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TOXICOLOGY INVESTIGATION

Opioid Receptor Polymorphism A118G Associated with Clinical Severity in a Drug Overdose Population

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Abstract Genetic variations in the human mu-opioid receptor gene (*OPRM1*) mediate individual differences in response to pain and opiate addiction. We studied whether the common A118G (rs1799971) mu-opioid receptor single nucleotide polymorphism (SNP) was associated with overdose severity in humans. In addition, we examined an SNP responsible for alternative splicing of *OPRM1* (rs2075572). We assessed allele frequencies of the above SNPs and associations with clinical severity in patients presenting to the emergency department (ED) with acute drug overdose. This work was designed as an observational cohort study over a 12-month period at an urban teaching hospital. Participants consisted of consecutive adult ED patients with suspected acute drug overdose for whom discarded blood samples were available for analysis. Specimens were linked with clinical variables (demographics, urine toxicology screens, clinical outcomes) then deidentified prior to genetic SNP analysis. Blinded genotyping was performed after standard DNA purification and whole genome amplification. In-hospital severe outcomes were defined as either respiratory arrest (RA; defined by mechanical ventilation) or cardiac arrest (CA; defined by loss of pulse). We analyzed 179 patients (61% male, median age 32) who overall suffered 15 RAs and four CAs, of whom three died. The 118G allele conferred 5.3-fold increased odds of CA/RA ($p<0.05$), while the rs2075572 variant allele was not associated with CA/RA. The 118G variant allele in the *OPRM1* gene is associated with worse clinical severity in patients with acute drug overdose. These findings mark the first time that the 118G variant allele is linked with clinical drug overdose vulnerability.

Keywords Opioid receptor · Polymorphism · Overdose · Addiction · Cardiac arrest

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Introduction

Poisoning is at an all-time high in the United States, having overtaken motor vehicle collisions as the leading cause of injury-related mortality [1]. Out of approximately 15 injury-related poisoning deaths per 100,000 population in the US, 90% are due to drug overdose [1, 2]. Furthermore, overdose deaths are increasingly associated with nonmedical use and diversion of pharmaceuticals, primarily opioid analgesics [3, 4]. The increase in opioid analgesic abuse is especially worrisome since at the doses that these substances are commonly abused, they can result in respiratory depression and death. In fact, during the period 1999–2008, overdose death rates, sales, and substance abuse treatment admissions related to prescription opioids all increased in parallel [5].

There is considerable interindividual variability in the clinical response to opioid analgesics [6, 7]. The principal target for endogenous and exogenous opioids is the mu-opioid receptor (MOR), a 7-transmembrane spanning, G-protein coupled receptor. Genetic variations in the mu-opioid receptor gene (*OPRM1*) can mediate individual differences in response to pain and opiate addiction. For example, *OPRM1* variants

were initially shown to be responsible for enhanced receptor affinity for beta-endorphin [8] and reduced tissue RNA expression of MOR in the postmortem human brain [9]. Over the past decade, a handful of promising OPRM1 genetic polymorphisms have been identified with relatively high allelic frequency that have now emerged as primary candidate sources for clinically relevant variability in baseline nociception and opioid sensitivity.

A common *OPRM1* single nucleotide polymorphism (SNP) that has been shown to have functional consequences is the A118G SNP (rs1799971). This missense SNP changes the N-terminal region amino acid asparagine to aspartic acid, which decreases the number of sites for *N*-linked glycosylation of the MOR from five to four (NCBI database). The A118G SNP is present in 5–30% of the general population with some racial variation [10]. Several studies have associated this SNP with elevated opioid requirements in patients with painful malignancies [11], postsurgical patients [12], and increased pain sensitivity in healthy human volunteers [13].

The human *OPRM1* gene consists of between 9 and 20 exons and codes for between 19 and 39 alternative-spliced forms [14, 15]. There is a growing body of evidence from rodent studies that demonstrates an important role of alternatively spliced forms of *OPRM1* in mediating opioid analgesia [16, 17]. One particular alternative-splicing variant SNP, rs2075572, has previously been associated with functional consequences regarding nicotine abuse behaviors [18]. Unpublished data from our laboratory has linked this SNP with MAP-kinase signaling in the striatum. We thus sought to investigate the rs2075572 SNP as another candidate for altered clinical severity in our drug overdose population.

Objectives

Given the effect of *OPRM1* SNPs on drug abuse behaviors and drug-effect thresholds, we hypothesized that rs1799971 and rs2075572 would be associated with more severe clinical outcomes due to acute drug overdose. Therefore, in order to evaluate the relationship between *OPRM1* SNPs and drug overdose severity, we studied an emergency department (ED) overdose population and assessed allele frequencies as well as associations with clinical overdose severity.

Methods

Study Design and Setting

This prospective cohort pilot study enrolled consecutive adult (18 years and older) ED patients with acute drug (medication and illicit) overdose over a 12-month period. The ED was located in an urban, tertiary-care medical center with annual visit volume in excess of 75,000 and staffed 24 h/day with board certified emergency physicians and on-call medical toxicologists. The study protocol was approved by the Institutional Review Board for all participating institutions with a waiver of informed consent.

Study Population

Patients with suspected acute drug overdose were initially screened for inclusion using one of two triggers: (1) consultation with the on-call toxicology service carrying a pager 24 h/day and 7 days/week or (2) by telephone referral of the case to the regional Poison Control Center (PCC) with Certified Specialists in Poisoning Information available to take calls 24 h/day and 7 days/week. All eligible patients underwent bedside medical toxicology consultation. Following screening of eligibility, we applied formal inclusion and exclusion criteria to determine whether patients would be included for data analysis. Inclusion criteria were both acute presentation (presentation within 24 h of exposure) and suspected overdose (i.e., illicit drug dose sufficient to cause symptoms or any drug exposure greater than its therapeutic dose). Exclusion criteria were the following: drug anaphylaxis, incomplete data, and do-not-resuscitate orders.

Clinical Data Collection

Data collection from the medical chart occurred in accordance with accepted guidelines for valid medical chart abstraction [19]. Data included demographics (gender, age), exposure information (timing of exposure, number of exposures, intent, suicidality), toxin identification (detail from chart text, serum drug concentrations if available), and toxicology screens. Toxicology screens sent as part of routine care were performed from the urine ELISA panel (Beckman Coulter Inc., AU680) and serum concentration (if any). Blood and urine toxicology screen results sent as routine part of clinical care were recorded in order to confirm exposure. Data were abstracted to a deidentified electronic database with password protection.

Clinical Protocol

Subjects were prospectively followed up to hospital discharge with data that included electronic medical records, paper medical records, consult records, and poison center electronic records. In-hospital overdose severity was defined as the occurrence of either respiratory arrest (RA; defined by mechanical ventilation) or cardiac arrest (CA; defined by loss of pulse requiring chest compressions) and in-hospital mortality (defined as death during the index

hospitalization). The PCC utilizes a daily follow-up system comprised by trained personnel, blinded to present study hypotheses, including: three to four clinical poisoning specialists, six medical toxicology fellows, and 15–20 resident rotators. Information from daily follow-up of all active cases is recorded into an electronic database which was reviewed for all patients. Additionally, hospital medical record follow-up for all patients was performed by one study investigator trained in medical abstraction and recorded using standardized data collection forms. Results (from electronic physician notes, laboratory records, radiology results, and discharge summaries) were prospectively available to the study investigators. Patients discharged from the hospital had no further follow-up.

Blood Sample Acquisition

Blood was drawn from subjects as part of routine clinical care at the time of the initial ED visit. Clinical laboratories at the study hospital routinely retain blood samples (generally for ~3 days) before discarding them. Programs were developed to screen blood samples from eligible patients that were obtained for routine clinical care and about to be discarded. Eligible samples were immediately transferred to cryostorage tubes and relabeled with a unique study code linking them to the deidentified clinical database. These blood samples were stored at −80°C until they were ready for SNP analysis.

Genetic Analysis

Two SNPs were chosen for this analysis based on likelihood of phenotypic impact on a drug overdose population. A118G (rs1799971) was chosen based on prior literature demonstrating associations with opioid tolerance [11, 12] and the response to the opioid antidote naloxone [20, 21]; a second SNP alternative splicing variant (rs2075572) was chosen based on prior literature implicating splicing variants with abuse behaviors [18] and based on unpublished data from our own laboratory implicating functional opioid signaling abnormalities in the striatum.

DNA was extracted and purified in batches using the Qiagen QIAamp DNA blood mini kit. Whole genome amplification was performed using the Qiagen REPLI-g kit from approximately 5 ng of initial genomic DNA per sample, with cycling and hybridization conditions set according to the manufacturer's instructions to produce approximately 1–2 μg amplified DNA per sample. The *OPRM1* polymorphisms (rs1799971 and rs2075572) were genotyped by the *Taqman* SNP Genotyping assay (Applied Biosystems, Foster City, CA, USA) by a blinded study investigator. Common and variant alleles were defined as follows for rs1799971 (AA homozygous common, AG heterozygous, GG homozygous variant) and rs2075572 (CC homozygous common, CG heterozygous, GG homozygous variant).

Statistical Analysis

Descriptive statistics included percentages (for nominal variables) and medians with interquartile ranges (IQR, for continuous variables). Fisher's exact test and Mann–Whitney *U*-test were calculated for categorical and continuous variables, respectively, with 5% alpha (two-tailed). We calculated severe outcome rates by dividing the number of patients with severe outcomes (defined above) in the study population by the total number of included drug overdoses. The PLINK 1.07 genetic association analysis program was used to verify SNP data quality, test for departure from Hardy–Weinberg equilibrium (HWE) and test individual SNPs for statistical univariate associations [22]. Computer analysis of univariate odds ratios, 95% confidence intervals (CI), and stepwise multivariable logistic regression were performed using SPSS v 19 software (IBM Inc., Chicago, IL, USA).

Results

Baseline Clinical Characteristics

Over the course of the study period, there were 359 individual subjects meeting inclusion/exclusion criteria, of whom 190 (mean age, 41.9±0.9 years, 62% male) had discarded blood samples available for analysis and 11 (3.1%) had incomplete follow-up data (i.e., left against medical advice, eloped from hospital, incomplete charts), allowing for analysis of 179 patients with both blood and outcome data. Overall, 15 patients experienced in-hospital severe outcomes: 15 RAs and four CAs, of whom three died. There were no substantial differences in demographics (age, gender) between those with and those without serious outcomes. Exposure intent was an important determinant of severe outcome with suicidal and recreational intent both associated with severe outcomes. There were no significant associations with outcome for any cardiopulmonary medical comorbidities (e.g., coronary artery disease) or number of drug exposures. Baseline clinical characteristics of all 179 patients with outcome data are summarized in Table 1.

Drug Exposures

In the SNP-analyzed cohort (n=179), the majority were intentional exposures (68%), and about half occurred with suicidal intent (47%). Numbers of exposures per subject ranged from 1 to 9 (median 2, IQR 1–2), and about half (51%) of all subjects presented with multidrug overdoses. The top five drug classes

Table 1 Baseline clinical characteristics of 179 overdoses with outcome determination

Clinical characteristic	Control[a]	Severe outcome
	No. (%) or median (IQR)	
Demographics		
Males	100 (61)	12 (80)
Median age	36 (25–52)	52 (31–64)
Exposure intent[b]		
Suicidal*	79 (48)	2 (13)
Recreational*	30 (18)	7 (47)
Therapeutic error	35 (21)	6 (40)
Unintentional	14 (9)	2 (13)
Undetermined	8 (5)	1 (7)
Number of drug exposures		
Single drug	56 (34)	3 (20)
Multidrug	92 (56)	7 (47)
Undetermined*	16 (10)	5 (33)
Median number exposed	2 (1–2.8)	2 (1–2)
Medical comorbidities		
CHF	9 (5)	3 (20)
COPD	3 (2)	0 (0)
Coronary artery disease	10 (6)	3 (20)
Diabetes mellitus	8 (5)	1 (7)
Totals	164 (100)	15 (100)

CHF congestive heart failure, *COPD* chronic obstructive pulmonary disease, *IQR* interquartile range, *%* percent of population within selected column with selected characteristic

[a] Controls defined as patients in the study without severe outcomes

[b] Exposure intent was not mutually exclusive, thus percentages in this category do not add up exactly to 100

*Univariate $p<0.05$ based on Fisher's exact test for nominal variables

exposed (based on self-reporting and clinical history in the medical record) were, in descending order: opioids (32, 18%), sympathomimetics (30, 17%), benzodiazepines (24, 13%), antipsychotics (13, 7%), and antidepressants (12, 7%). Ethanol was coingested in 58 patients (32%). Drug exposure confirmation through analytical serum/urine testing (in addition to suspicion of overdose) was positive in 118 (66%) patients. Urine toxicology was positive in 61 of 84 tested (73%), of which there were methadone, and two barbiturates. There were univariate associations with severe outcomes for overdoses involving opioids (OR=3.5; 95% CI, 1.1–10.8) and benzodiazepines (OR=3.8; 95% CI, 1.2–12.4). There was insufficient power to detect any univariate associations between any specific toxicology laboratory screening and either of the variant SNP alleles. The top five drug class exposures and associations with severe outcomes are summarized in Table 2.

SNP Results

SNP analysis was conclusive for the A118G allele in 161 subjects (11 with poor DNA quality) and for the rs2075572 allele in 148 subjects (seven poor DNA quality, 17 quantity not sufficient). The A118G SNP was wild-type AA in 115 (71%), heterozygous AG in 37 (23%), homozygous variant GG in nine (6%), yielding a G allele frequency of 18.5%, which was consistent with HWE (chi-square p=NS for inconsistency). The rs2075572 SNP was wild-type C/C in 75 (46%) patients, heterozygous C/G in 56 (34%) patients, homozygous G/G in 33 (20%) patients, yielding a G allele frequency 37.2%, which was consistent with HWE (chi-square p=NS for inconsistency). Allele frequencies and HWE analyses are summarized in Table 3.

Genetic Association with Clinical Outcomes

The overall incidence of severe in-hospital outcomes was 8.7% (95% CI, 5.4–13.9%). The 118G allele was associated with 2.5-fold increased odds of CA/RA (OR=2.5, $p<0.05$)

Table 2 Top five drug class exposures and severe outcomes

Drug class	Clinical suspicion	Toxicology screen positive[a]	Severe outcomes	OR[b] (CI)
	No. (% total)	No. (% in class)		
Opioids[c]	32 (18)	29 (91)	6 (19)	3.5 (1.1–10.8)
Sympathomimetics	30 (17)	27 (90)	2 (7)	0.8 (0.2–3.5)
Benzodiazepines[c]	24 (13)	21 (88)	5 (21)	3.8 (1.2–12.4)
Antipsychotics	13 (7)	13 (100)	0 (0)	0.9 (0.8–1.1)
Antidepressants	12 (7)	12 (100)	0 (0)	0.9 (0.8–1.1)
Totals	179 (100)	118 (66)	15 (8.4)	

CI 95% confidence intervals, *OR* odds ratios

[a] Patients were considered "toxicology screen positive" if any screen sent was positive

[b] Univariate association between drug class and severe outcomes based on clinical suspicion

[c] $p<0.05$ based on Fisher's exact test for nominal variables

Table 3 Allele frequencies and HWE analysis

SNP	Genotype	No. (%)	Allele	Count	Allele frequency	HWE *p* value
A118G[a]	AA	125 (70)	A	290	0.815	NS
	AG	40 (22)	G	66	0.185	
	GG	13 (7)				
Alternative splicing[b]	CC	75 (46)	C	206	0.628	NS
	CG	56 (34)	G	122	0.372	
	GG	33 (20)				

HWE Hardy–Weinberg equilibrium, *NS* not significant, *SNP* single nucleotide polymorphism

[a]rs1799971

[b]rs2075572

for all drug exposures. The rs2075572 variant allele was not associated with CA/RA (*p*=NS). To assess for an association between the A118G variant allele and severe clinical outcomes, we used stepwise multivariate logistic regression analysis. For the purposes of this analysis, G allele subjects were defined as either the GG or AG genotype on A118G SNP, in accordance with conventions from previous clinical studies [23, 24]. We derived a model that included covariates based on univariate factor analysis using the following variables in the final model: G allele (either GG or AG genotype), age, number of drug exposures, and recreational intent. Based on this model, independent predictors of severe clinical outcomes included 118G allele (adjusted OR=5.3; 95% CI, 1.2–23.8, $p<0.05$) and recreational intent (adjusted OR=12.9; 95% CI, 2.5–67, $p<0.01$). Neither age nor numbers of drug exposures were significantly associated with outcomes in the final model. Independent predictors of severe clinical outcomes from stepwise multivariable logistic regression analysis are summarized in Table 4.

Discussion

The main findings of this study are the 118G variant allele was associated with higher risk of a severe clinical outcome in a population of acute drug overdoses. In addition, we found high allele frequencies of opioid SNPs in our diverse overdose population which included non-opioid exposures and which did not violate assumptions under Hardy–Weinberg, suggesting these results are generalizable. These findings for the first time link the 118G variant allele with clinical drug overdose vulnerability and add to the growing body of evidence linking it to drug abusing populations. Therefore, the A118G SNP of the *OPRM1* gene may be a viable target for risk stratification and may drive future tailored prescription practices for populations at risk for drug overdose.

There are several mechanisms that can explain why SNP variants in *OPRM1* could result in changes in clinical severity of drug overdose. Anatomical changes in *OPRM1* gene expression (e.g., receptor density in the medulla respiratory center) could result in an increase or decrease in clinical response to opioid drugs. Alterations in affinity of the receptor for selected opioid ligands could change overall drug efficacy and lead to changes in drug use behaviors, drug tolerance, and pain perception. Analgesia thresholds may similarly vary in gene dose-dependent fashion, which has been demonstrated in rodent models for mice that lack *OPRM1* and subsequently have lower nociceptive thresholds than heterozygous knockouts that have 50% of wild-type receptor densities [16, 25]. Finally, it is possible that *OPRM1* SNPs could affect the dynamics of reward pathways which could affect drug abuse behaviors. For example, the opioid system is a convergent neuronal pathway for most drugs of abuse thus even opioid antagonist medications such as naltrexone are useful for treating alcoholics based on their *OPRM1* genotype [23, 26].

These data add to the diverse body of literature implicating the A118G SNP with clinical outcomes across varying pathological settings. Recently A118G has been found to potentially play a role in response to HIV treatment and breast cancer survival [27, 28]. There has also been conflicting evidence regarding the role of A118G and suicide related outcomes [29, 30]. Interestingly, we found no association with either SNP in the current study and suicidal ideation (*p*=NS for both); however, this study was underpowered to draw definitive conclusions, and further research is warranted to evaluate *OPRM1* variants with suicide related outcomes.

Our results have implications for personalized medicine regarding opioid prescriptions in high-risk subjects. Because MOR is the major site for the analgesic action of most

Table 4 Factors associated with severe clinical outcomes

Factor	Univariate OR (CI)	Adjusted OR[a] (CI)	*p* value[a]
118 G allele[b]	2.8 (1.0–8.2)	5.3 (1.2–23.8)	0.027
Recreational Intent	4.2 (1.3–12.8)	12.9 (2.5–67)	0.002

CI 95% confidence intervals, *OR* odds ratios

[a] Model for stepwise multivariable logistic regression adjusted for the following factors: age, presence of at least one 118G allele (i.e., patients with AG or GG genotype), recreational intent of overdose, and number of drug exposures

[b] G allele subjects defined as patients with either AG or GG genotype on A118G SNP, in accordance with conventions of previous clinical studies [23, 24]

clinically important opioid drugs, it follows that information about *OPRM1* genetic polymorphisms that can predict the likelihood of high or low expression in an individual could allow drug treatments to be tailored. For example, clinicians could prescribe alternate analgesics for those vulnerable and conceivably optimize dose ranges. Patients with chronic pain syndromes could benefit from better optimization of medication regimens. It may be possible to select a particular opioid with a safer therapeutic window for an individual based on her genotype and alternate opioids with safer profiles could be selected for high-risk populations. Finally, individuals could be genetically screened in pain clinics prior to initiation of an opioid to account for any known opioid sensitivity or vulnerability to overdose.

We performed multiple assessments for bias in our population that would undermine our central hypothesis. First, we assessed whether the presence of medical comorbidities (see Table 1) could explain the association with cardiorespiratory arrest; however we found no significant associations with chronic cardiovascular conditions (coronary artery disease, congestive heart failure, diabetes) or pulmonary conditions (chronic obstructive pulmonary disease). In addition, we performed multivariable logistic regression analysis to control for confounders in the association between 118G variant allele and outcome severity. Using this approach, 118G allele remained significantly associated with outcomes (see Table 4). Therefore, we do not believe that confounding explains any of the association between the 118G allele and severe outcomes in our results.

Future studies in animal models should evaluate changes in brainstem center expression based on particular *OPRM1* SNPs. There is also a need for human clinical studies evaluating *OPRM1* SNPs using bio-banks with increased population size than our study was able to achieve for more robust factor analysis. While the purpose of this study was to evaluate whether a potential association with clinical outcomes exists, it is premature to speculate which populations should be targeted for genetic testing. Future research will need to test specific at-risk populations to determine the impact on overdose prevention. In addition, future animal and human models should explore other *OPRM1* SNPs and SNPs related to other targets for drugs of abuse (e.g., benzodiazepines, stimulants) to further evaluate associations with drug overdose vulnerability.

Limitations

There are several limitations to our study that must be taken into account when considering our results. We only analyzed two SNPs out of >30 common *OPRM1* SNPs, which should be assessed with future studies. As this was a pilot study, we had access to only a small sample size which limited robust factor analysis and ability to evaluate for the association with prior overdose. Our database was limited by lack of racial information which prevented us from assessing for racial selection bias [31]; however our allele frequencies did not violate HWE which suggests this was not an issue. Technical limitations of our protocol included long storage time of samples, resulting in lower DNA quality and thus higher required sample volumes which was not possible for all cases; however this only led to a limited number of incomplete genetic data (approximately 5% of samples). And finally, our clinical outcomes were not adjudicated, but the composite endpoint (CA or RA) included easy-to-interpret outcomes that minimized the chance for bias in data collection or outcome classification. Defining RA using mechanical ventilation as our endpoint may have resulted in possible misclassification of a fraction of patients who were intubated for airway protection alone; however, subdividing the group based on such distinction is not useful in terms of severity, given that all intubated patients require intensive care unit admission.

Conclusions

OPRM1 SNP genetic screening in a population of acute drug overdose patients demonstrated that the 118G allele is highly prevalent and is significantly associated with worse clinical severity. These findings add to the growing body of evidence linking the A118G SNP with behavioral/physiologic drug abuse vulnerability. Validation studies and clinical feasibility programs are warranted to evaluate whether the A118G SNP may be a potential screen for personalized medical prescribing practices for the prevention of severe drug overdoses.

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