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Individual and Additive Effects of the *CNR1* and *FAAH* Genes on Brain Response to Marijuana Cues

Francesca M Filbey*[,1,2], Joseph P Schacht[1,3], Ursula S Myers[1], Robert S Chavez[1] and Kent E Hutchison[1,2]

[1]The Mind Research Network, Albuquerque, NM, USA; [2]Department of Psychology, The University of New Mexico, Albuquerque, NM, USA; [3]Departments of Psychology and Neuroscience, University of Colorado, Boulder, CO, USA

As previous work has highlighted the significance of the *cannabinoid receptor 1* (*CNR1)* and *fatty acid amide hydrolase* (*FAAH)* genes with respect to cannabis dependence (CD), this study sought to characterize the neural mechanisms that underlie these genetic effects. To this end, we collected DNA samples and fMRI data using a cue-elicited craving paradigm in thirty-seven 3-day-abstinent regular marijuana users. The participants were grouped according to their genotype on two single-nucleotide polymorphisms (SNPs) earlier associated with CD phenotypes: rs2023239 in *CNR1* and rs324420 in *FAAH*. Between-group comparisons showed that carriers of the *CNR1* rs2023239 G allele had significantly greater activity in reward-related areas of the brain, such as the orbitofrontal cortex (OFC), inferior frontal gyrus (IFG), and anterior cingulate gyrus (ACG), during exposure to marijuana cues, as compared with those with the A/A genotype for this SNP. The *FAAH* group contrasts showed that *FAAH* rs324420 C homozygotes also had greater activation in widespread areas within the reward circuit, specifically in the OFC, ACG, and nucleus accumbens (NAc), as compared with the *FAAH* A-allele carriers. Moreover, there was a positive correlation between neural response in OFC and NAc and the total number of risk alleles (cluster-corrected $p<0.05$). These findings are in accord with earlier reported associations between *CNR1* and *FAAH* and CD intermediate phenotypes, and suggest that the underlying mechanism of these genetic effects may be enhanced neural response in reward areas of the brain in carriers of the *CNR1* G allele and *FAAH* C/C genotype in response to marijuana cues.
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INTRODUCTION

The main psychoactive compound in marijuana, delta-9-tetrahydrocannabinol (Δ^9-THC), binds to central cannabinoid, or CB_1, receptors, in which it mimics the effects of endogenously produced cannabinoids. The administration of CB_1 antagonists in mice results in a decrease in reward behavior in response to cannabinoids and other substances of abuse (Arnone *et al*, 1997; Berrendero *et al*, 2003; Castane *et al*, 2002; Ledent *et al*, 1999), and the administration of the antagonist SR141716A (Rimonabant) extinguishes reward-related behaviors such as conditioned place preference and self-administration suggesting that CB_1 activation modulates these behaviors (Gardner *et al*, 2002). In the first study of cue- and drug-induced reinstatement of cannabinoid-seeking in non-human primates, it was found that continuous administration of rimonabant, but not naltrexone, decreased cue-induced drug seeking, THC-induced drug seeking, and the direct reinforcing effects of THC in squirrel monkeys (Justinova *et al*, 2003, 2008). Moreover, single-cell recordings in the ventral tegmental area (VTA), the origin of dopaminergic cell bodies, have shown that Δ^9-THC increases neuronal firing rates in this area (Cheer *et al*, 2000). More interestingly, increased dopamine (DA) neuronal firing rates are coupled with increased DA neuronal bursts, and these effects are blocked by SR141716A (Diana *et al*, 1998; French *et al*, 1997). These findings suggest that cannabinoids increase DA activity in the NAc and prefrontal cortex (PFC) by activating CB_1 receptors in the VTA, which increase DA neuronal firing and burst rates. In other words, CB_1 receptors increase DA activity by local disinhibitory mechanisms. The gene that encodes for CB_1, cannabinoid receptor 1 (*CNR1*), thus likely modulates endocannabinoid and DA-mediated reward signaling; consequently, it has attracted substantial attention in the search for genetic mediators of liability to substance use disorders (SUD).

Among human drug users, *CNR1* variants have been associated with both SUD phenotypes generally (eg, Ballon *et al*, 2006; Comings *et al*, 1997; Covault *et al*, 2001; Herman *et al*, 2006; Racz *et al*, 2003; Schmidt *et al*, 2002; Zhang *et al*, 2004; Zuo *et al*, 2007, 2009) and cannabis dependence (CD) specifically (Agrawal and Lynskey, 2009; Hopfer *et al*, 2006), although some groups have also reported null findings for this gene (eg, Covault *et al,* 2001; Hartman *et al*, 2009; Li *et al*, 2000*)*. A report by Zhang *et al* (2004) suggest that one variant, a G to A single-nucleotide polymorphism (SNP) in

*Correspondence: Dr FM Filbey, The Mind Research Network, 1101 Yale Boulevard, Albuquerque, NM 87106, USA, Tel: +505 272 8856, Fax: +505 272 8002, E-mail: ffilbey@mrn.org
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the third exon, may create an alternative *CNR1* transcript in the brain, and this SNP is associated with general liability to substance abuse (Zhang *et al*, 2004). Our group has also reported an association between this SNP (rs2023239) and differences in two intermediate CD phenotypes: withdrawal and craving after marijuana abstinence (Haughey *et al*, 2008). Specifically, withdrawal and craving among 105 young adults who reported smoking marijuana daily were assessed at baseline and again after 5 days of abstinence. The G allele of rs2023239 showed a significant abstinence by genotype interaction on withdrawal, as well as a main effect on overall levels of craving. However, results for this SNP have not been uniformly consistent; using a case–control design, Hartman *et al*, 2009 did not find an association between rs2023239 variance and number of CD symptoms in an adolescent sample.

Although significant effort has been devoted to analyzing CNR1 associations with CD, another critical gene in the endocannabinoid system, FAAH, has also shown associations with CD phenotypes. FAAH encodes for fatty acid amide hydrolase (FAAH), an enzyme expressed in the brain and liver that inactivates *N*-arachidonoyl-ethanolamine (anandamide), an endogenous CB1 agonist (Cravatt and Lichtmann, 2003). FAAH knockout mice (FAAH (−/−)) show exaggerated responses to anandamide, suggesting that FAAH is the primary regulator of anandamide signaling (Cravatt *et al*, 2001). Decreases in FAAH efficacy may increase sensitivity to anandamide (Cravatt *et al*, 2001), thereby increasing CB1 binding and affecting DA activity along reward pathways. Alterations in endocannabinoid signaling have been associated with a variety of SUD phenotypes in animal models (Wiskerke *et al*, 2008). Although no common human mutation causes FAAH deletion, a missense SNP, C385A (rs324420), results in a mutant form of FAAH with reduced expression and cellular stability (Chiang *et al*, 2004). Among human marijuana users, the C allele of this SNP has been associated with increased risk for progression to CD (Tyndale *et al*, 2007). Our study of young marijuana users also reported an association between the C allele and craving after marijuana abstinence (Haughey *et al*, 2008). In a follow-up study, we administered marijuana to 40 young adult daily users after 24 h of abstinence, and found that the C allele was associated with increased severity of withdrawal symptoms after abstinence, and increased happiness after smoking marijuana (Schacht *et al*, 2009).

In sum, the existing literature provides strong evidence for the relevance of CNR1 and FAAH with respect to SUD and CD phenotypes. However, much of this work has been conducted with dichotomous diagnostic phenotypes. This study aimed to use an imaging genomics approach (eg, Hariri and Weinberger, 2003) to examine the effects of variance in these genes on marijuana cue-elicited brain activation, a finer-grained phenotype that is arguably more proximal to the downstream effects of CNR1 and FAAH variance. As we have reported earlier that cue reactivity in chronic heavy marijuana users is associated with greater blood oxygenated level dependent (BOLD) response in reward-related areas of the brain (Filbey *et al*, 2009) and that among heavy drinkers, genetic variants, including rs2023239 in CNR1, modulate reactivity to the taste of alcohol in the form of increased activation (Filbey *et al*, 2008; Hutchison *et al*, 2008), we hypothesized that genetic risk, specifically, the G allele of rs2023239 in CNR1 and the C allele of rs324420 in FAAH, would be associated with an increased BOLD response to marijuana-related cues in reward-associated brain areas.

MATERIALS AND METHODS

Participants

For this study, we re-analyzed earlier reported data by genotype (Filbey *et al*, 2009). The earlier described participants were 43 self-reported regular marijuana users who were recruited through flyers and media advertisement in the Albuquerque, NM metro area and provided informed consent to participate in this study. Of these, six had movement >2 mm and were excluded from the analyses. All participants were right handed and free of MRI contraindications (ie, no metallic implants, claustrophobia, pregnancy, and so on). The participants included in this study were required to speak English, to report regular marijuana use at least four times per week over the previous 6 months, and to be willing to abstain from marijuana use for 3 days. Participants were compensated for their participation.

DNA was collected from buccal cells and extracted according to modified standard procedures (Walker *et al*, 1999). Samples were genotyped on the *CNR1* and *FAAH* polymorphisms (*CNR1*/rs2023239 and *FAAH*/rs324420) using Taqman PCR technology. Participants were grouped according to their *CNR1* and *FAAH* genotypes separately. As there were insufficient numbers of individuals who were homozygous for the rare allele for both SNPs, individuals who were homozygous for the rare allele were combined with the heterozygotes for the statistical analyses. For *CNR1*, because there were no homozygotes for the G allele, the heterozygotes ($n=10$) were compared with the A/A individuals ($n=24$); for *FAAH*, A/A individuals ($n=5$) were combined with heterozygotes ($n=15$), and compared with C/C individuals ($n=17$). Table 1 describes the characteristics of the 37 participants included in this study.

Procedure

The study took place in two sessions. During the first session, participants provided a saliva sample for DNA analysis and a urine sample for toxicological analysis. Participants were then scheduled for a second session, and were instructed to abstain from marijuana use for 72 h before this session. They were informed that a second urine drug screen administered at this session would confirm their abstinence. Although toxicological analysis was not sufficiently sensitive to detect abstinence-induced changes in urine levels of THC metabolites, bogus pipeline procedures have shown efficacy in increasing the accuracy of self-reports of drug use (eg, (Lowe *et al*, 1986)). During the second session, participants also completed a battery of neuropsychological tests (not reported here) and self-report measures of mood and craving. Participants were then placed in the MRI scanner. After collecting a high-resolution anatomical scan for registration and localization of the fMRI data, participants completed stop-signal and

Table 1 Characteristics of the Participants

	All	CNR1		FAAH	
		CNR1 G/A	CNR1 A/A	FAAH C/C	FAAH A/A or A/C
N	37	10	24	17	20
Age (mean ± SD)	23.27 ± 6.56	23.3 ± 7.54	23.54 ± 6.63	23.82 ± 7.04	22.8 ± 6.26
	Range = 18–46	Range = 18–44	Range = 18–46	Range = 18–46	Range = 18–44
Male (*n*, %)	29, 78.4%	8, 80%*	19, 79.2%*	11, 64.7%*	18, 90%*
Frequency of MJ use in days per week (mean ± SD)	5.91 ± 1.5	6.1 ± 1.52	5.89 ± 1.55	5.76 ± 1.48	6.05 ± 1.54
	Range = 3–7	Range = 3–7	Range = 3–7	Range = 3–7	Range = 3–7
Duration of regular MJ use in years (mean ± SD)	6.09 ± 5.95	5.05 ± 6.85	6.92 ± 5.86	5.54 ± 6.53	6.55 ± 5.53
	Range = 0.17–24	Range = 1–23	Range = 0.17–24	Range = 0.17–24	Range = 1–23
SCID MJ dependence (*n*, %)	25, 67.6%	4, 40%	19, 79.2%	11, 64.7%	14, 70%
SCID MJ abuse (*n*, %)	3, 8.1%	1, 10%	2, 8.3%	1, 5.9%	2, 10%

Abbreviation: MJ = marijuana. *$p < 0.05$.
This table summarizes the demographic and marijuana use characteristics of the current sample.
N.B. CNR1 genotypes were not available for three of the participants.

monetary incentive delay tasks (not reported here). Participants were then administered a cue-elicited craving paradigm, described below. The cue paradigm was the last task completed during a 105-m scanning session.

MRI images were collected using a 3 Tesla Siemens Trio. fMRI scans were collected using a gradient echo, echoplanar sequence with ramp sampling correction using the intercommissural line (anterior commissure/posterior commissure) as a reference (repetition time: 2.0 s, echo time: 27 ms, α: 70°, matrix size: 64×64, 32 slices, voxel size: $3 \times 3 \times 4\,mm^3$). A tilting acquisition was applied during the echoplanar imaging (EPI) sequence to compensate for the problems of B0 field spatial distortion in the orbitofrontal cortex (OFC). Slices were acquired higher than the anterior commissure/posterior commissure, approximately perpendicular to the sinuses (Deichmann *et al*, 2003; Weiskopf *et al*, 2007). The high-resolution anatomical MRI scan was collected with a multi-echo magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence with the following parameters: repetition time/echo time/inversion recovery time = 2300/2.74/900 ms, flip angle = 8°, field of view = 256×256 mm, slab thickness = 176 mm, matrix = $256 \times 256 \times 176$, voxel size = $1 \times 1 \times 1$ mm, number of echos = 4, pixel bandwidth = 650 Hz, total scan time = 6 min.

We used a previously described marijuana tactile cue-exposure paradigm (Filbey *et al*, 2009). Briefly, the paradigm was presented in two separate EPI runs of 12 pseudorandom tactile presentations of a marijuana pipe (marijuana cue × six trials) and a pencil (control cue × six trials). Each trial consisted of a 20-s cue exposure period, followed by a single 5-s urge question, and ended with a 20-s washout period to allow the hemodynamic response to return to baseline before the next trial. The total number of repetitions per run was 288 and the total task duration was 19 min and 12 s. The task was presented using a front projection to a mirror system mounted on the head coil. Responses were recorded using a fiber-optic pad. Stimulus presentations were delivered using E-Prime (Psychology Software Tools). The timing of the stimulus presentation was synchronized with trigger pulses from the magnet to ensure precise temporal integration of stimulus presentation and fMRI data acquisition. The University of New Mexico Human Research Review Committee approved all procedures used.

Analyses

Pre-processing of fMRI data followed a standard procedure. The images were realigned using INRIalign, a motion correction algorithm unbiased by local signal changes (Freire and Mangin, 2001; Freire *et al*, 2002). Next, using FEAT (fMRI Expert Analysis Tool) Version 5.98, part of FSL (fMRIB's Software Library, http://www.fmrib.ox.ac.uk/fsl/), the following pre-statistics processing was performed: non-brain tissue/skull removal using Brain Extraction Tool (BET); spatial smoothing using a Gaussian kernel of FWHM 8 mm^3; mean-based intensity normalization of all volumes by the same factor; and high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma = 50.0 s). Time-series statistical analysis was carried out using FILM (FMRIB's Improved Linear Model) with local autocorrelation correction. The first seven volumes of all EPI runs were discarded to allow the MR signal to reach steady state.

Explanatory variables were created by convolving the stimulus timing files with a double gamma hemodynamic response function in FEAT. A multiple linear regression analysis was performed to estimate the hemodynamic parameters for different explanatory variables (ie, active condition for marijuana cues, active condition for control cues) and a corresponding *t*-statistic indicates the significance of the activation of the stimulus. Contrast maps were created by contrasting marijuana active conditions *vs* control active conditions. These maps were then registered to a high-resolution image using FLIRT (FMRIB's Linear Image Registration Tool) (Jenkinson and Smith, 2001; Jenkinson *et al*, 2002). Group analyses were carried out using FLAME (FMRIB's Local Analysis of Mixed Effects) (Beckmann *et al*, 2003; Woolrich *et al*, 2004). Statistical

Table 2 Significant Clusters of Activation per Contrast

Z	x	y	z	Localization	Brodmann area
(a) *CNR1* G/A *vs CNR1* A/A					
3.29	24	30	18	R anterior cingulate	BA 32
3.24	24	26	22	R medial frontal gyrus	BA 9
3.19	−16	−16	−20	L parahippocampal gyrus	BA 28
3.17	−12	−36	10	L thalamus	—
3.04	34	26	28	R middle frontal gyrus	BA 9
3.04	2	−104	12	R cuneus	BA 18
(b) *FAAH* C/C *vs FAAH* A/A or A/C					
4.30	42	−40	30	R supramarginal gyrus	BA 40
4.22	8	−38	22	R posterior cingulate	BA 23
4.08	−6	−38	18	L posterior cingulate	BA 29
4.00	−4	−32	24	L posterior cingulate	BA 23
3.98	−6	46	−2	L anterior cingulate	BA 32
3.93	46	−12	−14	R middle temporal gyrus	BA 21

(a) *CNR1* G/A *vs CNR1* A/A (cluster-corrected $p<0.05$, $z>1.7$); (b) *FAAH* C/C *vs FAAH* A/A or A/C (cluster-corrected $p<0.05$, $z>1.9$).

maps were then registered to the Montreal Neurological Institute (MNI) template with a two-step process. First, EPI images were registered to the high-resolution MPRAGE image, which was subsequently registered to the 152 brain average MNI template. These registration steps were performed using FLIRT. After transformation of the masks into MNI space, higher-level analysis was carried out using FLAME. We set our threshold and multiple comparison correction using FEAT's cluster- thresholding method, which first defines contiguous clusters using a Z statistic maximum height threshold. Then, each cluster's estimated significance level (from Gaussian random field theory) is compared with the cluster probability threshold. Only clusters that meet these two levels of threshold are considered significantly active. Group analyses, using the genotype groups defined above, were carried out using FLAME (Beckmann *et al*, 2003; Woolrich *et al*, 2004). In addition, to determine possible additive effects of the *CNR1* and *FAAH*, we correlated number of risk alleles (ie, *CNR1* G and *FAAH* C) with BOLD response to the marijuana *vs* the control cue. Only participants with available genotypes for both *CNR1* and *FAAH* were included in these analyses ($N=34$). Of these, 6 had 3 risk alleles, 12 with 2 risk alleles, 13 with 1 risk allele, and 3 with no risk allele. There were no participants with four risk alleles.

Equivalence tests revealed that the *CNR1 and FAAH* genotype groups were significantly different on gender. Thus, all *CNR1 and FAAH* analyses controlled for the effects of gender. There were no other significant differences between any of the groups.

RESULTS

Comparisons of the *CNR1* groups showed that those with the G allele had significantly greater neural response to marijuana cues (compared with control cues) in a cluster of activation (volume = 10 719 voxels) encompassing the OFC, IFG, insula and dorsal anterior cingulate gyrus (ACG) as compared with the *CNR1* A/A group (cluster-corrected $p<0.05$, $z>1.7$). This cluster did not encompass other reward areas such as the striatum or the VTA. There was no significantly greater activation in the *CNR1* A/A group as compared with the G/A group.

Table 3 Areas of Significantly Positive Correlation Between Number of Risk Alleles and BOLD Response to Cues (Cluster-Corrected $p<0.05$, $z>1.9$)

Z	x	y	z	Localization	Brodmann area
4.21	36	28	26	R middle frontal gyrus	9
4	−34	−48	16	L superior temporal gyrus	22
3.89	−8	−40	16	L posterior cingulate	29
3.89	16	−36	30	R cingulate gyrus	31
3.84	16	−42	−34	R cerebellar tonsil	—

Comparisons of the *FAAH* groups showed that the *FAAH* C/C group had greater activation in a large cluster (volume = 42 161 voxels) encompassing several areas of the reward system including the OFC, IFG, ACG, striatum, and VTA during marijuana *vs* control cues as compared with the A/A or A/C group (cluster-corrected $p<0.05$, $z>1.9$) (see list of local maxima in Table 2). There were no significantly greater areas of activation in the *FAAH* A/A or A/C group as compared with the C/C group.

In these analyses, there were five participants who had both high-risk genotypes (ie, CNR1 G/A and FAAH C/C). To account for this non-independence of the FAAH and CNR1 risk groups, the previous analyses were also carried out while controlling for the other risk genotype (eg, controlling for FAAH genotype in the CNR1 analyses). These analyses found no significant difference between the groups.

Finally, correlation analyses indicated that there was a significant positive correlation between number of risk alleles (ie, *CNR1* G, *FAAH* C) and response to marijuana cues, such that the greater the number of risk alleles, the greater the BOLD response to marijuana cues (*vs* control cues) in one cluster (volume = 33 842 voxels) encompassing reward areas, such as the OFC and striatum in addition to cingulate, occipital, and cerebellar regions (see Table 3, Figures 1 and 2).

DISCUSSION

The overarching goal of this study was to characterize the mechanisms that underlie earlier described associations between SNPs in CNR1 and FAAH and cue reactivity in heavy marijuana users (Haughey *et al*, 2008; Schacht *et al*, 2009). Our results show that rs2023239 in CNR1 and rs324420 in FAAH are associated with differential neural response to marijuana cues, such that carriers of the CNR1 G allele and FAAH C homozygotes had greater neural response in structures along the reward pathways. Further, our results show that this pattern of heightened response to cues increases as the number of risk alleles increases. These findings provide further evidence for the relevance of CNR1 and FAAH to intermediate phenotypes of CD, and suggest

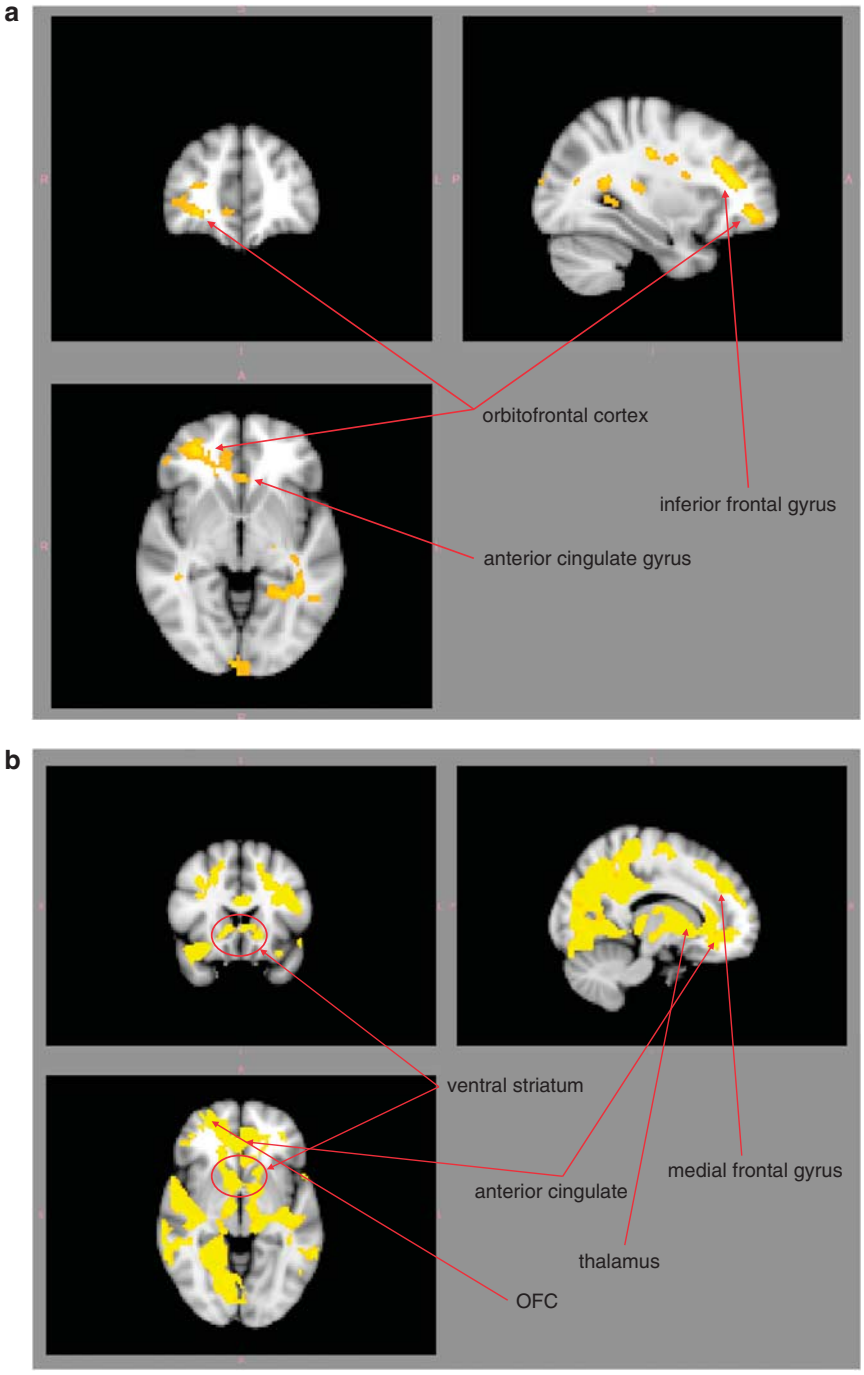

Figure 1 Differential neural response to cues by genotype groups. (a) *CNR1* G/A *vs CNR1* A/A; (b) *FAAH* C/C *vs FAAH* A/A or A/C (cluster-corrected $p<0.05$, $z>1.9$).

that a dysregulation in the reward system may mediate these genetic effects.

Although candidate genetic studies of CD to date are relatively sparse, these findings are congruent with the emergent literature (for a review, see (Agrawal and Lynskey, 2009)). Specifically, these findings support previous reports that CNR1 variants are associated with CD (Hartman *et al*, 2009; Agrawal *et al*, 2008), and that FAAH C allele carriers are more susceptible to marijuana dependence than FAAH A allele carriers (Haughey *et al*, 2008; Schacht *et al*, 2009; Tyndale *et al*, 2007). It is also noteworthy that the genetic effects were greater for the FAAH gene than for the CNR1 gene in terms of activation cluster size (ie, 42 161 *vs* 10 719 voxels) and maximum *z*-scores (4.3 *vs* 3.3). This is of particular interest given the recent reports from animal studies suggesting that FAAH blockade has complex effects that are similar to blocking CB1 receptors (Le Foll and Goldberg, 2004). The mechanism for this effect has been suggested to be not only increased anandamide levels but also elevated levels of other ethanolamides such as oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) (Solinas *et al*, 2006; Wise *et al*, 2008). Emergent

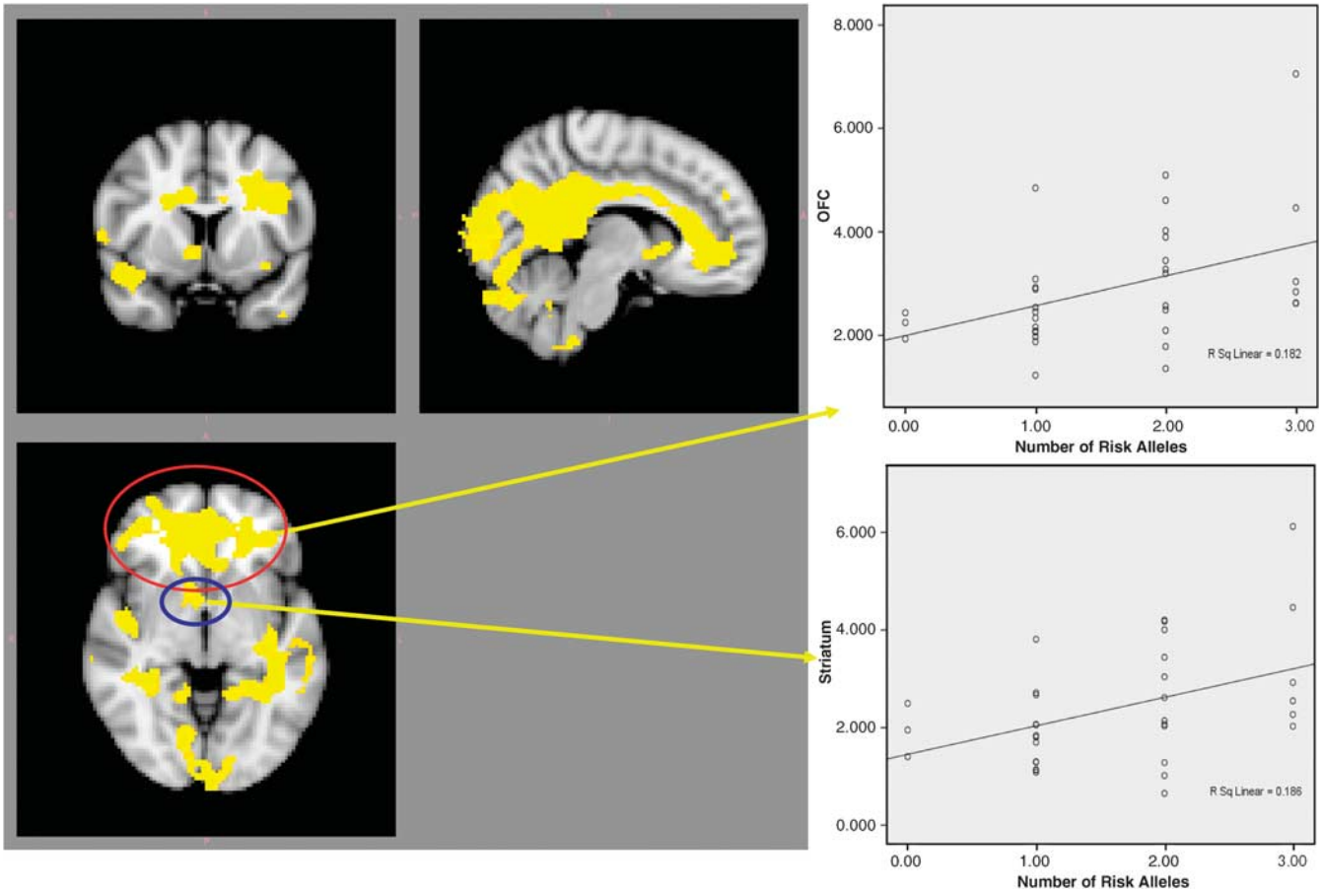

Figure 2 Positive correlations between number of risk alleles and BOLD response in reward-associated areas. Analyses showed that the greater number of risk alleles (ie, *CNR1* G, *FAAH* C, the greater the BOLD response to cues in several important areas related to reward processes such as the NAc, thalamus, ACG, OFC, and IFG (cluster-corrected $p<0.05$, $z>1.9$). Scatterplots illustrate the maximum *z*-scores for the OFC *($r=0.43$, $p=0.01$)* and striatum *($r=0.43$, $p=0.01$) (y axis)* against number of risk alleles *(x axis)*.

evidence for this effect particularly in response to nicotine has also been reported (Forget *et al*, 2009; Scherma *et al*, 2008). Unfortunately, this study does not allow for a direct investigation of the possible joint effects of cannabis and nicotine because there were only 12 smokers in our total sample.

The current findings add to this literature by elucidating the underlying mechanisms related to this increased sensitivity. The existing functional imaging study of cue reactivity among heavy marijuana users (Filbey *et al*, 2009), in addition to those of alcohol (Filbey *et al*, 2008), cocaine (Goldstein *et al*, 2009), and nicotine users (Franklin *et al*, 2007) show that the mechanism of cue reactivity is associated neural response in the pathways that underlie the anticipation of reward. These findings also add to a growing body of evidence suggesting that measures of neuronal response to cues are sensitive to genetic variation in genes such as *DRD4* (with smoking cues (McClernon *et al*, 2007); and alcohol cues (Filbey *et al*, 2008), and *CNR1* (Hutchison *et al*, 2008), although replications of these results are needed. This dysregulation of neural response in areas related to reward anticipation may have a critical role in the etiology of CD (Filbey *et al*, 2009), and genetic variation that influences this dysregulation may exacerbate or mitigate the development of dependence.

Our findings also suggest the presence of additive genetic effects, such that a greater number of risk alleles across *CNR1* or *FAAH* are associated with greater response in the reward system. A similar *CNR1* × *FAAH* interaction was also noted by Haughey *et al* (2008), such that individuals with the G/A–A/C genotype for *CNR1–FAAH* showed more severe negative affect than other genotype groups after abstinence and again after cue exposure. Additive genetic influences have been reported earlier on subjective effects of marijuana. Specifically, additive genetic influences (rather than because of a single gene with large effects or environmental influences) were found to explain the variance in subjective response to marijuana (Lyons *et al*, 1997). Similar findings of additive genetic effects in CD have also been reported in adolescents, which were particularly evident in late adolescence (Kendler *et al*, 2008).

These findings have several important clinical implications. Pharmacological agents that target CB1 receptors and/or elevate brain levels of endocannabinoids might alleviate cannabis withdrawal and dependence differentially by genotype. For instance, the FAAH inhibitor URB597, which selectively increases anandamide levels in the brain of rodents and primates, has been suggested as a possible therapeutic avenue for the treatment of cannabis withdrawal by significantly attenuating rimonabant-precipitated

withdrawal signs in THC-dependent mice although genetic mediators of this effect have not yet been analyzed (Schlosburg *et al*, 2009; Clapper *et al*, 2009). In addition, the determination of the genetic factors that are associated with CD holds promise for the future development of more targeted or personalized treatment. In light of the Tyndale *et al* (2007) finding that FAAH is associated with risk of progression to CD, as well as our own findings that both of the SNPs analyzed here show associations with intermediate CD phenotypes among fairly young users, determination of composite genetic risk for CD might allow intervention before progression to full-blown dependence.

Some caveats must be taken into consideration in the interpretation of these findings. First, the relatively small sample size does not allow for a three-group analysis (ie, with each allele combination for each SNP) or the ability to control for the effect of one gene on the other (ie, the same participants are used in both analyses and some overlap in the high-risk and low-risk groups of each set of analyses). Regarding the latter non-independence of the CNR1 and FAAH analyses, it is possible that the effects found in each are because of inclusion of persons with the alternative high-risk genotype being examined in the other set of analyses. The correlation analysis, to some extent, addresses the non-independence of these genotypes. Replication of these findings is necessary with a larger sample size that permits a more thorough interrogation of each allele contribution. There are also methodological issues that limit the interpretation of these findings. As we described in Filbey *et al* (2009), only 54% of the participants reported the marijuana pipe as their primary mode of use. However, keeping the cue consistent across participants controls for the effects of subject-specific cues. Another limitation is fatigue, which is a common caveat in fMRI studies. However, we believe that any effects are minimal because the task is not cognitively demanding.

To conclude, the present findings not only add to the growing literature on the effects of biologically relevant genes on the morbidity toward CD, but also suggest that these effects may be related to hyperactivity in reward pathways. Further, this study highlights the importance of analyzing families of genes (*vs* single genes) and their interactions to make better predictions of vulnerability to cannabis use disorders. Future studies should determine the mechanisms of *gene* $\times$ *gene* interactions, given that there is a biologically plausible pathway for their interaction.

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DISCLOSURE

The authors declare no conflict of interest.

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