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## **Translational studies support a role for serotonin 2B receptors 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 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 a mouse *Htr2b*<sup>-/-</sup> knockout with THC resulted in increased aggressive behavior in the resident-intruder paradigm, while the opposite effect was observed in wild type mice following THC administration, 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<sup>1</sup>. Genetic factors explain 50-63% of the variance<sup>2,3</sup>; several studies have identified genetic variants that influence aggressive behavior. Monoaminergic genes – identified via unbiased approaches or selected as biological candidates – have been extensively studied in relation to aggression-related traits<sup>3,4</sup>. 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<sup>5</sup> after first being localized by genetic linkage analysis<sup>6</sup>. Caspi et al.<sup>7</sup> 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<sup>8</sup>. 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<sup>9</sup>. In a subsequent study, *HTR2B* Q20\* carriers showed aggressive behavior, alcohol-related impulsivity, and emotional dysregulation<sup>10</sup>.

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<sup>11</sup>, 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<sup>12</sup> and decreased behavioral inhibition<sup>13,14</sup>. The relationship between cannabis use and aggression has been established<sup>2,15-17</sup>; cannabis use is associated with a 7-fold risk of subsequent violent and aggressive behavior<sup>2</sup>. 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)<sup>17</sup>.

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<sup>18-21</sup>. 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<sup>22</sup>. All subjects endorsed lifetime prevalence of cannabis use, assessed using the Structured Clinical Interview for the DSM-IV (SCID)<sup>23</sup>. 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<sup>24</sup>. The aggression measure was examined as a quantitative trait among these cannabis-exposed subjects.

### **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<sup>25,26</sup>. 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<sup>27</sup>, 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<sup>28</sup>. 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 ( $\lambda$ ) and combined by meta-analysis using the inverse variance method in the program METAL<sup>29</sup>. 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 a continuous 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)<sup>30</sup>. 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 mice (20 wild type, 14 *Htr2b*<sup>-/-</sup> 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. The selected THC dose was based on a previous study showing that THC (10 mg/kg) decreases social interaction in mice<sup>31</sup>. 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 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<sup>32</sup> and performed 1 hour after i.p. injection. 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  $p < 0.05$ . 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<sup>27</sup>. 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 ( $\beta=0.54$ ,  $P=1.79 \times 10^{-8}$ ) in the proteasome 26S subunit, non-ATPase 1 gene (*PSMD1*) and rs17440378 ( $\beta=0.57$ ,  $P=2.16 \times 10^{-8}$ ) 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. A 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 size, and effect direction 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<sup>33</sup>; 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 4; Supplementary Table 7). Extraversion PRS predicted greater CRA risk ( $p=5.70 \times 10^{-3}$ ,  $\beta=0.67$ , S.E.=0.24).

#### **THC increases aggressive behavior in *Htr2b*<sup>-/-</sup> mice.**

*Htr2b*<sup>-/-</sup> mice exhibited more impulsive behaviors and greater novelty-induced locomotion than wildtype (WT) mice<sup>9</sup>. In the present study, *Htr2b*<sup>-/-</sup> mice were abnormally aggressive ( $p < 0.001$  vs. WT), as shown by the significant effect of genotype on Aggressive Behavior ( $F_{S(1,30)} = 413.6$  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 treatment x genotype interaction for both Aggressive Behavior ( $F_{S(1,30)} = 21.25$  and  $17.26$ , respectively for frequency and duration,  $P < 0.0001$  and  $P = 0.0002$ ) and Social Investigation ( $F_{S(1,30)} = 7.553$  and  $6.017$ , respectively for frequency and duration,  $P < 0.0001$  for both) suggested a different response to THC treatment on the two genotype groups. Indeed, acute THC treatment (10 mg/kg, i.p.) induced an opposite effect on aggressive response by genotype: *post-hoc* comparisons showed increased aggression in *Htr2b*<sup>-/-</sup> mice ( $p < 0.05$  vs. *Htr2b*<sup>-/-</sup> 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 *Htr2b*<sup>-/-</sup> mice.

*Htr2b*<sup>-/-</sup> mice were also hyperactive, as shown by the statistically significant effect of treatment x genotype interaction for crossing frequency ( $F_{(1,30)} = 16.55$ ,  $P = 0.0003$ ; Supplementary Figure 8). In particular, *post-hoc* analysis revealed that saline *Htr2b*<sup>-/-</sup> mice crossed more the cage surface compared saline WT mice ( $p < 0.001$ ). Furthermore, THC treatment decreased locomotion in both WT and *Htr2b*<sup>-/-</sup> mice (respectively,  $p < 0.01$  and  $p < 0.001$  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)<sup>9,10</sup> 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<sup>34</sup>, 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*<sup>-/-</sup> 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<sup>12</sup> and less behavioral inhibition<sup>14</sup>, critical contributors to risk-taking, substance use, and aggressive behavior. Cannabis use and cannabis withdrawal also contribute to aggression and related traits<sup>35</sup>; cannabis use is associated with increased subsequent violent behavior<sup>2,17</sup>. 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<sup>9</sup>. 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<sup>36</sup>, and cingulate gyrus, implicated in schizophrenia<sup>37</sup> and threat processing in humans<sup>38</sup>. 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<sup>9,10</sup>, schizophrenia-like behaviors<sup>39</sup>, impaired social interaction<sup>39</sup>, and resistance to selective serotonin reuptake inhibitor antidepressants<sup>40</sup>. We extended these findings by showing that *Htr2b*<sup>-/-</sup> 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<sub>2B</sub> receptors diminishes the reinforcing effects of psychoactive drugs by modulating serotonin<sup>41</sup> and dopamine<sup>42</sup> signaling. In this study, we found that THC induces opposite effects in aggressive behavior and social interaction by *Htr2b* status: THC increased aggression and no change in social investigation in *Htr2b*<sup>-/-</sup> mice, while it decreased aggression and increased social investigation in WT mice.

Personality traits may contribute to the comorbidity of psychiatry disorders, so we conducted a PRS analysis to determine the genetic overlap of CRA and personality traits. Our results indicated that PRS for extraversion were associated with CRA, with higher Extraversion also shows high genetic correlation with bipolar disorder and attention deficit hyperactivity disorder (ADHD). Further, extraversion is positively associated with externalizing behavior<sup>43</sup>, and it has been linked to excitement- and attention-seeking behavior, as well as social and interpersonal dysfunction<sup>44</sup>.

Our human study is limited by the sample size, especially among EAs. Significant results were observed in AAs only, an effect that may be population-specific. Although the association is not statistically significant in EAs, the effect direction is the same; the meta-analysis of both populations yielded a  $p$ -value of  $3.06 \times 10^{-7}$ , 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 possibly-correlated or confounding factors such as testosterone levels, psychosocial environment, and socio-economic status. 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<sup>45</sup>. This limited our ability to identify an AA cohort of adequate size with the phenotypic assessment necessary to replicate our findings. However, we found a significant association of *HTR2B*\*rs17440378 and aggression in AA subjects with a history of cannabis use. The lack of direct assessment of the effects of *HTR2B*\*rs17440378 on protein function, including regulation, is a limitation of our study; however, eQTL analysis helped us to understand the functionality of this risk variant in 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 this gene in CRA.

Our findings support a role of 5-HT<sub>2B</sub> 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<sub>2B</sub> receptors may be relevant in modifying such behaviors acutely. For example, an 5-HT<sub>2B</sub> 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<sup>46</sup> and in the treatment of depression<sup>47</sup>, schizophrenia<sup>48</sup>, and agitation in autism<sup>49</sup>.

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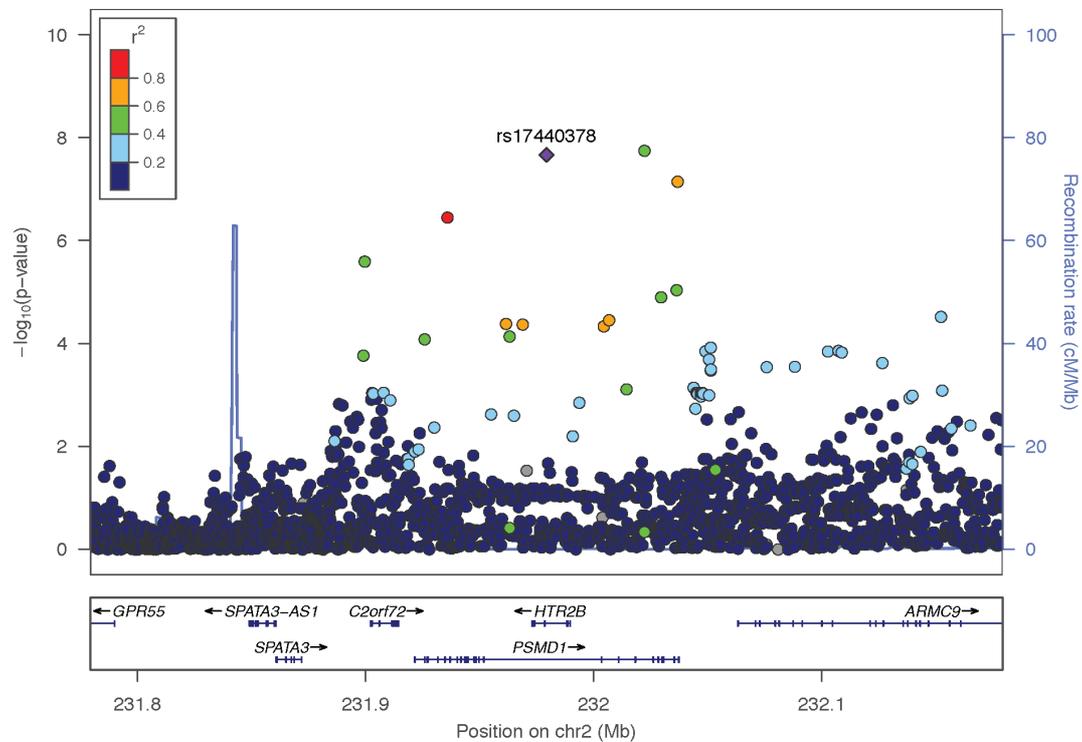
## References:

1. Siever LJ. Neurobiology of aggression and violence. *Am J Psychiatry*. 2008;165(4):429-442.
2. Schoeler T, Theobald D, Pingault JB, et al. Continuity of cannabis use and violent offending over the life course. *Psychol Med*. 2016;46(8):1663-1677.
3. Veroude K, Zhang-James Y, Fernandez-Castillo N, Bakker MJ, Cormand B, Faraone SV. Genetics of aggressive behavior: An overview. *American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*. 2016;171B(1):3-43.
4. Waltes R, Chiocchetti AG, Freitag CM. The neurobiological basis of human aggression: A review on genetic and epigenetic mechanisms. *American journal of medical genetics. Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*. 2016;171(5):650-675.
5. Brunner HG, Nelen M, Breakefield XO, Ropers HH, van Oost BA. Abnormal behavior associated with a point mutation in the structural gene for monoamine oxidase A. *Science*. 1993;262(5133):578-580.
6. Brunner HG, Nelen MR, van Zandvoort P, et al. X-linked borderline mental retardation with prominent behavioral disturbance: phenotype, genetic localization, and evidence for disturbed monoamine metabolism. *Am J Hum Genet*. 1993;52(6):1032-1039.
7. Caspi A, McClay J, Moffitt TE, et al. Role of genotype in the cycle of violence in maltreated children. *Science*. 2002;297(5582):851-854.
8. Weder N, Yang BZ, Douglas-Palumberi H, et al. MAOA genotype, maltreatment, and aggressive behavior: the changing impact of genotype at varying levels of trauma. *Biological psychiatry*. 2009;65(5):417-424.
9. Bevilacqua L, Doly S, Kaprio J, et al. A population-specific HTR2B stop codon predisposes to severe impulsivity. *Nature*. 2010;468(7327):1061-1066.
10. Tikkanen R, Tiihonen J, Rautiainen MR, et al. Impulsive alcohol-related risk-behavior and emotional dysregulation among individuals with a serotonin 2B receptor stop codon. *Translational psychiatry*. 2015;5:e681.
11. Karberg JCJ, D.J. Substance Dependence, Abuse, and Treatment of Jail Inmates, 2002. In: Statistics BoJ, ed2005.
12. Solowij N, Jones KA, Rozman ME, et al. Reflection impulsivity in adolescent cannabis users: a comparison with alcohol-using and non-substance-using adolescents. *Psychopharmacology (Berl)*. 2012;219(2):575-586.
13. Gruber SA, Dahlgren MK, Sagar KA, Gonenc A, Killgore WD. Age of onset of marijuana use impacts inhibitory processing. *Neurosci Lett*. 2012;511(2):89-94.
14. Bhattacharyya S, Atakan Z, Martin-Santos R, et al. Impairment of inhibitory control processing related to acute psychotomimetic effects of cannabis. *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology*. 2015;25(1):26-37.
15. Renard J, Krebs MO, Le Pen G, Jay TM. Long-term consequences of adolescent cannabinoid exposure in adult psychopathology. *Front Neurosci*. 2014;8:361.
16. Barthelemy OJ, Richardson MA, Cabral HJ, Frank DA. Prenatal, perinatal, and adolescent exposure to marijuana: Relationships with aggressive behavior. *Neurotoxicol Teratol*. 2016;58:60-77.
17. Dugre JR, Dellazizzo L, Giguere CE, Potvin S, Dumais A. Persistency of Cannabis Use Predicts Violence following Acute Psychiatric Discharge. *Frontiers in psychiatry*. 2017;8:176.
18. Gelernter J, Kranzler HR, Sherva R, et al. Genome-wide association study of opioid dependence: multiple associations mapped to calcium and potassium pathways. *Biological psychiatry*. 2014;76(1):66-74.
19. Gelernter J, Sherva R, Koesterer R, et al. Genome-wide association study of cocaine dependence and related traits: FAM53B identified as a risk gene. *Molecular psychiatry*. 2014;19(6):717-723.
20. Gelernter J, Kranzler HR, Sherva R, et al. Genome-wide association study of nicotine dependence in American populations: identification of novel risk loci in both African-Americans and European-Americans. *Biological psychiatry*. 2015;77(5):493-503.

21. Gelernter J, Kranzler HR, Sherva R, et al. Genome-wide association study of alcohol dependence: significant findings in African- and European-Americans including novel risk loci. *Molecular psychiatry*. 2014;19(1):41-49.
22. Gillespie CF, Bradley B, Mercer K, et al. Trauma exposure and stress-related disorders in inner city primary care patients. *Gen Hosp Psychiatry*. 2009;31(6):505-514.
23. First MB, Spitzer RL, Gibbon M, JBW W. *Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I Research Version 2.0)*. New York: Biometrics Research; 1998.
24. Straus MA, Douglas EM. A short form of the Revised Conflict Tactics Scales, and typologies for severity and mutuality. *Violence Vict*. 2004;19(5):507-520.
25. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nature genetics*. 2006;38(8):904-909.
26. Patterson N, Price AL, Reich D. Population structure and eigenanalysis. *PLoS genetics*. 2006;2(12):e190.
27. Sherva R, Wang Q, Kranzler H, et al. Genome-wide Association Study of Cannabis Dependence Severity, Novel Risk Variants, and Shared Genetic Risks. *JAMA Psychiatry*. 2016;73(5):472-480.
28. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. *Biometrics*. 1986;42(1):121-130.
29. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics (Oxford, England)*. 2010;26(17):2190-2191.
30. Costa PT, Jr., McCrae RR. Stability and change in personality assessment: the revised NEO Personality Inventory in the year 2000. *J Pers Assess*. 1997;68(1):86-94.
31. Long LE, Chesworth R, Huang XF, McGregor IS, Arnold JC, Karl T. Transmembrane domain Nrg1 mutant mice show altered susceptibility to the neurobehavioural actions of repeated THC exposure in adolescence. *The international journal of neuropsychopharmacology*. 2013;16(1):163-175.
32. Koolhaas JM, Coppens CM, de Boer SF, Buwalda B, Meerlo P, Timmermans PJ. The resident-intruder paradigm: a standardized test for aggression, violence and social stress. *Journal of visualized experiments : JoVE*. 2013(77):e4367.
33. Powers A, Almli L, Smith A, et al. A genome-wide association study of emotion dysregulation: Evidence for interleukin 2 receptor alpha. *Journal of psychiatric research*. 2016;83:195-202.
34. Muller CP, Homberg JR. The role of serotonin in drug use and addiction. *Behavioural brain research*. 2015;277:146-192.
35. Smith PH, Homish GG, Leonard KE, Collins RL. Marijuana withdrawal and aggression among a representative sample of U.S. marijuana users. *Drug Alcohol Depend*. 2013;132(1-2):63-68.
36. Hikosaka O, Kim HF, Yasuda M, Yamamoto S. Basal ganglia circuits for reward value-guided behavior. *Annu Rev Neurosci*. 2014;37:289-306.
37. Bersani FS, Minichino A, Fojanesi M, et al. Cingulate Cortex in Schizophrenia: its relation with negative symptoms and psychotic onset. A review study. *Eur Rev Med Pharmacol Sci*. 2014;18(22):3354-3367.
38. Fiddick L. There is more than the amygdala: potential threat assessment in the cingulate cortex. *Neurosci Biobehav Rev*. 2011;35(4):1007-1018.
39. Pitychoutis PM, Belmer A, Moutkine I, Adrien J, Maroteaux L. Mice Lacking the Serotonin Htr2B Receptor Gene Present an Antipsychotic-Sensitive Schizophrenic-Like Phenotype. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. 2015;40(12):2764-2773.
40. Diaz SL, Narboux-Neme N, Boutourinsky K, Doly S, Maroteaux L. Mice lacking the serotonin 5-HT2B receptor as an animal model of resistance to selective serotonin reuptake inhibitors antidepressants. *European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology*. 2016;26(2):265-279.
41. Doly S, Bertran-Gonzalez J, Callebert J, et al. Role of serotonin via 5-HT2B receptors in the reinforcing effects of MDMA in mice. *PLoS one*. 2009;4(11):e7952.
42. Doly S, Quentin E, Eddine R, et al. Serotonin 2B receptors in mesoaccumbens dopamine pathway regulate cocaine responses. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2017.

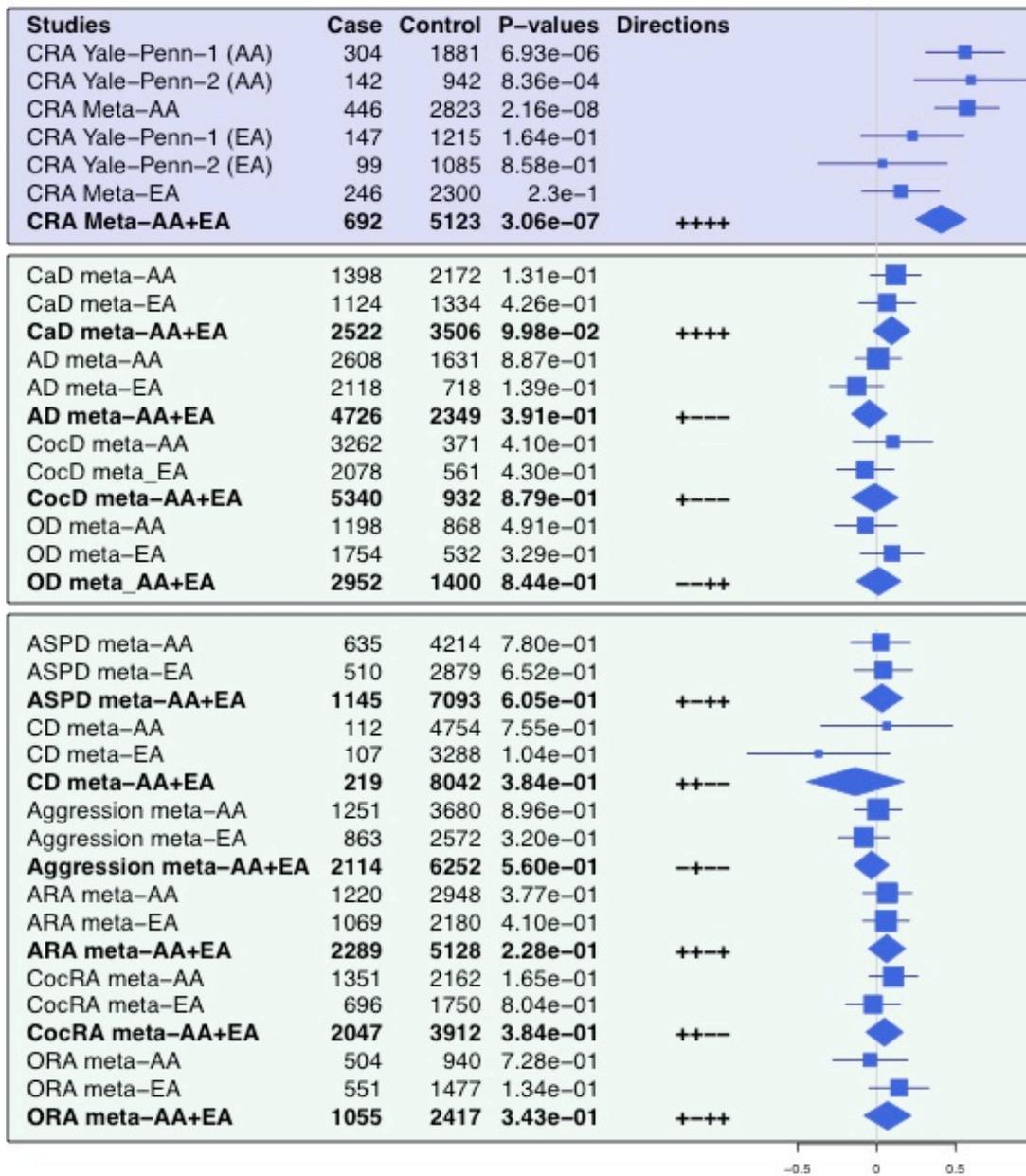
43. Muris P, Meesters C, Blijlevens P. Self-reported reactive and regulative temperament in early adolescence: relations to internalizing and externalizing problem behavior and "Big Three" personality factors. *J Adolesc.* 2007;30(6):1035-1049.
44. Trull TJ, Widiger TA. Dimensional models of personality: the five-factor model and the DSM-5. *Dialogues in clinical neuroscience.* 2013;15(2):135-146.
45. Need AC, Goldstein DB. Next generation disparities in human genomics: concerns and remedies. *Trends Genet.* 2009;25(11):489-494.
46. Stoops WW, Bennett JA, Lile JA, Sevak RJ, Rush CR. Influence of aripiprazole pretreatment on the reinforcing effects of methamphetamine in humans. *Progress in neuro-psychopharmacology & biological psychiatry.* 2013;47:111-117.
47. Chen J, Gao K, Kemp DE. Second-generation antipsychotics in major depressive disorder: update and clinical perspective. *Curr Opin Psychiatry.* 2011;24(1):10-17.
48. Fleischhacker WW, McQuade RD, Marcus RN, Archibald D, Swanink R, Carson WH. A double-blind, randomized comparative study of aripiprazole and olanzapine in patients with schizophrenia. *Biological psychiatry.* 2009;65(6):510-517.
49. Owen R, Sikich L, Marcus RN, et al. Aripiprazole in the treatment of irritability in children and adolescents with autistic disorder. *Pediatrics.* 2009;124(6):1533-1540.

## Figure legends



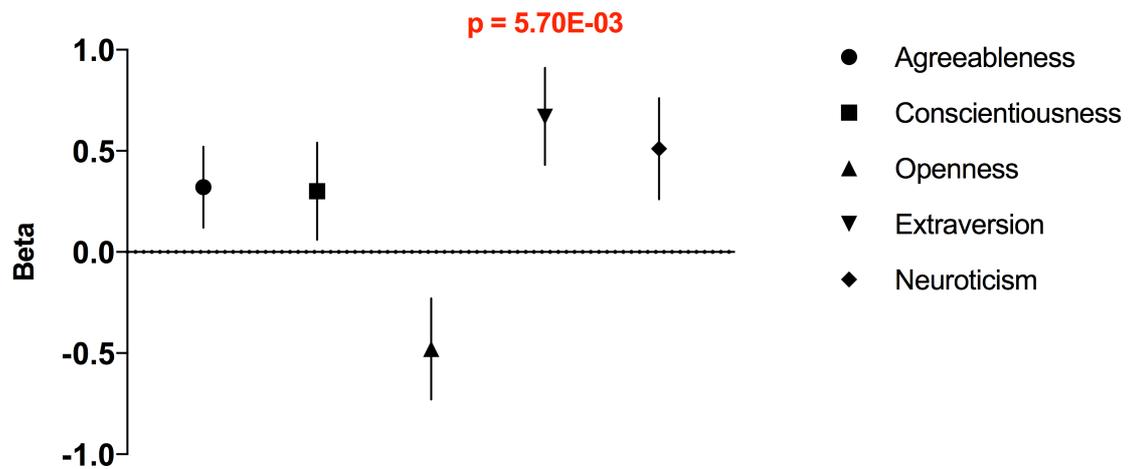
**Figure 1. Association results for cannabis-related aggression (CRA) in African Americans.**

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).

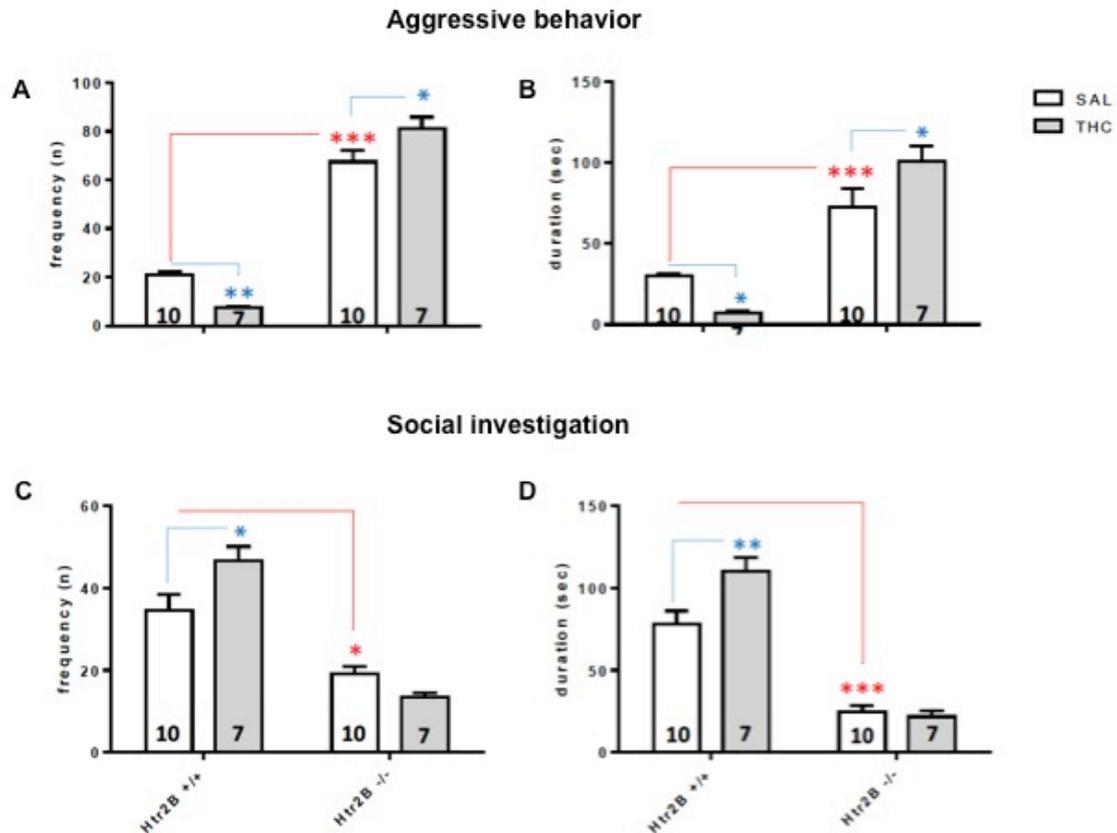


**Figure 2. Forest plot of rs17440378 (chr2:231979355), a genome-wide significant SNP for cannabis-related aggression (CRA).**

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 ( $p = 5.70 \times 10^{-3}$ ,  $\beta = 0.67$ , S.E. = 0.24). Error bars represent data  $\pm$  standard error (S.E.).



**Figure 4. THC increases aggressive behavior in *Htr2b*<sup>-/-</sup> mice.**

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

**Table 1. Demographic characteristics of the study cohorts.**

<b>Characteristic</b>	<b>Yale-Penn 1</b>		<b>Yale-Penn 2</b>		<b>GTP</b>
	<b>African American</b> ( <i>n</i> =2185)	<b>European American</b> ( <i>n</i> =1362)	<b>African American</b> ( <i>n</i> =1084)	<b>European American</b> ( <i>n</i> =1184)	<b>African American</b> ( <i>n</i> =89)
Age, mean (SD), y	41.2 (8.4)	36.8 (10.4)	40.6 (10.6)	37.5 (12.2)	45.0 (10.7)
Female sex, No. (%)	852 (39.0)	501 (36.8)	331 (30.5)	374 (31.6)	44 (49.4)
CRA, <i>n</i> (%)	304 (13.9)	147 (10.8)	142 (13.1)	99 (8.4)	N/A
CaD, <i>n</i> (%)	895 (49.4)	566 (55.2)	503 (55.6)	506 (61.3)	N/A
AD, <i>n</i> (%)	1322 (71.2)	865 (82.2)	741 (80.4)	787 (87.7)	N/A
CocD, <i>n</i> (%)	1892 (87.6)	1070 (82.1)	675 (64.3)	621 (61.1)	N/A
OD, <i>n</i> (%)	637 (29.8)	868 (65.9)	295 (27.7)	573 (55.2)	N/A
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, <i>n</i> (%)	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), <i>n</i> (%)	1969 (90.1)	1259 (57.6)	970 (44.4)	930 (42.6)	N/A
Aggression (physical fights), <i>n</i> (%)	1362 (62.6)	791 (58.2)	702 (64.8)	611 (56.4)	N/A
Sober Aggression (physical fights), <i>n</i> (%)	322 (14.7)	78 (5.7)	201 (18.5)	65 (6.0)	N/A
ASPD, <i>n</i> (%)	340 (15.9)	232 (17.4)	207 (19.6)	213 (19.9)	N/A
CD, <i>n</i> (%)	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.

**Table 2. Results for *HTR2B*\*rs17440378 from the analysis of cannabis-related aggression (CRA) and replication.**

Sample	<i>n</i>	<i>n</i> Cases	Direction	Effect	SE	MAF	MAF Controls	MAF Cases	<i>P</i> -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	NA	+	0.48*	0.27	0.13	NA	NA	4.00E-02**

Abbreviations: SE, standard error; MAF, minor allele frequency; NA, not applicable

\* This represents the odds ratio.

\*\* One-sided *P*-value

