Effects of long-term adolescent alcohol consumption on white matter integrity and their correlations with metabolic alterations

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ABSTRACT

Alcohol-related white matter (WM) microstructural changes have not been fully elucidated in adolescents. We aimed to investigate influences of subclinical alcohol use during adolescence on WM microstructure and to characterize those with serum metabolic alterations. 35 moderate-to-heavy drinkers (15 males, 20 females) and 27 controls (12 males, 15 females) were selected based on their ten-year Alcohol Use Disorders Identification Test scores measured at three time points. Magnetic resonance imaging was conducted at endpoint time. Whole brain analysis of fractional anisotropy (FA) was performed. Diffusivity indices in the significant regions were computed for between-group comparisons and correlation analyses with serum metabolite concentrations. Decreased FA was found in moderate-to-heavy drinking men in anterior corpus callosum, superior/anterior corona radiata and right inferior fronto-occipital fasciculus, accompanied by increased radial diffusivity and a smaller area of reduced axial diffusivity, which correlated with serum metabolites playing roles in energy metabolism, myelination and axonal degeneration. No significant difference in FA was detected between female or mixed-gender moderate-to-heavy drinking subjects and controls, supporting gender differences in the relationship between adolescent alcohol use and neurodevelopmental trajectories. Future researches with longitudinal imaging data are warranted for comprehensive evaluation on potentially reversible effects of alcohol use over adolescent brain.

1. Introduction

Alcohol is the most commonly used substance during adolescence (Organization, 2014). Chronic alcohol exposure is known to be associated with neurocognitive deficits, behavioral alterations, and functional and macrostructural brain changes (de la Monte and Kril, 2014; Galandra et al., 2018; Zahr and Pfefferbaum, 2017). However, influence of alcohol on white matter (WM) microstructure has not been fully elucidated during adolescence, a time period during which the brain undergoes specific structural and functional changes (Pfefferbaum et al., 1994).

Diffusion tensor imaging (DTI) is a non-invasive technique for the assessment of WM. The most commonly used DTI scalar, fractional anisotropy (FA), describes the degree of non-randomness of diffusion and is highly sensitive for detecting the overall alterations of axonal diameter, axonal density, and myelination (Smith et al., 2006). Supplementary metrics, i.e. radial diffusivity (RD), axial diffusivity (AD)

Table 1
Alcohol-related white matter microstructural changes during adolescence reported in previous DTI-based studies.

Author and year	Subjects	Age range at the last MRI scan (y)	Design	Comorbid other SUDs	Method	DTI results FA	MD	AD	RD
De Bellis et al. (2008)	32 AUD (25 M, 7F), 28 C (17 M, 11F)	12 - 19	Cross-sectional	Marijuana	ROIs	AUD > C in 8 regions of CC	AUD < C in 1 region of CC	/	/
Jacobus et al. (2009)	14 BG (12 M, 2 F), 14 BG + MJ (12 M, 2 F), 14 C (12 M, 2 F)	16 - 19	Cross-sectional	Marijuana	TBSS	BG < C in 8 regions; BG < BG + MJ in 4 regions; BG + MJ < C in 2 regions	-	/	/
Bava et al. (2009)	36 ALC + MJ (26 M, 10 F), 36 C (26 M, 10 F)	16 - 19	Cross-sectional	Marijuana	TBSS	ALC + MJ < C in 10 regions; ALC + MJ > C in 3 regions	ALC + MJ > C in right OL; ALC + MJ < C in left ILF	/	/
McQueeny et al. (2009)	14 BG (12 M, 2 F), 14 C (12 M, 2 F)	16 - 19	Cross-sectional	None	TBSS	BG < C in 18 regions	/	/	/
Thatcher et al. (2010)	24 SUD (12 M, 12 F), 12 C (6 M, 6 F)	14 - 18	Cross-sectional	Marijuana; Cigarettes; Cocaine	TBSS	SUD < C in right SLF	-	SUD < C in right SLF	SUD > C in right SLF
Clark et al. (2012)	35 SUD (19 M, 16 F), 20 C (9 M, 11 F)	14-19	Cross-sectional	Marijuana; Cigarettes; Cocaine	ROIs	SUD < C in PFC and parietal white matter	/	/	/
Luciana et al. (2013)	30 ALC (16 M, 14 F), 25 C (14 M, 11 F)	14 - 19	Longitudinal DTI data (2y follow-up)	None	TBSS	ALC < C in left caudate/thalamic region and right IFOF	-	-	-
Cardenas et al. (2013)	50 AUD (22 M, 28 F), 50 C (22 M, 28 F)	AUD: 15.0 (0.7), C: 14.8 (0.8)[a]	Cross-sectional	None	ROIs	AUD > C in the limbic system	-	/	/
Jacobus et al. (2013)	17 BG (11 M, 6 F), 21 BG + MJ (13 M, 8 F), 16 C (8 M, 8 F)	19 - 22	Longitudinal DTI data (3y follow-up)	Marijuana	TBSS	BG < C in 14 regions; BG < BG + MJ in 14 regions; BG < BG + MJ in right UF	/	/	/
Thayer et al. (2013)	74 ALC (66 M, 8 F), 51 C (35 M, 16 F)	14 - 18	Cross-sectional	Marijuana; Tobacco	TBSS	ALC < C in 2 regions; ALC > C in right ACR	-	/	/
Smith et al. (2017)	20 BG (10 M, 10 F), 20 C (10 M, 10 F)	18 - 25	Longitudinal DTI data (1y follow-up)	None	TBSS, ROIs, Tractography	Male BG < C in 4 regions of CC; Female BG > C in 5 regions of CC	/	/	/

Note: Sample in Thatcher et al. (2010) was a subsample of Clark et al. (2012). The participants in Jacobus et al. (2013) are mostly the 3-year follow-up of those in Jacobus et al. (2009). Jacobus et al. (2009) include the same sample as McQueeny et al. (2009) but with an additional BG + MJ group. M = male, F = female, AUD = alcohol use disorder, SUD = substance use disorder, BG = binge drinkers, ALC: alcohol users, C = Control subjects, ALC + MJ = marijuana and alcohol users, BG + MJ = binge drinkers who are also marijuana users, FA = fractional anisotropy, MD = mean diffusivity, AD = axial diffusivity, RD = radial diffusivity, ROIs = regions of interest, TBSS = tract-based spatial statistics, CC = corpus callosum, OL = occipital lobe, ILF = inferior longitudinal fasciculus, SLF = superior longitudinal fasciculus, PFC = prefrontal cortex, IFOF = inferior fronto-occipital fasciculus, UF = Uncinate fasciculus, ACR = anterior corona radiata, - represents "No significant difference", / represents "Not investigated", [a]presented with Mean (Standard Deviation) for age range not reported.

and mean diffusivity (MD) are recommended to better characterize the mechanisms of WM microstructure alteration. It is understood that AD mainly reflects axonal integrity, whereas RD is more related to the integrity and thickness of the myelin sheaths covering the axons. MD, characterizing the average magnitude of overall molecular displacement by diffusion, is thought to represent the integrity of the cellular matrix (Le Bihan et al., 2001).

Previous DTI-based studies on alcohol-related brain WM changes have largely focused on adults with a diagnosed alcohol use disorder (AUD) or alcohol dependence. Most studies have observed most pronounced microstructural abnormalities within frontal WM (Harris et al., 2008; Liu et al., 2010; Pfefferbaum et al., 2006, 2009; Yeh et al., 2009), the posterior and inferior bundles remaining relatively spared (Pfefferbaum et al., 2009). However, DTI studies on the effects of alcohol in adolescents or young adults have thus far failed to provide convincing and constant results (Table 1). Reduced FA values have been reported in association tracts (Jacobus et al., 2009, 2013; McQueeny et al., 2009), projection fibers (Jacobus et al., 2009, 2013; Luciana et al., 2013; McQueeny et al., 2009; Thayer et al., 2013), interhemispheric tracts (Jacobus et al., 2013; McQueeny et al., 2009; Smith et al., 2017), internal and external capsules, limbic fibers, brainstem (McQueeny et al., 2009), and subcortical areas (Luciana et al., 2013) in adolescent drinkers compared with controls. In contrast, others have reported higher FA within some WM tracts in adolescent drinkers (Cardenas et al., 2013; De Bellis et al., 2008; Thayer et al., 2013). In addition, some studies revealed sex-dependent influence on alcohol-related WM microstructural changes (De Bellis et al., 2008; Sawyer et al., 2018; Smith et al., 2017; Thatcher et al., 2010), while some did not (Bava et al., 2009; Cardenas et al., 2013; Pfefferbaum et al., 2010).

Accumulative evidence has suggested that alcohol-mediated brain injury is associated with alcohol's metabolic effects (de la Monte and Kril, 2014). Recently, correlations between serum metabolite concentrations and decreased brain grey matter volume in moderate-to-heavy drinking young adults were reported (Heikkinen et al., 2018). Conceivably WM microstructural alterations could be related to metabolic findings. Studies combining brain imaging and metabolomics might thus help to understand the underlying mechanisms of WM microstructural changes caused by alcohol.

Notably, previous studies on adolescents have been cross-sectional with retrospective data, or longitudinal studies with limited prospective data on alcohol. Furthermore, most adolescent studies have observed drinkers with a diagnosed AUD or comorbid other substance abuse. In addition, some studies used overlapping samples (Bava et al., 2009; Jacobus et al., 2009; McQueeny et al., 2009) or applied region of interest (ROI)-based method (Clark et al., 2012; De Bellis et al., 2008; Jacobus et al., 2009; Smith et al., 2017) (Table 1) lacking whole-brain information.

Therefore, DTI studies based on whole-brain analysis and with more detailed information on alcohol use are warranted. Using prospective data of over 10 years on light-drinking/abstinent vs. moderate-to-heavy-drinking adolescents not reaching the threshold for an AUD diagnosis, the present study aimed to: (1) investigate the influence of alcohol use during adolescence on WM microstructure via tract-based spatial statistics (TBSS); (2) explore the associations of regional DTI metrics with AUDIT-C scores and serum metabolite concentrations. We hypothesized that long-term moderate-to-heavy drinking during adolescence would impair WM integrity, and this compromise would link to drinking severity and specific serum metabolic alterations.

2. Methods

2.1. Participants

This study is part of the Youth Wellbeing Study, which aims at following Finnish adolescents' psychological and lifestyle variants on their health wellbeing. The original cohort was gathered in 2004–2005, when the participants were aged 13 to 17 years. Demographic data, health,

hobbies, lifestyle and substance use (including alcohol, current smoking status and lifetime drug use) were collected via questionnaire over a 10-year follow-up at three time points (time point 1: 2004–2005, $n = 4127$; time point 2: 2010–2011, $n = 797$; time point 3: 2013–2015, $n = 80$). The questionnaire included the Alcohol Use Disorders Identification Test (AUDIT), a structured questionnaire originally designed by the World Health Organization. In the present study, we used a shortened version known as AUDIT-C measuring in particular the amount of alcohol consumption (Reinert and Allen, 2007). Moderate-to-heavy alcohol use was defined as an AUDIT-C score of 4 or more in males and 3 or more in females. The light-drinking controls had low AUDIT-C scores (maximum 2), and had no binge drinking episodes. Alcohol users were listed based on their AUDIT-C scores at time point 2 and time point 1, and were invited in descending sum-score order for the MRI study. Light-drinking controls were recruited in parallel to those alcohol users agreeing to participate in this trial, matching them on age, gender and education. The exclusion criteria included a diagnosis of any central nervous system disease, severe mental disorder, regular use or abuse of other intoxicating substances. We sought to recruit a total of 80 subjects (40 moderate-to-heavy drinkers and 40 light-drinking controls) from those who completed all the follow-up questionnaires at time point 2. With this sample size, with power of 0.8, and an alpha level of 0.05, we estimated to be able to detect effects at the level of effect size (ES) 0.6. A more detailed description of the study setting has been published previously (Heikkinen et al., 2017; Laukkanen et al., 2009).

This study was approved by the Ethical committee of Kuopio University Hospital and University of Eastern Finland, the Finnish National Supervisory Authority for Welfare and Health, and the Finnish Ministry of Social Affair and Health. Written informed consent was given by all participants after complete description of the study protocol.

2.2. Psychiatric assessment

At time point 3, all participants underwent a structured clinical interview for DSM-IV axis I and II psychiatric disorders (SCID-I and SCID-II) by specialists in adolescent psychiatry. Information on clinical evaluation of previous and current psychiatric disorders, personality disorders, substance and alcohol use were gathered. The participants filled out the AUDIT questionnaire and reported using status of other substances. Their answers were then discussed and confirmed in the interview.

2.3. MRI data acquisition and DTI pre-processing

The structural MR imaging protocol (Philips Achieva 3.0T TX, Philips N.V., Eindhoven, The Netherlands) included high-resolution 3D-T1W, T2W and FLAIR images that were evaluated by an experienced neuroradiologist to exclude focal abnormalities. DTI was acquired using single-shot echo planar imaging and acquisition parameters as follows: TR = 6400 ms, TE = 56 ms, acquisition matrix = 128×128, field of view = 256×256 mm^2, slice thickness = 2 mm, with a 2 mm gap, diffusion directions: 16 unique directions at b0 = 1000 s/mm^2 with 1 repetition at b0 = 0 s/mm^2.

Diffusion-weighted images were analyzed using the FMRIB Software Library (FSL, version 5.0.4; http://www.fmrib.ox.ac.uk/fsl) (Smith et al., 2004). DTI data were firstly corrected for eddy current distortions and head movements, then masked to remove nonbrain voxels using Brain Extraction Tool. Diffusion tensors were then reconstructed and diffusion parameters calculated for the subsequent analyses.

2.4. TBSS analyses

Whole-brain voxelwise statistical analysis of the FA data was carried out using TBSS implemented in FSL (Smith et al., 2006). Each subject's FA images were non-linearly aligned into 1 mm × 1 mm × 1 mm MNI standard space using a FMRIB58 FA template, then a mean FA image

Table 2
Demographic information of the participants.

	All		Female		Male	
	LD (*n* = 27)	HD (*n* = 35)	LD (*n* = 15)	HD (*n* = 20)	LD (*n* = 12)	HD (*n* = 15)
Age at time point 3, mean (SD)[a]	24.6 (1.6)	24.9 (1.4)	24.7 (1.9)	24.9 (1.6)	24.4 (1.3)	24.9 (1.2)
Education, *n* (%)[b]						
Without H.S. diploma	1 (3.7)	0 (0.0)	1 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)
H.S. graduate without college education	3 (11.1)	2 (5.7)	1 (6.7)	1 (5.0)	2 (16.7)	1 (6.7)
Some college education	23 (85.2)	31 (88.6)	13 (86.7)	17 (85.0)	10 (83.3)	14 (93.3)
Degree from 4-year college or more	0 (0.0)	2 (5.7)	0 (0.0)	2 (10.0)	0 (0.0)	0 (0.0)
AUDIT-C, median (IQR)[c]						
Time point 1	0.0 (0.0)	8.0 (1.0)**	0.0 (0.0)	5.0 (4.0)**	0.0 (0.0)	6.0 (3.0)**
Time point 2	0.0 (0.0)	8.0 (1.0)**	0.0 (0.0)	8.0 (1.0)**	0.0 (0.8)	8.0 (2.0)**
Time point 3	1.0 (1.0)	7.0 (3.0)**	1.0 (1.0)	7.0 (3.7)**	0.5 (1.0)	6.0 (3.0)**
Mean AUDIT-C, median (IQR)[c]	0.3 (0.7)	6.9 (2.0)**	0.3 (0.4)	6.8 (2.0)**	0.2 (0.6)	7.0 (1.4)**
Smokers *n* (%)[b]	2 (7.4)	16 (45.7)*	0 (0)	11 (55.0)*	2 (16.7)	5 (33.3)
Smoking, pack years, median (IQR)[c]	0.01 (0.04)	2.4 (2.4)**	0.0 (0.0)	3.2 (2.5)**	0.02 (0.06)	1.25 (2.00)*
Lifetime marijuana use, *n* (%)[b,d]	0 (0.0)	5 (14.3)	0 (0.0)	4 (20.0)	0 (0.0)	1 (6.7)
Psychiatric diagnosis, *n* (%)[b], (Present; past)[e]						
Alcohol abuse	0 (0.0);	2 (5.7);	0 (0.0);	2 (10.0);	0 (0.0);	0 (0.0);
	0 (0.0)	4 (11.4)	0 (0.0)	3 (15.0)	0 (0.0)	1 (6.7)
Mood disorders	2 (7.4);	2 (5.7);	2 (13.3);	1 (5.0);	0 (0.0);	1 (6.7);
	6 (22.2)	8 (22.9)	6 (40.0)	5 (25.0)	0 (0.0)	3 (20)
Anxiety disorders	3 (11.1);	5 (14.3);	2 (13.3);	2 (10.0);	1 (8.3);	2 (13.3);
	4 (14.8)	5 (14.3)	3 (20.0)	4 (20.0)	0 (0.0)	1 (6.7)
Eating disorders	0 (0.0);	0 (0.0);	0 (0.0);	0 (0.0);	0 (0.0);	1 (6.7);
	2 (7.4)	3 (8.6)	2 (13.3)	2 (10.0)	0 (0.0)	1 (6.7)
Personality disorders	2 (7.4);	3 (8.6);	2 (13.3);	2 (10.0);	0 (0.0);	1 (6.7);
	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Note: LD = light drinking, HD = moderate-to-heavy drinking, H.S. = high school, AUDIT-C = Alcohol Use Disorders Identification test, the first three questions, SD = standard deviation, IQR = interquartile range, [a]2-tailed independent samples *t*-test, [b]Chi-squared test, [c]Mann-Whitney *U test*, [d]Reported use of more than 6 times during lifetime, [e]When comparing the number of participants with at least one diagnosis of any of the diagnostic groups between the alcohol users and controls.

  ** : $p < 0.001$.

  * : $p < 0.01$.

was created and the threshold of FA value was set on 0.2 to exclude peripheral tracts and GM regions. Finally, each subject's aligned FA images were projected onto the skeleton and the resulting images fed into voxel-wise cross-subject statistical analysis. Between-group comparisons were performed using independent two-sample *t*-test, by the FSL randomize procedure with 5000 permutations. Threshold-Free Cluster Enhancement (TFCE) was used to correct for family-wise error (FWE) of multiple comparisons. Age, gender, current smoking status and marijuana use were included as covariates via general linear model (GLM). Given the reported sex differences in adolescent brain maturation, group comparisons on TBSS were performed among men and women separately. Values of $p < 0.05$ and a contiguous voxel size > 50 were considered to indicate a statistically significant difference. To localize significant voxels, contrast maps were subdivided according to the John Hopkins University ICBM-DTI-81 White-Matter Labels.

When significant differences in FA were identified by TBSS, the average values of FA and supplemental indicators of WM integrity MD ([λ1 + λ2 + λ3] / 3), RD ([λ2 + λ3] / 2), and AD (λ1) within each cluster were computed for each individual. After confirming normal distribution of the data by a one sample Kolmogorov–Smirnov test, one-way analysis of covariance (ANCOVA) was performed to compare between-group differences in DTI indices, controlling for age, smoking status and marijuana use.

2.5. Metabolic profiling analysis

Venous blood samples were obtained via venipuncture and centrifuged to separate serum. The serum samples were frozen at −80 C until analyzed. Metabolites were extracted from the serum samples using acetonitrile (1:4, sample: solvent) and analyzed using an ACQUITY UPLC-MS/MS system (Waters Corporation, Milford, MA, USA). A detailed protocol and instrument conditions have been published elsewhere (Heikkinen et al., 2018).

2.6. Correlation analyses between DTI metrics, AUDIT-C scores and serum metabolite concentrations

Due to non-normal distribution of the data, associations between average regional DTI metrics from each significant cluster on FA map and AUDIT-C scores were evaluated by using Spearman's correlation coefficient.

Associations between serum metabolite concentrations and the regional DTI measures of each significant cluster on FA map for each subject were analyzed using exploratory Spearman correlation analysis. Group comparisons on serum metabolite concentrations between moderate-to-heavy and light-drinking groups have previously been published elsewhere (Heikkinen et al., 2018). In the present study, male and female participants were evaluated separately and analyses were limited to metabolites, which had *p*-value below 0.05, Cohen's d-value above 0.5 (or below −0.5), and variable importance for projection (VIP) values above 1.5 (from partial least squares discriminant analysis, PLS-DA) in the previously published analysis (Heikkinen et al., 2018). Spearman's correlation coefficients above 0.35 or below −0.35 were considered large and α level was adjusted with Bonferroni method to account for multiple testing.

3. Results

3.1. Demographic information

Detailed demographic data in moderate-to-heavy and light-drinking groups are shown in Table 2. Of the invited 80 participants, 14 were excluded due to an unsuitable AUDIT-C score at the last time point and 4 due to congenital structural abnormalities in MRI (focal cortical dysplasia, large cyst, enlarged ventricles). Altogether 35 moderate-to-heavy drinking (15 male, 20 female) and 27 light-drinking (12 male, 15 female) participants were finally included. A detailed description of the participants inclusion in the study has been published previously

(Heikkinen et al., 2017; Laukkanen et al., 2009). Age, education and the number of psychiatric diagnoses did not significantly differ between the moderate-to-heavy and light-drinking groups neither when all the participants were analyzed together nor while male and female participants were analyzed separately. Smoking was more common among female moderate-to-heavy drinkers than light-drinkers ($p < 0.01$) but didn't significantly differ in male group. Only four moderate-to-heavy drinking women and one moderate-to-heavy drinking man had smoked marijuana more than six times. None of the participants had other drug abuse or severe mental disorder or had any recreational drug use during the preceding month prior to MRI acquisition.

3.2. TBSS results

There was no significant difference in FA values between the moderate-to-heavy drinking and light-drinking participants either in women or in the whole mixed-gender group ($p < 0.05$, FWE corrected).

The male moderate-to-heavy drinkers showed significantly decreased FA values ($p < 0.05$, FWE corrected) in several brain regions (Fig. 1, Table 3) compared to the male controls. These regions of FA reduction in moderate-to-heavy drinking men were further divided into four clusters via FSL Cluster program. The corresponding anatomical locations were as follows: cluster 1: right inferior fronto-occipital fasciculus (IFOF), cluster 2: right body of corpus callosum (CC) and right superior corona radiata (CR), cluster 3: right body and genu of CC, right superior and anterior CR, cluster 4: left body and genu of CC, left superior and anterior CR. Within all these regions, FA was decreased and RD increased, while AD was reduced in two clusters (cluster 2 and 4) mainly involving the anterior CC ($p < 0.05$, Fig. 2). No significant difference was found in MD values. No region exhibited higher FA values in moderate-to-heavy drinking men ($p < 0.05$, FWE corrected).

3.3. Correlation results

3.3.1. Correlations between DTI metrics and AUDIT-C scores
None of the DTI metrics from the significant clusters significantly correlated with the AUDIT-C scores in male moderate-to-heavy drinking group. When correlations were conducted across the whole male sample, AUDIT-C scores were inversely correlated with FA values (cluster 1: $r = -0.557$, $q = 0.003$; cluster 2: $r = -0.441$, $q = 0.028$; cluster 4: $r = -0.494$, $q = 0.01$) and AD values (cluster 1: $r = -0.411$, $q = 0.048$; cluster 4: $r = -0.623$, $q = 0.001$) while the correlation was positive with RD values (cluster 1: $r = 0.461$, $q = 0.018$).

3.3.2. Exploratory correlation analysis between DTI metrics and metabolites
We limited the exploratory Spearman correlation analyses on metabolomics for males (Supplementary material), since significant DTI changes were not observed in females. Results are shown in Fig. 3. Substantial correlations were observed between homogentisic acid and FA (clusters 2 and 3, $r = -0.45$ and -0.46, $p = 0.018$ and 0.015, respectively), between aminoadipic acid and RD (cluster 1, $r = -0.41$, $p = 0.032$), between inosine monophosphate (IMP) and RD (cluster 1, $r = -0.38$, $p = 0.053$), and between glutamine and AD (cluster 2, $r = 0.45$, $p = 0.018$). None of these correlations were significant after correction for multiple testing (Bonferroni adjusted α level 0.00052).

4. Discussion

The key finding of this prospective ten-year longitudinal study was detection and characterization of regional WM disruption related to moderate-to-heavy alcohol consumption during adolescence without a diagnosed AUD. Via TBSS, we found disrupted WM integrity within bilateral genu and body of the CC, superior and anterior CR, and right

IFOF in moderate-to-heavy drinking men compared to controls. DTI metrics within significant regions on FA map showed correlations with levels of some serum metabolites among male participants. No significant difference in FA was detected in females, supporting different sensitivity to alcohol on male and female adolescent WM.

Importantly, the present study demonstrated interrupted WM microstructure in the anterior CC (genu and anterior body of CC) and the projection fiber tracts (superior and anterior CR), with a larger area within frontal region in male moderate-to-heavy drinkers. These findings are consistent with previous multi-modality MRI studies suggesting that frontal lobes are particularly susceptible to alcohol (Galandra et al., 2018; Harris et al., 2008; Spear, 2018). Frontal WM, including genu of CC and frontal-parietal portion of the CR, are among the brain regions that continue pruning and maturation after adolescence (Asato et al., 2010), and such maturation is likely to be particularly vulnerable to repeated exposure to alcohol (Crews et al., 2016; Spear, 2018). However, reduced FA was also found within a small area of the right IFOF that is known to mature earlier (Asato et al., 2010), in line with findings of Jacobus et al. (2009). In a study on middle-aged men (McEvoy et al., 2018), FA in IFOF exhibited the strongest associations with alcohol intake, also in line with our findings indicating that this long matured fiber might be particularly sensitive to alcohol in men.

As the most important frontal inter-hemispheric bundle, the anterior CC connects the key regions in cognition and execution processes, such as bilateral orbitofrontal and ventral prefrontal cortices (Aboitiz et al., 1992). The frontal-parietal portion of CR, which connects to the prefrontal cortex and the mammillothalamic tract, comprises a part of intra-hemispheric limbic–thalamic–frontal loop. Microstructural interruption within this loop is recognized in some psychiatric disorders (Jenkins et al., 2016). Macrostructural abnormality i.e. atrophy of CC seen in some complicated AUD such as Marchiafava-Bignami disease is accompanied by cognitive, emotional and psychotic symptoms (Dong et al., 2018).

In drinkers with alcohol dependence or diagnosed AUD, microstructural WM disruption within anterior CC and CR have been linked to impaired impulse control (Liu et al., 2010; Thayer et al., 2013) and cognition deficit (Smith et al., 2017). In our study, focusing on the moderate-to-heavy drinking adolescents without clinically recognized AUD, we found no significant increase in the prevalence of diagnosed psychiatric disorders. If excessive alcohol exposure is repeated, WM microstructural damage tends to deteriorate on a continuum (Jacobus et al., 2013; Smith et al., 2017; Zahr and Pfefferbaum, 2017). Our previous study with the same cohort observed significant GM volume loss in moderate-to-heavy-drinking adolescents within the orbitofrontal and frontopolar cortex (Heikkinen et al., 2017). Hence, it is possible that the observed microstructural damage within the frontal inter- and intra-hemispheric fibers, paralleling the frontal GM atrophy, might indicate an elevated vulnerability to psychiatric disorders and alcohol dependence in their later adult life (Crews et al., 2016; Jones et al., 2017; Zou et al., 2018).

Further, decreased FA in moderate-to-heavy drinking men was accompanied by increased RD in all the clusters while reduced AD was found in only two clusters, suggesting a more pronounced alteration in RD than AD. Despite the scarcity of literature on RD and AD in adolescence, our findings agree well with studies on adults with AUD (Pfefferbaum et al., 2009, 2010; Yeh et al., 2009), indicating that the underlying causes of alcohol-related compromise of WM integrity are more likely based on disruption in myelination or demyelination rather than axonal degeneration. Similar to most previous studies (Cardenas et al., 2013; Jacobus et al., 2009; Thayer et al., 2013), no difference in MD was found in the regions presenting decreased FA, suggesting that the integrity of cellular matrix remains unaltered despite the reorganization of myelin and/or axon within the affected WM (Le Bihan et al., 2001). This distinct pattern of the DTI metrics might
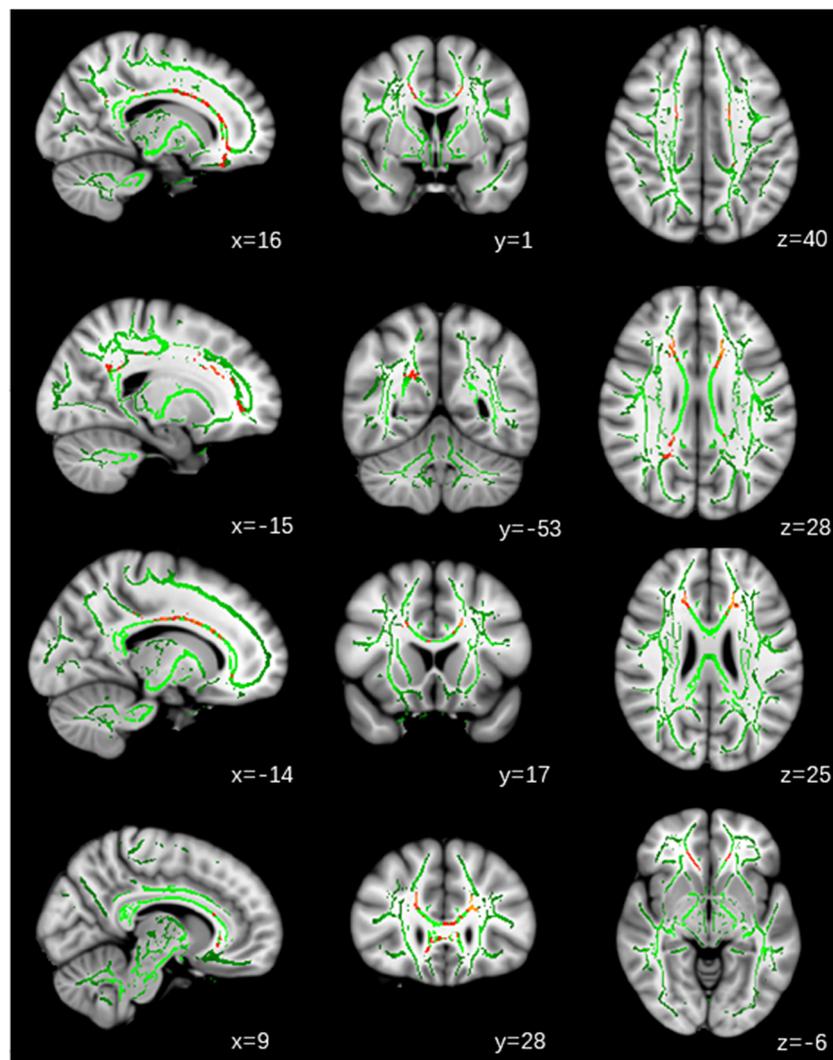
Fig. 1. TBSS analysis revealed decreased fractional anisotropy (FA) in moderate-to-heavy drinking men vs light-drinking men in bilateral genu and body of corpus callosum, superior and anterior corona radiata, and right inferior longitudinal fasciculus. The background image is the standard MNI152 brain template. Green voxels represent the white matter skeleton of all male subjects and red regions show significant voxels ($p < 0.05$, FWE controlled). Brain images are in radiologic convention (right hemisphere appears on left).

provide an in vivo tool for the assessment of the severity of alcohol-related WM damage, as well as its reversibility induced by abstinence.

With the hypothesis that alcohol-mediated WM microstructural alterations could be pathogenically related to metabolic changes, we further implemented exploratory correlation analyses between DTI metrics from the significant regions on FA and the serum concentration of metabolites that showed significant alterations in moderate-to-heavy drinking males in a previous study (Heikkinen et al., 2018). We found a negative correlation between homogentisic acid and FA in two clusters. Homogentisic acid is an intermediate metabolite of tyrosine and

Table 3
Areas of reduced fractional anisotropy (FA) values in moderate-to-heavy drinking men vs. light drinking men in TBSS analysis.

Cluster	Cluster size	Anatomical region	FWE- corrected p	MNI coordinates		
				x	y	Z
1	94	Right inferior fronto-occipital fasciculus	0.042	21	−53	28
2	207	Right body of corpus callosum	0.038	18	−28	35
		Right superior corona radiata	0.038	18	−26	36
3	1319	Right body of corpus callosum	0.027	16	1	27
		Genu of corpus callosum	0.027	5	21	17
		Right superior corona radiata	0.027	18	−2	38
		Right anterior corona radiata	0.035	17	18	30
4	1475	Left body of corpus callosum	0.017	−15	3	33
		Genu of corpus callosum	0.017	−16	29	19
		Left superior corona radiata	0.026	−18	−2	38
		Left anterior corona radiata	0.029	−17	18	30

Note: TBSS = Tract-based Spatial Statistics, FWE = Family-wise error, MNI = Montreal Neurologic Institute.
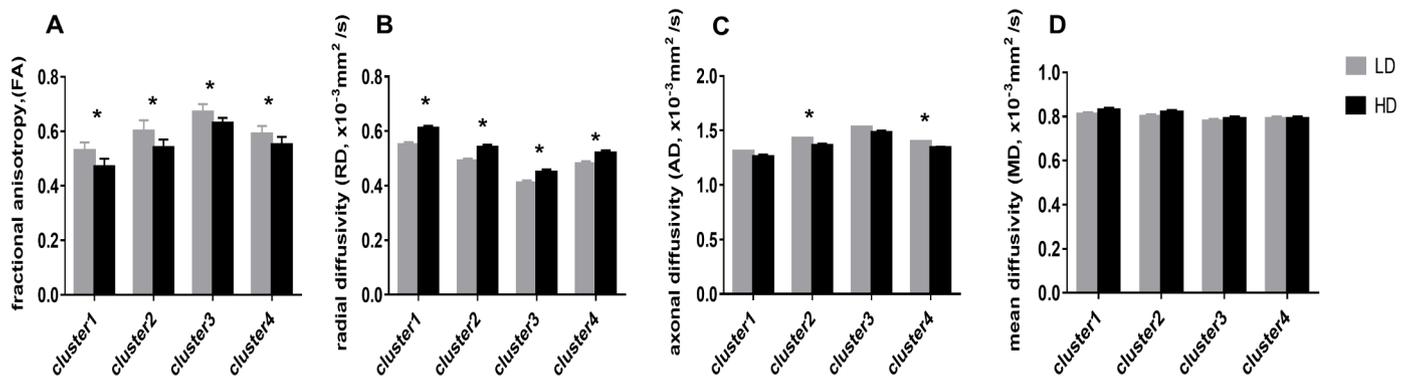
Fig. 2. White matter regions with lower fractional anisotropy (FA) in moderate-to-heavy drinking (HD) than light-drinking (LD) men analyzed by more detailed DTI measures. (A) FA was decreased in 4 clusters; (B) Radial diffusivity (RD) was increased in 4 clusters; (C) Axonal diffusivity (AD) was decreased in 2 clusters; (D) Mean diffusivity (MD) showed no significant difference between the two groups. The bars and whiskers represent mean values of the DTI metrics and standard deviations respectively. Cluster 1: right inferior longitudinal fasciculus, cluster 2: right body of CC and right superior corona radiata, cluster 3: right body and genu of CC, right superior and anterior corona radiata, cluster 4: left body and genu of CC, left superior and anterior corona radiata. *indicates $p < 0.05$.

phenylalanine, catabolized in the citric acid cycle. This finding could be associated with alcohol-related alteration in energy metabolism influencing WM integrity. A negative correlation was also detected between IMP concentrations and RD in one cluster. IMP is the first step in purine metabolism which is a key regulator of myelination (Fumagalli et al., 2017). Thus, decreased serum IMP level in the moderate-to-heavy drinking men might contribute to dysmyelination or demyelination in these fibers, corresponding to increased RD discussed above. Furthermore, the positive correlation between glutamine concentration and AD in one cluster suggests that low glutamine levels associated with alcohol use (Lehikoinen et al., 2018; Wurtz et al., 2016) might be linked to axonal degeneration, an observation in line with evidence from animal studies (Zhang et al., 2018). In addition, aminoadipic acid is a lysine metabolite and antagonist of the N-methyl-D-aspartate (NMDA) receptor. Even though oligodendrocytes express NMDA receptors, they do not seem to be essential for normal myelination (De Biase et al., 2011). Despite the exploratory nature of the present correlation analyses, these results suggest a new path in the efforts to elucidate the pathophysiology of alcohol-mediated influence on WM microstructure in adolescents.

Though correlations between reginal DTI metrics and AUDIT-C scores were found across the whole male subjects, no significant association between any DTI measures and AUDIT-C was detected within the moderate-to-heavy drinking male group. Alcohol drinking severity has been reported to correlate negatively with FA in different WM bundles in adolescents (McQueeny et al., 2009; Smith et al., 2017). Our results seemed to argue against the observed difference being a consequence of the direct toxic effects of alcohol. One explanation would be that the drinking subjects in our study were with subclinical alcohol use rather than AUD, and the drinking severity within the moderate-to-heavy drinking males was too close (with AUDIT-C score median 7.0 and interquartile range 1.4) to produce statistically significant associations.

Notably, although significant FA alteration was detected in the male subgroup analysis, no between-group difference was found in the combined mixed-gender group even after adjusting for gender variable. This finding implicates that excessive alcohol use has different and possibly more detrimental effects on male than female adolescents, in accordance with some previous brain morphometric and DTI studies (Pfefferbaum et al., 2001; Sawyer et al., 2018; Seitz et al., 2017; Smith et al., 2017). On the other hand, it indicates that controlling gender statistically as a covariate might reduce, but does not eliminate the possible confounding consequences of gender to alcohol-specific effects (Spear, 2018). When trying to thoroughly understand the influence of gender on alcohol-related WM changes, one of the difficulties is that even healthy girls and boys comply with different trajectories in their WM development and maturation (Asato et al., 2010; Bava et al., 2011). In future studies on alcohol-mediated WM changes in adolescents, careful evaluation of gender differences is of importance, and separate male and female subgroup analyses should be advocated, rather than simply adjusting gender as a covariate.

Our study has limitations. Cigarette smoking was more prevalent in the moderate-to-heavy drinking (45.7%) than light-drinking (7.4%) group, and this between-group difference tended to be larger in females in our sample (0 vs 55% in female and 16.7% vs. 33.3% in male light vs. moderate-to-heavy drinkers, respectively). Smoking has been reported to be associated with functional and structural brain abnormalities, especially within the fronto-striatal circuits (Hudkins et al., 2012; Yu et al., 2015; Yuan et al., 2016, 2017, 2018a, 2018b; Liu et al., 2019). Although all participants in our study underwent a structured interview to exclude regular use or abuse of cigarette and smoking status was statistically controlled for during the DTI analyses, this confounding factor could potentially mitigate the alcohol-specific influence, especially within women groups in our sample. While the groups primarily divided by drinking were reasonably sized, the sample size of the subgroups further divided by gender was relatively small, which makes the results difficult to be replicated. Future studies with larger sample size and better control for confounding factors are warranted to confirm these results. In addition, studies incorporating functional data and more detailed neuropsychological data will help to determine how WM impair is associated with altered neural connectivity, as well as behavioral and cognitive changes. These investigations are best performed within a framework with longitudinal structural and functional imaging data where both the persistent and potentially reversible effects of alcohol use over adolescence can be comprehensively evaluated.

5. Conclusion

Long-term moderate-to-heavy alcohol use during adolescence was associated with decreased WM integrity in frontal inter- and intrahemispheric fibers in men even before the AUD diagnose, and those WM abnormalities were linked with specific serum metabolites related to energy metabolism, myelination and axonal degeneration. No such observations were found in women, supporting gender differences in the relationship between WM organization and adolescent alcohol use. These findings might be crucial for elucidating the neuropathological basis underlying the alcohol-related behavioral changes and its sexual dimorphism, as well as for promoting reliable, evidence-based health

	Aminoadipic acid	Glutamine	IMP	Aspartate	Cotinine	Homogentisic acid
FA_cluster1	0.24	0.12	0.32	-0.19	-0.25	-0.19
FA_cluster2	0.14	0.20	0.32	-0.24	-0.27	-0.45
FA_cluster3	0.06	0.00	0.12	-0.02	-0.13	-0.46
FA_cluster4	-0.03	-0.11	0.06	-0.24	-0.24	-0.33
MD_cluster1	-0.24	-0.11	-0.23	0.05	0.22	0.12
MD_cluster2	-0.14	0.28	-0.24	-0.22	0.33	0.06
MD_cluster3	0.20	0.25	0.16	-0.09	-0.02	0.00
MD_cluster4	0.06	0.13	0.01	-0.10	0.24	0.17
RD_cluster1	-0.41	-0.23	-0.38	0.18	0.20	0.18
RD_cluster2	-0.16	-0.11	-0.32	0.11	0.36	0.33
RD_cluster3	0.08	0.10	0.03	-0.03	0.11	0.31
RD_cluster4	0.04	0.13	-0.02	0.10	0.23	0.29
AD_cluster1	-0.07	0.05	0.00	-0.21	-0.03	0.00
AD_cluster2	0.11	0.45	0.14	-0.34	0.08	-0.23
AD_cluster3	0.32	0.25	0.30	-0.13	-0.14	-0.33
AD_cluster4	0.05	-0.02	0.12	-0.27	-0.01	-0.13

Fig. 3. Correlation matrix between DTI metrics and metabolites in male participants. Exploratory Spearman correlation analysis was done between DTI metrics (FA, fractional anisotropy; MD, mean diffusivity; RD, radial diffusivity; AD, axial diffusivity) and serum concentrations of metabolites, which were significantly altered in moderate-to-heavy drinking (HD) males when compared to light-drinking (LD) controls. The figures in the matrices represent the correlation coefficients. Orange, blue and white matrices indicate positive correlations, negative correlations and no correlation respectively, and the darker the color, the greater the correlation. Cluster 1: right inferior longitudinal fasciculus, cluster 2: right body of CC and right superior corona radiata, cluster 3: right body and genu of CC, right superior and anterior corona radiata, cluster 4: left body and genu of CC, left superior and anterior corona radiata. IMP, inosine monophosphate. $n = 27$.

guidance on alcohol drinking for boys and girls during their vulnerable years of development.

Contributors

R. Vanninen and T. Tolmunen were responsible for the study concept and design. N. Heikkinen and O. Kaarre contributed to the acquisition of clinical data. Q. Shen, H. Gröhn, M. Könönen and Y.W. Liu contributed to the data analysis. O. Kärkkäinen, Z.S. Zhang and C.L. Tan assisted with interpretation of findings. Q. Shen drafted the manuscript.

R. Vanninen and T. Tolmunen provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication and participated in writing, review and editing.

Declaration of Competing Interest

None.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.pscychresns.2019.111003.

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