Pharmacogenetics of lethal opioid overdose: Study protocol and preliminary findings

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Article

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Abstract

There has been a worldwide substantial increase in accidental lethal opioid-overdose (ALOO). In this project, we will examine the role of genetic variation in opioid metabolism, transport, or opioid receptors, in contributing to opioid-related overdose deaths by 1) comparing the frequency of those variants to a corresponding reference population and exploring sex differences; 2) investigating the association between the metabolizer type (i.e., CYP2D6 poor metabolizers) and plasma concentrations; and 3) generating a series of polygenic risk scores (PRS) for predicting ALOO by using summary statistics from several large-scale genome-wide association studies (GWAS) of phenotypes relevant to opioid use disorder. This sample is currently being collected; however, we have analyzed the frequency of \textit{CYP2B6}*4, \textit{CYP2B6}*9 and \textit{OPRM1} A118G variants in methadone-only fatalities (n = 41). Findings showed a higher frequency of impaired CYP2B6 metabolism in males compared to females (p = 0.009, chi sq = 9.455), which suggests a potential genetic risk factor for lethal overdose in males.

1. Introduction

Opioids, commonly used as pain medication but abused as illicit drugs, bear a high risk for development of severe adverse effects and lethal intoxications. Opioid-related mortality caused by adverse drug reactions and unintentional overdose, is a serious and global problem. According to the World Health Organization (WHO), the number of opioid overdose deaths has increased substantially in recent years \cite{1}. Specifically, recent statistics from the Public Health Agency of Canada show that between April and December 2020, there were at least 5000 apparent opioid-related deaths in Canada, of which 96% were accidental \cite{2}. With the emergence of the coronavirus (COVID-19) pandemic, opioid-related mortality has increased by 89% in April to December 2020, compared to the same time period in 2019 in Canada alone \cite{2}. Provisional data from the National Center for Health Statistics in the U.S. indicate a 26% increase in opioid overdose deaths from April 2020 to April 2021, compared to the same period the year before \cite{3}. These statistics highlight the worsening of the opioid crisis as it affects a much wider population: from individuals who consumed drugs for the first time, to those living with chronic pain, and individuals with more substance use experience.

Unfortunately, there are still no clinical, demographical, or biological factors available to predict which individuals are at a higher risk for accidental lethal adverse effects. In this project, we hypothesize that genetic variations in opioids’ metabolizing enzymes and drug receptors will predict individuals’ response and tolerance to opioids.

Our first aim is to investigate single nucleotide polymorphisms (SNPs) or copy number variations (CNV), in genes with known functional relevance in opioids’ metabolism. Opioids, mainly tramadol, codeine, and oxycodone, are metabolized by CYP2D6, while methadone is metabolized by both CYP2B6 and CYP2C19, and fentanyl by CYP3A4/5 (see Table 1). In regard to fentanyl’s metabolism, there’s limited evidence linking CYP3A4/5 genotypes with variability in fentanyl’s adverse side-effects (see Table 1). CYP3A4 poor metabolizers are rare \cite{4}, and only a small number of SNPs were found to be common in at least one of these populations: Europeans, Africans, East Asians, and Admixed Americans \cite{5}. In contrast, variations in the \textit{CYP2B6} genes have been extensively shown to alter methadone’s metabolism, plasma levels, and toxicity \cite{6, 7}. Clinical studies have indicated that \textit{CYP2B6} slow metabolizers (*6/*6 genotype) show greater (S)-methadone plasma concentration and greater risk for QT prolongation and cardiac arrhythmias, compared with normal metabolizers (*1/*10) \cite{8, 9}. Furthermore, genetic variants in \textit{CYP2D6} predict four metabolizer phenotypes with differing rates of opioid metabolism, which are: 1) poor metabolizers (PM), 2) intermediate metabolizers (IM), 3) normal (formerly extensive) metabolizers (NM), and 4) ultrarapid metabolizers (UM) \cite{10}. According to the Clinical Pharmacogenetics Implementation Consortium (CPIC), when prodrugs such as codeine or tramadol are used for analgesia, individuals who are \textit{CYP2D6} ultrarapid metabolizers (UMs) produce higher levels of active metabolites and, therefore, are at a greater risk for lethal side effects (such as respiratory depression) \cite{10}. In addition, a recent review has found that approximately 7% of individuals who died due to an opioid overdose carried an UM phenotype \cite{11}.
<table>
<thead>
<tr>
<th>Medication</th>
<th>Pharmacokinetic genes</th>
<th>Pharmacodynamic genes</th>
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<tbody>
<tr>
<td><strong>Metabolism</strong></td>
<td><strong>Transport</strong></td>
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<tr>
<td><strong>Primarily metabolized</strong></td>
<td><strong>Substantially metabolized</strong></td>
<td><strong>Minimally metabolized</strong></td>
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<td>Fentanyl</td>
<td>CYP3A4</td>
<td>CYP3A5</td>
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<td><em>CYP3A4</em>1G decreases the metabolism of fentanyl and thus there is a higher plasma concentrations of fentanyl. [20]</td>
<td>Fentanyl plasma concentration in the CYP3A5*3/<em>3 group was higher than in the CYP3A5</em>1/<em>1 and CYP3A5</em>1/<em>3 groups after transdermal fentanyl administration [21]. The central adverse effects were slightly higher in the CYP3A5</em>3/<em>3 group than in the CYP3A5</em>1/*1, *1/*3 group [21].</td>
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<tr>
<td>Methadone</td>
<td>CYP2B6</td>
<td>CYP2D6</td>
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<td><em>CYP2B6</em>1/*4 + <em>4/</em> carriers have increased clearance of methadone compared to CYP2B6 *1/*1 carriers [7]. <em>CYP2B6</em>1/*6 + *6/*6 carriers have decreased clearance of methadone compared to CYP2B6 *1/*1 carriers [8].</td>
<td>CYP2D6 ultrarapid metabolizers have 0.7-fold decrease in trough (S) and a 0.8-fold decrease in (R)-methadone plasma levels compared with the extensive or intermediate metabolizers. PMs did not present significantly different methadone plasma levels compared with the EM/IM group [9].</td>
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<th>Medication</th>
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<tr>
<td>Morphine</td>
<td>UGT2B7</td>
<td>ABCB1</td>
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<td></td>
<td>*UGT1A1 and UGT1A8</td>
<td>C3435T:</td>
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<td>contribute minimally to the variation in morphine metabolic ratios after oral morphine administration in subjects affected by cancer [26].</td>
<td>Studies involving postoperative, or cancer cohorts have shown higher, lower, or no changes in dosing requirement for the TT genotype carriers compared with the CC/CT genotypes [27–29].</td>
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<td>OCT1</td>
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<td>SLC22A1</td>
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<td>*2/*2 + *3 + *4 + *5 + *6 genotypes is associated with decreased clearance of morphine in children as compared to SLC22A1 *1/*1 genotype [30, 31].</td>
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<td>OPRM1</td>
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<td>A118G: G-allele in OPRM1 was strongly associated with increased morphine requirement. [27]</td>
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Genotype GG is associated with increased dose of morphine in women with postoperative pain compared to genotypes AA + AG [32].

COMT: Val158Met: Higher morphine dosing requirements in individuals with higher COMT activity (Val/Val) and a lower morphine dosing requirement in patients with a lower COMT activity (Met/Met) [33].

### Medication | Pharmacokinetic genes | Pharmacodynamic genes
--- | --- | ---
**Oxycodone** | CYP3A4 and CYP3A5  Noroxycodone and its ratio to oxycodone were significantly higher in the CYP3A5*1/*1 + *1/*3 group than in the *3/*3 group. Incidence of dose escalation was significantly higher in CYP3A5*3/*3 than in *1/*1 + *1/*3 [34]. | CYP2D6  *CYP2D6* poor metabolizer phenotype is associated with decreased plasma oxymorphone/oxycodone ratio and decreased reduction in pain when treated with oxycodone in healthy individuals compared to CYP2D6 extensive metabolizer phenotype [35].  *CYP2D6* ultrarapid metabolizer phenotype is associated with increased side effects when treated with oxycodone in healthy individuals compared to the normal metabolizer phenotype [35]. | ABCB1  3435C>T: TT homozygous patients received higher 24h- and weight-surface area-adjusted-24h-opioids doses. [36] | COMT  Val158Met: Val/Val group demonstrating a higher 6-48hour opioid consumption when compared with that of the Met/Met group. [37]
**Heroin** | Heroin is metabolized into morphine, thus, for further details regarding the pharmacokinetic and pharmacodynamic genes of heroin please refer to”Morphine”.
 | | |
**Carfentanil** | Carfentanil is an analogue of the synthetic opioid fentanyl and pharmacokinetic information is limited to animal and in vitro studies as well as a few scattered case reports of intentional or unintentional human exposures. While the specific P450 isoforms responsible are not known, CYP3A4 is the most likely isoform based upon analogy of carfentanil with fentanyl [38].
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<tr>
<td><strong>Codeine</strong></td>
<td>CYP2D6</td>
<td>ABCB1</td>
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<td>Genetic variations in <em>CYP2D6</em> are critical for codeine as they affect drug response and adverse events. CPIC recommendation for opioids is to avoid codeine for poor and ultrarapid metabolizers, and to monitor intermediate metabolizers for less-than-optimal response [4].</td>
<td><em>ABCB1</em> 2677 T/T and <em>CYP2D6</em> EM or UM genotypes were highly associated with codeine toxicity resulting from increased morphine in the brain in both infants and their mothers [40].</td>
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<tr>
<td>UGT2B7</td>
<td>Codeine is metabolized to morphine, therefore, please see the &quot;Morphine&quot; section for more details regarding pharmacodynamic genes of Codeine.</td>
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<tr>
<td><strong>Tramadol</strong></td>
<td>CYP2D6</td>
<td>ABCB1</td>
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<tr>
<td>Genetic variations in <em>CYP2D6</em> are critical for tramadol as they affect drug response and adverse events. CPIC recommendation for opioids is to avoid codeine and tramadol for poor and ultrarapid metabolizers, and to monitor intermediate metabolizers for less-than-optimal response [4].</td>
<td><em>G2677T</em>/A: A-allele is associated with increased likelihood of a decrease in Visual Analog Scale (VAS) of more than 30 mm within 6 hours when treated with tramadol in people with bone fractures as compared to allele G [41].</td>
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<td></td>
<td></td>
<td>OPRM1</td>
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<tr>
<td></td>
<td></td>
<td>A118G: G-carriers had lower response to tramadol for treatment of cancer pain [42].</td>
</tr>
<tr>
<td><strong>Utopics</strong> (U-47700)</td>
<td>There is very little pharmacological data published for Utopics. There seems to be no pharmacokinetic gene data.</td>
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Our second aim is to investigate variants in pharmacokinetic genes related to opioid transport. The P-glycoprotein protein pump, encoded by the *ABCB1* gene, regulates the concentration of certain opioids (e.g., fentanyl) in the brain [7]. However, genetic variants in the *ABCB1* gene have inconsistently been shown to influence plasma levels and response of opioids in previous studies (see Table 1). The association between *ABCB1* variations and opioids pharmacokinetic variability remains complex, thus there's still a need to determine the role of P-gp functionality on opioids' plasma levels and response.

Our third aim is to investigate variants in genes with known functional relevance in opioid-receptor binding and opioid response. The A118G SNP in the mu-opioid receptor-encoding gene, *OPRM1*, is of particular interest as it has shown consistent association

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with morphine dosing requirements. A meta-analysis with 4,607 postoperative patients showed that the OPRM1 118G-allele carriers were associated with higher postoperative morphine dose requirements compared to the AA homozygotes [12]. The OPRM1 A118G variant has also been linked with susceptibility to drug addiction [13]. Other genes known to be involved in development of addiction and response to opioids, such as the dopamine receptor-encoding gene, DRD2, and the Catechol-o-methyltransferase-encoding gene, COMT, have shown preliminary but limited association between their common genetic variants and opioid dosing variability (see Table 1).

One future aim will be to explore genome-wide analyses (e.g., whole-exome/genome sequencing), for example testing the relevance of polygenic risk scores to ultimately develop comprehensive predictive models to identify individuals at high risk of opioid overdose.

**Objectives**

1. Our first objective will be to investigate the frequency of metabolizer status in drug metabolizing enzymes (CYP2D6, CYP2B6, CYP3A4/5, CYP2C19) in our sample as these likely contributed to excessive opioid serum concentrations.
   
   a. *We hypothesize that our sample will be enriched for non-normal metabolizers compared to the frequency of a reference population. The CYP2B6/CYP2C19 poor metabolizer phenotype will be enriched in our methadone-overdose cases and the CYP3A4 poor metabolizer phenotype will be enriched in the fentanyl-overdose cases. In contrast, we hypothesize that the CYP2D6 UM phenotype will be enriched in our codeine- or tramadol-overdose cases.*

2. Our second and exploratory objective will be to compare the frequency of ABCB1 gene variants in our sample to the frequency of the reference population.

   a. *We hypothesize that the ABCB1 TTT haplotype (C3435T, C1236T and G2677T) will be enriched in our sample with opioid overdose.*

3. Our third and exploratory objective will be to compare the frequency of OPRM1 gene variants in our sample to the frequency of the reference population.

   a. *For the OPRM1 A118G variant, we hypothesize that the presence of the G-allele will be enriched in our sample with opioid overdose.*

**2. Materials And Methods**

**2.1 Recruitment**

In this study, we aim to include 200 cases of unintentional opioid-related fatalities. Identification of appropriate cases will be through our collaboration with the Regional Supervising Coroner, RW, and Deputy Chief Coroner, RJ, from the office of the Chief Coroner of Ontario and Ontario Forensic Pathology Service (OCC/OFPS). Inclusion of cases into our study will involve the next-of-kin approval in the consent process. After which, anonymized blood samples will be released to our research team for investigation.

**2.2 Toxicological report**

We will receive data pertaining to sex and ancestry, in addition to variables that will be collected at time of death including age, plasma levels of opioids, and co-intoxication. All clinical and demographical data obtained will be anonymized by RW from the OCC.

**2.3 Blood sample collection**

Blood samples are stored at the OCC/OFPS. Blood samples will be used for DNA extraction and genotyping, which will be performed in the CAMH Biobank and Molecular Core Facility.

**2.4 Selection of gene variants for genotyping**
We will prioritize the genetic markers based on previous association findings in the literature, their functional significance according to online databases (PharmGKB) [14].

5. *CYP3A5* (*1, *3)
6. *ABCB1* variants (C1236T, G2677T and C3435T)
7. *OPRM1* variants (A118G)

### 2.5 Genotyping and quality control

Sample will be sent for genotyping at the CAMH Biobank and Molecular Core Facility (Centre for Addiction and Mental Health, Toronto, Canada). Genotyping will be performed using standard TaqMan® Assays (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol. CNVs including deletion (*5) and duplications of *CYP2D6* will be assessed using a copy-number assay and CopyCaller Version 1.0 (Applied Biosystems, Burlington, ON, Canada). The overall phenotype for *CYP2D6* duplications will be determined using the results from the SNP and CNV assays (e.g., genotype is reported as *1/*3 (xN) if SNP assays revealed *1 and *3 and CNV assay showed more than two copies of the *CYP2D6* gene for the same participant).

Predictions of *CYP2D6* and *CYP2C19* metabolizer phenotypes will be based on the expected enzyme activity of the alleles as reported in CPIC guidelines for CYP450 genes (https://www.pharmgkb.org/guidelines). *CYP2B6* *4 (rs2279343) and *9 (rs3745274) will be genotyped using standard PCR amplification followed by restriction enzyme digest. The digested products will be visualized on an agarose gel. Genotyped SNPs will undergo standard quality control (QC) steps including minor allele frequency (MAF) > 5%, Hardy-Weinberg equilibrium (p > 0.05, genotype call rates > 99%, and percentage of missing genotypes individual < 10%. CNVs will be called using PennCNV.

### 2.6 Statistical analyses

All analyses will be conducted using R Version 4.0.4. (R Foundation for Statistical Computing Platform, 2021) and RStudio Version 1.4.1106 (RStudio Inc, 2021). The normality of variables was tested using the Shapiro-Wilk test. Descriptive statistics for demographic and clinical characteristics will be generated using the chi-squared test for categorical variables and the Kruskal-Wallis test for continuous variables. We will apply Wilcoxon's test to compare genotype frequency between overdose cases and frequency in the reference population (using the 1000 genome sample). Our proposed sample of 200 cases has more than 80% power to detect an effect size of 1.50 (minor allele frequency 0.2, alpha 0.05, additive model).

### 3. Results

#### 3.1 Preliminary data

To test the feasibility of this project and for quality control purposes, we have obtained and analyzed the first 41 blood samples from post-mortem methadone-overdose cases. The majority (75%) of the cases were male. We have genotyped 25 samples for the *CYP2B6* *4, CYP2B6* *9 variants and 41 samples for the *OPRM1*A118G variant. Our initial results suggested a minor allele frequency of 0.3 for *CYP2B6* *4 and *9 variants, which is consistent with the reported global frequency (shown in Fig. 1–2). Notably, we detected a difference in the distribution of *CYP2B6* *9 between males and females in our sample (p = 0.009, chi sq = 9.455) (shown in Fig. 3). With respect to the *OPRM1* gene, we observed a minor allele frequency of 0.05 in our sample of methadone-overdose (shown in Fig. 4–5).

### 4. Discussion And Conclusions

This study will aim to discover genetic variants associated with opioid overdose in post-mortem cases obtained from the Chief Coroner of Ontario (OCC). Our preliminary analysis examined the frequency of *CYP2B6* *4, CYP2B6* *9 and *OPRM1*A118G SNPs in methadone-only overdose to test the feasibility of the project and for quality control, while our future analysis will be inclusive of
all opioids. Our preliminary results show a minor allele frequency (MAF) for the *CYP2B6*4 to be 0.3 (30%). The global minor allele of the *4-allele frequency has been reported to be 29% (www.ensembl.org). *CYP2B6*4 is an increased function allele, and it has been reported to increase R- and S-methadone oral clearance after a single dose intake of oral methadone in healthy volunteers [7].

As for *CYP2B6*9, we have obtained a minor allele frequency of 0.3 (30%). The global *9 minor allele frequency has been reported to be 32% (www.ensembl.org). The *CYP2B6*9 is known to decrease methadone metabolism. Ahmad et al., 2017 has found that the *9-allele was enriched in a sample of methadone-overdose cases (N = 125) compared to a control group from the same population [15]. Our data is inconsistent with the results obtained by Ahmed et al., 2017. However, it should be noted that the *9-allele frequency varies across populations of different ancestry. While population stratification was not part of this preliminary analysis, we are planning to use ancestry-informative markers at a later stage in this study. Furthermore, since our recruitment is still ongoing, we may observe different results as the sample size increases. Nonetheless, we did observe an interesting finding with regards to the differences in the distribution of the *9-allele between males and females. Males were more likely to carry the decreased function *9-allele, which could explain the over-representation of males in our sample of lethal methadone overdose cases. The *9-allele may be one of many risk factors for overdose in males only, since females are protected through the upregulated normal CYP2B6 gene by estrogen [16–18]. This was shown in studies that reported higher methadone clearance in pregnant females undergoing methadone maintenance treatment during pregnancy contributed by the increase in estradiol circulation [16, 17].

With respect to the *OPRM1 A118G variant, we have found that the minor allele frequency (G-allele) in our sample to be 0.05 (5%). The frequency of the 118G-allele varies greatly amongst populations of different ancestry, with 1% in Africans, 16% in Europeans, to 42% in South Asians (www.ensembl.org). Bunten et al., reported a frequency of 10% for the 118G-allele in post-mortem methadone-related fatalities of Caucasian (European) ethnicity [19]. Therefore, without information on ancestry in our preliminary analysis, our results cannot be compared to that of Bunten et al., 2011 [19].

There are certain limitations to our preliminary analyses. First, we have limited information, i.e., only obtained demographic data on sex from the 41 post-mortem samples that we have genotyped. For our future analysis, we plan to collect additional demographics, including ancestry, age at death and plasma levels. Second, we have obtained samples of methadone-overdose only, however, in future case recruitment, we will collect samples from a wide array of opioids involved in accidental overdose in addition to corresponding plasma levels. Furthermore, we are planning to increase our sample size to connect with future consortia, such as the substance use disorder (SUD) working group part of the psychiatric genomic consortium (PGC).

The ongoing analysis in this project is unique because, first, it will explore the association of genetic contributions in overdose caused by several opioid medications. Second, it will provide an insight into new variants and their implication in opioid overdose by performing whole-exome sequencing. Finally, we expect to validate our findings through collaborators in global consortiums. Given the complexity of this study design, this work could lead to generations of new hypotheses that will be validated in future studies with larger samples.

**Declarations**

**Acknowledgments**

We would like to thank our CAMH Biobank and Molecular Core Facility for sample genotyping.

**Funding**

This work was supported by the CAMH-AHSC AFP Innovation fund (Centre for Addiction and Mental Health, Toronto, Canada), Project name: Pharmacogenetics of Lethal Opioid Overdose.

**Author contributions:**

IG was responsible for project supervision, project administration, funding acquisition, design and review of protocol, writing original draft, manuscript review & editing. LM was responsible for project coordination and administration, design of study methodology, writing and review of protocol, extracting and analysing data, interpreting results, and creating tables and figures,
RW was responsible for project recruitment, sample and data acquisition, consent-form collection, next-of-kin contact, manuscript review and editing. RJ was responsible for project recruitment, manuscript review and editing. BLF was responsible for Project conceptualization, supervision, manuscript review & editing. DJM was responsible for project conceptualization, supervision, manuscript review & editing.

Statement of Ethics

This study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. This study protocol was reviewed and approved by the Centre for Addiction and Mental Health Institutional Ethics Review Board (REB), project name: Pharmacogenetics of lethal opioid overdose; approval number [096-2013]. Written informed consent was obtained from next-of-kin to participate in the study.

Competing Interests

The authors have no conflicts of interest to declare.

Data Availability Statement

The data that support the findings of this study are not publicly available because they contain genetic information that could compromise the privacy of research participants but are available from the corresponding author [D.J.M] upon reasonable request.

References


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**Figures**

**Figure 1**

Distribution of *CYP2B6* genotype in the whole sample of lethal methadone overdose cases (n=23)
Figure 2

Distribution of *CYP2B6*<sup>*9*</sup> genotypes in the whole sample of lethal methadone overdose (n=25).

Figure 3

Distribution of *CYP2B6*<sup>*9*</sup> genotypes in the whole sample, stratified by sex (n=24).
Figure 4

Distribution of *OPRM1* genotypes in the whole sample of lethal methadone overdose (n=41).
Figure 5

Frequency of *OPRM1* A118G genotypes in the whole sample, stratified by sex (n=24).