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Original Article

Mu opioid receptor gene variant modulates subjective response to smoked cannabis

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Abstract: The mu-opioid receptor (MOR) mediates the rewarding properties of many psychoactive drugs and is an important target in the treatment of addictions. Functional interactions between the opioid and endocannabinoid systems are established and have been hypothesized to contribute to the effects of cannabis. We investigated associations between three single nucleotide polymorphisms in the MOR gene *OPRM1* (rs1799971, rs2281617, and rs510769) and subjective responses to smoked cannabis. Fifty-two regular cannabis users (1-4 days/week) were given a cannabis cigarette (12.5% THC) and rated their subjective responses on visual analog scales at baseline and at multiple time points after smoking. Blood samples were collected for THC quantification. There was a significant impact of the intronic variant rs510769 on subjective cannabis effects and THC blood levels. The influence of this gene variant may thus be mediated by pharmacodynamics and/or pharmacokinetic factors. We provide novel evidence that variability in *OPRM1* contributes to individual responses to cannabis and may affect risk of cannabis use disorder. Our findings add to the growing body of literature on the genetic basis of individual responses to cannabis and may have implications for targeting the endogenous opioid system in the treatment of cannabis use disorder.

Keywords: Cannabis, genetics, mu opioid receptor, subjective effects

Introduction

Acute subjective effects of cannabis include euphoria, sedation, cognitive and psychomotor impairment, and sensory distortion; however self-reported experiences vary considerably between individuals [1]. Early positive reactions to cannabis have been associated with progression to heavier use, including cannabis use disorder [2-4]. There is evidence suggesting that individual subjective responses to cannabis are partially heritable and may be mediated by genetic factors [5]. However, further research is required to determine whether there is a genetic contribution to individual differences and identify which genes or genetic polymorphisms are involved.

Δ9-Tetrahydrocannabinol (THC) induces the subjective effects of cannabis primarily by the activation of CB1 receptors (CB1R) in the endocannabinoid system. Endogenous opioid signaling is also thought to contribute to the rewarding properties of cannabis, through func-

tional interactions with the endocannabinoid and dopamine systems [6]. The mu opioid receptor (MOR), encoded by *OPRM1*, indirectly modulates the rewarding effects of many drug classes, including cannabinoids, through the mesolimbic dopamine system [6]. Early studies provided initial evidence of this, showing that cannabinoid-induced increases in dopamine release are blocked by MOR antagonism [7, 8]. Moreover, MORs and CB1Rs co-localize within the reward system [9, 10], and there is evidence suggesting that the two receptor subtypes form heterodimers and act synergistically [11].

The implication of the MOR in THC reinforcement is further supported by preclinical research. In rodent studies, the MOR antagonist naloxone fully abolishes conditioned place preference (CPP) to the cannabinoid agonist CP 55,940 [12] and reduces self-administration of the same compound [13]. Similarly, THC CPP is completely eliminated in MOR knockout mice [14]. MOR antagonism by naloxone also blocks THC-induced hyperphagia in rats [15, 16]. In squirrel monkeys, pretreatment with the opioid antagonist naltrexone reduced intravenous self-administration of THC on a fixed-ratio schedule [17]. Drug-taking responses were markedly attenuated, but remained significantly elevated compared to those of animals self-administering saline. Under the same experimental conditions, pretreatment with the CB1 antagonist SR141716A completely eliminated THC self-administration and flattened the dose-response curve to vehicle-control levels [18]. The rewarding effects of THC are thus attributed to CB1 activation and are subject to modulation by endogenous opioids.

Cannabinoid-opioid interactions appear to be more complex in humans. Low doses of naltrexone can reduce intoxication to low doses of THC in regular cannabis smokers; however, this effect was not found at a higher dose of THC. Interestingly, the opposite effects were found in non-cannabis smokers, suggesting that chronic THC exposure modifies the interactions between cannabis and endogenous opioids [19]. A subsequent study found that a wide range of therapeutic naltrexone doses increased the subjective effects and self-administration of cannabis in heavy cannabis users [20]. In this population, endogenous opioids may counteract cannabinoid reinforcement rather than mediate subjective reward. However, when administered repeatedly to daily cannabis users on a maintenance schedule, naltrexone reduces cannabis intoxication and self-administration [21]. Although the nature of the relationship is not fully understood, these studies provide evidence for the implication of MOR in cannabinoid reward and abuse liability in humans.

Given the importance of the MOR in THC reward, we investigated the effect of three *OPRM1* single nucleotide polymorphisms (SNPs) on subjective responses to cannabis. The most extensively studied *OPRM1* variant is rs1799971, which is an A118G (Asn40Asp) substitution in exon 1. The G allele results in reduced MOR expression *in vitro* [22] and an increased affinity of the receptor for the endogenous opioid substrate beta-endorphin [23]. It has been investigated as a candidate gene for drug addiction, but association studies have reported inconsistent findings. The G allele has been associated with increased risk of alcohol, heroin, and general substance dependence [24-26], but some studies have reported the opposite effect [27], or shown no effect at all [28-30]. We also examined two intronic variants: rs2281617 and rs510769. Both are C/T polymorphisms located in intron 1 and have unclear effects on gene expression and protein function [31]. The rs2281617 T-allele has been associated with lower dietary fat preference and body fat mass [32], as well as reduced energy and stimulation in response to amphetamine [31]. These findings could suggest a loss of function of *OPRM1* and reduced subjective reward in minor allele carriers. The rs510769 T-allele has been associated with an increased risk of heroin dependence [33], increased smoking behavior in patients undergoing methadone therapy [34], and decreased subjective responses to amphetamine [31].

To determine whether the rewarding properties of cannabis are modulated by *OPRM1*, we investigated the impact of SNPs on visual analogue scale (VAS) ratings of subjective drug effects in healthy regular (1-4 days/week) cannabis users.

Methods

This study was conducted in follow-up to a previous study conducted at the Centre for

Table 1. Allelic frequencies of *OPRM1* polymorphisms

Polymorphism	Location	Alleles (WT/SNP)	Frequency (N)		
			WT/WT	WT/SNP	SNP/SNP
rs1799971	154,360,797	A/G	29	21	2
rs2281617	154,529,113	C/T	30	20	2
rs510769	154,362,019	C/T	38	12	2

Addiction and Mental Health (CAMH) [35], during which participants had the option of being included in an additional genetic investigation. Those who consented provided a 20 mL blood sample from which DNA was extracted for genotyping. All study procedures were conducted in accordance with the Declaration of Helsinki and approved by the CAMH Research Ethics Board (Protocol #097-2019), and the Health Canada Research Ethics Board (Protocol #2011-0024). All participants provided written informed consent prior to participating in any study procedures.

Participants

Participants included in the study were male and female active cannabis users (using 1-4 days per week) between the ages of 19-25 years. Current cannabis use was confirmed by a positive urine toxicology screen for THC. Those who met criteria for a severe psychiatric disorder, DSM-IV cannabis dependence or any current or lifetime substance dependence (with the exception of nicotine dependence) were excluded. Participants were also excluded if they regularly used medications affecting brain function (e.g., antidepressants, stimulants, benzodiazepines), were pregnant, breastfeeding, or trying to become pregnant.

Out of the 99 participants enrolled in the original study, 70 completed the trial and consented to the genetic analysis. Fifty-two of them were randomized to the active cannabis group and were included in the present analysis.

Study procedure

Participants were required to abstain from alcohol and recreational drugs 48 hours prior to and for the duration of the study. This was verified by alcohol breathalyzer tests and point-of-care urine toxicology prior to each study session. Participants who were randomized to the active cannabis group received one cannabis cigarette with a mass of 750 mg and a potency of 12.5% THC. They were instructed to smoke ad libitum, in an externally ventilated reverse airflow room over a duration of 10 minutes. Total smoking duration was timed, and cannabis cigarettes were weighed before and after smoking. To obtain an estimate of total THC dose for each participant, the potency of the cannabis (0.125) was multiplied by the change in mass of the cigarette. Ratings of subjective drug effects were collected at baseline, 5, 15, 30 minutes and 1, 2, 3, 4, 5, 6, 24, 48 hours after cannabis administration. Participants reported the intensity of drug effects at each time point on a seven-item visual analog scale. The scale assessed ratings of "I feel a drug effect", "I feel this high", "I feel the drug's good effects", "I feel the drug's bad effects", "I like the drug", "I feel a rush", and "It feels like cannabis". A blood sample was drawn at each data collection time point for the measurement of THC concentrations. More details regarding procedures used for blood sample collection and THC quantification can be found in our previous manuscript [35]. It should be noted that THC was measured in whole blood (typically leading to lower values as compared to plasma measurements).

Genotyping

Approximately 650,000 polymorphic sites were genotyped using the Infinium Global Screening Array (Illumina, Inc., San Diego, CA, USA) at the CAMH Biobank and Molecular Core Facility. The array data underwent standard quality control procedures as described previously [36], and genotypes were extracted for the three *OPRM1* polymorphisms. The cluster plots for these three polymorphisms are shown in Supplementary Figure 1. As verified in the quality control steps, SNP genotypes did not deviate significantly from Hardy-Weinberg Equilibrium (P>5e-8). For each polymorphism, only two individuals in our sample were homozygous for the minor allele. Therefore, they were pooled with the heterozygous genotype as one group and compared against the homozygous wild type (WT) genotype. Allele frequencies are presented in **Table 1**.

Data analysis

All data were analyzed using SPSS version 25. Differences in demographic characteristics

Table 2. Participant characteristics by genotype (Mean (SD))

	rs1799971		rs2281617		rs510769	
	AA (N = 29)	AG + GG (N = 23)	CC (N = 30)	CT + TT (N = 22)	CC (N = 38)	CT + TT (N = 14)
Age	22.55 (1.70)	22.13 (2.14)	22.43 (1.72)	22.27 (2.16)	22.21 (2.00)	22.79 (1.58)
Sex (% female)	24.14	39.13	36.67	22.72	34.21	21.43
BMI	25.33 (5.50)	23.63 (2.89)	25.54 (5.30)	23.25 (2.99)	25.02 (4.98)	23.48 (3.31)
Cannabis use (times per week)	2.55 (0.93)	2.52 (0.83)	2.65 (0.97)	2.39 (0.74)	2.57 (0.89)	2.46 (0.89)

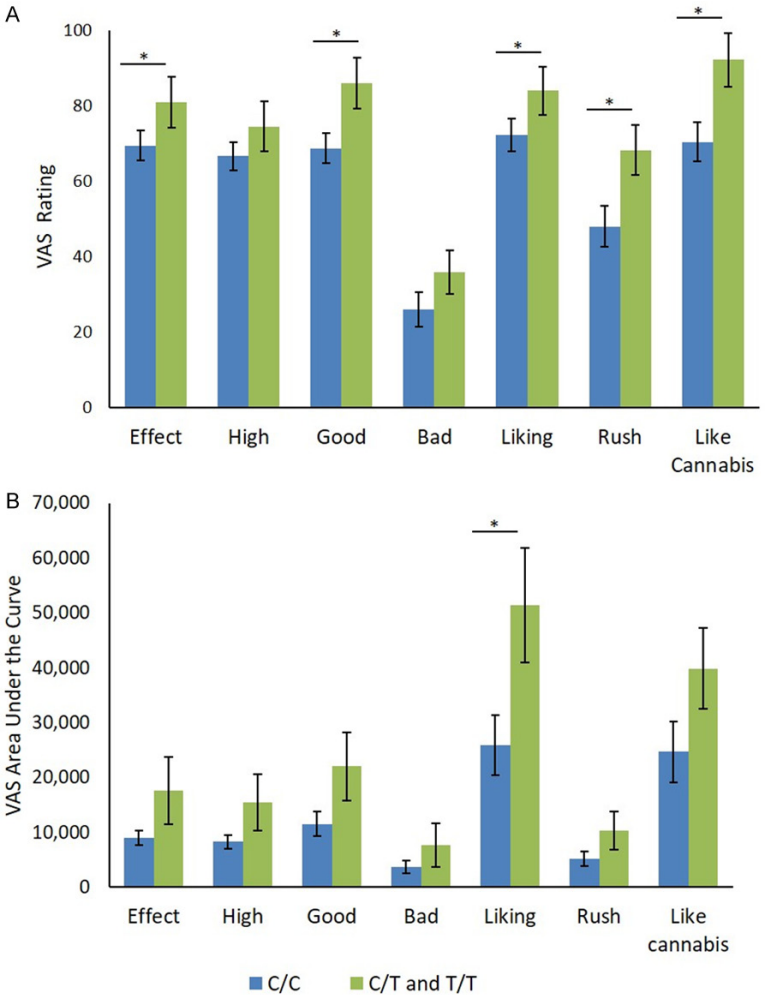

Figure 1. Effects of rs510769 genotype on subjective responses to cannabis measured by visual analog scales. Data expressed as mean ± SEM of (A) maximum VAS ratings by rs510769 genotype (C/C: N = 38; C/T and T/T: N = 13) and (B) area under the curve of VAS ratings over time (C/C: N = 34; C/T and T/T: N = 11). C/C genotype compared to C/T and T/T genotypes using independent sample t-tests. *P<0.05.

and cannabis use between genotype groups were analyzed by independent-sample T-tests and Chi square tests. Maximum ratings and area under the curve (AUC) for each VAS item were determined, and differences between groups were compared using independent-sample t-tests.

Results

Participant characteristics

Fifty-two healthy regular cannabis users were included in the study. Demographic characteristics and cannabis use frequency did not differ between genotype groups (**Table 2**).

rs510769 and subjective drug effects

rs510769 C/T and T/T genotypes reported significantly higher maximum VAS ratings of "Effect" t(48.44) = -2.15, P = 0.037, "Good" t(47.59) = -3.28, P = 0.002, "Liking" t(48.11) = -2.17, P = 0.035, "Rush" t(36.98) = -2.85, P = 0.007 and "Feels like cannabis" t(48.99) = -3.55, P = 0.001, compared to the C/C genotype. Maximum ratings of "High" and "Bad" drug effects were not statistically different between the groups (**Figure 1A**).

Mean area under the curve (AUC) of "Liking" was significantly elevated in rs510769 T-allele carriers compared to C allele homozygotes t(43) = -2.25, P = 0.029. AUC of all other VAS items did not differ significantly between groups (**Figure 1B**).

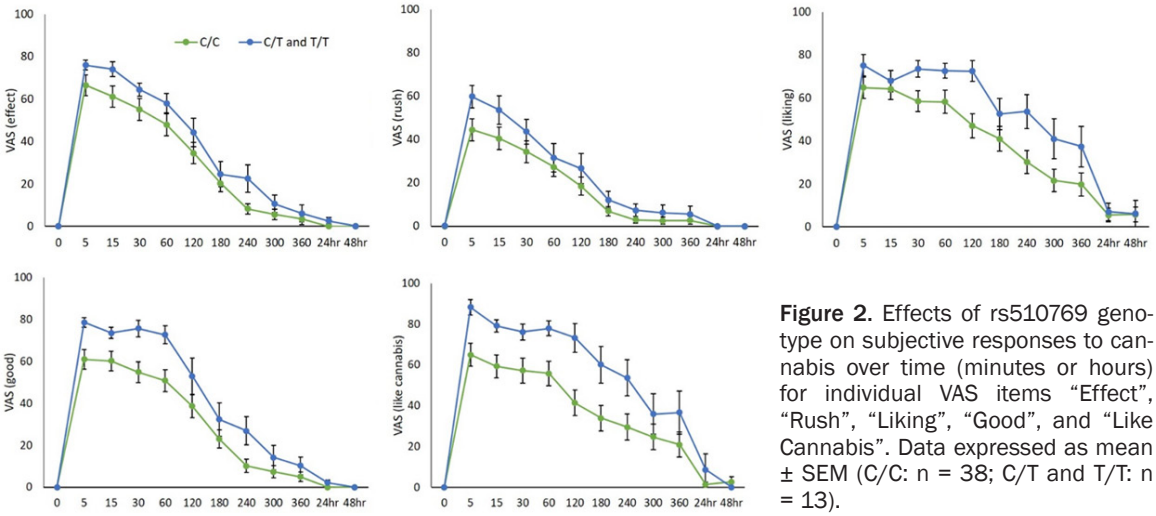

Figure 2. Effects of rs510769 genotype on subjective responses to cannabis over time (minutes or hours) for individual VAS items "Effect", "Rush", "Liking", "Good", and "Like Cannabis". Data expressed as mean ± SEM (C/C: n = 38; C/T and T/T: n = 13).

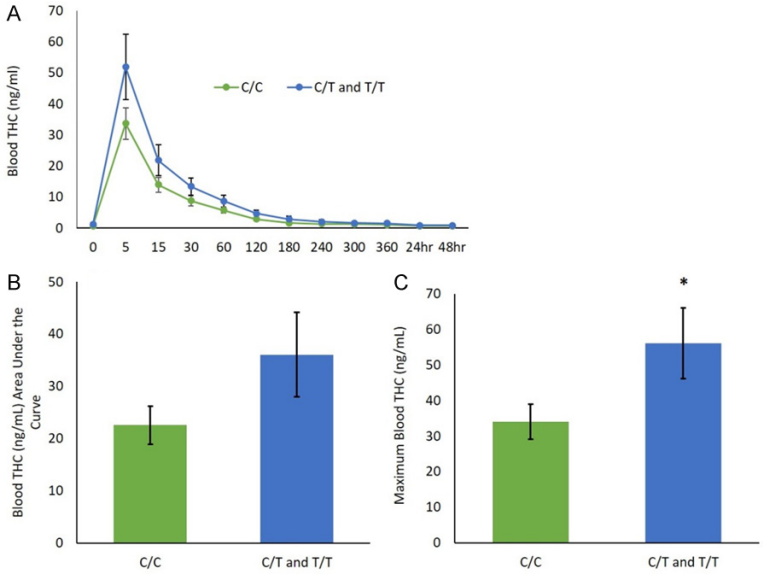

Figure 3. Effect of rs510769 genotype on THC pharmacokinetics. Mean ± SEM of (A) blood THC concentration (ng/ml) over time (minutes or hours) analyzed across genotypes by repeated measures ANOVA, (B) blood THC area under the curve using independent sample t-test, (C) maximum blood THC concentration (ng/ml) using independent sample t-test. C/C genotype compared to C/T and T/T genotypes (C/C: N = 34, C/T and T/T N = 12). *P<0.05.

Mean ratings over time of significant VAS items are presented in **Figure 2**.

rs510769 and THC pharmacokinetics

The mean estimated THC dose was 81.64 mg (SD = 23.06) in the C/C group and 85.18 mg (SD = 17.70) in the C/T and T/T group. This was not a statistically significant difference (t(46) = -0.50, P = 0.619).

Blood THC pharmacokinetics for both groups over time are presented in **Figure 3**. Repeated measures ANOVA of blood concentration over time revealed no statistically significant effect of genotype (F(1,42) = 2.35, P = 0.133), or time by genotype interaction (F(1.07,44.96) = 2.59 P = 0.113). THC concentrations peaked at five minutes and decreased over time in both groups. There was no significant difference in the area under the THC concentration/time curve between groups (C/C: M = 22.55, SD = 21.52, C/T and T/T: M = 36.05, SD = 27.99); (t(44) = -1.73, P = 0.091). The maximum blood THC concentration was significantly elevated in T-allele carriers (M = 56.17, SD = 34.46) compared to C/C individuals (M = 34.04, SD = 28.83); t(44) = -2.17, P = 0.035.

rs2281617 and rs1799971

No significant associations were found between rs2281617 or rs1799971 and any VAS measures (**Table 3**).

Discussion

We provide preliminary evidence that *OPRM1* contributes to the variability in subjective responses to smoked cannabis. Out of the three investigated SNPs, the rs510769 T-allele was associated with increased positive responses

Table 3. Maximum VAS scores over time by rs2281617 and rs1799971 genotype groups (Mean (SEM))

	rs1799971		rs2281617	
	AA (N = 28)	AG + GG (N = 23)	CC (N = 29)	CT + TT (N = 22)
Effect	74.79 (3.89)	69.61 (6.67)	75.17 (4.19)	68.86 (6.51)
High	70.07 (3.68)	67.04 (6.65)	71.24 (4.18)	65.36 (6.27)
Good	76.75 (3.94)	68.78 (6.78)	77.00 (4.35)	68.09 (6.49)
Bad	28.71 (4.57)	28.30 (5.79)	27.03 (4.77)	30.50 (5.53)
Liking	79.11 (4.35)	70.74 (6.36)	79.69 (4.28)	69.59 (6.49)
Rush	54.18 (5.37)	52.13 (6.59)	53.79 (5.46)	52.55 (6.51)
Like Cannabis	79.78 (5.11)	71.43 (7.11)	77.59 (5.38)	73.95 (6.99)

to cannabis and higher blood THC levels compared to C-allele homozygotes. Genotypes at rs1799971 and rs2281617 had no significant effect on subjective ratings. Variation in *OPRM1* has previously been shown to affect responses to alcohol, opioids, and amphetamine [31-33], and may also affect substance dependence liability [25-27]. Our results show an effect of the intronic variant rs510769. T- allele carriers reported significantly higher maximum VAS ratings of "Effect", "Good", "Liking", "Rush", and "Feels like cannabis", suggesting increased sensitivity to the drug's positive effects. This SNP may have a regulatory effect on receptor expression in the brain [37] and has previously been associated with reduced *OPRM1* expression in the cerebellum [38]. The C/T and T/T group also had significantly higher maximum levels of blood THC concentrations, indicating that the increased drug effects may be due to pharmacokinetic differences between genotypes. This SNP was previously shown to have the opposite effect on subjective responses to amphetamine, to which T-allele carriers reported reduced euphoria and stimulation [31]. Further research is required to determine how this SNP affects opioid receptor function and expression, THC pharmacokinetics, and the mechanisms by which it affects subjective responses to different drugs.

We found no association between rs1799971 and ratings of subjective effects. This functional polymorphism leads to a change in MOR function, which has unclear effects on intoxication and risk of addiction to different substances. The G allele has been associated with increased alcohol intoxication [39] and found to have no effect on responses to amphetamine [31]. One study that associated the G allele with increased risk of dependence to four pooled substances found no effect on cannabis dependence alone; however, this may have resulted from limited sample size [26]. Due to the small number of G allele homozygotes in our study (N = 2), A/G and G/G genotypes were combined into one group and compared to A allele homozygotes. Interestingly, both G/G individuals in our sample had peak ratings of "Effect" and "Good" over 25% higher than those reported by A/A individuals. Previous studies have associated the G/G genotype with dosage and response to opioid analgesics compared to A/A and A/G genotypes [40-42].

rs2281617 did not affect subjective responses to cannabis. It has been investigated by very few studies which have indicated possible reduced subjective reward in minor allele carriers [31, 32]. It is a non-coding SNP with unknown consequences on gene or protein expression. Further research is required to characterize its function and determine its effects on reward processing.

Our findings may have implications for the therapeutic use of naltrexone in cannabis use disorder (CUD). MOR antagonism by naltrexone results in the blunting of rewarding drug effects and a reduction in cravings, thereby reducing drug use and rates of relapse [43]. It is one of the most effective pharmacologic treatments for alcohol use disorder. Polymorphisms in *OPRM1* affect subjective alcohol intoxication, the effectiveness of naltrexone in blocking subjective effects of alcohol, and rates of relapse after treatment [43-45]. Our findings may suggest a similar implication of the gene in the treatment of CUD. In addition to affecting therapeutic outcomes, *OPRM1* may contribute to the initial development of cannabis dependence. An increased risk of problematic cannabis use has been reported in users with stronger positive reactions to the drug [2-4]. Based on our findings, it is possible that rs510769 T-allele carriers are more susceptible to developing CUD. The association between this SNP and CUD as well as potential prevention strategies require further study.

The results of this study should be interpreted in light of certain limitations. Although demographic characteristics and cannabis use frequency did not vary between genotype groups, our findings should be confirmed in a larger sample, using an adjusted statistical model to control for potential confounding variables. Participant ancestry would be an important variable to control for, as minor allele frequencies for rs1799971 and rs2281617 differ considerably between ethnic groups [46-48]. Importantly, this limitation may have led to false negative results, especially for rs1799971 [48]. In addition, we did not correct for multiple comparisons in our statistical analyses and cannot exclude the possibility of type I error. A larger sample would allow the detection of potential effects with greater statistical power and an adjusted significance level. Despite limitations related to small sample size, our results provide novel, preliminary evidence for the possibility that variation in *OPRM1* contributes to differences in subjective responses to smoked cannabis.

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Disclosure of conflict of interest

None.

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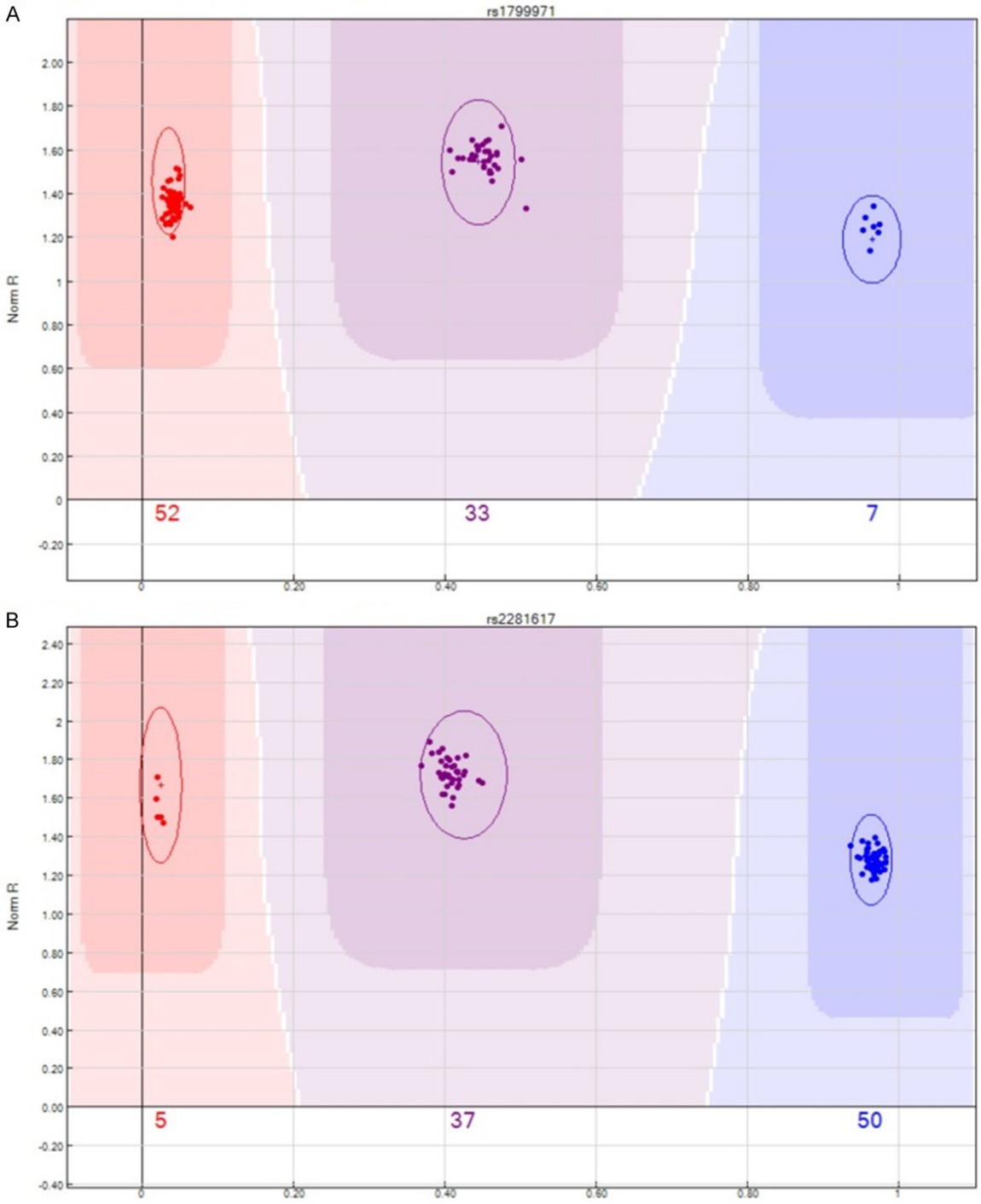
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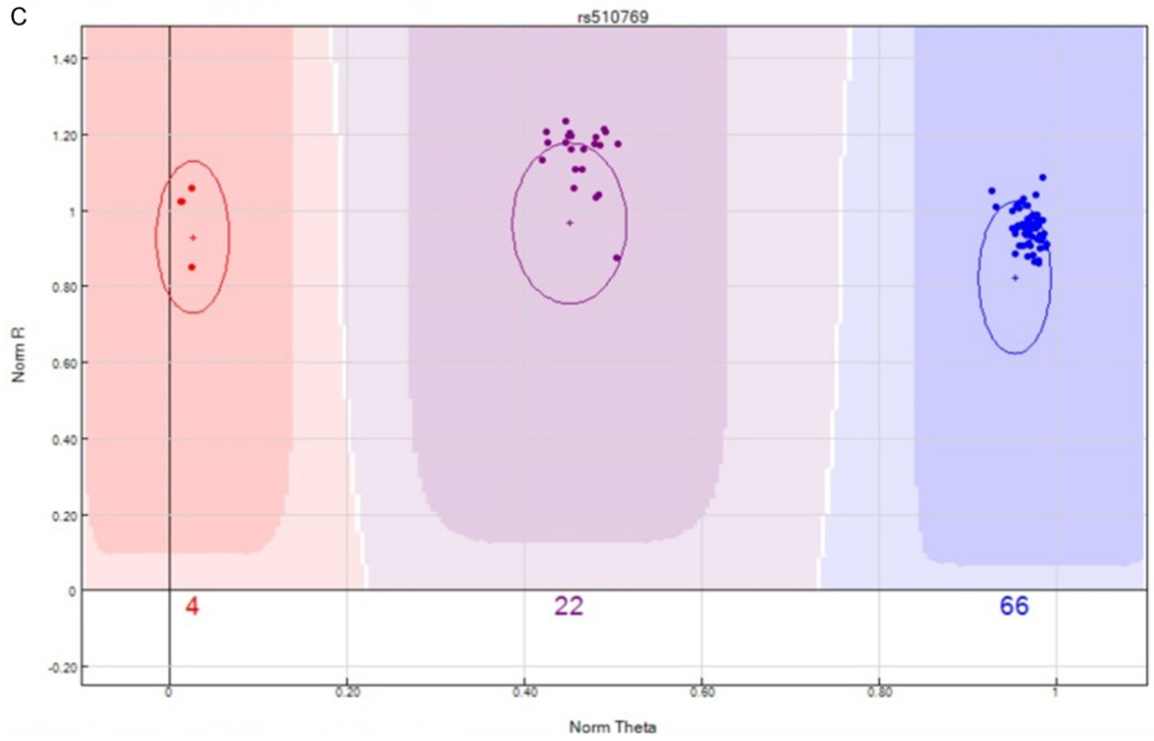

Supplementary Figure 1. The cluster plots for *OPRM1* single-nucleotide polymorphisms (SNPs) (A) rs1799971, (B) rs2281617, and (C) rs510769 extracted from the Genome Studio. The x-axis (theta) indicates frequencies of the assigned alleles and the y-axis (R) represents the fluorescence intensity for the allele-specific probes. For each plot, each data point represents a research participant. The color clusters indicate the three genotypes, with the number of each genotype shown below each cluster. The genotype calls for rs1799971 from left to right are AA, AG, and GG. The genotype calls for rs2281617 from left to right are TT, CT, and CC. The genotype calls for rs510769 from left to right are TT, CT, and CC.