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Intermediate cannabis dependence phenotypes and the *FAAH* C385A variant: an exploratory analysis

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Abstract

Rationale—Cannabis dependence is a growing problem among individuals who use marijuana frequently, and genetic differences make some users more liable to progress to dependence. The identification of intermediate phenotypes of cannabis dependence may aid candidate genetic analysis. Promising intermediate phenotypes include craving for marijuana, withdrawal symptoms after abstinence, and sensitivity to its acute effects. A single nucleotide polymorphism (SNP) in the gene encoding for fatty acid amide hydrolase (FAAH) has demonstrated association with substance use disorder diagnoses, but has not been studied with respect to these narrower phenotypes. FAAH is an enzyme that inactivates anandamide, an endogenous agonist for CB₁ receptors (to which Δ⁹-tetrahydrocannabinol binds). CB₁ binding modulates mesocorticolimbic dopamine release, which underlies many facets of addiction.

Objectives—The SNP, *FAAH* C385A (rs324420), was examined to determine whether its variance was associated with changes in craving and withdrawal after marijuana abstinence, craving after cue exposure, or sensitivity to the acute effects of marijuana.

Materials and methods—Forty daily marijuana users abstained for 24 h, were presented with a cue-elicited craving paradigm and smoked a marijuana cigarette in the laboratory.

Results—C385A variance was significantly associated with changes in withdrawal after abstinence, and happiness after smoking marijuana in the predicted directions, was associated with changes in heart rate after smoking in the opposite of the predicted direction, and was not associated with changes in craving or other acute effects.

Conclusions—These data lend support to some previous association studies of C385A, but suggest that further refinement of these intermediate phenotypes is necessary.

Keywords

Marijuana; Cue-elicited craving; Withdrawal; Acute effects; Gene

Introduction

Marijuana is the most commonly used illicit drug (Substance Abuse and Mental Health Services Administration 2007), and the proportion of users who meet *DSM-IV* (American Psychiatric Association 1994) diagnostic criteria for cannabis dependence (CD) is increasing (Compton et al., 2004). However, marijuana users are not equally likely to progress to CD.

Genetic variance exerts a significant influence on the liability for substance use disorders (SUD; e.g., Gynther et al. 1995; van den Bree et al. 1998; Rhee et al. 2003). Yet, candidate gene association studies of SUD have proved difficult to replicate, in part, because they have often attempted to associate a genetic variant with a dichotomous diagnostic variable (see Hutchison et al. 2004). A better approach, and one that is more reflective of the underlying pathology of SUD, is the use of an intermediate phenotype (Gottesman and Gould 2003), whereby a narrowly defined phenotype that is associated with the clinical presentation of SUD and represents an underlying biological mechanism is substituted for the dichotomous variable. However, intermediate phenotypes of CD remain unexplored.

One candidate gene of interest for CD is *FAAH* (alias *FAAH-1*). *FAAH* encodes for fatty acid amide hydrolase (FAAH), an enzyme expressed in the brain and liver that inactivates *N*-arachidonoyl-ethanolamine (anandamide), an endogenous agonist for CB₁ cannabinoid receptors (Cravatt and Lichtman 2003). Delta-9-tetrahydrocannabinol (Δ^9 -THC), the main psychoactive component in marijuana, is also a CB₁ agonist. CB₁ binding increases dopamine transmission along mesocorticolimbic pathways (Gessa et al. 1998; Mascia et al. 1999), the activation of which underlies many facets of addiction (e.g., Robinson and Berridge 1993). Decreases in *FAAH* efficacy may increase sensitivity to anandamide (Cravatt et al. 2001), and thereby alter mesocorticolimbic dopaminergic activity. Alterations in endocannabinoid signaling have been associated with a variety of SUD phenotypes in animal models (Wiskerke et al. 2008).

FAAH knockout mice [*FAAH* (–/–)] display exaggerated responses to anandamide, suggesting that *FAAH* is the primary regulator of anandamide signaling (Cravatt et al. 2001). Although no common human mutation causes *FAAH* deletion, a missense single nucleotide polymorphism (SNP), C385A (rs324420), results in a mutant form of *FAAH* with reduced expression and cellular stability (Chiang et al. 2004). The A allele of this SNP has demonstrated association with street drug use, problem drug and alcohol use (Sipe et al. 2002), and multiple substance dependences (Flanagan et al. 2006). However, one group did not find any association with methamphetamine dependence (Morita et al. 2005), and the C allele has also been associated with increased risk for progression to CD, although this result has not yet been replicated (Tyndale et al. 2007). Our group has reported an association between the C allele and craving after marijuana abstinence (Haughey et al. 2008). To our knowledge, no other studies have examined *FAAH* C385A and intermediate phenotypes of CD.

Three promising intermediate phenotypes are craving, withdrawal, and sensitivity to the acute effects of marijuana. Each is mediated by mesocorticolimbic dopamine transmission, which is modulated by CB₁ binding (Gessa et al. 1998; Mascia et al. 1999). Craving and loss of control over drug-seeking behavior drive end-stage addiction (e.g., Kalivas and Volkow 2005) and cue-elicited craving, initiated by exposure to drug-related cues, is especially potent (e.g., Rohsenow et al. 1990; Payne et al. 1991; Johnson et al. 1998). Marijuana-related imagery scripts (Singleton et al. 2002), visual cues (Wölfling et al. 2008), and tactile cues (Haughey et al. 2008) induce craving in heavy users. Craving is mediated by the repeated pairing of drug cues with activation of mesocorticolimbic dopamine pathways (Robinson and Berridge 1993; Berridge and Robinson 1998). Nearly all frequent marijuana users experience cannabis withdrawal symptoms when they abstain from use (Budney et al. 1999, 2003, 2004), and the experience of these symptoms during abstinence is associated with rapid relapse to dependence (Cornelius et al. 2008). Withdrawal is accompanied by decreased mesocorticolimbic dopamine transmission, an effect mediated by decreased CB₁ binding (Diana et al. 1998). Sensitivity to the acute effects of marijuana is genetically influenced (Lyons et al. 1997), and positive subjective reactions before the age of 16 predict later CD (Fergusson et al. 2003). Marijuana acutely increases heart rate (HR) (Kanakakis et al., 1976), and heightened HR reactivity to other drugs is related to the genetic predisposition to SUD (Conrod et al. 1998). The acute effects of

marijuana are mediated by CB₁-induced increases in mesocorticolimbic dopamine release (Cooper and Haney 2008).

This study investigated whether *FAAH* C385A variance was associated with craving, withdrawal, and sensitivity to the acute effects of marijuana. From the intermediate phenotype perspective, each of these more narrowly defined phenotypes is influenced by CB₁-regulated changes in mesocorticolimbic dopamine transmission, and CB₁ binding by anandamide is affected by changes in *FAAH* efficacy. Frequent marijuana users homozygous for the C allele, were predicted to report more severe withdrawal symptoms after marijuana abstinence, greater craving after abstinence, and after exposure to a marijuana-related cue, and more positive and fewer negative reactions to the acute effects of marijuana than frequent users with at least one copy of the A allele. Additionally, C/C individuals were predicted to demonstrate greater HR increases after smoking marijuana than A/C and A/A individuals.

Materials and methods

Participants

All participants gave their written, informed consent before participating in this study. Participants were 40 daily marijuana smokers (ten females, mean age=20.2) recruited from the Boulder, Colorado area. Thirty-five participants identified as Caucasian (of whom six identified as Hispanic), one as Black, three as Asian, and one as Native American. Individuals were excluded if they were younger than 18 or older than 25, taking psychotropic medication, currently using any substance other than marijuana, attempting to stop smoking marijuana, or currently in substance abuse treatment. Individuals who met *DSM-IV* diagnostic criteria for other SUD (e.g., alcohol dependence) were excluded, as were individuals who reported any conditions that might interfere with smoked marijuana administration. Women were excluded if they were currently pregnant or nursing. Individuals were compensated for their participation. The University of Colorado Human Research Committee approved all procedures.

Procedure

Baseline assessment—Eligible participants attended a baseline session, before which they were instructed to abstain from alcohol and marijuana for 6 h and from caffeine and nicotine for 1 h. After reading and signing an informed consent form, participants gave a saliva sample, for DNA analysis, and Breathalyzer and urine samples, to ensure they had abstained from alcohol, had recently used marijuana, and had not used any other substances. All participants tested positive for Δ^9 -THC metabolites, but negative for metabolites of other drugs. Participants completed the *Marijuana Withdrawal Checklist* (MWC), *Marijuana Craving Questionnaire* (MCQ), and *Profile of Mood States* (POMS; see below for descriptions of these measures), and were then scheduled to return in 24 h. They were instructed to abstain from marijuana and alcohol between the two sessions and from caffeine and nicotine for 1 h prior to the second session and were informed that another toxicology screen would be performed to confirm their abstinence.

Cue exposure—During the second session, after again providing Breathalyzer and urine samples, participants completed the MWC, MCQ, POMS, and the *Biphasic Alcohol Effects Scale* (BAES). Electrodes were placed on the participant's chest and lower back to monitor HR using the BIOPAC 100 system (BIOPAC Systems, Goleta, California, USA), and a baseline HR was recorded. Then, based on their preference, participants were exposed to either a used marijuana bong or pipe while sitting alone at a desk. Via audio tape, they were instructed to focus on the cue, smell it, and imagine what it would be like to smoke marijuana from it. The exposure lasted 2.5 min, after which participants completed the MCQ, POMS, and BAES.

Marijuana administration—After cue exposure, participants received a 1-g marijuana cigarette (3.1% Δ^9 -THC by weight), provided by the National Institute on Drug Abuse and were instructed to smoke it following paced puffing procedures, which produce significant, concentration-dependent increases in psychophysiological and subjective measures (Hart et al. 2001, 2002; Lukas and Orozco 2001). Via audio tape, participants were instructed to “light the cigarette” (30 s), “get ready” (5 s), “inhale” (3 s), “hold smoke in lungs” (5 s), and “exhale.” The interpuff interval was 30 s. Instructions continued until 10 mm of the cigarette remained; participants typically finished the cigarette in 5 m. Participants completed the POMS and BAES 5, 10, 15, 30, and 60 m after smoking, and HR was recorded at these time points.

DNA extraction and genetic analysis—DNA was collected using the Oragene DNA Self-Collection Kit (DNA Genotek, Ottawa, Ontario, Canada). After DNA extraction, *FAAH* C385A was assayed using a commercially available 5'-nuclease (TaqMan) assay and an ABI 7500 thermocycler (Advanced Biotechnologies, Columbia, MD, USA). To confirm genotype, samples for each participant were run in triplicate. Primer sequences were, forward, 5'-TCTGGCTGACTGTGAGACTCA-3' and reverse, 5'-CTCACAGGGACGCCATAGAG-3'.

Baseline measures

The *Timeline Follow-Back* (TLFB; Sobell and Sobell 1992) was used to assess past-month substance use. The TLFB is a calendar-assisted structured interview that provides temporal cues to increase recall accuracy and has demonstrated good test-retest reliability and validity (Sobell et al. 1979).

The *Marijuana Dependence Checklist* was used to assess *DSM-IV* Cannabis Dependence diagnostic criteria. The number of items endorsed is a proxy measure of CD.

Experimental measures

Withdrawal—A short form of the MWC (Budney et al. 1999) was used to assess withdrawal symptoms at baseline and after abstinence. The MWC is sensitive to the effects of marijuana abstinence (Budney et al. 1999) and has demonstrated good inter-item reliability (Budney et al. 2003). The short form used asked participants to rate nine symptoms on a scale from 0 (“none”) to 6 (“severe”).

Craving—The MCQ (Heishman et al. 2001) was used to assess craving at baseline and before and after cue exposure. The MCQ was created from a factor analysis of marijuana craving and has demonstrated good test-retest reliability and validity (Singleton et al. 2002). All analyses used the total score on the MCQ, rather than its subscale scores.

Acute effects—The BAES (Martin et al. 1993) was used to assess sedation and stimulation, and was administered after cue exposure and after marijuana administration. The BAES has been used previously to assess the effects of marijuana and has demonstrated good reliability (Erblich and Earleywine 1995). Sedation and stimulation both increase significantly after marijuana use (Hart et al. 2001, 2002); increases in both states were considered positive reactions.

The POMS (McNair et al. 1971) was used to assess mood after cue exposure and after marijuana administration. The POMS is a reliable and valid measure of affect (Johanson and Uhlenhuth 1980). Its four primary subscales are Happiness, Vigor, Tension, and Depression; increases in Happiness and Vigor were considered positive reactions, and increases in Tension and Depression negative reactions.

Statistical analysis

Genotype frequencies for *FAAH* C385A were consistent with previous reports (see Table 1; expected frequencies are from dbSNP for rs324420). Two subjects could not be genotyped. For each intermediate phenotype tested, A/A individuals were combined with heterozygotes, and this group was compared with C/C individuals. We chose to combine groups in this manner because past reports (Sipe et al. 2002; Flanagan et al. 2006) have suggested that one copy of the A allele is associated with increased risk for substance dependence. Prior to the main analyses, tests were conducted to ensure that genotype groups did not differ on baseline variables (see Table 2).

A series of analyses was conducted to determine whether withdrawal and craving changed after abstinence or cue exposure, whether subjective effects and HR changed after marijuana administration, and whether these changes were associated with *FAAH* C385A variance. The analyses of the effects of abstinence and cue exposure utilized 2 (Trial: Pre- vs. Post-Abstinence or Pre- vs. Post-Cue Exposure) $\times$ 2 (Genotype: *FAAH* C/C vs. A/C and A/A) mixed analyses of variance (ANOVAs), where Trial was a within-subjects factor, and Genotype was a between-subjects factor. The analyses of the effects of marijuana utilized 2 (Trial) $\times$ 2 (Genotype) mixed analyses of variance (ANCOVAs), with baseline scores on the outcome measures co-varied, where Trial was a within-subjects repeated factor, and Genotype was a between-subjects factor. The baseline score co-varied for each outcome measure was the score recorded after cue exposure, to avoid conflating cue-induced effects. Results were deemed significant at $p < 0.05$.

Results

Effects of Abstinence

Analysis of the MWC revealed a significant main effect for trial ($F_{(1, 37)} = 7.95, p < 0.01$), indicating that withdrawal symptom severity increased after abstinence. A 2 (Trial) $\times$ 2 (Genotype) mixed ANOVA revealed a significant Genotype $\times$ Trial interaction ($F_{(1, 37)} = 4.99, p = 0.03$), such that C/C individuals reported more severe withdrawal symptoms than A/A and A/C individuals (see Fig. 1). Because the genotype groups differed with respect to this variable, it was co-varied in all subsequent genotype analyses, to account for the possibility that changes in craving or sensitivity to acute effects were caused by differences in withdrawal symptom severity.

Analysis of the MCQ revealed a significant main effect for trial ($F_{(1, 37)} = 6.74, p = .01$), indicating that craving increased after abstinence. There were no significant genotype interactions. Withdrawal severity predicted changes in craving ($F_{(1, 37)} = 4.07, p = 0.05$), such that individuals who experienced more severe withdrawal symptoms also experienced greater craving.

Effects of Cue Exposure

Analysis of the MCQ revealed a significant main effect for trial ($F_{(1, 37)} = 24.37, p < .0001$), indicating that craving increased after cue exposure. There were no significant genotype interactions. Withdrawal severity did not predict changes in craving after cue exposure.

Effects of marijuana

Analyses of the POMS subscales did not reveal any significant main effects for Happiness, Tension, Vigor, or Depression. However, a 2 (Trial) $\times$ 2 (Genotype) mixed ANCOVA analysis of the Happiness subscale revealed a significant Genotype $\times$ Trial interaction ($F_{(4, 136)} = 2.51, p = .04$). A/A and A/C individuals reported greater happiness than C/C individuals at baseline, a trend that approached significance ($t_{(36)} = -1.89, p = 0.07$). After co-varying this baseline difference, A/A and A/C individuals' happiness decreased after smoking, while C/C

individuals' happiness increased (see Fig. 2). Analyses of the Tension, Vigor, and Depression subscales did not reveal any significant genotype interactions. Withdrawal severity did not predict changes on any POMS subscales after marijuana.

Analysis of the BAES Stimulation and Sedation sub-scales did not reveal any significant main effects or genotype interactions. Withdrawal severity did not predict changes in BAES subscales after marijuana.

Analysis of HR measurements revealed a significant main effect for trial ($F_{(4, 136)}=6.75$, $p<0.0001$). A 2 (Trial) $\times$ 2 (Genotype) mixed ANCOVA with baseline HR included as a covariate indicated that, 15 m after smoking, A/A and A/C individuals had significantly higher HR than C/C individuals ($F_{(1, 37)}=4.36$, $p=0.04$; see Fig. 3). HR did not differ between genotype groups 5, 10, 30, or 60 m after smoking. Withdrawal severity did not predict HR changes after marijuana.

Discussion

Variation at *FAAHC385A* was not associated with most of the phenotypes assessed, including craving and most of the acute effects measures. However, C385A variance was associated with several intermediate phenotypes. As predicted, after marijuana abstinence, C/C individuals reported more severe withdrawal symptoms than A/A and A/C individuals. Furthermore, C/C individuals displayed an increase in happiness after smoking marijuana, compared to A/A and A/C individuals, whose happiness decreased. Together, these results suggest that C/C individuals experience more negative effects when they are abstinent and more positive effects after they smoke marijuana; these differences might make them more liable to progress to CD than A/A and A/C individuals.

We did not predict that A/A and A/C individuals would display higher HR after smoking marijuana than C/C individuals. Although individuals with a genetic predisposition to dependence on other drugs display heightened HR reactivity (Conrod et al. 1998), this result suggests that either the “risk” allele of the SNP is actually the A allele (as Sipe et al. 2002 and Flanagan et al. 2006 found), or that *reduced* HR reactivity to marijuana may be related to risk for CD.

Craving did not differ between genotype groups. Previously, we had found that, after marijuana abstinence, C/C individuals exhibited a greater increase in craving than A/A and A/C individuals (Haughey et al. 2008). It is possible that the current sample was too small to detect a change in this phenotype. Alternatively, the required period of abstinence may not have been long enough to induce changes in craving large enough to reveal this genotype interaction. While participants in our past study abstained for 5 days, we chose to require 24 h of abstinence in order to avoid conflating withdrawal with acute effects, and to maximize the magnitude of these effects. We wanted to ensure participants were “primed” to respond to marijuana, but were not overwhelmed by withdrawal symptoms.

Limitations include sample size and choice of population. The rarity of the minor (A) allele prevented the attainment of a sample large enough to assess the effects of homozygosity. Thus, A/A and A/C individuals were combined into one group. Future studies should examine whether either allele has an additive effect on liability for CD. Additionally, although participants' daily marijuana use allowed us to induce significant increases in withdrawal and craving after only 24 h of abstinence, these phenotypes, as well as sensitivity to the acute effects of marijuana, need to be examined in less frequent users, who may demonstrate different phenotypic association.

These results partially support past findings that the C allele of *FAAH* C385A confers risk for CD (Tyndale et al. 2007; Haughey et al. 2008). However, the lack of association with many of the measures examined and the association between the A allele and HR reactivity necessitate further phenotype refinement. Craving, withdrawal, and sensitivity to the acute effects of marijuana are promising intermediate phenotypes of CD and represent targets of opportunity in the search for candidate genes that may influence this disorder.

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Fig. 1.

Mean severity of withdrawal symptoms reported before and after marijuana abstinence for each genotype group. *Vertical lines* represent 1 SEM. Individuals with the *FAAH C/C* genotype display significantly more severe withdrawal symptoms after abstinence than do *A/C* and *A/A* individuals

Fig. 2. Mean happiness at baseline and mean-adjusted happiness at each post-smoke time point for each genotype group. *Vertical lines* represent 1 SEM. Individuals with the *FAAH C/C* genotype display significantly increased happiness after smoking compared to *A/C* and *A/A* individuals, who display decreased happiness

Fig. 3. Mean heart rate at baseline and mean-adjusted heart rate at each post-smoke time point for each genotype group. *Vertical lines* represent 1 SEM. Individuals with the *FAAH C/C* genotype display significantly lower heart rates than A/C and A/A individuals 15 m after smoking

Table 1

Genotype frequencies for FAAH C385A (rs324420)

	<i>C/C</i>	<i>A/C</i>	<i>A/A</i>
Number of participants	21	12	5
Genotype frequency	0.55	0.32	0.13
Expected genotype frequency (from dbSNP)	0.60	0.35	0.05

The HapMap allelic distribution of rs324420 is: $A=0.225$, $C=0.775$ for those of European descent, $A=0.193$, $C=0.807$ for Asian (Japanese) descent, and $A=0.442$, $C=0.558$ for Sub-Saharan African descent. Because the sample in this study was primarily Caucasian, and because there were no differences in ethnicity between genotype groups, there is little risk of population stratification. See Hutchison et al., 2004, for further discussion of this issue.

Table 2

Pretest differences in FAAH (C385A) C/C versus A/C and A/A participants

Variable ^a	C/C (n=21)	A/C and A/A (n=17)	Test for difference
Gender (% male)	76.2%	70.6%	$\chi^2_{(1)}=0.15$, ns
Race (% Caucasian)	85.7%	64.7%	$p=0.25$ (Fisher's exact test)
Age	21.41 (2.04)	20.88 (1.60)	$t(36)=-0.69$, ns
Marijuana dependence checklist score (Possible range of scale: 0–0)	4.71 (2.55)	4.35 (2.80)	$t(36)=-0.42$, ns
Frequency of past month marijuana use (Possible range of scale: 0–0 days)	27.81 (4.25)	26.71 (5.92)	$t(36)=-0.67$, ns
Quantity of past month marijuana use (No. of joints)	48.38 (33.39)	48.93 (27.48)	$t(36)=0.05$, ns
Frequency of marijuana use per day (No. of times used per day)	3.36 (2.21)	3.94 (2.49)	$t(36)=0.76$, ns
Years of regular marijuana use	3.76 (2.43)	4.00 (2.04)	$t(36)=0.32$, ns

ns not significant

^aSDs appear in parentheses below the means of continuous variables