Smoking and the Developing Brain: Altered White Matter Microstructure in Attention-Deficit/Hyperactivity Disorder and Healthy Controls

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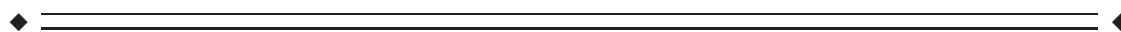
Abstract: Brain white matter (WM) tracts, playing a vital role in the communication between brain regions, undergo important maturational changes during adolescence and young adulthood, a critical period for the development of nicotine dependence. Attention-deficit/hyperactivity disorder (ADHD) is associated with increased smoking and widespread WM abnormalities, suggesting that the

developing ADHD brain might be especially vulnerable to effects of smoking. This study aims to investigate the effect of smoking on (WM) microstructure in adolescents and young adults with and without ADHD. Diffusion tensor imaging was performed in an extensively phenotyped sample of non-smokers ($n = 95$, 50.5% ADHD), irregular smokers ($n = 41$, 58.5% ADHD), and regular smokers ($n = 50$, 82.5% ADHD), aged 14–24 years. A whole-brain voxelwise approach investigated associations of smoking, ADHD and their interaction, with WM microstructure as measured by fractional anisotropy (FA) and mean diffusivity (MD). Widespread alterations in FA and MD were found for regular smokers compared to irregular and nonsmokers, mainly located in the corpus callosum and WM tracts surrounding the basal ganglia. Several regions overlapped with regions of altered FA for ADHD versus controls, albeit in different directions. Irregular and nonsmokers did not differ, and ADHD and smoking did not interact. Results implicate that smoking and ADHD have independent effects on WM microstructure, and possibly do not share underlying mechanisms. Two mechanisms may play a role in the current results. First, smoking may cause alterations in WM microstructure in the maturing brain. Second, pre-existing WM microstructure differences possibly reflect a risk factor for development of a smoking addiction. *Hum Brain Mapp 36:1180–1189, 2015.* © **2014 Wiley Periodicals, Inc.**

INTRODUCTION

Brain white matter (WM) tracts, playing a vital role in communication between brain regions, undergo important maturational changes during adolescence and young adulthood [Lebel and Beaulieu, 2011], a period that is also critical for the development of nicotine dependence [Duncan et al., 2012]. In addition, Attention-Deficit/Hyperactivity Disorder (ADHD) in adolescents and young adults is associated with an increased risk of developing nicotine dependence [Groenman et al. 2013], and widespread disturbances in WM microstructure [van Ewijk et al., 2012]. Importantly, no studies to date have investigated the effect of smoking on WM microstructure of the developing brain, and it is unclear whether this effect is different in ADHD, or whether smoking and ADHD may share underlying mechanisms.

Diffusion tensor imaging (DTI) provides the opportunity to measure microstructural properties of brain WM in vivo. Most commonly used DTI measures include fractional anisotropy (FA) and mean diffusivity (MD), representing the direction and strength of water diffusion in the brain, respectively [Alexander et al., 2007; Beaulieu 2002]. As such, FA can be seen as indicative of axonal integrity and axon orientation and organization, while MD is more sensitive to changes in intercellular space such as tissue necrosis or edema [Alexander et al., 2007].

DTI studies of nicotine dependence have generally shown that regular smoking compared to nonsmoking is associated with differences in both FA and MD. Most studies adopting a region of interest (ROI) approach showed higher FA in the corpus callosum [Paul et al., 2008; Zhi et al., 2012], prefrontal WM, cingulum [Zhi et al., 2012], and the superior longitudinal fasciculus [SLF; Liao

et al., 2011] in adult smokers versus nonsmokers. Although few hypothesis-free whole-brain studies have been performed, the available studies reported altered FA in the same regions as ROI-studies [Lin et al., 2013; Umene-Nakano et al., 2014], albeit in a different direction (i.e., smoking associated with lower FA). Only one study reported on MD differences between smokers and non-smokers, and found higher MD in smokers [Gons et al., 2011]. However, as no prospective longitudinal studies have been performed, the causality of these findings remains uncertain. In other words, it is unclear whether smoking causes alterations or damage to WM microstructure, or whether pre-existing differences in WM microstructure are a risk factor for the development of a smoking addiction.

Studies of continuous measures of smoking showed associations between lower FA and stronger physical dependence [Zhang et al., 2011], longer smoking duration [Lin et al., 2013], and higher amounts of tobacco use [Umene-Nakano et al., 2014]; these findings are in line with the categorical whole-brain studies, but contrast categorical ROI-studies. Also, whole brain results indicate an association between the duration of smoking cessation and lower MD [Gons et al., 2011]. Taken together, in adulthood, smoking is associated with altered WM microstructure in the corpus callosum, although the direction of findings (i.e., higher vs. lower FA) differs between studies, possibly due to differences in sample selection (e.g., age and severity of dependency) and analysis parameters (e.g., motion correction and thresholds).

Converging evidence suggests that ADHD is associated with microstructural abnormalities in a variety of WM tracts and subcortical structures. Meta-analytic evidence shows altered FA in the anterior corona radiata, genu of

the corpus callosum, internal capsule, and cerebellar WM in ADHD [van Ewijk et al., 2012]. Combined with more recent evidence, WM microstructure in ADHD appears to be disturbed in a widespread network, rather than local deficits [Cortese et al., 2013; van Ewijk et al., in press]. Importantly, current literature on WM microstructure in ADHD is highly inconsistent in terms of the location and direction of findings, possibly due to differences in sample characteristics such as the inclusion of comorbid problems, hampering the generalizability and interpretation of findings.

An unexplored area of research is the relation between smoking and WM microstructure in the developing brain, in typically developing children as well as ADHD. WM microstructure develops throughout adolescence and continues to develop in young adulthood [Lebel and Beaulieu, 2011] and adolescence is a critical period for the development of nicotine dependence [Duncan et al., 2012]. Additionally, adolescents and young adults with ADHD are at increased risk of smoking [Groenman et al., 2013]. In schizophrenia, it was shown that both smoking and schizophrenia had independent, but also additive effects on WM microstructure [Zhang et al., 2010]. If generalizable, this could indicate that vulnerable WM microstructure, as previously described in ADHD, may be more sensitive to the effects of smoking. Possibly, such an additive effect could play an important role in the inconsistencies in the current DTI literature in ADHD, due to differing percentages of smokers between the samples.

The aim of this study was twofold: (a) investigate the effect of smoking on WM microstructure in adolescence and young adulthood, and (b) test whether this association is different in individuals with ADHD compared to controls. We hypothesized that smoking would have a stronger negative effect on the already fragile WM microstructure of the ADHD brain, compared to controls.

METHODS

Participants

In short, participants were selected from the Neuro-IMAGE cohort (the Dutch follow-up of the IMAGE study [von Rhein et al., in press]; also see www.neuroimage.nl). For this study, inclusion criteria were: age between 14 and 30 years, European Caucasian descent, IQ $\geq$ 70, and no known neurological or genetic disorder. Comorbid psychiatric disorders reported by parents (such as classical autism, Asperger's, or bipolar disorder) were excluded, except for oppositional defiant disorder (ODD), conduct disorder (CD), and pervasive developmental disorder not otherwise specified (PDD-NOS), given their high co-occurrence in ADHD. Complete data (i.e., a DTI scan and information on ADHD and smoking behavior) were available of 186 participants who met inclusion criteria, including 113 ADHD patients and 73 healthy controls, aged between 14 and 24 years (mean age 17.8 years). Participants were divided into three groups: nonsmokers (never smoked; $n = 95$, 50.5% ADHD), irregular smokers (smoked weekly or less during the past 6 months; $n = 41$, 58.5% ADHD), and regular smokers (smoked daily during the past 6 months; $n = 50$, 82.5% ADHD). Previously, we have reported higher rates of nicotine dependence and altered WM microstructure in a partially overlapping sample [Groenman et al., 2013; van Ewijk et al., in press]. For a detailed description of the sample, see previous work [von Rhein et al., in press]. After complete description of the study to the subjects, written informed consent was obtained.

Phenotypic Measures

To determine ADHD diagnoses, all participants were assessed with the Schedule for Affective Disorders and Schizophrenia for School-Age Children—Present and Lifetime Version (K-SADS-PL) [Kaufman et al., 1997] and Conners' ADHD questionnaires from multiple informants. Participants were diagnosed with ADHD when they met full DSM-IV-TR criteria for the disorder. Controls were required to score $\leq$ three symptoms on both ADHD symptom dimensions, to ensure they did not show signs of (subthreshold) ADHD. For a full description of the diagnostic procedures for ADHD, see Supporting Information 1.

Smoking and drug use were assessed using a rating scale version of the self-report of antisocial behavior interview [Loeber et al., 1989]. Participants indicated whether they had ever smoked cigarettes, or used marijuana or other drugs (such as Ecstasy (XTC)) and if so, how often this occurred during the past 6 months.

Additional information was collected to assess subclinical autism spectrum symptoms using the Children's Social Behavior Questionnaire (CBSQ) [de Bildt et al., 2009], the presence of ODD or CD (using the relevant K-SADS-PL modules), and socioeconomic status (SES; reflected by the average of the completed level of education of both parents, recoded into a measure reflecting years of education) [Buis, 2010].

Procedure

This study was part of a comprehensive assessment protocol [von Rhein et al., in press], including a DTI-scan. IQ was estimated by the Vocabulary and Block Design subtests of the Wechsler Intelligence Scale for Children-III (WISC-III) or Wechsler Adult Intelligence Scale-III (WAIS-III; for participants $\geq$17 years). Participants were asked to withhold the use of psychoactive medication for 48 h before measurement. Twelve participants with ADHD (using stimulants, antidepressants, and/or antipsychotics) were not able to comply, resulting in eight participants with a 24-h washout and four participants using medication during measurement (equally distributed between the three smoking groups; $P = 0.90$). All participants were familiarized with the scanning procedure using a mock-

scanner. Data acquisition was carried out at two sites in The Netherlands, and the Dutch local medical ethics committees approved the study. Afterward, participants received a reward of €50 and a copy of their MRI-scan.

Imaging Acquisition and (Pre)Processing

MRI-scanning was carried out on either a 1.5 T Sonata or a 1.5 T Avanto MRI-scanner (Siemens, Erlangen, Germany), using the same Siemens 8-channel head coil. Whole-brain, high-resolution T1-weighted anatomical images were acquired in the sagittal plane (MP-RAGE, 176 slices, acquisition matrix 256×256, voxel size $1 \times 1 \times 1$ mm; TE/TR = 2.95/2,730 ms, TI = 1,000 ms, FA = 7°, GRAPPA-acceleration 2). Eddy-current compensated diffusion-weighted Spin-echo echo planar imaging (SE-EPI) images were collected during one acquisition consisting of five volumes without directional weighting (b-value of zero), followed by 60 volumes with noncollinear gradient directions (60 interleaved slices, matrix 64×64, voxel size $2 \times 2 \times 2.2$ mm, TE/TR = 97/8,500 ms, b-value 1,000 s/mm^2, GRAPPA-acceleration 2).

Preprocessing included eddy-current correction, realignment (using affine transformations and mutual information as a cost function), unwarping image distortions, and correction of motion-induced artifacts, using SPM8 (Wellcome Trust Centre for Neuroimaging) functionality and in-house developed methods. Tensor images were estimated, and FA and MD maps were derived for each participant, which were further processed using FSL's tract-based spatial statistics (TBSS) [Smith et al., 2006] using standard settings with a threshold of FA > 0.3. For a full description of image (pre)processing, see Supporting Information 1.

Data Analysis

Differences in sample characteristics of the three groups were investigated using analysis of variance and χ^2 tests in Statistical Package for the Social Sciences (SPSS) (version 21, IBM, Chicago, IL). The main TBSS analysis was set up to investigate the effect of smoking on WM microstructure. A general linear model was built with smoking (regular, irregular, or never) and ADHD (yes or no) as predictors, while correcting for age, gender, and scan site. A smoking by ADHD interaction was added to investigate whether the effect of smoking differed between individuals with ADHD and controls. Voxelwise analyses were performed on FA and MD in FSL, using the randomize tool (based on permutation testing). Results were obtained using threshold-free cluster enhancement [Smith and Nichols, 2009], providing results at $P < 0.05$ following familywise error correction for multiple testing. Anatomical labels of voxels showing significant effects were identified using the built-in Juelich Histological Atlas (JHU) atlases for WM tracts in FSLview.

Subsequently, several follow-up analyses were conducted in SPSS. Second, in regions in which regular smoking was associated with WM microstructure, we tested whether the following confounders had influenced our main effect: IQ, SES, ODD/CD, and the use of marijuana and other drugs. Third, to ensure that our main effect was not confounded by the broad age range of our sample, we tested (a) whether our main effect interacted with age, and (b) whether the main effect remained significant in two different age groups: adolescents (<19 years) and young adults (≥19 years). Fourth, we tested whether the effect of smoking was dependent on age of first cigarette. For a detailed description of follow-up analyses, see Supporting Information 1.

RESULTS

Sample characteristics are summarized in Table I and Supporting Information Table S1. The main TBSS analysis revealed higher FA and lower MD for regular smokers compared to irregular smokers and nonsmokers in structures and WM tracts that are mainly located in and around the basal ganglia (Fig. 1). More specifically, regular smoking compared to nonsmoking was associated with higher FA and lower MD in the thalamus, internal and external capsule, corpus callosum, anterior corona radiata, SLF, and corticospinal tract. These findings were bilateral for FA (except for the SLF, for which effects were located in the right hemisphere) and mostly unilateral (right hemisphere) for MD. However, with more liberal thresholds, MD findings also extended to the left hemisphere. Regular smoking compared to irregular smoking showed an almost identical pattern, although slightly less widespread. With a more liberal threshold, these effects became more widespread, largely overlapping with findings of regular smokers versus nonsmokers.

The TBSS analysis revealed lower FA for ADHD compared to controls (Fig. 2), very similar to previous findings in a partly overlapping sample [van Ewijk et al., in press]. In contrast to our previous findings, no group differences were found for MD. This appeared to be the result of reduced power in the current study due to a smaller sample size, as exploratory analysis with a more liberal threshold did reveal the same regions of higher MD for ADHD as in the previous study (results not shown). No significant interaction was found between smoking and ADHD. Given the relatively low number of regularly smoking controls ($n = 9$), we conducted exploratory analyses to explore possible subthreshold interaction effects. The interaction was tested at the more liberal threshold of $p < .10$, which showed no subthreshold interaction between smoking and ADHD.

Additionally, the main findings were robust to the influence of possible confounders (IQ, SES, ODD/CD, and marijuana and other drugs use), did not interact with age ($P = 0.906$), and were present in both adolescents (<19 years) and young adults (>19 years; both $P = 0.001$). Furthermore, the association between regular smoking and WM microstructure was not dependent on age of first cigarette ($P = 0.940$ for FA and $P = 0.321$ for MD). More

TABLE I. Sample characteristics

	Nonsmoker (n = 95)	Irregular smoker (n = 41)	Regular smoker (n = 50)	Test statistic	Post hoc comparisons
Age (M, SD)	16.7 (2.2)	19.1 (2.5)	18.8 (2.0)	$F_{1,183}=22.6$***	NS < IS = RS
Gender (N, % male)	59 (62.1%)	23 (56.1%)	39 (78.0%)	$\chi^2(2)=5.5$	—
IQ (M, SD)	102.9 (15.3)	99.1 (14.3)	94.9 (15.3)	$F_{1,183}=4.9$**	NS > RS
Scan site (N, % Amsterdam)	46 (48.4%)	25 (61.0%)	25 (50.0%)	$\chi^2(2)=1.9$	—
ADHD status (N, % diagnosed)	48 (50.5%)	24 (58.5%)	41 (82.0%)	$\chi^2(2)=13.7$***	NS = IS < RS
SES (M, SD)	12.6 (2.7)	12.1 (2.3)	11.3 (2.0)	$F_{1,180}=4.1$*	NS > RS
ODD/CD (N, %)	11 (11.6%)	6 (15.0%)	14 (28.0%)	$\chi^2(2)=6.4$*	NS < IS = RS
PDD-NOS (N, %)	5 (5.3%)	1 (2.4%)	2 (4.0%)	$\chi^2(2)=0.6$	—
Autism spectrum symptom score (M, SD)	7.9 (8.7)	6.1 (7.5)	8.8 (7.6)	$F_{1,181}=1.2$	—
Age of first cigarette	—	15.1 (1.6)	13.5 (2.1)	$F_{1,86}=16.3$***	IS > RS
Marijuana users (N, %)	0 (0.0%)	16 (39.0%)	38 (76.0%)	$\chi^2(1)= 93.675$***	IS < RS
Other drug users (N, %)	1.1%	12.2%	16.0%	$\chi^2(2)=12.2$**	NS < IS = RS

ADHD, attention deficit/hyperactivity disorder; CD, conduct disorder; IS, irregular smoker; NC, normal controls; NS, nonsmoker; ODD, oppositional defiant disorder; RS, regular smoker; SES, socioeconomic status
Significance levels: *$P < 0.05$ **$P < 0.01$ ***$P < 0.001$

details on follow-up analyses are provided in Supporting Information 1.

Effects of smoking and ADHD on FA were found in partly overlapping regions (overlap was ~10%), including the corpus callosum, bilateral internal capsule, corticospinal tract, right external capsule, SLF, and anterior corona radiata, as shown in Figure 3. This overlap indicates that FA in these regions was associated with smoking as well as ADHD, which could signify an additive effect, although effects were in opposite directions (Fig. 2).

DISCUSSION

In this study, we investigated the association between smoking and WM microstructure, and whether this association was different in individuals with ADHD. We hypothesized that smoking would have a stronger negative effect on the already fragile WM microstructure of the ADHD brain, relative to controls. Although several regions were found in which WM microstructure was associated with ADHD as well as smoking, we found no interaction between the effects of ADHD and smoking and both effects were in opposite directions. Hence, smoking and ADHD appear to have independent effects on WM microstructure, and possibly do not share underlying mechanisms.

Regular smoking was associated with higher FA and lower MD compared to irregular smoking and nonsmoking, mainly in the corpus callosum and WM tracts located in and around the basal ganglia (Fig. 1). More specifically, regions included the thalamus, internal and external capsule, corpus callosum, anterior corona radiata, SLF, and corticospinal tract, and were mostly bilateral.

Our finding of higher FA in smokers concurs with previous studies [Paul et al., 2008; Zhi et al., 2012], although some other studies found lower FA [Lin et al., 2013; Umene-Nakano et al., 2014]. These differences appear to be related to participant characteristics, where higher FA has been reported in young adulthood and low frequent smokers, and lower FA is reported in chronic smokers and adults. A novel discovery is our finding of lower MD in regular smokers. Only one study reported MD differences between adult smokers and nonsmokers, and found higher MD in smokers [Gons et al., 2011], but their sample was much older (mean age 65.6 years), possibly explaining the difference in direction.

Two explanations of the association between higher FA/lower MD and smoking are possible. First, smoking may cause changes in WM microstructure, represented by higher FA and lower MD. This finding could be related to the neurogenic properties of nicotine. For example, nicotine is thought to upregulate nerve growth factor, and may be neuroprotective. Also, there is evidence that nicotine exposure increases glial cell density [Liu et al., 2005] and number [Bruijnzeel et al., 2011], possibly through the induced mRNA expression of glial cell line-derived neurotropic factor [Takarada et al., 2012]. Glial cells are important in myelin development and the development of WM tracts. Hence, glial cell number and density can have a direct effect on the extracellular space and consequently FA and MD. This hypothesis is supported by the finding that acute nicotine administration causes higher FA [Kochunov et al., 2013]. However, in more chronic phases of smoking, it is likely that smoking-induced blood desaturation causes damage to WM [Borson et al., 2008]. Thus, the higher FA found in our adolescent and young adult sample could, with future progressive smoking, change into lower FA at adult age. Our results showed no difference between irregular smokers and nonsmokers, which could signify a dose–response effect, indicating that only

Figure 1.

TBSS results for smoking. Figures show higher fractional anisotropy (FA) and lower mean diffusivity (MD) for regular smokers compared to nonsmokers (panel **A**, **B**) and compared to irregular smokers (panel **C**, **D**). Results are overlaid on a standard MNI152 template, and for visualization purposes, skeletonized results were "thickened" toward the full width of the tract (using the FSL tool tbss_fill). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

higher or more regular doses of smoking would cause this effect. However, our adolescent and young adult sample had not yet been smoking for a long time (regular smokers $M = 5.3$ and irregular smokers, $M = 3.9$ years since their first cigarette) and it is possible that continued irregular smoking would have an effect on WM microstructure. Taken together, it is possible that on a neurobiological level, regular nicotine intake leads to higher FA through upregulation of nerve growth factor and increase of number of glial cells and density, which could have a neuroprotective effect on WM tracts during adolescence and young adulthood [also

suggested by Paul et al., 2008; Zhi et al., 2012]. It is important to note that the interpretation of differences in FA and MD remains speculative. While FA and MD are overall sensitive to a wide range of tissue properties, neither one is sensitive to one specific tissue property [Jones et al., 2013]. Based on the current data, it is not possible to interpret findings in terms of neurobiological properties, or to determine whether higher FA indicates a positive (e.g., neuroprotective) or negative (harmful) effect.

A second explanation is that higher FA and lower MD could be premorbid to smoking, and perhaps reflect risk
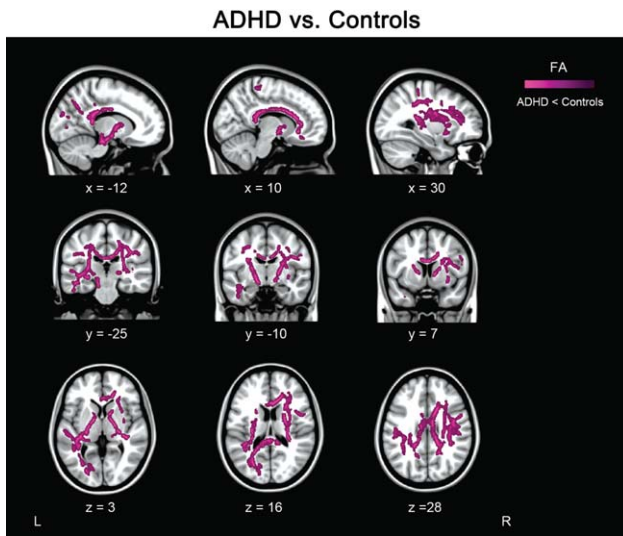
ADHD vs. Controls

Figure 2.

TBSS results for ADHD. Purple colors represent regions of decreased fractional anisotropy (FA) for ADHD compared to controls. No differences in mean diffusivity (MD) were found. Results are overlaid on a standard MNI152 template, and for visualization purposes, skeletonized results were "thickened" toward the full width of the tract (using the FSL tool tbss_fill). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

factors for developing a smoking addiction. Importantly, we found smoking-related alterations in WM microstructure in regions surrounding the striatum (also see Supporting Information Fig. 1), structures crucially involved in the pathogenesis of addictions [Bobzean et al., 2014]. Nicotinic acetylcholine receptors, the main binding site for nicotine, are available in the same regions [Ding et al., 2004]. Furthermore, longitudinal functional and structural imaging studies have shown striatal microstructure and functional activity to be predictive of later risk-taking [Heitzeg et al., 2010] and substance use [Heitzeg et al., 2010; Stice et al., 2013]. Given that we found no difference between irregular smokers and nonsmokers, pre-existing alterations in WM microstructure possibly reflect a risk factor for smoking addiction, and not necessarily a consequence of smoking. Future prospective studies are needed to provide more insight in whether pre-existing WM alterations are generalizable to an addictive personality, or are specific to smoking addiction.

The effects of smoking and ADHD were located in partly overlapping areas, but in opposite directions (i.e., higher vs. lower FA). On the one hand, this could indicate that the associations of ADHD and smoking with WM microstructure lie on opposite ends of one continuum. For example, if altered FA-values are indicative of myelin thickness, ADHD would be associated with less myelin, while smoking is associated with more myelin. However, given that FA is sensitive to a wide range of tissue properties, such as

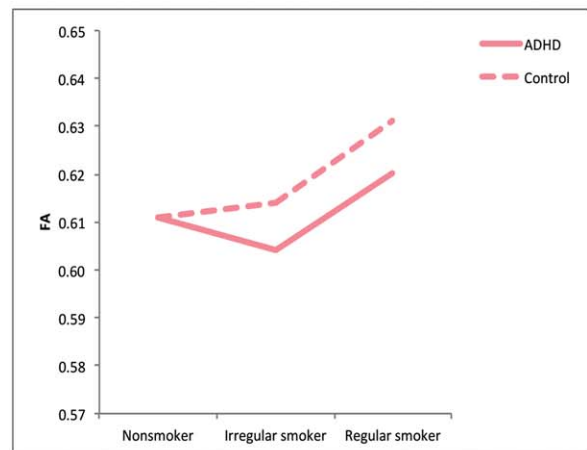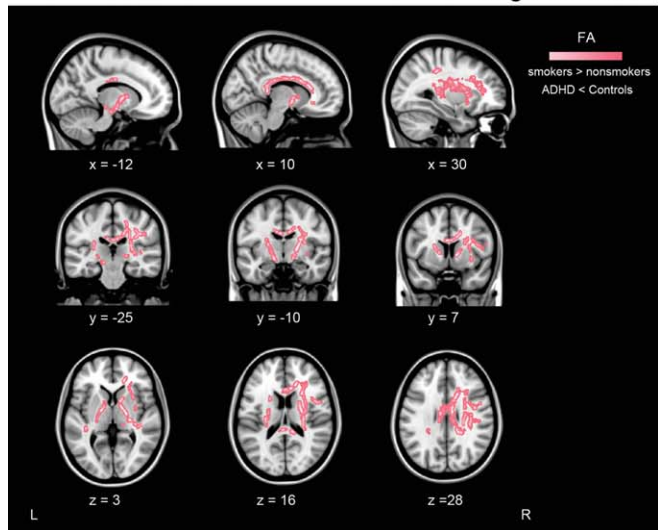## Regular smoking and ADHD (overlapping regions)

Figure 3.

Overlapping regions between the main effects of both TBSS analyses (i.e., regular smoking and ADHD). The left panel shows regions in which regular smoking was associated with higher fractional anisotropy (FA) compared to nonsmokers, and in which ADHD was associated with lower FA compared to healthy controls. No significant interaction between smoking and ADHD was found ($P > 0.10$). Results are overlaid on a standard MNI152 template, and for visualization purposes, skeletonized results were "thickened" toward the full width of the tract (using the FSL tool tbss_fill). The right panel shows mean FA values for each group in these voxels. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

axonal orientation, axonal and cell density, and myelination [Alexander et al., 2007], FA changes associated with smoking and ADHD do not necessarily represent the same underlying tissue property. It is possible, and even probable, ADHD and smoking are associated with WM microstructure through different neurobiological mechanisms. For example, lower FA in ADHD could indicate less myelin, while higher FA in regular smokers could indicate a higher cell density [Beppu et al., 2005].

The opposing effects of ADHD and smoking in overlapping regions have important implications for the interpretation of current DTI literature in ADHD. ADHD is associated with regular smoking [Groenman et al., 2013], but no DTI studies in ADHD have taken smoking into account. Some studies reported higher FA or lower MD in regions associated with ADHD and smoking in our study [e.g., Li et al., 2010; Silk et al., 2009]. It is possible that smoking may have confounded some of the findings in the current DTI literature in ADHD. Consequently, smoking in ADHD should be taken into account in future studies, as it may be a source for inconsistencies between studies.

Our findings should be viewed in light of some strengths and limitations. We are the first to examine the effects of smoking on WM microstructure in adolescence and young adulthood, and the effects of smoking on WM microstructure in ADHD. Another strength is our large sample size, although the number of regularly smoking controls was possibly too small to draw robust conclusions on the absence of an interaction between (regular) smoking and ADHD. We did not have information indicating the exact number of cigarettes smoked by our participants, and no measure of dependency among our regular smoking group, while lifetime tobacco exposure, that is, pack-years [Zhi et al., 2012], as well as dependency [Zhang et al., 2011] may affect the association between smoking and WM microstructure, and are important factors to take into account in future studies. Nevertheless, we found widespread subcortical WM microstructural alterations between regular smokers and nonsmokers. Also, while we had the opportunity to assess possible confounding effects of illicit drug use, we did not have information regarding alcohol use.

Despite these limitations, our study adds significantly to the scarce literature concerning the relation between smoking and the developing brain, by showing that daily smoking is associated with alterations in WM microstructure already in adolescence and young adults. Our results suggest no difference in the relation between smoking and WM microstructure between ADHD and controls, although more research is warranted. Regular smokers in both groups show higher FA and lower MD in several regions, including the corpus callosum and WM tracts surrounding and intersecting structures of the basal ganglia. Furthermore, our results provide a possible explanation for heterogeneity in DTI studies looking at ADHD. The two most likely explanations of our results are possible the following. First, smoking may cause alterations in WM microstructure in the developing brain. Second, pre-existing differences in structural connectivity between striatal areas may predispose to the development of smoking addiction. Longitudinal studies are needed to differentiate between these two possibilities.

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