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Searching for Evidence of Genetic Mediation of Opioid Withdrawal by Opioid Receptor Gene Polymorphisms

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Abstract

Background—Previous research has identified many genetic polymorphisms that appear to mediate the effects of opioid drugs. However, the relationship between genetic polymorphisms and the severity of opioid withdrawal has not yet been characterized.

Methods—Data were collected from 48 daily heroin users who previously completed a standardized abstinence-induced or naloxone-precipitated withdrawal procedure to assess opioid dependence. The total withdrawal severity score (based on the COWS) from this procedure was correlated with genotype information for variants of *OPRM1* (rs1799971; rs6848893), *OPRD1* (rs10753331; rs2234918; rs581111; rs678849; rs1042114) and *OPRK1* (rs6473797; rs963549). Genotype and other participant variables (age, race, sex, duration of drug use, concomitant drug use, route of opioid use) were used as predictors.

Results—Of these variables, those individually correlated with a $p<0.2$ were entered into a multivariate regression in order to identify the most predictive model. Three polymorphisms were significantly associated with severity of abstinence-induced withdrawal (n = 19) in the bivariate analysis (R): *OPRM1* rs6848893 (0.45), *OPRD1* rs10753331 (0.03) and rs678849 (0.08), but only the *OPRM1* rs6848893 was retained in the multivariate model ($p <.001$). For participants who underwent naloxone-precipitated withdrawal (n = 29) only *OPRK1* rs6473797 (−0.23) was significant in the bivariate analysis, though not retained in the final model.

Conclusions—These data provide evidence for genetic modulation of opioid withdrawal severity, and suggest there may be qualitative differences between withdrawal resulting from abstinence and antagonist-precipitated withdrawal.

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Contributors: JDJ designed and planned the study. JDJ and SDC, were responsible for screening and assessing study participants. JDJ and RL and JLV performed analyses of study results. JDJ developed the first draft of the manuscript that all authors edited and approved the submitted draft.

Introduction

Opioid abuse continues to be a major social, economic, and medical concern (1). The annual global prevalence of opioid abuse was estimated at between 28 and 38 million users (2). Physiological dependence is one of the key features that maintain repeated opioid use, contributing significantly to the cycle of chronic use/abuse (3, 4). The withdrawal syndrome individuals experience when they cease opioid use or when an opioid antagonist is administered is characterized by: dilated pupils, lacrimation, yawning, sweating, and agitation/anxiety; and as it progresses: muscle pain, abdominal cramping, nausea, vomiting, and diarrhea (5, 6, 7). Although the symptomology of opioid withdrawal is among the most predictable of all the drug classes, variability in the severity of withdrawal may be due in part to an individual's genetic makeup.

The genetic contribution to opioid abuse has been estimated at up to 80%, greater than for any other drug class (8, 9, 10, 11, 12). Previous research has identified many genetic polymorphisms that appear to mediate the binding efficacy of opioid drugs, along with their analgesic response, and subjective effects (13, 14, 15, 16, 17; See 18 for a review). Therefore, pharmacogenetic research may also identify genetic variants that mediate the severity of opioid withdrawal.

In searching for pertinent gene polymorphisms, many studies have focused on the genes that encode for the endogenous opioid receptors (19, 20). The mu subtype of opioid receptors is thought to primarily mediate opioid analgesia, reward and withdrawal (21, 22). As such, variation in the *OPRM1* gene has been the most extensively studied in opioid candidate gene research. Opioids also activate the kappa and delta opioid receptors (23, 24, 25, 26, 27). Like mu, the kappa opioid receptors also release pain (28) but oppose mu receptors in the regulation of hedonic homeostasis (29). Kappa receptors may also mediate the aversive effects of stress (30, 31). The delta receptor is also thought to contribute to anxiolysis, and regulate inhibitory control and emotional reactivity (32, 33, 34, 35). Although variation in the delta and kappa opioid receptor genes (*OPRD1 & OPRK1,* respectively) has been the target of fewer pharmacogenetic studies, research has found associations with the risk of opioid abuse (36, 37, 38, 39, 40).

The application of genetic approaches has allowed us to clarify the role of each opioid receptor in many aspects of opioid-related responses. The goal of the present analysis was to investigate the nature of the relationship between genetic variation of the mu, delta and kappa opioid receptor genes and severity of opioid withdrawal. If significant genetic associations are found, it may indicate important genetic contributions to the severity of withdrawal individuals experience when they discontinue opioid use. This study will assess two types of opioid withdrawal. Opioid withdrawal resulting from short-term abstinence from opioid use (abstinence-induced) and withdrawal precipitated by the administration of a mu opioid receptor antagonist (naloxone-precipitated). Although the symptomology and severity of the two are assumed to be the same, and they are often thought of as interchangeable in clinical research, differences in their physiological underpinnings make it possible that genetic factors mediate them differentially.

Methods

Design Overview

This study is a secondary analysis utilizing data from heroin users screening for clinical research studies who were subjected to a standardized, naloxone-precipitated-withdrawal procedure or observed abstinence-induced withdrawal to verify opioid dependence. These participants were genotyped for various common and previously studied *OPRM1, OPRD1* and *OPRK1* polymorphisms (17, 36, 41, 42, 43). The severity of withdrawal was then correlated with the presence or absence of: *OPRM1* (rs1799971; rs6848893), *OPRD1* (rs10753331; rs2234918; rs581111; rs678849; rs1042114) *and OPRK1* (rs6473797; rs963549). These predictor variants were selected based upon functional relevance to receptor function or gene expression, a minor allele frequency of >10% and/or evidence of modulation of the aspects of opioid abuse from previous studies (44). The authors hypothesized that the presence or absence of one or more of the target genetic variants will be significantly associated with and predict withdrawal severity.

Participant Screening and Selection

Data for this study were collected as a part of screening procedures for clinical research studies within the Opioid Research Laboratory at Columbia University College of Physicians and Surgeons/New York State Psychiatric Institute (see 45 for an example of this work). For various protocols, active heroin users were recruited from the New York City metropolitan area through various print media and online advertisements. Respondents were then scheduled to come to the New York State Psychiatric Institute for additional screening procedures.

Screening consisted of both self-report and clinical interviews administered by a team of research assistants, psychologists, nurses, and physicians. The Beck Depression Inventory (46) and Structured Clinical Interview for DSM-IV (SCID; 47) and a psychiatric evaluation with a division psychiatrist were used to exclude participants with severe psychiatric symptomology. Other assessments included: assessment of drug use, general health, and medical history, and multiple laboratory tests were performed. Rapid urine drug screens were also conducted and tested at each visit using an 11-Panel DrugCheck® Dip Drug Tests. This commercially available test has the following positive result cut-offs: Amphetamine: 1000 ng/mL, Barbiturate: 300 ng/mL, Benzodiazepine: 300 ng/mL, Buprenorphine (Bup): 10 ng/mL, Cocaine: 150 ng/mL, Methamphetamine: 500 ng/mL, Methadone: 200 ng/mL, Opiates (morphine, codeine, heroin): 300 ng/mL, Oxycodone: 100 ng/mL, PCP: 25 ng/mL, THC: 50 ng/mL.

Participants were required to be physically healthy users of heroin between the ages of 21 and 60. Physical health was assessed using medical history, clinical interview performed by a nurse and/or a physical examination by a study physician. Participants were excluded for psychiatric symptomatology that required treatment with a psychotropic medication, may have impaired their ability to provide informed consent (e.g., active bipolar or schizophrenia), or make participation hazardous (e.g., significant history of violence). Participants were also required to be currently physiologically dependent on opioids.

Withdrawal Assessment

In order to confirm physiological dependence on opioids, participants could either present to study staff in a state of withdrawal as a result of not having used opioids recently, or undergo a naloxone challenge procedure, which is type of withdrawal assessment. Participants were asked not to change their pattern of drug use in order to prepare for the challenge. The challenge began with pre-test measurements of pupil diameter (using a NeurOptics™ Pupillometer under ambient lighting conditions), vital signs (HR, BP, pulse oximetry), and symptoms of opioid withdrawal (gooseflesh, vomiting, tremor, seating, restlessness, lacrimation/nasal congestion, yawning, warming/cooling sensations, stomach pain and muscle ache). One of two research nurses rated symptoms of opioid withdrawal as being either “absent” or “present.” Points were added to the outcome score for “present” symptoms, while no points were added for “absent” symptoms (Table 1).

After pre-test assessments, the nurse began with a 0.2 mg intramuscular dose of naloxone. If no withdrawal signs were present after 10 minutes (score = 0), an additional 0.2 mg was administered. If no withdrawal signs were present after an additional 10 minutes, an additional 0.4 mg was administered. In order to calculate the final score [based on the clinical opioid withdrawal scale (COWS) 48, 49], the total score across the entire testing phase was multiplied by 4 if only 0.2 mg of naloxone was used, by 2 if a total dose of 0.4 mg was used, and was unaltered if 0.8 mg was needed. Although naloxone was not administered to participants who underwent the abstinence-induced withdrawal observation, the same timeline and scoring procedures were used. One of the two research nurses completed the withdrawal assessments. Both nurses were trained by the lab director, have worked with the Opioid Lab for over 8 years, and are intimately familiar with the assessment procedure and proper scoring. Withdrawal data were compiled across prospective participants who underwent either procedure from 2012–2014 and combined with genotyping data. All study procedures were approved by the Institutional Review Board of the New York State Psychiatric Institute.

Genotyping

Whole blood samples (1 tablespoon) were collected in blood collection tubes with acid citrate dextrose (ACD) solution and delivered within 48 hours to the Human Genetics Research Core of Columbia University, where DNA was isolated and stored. Batches of isolated DNA were sent to the Genomics Core of the Taub Institute/Columbia University Medical Center for genotyping. SNP-marker genotyping was performed using matrix-assisted laser desorption/ionization time of flight mass spectrometry (Sequenom, San Diego, CA, USA). PCR assays and mass extend reactions were designed using mass array assay design software (Sequenom). PCR assays were performed using Applied Biosystems (Foster City, CA, USA) Geneamp PCR thermocyclers according to iPlex PCR Protocol (Sequenom). Extension products were analyzed using the mass array compact mass spectrometer (Bruker Daltonik, Billerica, MA, USA), and spectra were analyzed using TYPER 4.0 software (Sequenom).

Statistical Analyses

Expected gene frequencies were calculated from respective single-allele frequencies, according to the Hardy–Weinberg equation. Observed and expected gene frequencies were compared using the chi-square (X^2) goodness-of-fit test and the Hardy–Weinberg proportion.

For the primary analysis, opioid receptor genotype information and other participant variables (age, race, duration of drug use, concomitant drug use, route of opioid use) were used as predictors of the severity naloxone-precipitated and abstinence-induced withdrawal. Single logistic regression (SLR) analyses were performed to identify which variables were associated withdrawal severity. These analyses were used to select eligible factors for a multivariate model. To avoid situations where strong confounding could hide important predictors, a liberal cutoff of p value <0.20 were defined in the SLR to select eligible factors to be included into the multiple regression. A stepwise selection procedure was then used, to enter the eligible predictors in the regression equation. The stepwise method was selected because it combines a forward and backward entry procedure to account for the complexity of intercorrelations between the variables. For the final regression model, only variables that were significant at the $p < 0.05$ level were retained. In order to control for the small sample sizes, Firth's bias adjustment was applied to the SLR (48). Bias adjustment did not change the factors selected for inclusion in the multiple regression model, as such unadjusted figures are reported.

Results

Participants

In total, data from 48 heroin users (without chronic pain) were used in the current analysis. Participants were predominately male (>94%), and in their early 40s. On average, participants had been using heroin for ~16 years and all reported daily heroin use with an average of 7 bags per day. Depression and anxiety were the most common psychiatric symptomology with 20% of participants who passed screening found to have a history or current (yet mild) symptoms. Table 2 presents a more extensive list of demographic information separated by participants who underwent the abstinence-induced (average 10.5 hrs since the last opioid use) and naloxone-precipitated procedures. Also shown in Table 2 are the final withdrawal severity scores for both groups. The final score was significantly greater for participants who underwent the naloxone-precipitated procedure ($p<0.05$).

Observed Gene Frequencies

Allele frequencies for the *OPRM1, OPRK1* and *OPRD1 alleles* were calculated and assessment for violation of Hardy–Weinberg equilibrium (HWE) resulted in no significant chi-square values (all p's>0.05). One OPRD1 SNP rs1042114 was removed from subsequent analyses due to a high rate of genotyping failure (38%). Without this SNP, the overall genotyping failure rate was >1%. Consistent with most related research, we employed a carrier vs. non-carrier categorization (44). For each genotype, participants were grouped as homozygous major allele carriers (e.g., AA), while minor allele homozygotes and heterozygotes were combined (e.g., AG and GG).

Regression Analysis

Table 3 displays the demographic, drug use and genetic variables individually associated with the final severity score at $p<0.20$. These variables were subsequently entered into the multivariate model. Three alleles were significantly associated with severity of abstinence-induced withdrawal *OPRM1* rs6848893, *OPRD1* rs2234918, *OPRD1* rs678849. The additional use of buprenorphine (Bup), prescription opioids and nicotine, were also significant predictors. However, only the *OPRM1* rs6848893 variant and nicotine use were retained in the final model (Adjusted $R^2 = 0.68$, $p<0.001$; Table 4).

In comparison to abstinence-induced withdrawal, many more participant variables were significantly associated with the severity of naloxone-precipitated withdrawal (in the univariate analyses). The *OPRK1* allele rs6473797 was the only genetic factor that met eligibility criterion ($p <0.20$) for inclusion in the multivariate analysis. However, this genetic variant was not retained in the final model. Various concomitant drug use factors were found to be the most predictive (Adjusted $R^2 = 0.76$, $p<0.001$).

Discussion

In this study we were able to identify several demographic and genetic correlates of withdrawal due to opioid abstinence and administration of the opioid antagonist naloxone. For participants whose withdrawal severity was measured following brief opioid abstinence, nicotine use and the presence of the *OPRM1* (rs6848893) were found to be significant predictors of withdrawal severity. The adjusted R^2 of the multivariate model found that 68% of the variance in the severity of abstinence-induced withdrawal was explained by these two predictors. Although the functional significance of this intron 3 variant is unknown, it has been associated with opioid and alcohol abuse (17, 51), two drugs with effects on the mu opioid receptor (22, 52). Nicotine use also was positively correlated with withdrawal severity. Other studies have found that smoking during opioid detoxification is associated with increased withdrawal discomfort (53) and smoking is a predictor of negative opioid detoxification outcome (54, 55). As such, these data argue for the need to study the impact of tobacco on opioid withdrawal.

Surprisingly, concomitant use of long-acting opioids (i.e., buprenorphine and methadone) was not significantly associated with the severity of withdrawal. However, our sample consisted mainly of sporadic users of these opioids. More frequent use or physiological dependence on buprenorphine or methadone may have resulted in less withdrawal severity following acute abstinence. In contrast, regular use of buprenorphine was associated with severity of naloxone-precipitated withdrawal (though not retained in the final analysis). Because Bup binds to the opioid receptor with greater affinity than other mu agonists/antagonists, it's possible that recent use of Bup may have made participants less reactive to the effects of naloxone (56).

Data obtained from naloxone-precipitated withdrawal found more influence of participant variables such as demographics and current drug use. In this model, concomitant use of non-opioid drugs was negatively associated with the severity of withdrawal, while the use of prescription opioids was positively correlated with severity of withdrawal. Combined, these

factors accounted for 75% of the variance in withdrawal scores. Although not retained in the final model, the bivariate analysis found that the *OPRK1* allele rs6473797 was significantly associated with withdrawal severity.

The finding of kappa involvement in an aversive experience such as opioid withdrawal is not surprising. Other investigations have suggesting that the kappa receptor may mediate aversive effects (30) with the endogenous ligand for these receptors (dynorphins) being released during stress exposure (57). Another investigation also found an association between the *OPRK1* polymorphisms (rs7832417, rs16918853, rs702764, and rs7817710) and the opioid withdrawal symptoms of bone or joint aches, gooseflesh skin, yawning, and restlessness (58). Reports have identified the dynorphin/κ-opioid system as critical in reinstatement of drug seeking behavior (in rodent models), so the moderation of withdrawal severity may be the means by which genetic variation in this system affects this outcome (59, 60). Between the two groups under investigation in the current study, the findings suggest that there may be distinct physiological and genetic factors affecting abstinence-induced and antagonist-precipitated withdrawal, which may be important for clinical investigators who may need to assess and/or model opioid withdrawal.

Though the findings of the current study are provocative, there are several limitations that warrant discussion. First and foremost, as a secondary data analysis we were unable to control for the severity/duration of opioid use and concomitant drug use, prior to completing assessment of withdrawal. Additionally, we were unable to control for differential scoring between the two raters. Also, as a racially diverse sample was employed, we are unable to account for population genetic differences in the allele frequencies. Population admixture is a significant concern for all genetic investigations and as such, these findings should be viewed with caution. Finally, the sample size of the study is small for a genetic analysis and the conclusions drawn from this study should be viewed as preliminary. A prospective study observing these methodological considerations is recommended to further replicate the current findings.

Despite the limitations, the current data suggest that there may be significant genetic mediation of the severity of opioid withdrawal and their biochemical mechanisms warrant further investigation. Opioid withdrawal treatment is often used to facilitate entry into long-term psychosocial and pharmacological treatment. Withdrawal severity has been shown to be inversely related to retention in detoxification (61, 62). Therefore, retention in detoxification is of great value to subsequent, longer-term therapy and treatment prognosis. The ability to predict which individuals may experience greater opioid withdrawal (either during detoxification or subsequent periods of abstinence) may increase likelihood of treatment success.

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Table 1

Withdrawal Symptom Observation and Scoring Sheet

	Presence or absence of signs and symptoms (circle number)											
	(Pre-test)		Time after first naloxone dose									
	0 min		10 min		20 min		30 min		40 min		50 min	
Signs and Symptoms	**Present**	**Absent**	**Present**	**Absent**	**Present**	**Absent**	**Present**	**Absent**	**Present**	**Absent**	**Present**	**Absent**
Gooseflesh	6	0	3	0	3	0	2	0	1	0	1	0
Vomiting	6	0	3	0	3	0	2	0	1	0	1	0
Tremor	6	0	3	0	3	0	2	0	1	0	1	0
Sweating	6	0	3	0	3	0	2	0	1	0	1	0
Restlessness	4	0	2	0	2	0	1	0	1	0	1	0
Lacrimation/Nasal Congestion	4	0	2	0	2	0	1	0	1	0	1	0
Yawning	4	0	2	0	2	0	1	0	1	0	1	0
Feeling of Change in Temperature	3	0	1	0	1	0	1	0	1	0	1	0
Stomach Pain	3	0	1	0	1	0	1	0	1	0	1	0
Muscle Ache	3	0	1	0	1	0	1	0	1	0	1	0
Actual time of observation												
Scores												
Pupil Diameter												

Table 2

Sample Demographics and Withdrawal Severity

Demographics			
	Participants (%) or Median (Std. Dev.)		
	Abstinence-Induced Withdrawal (n=19)	***Naloxone-Precipitated Withdrawal (n=29)***	***Statistic (p-value)***
Age	41 (12)	43 (14)	$t = 0.4$, p =.7
Sex			
Male	18 (95)	28 (97)	$X^2 = 0.5$, p = .5
Female	1 (5)	1 (3)	
Ethnic/Racial Category			
African-American	8 (42)	10 (35)	$X^2 = 1.1$, p = .2
Caucasian	2 (11)	6 (21)	
Latino	9 (47)	12 (41)	
More Than One Race	--	1 (3)	
Heroin Use			
Heroin Use (bags/day)	8.2 (3.9)	5.9 (2.7)	$t = 0.7$, p =.5
Years of Use	19.0 (13.0)	14.3 (10.1)	$t = 0.3$, p =.7
Route of Administration Preference			
Intranasal	14 (64)	15 (52)	$X^2 = 2.3$, p = .1
Intravenous	8 (36)	14 (48)	$X^2 = 0.2$, p = .7
Concomitant Substance Use			
Cocaine (Yes/No)	7 (37)	16 (55)	$X^2 = 1.5$, p = .2
Years of Use	7.7 (8.9)	15.7 (10.7)	$t = 0.5$, p = .6
Nicotine (Yes/No)	17 (90)	25 (86.2)	$X^2 = 0.1$, p = .8
Cigarettes per Day	12.6 (7.4)	12.1 (5.9)	$t = 0.5$, p =.9
Sedatives (Yes/No)	5 (26)	12 (41)	$X^2 = 1.4$, p = .3
Rx Opioids (Yes/No)	7 (37)	10 (35)	$X^2 = .02$, p = .8
Methadone (Yes/No)	3 (16)	11 (38)	$X^2 = 9.9$, p = .01
Buprenorphine (Yes/No)	5 (26)	9 (31)	$X^2 = 0.2$, p = .7
Severity Withdrawal Score			
	Mean (Std. Dev.)		
	Abstinence-Induced Withdrawal	***Naloxone-Precipitated Withdrawal***	
	19.3 (7.0)	67.2 (37.7)	$t = 2.0$, p = .04

Table 3

Predictors (Bivariate Analysis)

	Abstinence-Induced Withdrawal (n=19)		Naloxone-Precipitated Withdrawal (n=29)	
	R (p-value)	***B (95% CI)***	***R (p-value)***	***B (95% CI)***
Age	--	--	−0.29 (0.13)	−1.41 [−3.23–0.42]
Years of Heroin Use	--	--	−0.41 (0.03)	−1.54 [−2.88–−0.19]
Cocaine Use (Yes/No)	--	--	−0.39 (0.04)	−29.0[−56.1–−1.98]
Years of Cocaine Use	0.95 (0.20)	1.43 [−4.56–7.41]	--	--
Nicotine Use (Yes/No)	0.59 (0.01)	13.1 [3.94–22.3]	--	--
Cigarettes per Day	--	--	−0.51 (0.01)	−3.92 [−6.50– −1.31]
Rx Opioid Use (Yes/No)	0.07 (0.14)	4.94 [−1.84–11.7]	0.35 (0.06)	27.4 [−1.34–56.2]
Rx Opioid Use (Route of Administration)	--	--	−0.61 (0.06)	−12.3[−36.1–11.5]
Bup Use (Yes/No)	--	--	0.35 (0.07)	14.5 [−2.00–57.3]
Bup Use (Frequency)	−0.95 (0.01)	−13.5 [−21.7– −5.25]	−0.50 (0.17)	−7.13 [−22.3–8.05]
Bup Use (Dose per Occasion)	--	--	−0.59 (0.09)	−4.78 [−13.4–3.82]
Last Use of Heroin (hrs)	--	--	−0.34 (0.08)	−6.12 [−12.84–.60]
OPRM1: rs6848893	0.45 (0.06)	5.21 [−0.29–10.7]	--	--
OPRD1: rs10753331	0.03 (0.07)	4.64 [−0.39–9.68]	--	--
OPRD1: rs678849	0.08 (0.16)	−3.38 [−8.23–1.48]	--	--
OPRK1: rs963549	--	--	−0.23 (0.22)	−13.5 [−35.8–8.8]

Table 4

Predictors (Multivariate Analysis)

	Abstinence-Induced Withdrawal (n=19)		Naloxone-Precipitated Withdrawal (n=29)	
	Beta	***p-value***	***Beta***	***p-value***
Years of Heroin Use	--	--	−.386	.001
Cocaine Use (Yes/No)	--	--	−.377	.002
Cigarettes per Day	--	--	−.537	.001
Rx Opioid Use (Yes/No)	--	--	.246	.027
OPRM1: rs6848893	.434	.009	--	--
Nicotine Use (Yes/No)	.468	.007	--	--
	R^2= 0.68		R^2=0.75	