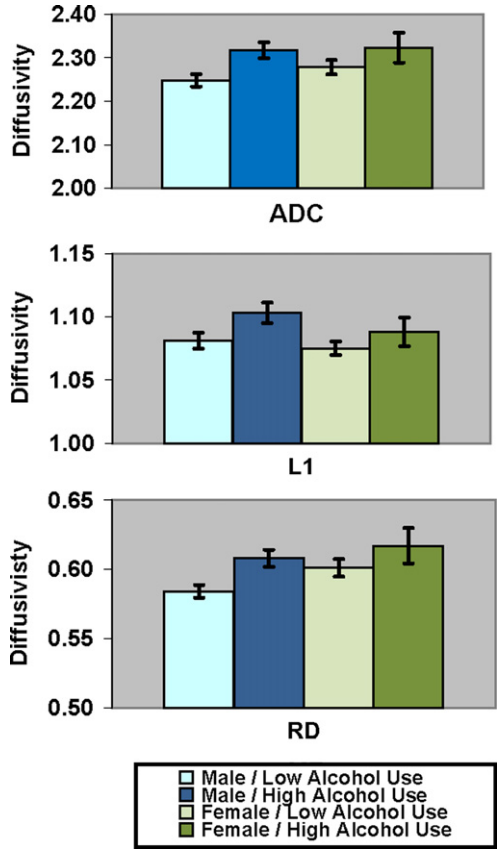

Fig. 4. Diffusivity metrics by familial risk group and lifetime alcohol exposure.

size that could reduce power to detect differences for the major variables of interest (risk, exposure and risk × exposure), the results seen in Tables 4A and 4B were supported.

4. Discussion

The results of the present study need to be interpreted in view of two separate goals: (1) to assess the effects of familial risk effects on the SLF and ILF association tracts and the forceps major and the effects of alcohol exposure, alone and in combination with familial risk effects, and (2) to explore these variables in other tracts. Accordingly, two different phases of our statistical analysis are reported. Our first goal was to determine if white matter integrity would differ in SLF, ILF and forceps major, tracts previously identified as being affected by either exposure to alcohol or other drugs or familial risk for alcohol dependence. Because this portion of the study was hypothesis-driven, the nominal p values can be taken largely at face value. Accordingly, the present results show significant familial risk group by alcohol exposure changes in FA for the ILF (left) and SLF (left) that are consistent with a previous report showing reduced FA in these tracts in 15–18 year olds with varying familial risk group membership (Herting et al., 2010). The present results are also consistent with findings in which binge-drinking youth differed from controls in FA in these tracts (McQueeny et al., 2009). Reduction of FA for ILF right and SLF left has been reported for youth with both heavy alcohol exposure and marihuana use in contrast to those without such history (Bava et al., 2009). Long-term alcohol exposure at a level that results in diagnosed alcohol dependence is clearly associated with reduced FA in the SLF in individuals studied at ages 28–59 (Pfefferbaum et al., 2009) and even in those with much shorter drinking histories who have significant exposure with early onset of alcohol dependence (mean=16.7 years) (Thatcher et al., 2010). Clearly, there are major age-related changes in WM microstructure (Lebel et al., 2008; Tamnes et al., 2010; Schmithorst and Yuan, 2010) as well as sex differences (Schmithorst and Yuan, 2010; Bava et al., 2011). Taking all of the studies reviewed in Table 5 together suggests a remarkable convergence in findings with respect to the effects of alcohol exposure, familial risk effects, and microstructural changes in the ILF and SLF tracts in view of variations in age, gender, and exposure histories across studies. Previous studies have typically not studied both variables and their interaction, however.

The ILF tract is a major pathway between the occipital lobe and the temporal lobe (Craig et al., 2009). It has been suggested that the ILF may be responsible for transmitting signals back to visual areas, signals that may pertain to the salience of observed stimuli so that visual processing of emotionally significant stimuli may be enhanced (Catani et al., 2003). Others have suggested that occipital-temporal connectivity may contribute to individual differences in social cognition, refinement of form, face and object representation (Barnea-Goraly et al., 2005). Previously we observed differences between high- and low-risk subjects in an fMRI paradigm designed to assess social cognition (Hill et al., 2007b). We have suggested that deficits in social cognition may be one mechanism whereby individuals may have greater susceptibility for developing alcohol use disorders.

Table 5
Summary of DTI studies reporting alcohol or marihuana exposure or family history effects. Fiber integrity is noted for fractional anistropy (FA) and transverse or radial diffusivity (λT), a putative index of myelin integrity.

Study	Method	Sample	Mean age/ range	ILF[d]	SLF[e]	Frontal forceps	Corpus callosum	Family history effects found
Hill et al (present study)	QFT [b]	44 HR; 37LR	13–34 years	↓ FA (Left) ↑RD (Left)	↓ FA (Left)	↓ FA↑RD		Yes
Herting et al. (2010)	Voxel-wise WB	15 HR; 18 LR	11–15 years	↓ FA (Left)	↓ FA (Left)			Yes
Thatcher et al. (2010)	Voxel- wise WB	24 SUD;12 Controls	16.7 years		↓ FA (RT)↑RD (RT)			Not studied
Pfefferbaum et al. (2009)	QFT[c]	94 AD;70 Controls	28–59 years	↔ FA	↓ FA↑ ADC ↑ RD	↓ FA	↓ FA (Pfefferbaum et al., 2006; Sullivan and Pfefferbaum, 2005)	Not studied
McQueeny et al. (2009)	Voxel-wise WB	14 BD; 14 Controls	18 years	↓ FA (Left)	↓ FA (Left)		↓ FA	Not studied
Bava et al. (2009, 2010)	Voxel-wise WB	36 heavy alcohol/MJ users	17.9 years	↓ FA (Right)	↓ FA (Left)		↓ FA	Qualified yes
Medina et al. (2008)	Voxel-wise ROI	14 AUD; 17 Controls	15–17 years				PFC abnormalities anterior to genu of CC	No
De Bellis et al. (2008)	Voxel-wise ROI	32 AUD; 28 Controls	15.9 years; 16.9 years				↑ FA↓ RD	No

[a]WB=Whole brain.
[b] Similar to quantified fiber tracking but uses tract as ROI.
[c] Quantified fiber tracking—fibers identified that pass through target and source voxels.
[d] Inferior longitudinal fasciculus.
[e] Superior longitudinal fasciculus.

The SLF tract contains bidirectional association fibers that link the frontal lobe to the occipital lobe and parts of the parietal and temporal lobes. The following three distinct components have been identified (1) a dorsal component originating in the parietal cortex and terminating in the frontal lobe and in the supplementary motor cortex; (2) a portion that originates in the caudal portion of the parietal cortex and ends in the dorsolateral prefrontal cortex; and (3) a ventral component that originates in the inferior parietal lobe and ends in the ventral premotor and prefrontal cortex. Although the SLF has extensive connections and shows a variety of branching patterns across its extent, nevertheless the primary connections appear to be between the parietal and frontal lobes. Functional relationships have been identified between FA in the SLF and neuropsychological performance (Karlsgodt et al., 2008; Hoeft et al., 2007). These appear to be lateralized with verbal performance being poorer in those with reduced FA in the left hemisphere (Karlsgodt et al., 2008) and visuospatial construction deficits in those showing abnormal white matter integrity in the right SLF. The present study identified less FA in the left SLF in association with familial risk and alcohol exposure.

Because neuropsychological data were not included in the present analysis, it is uncertain if impaired verbal performance was present in the subset of individuals with reduced SLF FA. Future analysis with a larger sample is planned for assessing this possibility.

Previous studies have reported impairment in frontal and parietal networks in long-term alcoholics (Pfefferbaum et al., 2010). Also, youth with a family history of alcohol dependence have been shown to have weaker frontal/parietal connectivity (Wetherill et al., 2012). The present results which find reduced FA in the left SLF in association with familial risk and greater exposure to alcohol appear to be consistent with these previous reports.

The significant RD effect found for the forceps major appears to be a new finding as previous reports addressing WM integrity by familial risk or exposure to alcohol and drugs have not noted this effect. The forceps major is a fiber bundle that connects the occipital lobes via the splenium of the corpus collosum. FA in the forceps major has been associated with the general factor of intelligence (Tang et al., 2010), although the relationship varies by gender, with males showing an inverse relationship and females a positive relationship.

The present study did not find main effects of exposure or familial risk alone for either the ILF or SLF but rather uncovered a significant interaction between risk and exposure. However, a significant main effect of alcohol exposure was seen for the anterior thalamic radiation (right). The average diffusion coefficient and both the radial and longitudinal diffusivity showed associations with the alcohol exposure variable. These results are of interest because further analysis by gender revealed that the effect was seen only in males and not females. This may not be surprising in view of the fact that males drank almost twice as much alcohol by the time of their scans as did females. Why this tract may be especially vulnerable to effects of alcohol is currently unknown. However, Pfefferbaum et al., (2009) have noted that fiber bundles showing the most consistent alcohol effects across DTI metrics in alcoholic men and women include the anterior association fibers with posterior fibers being relatively more spared.

A second phase of our statistical analyses was focused on determining if any other major tract would show differences by familial risk, exposure or their interaction. Here, a large number of exploratory tests were run so that nominal p values observed when correctly interpreted against an adjusted significance level could be interpreted as statistically nonsignificant. The additional would be viewed as tracts tested included the ATR, CST, CG, CH, IFOF, forceps minor, and uncinate. Only one of these tracts (ATR) was significant. The analysis did not show a familial risk by alcohol exposure interaction but showed a nominally significant effect due to alcohol exposure. Because this tract was part of the exploratory phase of our study, the result should be viewed with caution. However, detailed analysis of this tract did reveal changes in DTI metrics that would suggest that the association between alcohol exposure and white matter that was observed may be valid. Specifically, greater ADC and radial diffusivity were seen in association with level of alcohol exposure (highest tercile of consumption versus lower terciles).

In view of the many studies reporting main effects of either exposure or familial risk, it is of interest to examine why the present results only find interactive effects. First, most studies have not examined both familial risk and exposure within the same investigation (Table 5) so that some results reported as exposure effects may also reflect familial risk group differences as well. Second, although we attempted to characterize the exposure level by quantifying the estimated lifetime exposure for analysis of the main effect of this variable, there are collinear effects with familial risk that complicate analyses. Although our high- and low-risk subjects were studied at similar ages, the number of years drinking by the time they were scanned was significantly greater in the high-risk males studied than their low-risk counterparts (10 years versus 3.3 years). A greater number of high-risk individuals were also showing more extreme drinking and marihuana use with twice as many drinking in the top tercile of the sample distribution, and almost six times the number of high-risk subjects reporting marihuana use that would place them in the top tercile. A third possible explanation for the interactions found might be that those with a familial risk for alcohol dependence are somehow more sensitive to effects of alcohol exposure in these specific tracts (ILF and SLF). Recent attention to the important role that epigenetic factors can play across generations suggests that alcohol exposure in previous generations might confer changes in gene expression through methylation or histone modification of genes associated with white matter integrity (Feil and Fraga, 2012).

4.1. Limitations

There are limitations to the present study that should be mentioned. First, the study sample consisted of individuals who were between the ages of 13 and 34 years at the time of scanning. This represents a period of both rapid and less accelerated development in major white matter tracts. In a sample of 168 individuals between the ages of 8–30, increases in WM volume were seen across many, though not all, brain regions (Tamnes et al., 2010). Similar findings have been noted by others (Lebel et al., 2008). Although significant age-related differences were not seen for most of the tracts examined, this may have been due to the wide variation in age across the subjects assessed. Additionally, the study did not include repeated scans of the same subjects so age-related effects were obtained from cross-sectional data.

With the use of standard TBSS procedures for developing a template, study scans from both high- and low-risk subjects were included. This may have made significant risk-group differences more difficult to detect because high-risk subjects with more substantial exposure were included. Because our goal was to perform an exploratory analysis aimed at identifying risk and exposure variation for 20 JHUA tracts with hypothesis-driven tests for specific tracts (ILF,SLF, and forceps major) FA, it appeared prudent to take a more conservative approach with respect to template formation. Our statistical approach to data analysis was not conservative (no corrections were applied). Therefore, it is possible that some of the differences seen might have been type I errors. We chose to report results without correction to best inform future studies, our own and those of others, by identifying regions most susceptible to alcohol effects and reporting all nominal p values.

Determination of lifetime exposure for alcohol presents challenges even in longitudinal studies where prospective data collection assures that subjects have minimal retrospective recall of quantity consumed. Our study design included a plan for yearly follow-up between the ages of 8 and 18 years and biennial follow-up during young adulthood (19 years and beyond). Nevertheless, subjects started drinking at varying ages, and some were unable to come in at exactly 1-year intervals. Therefore, we choose a drinking metric that was based on total reported quantity at all of the visits and adjusted to meet the number of years they had been regularly drinking. A limitation of this measure is that it assumes an equal quantity of drinking across the time period of interest, namely from drinking onset to time of scan. Clearly, quantity consumed may have shown an increase over time. However, yearly drinking density has the advantage of approximating the impact that alcohol use may have on white matter integrity by adjusting for the varying ages of onset seen in adolescent samples and the fact that timing of visits often does not conform to one's study design.

Another limitation of the study was that we were unable to conclusively determine if marihuana use is detrimental to WM integrity due to the collinearity between marihuana and alcohol use. More than half of the subjects who were the highest consumers of alcohol were also the highest consumers of marihuana making it difficult to conclude that alcohol or marihuana was causative in the observed lesser FA seen in association with such exposure. The absence of a main effect of marihuana use may reflect the fact that approximately half of the sample had never used marihuana. It is likely that years of marihuana smoking showed significance as a covariate in our analyses because those who regularly used alcohol were most likely to have used marihuana extensively.

Another possible limitation of our analysis was that some offspring were exposed to alcohol, cigarettes, or other drugs used by their mothers while they were *in utero*. The ideal situation would be to have offspring without *in utero* exposure when evaluating the effects of familial risk and personal exposure. The base rate of alcohol and cigarette use during pregnancy suggests this would probably not be possible even if a national sample were studied. Using data from a survey of eight obstetric clinics in Southeastern Michigan, Flynn et al. (2003) found that 81.9% of the mothers reported drinking less than one drink per week. In our sample, 81.1% reported no drinking during pregnancy. Thus, we find the rate of alcohol use reported by the mothers during their pregnancies is similar to that seen in the general population. Smoking during pregnancy was reported by 22.8% of the mothers in our sample. Survey data for the US and England reveal that 12–20% of mothers continue to smoke during pregnancy (Gilman et al., 2008; Office for National Statistics, 2006).

While it may be argued that mothers may not recall use during pregnancy accurately or simply choose to not disclose the full extent of their use, it appears that was not the case in our sample. Multiple points of inquiry were available for these mothers because they were most often the parent bringing in the child for follow-up evaluation. At each follow-up visit, a parent update interview covering major psychiatric disorders including alcohol and drug dependence was performed to determine the clinical status of the parent. With this information in hand, a time-line could be constructed that enabled us to determine if reported use during pregnancy was consistent with the overall picture emerging over several years of follow-up.

Moreover, performing the analysis using the mothers' lifetime history of alcohol or drug dependence as a covariate on the assumption that those with a lifetime history may have underestimated their pregnancy use did not alter our results. These considerations suggest that the risk-group differences seen in combination with the subject's own personal exposure was minimally influenced by the effects of prenatal use of substances by the mothers of these offspring.

The absence of a familial risk group effect might be considered a limitation of the present study in view of reported volumetric differences for specific brain regions (Hill et al., 2001; Hill et al., 2009a) and for total brain volume (Gilman et al., 2007). Also, family history effects on white matter integrity have been

assessed previously (De Bellis et al., 2008; Medina et al., 2008; Bava et al., 2009) with one study (Herting et al., 2010) finding significant family history effects in youngsters between the ages of 11 and 15 years. However, one study that addressed family history did not find reduction in FA in the regions assessed (Medina et al., 2008) while another found only limited support for a family history effect with no significant familial risk by exposure effects (Bava et al., 2009), and one study found an increase in FA (De Bellis et al., 2008) in the corpus callosum. Another study (Bava et al., 2010) reported a correlation between ILF (right) FA and parental history of alcohol dependence in 16–19 year olds. Possibly white matter metrics associated with familial alcohol dependence risk are most apparent in younger children where exposure does not contaminate results. Alternatively, biological markers for alcohol dependence risk appear to show developmental effects so that they are most apparent in younger children as is the case for P300 amplitude, which has been shown to predict later SUD outcome best in 9-year-old children (Hill et al., 2009b).

Significant effects of age on FA were not seen for any of the tracts studied with the exception of the corticospinal tract. This may be viewed as a limitation of our study in view of previously reported increases in FA across adolescence and young adulthood (for review, see Schmithorst and Yuan, 2010). However, it should be noted that Lebel et al. (2008) reported substantial variability in age-related changes in tracts showing FA increases over the age range of 5 to 30 years. They note that the association fibers such as the SLF, and the superior and inferior fronto-occipto fasciculi reach 90% of maximum FA between ages 13 and 20 years. It should be noted that the median age for our sample was 22 years, making it more likely that across the age range studied (13–34 years) these tracts would not show age-related changes. In contrast, previous studies have consistently reported that continued brain maturation occurs during adolescence in the corticospinal tracts (Schmithorst and Yuan, 2010; Barnea-Goraly et al., 2005; Ben Bashat et al., 2005). Our findings indicate a significant nonlinear age-related change in FA for the CST in the full sample of 81 cases, a finding that is consistent with an exponential fit for CST age-related changes observed in 202 subjects, ages 5.6 to 29.2 years, by Lebel et al. (2008). In our study, a separate analysis within each risk group indicated that this pattern was only seen in the low-risk controls and not the high-risk sample. This finding merits further study in a larger sample to determine if familial risk confers a differing trajectory of FA development in the CST.

4.2. Conclusions

In spite of these limitations, there are a number of strengths that should be mentioned. Prospective collection of data on alcohol and drug exposure results in better recall of actual use than retrospective recall. Attempts to find familial risk factors that may influence FA are more likely to be revealed in studies of offspring from multiplex families selected for multiple cases of alcohol dependence within the family. Finally, it appears essential to address familial risk and lifetime exposure to drugs and alcohol to the degree it is possible within the same study. Herting et al. (2010) provided important data showing family history effects in adolescents but had minimal opportunity to find risk by exposure effects because of study design (heavier drinking youth [> than 10 drinks by lifetime history] were excluded from the study). Three important DTI studies demonstrating exposure effects (Pfefferbaum et al., 2009; McQueeny et al., 2009; Thatcher et al., 2010) did not investigate family history and so could not have found an interaction.

Future studies need to address larger samples so that a sufficiently large number of subjects can be included by age and gender to identify critical periods when exposure is most likely to affect WM integrity and to provide an opportunity to determine the extent to which familial risk and alcohol exposure contribute to WM changes.

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