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Acute effects of cannabinoids on addiction endophenotypes are moderated by genes encoding the CB1 receptor and FAAH enzyme

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Abstract

Understanding genetic factors that contribute to cannabis use disorder (CUD) is important, but to date, findings have been equivocal. Single nucleotide polymorphisms (SNPs) in the Cannabinoid receptor 1 gene (CNR1; rs1049353, rs806378) and Fatty Acid Amide Hydrolase (FAAH) gene (rs324420) have been implicated in CUD. Their relationship to addiction endophenotypes such as cannabis-related state satiety, the salience of appetitive cues and craving after acute cannabinoid administration has not been investigated. Forty-eight cannabis users participated in a double-blind, placebo-controlled, four-way crossover experiment where they were administered 4 treatments in a randomised order via vaporisation: placebo, Δ^9-tetrahydrocannabinol (THC) (8mg), THC+Cannabidiol (THC+CBD) (8mg + 16mg), CBD (16mg). Cannabis-related state satiety, appetitive cue salience (cannabis, food), and cannabis craving were assessed each day. Participants were genotyped for rs1049353, rs806378 and rs324420. Results indicated CNR1 rs1049353 GG carriers showed increased state satiety after THC/THC+CBD administration, in comparison to placebo and reduced the salience of appetitive cues after THC in comparison to CBD administration; A carriers did not vary on either of these measures indicative of a vulnerability to CUD. CNR1 rs806378 CC carriers showed greater salience to appetitive cues in comparison to T carriers but there was no evidence for changes in state satiety. FAAH rs324420 A carriers showed greater bias to appetitive cues after THC, in comparison to CC carriers. FAAH CC carriers showed reduced bias after THC in comparison to CBD. No SNPs modulated craving. These findings identify candidate neurocognitive mechanisms through which endocannabinoid system genetics may influence vulnerability to CUD.

Introduction

Problematic drug use is influenced by both environmental and genetic factors with genetic variation accounting for ~40 to 60% of the variance of the total risk in vulnerable individuals(1). Policies regarding cannabis use worldwide are becoming more liberal. Investigating individual differences in the vulnerability and resilience to the harmful effects of cannabis has become a priority area of research. This is particularly important because cannabis currently stands poised to join alcohol and tobacco as a legal drug across the globe, meaning rates of cannabis use disorders (CUDs) may also rise. CUDs account for the largest global burden of disease related to cannabis(2). The endocannabinoid system is fundamental in drug use/abuse(3). Genetic differences in the endocannabinoid system may contribute to an individual's vulnerability or resilience to cannabis dependence.

The primary psychoactive cannabinoid in cannabis, Δ^9-tetrahydrocannabinol (THC), is a partial agonist at the cannabinoid receptor type 1 (CB1R). Using cannabis can lead to the development of CUD which affects ~9% of those who initiate use(4). The percentage of THC in cannabis has been increasing over the past two decades mirroring increased demand for treatment for CUD(5). Cannabidiol (CBD), the second most abundant cannabinoid found in many cannabis plants is neither intoxicating(6) nor rewarding(7) and has several psychopharmacologically opposite effects to THC, but its mechanism of action has not fully been determined(8). CBD acts as a negative allosteric modulator of the CB1R; and/or increases the inhibition of fatty acid amide hydrolase (FAAH); the main degradative enzyme of anandamide, thus indirectly regulating the activity of the CB1R. The ratio of THC:CBD is important as CBD protects against the development of CUD and psychotic-like effects of THC(9-11). Moreover, preparations of CBD are commonly used alone and are available commercially, therefore it is important to investigate the interaction between CBD and genetic variation in the endocannabinoid system.

The CNR1 gene encodes CB1R and is located on chromosome 1(12). Meta-analyses have found that polymorphisms in CNR1 have been associated with cannabis, alcohol, nicotine and cocaine dependence(12, 13). These polymorphisms are also associated with potential CUD endophenotypes such as functional reward-related brain activity during exposure to cannabis cues(14). As such, genetic influences may therefore alter other mechanisms related to CUD – such as craving, satiation and drug-cue salience.

Endocannabinoid signalling is terminated by enzymes such as FAAH. FAAH inhibition is a mechanism that is currently being investigated as a treatment of CUD in humans(15, 16). The rs324420 Single Nucleotide Polymorphism (SNP) of the FAAH enzyme is a C to A polymorphism which results in a proline to a threonine substitution at codon 129(12). Those with the A allele have reduced FAAH expression (17, 18). This reduction has been associated with problematic cannabis use(17, 19) and

putative endophenotypes such as craving and withdrawal after short-term abstinence(20). However, the C allele has also been associated with cannabis dependence in Genome Wide Association Studies (GWAS) (19) as well as other potential endophenotypes such as greater craving and withdrawal after short-term abstinence (20, 21). Filbey, et al. (14) found that those who were homozygous for the C allele showed greater activation in reward circuitry (which included the orbitofrontal cortex, anterior cingulate gyrus and the nucleus accumbens) after a cannabis cue reactivity paradigm, in comparison to A allele carriers. However, no studies have investigated how genes related the endocannabinoid system predict CUD-related endophenotypes after controlled acute administration of cannabinoids.

Our innovative approach was to study, in depth, the neurocognitive endophenotypes of CUD after acute cannabinoid administration, which may be more valid than a single dichotomous variable such as a diagnosis of CUD itself(22). We focussed upon three endophenotypes of CUD which are distinct but related processes in CUD. Firstly, attentional processing is an important transdiagnostic marker for depression, anxiety and drug dependence(15, 23). THC:CBD ratio predicts attentional bias to cannabis cues when intoxicated, with those using more CBD in their cannabis strains showing reduced attentional bias (9). The salience of appetitive cannabis stimuli is also related to *frequency* of cannabis use; dependence on the drug itself, and craving(24-26). Secondly, craving, or the intense desire for a reward, is a primary behavioural component of CUD which motivates drug use and predicts cannabis use after 6 months in adults and adolescents(27, 28). Thirdly, cannabis-related satiety after acute ingestion of the drug is a key element of CUD. In line with common theories of addiction(29), after acute intoxication, there is reduced incentive value of cannabis i.e. satiety temporarily devalues the rewarding effect of cannabis and reduces "wanting" which would be evidenced by an increase in reported state satiety, a reduction in craving and attentional bias to drug cues. Therefore, when these related processes are unaffected by acute intoxication then this is a key indicator of CUD. Satiety is related to loss of control and tolerance measures which is reflected in DSM-5 CUD criterion "taking the drug in larger amounts for longer than intended"(30).

Our primary aim was to investigate if and how genetic variants in the endocannabinoid system, in particular the CB1 receptor (rs1049353 and rs806378) and the FAAH enzyme (rs324420) would modulate the acute response to cannabinoids, in relation to promising endophenotypes: cannabis-related satiation, the salience of appetitive cues and craving. To this end, in this preliminary candidate gene investigation, we carried out a randomised, double-blind, crossover study where participants were administered THC(8 mg), THC(8 mg)+CBD(16 mg), CBD(16 mg) and placebo (ethanol vehicle) across four separate sessions. We hypothesise that CNR1 rs1045393 A allele carriers (versus G carriers), rs806378 T carriers (versus C carriers) and FAAH 324420 C carriers (versus A carriers) would show greater indicators of CUD which would be evidenced by greater drug cue salience, lower satiation and greater craving after intoxication with THC. Moreover, given research that suggests that

CBD protects against the dependence-related effects of THC (Morgan et al 2010), differential effects of drug conditions were expected on CUD-endophenotypes, although how this would interact with genotype was exploratory, given the paucity of research in this area. The main outcome of interest was interactions between drug and genotype.

Material and Methods

Participants

Forty-eight participants were recruited for the acute drug challenge study on the basis of having previously volunteered in a large scale study of over 400 cannabis users where genotyping was conducted(31). Participants were aged between 16 and 23 years wherein participants had a wide range of cannabis exposure from 1 day per month to daily use and were recruited via word of mouth and snowball sampling(11). Participants for this study were recruited based on 1) schizotypy as measured by the Schizotypal Personality Questionnaire score (top and bottom quartiles) and 2) Frequency of cannabis use ("light"=1-24 days per month; "heavy"=25+ days per month). This study is a secondary analysis concerned with genetic associations across the whole sample regardless of sub-group. For this study, which had a sample size of 48, we conducted a sensitivity power analysis (32) for a repeated measures ANOVA (within-between interaction) which showed we had 80% power to detect a small-to-medium effect size (Cohen's d=0.34) at an alpha of 0.05 with 2 groups, 4 measures and an r=0.5 between measures. Additional data from this study on facial affect recognition and visual analogue scales(6) and psychotomimetic symptoms and memory function have been reported elsewhere(33).

Participants were matched for age and Spot the Word score (as a correlate of premorbid verbal IQ) (34) scores across frequency groups. Inclusion criteria were: (i)self-reported abstinence (confirmed with saliva and urinary tests on each experimental day) from cannabis, other drugs and alcohol use for 24h prior to each test day; (ii)English fluency, (iii)normal/corrected to normal vision. Exclusion criteria were: current self-reported (i)respiratory/physical health problems, (ii)pregnancy or the risk of being pregnant, (iii)clinically diagnosed learning impairments, (iv)clinically diagnosed schizophrenia/psychosis or substance abuse problems, and (v)illicit drug use other than cannabis more than once a week.

Design

A four session, randomised, double-blind crossover design was used to compare the acute effects of THC (8mg), CBD (16mg) and their combination (8mg THC+16mg CBD) with placebo (ethanol vehicle). Both cannabinoids were formulated in alcohol solution and were purchased from STI Pharmaceuticals (Brentwood, Essex, UK). Treatment order across the 4 sessions was determined by a balanced Latin square resulting in 12 combinations.

Drug administration

Cannabinoids and placebo (ethanol vehicle) were administered using a Volcano Medic Vaporisor (Storz & Bickel, Tuttlingen, Germany). 8mg THC dissolved in ethanol and 16mg of CBD dissolved in ethanol were administered on a 10-second inhalation cycle wherein participants was instructed to first fully exhale, next fully inhale from the balloon, hold their breath for 10 seconds and then fully exhale; this was repeated until the balloon was empty. This inhaled dose of THC has been found to produce effects on human brain and behaviour, including psychotic-like symptoms, memory impairment and cannabis reward processing(35, 36). The 2:1 ratio of CBD:THC reflects the upper limit (mean+ 3 SD) found in high CBD/low THC cannabis preparations in the UK(37). Detailed information about concealment and blinding can be found in Hindocha, et al. (6) and Morgan et al.(33).

Genotyping

DNA was obtained from cheek swabs of all participants who completed the assessments described above. DNA extraction was performed using standard phenol–chloroform methods. Analyses were performed on two SNPs of CNR1: rs1049353, rs806378 and one single-nucleotide polymorphism of the FAAH gene (rs324420). Off the shelf Taqman assays for these polymorphisms are available as a kit (Applied Biosystems, Life Technologies, Paisley, UK). Genotype calls were discriminated on the basis of algorithmic membership of three clusters representing homozygote A/A, heterozygote A/G, and homozygote G/G genotype classes for CNR1 rs1049353, C/C, C/T and T/T for CNR1 rs806378 and CC/AC/AA for FAAH rs324420. Individuals with the minor allele of these SNPS were combined for power and due to the rarity of these alleles. For rs1049353, those with the minor allele of A, were combined with heterozygotes AG according to convention(38). For CNR1 rs806378, those with the minor allele T were combined with heterozygotes CT and for FAAH rs324420, the minor allele A, was combined with the heterozygote AC. Data was missing for 6 individuals for rs1049353, 3 individuals for rs806378 and 4 individuals for rs324420.

Baseline Assessments

Under non-intoxicated conditions, participants completed the Beck Depression Inventory(39), Spielberger Trait Anxiety Inventory(40), Schizotypal Personality Questionnaire(41), Severity Of Dependence Scale(42) and a drug history(6, 33) (see table 1).

Assessment of Endophenotypes

Bodily Symptoms Scale (BSS)(43): "want to smoke a joint" single item to assess state satiation.
The BSS was designed to detect physical symptoms of acute cannabinoids administration. Participants rated on scale from 0 (do not want to smoke a joint) to 10 (really want a joint), how much they wanted to smoke a joint. This measure was assessed twice, both 10 and 70 minutes post-drug administration (taking approximately 2 minutes). This measure was used to index state satiation.

Dot Probe task (Fig 1)

Adapted from Morgan, et al. (9), this computer-based dot-probe paradigm was used to assess attentional bias to both cannabis- and food-related stimuli. Ten colour photographs of cannabis-related stimuli and 10 colour photographs of food-related stimuli were used, with each image simultaneously paired with a neutral photograph matched as closely as possible for visual composition and complexity. A total of 80 of the 160 total trials were critical trials of which 40 featured cannabis-related and 40 food-related stimuli, each presented twice for 250ms. Only the short exposure time was chosen to index automatic (250ms) processing. The critical (food- or cannabis-related) images appeared once on the left and once on the right at each time interval. The side at which the probe appeared was counterbalanced across all the trials. An asterisk was used as the probe. A total of 10 neutral practice trial pairs were used as training, followed by two blocks of 80 experimental trials. Short break occurred between blocks. Versions were randomised across testing days. Each trial began with a central fixation cross shown for 1000ms, after which a pair of matched images would appear, one on each side of the fixation cross, for 250ms durations. Both images then disappeared revealing the probe behind one of the two images. Participants were required to respond to the probe as quickly as possible by pressing a button corresponding to the relevant side of the screen. The task took place approximately 25 minutes after drug administration and lasted approximately 10 minutes. Attentional bias was calculated as the difference in reaction time between when the probe replaced the neutral compared with the incentive (cannabis/food) stimulus [Rtneutral-RTincentive], such that a greater difference indicated greater bias toward that stimulus.

Marijuana Craving Questionnaire (44)

A short 12-item questionnaire was given to assess current craving for cannabis. Participants completed the MCQ immediately after the attentional bias task; approximately 35 minutes post-drug and taking approximately 2 minutes. The MCQ is reliable for assessing craving in cannabis users not seeking treatment.

Procedure

Experimental sessions occurred on four occasions each separated by a one-week washout to minimize carry-over effects (>3 times elimination half-life of THC (6)). On each (identical) session, 10 minutes after drug administration, participants completed the BSS to assess cannabis related state satiety (T1), they completed the dot probe task to assess appetitive cue salience at 25 minutes post-drug administration, the MCQ at 35 minutes and the BSS again (T2) at 70 minutes post-drug administration. The full test battery took approximately 1.5 hours on each test day. Other tasks conducted during the session are reported elsewhere (6, 33). Acute drug effects were monitored throughout the session (see supplementary figure 1 reproduced from (6)). Participants were reimbursed £120 for their time on the last testing day and debriefed fully. All participants provided written, informed consent on each

occasion and ethical approval was given by the UCL Research Ethics Committee and was conducted in accordance with the Declaration of Helsinki.

Statistical analysis

All analyses were conducted with SPSS 24.0. Syntax are available from CH on request. Outliers and normality were assessed via diagnostic plots. Extreme outliers (>3 times interquartile range) were winsorized within-group to the next highest/lowest value +/- 1. Descriptive statistics (table 1) based on genotype were conducted with one way analysis of variance (ANOVA) and Chi-Squared tests. When variances were not equal between groups (assessed using Levene's test) then unequal variances (Welch's) t-tests were used.

Analyses were completed using the general linear model repeated measures ANOVA (RMANOVA) with each model containing a within-subjects factor of drug (placebo, THC, THC+CBD, CBD) and a between-subjects factor of genotype i.e. a 4 (drug) x 2 (genotype) RMANOVA. For attentional bias, there was an additional within-subjects factor of stimulus type (cannabis, food) which led to a 4 (drug) x 2 (stimuli) x 2 (genotype) RMANOVA. For BSS "want to smoke a joint", there was additional within-subject factor was time as the assessment was taken twice (T1 – 10 minutes post-drug, T2 – 70 minutes post drug) which led to a 4 (drug) x 2 (genotype) x 2 (time) RMANOVA. Greenhouse Geisser corrections were applied for violations of sphericity (degrees of freedom are rounded to the nearest integer Main effects of drug were explored with *a priori* simple contrasts versus placebo to reduce the number of comparisons (i.e. placebo vs. THC, placebo vs. THC+CBD and placebo vs. CBD). Interactions between factors were explored with Bonferroni-corrected pairwise comparisons locally within each omnibus term. The main factor of interest was a drug x genotype interaction. η^2 was calculated as the SSeffect/SStotal.

Results

Sample Characteristics (Table 1)

CNR1 rs1049353 genotypes were in Hardy-Weinberg equilibrium (G/G=20, A/G=17, AA=5; χ^2 (2)= 0.21, p=0.64), as was CNR1 rs806378 (C/C=18, C/T=24, T/T=3; $\chi^2(2)$=1.04, p=0.17) but there was some evidence that FAAH rs324420 (C/C=30, A/C=10 and A/A=4; $\chi^2(2)$=4.00, p=0.05) was not. As seen in table 1, participants did not differ on demographics or trait mood scales based on genotype groupings for CNR1 rs1049353, CNR1 rs806378, or FAAH rs324420. Genotype groups differed significantly on the SDS where those who were homozygote GG for the CNR1 rs1039353 SNP, had a higher cannabis dependence score than A carriers, but groups did not differ on cannabis use variables. Additionally, a significant difference was observed for FAAH rs324420 CC homozygotes who had used cannabis more recently than A carriers.

BSS "Want to smoke a joint"

CNR1 rs1049353 (Fig 2)

There was a drug x genotype interaction ($F(3,105)=4.192$, $p=.008$, $\eta^2=.05$). The interaction was explored with Bonferroni-corrected pairwise comparisons which showed those with the GG genotype had *decreased* wanting to smoke a joint after both THC ($p=.016$; d=.52) and THC+CBD ($p<.001$, d=.52), but not CBD ($p=.137$, d=.31) in comparison to placebo. Those with the A allele did not experience this reduction after THC/THC+CBD administration (both $p's=1.000$). There was a main effect of drug ($F(3,105)=4.206$, $p=.007$, $\eta^2=.05$). Simple contrasts showed lower scores for THC (M:4.73 SE:.41, $p=0.047$) and for THC+CBD (M: 4.45, SE:.42; $p=0.001$), in comparison to placebo, but not for CBD (M: 4.95, SE:.43; p=.182). There was a main effect of time ($F(1,36)=12.945$, $p=.001$, $\eta^2=.03$) which showed that wanting to smoke a joint increased across the two time-points ($p<.001$). There was no main effect of genotype ($F(1,36)= .176$, $p=.675$, $\eta^2=0.00$), there were no other two-way or three-way interactions.

CNR1 rs806378

There was a main effect of drug ($F(3,114)=3.784$, $p=.012$, $\eta^2=.005$). Simple contrasts show lower wanting to smoke a joint after THC (M:4.730, SE:.40; $p=.043$) and THC+CBD (M:4.55, SE:.43; $p=.004$) in comparison to placebo (M=5.36, SE:.43) but no differences emerged CBD (M:5.06, SE:.43; $p=.254$). A main effect of time emerged ($F(1,28)=16.069$, $p<.001$, $\eta^2=.04$) which showed that wanting to smoke a joint increased across the two time-points (p<.001). There were no main effects or interactions with genotype.

FAAH rs324420

Only a main effect of time emerged ($F(1,27)=11.738$, $p=.002$, $\eta^2=.04$) which showed that wanting to smoke a joint increased across the two time-points (p<.001).

Attentional bias

CNR1 rs1049353 (Fig 3)

There was a drug x genotype interaction ($F(3,120)=3.108$, $p=.029$, $\eta^2=.03$). Within the GG group, attentional bias was significantly lower after acute THC administration (M:4.25, SE: 4.79), in comparison to CBD administration (M:25.93, SE: 4.88; $p=.011$; d=.74), but this was not significant for the THC+CBD ($p=.066$; d=.55), or placebo (p=.291; d=.47) conditions. A carriers show no differences in attentional bias between drug administration conditions ($p's=1.000$). No Bonferroni-corrected pairwise comparisons met significance between genotypes in each drug condition. There was no main effect of drug ($F(3,120)=2.002$, $p=.177$, $\eta^2=.20$), stimulus type ($F(1,40)=.232$, $p=.129$, $\eta^2=.005$) or genotype ($F(1,40)=.723$, $p=.40$, $\eta^2=.00$) or any other two way or three way interactions.

CNR1 rs806378

A main effect of genotype emerged ($F(1,43)=5.679$, $p=.022$, $\eta^2=.047$) which showed homozygote CC carriers (M:20.21, SE:2.87) had a greater attentional bias than T carriers (M:11.38, SE:2.34), regardless of stimuli type and drug. There was no main effect of drug ($F(3,129)=1.674$, $p=.176$, $\eta^2=.002$), stimulus type ($F(1,43)=.523$, $p=.474$, $\eta^2=.00$) or other two way or three way interactions.

FAAH rs324420 (Fig 4)

A drug x genotype interaction emerged (F(3,126)=3.385, p=.020, η^2=.003). Bonferroni-corrected pairwise comparisons reveal lower attentional bias, irrespective of stimuli, in the homozygote CC group (M:5.56, SE:3.71) compared to A carriers (M:21.41, SE:5.42) after THC only (p=0.02; d=.78). No differences emerged between genotype groups for placebo (p=.518,d=.20), THC+CBD (p=.321; d=.32) or CBD (p=.261; d=.37). Within the CC group, there was a significantly lower attentional bias after THC in comparison to CBD (M:21.14, SE:3.81; p=.018; d=.56). There was no main effect of drug (F(3,126)=.418, p=.740, η^2=.004) or stimulus type (F(1,42)=1.089, p=.303, η^2 =.002) or genotype (F(1,42)=.169, p=.683, η^2=.001). There were no other two way or three way interactions.

Marijuana Craving Questionnaire

CNR1 rs1049353

There was no main effect of drug, genotype or drug x genotype interaction.

CNR1 rs806378

There was no main effect of drug, genotype or drug x genotype interaction.

FAAH rs324420

There was no main effect of drug, genotype or drug x genotype interaction.

Sensitivity Analysis

We included several continuous covariates to our analyses which can be found in the Supplementary Materials.

Discussion

This preliminary study, to our knowledge, is the first to show that the acute effects of cannabinoids on addiction endophenotypes are moderated by genes encoding components of the endocannabinoid system. Specifically, we found drug by genotype interactions for cannabis satiety and salience of appetitive cues for the CNR1 rs1049353 SNP. GG carriers of rs1049353 showed increased satiety and lower salience of cues after THC conditions (vs. placebo/CBD) indicative of intoxication. *A carriers* did not show this suggesting A carriers may be more liable to develop CUD. In regards to CNR1 rs806378, we found a main effect of genotype on the salience of appetitive cues wherein CC carriers showed greater salience to appetitive stimuli, regardless of cue type (cannabis/food) and drug condition. This suggests CC carriers may be more biased to appetitive cues. Finally, in regards to FAAH rs324420, we found a drug by genotype interaction for the salience of appetitive cues showing that *A carriers* showed a greater bias towards appetitive stimuli in comparison to CC carriers suggesting low FAAH functioning is influencing automatic processes related to appetitive cues. Across all three SNPs, genotype did not modulate craving on the marijuana craving questionnaire. These data have important implications. The acute response to cannabis is thought to be a marker of the development of CUD and psychosis from smoking the drug(31). These results may further helps us understand the role of the endocannabinoid system in individual differences in risk and resilience for CUD.

CNR1 genes modify the binding of cannabis and endogenous cannabinoids to the CB1R, thus altering the signalling of the endocannabinoid system which is known to play a key role in substance use disorders(12, 17). In the brain, CB1Rs are found on GABAergic and glutamatergic interneurons in areas of the brain associated with reward processing where they regulate the mesolimbic dopaminergic pathway leading to modulation of dopamine release in the nucleus accumbens; a key mechanism in incentive salience attribution(29). In this study, CNR1 genes were found to modulate cannabis users' response to acute administration of cannabinoids on putative endophenotypes such as appetitive cue salience(24) and satiety(30) but not craving and as such, does not support common models of addiction(29). It may be that A carriers of the CNR1 rs1049353 are more liable for CUD because they continue to show attentional bias to appetitive cues and wanting to smoke a joint after acute intoxication. In contrast, the GG carriers showed reductions in these endophenotypes in response to THC administration, as hypothesised. However, GG carriers had greater self-reported cannabis dependence, but the groups did not differ on other drug use measures such as frequency of use, last use of cannabis or years of cannabis use. When we adjusted for frequency of use and severity of dependence, it had no effect on the results, suggesting that this effect was not explained by variation in cannabis use. CC carriers of CNR1 rs806378 showed increased bias for both cannabis and food related cues regardless of drug condition suggesting that CC carriers may be more susceptible to appetitive cues. However, no rs806378-specific effects were seen on cannabis state satiety or craving.

In regards to FAAH, those who are homozygote for the A allele have a 30% reduction in FAAH activity and are a minority of the population (5%)(17, 18), however, it should be noted that in this study, we combined AA and AC carriers to increase power. As a result, A carriers can be used as a human genetic model of elevations in anandamide which may be able to inform whether FAAH inhibitors would have an effect on these intermediate endophenotypes(45). Indeed, FAAH inhibitors have been shown to be effective for treating CUD (16). However research has also shown that those with the C allele have an increased risk of cannabis dependence and related endophenotypes (14, 19-21). In this study, *A carriers* showed a greater attentional bias towards appetitive stimuli in comparison to CC carriers – which would be consistent with some previous research suggesting this polymorphism is associated with emotional-motivational reactivity(46) but contradicts others(47). However, FAAH genotype did not modulate state satiation or craving suggesting that FAAH is modulating attentional processes towards motivationally salient cues, which would support previous research in anxiety disorders(47). This dissociation between measures is not in concordance with common models of addiction that suggest craving, attentional bias and satiety are related so a change in attentional bias would be accompanied with a change in craving(29). FAAH A carriers had significantly fewer years of cannabis use; but when we adjusted for this in the model, results remained unchanged. In this study, low FAAH functioning may be influencing the implicit processes associated with salience of drug cues but did not

influence satiation or craving after acute drug administration – which are arguably more explicit measures of CUD.

In genetic association research, there have been equivocal findings with variants in CNR1 and FAAH genotypes on CUD and this study adds to the data regarding the relationship between these genes and CUD endophenotypes. Future research should investigate the role of genetic variants in the endocannabinoid system on transdiagnostic markers for mental health found in the National Institute of Mental Health (NIHM) Research Domain criteria (RDoC) initiative including neuroimaging and plasma biomarkers - which may be reliable indicators(22). Additionally, the CNR1 and FAAH SNPs noted in this study should be investigated in relation to other cannabis-related harms such as psychotic-like experiences, depression and anxiety as they have already been showed to contribute to psychiatric problems(48). Longitudinal studies are imperative to clarify whether genetic variation influences cannabis dependence – such is the focus of the ABCD study(49). Moreover, the development of polygenic risk scores for cannabis dependence, that can capture a wider range of common genetic variants, should be developed and utilised.

Strengths and Limitations

Strengths of this study, include a controlled design of a four-way crossover with THC, CBD and their combination on CUD-related outcomes. One criticism levied at GWAS is that they tend to utilise a dichotomous diagnostic cut off, such as CUD only (22), for which the causes are likely to be complex and involve many mechanisms and predictors . The NIHM RDoC initiative supports research about the biobehavioural dimensions that cut across these prescriptive diagnostics(50). However, such intermediates have remain unexplored for substance use disorders until recently. They are important for understanding biological pathways through which genes shape behaviour. In this study, we took endophenotypes that have strong theoretical and empirical clinical relevance to CUD, potentially more than diagnostic criteria alone, which is a key strength of this highly controlled, experimental study. However, the behavioural genetics approach has also been heavily criticised for its lack of replicability. An inevitable trade-off of this rich phenotyping approach is that the sample size of this study was small and there were unequal numbers of each genotype, with a small amount of missing genetic information. The sample size calculation was based on the effects of THC, not on genetic differences but we strictly corrected for multiple comparisons. Given the small cell sizes, this study was only powered to detect small-to-medium effect sizes. It would be important to replicate these findings with a larger sample size allowing for analysis of a dose-response relationship between genotype and risk. Therefore it is important to consider these results as preliminary. Moreover, we did not use prospective genotyping or account for the effects of ancestry and ethnicity. Finally, we were not able to externally validate the consequences of the SNPs, for example, but assessing anandamide levels in the plasma.

Conclusions

In conclusion, we report for the first time that the genes that code for the CB1 receptor and FAAH enzyme are implicated in the acute CUD-related response to acute consumption of cannabinoids. This was found for the salience of appetitive cues and state satiety, but not for craving. These results have important pharmacogenetic implications in regards to recreational users of cannabis who may be more vulnerable to the effects of THC and who may therefore be at greater risk of transitioning into CUD.

Author Contributions

CJAM and VC designed the protocol. GS and CG conducted the testing assessments. CH, TPF and EB and MB conducted the statistical analysis. CH wrote the manuscript. All authors approved the final version of the manuscript.

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Table 1: Means (SD) for demographic, mental health and cannabis use variables for each of the genotype groups.

	CNR1 rs1049353			CNR1 rs806378			FAAH rs324420		
	GG	AA/AG	Test statistic	CC	CT/TT	Test Statistic	CC	AA/AC	Test statistic
Total N (N female)	20 (7)	22 (6)	$\chi^2(1)=.293$, ns	18 (6)	27 (8)	$\chi^2(1)=.069$, ns	30 (7)	14 (7)	$\chi^2(1)=3.129$, ns
Age	21.90 (1.94)	21.59 (1.94)	$F(1,40)=.265$, ns	21.44 (1.98)	22.00 (1.79)	$F(1,43)=.953$, ns	21.87 (1.92)	21.79 (1.72)	$F(1,43)=.018$, ns
Race/Ethnicity (self-reported)									
White British	*14*	*17*		*12*	*20*		23	8	
Other Ethnic Group	*6*	*5*	$\chi^2(1)=.28$, ns	*6*	*7*	$\chi^2(2)=.005$, ns	7	5	$\chi^2(1)=1.03$, ns
Frequency of cannabis use	19.75 (10.95)	17.72 (10.21)	$F(1,40)=.394$, ns	20.36 (10.15)	17.98 (10.82)	$F(1,43)=.548$, ns	19.53 (17.21)	17.21 (10.21)	$F(1,42)=.452$, ns
Severity of Dependence	4.05 (3.62)	2.09 (2.21)	**F(1,40)=4.585,p=0.038 ***	3.55 (3.70)	2.56 (2.47)	$F(1,43)=1.187$, ns	3.47 (3.26)	1.71 (2.16)	$F(1,42)=3.345$, ns
Last use of cannabis	3.25 (3.17)	7.81 (25.09	$F(1,40)=.652$, ns	2.94 (1.98)	8.00 (23.14)	$F(1,43)=.848$, ns	2.63 (1.87)	13.43 (31.67)	$W(1,12)=1.624$, ns[a]
Years of cannabis use	6.80(2.31)	6.02 (3.05)	$F(1,40)=.854$, ns	6.00 (2.57)	6.31 (2.91)	$F(1,43)=.138$, ns	6.83 (2.64)	4.96 (2.68)	**F(1,42)=3.557,p=.035 ***
SPQ total	19.05 (12.41)	16.55 (15.86)	$F(1,40)=.320$, ns	19.83 (13.43	15.15 (14.32)	$F(1,43)=1.214$, ns	14.07 (9.92)	22.36 (19.46)	$F(1,42)=3.542$, ns
BDI	13.30 (9.42)	7.91 (8.87)	$F(1,40)=3.651$, ns	11.96 (10.79)	8.48 (8.25)	$F(1,43)=1.485$, ns	9.23 (9.16)	10.79 (10.32)	$F(1,42)=.253$, ns
STAI	43.50 (11.40)	40.41 (8.81)	$F(1,40)=.976$, ns	42.44 (11.55)	40.04 (9.63)	$F(1,43)=.575$, ns	40.47 (10.95)	42.14 (10.95)	$F(1,42)=.239$, ns

Notes: [a] - Welch's Test, [b] Includes White Other, mixed white and black Caribbean, mixed white and black African, any other mixed background, Asian/British Asian, any other Asian/British Asian background, Black/British Caribbean, Chinese and any other ethnic group, * indicated significant difference at p ≤0.05

Figure legends

Figure 1. Trial structure for the visual probe task. Example of Cannabis (right) and matched neutral stimuli (left) provided

Figure 2: Mean (±Standard Error) of the single item of the Bodily Symptoms Scale: "want to smoke a joint" averaged across the two time-points. Bonferroni corrected p values are displayed for the drug x genotype interaction. Homozygote GG carriers of CNR1 rs1049353 showed reduced wanting after both THC measures, but A carriers show no such reduction in state satiety.

Figure 3: Mean (±Standard Error) attentional bias, as assessed by the dot probe task, to drug and food stimuli (ms) after drug administration for each genotype group. Bonferroni corrected p values are displayed for the drug x genotype interaction. CNR1 rs1049353 "A" carriers' attentional bias remains relatively constant whilst GG homozygotes vary by cannabinoid administration.

Figure 4: Mean (±Standard Error) attentional bias, as assessed by the dot probe task, after drug administration for each genotype group for FAAH rs324420. Bonferroni corrected p values are displayed for the drug x genotype interaction. FAAH rs324420 "A" carriers' attentional bias remains relatively constant whilst CC homozygotes vary by cannabinoid administration.