## **Developmentally Specific Associations Between** *CNR1* **Genotype and Cannabis Use Across Emerging Adulthood**

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**ABSTRACT. Objective:** Previous studies have found preliminary evidence for associations between common single-nucleotide polymorphisms (SNPs) in the cannabinoid receptor gene *CNR1* and cannabis use and dependence. The present study examined a set of eight independent SNPs in or near *CNR1* in relation to cannabis use measured longitudinally across emerging adulthood. **Method:** Using latent growth curve modeling of 10 waves of longitudinal data spanning mean ages 18.4–23.8 years in a sample of non-Hispanic White individuals (n = 334), we tested if genotype at each *CNR1* SNP was associated with both level and growth of cannabis use over time. Peer group drug use, a known correlate of individual use, was evaluated as a time-varying predictor of cannabis use and as a moderator of the relationship between SNPs and individual use. **Results:** After correction for multiple comparisons,

ANNABIS (MARIJUANA) is the most commonly used villicit drug in the United States (Center for Behavioral Health Statistics and Quality, 2016), with emerging adults (i.e., those between 18 and 25 years old; Arnett, 2000) endorsing a higher rate (around 20%) of past-month use than any other demographic segment in the United States (Center for Behavioral Health Statistics and Quality, 2016). A meta-analysis across 28 twin studies (Verweij et al., 2010) indicated that, for males and females, respectively, 48% and 40% of the variation in cannabis use initiation is explained by heritable factors. Longitudinal twin modeling has demonstrated developmental fluctuations in additive genetic effects on cannabis use across adolescence through emerging adulthood (Kendler et al., 2008). Heritability is close to zero from age 14 to 16, then increases up to nearly 40% around age 17 to 18, followed by a decline to near 10% by age 21. After this, heritability increases to 45% by age 25 and finally plateaus at 60% by age 32. Developmental fluctuations in heritability suggest that genetic association studies will profit from using longitudinal measurement, one SNP, rs806374, was significantly associated with individual differences in level—but not growth—of cannabis use over time, such that C carriers were more likely to use cannabis more frequently at study onset (around age 18). Peer drug use was a predictor of individual cannabis use that grew in terms of effect size with time, but did not significantly moderate the effect of rs806374 genotype. **Conclusions:** C carriers at rs806374 may be at specific risk for increased odds of use during the transition out of high school (around age 18). Future studies should investigate potential mechanisms at this developmental stage, including individual differences in subjective response, innate tolerance, reinforcement mechanisms, or general liability for substance misuse. (*J. Stud. Alcohol Drugs, 78,* 686–695, 2017)

as the effects of certain genes may be specific to particular developmental periods.

Initial targets for candidate gene studies of cannabis use (Agrawal & Lynskey, 2009) included the *CNR1* gene, located on chromosome 6q14-q15 (Hoehe et al., 1991), which encodes the G-protein coupled cannabis receptor type 1 (CB1). The CB1 receptor binds a psychoactive constituent of cannabis smoke,  $\Delta^9$ -tetrahydrocannabinol (THC; Pertwee, 1997). The endocannabinoid system has been implicated in an array of processes, including movement, memory, appetite, mood, and pain (Elphick & Egertová, 2001; Porter & Felder, 2001).

A few candidate gene studies have shown that common single-nucleotide polymorphisms (SNPs) within *CNR1* are associated with both clinical diagnoses and cannabis-related behavioral phenotypes. Specifically, rs806368 (Agrawal et al., 2009), rs806380 (Agrawal et al., 2009; Hopfer et al., 2006), and rs1049353 (Hartman et al., 2009) are associated with presentation of cannabis dependence symptoms. In terms of nondiagnostic phenotypes, rs2023239 was shown to interact with cannabis use status to predict lower bilateral hippocampal volume (Schacht et al., 2012), to predict neural response to cannabis cues in the orbitofrontal cortex, inferior frontal gyrus, and anterior cingulate gyrus (Filbey et al., 2010), and is associated with levels of self-reported craving (Haughey et al., 2008).

Importantly, these studies do not conclusively point to a single specific site of variation in *CNR1* (Agrawal et al., 2011, 2014), and to our knowledge, only one SNP (in the *CSMD1* gene) has shown genomewide significance of association with cannabis dependence (Sherva et al., 2016).

Received: June 16, 2016. Revision: February 15, 2017.

This work was conducted at The University of Texas at Austin, with support by National Institute on Alcohol Abuse and Alcoholism (NIAAA) training grant T32-AA007471 (principal investigator: Rueben A. Gonzales), as well as NIAAA R01-AA013967 (principal investigator: Kim Fromme) and R01-AA020637 (principal investigator: Kim Fromme).

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Furthermore, in a large genomewide meta-analysis, no single SNP was significantly associated with lifetime cannabis use (Stringer et al., 2016). Nonetheless, dependence and use per se may be influenced by distinct sets of genes, and no study has examined if SNPs in *CNR1* associate with cannabis use across time, taking into account developmental trajectories of individual use.

Variation in cannabis use is also attributable to social factors, including peer group substance use. Greater perceived peer use is positively associated with personal cannabis use (Jenkins, 1996) and a shorter time lag in the transition from initiation to subsequent use (Hines et al., 2015). Actual use from measured friend dyads also shows associations with personal use (Maxwell, 2002). Whether operating by peer selection or socialization (both of which can also be genetically influenced), peer group behavior is clearly an important factor in cannabis use.

Last, it is plausible that genetic differences and peer group environment do not operate in isolation but instead interact to explain developmentally specific genetic effects on cannabis use. Several studies have reported that genetic effects on substance use phenotypes are potentiated in riskpromoting peer contexts (Agrawal et al., 2010; Harden et al., 2008; Salvatore et al., 2014), although other studies have suggested that peer influences are less important for individuals at high levels of genetic risk (Johnson et al., 2010).

The goals of the current study were (a) to determine if SNPs covering variation across *CNR1* associate with the trajectory of cannabis use across ages 18-24 in a target sample of non-Hispanic White individuals, (b) to test for time-varying main effects of peer group drug use, and (c) to assess Gene × Peer Drug Use interactions across this same time span. We expected that, overall, greater peer group drug use, but that the effect of peer group might differ between genotypes. To our knowledge, these analyses represent the first longitudinal examination of cannabis use, *CNR1*, and peer group drug use.

#### Method

#### Participants

Study participants were from an entering freshman class at a large Southwestern university beginning in 2004, as described previously (Ashenhurst et al., 2015). Of those invited (N = 6,391), about 76% agreed to complete survey data (n = 4,832), and a subset (n = 3,046) were invited to complete a series of surveys beginning at the end of high school and continuing over the following 6 years. Of those invited, about 74% (n = 2,245) provided informed consent and completed the first survey. The majority of these respondents were female (n = 1,345, 59.9%).

TABLE 1. Descriptive statistics of cannabis use across time

Wave	Time point	п	$M_{age}$ , in years	% endorsing use (SD)
1	Summer 2004	2.245	18.4	14.9% (35.6%)
2	Fall 2004	2,077	18.8	17.0% (37.6%)
3	Spring 2005	2,026	19.2	19.6% (39.7%)
4	Fall 2005	1,896	19.8	18.9% (39.2%)
5	Spring 2006	1,790	20.2	20.7% (40.6%)
6	Fall 2006	1,675	20.8	18.6% (38.9%)
7	Spring 2007	1,639	21.2	18.4% (38.8%)
8	Fall 2007	1,539	21.8	17.0% (37.5%)
9	Fall 2008	1,429	22.8	13.8% (34.5%)
10	Fall 2009	1,407	23.8	14.3% (35.0%)

From the full longitudinal sample, a targeted subsample (n = 1,060) was invited to provide salivary DNA. Only a subset of the original sample was invited for the genetic study because of budgetary constraints. Criteria for invitation to the genetic study included permission to re-contact and completion of W1 plus at least one other survey. To date, 601 individuals have provided saliva samples. To minimize any confounding effects of population stratification, the target sample set used in analyses was the largest ancestral segment of the available data, comprising non-Hispanic White individuals. After quality control procedures (see Genotyping Procedures), the final sample size was 334. The longitudinal data used for the present analysis are drawn from 10 assessment waves at the time points provided in Table 1. The university institutional review board approved all study surveys and procedures.

## Measures

Cannabis and peer drug use. One item assessed individual cannabis use, as follows: "During the last 3 months, how many times did you smoke marijuana?" The available responses were 0 (0 times), 1 (1 time), 2 (2 times), 3 (3–5 times), 4 (6–10 times), 5 (11–20 times), and 6 (>20 times). A second question assessed peer group drug use, as follows: "During the last 3 months, how many members of your social group do you think used illegal drugs (e.g., marijuana, stimulants, Ecstasy, etc.)?" Available responses were coded 0 (none), 1 (some), 2 (half), 3 (most), and 4 (all). Both measures were treated as ordinal categorical variables.

#### Genotyping procedures

Saliva was collected in Oragene-Discover (Oragene<sup>TM</sup>, DNAgenotek, Ottawa, Ontario, Canada) kits distributed to participants through the mail. DNA extraction and purification were conducted at the Institute for Behavior Genetics at the University of Colorado Boulder. Samples were standardized to 50 ng DNA/µl for chip genotyping. Purified and diluted samples were sent to the Neuroscience Genomics Core at the University of California, Los Angeles, for SNP

TABLE 2. Target single-nucleotide polymorphisms (SNPs) in CNR1 region

SNP	Minor allele	Major allele	Minor/het/ major <sup>a</sup>	MAF	HW p value
rs10485171	G	А	74/159/108	0.45	.28
rs806365	Т	С	59/165/117	0.42	1.00
rs806374	С	Т	41/157/142	0.35	.91
rs806376	С	Т	77/156/108	0.45	.16
rs6928813	G	А	9/87/244	0.15	.68
rs12205430	С	Т	18/105/218	0.21	.25
rs2180619	G	А	55/159/126	0.40	.73
rs10485170	С	Т	2/60/279	0.09	.75

*Notes:* MAF = minor allele frequency; HW = Hardy–Weinberg. <sup>*a*</sup>Observed allele frequencies of SNPs in the *CNR1* gene in the sample of non-Hispanic Whites. Minor/het/major refers to the number of minor or major allele homozygotes versus the number of heterozygotes. No SNPs violated Hardy–Weinberg equilibrium.

genotyping assay. Samples were run on an Illumina BeadLab platform using an Infinium PsychArray BeadChip (Illumina, Inc., San Diego, CA). Chips were scanned on an Illumina iScan confocal laser, with genotypes called in GenomeStudio (Illumina, v 2011.1, genotyping module v1.9.5). Data were converted to PLINK (v1.90b3v) format for subsequent analyses.

Of the samples sent for DNA extraction (n = 601), a subset yielded insufficient concentrations of DNA for further processing (n = 28) or suffered from poor amplification (n = 8). Furthermore, three randomly selected samples were not assayed in order to run full plates only. Thus, the total sample with available genetic information was 93.5% (n = 562) of the total collection sample.

Next, we followed quality control procedures recommended for the chip-based genomic data (Turner et al., 2011), including sex check, identity by descent, and genotyping efficiency analyses (Purcell et al., 2007), resulting in the removal of 11 samples. Although the target sample self-identified as non-Hispanic White (quality controlled n= 338), we controlled for any additional population stratification (Turner et al., 2011) using principal components extracted within this subsample by EIGENSTRAT (Price et al., 2006). This analysis resulted in the elimination of four individuals as ancestral outliers. The first two principal components were included as covariates in all analyses. After all quality control procedures, the final sample set included in analysis was n = 334.

CNR1 single-nucleotide polymorphism selection. Our goal was to capture the available variation across the gene with coverage both upstream and downstream of the gene. To select from available SNPs, we first pruned the full data set of SNPs that showed strong pairwise linkage disequilibrium (LD) using PLINK (a window of 50 SNPs, a window step size of 5 SNPs, and an  $R^2$  LD threshold of .5). The target region was within the boundaries of the 26kb *CNR1* gene in GRCh37 coordinates plus a window of 10kb both upstream and downstream. Allele frequencies at the resulting eight SNPs and Hardy–Weinberg statistics are present-

ed in Table 2. Haploview (Barrett et al., 2005) identified three SNP pairs with high LD in terms of D' (Figure 1). Nonetheless, given the relatively low LD values in terms of  $R^2$ , the planned analysis was to consider each SNP as independent.

To determine if the eight SNPs selected adequately captured CNR1, we used the Tagger program within Haploview (Barrett et al., 2005) to assess European-ancestral (CEU+TSI) reference panel data downloaded from HapMap (International HapMap Consortium, 2003). Reference data in the target region included 34 SNPs with minor allele frequencies over 5%. The eight SNPs captured 23/34 (67%) available alleles at  $R^2 > .5$  and 16/34 (47%) at  $R^2 > .8$ . Pairwise average  $R^2$  between the eight target SNPs and the 34 known SNPs was .675. Last, we examined the degree to which our SNPs could serve as proxies for SNPs with prior evidence of association (Agrawal et al., 2009; Filbey et al., 2010; Hartman et al., 2009; Haughey et al., 2008; Hopfer et al., 2006; Schacht et al., 2012) using the SNAP web tool (Johnson et al., 2008) and data from the 1000 Genomes CEU reference panel (Abecasis et al., 2012). Table 3 displays the pairwise  $R^2$  and D' values from these reference data for pairs with  $R^2 > .30$ .

For association analyses, all SNPs were coded as 0 = nominor alleles, 1 = minor allele carrier. To account for multiple comparisons, we applied a studywide Bonferroni correction such that the significance threshold was p < .00625.

## Analyses

Latent growth analyses of cannabis use. Growth analyses of cannabis use over assessment Waves 1-10 were conducted in Mplus Version 7.2 (Muthén & Muthén, Los Angeles, CA). Repeated measures of cannabis use were modeled as a function of three latent growth factors: intercept (I), linear slope (S), and quadratic slope (O) (McArdle & Nesselroade, 2003). The intercept provides an estimate of level of cannabis use at the first wave of assessment, whereas the linear and quadratic slopes capture linear growth and acceleration or deceleration in use, respectively. Fit statistics for such models included Akaike Information Criterion (AIC; Akaike, 1987) and Bayesian Information Criterion (BIC; Schwarz, 1978; Sclove, 1987). In separate models for each SNP, these latent I, S, and Q growth factors were regressed on demographic variables (biological sex, principal components) and on CNR1 genotypes.

Influence of peers over time. The next step of the planned analyses was to examine the main effects of peer drug use and SNP × Peer Use interactions for SNPs showing preliminary indication of significant association with use. Peer drug use was entered as a time-varying covariate at every available wave (Waves 1, 3, 5–10). To account for missing peer use data, a secondary variable was coded as 0 = available, 1 = missing peer use data at each wave. Individual cannabis



FIGURE 1. Linkage disequilibrium plot. Calculated linkage disequilibrium values based on observed allele frequencies within a non-Hispanic White sample. Values presented are (A)  $R^2$  and (B) D'. The observed pattern of allele frequencies indicated sufficient independence between SNPs in terms of  $R^2$ , although several SNP pairs showed high LD in terms of D'. Blank boxes indicate D' = 100%.

SNP in present panel <sup>a</sup>	SNP from literature <sup>b</sup>	Key references	Distance (bp)	$R^{2c}$	D′
rs10485170	rs2023239	(Filbey et al., 2010; Haughey			
		et al., 2008; Schacht et al., 2012)	22,170	.36	1.0
rs10485171	rs1049353	(Hartman et al., 2009)	10,245	.36	1.0
rs12205430	rs806380	(Agrawal et al., 2009; Hopfer			
		et al., 2006)	3,272	.46	1.0
rs6928813	rs2023239	(Filbey et al., 2010; Haughey			
		et al., 2008; Schacht et al., 2012)	1,216	1.0	1.0
rs806374	rs806368	(Agrawal et al., 2009)	7,220	.50	.84
rs806376	rs806380	(Agrawal et al., 2009; Hopfer	,		
		et al., 2006)	6,005	.75	.96

TABLE 3. Proxy single-nucleotide polymorphism (SNP) evaluation

*Notes:* bp = base pairs. *a*Proxy-SNP evaluation of SNPs in the current analysis with reference to *b*SNPs previously indicated in the literature. Statistics are calculated from 1000 Genomes reference panel data (Abecasis et al., 2012) for individuals with European White ancestry (CEU panel). *c*Linkage disequilibrium statistics for pairwise comparisons are presented in terms of  $R^2$  and D'. Most pairs show a weak proxy relationship, with strong D' values but most  $R^2$  values falling below .8.

use was regressed on this time-varying missingness variable with regression coefficients constrained to be equal across waves. The percentage of respondents with missing peer data at a given wave ranged from 0.6% to a maximum of 18.6%. To model interactions between genotypes and illicit drug use, we generated time-varying cross-product interaction terms and entered them into the model.

#### Results

We compared the characteristics of those included in this analysis to those who did not provide DNA or were dropped from analysis for quality control issues among non-Hispanic White individuals (total pool of non-Hispanic Whites, n =1,211; non-analysis sample, n = 877). The proportion of females in the analysis sample (64.7%) was significantly greater than in the non-analysis sample (56.8%),  $\chi^2(1) =$ 6.22, p = .013. Endorsement of cannabis use did not significantly differ at each wave (*ps* > .05) except Wave 5,  $\chi^2(1) =$ 4.36, p = .037. Ashenhurst et al. (2016) presented additional results comparing the non-Hispanic White genetic sample to non-Hispanic Whites without genetic data on a variety of other externalizing phenotypes and found that the groups did not differ with regard to alcohol use, property crime, tobacco use, risky sex, or sensation seeking and impulsive personality traits.

## Overall growth model

Our first step was to examine trends in an unconditional growth model of cannabis use. The overall growth pattern, as shown in Figure 2A, was an inverted U shape, peaking at around age 20. Fit parameters were AIC = 4,322.875, BIC = 4,410.532. There was no effect of sex on growth parameters (all ps > .20). Importantly, the standardized correlations between I and S or Q were not significant (ps > .25), indicating independence between initial level of use and growth in

use. We tested if the model could be simplified to a linear rather than quadratic model, but this resulted in a significant decrement in model fit, AIC = 4,357.986, BIC = 4,426.587,  $\chi^2_{\text{diff}}(5) = 53.61, p < .001.$ 

# Growth models influenced by CNR1 single-nucleotide polymorphisms

As a first set of tests, CNR1 genotypes at each SNP were modeled separately as predictors of latent growth factors I, S, and Q, controlling for population stratification and biological sex. After correction for multiple comparisons, one SNP, rs806374 (Table 4; Figure 2B), had a significant effect on the latent intercept factor ( $\beta = .219$ , SE = .080, p = .0061) but not linear slope ( $\beta = -.164$ , SE = .098, p = .094), or quadratic slope ( $\beta = .110$ , SE = .096, p = .25) factors. The main effect of this SNP on level and growth of cannabis use is presented in Figure 2B. Again, there were no effects of biological sex on intercept or growth factors (all ps > .25). We queried this SNP in RegulomeDB (Boyle et al., 2012); rs806374 does not have any clear regulatory significance (not in or near any known region of DNase hypersensitivity, transcription factor binding side, or promoter region).

As a post hoc power analysis, we conducted a series of Monte Carlo simulations (Muthén & Muthén, 2002). Results from the growth model testing the association with rs806374 were used to generate 100 simulated data sets of n = 334, and then the genetic association model was fit to a simulated data set. Ninety-six (96) of 100 replications converged, and the SNP association was nominally significant at p < .05 in 78 replications. Of course, the effect size of the generating model, which was based on the effect size observed in the current study ( $R^2 \sim 4\%$ ), is unrealistically large. As our results were based on a small sample, the observed genetic association with rs806374 should be considered preliminary until directly replicated.



FIGURE 2. Latent growth models of cannabis use. Observed and estimated values for the series of latent growth curve models. Error bars for observed measures are standard error of the mean. (A) Unconditional growth model capturing change in cannabis use over time across not accounting for genotypes or peer group drug use. (B) Observed and estimated values from a model examining the main effect of genotype at rs806374 only. (C) Conditional estimated probabilities for those with either no or most members of their social group using illicit drugs, and the sample mean.

TABLE 4. Single-nucleotide polymorphisms (SNPs) as predictors of growth in cannabis use

SNP	Ι	S	Q
rs10485171	038 [187, .112]	.026 [170, .221]	059 [251, .133]
rs806365	032 [186, .122]	.011 [177, .199]	.006 [181, .193]
rs806374	.219 [.062, .376]	164 [355, .028]	.110 [077, .298]
rs806376	013 [165, .139]	008 [208, .193]	.038 [163, .239]
rs6928813	018 [174, .137]	028 [221, .164]	.047 [140, .234]
rs12205430	002 [160, .155]	070 [264, .124]	.088 [097, .273]
rs2180619	.047 [106, .200]	.182 [027, .391]	097 [310, .115]
rs10485170	014 [180, .151]	025 [211, .161]	.062 [113, .236]

*Notes:* Standardized betas and 95% confidence intervals [95% CI] for each SNP as predictors of latent growth parameters in models evaluating the main effects of SNPs among non-Hispanic Whites. One SNP (**bolded**) showed significant effects on intercept after Bonferroni correction for multiple comparisons (p < .00625).

#### Peer influence over time

There was a significant main effect of peer drug use at all waves. MODEL TEST functions of Mplus indicated that the model could not be simplified by constraining the main effect of peer group drug use to be equal across time, Wald  $\chi^2(7) = 49.23$ , p < .001. Those endorsing a greater proportion of friends who used illicit drugs were more likely to use cannabis themselves (Figure 2C,  $\beta$  range: .139–.559). The main effect of rs806374 genotype on the intercept also remained significant ( $\beta = .232$ , SE = .084, p = .005).

*Peer* × CNR1 *interactions.* To evaluate if genotype at rs806374 moderates the effect of peer group use, we entered a time-varying genotype by peer group cross-product term at each wave. There were no significant interactions beyond the studywise correction factor (all ps > .00625), although interactions at Waves 5 ( $\beta = .103$ , SE = .048, p = .03) and 10 ( $\beta = .206$ , SE = .105, p = .049) were statistically significant without correction.

#### Discussion

The goals of this study were to test for effects of *CNR1* SNPs on individual differences in level and growth in cannabis use across emerging adulthood, as well as time-varying effects of peer group drug use and potential *CNR1* × Peer Group Drug Use interactions. The percentage of young adults using cannabis at least once in the past 3 months increased from 17% at mean age 18.4 up to 26% by mean age 20.2 and decreased thereafter to 18% at mean age 23.8 (Figure 2A). This overall pattern is consistent with U.S. population data showing 22.3% past-month cannabis use among 18- to 25-year-olds (Center for Behavioral Health Statistics and Quality, 2016).

## Developmentally specific genetic effects of CNR1 singlenucleotide polymorphism rs806374

Genotype at one SNP within *CNR1*, rs806374, had a significant main effect on level of use (intercept), but not on

linear or quadratic growth (Figure 2B). Carriers of the minor C-allele showed a higher level of use limited to the early assessment waves. By mean age 20.8, use patterns by geno-type were entirely overlapping. These results converge with evidence from twin studies on the heritability of cannabis use across emerging adulthood, which demonstrate peaks in heritability around age 18, relative to middle adolescence or the early 20s (Kendler et al., 2008). The overall implication is that genetic effects of *CNR1* are most prominent around the time that individuals in the United States are transitioning out of high school, around age 18–19. This finding of developmental specificity could result from a number of possible mechanisms, as multiple personal and social changes are occurring as this point in the life span, and cannabis use at this age represents relatively early use.

This finding also has relevance for genome-wide association studies (GWAS). The recent GWAS of cannabis use by Stringer et al. (2016), for example, used a meta-analytic sample with participants ranging in age from 16 to 87 years; if the strength of genetic associations differs by age, such age-heterogeneity may obscure genetic signals. Consistent with this idea, a GWAS of another psychiatric phenotype, depression, found differing genetic etiologies between adultand adolescent-onset major depressive disorder (Power et al., 2017). In the continuing effort to understand the genetic etiology of cannabis use and cannabis use disorders, longitudinal studies that track cannabis use over development will be particularly valuable.

No other SNPs tested were significant predictors of level or growth in cannabis use. According to proxy-SNP analysis (Johnson et al., 2008) of European-ancestry population (CEU) reference data from 1,000 Genomes (Table 2; Abecasis et al., 2012), rs806374 can serve as a relatively weak proxy-SNP for the nearby SNP rs806368, which has previously been associated with cannabis dependence symptoms (Agrawal et al., 2009). Of note, two available SNPs that could serve as weak proxies for the previously identified SNP rs806360 (Agrawal et al., 2009; Hopfer et al., 2006) were not significant predictors in the overall growth model.

#### Peer illicit drug use

Greater peer group illicit drug use was strongly associated with greater odds of personal cannabis use across the time window examined, as was expected. Over time, the effect size of peer group behavior generally grew in terms of standardized betas, and these estimates could not be constrained to be equal. As such, it appears that peer group drug use becomes a stronger correlate of individual use across emerging adulthood (unstandardized model presented in Figure 2C), perhaps as a consequence of ongoing peer selection or socialization processes. There were no robust interactions between genotype and peer group use at any wave, suggesting that the effect of peers was not different between C carriers versus noncarriers.

#### Mechanisms and future directions

There is limited information about any functional consequences of variation at the intronic SNP, rs806374. Given the role of *CNR1* and the CB1 receptor in numerous systems (Elphick & Egertová, 2001; Hoehe et al., 1991; Pertwee, 1997), there is a broad array of potential explanatory mechanisms. Furthermore, it is unclear if the effect should depend on experience with cannabis or THC, given that the gene encodes a protein that binds the substance (Pertwee, 1997). In other words, SNPs in CNR1 may be more relevant for the transition from initial use to habitual use than for use per se. Nonetheless, both the transition from nonuser to user and that from user to heavy/problem user are heritable, albeit with stronger additive genetic contribution for the latter transition (Agrawal & Lynskey, 2006). The present study did not include questions about lifetime or initiation of use, however, so we are unable to examine differences between ever and never users.

The substance abuse literature provides examples of potential mechanistic endophenotypes (Gottesman & Gould, 2003), as investigated in experimental animals (Belin et al., 2016) and human behavioral and neuroimaging paradigms (Jupp & Dalley, 2014; Müller et al., 2010; Ray et al., 2010). These could include individual differences in (a) subjective response to cannabis in terms of positive or negative affect, (b) reinforcement learning processes, (c) innate or acquired tolerance processes, or (d) disinhibitory or impulsive traits. It is also possible that *CNR1* contributes to a general vulnerability to substance use not specific to cannabis, since much of the genetic vulnerability to substance misuse is shared across substances (Agrawal & Lynskey, 2006; Palmer et al., 2012).

## Strengths and limitations

Our findings must be considered within the unique strengths and limitations of this study. First, the longitudi-

nal assessment of cannabis use was a major strength of the study, as it allowed us to estimate time-varying effects of genetic influences in a person-centered framework. Although any single genetic association study should be considered preliminary until directly replicated, our analytic methods, which combine genetic data with methods for modeling intraindividual growth in cannabis use, illustrate one approach for integrating genetic and developmental methods. In particular, future work can examine the developmental specificity of associations between cannabis use and polygenic scores that use GWAS results (e.g., Sherva et al., 2016; Stringer et al., 2016) to aggregate risk across the genome, rather than focusing on single genes. Such an approach would also help to mitigate the biggest limitation of the current study, which is the modest sample size.

The present target sample was also not representative of the broader U.S. population, being composed of non-Hispanic White individuals who attained admission to a large public university. Although the participants' university is economically diverse, the sample is positively selected on cognitive abilities and personality factors predictive of academic achievement, compared with the nation as a whole. Results may not generalize to other populations, such as young adults who are not in college or who are seeking treatment. Finally, the peer group drug use variable was not specific to cannabis, and lifetime cannabis use information was unavailable, precluding analyses of initiation versus maintenance of use.

#### Conclusions

This study presented the first examination of *CNR1* in the context of longitudinal assessments over late adolescence through emerging adulthood among non-Hispanic White individuals. Our models indicated an effect of one SNP in the *CNR1* gene, rs806374. Minor allele carriers (C carriers) had greater odds of using across the transition from high school to college, around age 18–19, but not thereafter. Overall, peer group drug use was a strong predictor of individual cannabis use that strengthened over time.

#### Acknowledgments

The authors thank Dr. Andrew Smolen, Dr. Alexis Frazier-Wood, Dr. Donna Werling, Emily Wilhite, Elise Marino, Dr. Elliot Tucker-Drob, Frank Mann, Marie Carlson, Andrew Grotzinger, Laura Engelhardt, Stephanie Savicki, and Megan Patterson for their assistance in the development of this work.

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