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Translational studies support a role for serotonin 2B receptor (*HTR2B*) gene in aggression-related cannabis response

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Abstract

Cannabis use is increasing in the United States, as are its adverse effects. We investigated the genetics of an adverse consequence of cannabis use: cannabis-related aggression (CRA) using a genome-wide association study (GWAS) design. Our GWAS sample included 3,269 African-Americans (AAs) and 2,546 European Americans (EAs). An additional 89 AA subjects from the

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Conflict of interest statement

Although unrelated to the current study, HRK has been a consultant, advisory board member, or CME speaker for Indivior and Lundbeck. He is also a member of the American Society of Clinical Psychopharmacology's Alcohol Clinical Trials Initiative, which in the last three years was supported by Abbvie, Alkermes, Amygdala Neurosciences, Arbor, Ethypharm, Indivior, Lilly, Lundbeck, Otsuka, and Pfizer. No other authors declare possible conflicts.

Grady Trauma Project (GTP) were also examined using a proxy-phenotype replication approach. We identified genome-wide significant risk loci contributing to CRA in AAs at the serotonin receptor 2B receptor gene (*HTR2B*), and the lead SNP, *HTR2B**rs17440378, showed nominal association to aggression in the GTP cohort of cannabis-exposed subjects. *A priori* evidence linked *HTR2B* to impulsivity/aggression but not to cannabis response. Human functional data regarding the *HTR2B* variant further supported our finding. Treating an *Htr2b*$^{-/-}$ knockout mouse with THC resulted in increased aggressive behavior, whereas wild type mice following THC administration showed decreased aggression in the resident-intruder paradigm, demonstrating that *HTR2B* variation moderates the effects of cannabis on aggression. These concordant findings in mice and humans implicate *HTR2B* as a major locus associated with cannabis-induced aggression.

Introduction

Violent behavior is a major public health problem, resulting annually in approximately 1.43 million deaths worldwide[1]. Genetic factors explain 50-63% of the variance in aggressive behavior and several genetic variants that influence aggressive behavior have been identified[2,3]. Monoaminergic genes – identified via unbiased approaches or selected as biological candidates – have been extensively studied in relation to aggression-related traits[3,4]. An apparently unique mutation at the monoamine oxidase A locus (*MAOA*), which encodes an enzyme that catabolizes monoamines (including serotonin), was associated with aggressive behavior in a Dutch kindred[5] after first being localized by genetic linkage analysis[6]. Caspi et al.[7] subsequently reported that carriers of a different low-activity *MAOA* variant exhibited violent behavior only after exposure to moderate or severe levels of child abuse[8]. Sequencing of serotonin-system genes in a Finnish population of impulsive individuals revealed association between a stop codon in the serotonin 2B receptor gene (*HTR2B* Q20*) and risk of committing violent acts[9]. In a subsequent study, *HTR2B* Q20* carriers showed aggressive behavior, alcohol-related impulsivity, and emotional dysregulation[10].

Aggressive behavior is influenced by a combination of genetic and environmental factors, including substance use. A high proportion of all crimes are committed under the influence of substances of abuse[11], and thus aggression while intoxicated is an important subtype of all aggressive behavior. Cannabis, one of the most widely used drugs worldwide, has been linked to increased impulsivity[12] and decreased behavioral inhibition[13,14]. The relationship between cannabis use and aggression has been established[2,15-17]; cannabis use is associated with a 7-fold risk of subsequent violent and aggressive behavior[2]. A recent study of 1,136 subjects from the McArthur Risk Assessment study also found that continuity of cannabis use is associated with increased risk of future violent behavior (OR=2.44)[17].

We first conducted a genome-wide association study (GWAS) of physical aggression occurring under the influence of cannabis in African Americans (AA) and European Americans (EA) and a polygenic risk score (PRS) analysis to determine whether genetic risk factors for personality traits could predict cannabis-related aggression in humans. The GWAS implicated the serotonin 2B receptor gene (*HTR2B*), which was not previously

considered a key cannabis target. We then evaluated the cannabis/*Htr2b* interaction in a mouse knockout model.

Methods

Subjects and Diagnostic Procedures

Discovery cohort—Our GWAS discovery sample included 2,185 AA and 1,362 EA subjects selected from the Yale-Penn sample, all of whom endorsed having used cannabis 10 or more times. The initial sample (Yale-Penn 1) consisted of small nuclear families and unrelated individuals recruited at five US clinical sites: Yale University School of Medicine (APT Foundation, New Haven, CT), the University of Connecticut Health Center (Farmington, CT), the University of Pennsylvania, Philadelphia, PA), the Medical University of South Carolina (Charleston, SC) and McLean Hospital (Belmont, MA). Subjects were recruited for studies of the genetics of drug (opioid or cocaine) or alcohol dependence[18–21]. All subjects provided written informed consent as approved by the institutional review boards at each site, and certificates of confidentiality were obtained from NIDA and NIAAA. Subjects were interviewed using the Semi-Structured Assessment for Drug Dependence and Alcoholism (SSADDA), which yield reliable DSM-IV and DSM-5 criteria and diagnoses. Cannabis-related aggression (CRA) was assessed with the question, “Did you ever get into physical fights while using marijuana?” Results from the Yale-Penn 1 sample were combined via meta-analysis with results from 1,084 AA and 1,184 EA cannabis-exposed subjects who were identically ascertained (Yale-Penn 2 sample). The study cohorts are described in Table 1.

Replication cohort: Grady Trauma Project (GTP)—The subjects for this study were part of a larger investigation of genetic and environmental factors that predict response to stressful life events in a predominantly African American, urban population of low socioeconomic status[22]. All subjects endorsed lifetime prevalence of cannabis use, assessed using the Structured Clinical Interview for DSM-IV (SCID)[23]. Aggressive behavior was assessed using the “Beat some up” item from the Aggressive Behavior Questionnaire (ABQ), a 48-item, self-report inventory of physical and verbal aggression, and criminal offenses including drug use and theft. This scale is based on the Conflict Tactics Scale, a commonly used measurement of relationship conflict behaviors[24]. The aggression measure was examined as a quantitative trait among these cannabis-exposed subjects. Additional details are provided in Table 1.

Genotyping and Quality Control

Discovery cohort—The Yale-Penn samples were genotyped using one of two genotyping arrays: 1) for the Yale-Penn 1 sample, the Illumina HumanOmni1-Quad v1.0 microarray was used (988,306 autosomal SNPs; Illumina, San Diego, CA, USA) at the Center for Inherited Research (CIDR) and the Yale Center for Genome Analysis (YCGA) and 2) for the Yale-Penn 2 sample, the Illumina Infinium Human Core Exome microarray (265,919 exome-focused SNPs and 243,345 tagging SNPs which allow genome-wide imputation) at our lab. All QC and subsequent analyses were performed separately within individuals genotyped on the two platforms. Genotypes were called using GenomeStudio software V2011.1 and

genotyping module V1.8.4 (Illumina, San Diego, CA, USA). SNPs with significantly different allele frequencies (within population) across genotyping centers were set to missing prior to imputation. SNPs were filtered by minor allele frequency (MAF) < 0.01 and imputation score threshold of 0.8. After imputation, data cleaning, and QC, in AAs 15,101,332 SNPs and in EAs 8,365,931 SNPs were successfully meta-analyzed.

To verify and correct the misclassification of self-reported race, we compared the GWAS data from all subjects with the genotypes from the HapMap 3 reference CEU, YRI and CHB, and 1000 Genomes reference AFR, EUR, and ASN populations. Principle components (PC) analysis was conducted in the merged sample using Eigensoft[25,26]. The first 10 PCs were used to remove outliers and to distinguish AAs and EAs; these groups were subsequently analyzed separately. We then conducted PC analyses within the two remaining groups as previously described[27], and the first three PCs were used in all subsequent analyses to correct for residual population stratification.

Grady Trauma Project (GTP) sample: Quality control was performed using the Psychiatric Genomics Consortium PTSD Workgroup guidelines. Additional details are provided in Supplementary Notes.

Statistical Analysis Methods

Association tests were performed using logistic regression models embedded in generalized estimating equations (GEE) to correct for correlations among related individuals[28]. We modeled CRA as a binary variable, analyzed in a standard logistic regression and adjusted for age, sex, and three PCs of ancestry. Analyses were conducted separately within each population group, corrected for the genomic-inflation factor (λ) and combined by meta-analysis using the inverse variance method in the program METAL[29]. Conditional analysis was conducted to determine whether closely mapped genome-wide significant (GWS) SNPs represent independent signals. We conducted statistical testing as previously described, using a logistic regression model embedded in GEE with age, sex, three PCs, and the SNP of interest as covariates.

For the GTP cohort, we also conducted a logistic regression analysis to determine the association between rs17440378 and aggression-related trait in AA subjects with a lifetime prevalence of cannabis. The aggression-related trait used was “Beat someone up”, and modeled as an ordinal variable (Never=0, Once=1, Sometime=2, Many times=3, More than I can count=4) with age, sex, and first 3 PCs as covariates.

We used publicly available bioinformatics tools to identify the functional effects of the GWS variants found in our CRA GWAS. Additional details are provided in Supplementary Notes.

Polygenic risk score analysis

To test for pleiotropy between the NEO personality factors and CRA, we conducted a PRS analysis using GWAS data from the five-factor model traits of neuroticism, extraversion, openness, agreeableness and conscientiousness, measured with the NEO Personality Inventory (NEO PI-R)[30]. The public summary data were downloaded from the website of

the Genetics of Personality Consortium (GPC, http://www.tweelingenregister.org/GPC/). Additional details are provided in Supplementary Notes.

Htr2b knockout mice, THC administration and behavioral phenotyping

A total of 34 adult mice (20 wild type, 14 *Htr2b*$^{-/-}$ knockout) of 129SvPas background were used in the study; wild type (WT) mice were used as a control group. Saline or THC (10 mg/kg) was administered by intraperitoneal (i.p.) injection, 1 hour before test. Animals were randomly balanced in agreement to genotype and treatment during the experiment. The selected THC dose was based on a previous study showing that THC (10 mg/kg) decreases social interaction in mice[31]. Behavioral test, and animal care were conducted in accordance with the standard ethical guidelines (National Institutes of Health's 'Guide for the care and use of laboratory animals', and European Directive 2010/63/UE). All of the experiments involving mice were approved by the local ethical committee (N°1170.02). Aggressive behavior was investigated using the resident-intruder test adapted from Koolhaas and colleagues[32] and performed 1 hour after i.p. injection. Behavioral scoring was carried out by blinded investigators using the JWatcher™ software (University of California, LA, USA, and Macquarie University, Sidney, Australia). Additional details are provided in Supplementary Notes.

Behavioral data were analyzed using two-way ANOVA with genotype (knockout vs. WT) and treatment (saline vs. THC) as between-subjects factors. Tukey's test was used for post hoc comparisons with significance set at $p < 0.05$. Statistical outliers, detected by Grubb's test, were excluded from the final analysis. Analyses were carried-out using GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA, USA).

Results

We studied two samples, Yale-Penn 1 and Yale-Penn 2[27]. In Yale-Penn 1, the prevalence of cannabis-related aggression (CRA) was 13.9% in AAs and 10.8% in EAs, while in Yale-Penn 2 it was 13.1% in AAs and 8.4% in EAs (Table 1). GWAS results are summarized in Manhattan plots (Supplementary Figure 1*), Table 2, and* Supplementary Tables 1 and 2. Quantile-Quantile plots are shown in Supplementary Figures 2 and 3.

GWAS of cannabis-related aggression identified a GWS locus at *HTR2B/PSMD1*.

We identified one genome-wide significant (GWS) region on chromosome 2 in AAs (Regional Manhattan plot, Figure 1). The top SNPs included rs35750632 (β=0.54, $P=1.79\times10^{-8}$) in the proteasome 26S subunit, non-ATPase 1 gene (*PSMD1*) and rs17440378 (β=0.57, $P=2.16\times10^{-8}$) in *HTR2B*. The coding region of *HTR2B* is contained within that of *PSMD1* (Figure 1); the lead variants are ~43 kb apart and in high LD (r^2= 0.42, D'= 0.98). Conditional analysis confirmed that both SNPs reflect the same association signal. There was no GWS SNPs in EAs.

In AAs only, there was a dose-dependent relationship between CRA and *HTR2B**rs17440378 genotype (Supplementary Figure 4), with TT genotype (MAF=0.1) associated with higher CRA: CC (n=2587), CRA=12.1%; CT (n=640), CRA=18.9%; TT (n=42), CRA=28.6%. Genome-wide meta-analysis of AA and EA samples showed no

additional GWS SNPs. P-values of association, effect sizes, and effect directions of *HTR2B**rs17440378 for CRA are shown in Figure 2 (upper panel).

Annotation analysis was conducted using publicly available bioinformatics tools (Braineac, GTEx, and HaploReg v4.1) to identify the functional effects of the GWS variants found in the CRA GWAS. We found that *HTR2B**rs17440378 is an eQTL for *HTR2B* and nearby genes (*PSMD1, C2orf72, DIS3L2, SP140, B3GNT7)* across several brain regions, (e.g., medulla, substantia nigra, and putamen) and peripheral tissue (Supplementary Tables 3 and 4; Supplementary Figures 5-7). Multiple epigenetic marks were also identified for *HTR2B**rs17440378 (Supplementary Tables 5 and 6), suggesting a functional regulatory role for this GWS SNP.

Grady Trauma Project (GTP) sample: A total of 89 AA subjects with data available for cannabis and aggression had previously been genotyped using the Illumina Omni-Quad 1M Array[33]; the *HTR2B**rs17440378 variant was imputed (r^2=0.99). *HTR2B**rs17440378 show a significant association (p=0.04, one sided) with aggression in subjects with a lifetime prevalence of cannabis, with an effect size similar to that in the discovery sample (Table 2).

Polygenic risk score analysis for CRA and NEO factors.

Using *P*-value thresholds of 0.001 and 0.005, polygenic risk scores (PRS) of extraversion were significantly associated with CRA, with the most significant result obtained for the SNP set (n=6,772) with a *P*-value threshold of 0.001 (Figure 3; Supplementary Table 7). Extraversion PRS predicted greater CRA risk (p=5.70×10^{-3}, β=0.67, S.E.=0.24).

THC increases aggressive behavior in Htr2b$^{-/-}$ mice.

Htr2b$^{-/-}$ mice exhibited more impulsive behaviors and greater novelty-induced locomotion than wildtype (WT) mice[9]. In the present study, *Htr2b*$^{-/-}$ mice exhibited highly aggressive behavior (p<0.001 vs. WT), as shown by a significant effect of genotype on Aggressive Behavior ($Fs_{(1,30)}$ = 413.6 and 118.7, respectively for frequency and duration, P < 0.0001 for both) and had less social interaction (p<0.001 vs. WT) in the resident-intruder paradigm (Figure 4).

A significant effect of the treatment × genotype interaction on both Aggressive Behavior ($Fs_{(1,30)}$ = 21.25 and 17.26, respectively for frequency and duration, P < 0.0001 and P =0.0002) and Social Investigation ($Fs_{(1,30)}$ = 7.553 and 6.017, respectively for frequency and duration, P < 0.0001 for both) showed a differential response to THC treatment for the two genotype groups. Indeed, acute THC treatment (10 mg/kg, i.p.) induced an opposite effect on aggressive response as a function of genotype: *post-hoc* comparisons showed increased aggression in *Htr2b*$^{-/-}$ mice (p<0.05 vs. *Htr2b*$^{-/-}$ saline control) and reduced aggression in WT mice (p<0.01 vs. WT saline control) (Figure 4A and B). Further, THC significantly increased the propensity to social investigation only in WT mice (p<0.05 vs. WT saline control; Figure 4C and D), but not in in *Htr2b*$^{-/-}$ mice.

Htr2b$^{-/-}$ mice were also hyperactive, as shown by the statistically significant interaction of treatment × genotype on crossing frequency ($F_{(1,30)}$ = 16.55, P = 0.0003; Supplementary Figure 8). *Post-hoc* analysis revealed that saline-treated *Htr2b*$^{-/-}$ mice crossed the cage

surface more than saline-treated WT mice ($p < 0.001$). THC treatment decreased locomotion in both WT and *Htr2b*$^{-/-}$ mice ($p < 0.01$ and $p < 0.001$, respectively, vs. each respective saline control) (Supplementary Figure 8).

Discussion

In this first GWAS of CRA, we report GWS evidence linking *HTR2B* to cannabis response. We identified one GWS region mapped to *HTR2B*, which overlaps *PSMD1*. Functional analysis revealed genomic regulatory roles for *HTR2B**rs17440378, mainly in brain tissue. *HTR2B**rs17440378 also showed nominally significant association in the GTP sample with a related (but non-identical) phenotype. This specific locus is one of the best supported in previous studies of aggression phenotypes (that unlike the present study, used a candidate-locus approach)[9,10] and the serotonin system is very well established as being related to traits involving violence and impulsivity. However, although serotonergic function is strongly implicated in cannabis response[34], we are aware of no prior evidence implicating this particular receptor. PRS analysis showed that extraversion was significantly associated with CRA; higher extraversion predicted greater risk for CRA. Further, *Htr2b*$^{-/-}$ mice that received THC exhibited a greater aggressive response than WT mice, with the latter showing a reduced aggressive response, suggesting that *Htr2b* modulates aggression-related cannabis response.

Aggressive behavior is influenced by both genetic and environmental factors, including substance use. Cannabis use is associated with greater impulsive decision making[12] and less behavioral inhibition[14], critical contributors to risk-taking, substance use, and aggressive behavior. Cannabis use and cannabis withdrawal also contribute to aggression and related traits[35]; cannabis use is associated with increased subsequent violent behavior[2,17]. The identification of genetic factors contributing to the risk for aggressive behavior following cannabis use provides opportunities both for prevention and treatment.

Our CRA GWAS identified GWS loci at and near *HTR2B*. It is noteworthy that this GWAS, by nature hypothesis-free, landed on this gene, as it has previously been closely associated with impulsivity and aggressive behaviors in both humans and animal models. A stop codon variant of *HTR2B* (*HTR2B* Q20*) was associated to impulsivity in Finns[9]. A follow-up functional annotation analysis of rs17440378, the *HTR2B* intronic polymorphism, provided the best evidence of functionality based on its numerous regulatory effects in brain and peripheral tissues. *HTR2B**rs17440378 is an eQTL for *HTR2B* and nearby genes. Multiple regulatory epigenetic marks were identified for rs17440378 in brain and peripheral tissue, including anterior caudate, involved in reward and cognitive function[36], and cingulate gyrus, implicated in schizophrenia[37] and threat processing in humans[38]. Based both on its demonstrated contribution to aggressive behavior and our functional annotation analysis, *HTR2B* appears to be the relevant gene rather than *PSMD1*.

The risk effect of *HTR2B**rs17440378 appeared to be specific to individuals who are aggressive under the influence of cannabis, rather than being driven by drug dependence alone, aggression alone, or aggression referable to drugs other than cannabis (Figure 2). Decreased or null expression of *HTR2B* has been linked to impulsivity[9,10], schizophrenia-

like behaviors[39], impaired social interaction[39], and resistance to selective serotonin reuptake inhibitor antidepressants[40]. We extended these findings by showing that *Htr2b*$^{-/-}$ mice exhibit greater aggressive behavior and decreased social interaction than WT mice in the resident-intruder test. *HTR2B* variation may therefore interact with cannabis use to induce aggression by reducing brain monoaminergic tone. Interestingly, null expression or blockade of 5-HT$_{2B}$ receptors diminishes the reinforcing effects of psychoactive drugs by modulating serotonin[41] and dopamine[42] signaling. In this study, we found that THC induces opposite effects in aggressive behavior and social interaction by *Htr2b* status: THC increased aggression and produced no change in social investigation in *Htr2b*$^{-/-}$ mice, while it decreased aggression and increased social investigation in WT mice.

Because personality traits may contribute to the comorbidity of psychiatric disorders, we conducted a PRS analysis to estimate the genetic overlap of CRA with personality traits. PRS for extraversion was associated with CRA, such that higher extraversion showed a higher risk for cannabis-related aggression. Extraversion is positively associated with externalizing behavior [43]. Further, extraversion has previously been linked to excitement- and attention-seeking behavior, as well as social and interpersonal dysfunction[44].

Our human study is limited by the comparatively small sample size, especially among EAs. Significant results were observed in AAs only, an effect that may be population-specific. Although the association was not statistically significant in EAs, the effect direction was the same, such that a meta-analysis of both populations yielded a *p*-value of 3.06×10^{-7}, i.e., it reduced significance. Thus, the effect may also be present in EAs, but a larger sample is needed to evaluate this possibility. Larger samples with additional phenotypic information would also be required to assess additional relevant correlated or confounding factors such as testosterone levels, psychosocial environment, and socioeconomic status, as well as impulsivity traits. In addition, human genomic and precision medicine research is limited by a lack of racial diversity, with 96% of GWAS participants reportedly being of European descent[45]. This limited our ability to identify an AA cohort of adequate size with the phenotypic assessment necessary to replicate our findings.

However, by using a proxy phenotype approach and a within-trait assessment, we found a significant association of *HTR2B**rs17440378 and aggression in AA subjects with a history of cannabis use. This proxy approach supported out major GWAS finding, but is inherently limited (in this case, by both the phenotype definition and the sample size), and we emphasize the need for stricter replication. The lack of direct assessment of the effects of *HTR2B**rs17440378 on protein function, including regulation, is a limitation of our study. Nonetheless, eQTL analysis enabled us to understand the functional effect of this risk variant on genomic regulation of *HTR2B* and nearby genes in peripheral and brain tissue. Further, there is clear biological relevance of the GWS locus, *HTR2B*, a gene previously shown to play an important role in aggression and impulsivity traits. The prior evidence for this gene and the findings from the animal model further support the role of the gene in CRA.

Our findings support a role of 5-HT$_{2B}$ receptors in the modulation of the aggression-related cannabis response, identify a specific mechanism that could result in violence in the context

of cannabis use, and provide the first evidence that this is an important site for cannabis response. Medications acting at 5-HT_{2B} receptors may be relevant in modifying such behaviors acutely. For example, an 5-HT_{2B} receptor antagonist, such as the widely used antipsychotic aripiprazole, may, based on the evidence presented herein, exacerbate cannabis-related aggression, an interesting idea given its effects on the response to the acute administration of methamphetamine[46] and in the treatment of depression[47], schizophrenia[48], and agitation in autism[49].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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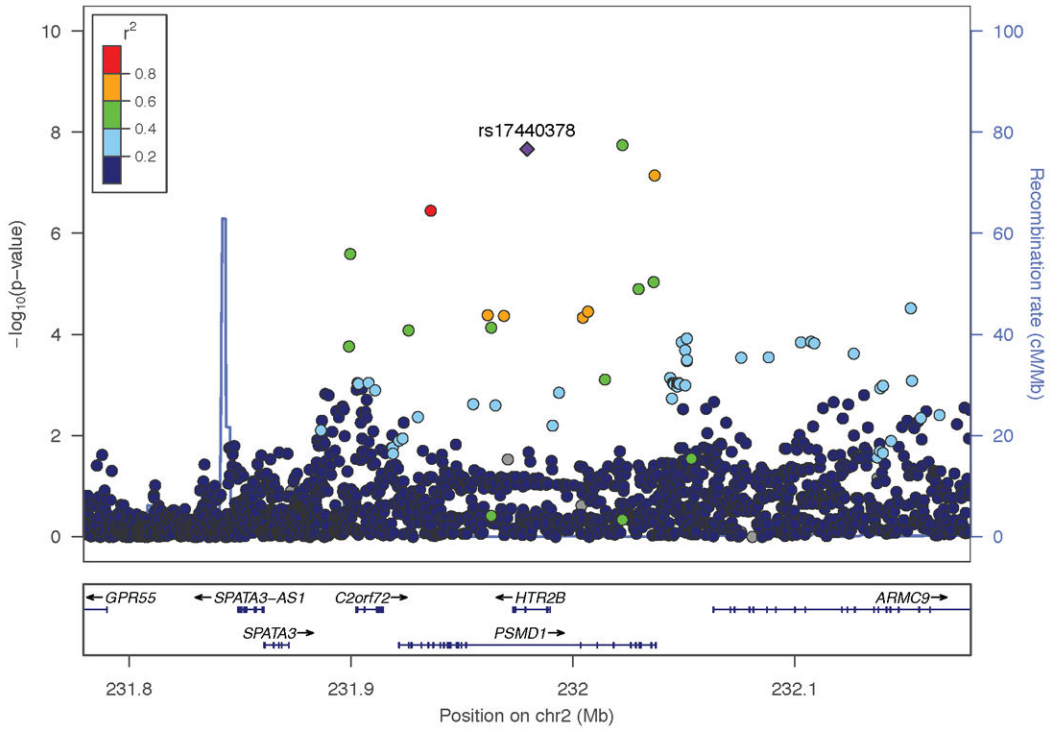

Figure 1. Association results for cannabis-related aggression (CRA) in African Americans.

Regional association plot of 231.8- to 232.1-MB region on chromosome 2 encompassing *HTR2B* and *PSMD1* in the Yale-Penn African-American participants after meta-analysis. This plot shows the genome-wide significant association between cannabis-related aggression and a single nucleotide polymorphism (SNP) rs17440378 (purple) at the *HTR2B* locus. Each circle represents a SNP, the left y-axis reflects the $-\log_{10}(P\text{ value})$, and the light blue line and right axis the observed recombination rate. Color coding (dark blue, blue, green, yellow, and red) shows the degree of linkage disequilibrium (r^2) between rs17440378 (purple, diamond shaped) and other SNPs in the region. Centimorgan (cM), megabase (Mb).

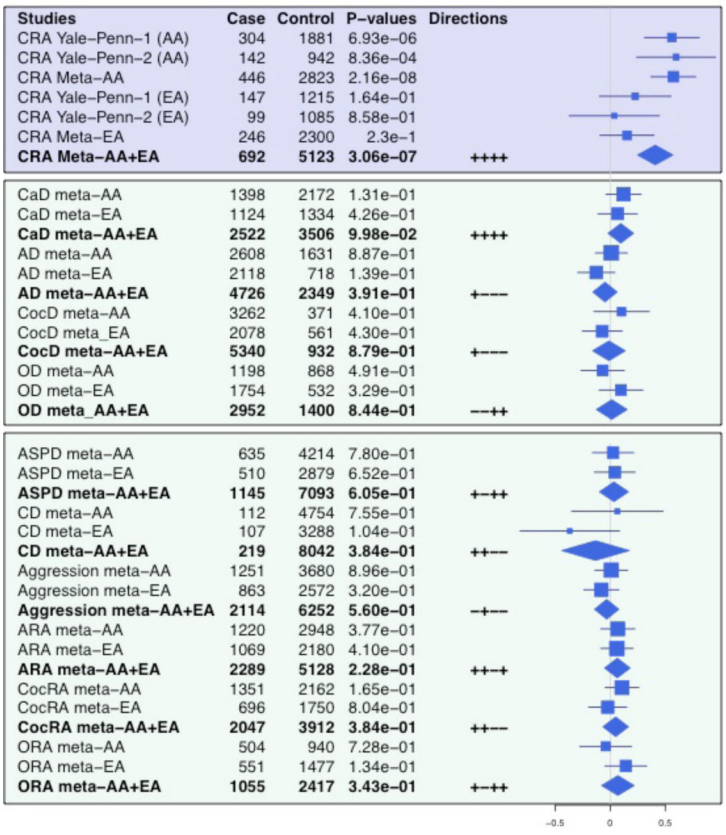

Figure 2. Forest plot of rs17440378 (chr2:231979355), a genome-wide significant SNP for cannabis-related aggression (CRA).

The blue lines represent 95% confidence intervals of the effect size estimates. The blue rectangles are proportional to the square-root of the sample size. The blue diamonds represent the meta-analysis estimate of AAs and EAs. *Abbreviations:* Cannabis related aggression (CRA), cannabis dependence (CaD), alcohol dependence (AD), cocaine dependence (CocD), opioid dependence (OD), antisocial personality disorder (ASPD), conduct disorder (CD), alcohol-related aggression (ARA), cocaine-related aggression (CocRA), opioid-related aggression (ORA).

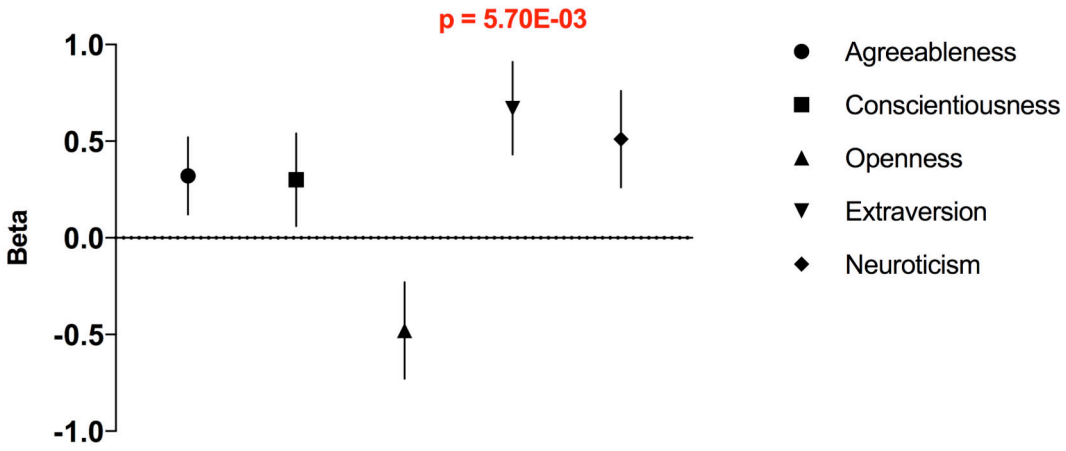

Figure 3. Results of polygenic risk score analyses predicting cannabis-related aggression (CRA) based on polygenic risk scores from NEO personality factors.

Polygenic risk scores (PRS) were calculated using *P*-value thresholds of 0.00001 – 0.5. (A) We show the PRS of *P*-value threshold of 0.0001 for agreeableness, and 0.001 for conscientiousness, openness, extraversion, and neuroticism. Extraversion polygenic risk scores were significantly positively associated with CRA ($p = 5.70 \times 10^{-3}$, β= 0.67, S.E. = 0.24). Error bars represent mean ± standard error (S.E.).

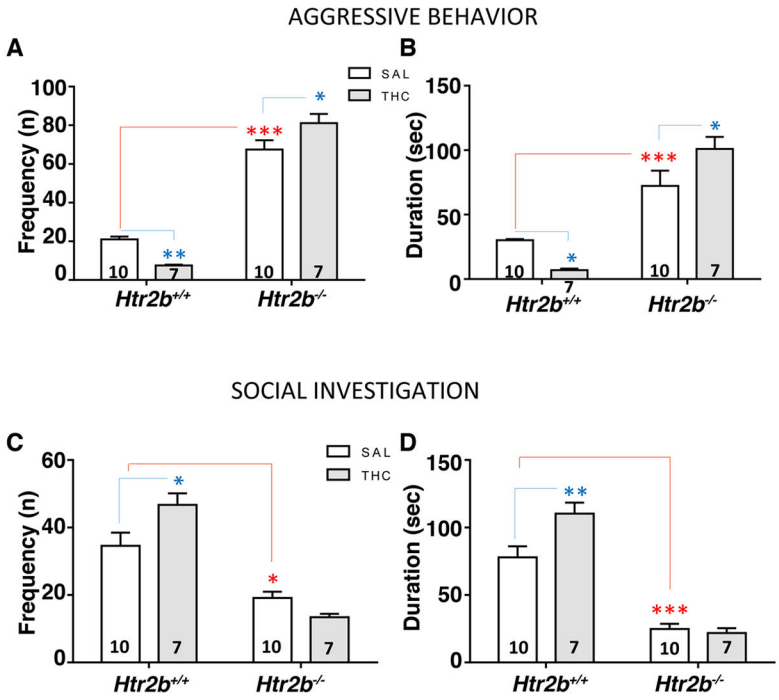

Figure 4. THC increases aggressive behavior in *Htr2b*$^{-/-}$ mice.

Effects of acute THC treatment (10 mg/kg, i.p.) on aggressive behavior and social interaction in *Htr2b*$^{-/-}$ and WT (*Htr2b*$^{+/+}$) mice. (A-B) *Htr2b*$^{-/-}$ mice showed more aggressive behavior than WT. THC treatment significantly increased the aggressive response in *Htr2b*$^{-/-}$ mice; an opposite and significant effect was observed in WT mice. (C-D) THC treatment reduced social investigation in *Htr2b*$^{-/-}$ mice. Genotype effect: *** and * = $P <$ 0.001 and 0.05, respectively. Treatment effect: ** and * = $P < 0.01$ and 0.05, respectively. Error bars represent mean ± standard error (S.E.).

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Table 1.

Demographic characteristics of the study cohorts.

	Yale-Penn 1		Yale-Penn 2		GTP
Characteristic	**African American (*n*=2185)**	**European American (*n*=1362)**	**African American (*n*=1084)**	**European American (*n*=1184)**	**African American (*n*=89)**
Age, mean (SD), y	41 (8.4)	36 (10.4)	40 (10.6)	37 (12.2)	45 (10.7)
Female sex, No. (%)	852 (39.0)	501 (36.8)	331 (30.5)	374 (31.6)	44 (49.4)
CRA, *n* (%)	304 (13.9)	147 (10.8)	142 (13.1)	99 (8.4)	N/A
CaD, *n* (%)	895 (49.4)	566 (55.2)	503 (55.6)	506 (61.3)	76 (85.4) *
AD, *n (%)*	1322 (71.2)	865 (82.2)	741 (80.4)	787 (87.7)	65 (73.0) *
CocD, *n* (%)	1892 (87.6)	1070 (82.1)	675 (64.3)	621 (61.1)	42 (47.7) *
OD, *n* (%)	637 (29.8)	868 (65.9)	295 (27.7)	573 (55.2)	5 (5.7) *
Age of onset cannabis use, mean (SD), y	15 (4.6)	14 (3.7)	15 (5.6)	14 (2.8)	N/A
More than 1 before 15, *n* (%)	945 (43.3)	720 (52.9)	438 (40.4)	527 (48.6)	N/A
Years exposed to cannabis, mean (SD), y	21 (13.0)	15 (11.7)	19 (13.1)	14 (12.8)	N/A
Frequency cannabis use (>100), *n* (%)	1969 (90.1)	1259 (57.6)	970 (44.4)	930 (42.6)	
Aggression (physical fights), *n* (%)	1362 (62.6)	791 (58.2)	702 (64.8)	611 (56.4)	N/A
Aggression (beat someone up), *n* (%)					
Never	N/A	N/A	N/A	N/A	27 (30.3)
Once	N/A	N/A	N/A	N/A	9 (10.1)
Several times	N/A	N/A	N/A	N/A	35 (39.3)
Many times	N/A	N/A	N/A	N/A	11 (12.4)
More than I can count	N/A	N/A	N/A	N/A	7 (7.9)
ASPD, *n* (%)	340 (15.9)	232 (17.4)	207 (19.6)	213 (19.9)	N/A
CD, *n* (%)	56 (2.6)	49 (3.7)	29 (2.7)	33 (3.1)	N/A

Abbreviations: SD, standard deviation; CRA, cannabis-related aggression; CaD, cannabis dependence; AD, alcohol dependence; CocD, cocaine dependence; OD, opioid dependence; ASPD, antisocial personality disorder; CD, conduct disorder; N/A, not applicable.

* = Substance abuse and/or dependence.

Table 2.

Results for *HTR2B**rs17440378 from the analysis of cannabis-related aggression (CRA) and replication.

Sample	*n*	*n* Cases	Direction	Effect	SE	MAF	MAF Controls	MAF Cases	*P*-value
Yale-Penn 1	2185	304	+	0.56	0.13	0.11	0.10	0.16	6.93E-06
Yale-Penn 2	1084	142	+	0.59	0.17	0.12	0.11	0.16	8.36E-04
Yale-Penn Meta	3269	446	+	0.57	0.10	0.11	0.10	0.16	2.16E-08
GTP	89	N/A	+	0.48*	0.27	0.13	N/A	N/A	4.00E-02**

Abbreviations: SE, standard error; MAF, minor allele frequency; N/A, not applicable

* This represents the odds ratio.

** One-sided *P*-value