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Low smoking-exposure, the adolescent brain, and the modulating role of CHRNA5 polymorphisms

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Abstract

Background: Studying the neural consequences of tobacco smoking during adolescence, including those associated with early light use, may inform the mechanisms that underlie the transition from initial use to nicotine dependence in adulthood. However, only a few studies in adolescents have appeared and those available include small samples. In addition, the neural mechanism, if one exists, that links nicotinic receptor genes to smoking behavior in adolescents is still unknown.

Methods: Structural and diffusion tensor magnetic resonance imaging data were acquired from a large sample of 14-year-old adolescents who completed an extensive battery of neuropsychological, clinical, personality and drug use assessments. Additional assessments were conducted at age 16.

Results: Exposure to smoking in adolescents, even at low doses, is linked to volume changes in the ventromedial prefrontal cortex and to altered neuronal connectivity in the corpus callosum. The longitudinal analyses strongly suggest that these effects are not pre-existing conditions in those who progress to smoking. There was a genetic contribution wherein the volume reduction effects were magnified in smokers who were carriers of the high-risk genotype of the alpha 5 nicotinic receptor subunit gene, rs16969968.

Conclusions: These findings give insight into a mechanism involving genes, brain structure and connectivity underlying why some adolescents find nicotine especially reinforcing.

Keywords

Neuroimaging; Low smoking exposure; Genetics; Grey matter volume; fMRI; adolescents

Introduction

Smoking is the leading cause of preventable death in the United States and other developed countries. According to the U.S. Centers for Disease Control and Prevention (www.cdc.gov), smoking causes nearly six million deaths per year globally; current trends predict this will reach eight million by 2030. Using the U.S. as an example, more than five million Americans under 18 today are expected to die from a smoking-related illness, which is higher than the number of deaths caused by HIV, drug misuse, suicide, murder and motor vehicle injuries, combined. Smoking starts primarily during adolescence, with about 90% of US smokers reporting that they tried smoking before the age of 18. Every day in the US alone, 3,800 adolescents smoke their first cigarette and 2,100 become daily smokers. These statistics are particularly troubling given that early use of cigarettes during adolescence has been associated with heightened risk for later dependence (1,2). Remarkably, even relatively

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low rates of cigarette consumption during adolescence (e.g., two to four cigarettes per week) increase the risk of becoming nicotine dependent by early adulthood (1,3).

Adolescence is a period of considerable brain development (4,5) and it has been hypothesized that nicotine use during this critical period produces neurobiological changes that promote tobacco dependence later in life (6). Thus, studying the neural consequences of smoking during adolescence, including those associated with early use, may inform the mechanisms that underlie the transition from initial use to nicotine dependence in adulthood. In contrast to the extensive literature on adult smokers, few studies in adolescents have appeared and those available include small samples.

Nicotine dependence is highly heritable (7) and genome-wide association studies have revealed a reliable association between dependence and single nucleotide polymorphisms (SNPs) at the 15q nicotinic acetylcholine receptor (nAChR) α5-α3-β4 gene cluster (8–10). The most replicated SNPs associated with smoking and lung cancer are the α3 subunit gene (CHRNA3) rs1051730 (11,12) and the α5 subunit gene (CHRNA5) rs16969968 (8), which both convert aspartic acid (G allele) to asparagine (A allele), with A being the risk-allele. However, the neural mechanisms, if any exist, that link these SNPs to smoking are poorly understood. One possible mechanism linking the polymorphism to nicotine dependence is reduced dopamine-mediated reward processing due to A allele nAChR expression (9). For example, Sherva et al. concluded that the A allele was significantly related to enhanced pleasurable responses to a person's first cigarette (10). Hong et al. showed that rs16969968 is associated with a dorsal anterior cingulate–ventral striatal/extended amygdala circuit, in which the risk-allele was associated with decreased intrinsic resting functional connectivity strength in smokers and, to a lesser extent, nonsmokers. Further, the connectivity strength of the circuit distinguished smokers from nonsmokers and predicted addiction severity in smokers (13).

In the present study, we sought to determine how these genetic predispositions might impact the morphometry of the developing adolescent brain and its relationship to smoking. We were specifically interested in the genetic influence of the CHRNA3 SNP rs1051730 and the CHRNA5 SNP rs16969968 on initial cigarette use in order to illuminate possible pathways that lead to heavier use and dependence. Using multimodal neuroimaging in a large cohort of 14 years old adolescents from the IMAGEN study (http://www.imagen-europe.com), we first determined grey matter volume (GMV) differences in both early smokers and nonsmokers followed by the influence of the above two SNPs. Then, we measured white matter connectivity to determine the anatomical structural connectivity that may have supported these genetic polymorphism findings.

We hypothesize, based on previous neuroimaging studies, to find a smoking association with GMV mainly in the prefrontal cortex (14–16), and with white matter connectivity mainly in the corpus callosum (17–19). We also hypothesize, based on previous GWAS findings (8), to observe a small but significant association between CHRNA5 and smoking behavior.

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Materials and Methods

Overview of IMAGEN protocols

Full details of the procedures employed by the IMAGEN study, including details on ethics, recruitment, standardized instructions for administration of the psychometric and cognitive behavioral measures are available in the standard operating procedures for the IMAGEN project (http://www.imagen-europe.com/en/Publications_and_SOP.php).

Participants

Data were acquired from 14-year-old adolescents. After complete description of the study to the participants and their parents/guardians, written informed consent was obtained. Individuals who provided assent completed an extensive battery of neuropsychological, clinical, personality and drug use assessments online and at the testing centers. Additional assessments were conducted at age 16. Participants were excluded if they had contraindications for MRI (metal or electronic implants and claustrophobia) or problematic medical history (e.g., diabetes, tumors, heart defects), neurological conditions (e.g., epilepsy, head trauma, neurodevelopmental disorders such as ADHD, OCD, depression and anxiety) or low IQ (<70). The Wechsler intelligence scale for children was used to measure IQ and was administered by experimenters at the study centers. The vocabulary and similarities subscales were employed to determine verbal IQ. The block design, matrix reasoning and digit span subscales were employed to determine non-verbal/performance IQ. A puberty score was calculated using the Pubertal Development Scale (PDS) scale (20), which consisted of asking adolescents about physical development traits, such as growth in height, body hair, skin changes, and other gender-specific traits (i.e. voice deepening, menstruation). The PDS category scores (answers) are as follows: 1 (No), 2 (Yes (Barely)), 3 (Yes (Definitely)), 4 (Development Completed). Data were normed according to the subject's age.

Smoking score

A cigarette-smoking score was calculated for the 14 year old adolescents from the European School Survey Project on Alcohol and Drugs (ESPAD) (21) questionnaire, which asked: "On how many occasions (if any) during your lifetime have you smoked cigarettes?". The ESPAD category scores are as follows: Score (Lifetime occurrences): 0 (0), 1 (1–2), 2 (3–5), 3 (6–9), 4 (10–19), 5 (20–39), 6 (>40). Follow-up smoking scores at age 16 were also collected. To account for second-hand smoke exposure, parent smoking scores were calculated using the same ESPAD questionnaire. These scores were not significantly different between smokers and non-smokers in the sample (1.6±0.5 vs 1.3±0.8, respectively), with p=0.23. Similar data using the same scoring system were obtained for alcohol use where an alcohol score was calculated from a similar ESPAD questionnaire which asked: "On how many occasions (if any) during your lifetime have you had any alcoholic beverage to drink?".

Different numbers of smokers and non-smokers were available for the neuroimaging and genetic analyses, which is graphically presented in Figure 1 and subject demographics detailed in supplementary Table 1.

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Neuroimaging

MRI acquisition—MRI scanning was performed at the eight IMAGEN assessment sites (London, Nottingham, Dublin, Mannheim, Dresden, Berlin, Hamburg, and Paris) with 3T whole body MRI systems made by several manufacturers (Siemens: 4 sites, Philips: 2 sites, General Electric: 1 site, and Bruker: 1 site). To ensure comparability of MRI data acquired on these different scanners, image-acquisition techniques were implemented using a set of parameters compatible with all scanners that were held constant across sites, for example, those directly affecting image contrast or fMRI preprocessing. The full details of the MRI acquisition protocols and quality checks have been described previously, including the extensive period of standardization across MRI scanners (22).

Structural MRI—High-resolution anatomical MRIs were acquired with a three-dimensional T1 weighted magnetization prepared gradient echo sequence (MPRAGE) based on the ADNI protocol (http://www.loni.ucla.edu/ADNI/Cores/index.shtml).

Diffusion MRI—Diffusion weighted images were acquired with a single-shot EPI sequence with a b value of 1300 s/mm^2, a TE of 104 ms and a voxel size of 2.4×2.4×2.4mm, with 60 slices providing whole-brain coverage.

MRI data preprocessing—Preprocessing of the structural T1-weighted data was performed centrally with the Statistical Parametric Mapping version 8 (Wellcome Department of Neuroimaging, London, United Kingdom, http://www.fil.ion.ucl.ac.uk/spm/software/spm8/), using standard automated pipelines (22). Structural T1-weighted MRI processing included image segmentation into grey matter (GM), white matter and cerebrospinal fluid tissue classes, preceded by an iterative registration to the Montreal Neurological Institute (MNI) template space, using SPM's optimized normalization routine (23). For voxel-based morphometry (VBM), gray matter images were smoothed with a Full Width at Half Maximum (FWHM) Gaussian kernel of 8 mm, warped to standard MNI space and modulated by multiplying the linear and non-linear component of the Jacobian determinants generated during spatial normalization. Thus, the dependent measure in subsequent statistical analyses was absolute grey matter volume (GMV), facilitating comparisons of volumetric, rather than tissue concentration differences (24).

The Diffusion Tensor Imaging (DTI) preprocessing was performed with FSL 4.1 (www.fmrib.ox.ac.uk/fsl). The pipeline consisted of the following steps: First, Eddy-current and motion correction were applied using an affine registration and the B0 volume of the DTI data as reference. After registration, a brain extraction was applied to remove non-brain tissues and a first estimation of the diffusion tensor was achieved for each voxel. After B0 unwarping using the magnitude and phase images from the fieldmap acquisition, a second estimation of the diffusion tensor was achieved for each voxel. Finally, Fractional Anisotropy (FA) maps were generated for each subject.

Genetics

Genotyping and Quality control—DNA purification and genotyping was performed by the Centre National de Génotypage in Paris. DNA was extracted from whole blood samples

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preserved in ethylene-eiamine-tetra-acetic acid vacutainer tubes (BD, Becton, Dickinson and Company, Oxford, United Kingdom) using Gentra Puregene Blood Kit (QIAGEN, Valencia, California) according to the manufacturer's instructions. Genotype information was collected at 582,892 markers using the Illumina HumanHap610 and HumanHap660 Genotyping BeadChips (San Diego, California). The SNPs with call rates of <95%, minor allele frequency < 1%, deviation from the Hardy-Weinberg equilibrium ($p \leq$ 1E-06), and non-autosomal SNPs were excluded.

Imputation of markers data—Markers data imputation and quality control for ambiguous SNPs, low MAF, missingness and HWE were done with MACH (25) following the ENIGMA2 guidelines (26). The 1000 Genomes project reference set of markers (http://www.1000genomes.org/data) was used for the imputation after decreasing the markers from ~41 million to ~13 million relevant genetic variants observed more than once in the European populations (26). Both rs16969968 and rs1051730 data were imputed and had good imputation quality (R^2 imputation quality metric ≥ 0.87) (supplementary Table 2).

Missing demographic data

Participants with missing data on sex and/or site were excluded. Missing values on continuous variables were replaced with the mean derived according to the participant's site and sex. Missing values on nominal data were replaced with the mode of that variable for the participant's site and sex. The maximal missing rate for each variable was lower than 10%.

Statistical Analyses

Single SNP association analysis—Genotype effects of rs16969968 and rs1051730 on smoking levels were examined using single SNP linear regression in the 1417 subjects included in the genetic analysis (described in supplementary Fig 1-c and supplementary Table 1-Genetic analysis). The frequencies of the high-risk, the intermediate risk and the normal (no risk) genotypes for the two SNPs are described in supplementary Table 2. Four multidimensional scaling components were calculated using a metric model to account for population stratification and were included, in addition to age and gender, as covariates in an additive regression model (genotypes coded as 0, 1, and 2 for the number of risk alleles). These analyses were performed using PLINK v1.9 (http://pngu.mgh.harvard.edu/~purcell/plink/). No other SNPs were investigated in this study.

Brain voxel-wise analyses—Whole brain voxel-wise multiple regression analyses were performed on GMV and FA maps to identify regions significantly correlated with the smoking score and test whether effects were observable across the full range of smoking exposure levels (i.e. scores 0–6). These analyses were carried out using the general linear model, performed with the VBM toolbox of SPM8. Age, sex, PDS, handedness, scanner site (dummy coded), Performance IQ, Verbal IQ, socio-economic status and total GMV (only for GMV analyses) were included as nuisance covariates in the design matrix in all analyses. Performance IQ was significantly lower and alcohol use significantly higher in smokers (p's<0.05) and were included in the subsequent ROI-level analyses. The other variables were not different between the two groups (p's>0.1).

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The resulting set of voxel values constituted a Statistical Parametric Map of the *t* statistic (SPM{*t*}). We used 3dClustSim, a cluster correction Monte Carlo procedure available in AFNI (http://afni.nimh.nih.gov/), to determine a minimum cluster size that achieves a corrected significance of $p < 0.05$ with a voxel-wise threshold of $p < 0.001$ and a Full Width at Half Maximum (FWHM) spatial blur that is empirically derived from the spatial autocorrelation in the datasets (residuals from the voxelwise statistical analyses). Clusters with a spatial extent threshold > 411 voxels were considered significantly related to smoking levels.

ROI-level analysis—The mean GMV was extracted with the Marsbar toolbox (27) from the ROI that the VBM regression analysis revealed to be significantly associated with smoking. GMV values were then included as a dependent variable in a 2 (smoking status) by 3 (genotype) ANCOVA model to test the interaction between smoking and genotype on GMV. In this model, subjects were grouped into smokers (score = 1–6) and non-smokers (score = 0) then into the three genotypes (AA, GA and GG). Further, the mean FA was extracted from the ROI obtained from the DTI regression analysis that was significantly associated with smoking and then included as a dependent variable in a similar ANCOVA model to test the interaction between smoking amount and genotype on FA.

Results

Smoking status and CHRNA genotype effects on structural variations in the cortex

First, we studied the neuroanatomical correlates of cigarette use in grey matter density in 211 adolescent smokers from the cohort who had VBM data passing quality control and very low to moderate smoking exposure (smoking scores 1–6), compared with 627 non-smokers (Figure 1–A). A whole brain VBM regression analysis showed a significant negative linear relation between GMV and smoking scores ($r = -0.2$, $p = 5E^{-06}$, df=822) in the ventromedial prefrontal cortex (vmPFC) (cluster size: 499 voxels; cluster peak coordinates: x = 6, y = 30, z = −12) after $p < 0.05$ cluster-wise correction (Figure 2–A).

We next replicated the association of the rs16969968 genotype previously reported in smokers (9) in 940 non-smokers and 477 smokers across all smoking ranges (Figure 1–C) (p=0.003), which explained ~ 3.5% of smoking behavior variance in the sample (Supplementary Table 2). Analyses of the rs1051730 SNP revealed a similar result to that seen for rs16969968, which is not surprising given their strong linkage disequilibrium (D'=1; $R^2 = 0.99$; supplementary Table 2). Assuming that rs16969968 is the functional locus (9), results from rs1051730 are not reported further and subsequent analyses are restricted to rs16969968.

Next, we studied smoking exposure * genotype interaction effects on the lower vmPFC volume derived from the whole brain regression analysis that provided the vmPFC ROI used for subsequent analyses. A 2 (smoking status) by 3 (genotype) ANCOVA indicated that smoking status (all smoking-exposure levels; $p < 0.0005$) and the smoking*genotype interaction ($p = 0.02$) had significant effects on the vmPFC volume (Figure 2–C); there was no significant main effect for genotype in either the whole sample or in non-smokers ($p > 0.09$). Notably, the ANCOVA showed that the vmPFC volume was significantly lower in

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smokers in each of the three genotypes, compared with non-smokers (p = 0.000012), with the largest effect in homozygote carriers of the high-risk alleles (AA genotype; n= 18 smokers and n = 58 non-smokers). Moreover, smoking levels did not differ significantly among the three genotype groups (p=0.1).

Alcohol use and Performance IQ effects in smokers

In our sample, alcohol use and performance IQ were significantly associated with smoking (supplementary Table 1), thus rendering it difficult to attribute the vmPFC anatomical and functional effects to smoking *per se*. To address this, we identified 341 non-smokers and assessed the correlations between alcohol use (alcohol scores ranging from 0–6 reflecting the same ranges of lifetime use as defined for smoking) and performance IQ with the volumetric measures for the vmPFC. The same covariates were included as above. The results yielded no significant associations with alcohol use ($R^2 < 0.0005$, $p > 0.1$, df=326) or performance IQ ($R^2 < 0.01$, $p > 0.05$, df=325) (Figure 3). These results strongly suggest that alcohol use and performance IQ, on their own (i.e., in non-smokers), do not significantly impact the volumetric effects in the vmPFC and thus are unlikely to be the source of the observed genetic effects in smokers.

Smoking status effects on white matter

Finally, we examined the connectivity of the white matter as a function of smoking. Using the same covariates as in the previous analyses (except for total GMV), we performed a whole-brain regression on a sample of 147 smokers and 529 non-smokers for a total of 676 subjects (Figure 1–B). The analysis yielded a significant negative correlation (r=−0.24, $p = 5E^{-06}$, df=661) between FA and smoking scores, in the anterior corpus callosum (359 voxels, x=−15, y=30, z=10) after p corrected<0.05 (Figure 4–A), reflecting altered inter-hemispheric axonal structural properties with smoking and revealing a linear relationship similar to what was observed between the vmPFC volume and smoking. Finally, a 2 X 3 ANCOVA revealed no significant interaction between smoking and genotype on FA extracted from the corpus callosum ROI (p>0.1). While this could be explained by the lack of statistical power due to the loss of a significant number of heavier smokers who did not have DTI data, it is also possible that the nicotinic receptor genetic influence was manifest on GMV but not white matter connectivity.

Discussion

In this study, we have shown that exposure to tobacco smoking in adolescents, even at low doses, is linked to a reduction in vmPFC gray matter volume and altered neuronal connectivity in the corpus callosum. Most notably, the regression analyses indicate linear reductions in vmPFC volumes and neuronal connectivity observable in the very light smoking group of young adolescents. Finally, there was a small yet interesting genetic contribution wherein the vmPFC volume reduction effects were magnified in smokers who were carriers of the high-risk polymorphisms of the alpha 5/alpha 3 nicotinic receptor subunits. The absence of both main effects of genotype and any genotype effects in non-smokers indicates a gene * exposure interaction such that the effects of the polymorphisms are only evident if the adolescent is a smoker.

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Our structural findings are in line with numerous VBM studies reporting negative dose-response correlations between the PFC volume/density in general (14–16) or the vmPFC volume in particular (28) and lifetime cigarette usage in adult heavy smokers. Notably, the present results show this relationship in a group of relatively inexperienced adolescent smokers and suggests the linear decrease is present even at the lightest levels of smoking. These results support a growing literature suggesting that smoking particularly affects the PFC, either from nicotine or one of the more than four thousand chemicals present in tobacco, about 400 of which, including nicotine and CO, are known toxins (29).

Despite the converging evidence of apparent brain atrophy in moderate and heavy adult smokers in the PFC (14–16), the dose-response relationship observed in our data is intriguing as it suggests that just one or two cigarettes can potentially alter adolescent cortex development, an observation that has not been previously reported. An alternative interpretation is that the lower vmPFC GMV preceded and predisposed toward smoking initiation. To address this possibility, we assessed the relationship between vmPFC volume at age 14 and future smoking at age 16 in 627 adolescents who were smoking naïve at age 14, using the same covariates included in the previous regression analyses. Within this sample of adolescents, 386 remained never-smokers at age 16 with the remaining 241 showing a similar use distribution in smoking levels at age 16 as was observed in the previous analysis of smokers at age 14 (see Figure 1). The vmPFC volume derived from the age 14 regression analysis did not predict future smoking at age 16 ($\beta = 0.02$, $p = 0.9$, df=611) (Figure 2–B), which does not support the hypothesis that the observed volume reduction predisposed towards adolescent use. Rather, this finding is consistent with the interpretation that even extremely low smoking exposure by age 14 may influence brain maturation in early adolescence.

The association of the rs16969968 genotype with smoking behavior is consistent with Hong et al (30) who showed that rs16969968 genotype significantly explains 3.3% and 4.6% of the variance of nicotine addiction severity and cigarettes per day, respectively. The gene*exposure vmPFC effect is consistent with a recent meta-analysis (31) of pharmacological neuroimaging studies which revealed that cigarette smoking and CHRNA agonist administration in adult smokers are both associated with lower neural activity in, among other regions, the vmPFC. In line with these findings, another meta-analysis, interrogating the neurobiological targets of pharmacological and cognitive-based treatments for addiction to nicotine, identified similar portions of the vmPFC to have lower activity in smokers (32). Moreover, the rs16969968 * smoking interaction provides evidence that nicotine, rather than the other chemicals in cigarettes, might be the basis of the association between smoking and GMV reductions modulated by the nicotinic acetylcholine receptor system.

The DTI findings are in line with previous data showing that smokers have lower white matter FA in the anterior corpus callosum (17–19), which has been interpreted to indicate possible axonal damage and disrupted myelin integrity in the region (19). Conversely, other studies have reported higher (33) or unchanged FA (34) in the corpus callosum of smokers. This discordance could be explained by the fact that the used sample differed by age, size and/or smoking exposure. Similar to the GMV findings, FA differences were observed even

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in very light smokers. To address the possibility that the reduced FA values, like the reduced vmPFC GMV, preceded smoking, we similarly assessed the relationship between anterior corpus callosum FA at age 14 and future smoking at age 16 in 531 adolescents who were smoking naïve at age 14 (348 never-smokers and 183 smokers), using the same covariates as in the previous FA analyses. The anterior corpus callosum FA, derived from the regression analysis, did not predict future smoking (r=0.02, p=0.89, df=516) (Figure 4–B), which supports the conclusion that the FA reduction did not precede adolescent use but rather that very low smoking exposure appears to alter adolescent brain development.

Since the anterior corpus callosum connects regions of the prefrontal cortex with morphologically similar regions in the opposite hemisphere (35), we next asked if the anterior corpus callosum FA reduction was related to the vmPFC volume reduction of the age 14 smokers. There was a significant positive correlation between the two in smokers and non-smokers (r=0.25, p<0.0001, df=615; Figure 4–C) within 630 adolescents (498 non-smokers and 132 smokers) from the sample used for the whole brain FA regression analysis who also had GMV data. Finally, while the absence of interaction between smoking and genotype on FA could be explained by the lack of statistical power due to the loss of a significant number of heavier smokers who did not have DTI data, it is also possible that the nicotinic receptor genetic influence was manifest on grey matter volume but not white matter connectivity.

The key limitation of the present study is the sample size for the genetic analyses. With 1417 participants on whom genetic data was available (940 non-smokers, 417 smokers), we are at the lower limit of the genetic-association approaches for estimating contributions of common SNPs to phenotypic variations, especially because individual SNPs only explain small amounts of this variance. The limited sample size also affected the significance values in the brain-genotype interaction analysis where we ended up with a small group (N=18) of smokers having the double risk allele. The genetic findings must be therefore interpreted in light of the sample size limitations. Nevertheless, this sample size limitation must be viewed in the context of the phenotype under study. In fact, the IMAGEN dataset is currently the largest longitudinal brain imaging and genetics study in adolescents worldwide, allowing us to detect unique relationships, even if small, between the brain, genetics and smoking behavior.

Combined, this study’s results indicate a structural and functional basis for dose-response changes in the brain of young adolescent smokers, which may underlie, at least in part, the known CHRNA genetic association with smoking. The longitudinal analyses suggest that these effects are not pre-existing conditions in those who progress to smoking, but rather may be, in the case of GMV for example, an initial phase of volume reductions of the PFC in general and the vmPFC in particular that have been observed in adult heavy smokers (14–16,28). Although adolescent experimentation with smoking is common, these results give insight into a mechanism involving genes, brain structure and brain connectivity underlying why some teens find nicotine especially reinforcing and transition to repeated use leading to increased risk of lifetime dependence.

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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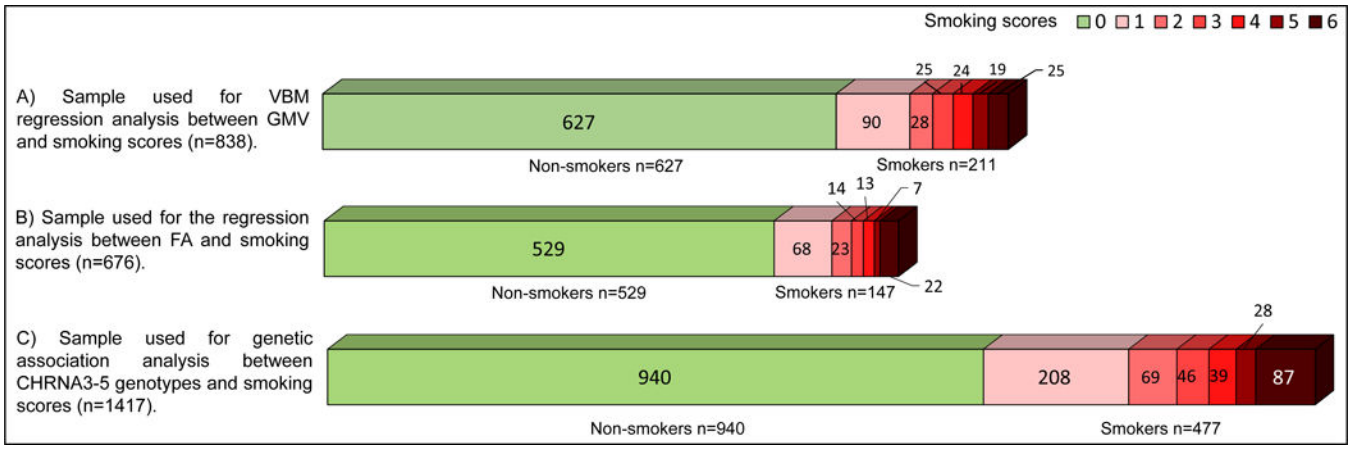

Figure 1.

The different participant groups available from the IMAGEN study and used for the neuroimaging regression analyses (A, B) as well as for the genetic analyses (C). Non-smokers are represented with green bars (smoking score = 0). Smokers included in regression and genetic analyses had very low, low and moderate smoking exposure levels (smoking scores 1 to 6). Numbers on bars represent the total number of participants for each smoking score.

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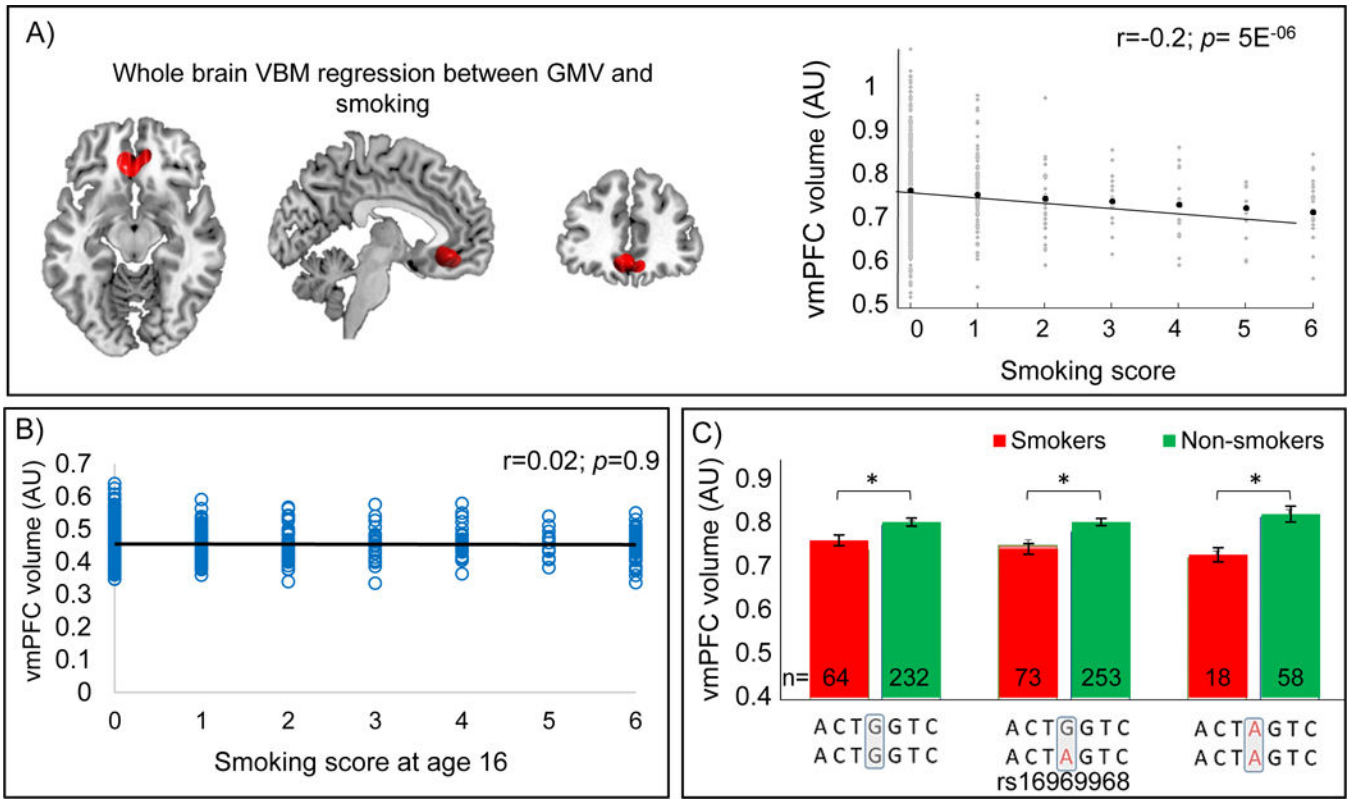

Figure 2.

A) Whole brain rendering of the T maps resulting from the brain voxel-wise regression analysis between GMV and smoking score. A significant negative correlation (initial threshold $p<0.005$; $p<0.05$ when corrected for multiple comparisons) was observed in the ventromedial prefrontal cortex (vmPFC). No positive correlations between GMV and smoking score were detected. B) The relationship between the vmPFC volume and future smoking at age 16 in 627 adolescents who were smoking naïve at age 14. No significant correlation was observed with r=0.02 and $p=0.9$. C) The rs16969968 genotype effects on structural GMV in the vmPFC. A 2×3 ANCOVA indicated that smoking status and the smoking-genotype interaction had significant effects on the vmPFC volume ($p<0.0005$ and $p=0.026$, respectively), where it was significantly decreased in smokers with the effect being largest in the carriers of the smoking-related high-risk genotype (AA). (*): significant difference with $p<0.05$.

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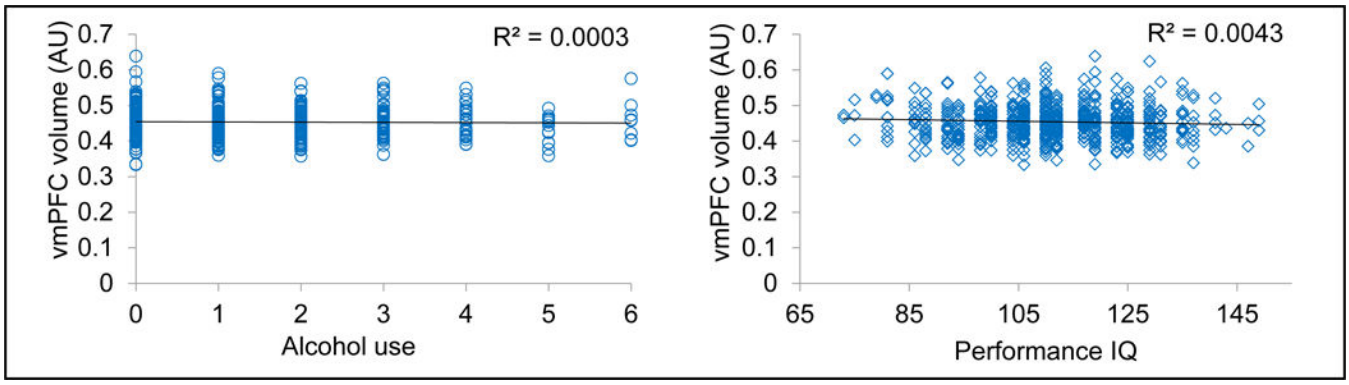

Figure 3.

Alcohol use and performance IQ association with vmPFC volume in non-smokers. No significant correlations were observed in any of the analyses with $R^2 \leq 0.0043$ and $p > 0.1$, suggesting that alcohol use and performance IQ are not correlated with vmPFC volume. vmPFC: ventromedial prefrontal cortex.

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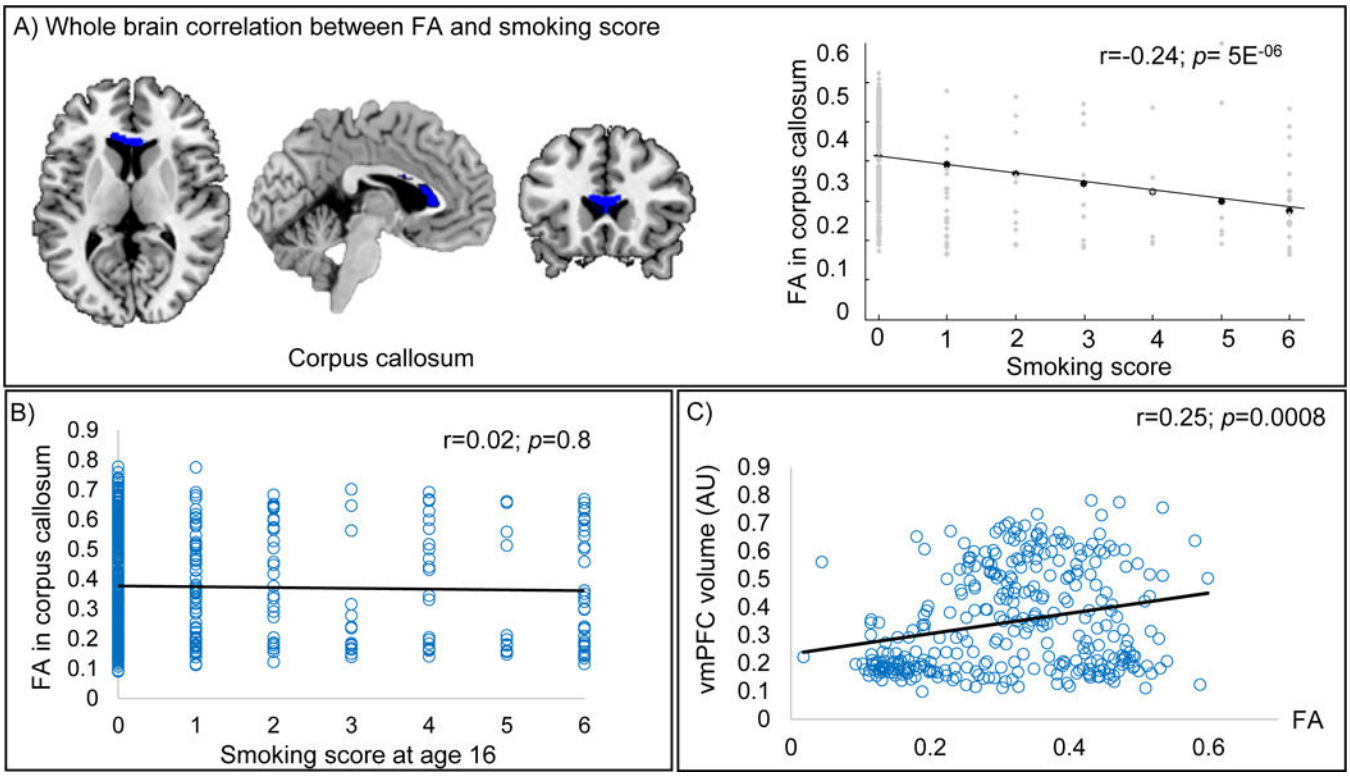

Figure 4.

Smoking status effects on white matter integrity. A) Whole brain rendering of the T map and Pearson's correlation showing significant negative correlation between FA values in the corpus callosum ROI and smoking occasions, with r =−0.24 and p=5E^{-06}. B) The regression between the ventromedial prefrontal cortex (vmPFC) volume and future smoking at age 16 in 531 adolescents who were smoking naïve at age 14 revealed no significant correlation relationship with r=0.02 and p=0.8. C) Pearson's correlation test highlighting the significant positive correlation between FA values in the corpus callosum cluster and the vmPFC volume, with r =0.25 and p=0.00008. (*): significant difference with p<0.05.