Drug and Alcohol Dependence 150 (2015) 183–187

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

Short communication

Are genetic variants for tobacco smoking associated with cannabis involvement?

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ARTICLE INFO

Article history:
Received 12 August 2014
Received in revised form 18 February 2015
Accepted 19 February 2015
Available online 4 March 2015

Keywords:
Cannabis
Tobacco
Chrna5

ABSTRACT

Background: Cannabis users are highly likely to also be tobacco cigarette smokers and a proportion of this comorbidity is attributable to shared genetic influences. Three large meta-analyses of genomewide association studies (GWAS) of tobacco smoking have identified multiple genomewide significant ($p < 5 \times 10^{-8}$) single nucleotide polymorphisms (SNPs). We examine whether these SNPs are associated with tobacco smoking and with cannabis involvement in an independent sample.
Method: Eleven SNPs associated with cigarettes per day (CPD), ever versus never smoking and current smoking/smoking cessation at $p < 5 \times 10^{-8}$ were selected from three published meta-analyses. Association analyses were conducted with similar tobacco smoking measures in 2716 European-American subjects from the Study of Addictions Genes and Environment (SAGE) and with lifetime and current cannabis use and DSM-IV cannabis abuse/dependence.
Results: Cannabis use and tobacco smoking correlated at 0.54. Rs16969968 in CHRNA5 (and its proxy, rs1051730 in CHRNA3) and rs1451240, a proxy for rs13280604 in CHRNB3, were associated with CPD after Bonferroni correction ($p < 0.006$). rs1451240 was also associated with DSM-IV cannabis abuse/dependence. Rs6265 in *BDNF* was associated with smoking initiation, as in the original meta-analysis and also with lifetime cannabis use. Associations with cannabis involvement were no longer significant upon adjustment for the tobacco smoking measures.
Conclusions: The modest associations between cannabis involvement and SNPs for tobacco smoking were not independent of the comorbidity between tobacco and cannabis involvement. Larger samples of individuals might be required to articulate the specific genetic architecture of cannabis involvement.

1. Introduction

Cannabis and tobacco smoking frequently co-occur (Grant et al., 2004). A proportion of this correlation between cannabis involvement and cigarette smoking has been attributed to genetic influences (Agrawal et al., 2012), with genetic correlations ranging from 0.31 in an adolescent sample to 0.82 (in adults) (Huizink et al., 2010; Neale et al., 2006; Young et al., 2006). One twin study also noted that while cannabis and nicotine dependence are influenced by different genetic factors, the correlation between these factors is 0.82 (Kendler et al., 2007).

Despite evidence for genetic overlap, few studies have examined whether genetic variants implicated for one substance also influence the other. Even though cannabis and tobacco have unique pharmacological profiles, it is possible that pleiotropic effects may be responsible for shared aspects of their use and misuse,

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http://dx.doi.org/10.1016/j.drugalcdep.2015.02.029
0376-8716/

such as experience of reward or attenuation of negative mood states. One approach for identifying such genetic variants is to focus on those that have been repeatedly identified in meta-analyses of genomewide association studies (GWAS; Duncan and Keller, 2011). As GWAS simultaneously examines a million or more single nucleotide polymorphisms (SNPs), in these analyses only SNPs with p-values of 5×10^{-8} are considered statistically significant. Consequently, the identification of such SNPs typically necessitates large sample sizes. While there have not been such meta-analyses for cannabis involvement, three large meta-analyses of cigarette smoking have identified such significant SNPs (Liu et al., 2010; Thorgeirsson et al., 2010; Tobacco and Genetics Consortium, 2010). In the present study, we selected 11 genomewide significant SNPs associated with CPD, smoking initiation and smoking cessation/current smoking and examined: (a) whether they were associated with equivalent cigarette smoking measures in European-American subjects from an independent sample, Study of Addictions Genes and Environment (SAGE); and (b) whether the same SNPs were associated with cannabis use, current use and DSM-IV cannabis abuse/dependence in SAGE, especially after accounting for comorbid cigarette smoking.

2. Methods

2.1. Sample

Data were extracted from SAGE, which drew data from three independent studies to create a sample of unrelated DSM-IV alcohol dependent cases and alcohol exposed controls (Bierut et al., 2010). Analyses focused on those of self-reported European-American ancestry who also had genotypic data ($N = 2716$).

2.2. Measures

All three parent studies (Begleiter et al., 1995; Bierut et al., 2007, 2008; Nurnberger et al., 2004) used modified versions of the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) to assess substance use and substance use disorders (Bucholz et al., 1994). The current analyses utilized data on nicotine and cannabis related measures. For *nicotine*, these included smoking initiation (defined as smoking 100 or more cigarettes during the lifetime; while this is also referred to as regular smoking, we use the term "smoking initiation" to be consistent with prior meta-analyses of this phenotype), current tobacco use (currently using tobacco or last using a tobacco product within the year prior to the interview) and cigarettes smoked per day (CPD), defined as a categorical variable (0 = 10 cigarettes or less; 1 = 11–20; 2 = 21–30; 3 = 31 or more). Those who did not report smoking initiation were coded as missing for current smoking and CPD. For *cannabis*, items included ever using cannabis, current use (currently using cannabis or last using cannabis with the year prior to the interview) and DSM-IV cannabis abuse/dependence, with lifetime never users coded as missing for current use and abuse/dependence.

2.3. Genotyping

As described in more detail in a previous publication (Laurie et al., 2010), genotypic data were obtained from the Illumina Human 1M beadchip. For the current analyses, 11 SNPs were selected based on evidence for genomewide significant association in three large meta-analyses (Table 1).

2.4. Data analysis

Analyses were conducted using linear (CPD) and logistic (smoking and cannabis initiation, current use of cigarettes and cannabis, cannabis abuse/dependence) regression models in PLINK (Purcell et al., 2007), with genotype coded log-additively (0, 1, 2 copies of the minor allele). Covariates included sex, age at interview, source of study data and two principal components that accounted for any residual admixture in those of European-American ancestry. Only SNPs associated with a specific tobacco smoking phenotype in the original meta-analyses were tested against that phenotype (e.g., rs6265 was only tested for smoking initiation). Likewise, only SNPs associated with any tobacco smoking measure were further tested with cannabis involvement measures.

3. Results

The mean age of the sample was 38.7 [range 18–77 years]. Ever smoking even one cigarette ($N = 2447$) and ever using cannabis ($N = 2017$) was reported by 90.1% and 74.2% of the sample, respectively. Of those who had ever smoked, 84.5% reported smoking 100 or more cigarettes (smoking initiation; $N = 2067$). Current smokers ($N = 1391$) and cannabis users ($N = 622$) comprised 67.3% and 30.1% of those who had smoked 100 or more cigarettes and ever used cannabis, respectively. Of those who had ever used cannabis ($N = 2017$), 822 met criteria for DSM-IV cannabis abuse/dependence. In addition, for tobacco smoking, of those who had smoked 100 or more cigarettes, 1030, 530 and 223 individuals reported smoking ≥10, 11–20 and 21–30 CPD, respectively, with the remainder reporting smoking >30 cigarettes. Of those who had initiated smoking ($N = 2067$), 39.4% were nicotine dependent by the Fagerström Test for Nicotine Dependence criteria (≥4 symptoms).

Among those who had ever used cannabis, 96.7% ever smoked cigarettes and 90.6% reported smoking initiation ($r = 0.67$). Of those who reported current cannabis use, 85% were also current tobacco users ($r = 0.56$). CPD scores ($r = 0.47$) and FTND ($r = 0.54$) were also correlated with DSM-IV cannabis abuse/dependence.

Table 2 presents association results for the tobacco smoking and cannabis involvement measures. After correction for multiple testing, the data confirmed previously reported associations between rs6265 (*BDNF*) and smoking initiation and between rs16969968 (*CHRNA5*), rs1051730 (*CHRNA3*) and rs1451240 (*CHRNB3*) with CPD. For cannabis, rs6265 was associated with lifetime cannabis use while rs1451240 was associated with DSM-IV cannabis abuse/dependence.

To examine whether the associations for cannabis involvement were attributable to the comorbidity between smoking initiation and ever using cannabis (for rs6265) and between nicotine dependence (FTND; see Rice et al., 2012) and DSM-IV cannabis abuse/dependence (for rs1451240), analyses were repeated while including the tobacco measures as covariates. In both instances, while effect sizes remained consistent (odds-ratios for rs6265 and rs1451240 were 0.85 and 0.88 respectively), the p-values were no longer significant (p-value of 0.07 and 0.15, respectively). Regardless of adjustment for tobacco smoking, rs6265 (adjusted $R^2 = 0.003$) and rs1451240 (adjusted $R^2 = 0.004$) explained only modest proportions of variance in cannabis use and abuse/dependence respectively.

4. Discussion

These analyses suggest that the association between cannabis involvement and SNPs previously associated with tobacco smoking in three large meta-analyses is modest and, quite likely, not specific to cannabis involvement. Additionally, while the association between rs16969968/rs1051730 and tobacco smoking is well-replicated (Bierut, 2011), these SNPs were not associated with cannabis involvement (Culverhouse et al., 2014).

In contrast, our own prior study (Rice et al., 2012) has found support for the association between rs1451240 and FTND. This SNP is a proxy for rs13280604 in *CHRNB3* which was associated at genomewide significant levels ($p = 2.4 \times 10^{-8}$) with CPD in one prior meta-analysis (Thorgeirsson et al., 2010) and has been implicated in studies of nicotine dependence (Hoft et al., 2009b; Saccone et al., 2007) as well as subjective reactions to nicotine (Ehringer et al., 2010; Zeiger et al., 2008). The present study finds support for a similar protective association for the same allele of this variant and cannabis abuse/dependence. Being intronic, the functional significance of this SNP is unknown. This SNP has also been linked to alcohol consumption (Hoft et al., 2009a) indicating its putatively pleiotropic role across a host of substance-related behaviors.

Similarly, rs6265 in *BDNF*, a missense SNP (Val66Met), was associated with smoking initiation and with lifetime cannabis use. In

Table 1
Genomewide significant single nucleotide polymorphisms (SNPs) from three meta-analyses of cigarette smoking.

SNP	Chr; gene	Phenotype; reference allele	*P*-value	Proxy required
TAG Consortium				
rs1051730[*,#]	15; CHRNA3	CPD; G	2.8×10^{-73}; 2.4×10^{-69}	
rs16969968[*]	15; CHRNA5	CPD; G	5.57×10^{-72}	
rs3733829	19; EGLN2	CPD; G	1.04×10^{-8}	
rs1028936	10; *LOC100188947*	CPD; C	1.29×10^{-9}	
rs4933206	10; *LOC100188947*	CPD; A	5.67×10^{-10}	Proxy for rs1329650 (T); $r^2 = 1.0$
rs6265	11; BDNF	Smoking initiation; T	1.8×10^{-8}	
rs3025316	9; near DBH	Smoking cessation/Current smoking; T	3.56×10^{-8}	Proxy for rs3025343 (G); $r^2 = 0.93$;
Thorgeirsson et al.				
rs1051730	15; CHRNA3	CPD; A	2.4×10^{-69}	
rs1451240	8; intergenic	CPD; A	1.3×10^{-8}	Proxy for rs13280604 (A) in CHRNB3; $r^2 = 1.0$
rs8102683	19; intergenic	CPD; C	2.2×10^{-12}	Proxy for rs4105144 (C); $r^2 = 0.87$
rs7937	19; RAB4B	CPD; T	2.4×10^{-9}	
rs215605	7; PDE1 C	CPD; G	5.4×10^{-9}	

No proxy found for rs6474412 ($p = 1.4 \times 10^{-8}$); $r^2 = 1.0$ with rs13280604.
[*] SNPs in high LD, $r^2 = 1.0$.
[#] Also identified by Liu et al.

the TAG meta-analysis, rs6265 was one of several genomewide significant SNPs associated with smoking initiation, however, its genomewide significance level was achieved only after sample sizes exceeded 143,000. In this context, the significant *p*-value in our sample served as replication. Carriers of the A (Met) allele were less likely to smoke 100 or more cigarettes during their lifetime, which is consistent with the meta-analysis which reported that the alternate G (or C) allele was associated with a 6% increase in relative risk for smoking initiation. This SNP has been implicated in a wide array of psychopathology, most notably affective disorders, with meta-analyses suggesting that the Met allele is associated with increased risk for major depressive disorder (Verhagen et al., 2010), schizophrenia and eating disorders but decreased risk for substance use disorders. We see a similar protective effect of the Met allele on cannabis use in this study (Table 2) and this decreased risk is consistent with neurobiological models of stress response that implicate this variant in blunted dopamine activity during reward processing (Pecina et al., 2014).

It is also possible that the effect of rs6265 reflects Mendelian randomization, in which the association between genotype and an outcome (i.e., cannabis use) relates to individuals being "randomized" at birth to a higher likelihood of environmental exposure (i.e., smoking initiation; Smith and Ebrahim, 2003). If carriers of the G (Val) allele of rs6265 are more likely to initiate cigarette smoking, and cigarette smoking, in turn, increases the likelihood of cannabis initiation via environmental pathways, then the association between rs6265 and cannabis use may reflect an environmental process rather than evidence for genetic overlap. Due to the rather general biological role of rs6265, these processes are difficult to disentangle.

Importantly, for both rs1451240 and rs6265, when smoking initiation and FTND were included as covariates, the associations with the cannabis measures were no longer statistically significant. While this might imply that the associations observed with cannabis involvement are not independent of the associations between these SNPs and tobacco-related outcomes, it is worth

Table 2
Association between previously identified genomewide significant SNPs and tobacco and cannabis phenotypes in European-American subjects from SAGE.

SNP	Reference allele	Smoking initiation	Current smoking	CPD	Cannabis use	Current cannabis use	DSM-IV cannabis abuse/dependence
rs1051730[*]	T	–	–	0.100 (.03) *P = 0.001*	1.03 [0.89–1.18]	0.98 [0.84–1.14]	0.91 [0.79–1.05]
rs16969968[*]	A	–	–	0.101 (0.03) *P = 0.0009*	1.03 [0.89–1.18]	0.97 [0.83–1.12]	0.92 [0.79–1.06]
rs3733829	C	–	–	0.067 (0.03)	–	–	–
rs1028936	C	–	–	0.069(.04)	–	–	–
rs4933206	C	–	–	0.046 (.03)	–	–	–
rs6265	A[#]	0.76 [0.63–0.91] *P = 0.003*	–	–	0.82 [0.70–0.97] *P = 0.02*	0.88 [0.73–1.07]	1.03 [0.86–1.24]
rs3025316	C	–	0.94 [0.74–1.19]	–	–	–	–
rs1451240	A[##]	–	–	−0.101 (.04) *P = 0.005*	1.17 [0.99–1.38]	0.83 [0.70–0.99] *P = 0.04*	0.81 [0.68–0.96] *P = 0.01*
rs8102683	T	–	–	−0.063 (.04)	–	–	–
rs7937	T	–	–	0.043 (.03)	–	–	–
rs215605	G	–	–	0.01 (.03)	–	–	–
Corrected *p*-value		0.05	0.05	0.006	.017		

[#] Alternate versus [##] same allele as prior studies of smoking (see Table 1).
[*] Perfect linkage disequilibrium, $r^2 = 1$; treated as one SNP for corrected *p*-value.

noting that the point estimates for and variance explained by each SNP remained relatively unchanged even after accounting for tobacco smoking. Therefore, it is possible that power to detect their specific/independent effects on cannabis involvement was attenuated in this sample that is enriched for nicotine dependence.

One other study has utilized results from TAG to predict cannabis involvement in an independent sample. Vink and colleagues (2014) used polygenic scores created by aggregating SNPs at varying p-value thresholds, up to $p = 0.5$, to predict tobacco, alcohol and cannabis phenotypes in an independent Dutch sample. Such an approach has the advantage of utilizing effects, even those that are not statistically significant, to create a genetic predictor that is more consistent with the polygenic view of complex behavioral traits. The TAG polygenic scores for CPD were associated with CPD ($p = 0.012$ for TAG SNPs with p-value < 0.5) and cannabis use ($p < 0.001$) but the extent to which the most significant SNPs (e.g. rs1051730) contributed to these findings is unclear. Our analyses studied these highly significant individual SNPs that were part of the polygenic scores and while we note a similar degree of association with rs1051730, we do not see any association with other TAG SNPs (e.g. rs3733829). We also extended the association to cannabis abuse/dependence, a phenotype that was not studied by Vink et al. (2014).

It is also worth noting that, for both tobacco smoking and cannabis involvement, SNPs associated with initiation (or lifetime use) were not associated with later stages of CPD or abuse/dependence. Prior twin analyses have suggested substantial overlap across genetic factors influencing initiation and problem use of both tobacco and cannabis (e.g., Gillespie et al., 2009; Neale et al., 2006). Nonetheless, evidence for genetic factors that only influence later stages of tobacco dependence have also been identified (Kendler et al., 1999; Neale et al., 2006) and is consistent with our results and those from the prior smoking meta-analyses (Table 1).

Some limitations of this study are worth noting. First, the evaluations focused only on European American subjects as the meta-analyses were restricted to similar samples. Second, these data did not have a quantitative "CPD-like" measure of cannabis use. Third, as the sample was enriched for alcohol dependence, rates of cigarette smoking and cannabis involvement are higher than those noted in the general population. This enrichment, particularly as one of the contributing samples was ascertained for nicotine dependence (COGEND), may have influenced our ability to detect these associations. Importantly, the associations with cannabis involvement should be viewed as preliminary and pending replication.

In conclusion, while analyses revealed some promising relationships of SNPs previously implicated in tobacco smoking with cannabis measures, these associations did not appear to be independent of their effects on smoking. As large meta-analyses of tobacco smoking have shown, by increasing sample sizes, common variants associated with cannabis involvement can also be successfully identified. Such discoveries will enhance our ability to identify such cross-disorder SNPs with greater precision, as has been witnessed for phenotypes such as schizophrenia, bipolar disorder, major depressive disorder and autism (Lee et al., 2013).

Role of the funding source

Funding sources did not participate in the preparation or writing of this study. R01DA23668 and K02DA32573 (AA); K02DA021237 (LJB); R01DA026911 (NLS); funding support for the Study of Addiction: Genetics and Environment (SAGE) was provided through the NIH Genes, Environment and Health Initiative [GEI] (U01 HG004422). SAGE is one of the genome-wide association studies funded as part of the Gene Environment Association Studies (GENEVA) under GEI. Assistance with phenotype harmonization and genotype cleaning, as well as with general study coordination, was provided by the GENEVA Coordinating Center (U01 HG004446). Assistance with data cleaning was provided by the National Center for Biotechnology Information. Support for collection of datasets and samples was provided by the Collaborative Study on the Genetics of Alcoholism (COGA; U10 AA008401), the Collaborative Genetic Study of Nicotine Dependence (COGEND; P01 CA089392), and the Family Study of Cocaine Dependence (FSCD; R01 DA013423, R01 DA019963). Funding support for genotyping, which was performed at the Johns Hopkins University Center for Inherited Disease Research, was provided by the NIH GEI (U01HG004438), the National Institute on Alcohol Abuse and Alcoholism, the National Institute on Drug Abuse, and the NIH contract "High throughput genotyping for studying the genetic contributions to human disease" (HHSN268200782096C).

Contributors

A.A., M.L. and M.K. conceived of analyses with significant input from H.J.E. and L.J.B.; A.A. and M.K. conducted analyses; H.J.E., A.B., N.S. and J.T. provided critical input on genetic components of analysis; K.K.B., V.H., J.K., N.S., M.S. and L.J.B. provided input on measurement of appropriate phenotypes. A.A. and M.L. prepared the 1st draft; all authors reviewed and approved the final version.

Conflict of interest

Laura J. Bierut and the spouse of Nancy Saccone is listed as an inventor on Issued U.S. Patent 8,080,371, "Markers for Addiction" covering the use of certain SNPs in determining the diagnosis, prognosis, and treatment of addiction.

Acknowledgement

None.

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