Implications of OPRM1 and CYP2B6 variants on treatment outcomes in methadone-maintained patients in Ontario: Exploring sex differences

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Abstract

Genetic variants in the OPRM1 and CYP2B6 genes, respectively coding for an opioid receptor and methadone metabolizers, have been linked to negative treatment outcomes in patients undergoing methadone maintenance treatment, with little consensus on their effect. This study aims to test the associations between pre-selected SNPs of OPRM1 and CYP2B6 and outcomes of continued opioid use, relapse, and methadone dose. It also aims to observe differences in associations within the sexes. 1,172 participants treated with methadone (nMale = 666, nFemale = 506) were included in this study. SNPs rs7356864 and rs7451325 from OPRM1 and all the tested CYP2B6 SNPs were detected to be in high linkage disequilibrium. Though no associations were found to be significant, noteworthy differences were observed in associations of OPRM1 rs7356864 and CYP2B6 rs3745274 with treatment outcomes between males and females. Further research is needed to determine if sex-specific differences are present.

Introduction

Background

Methadone maintenance treatment (MMT) targeted for patients with opioid use disorder (OUD) has been proven over time to decrease opioid cravings and use [1]. However, due to the chronic classification of OUD, MMT is not curative, but aims to maintain patients on a specific dose, controlling their opioid use and enabling them to regain stability [1–3]. Administered methadone binds to endogenous opioid receptors in the human brain, eliciting similar effects within the reward system as an opioid would, while suppressing withdrawal symptoms [4].
Though effective in reducing opioid use, MMT has been observed to have interindividual variability in methadone’s metabolism and methadone blood concentration for a given dose [5]. This can be potentially dangerous to patients, as prescribing physicians are unable to accurately predict the patient’s reaction to a methadone dose prior to administering it. If the methadone dose administered is too low, the patient can be at a high risk of relapse [6, 7]. Alternatively, if the dose is too high, the patient might be at a risk of overdosing, if supplementing with other opioids [8]. As such, a genetic predisposition for individual-based MMT outcomes has been the focus of much research [9–12].

The opioid receptor proteins, encoded by the mu opioid receptor 1 (OPRM1) gene, bind both endogenous and exogenous opioids, resulting in pain relief and feelings of euphoria [13]. Single nucleotide polymorphisms (SNPs) in OPRM1 have been associated with the number of opioid receptors present and their ability to function [14]. OPRM1 SNPs rs1799971 and rs1799972 have been previously implicated in opioid use disorder [15]. Interestingly, rs1799971, rs73568641, and rs10485058 have been associated with methadone plasma concentration, methadone dose, and opioid use changes [16].

The enzymes encoded by the cytochrome P450 family 2 subfamily B member 6 (CYP2B6) gene are involved in metabolizing 2 to 10% of clinically administered drugs, including methadone [17]. SNPs in this gene can lead loss or gain of function of the encoded proteins, possibly resulting in altered drug metabolism [18]. Many CYP2B6 SNPs have been implicated in altered methadone metabolism and plasma concentrations, most notably rs2279343 and rs10403955 [11, 19, 20]. Some studies have also found associations to adverse events in methadone patients, with rs8192719 and rs3745274 associated with overdose fatality [16, 21].

Disparities in opioid use patterns, health and social functioning, and polysubstance use in methadone patients have been observed between the sexes [22, 23]. Further, genetic differences between sexes have been detected in psychiatric disorders and traits, and studies have highlighted the presence of sex-dependent effects in models with common genetic variants [24, 25]. Though past studies have adjusted for sex in their analysis models, very few have been observed to assess the contribution of sex to the genetic predisposition to MMT outcomes using rigorous sex-based analyses, considering how findings might differ within males and within females.

Studying select OPRM1 and CYP2B6 SNPs in a European sample would allow us to not only confirm conclusions within the published literature but also test if the strength of these associations holds true to direct clinical MMT outcomes observable in patients, such as continued opioid use, relapse, and methadone dose. Additionally, having comparable male to female ratios within our sample enables us to robustly examine sex-based differences that have not been adequately highlighted in past studies.

Objectives

This study aims to report new genetic associations that have not been tested previously, as well as analyze associations with biological relevance from previous literature within a larger sample of European descent. The objectives of this study are to:

1. Test the association between pre-selected OPRM1 (rs73568641, rs7451325, rs10485058, rs1799971) and CYP2B6 (rs2279343, rs10403955, rs8192719, rs3745274) SNPs and continued opioid use, relapse, and methadone dose in MMT patients; and

2. Determine if there are differences in associations within and between the sexes through sex stratification and exploratory SNP x Sex interaction analyses.

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Methods
This candidate gene study is reported according to Strengthening the Reporting of Genetic Association studies guideline, an extension of Strengthening the Reporting of Observational studies in Epidemiology statement [26]. An accompanying guideline checklist could be found in S1 File.

Study design and setting
This research reports data collected by the Genetics of Opioid Addiction (GENOA) study, which is an observational cohort study of 1,536 participants recruited from Canadian Addiction Treatment Centres across Ontario, Canada [27]. Data collected at the baseline (enrollment in the study) are the primary sources of information used. The data used include socio-demographic, opioid use-related, and treatment-related information, as well as information obtained from urine toxicology screen (UTS) results and blood samples. UTS results were also collected 3 months prior to study enrollment and up to a 12-month follow up period for measuring treatment outcomes. UTSs testing for opioid use were conducted regularly, on a weekly/biweekly basis, with results reported at 3-month intervals for the GENOA study. The GENOA study was approved by the Hamilton Integrated Research Ethics Board (#11056). All the participants enrolled in the study provided written informed consent.

Eligibility criteria
The participants selected for this study are those deemed eligible by the GENOA study eligibility criteria [27]. These required participants to be 18 years of age or older, have a Diagnostic and Statistical Manual of Mental Disorders [5th edition] OUD diagnosis, undergo an opioid substitution or antagonist therapy for OUD, and provide informed consent. Further inclusion criteria for all research questions addressed in this study include only participants who have provided a DNA sample and have received methadone as the primary opioid substitution or antagonist therapy.

For the measures of continued opioid use and relapse, participants must have had UTSs assessing the presence of opioids for a minimum duration of 3 months and 6 months, respectively. Participants taking prescription opioid medications were excluded due to the uncertainty of the opioid origin when reviewing the UTSs in these participants. These exclusion criteria did not apply to the methadone dose outcome measure as no UTSs were used for that set of analyses.

Outcomes and quantitative variables
Outcomes measured in this study include the following:

1. Continued opioid use while on MMT, defined as any opioid positive UTS (including opiates and oxycodone) observed over a duration of 3 to 15 months. It was measured as a binary variable.

2. Relapse while on MMT, defined as an event of opioid positive UTS following at least 3 months of opioid negative UTSs. It was measured as a binary variable.

3. Methadone dose while on MMT, defined as the amount of methadone a patient is administered at the time of study recruitment in milligrams. It was measured as a continuous variable.
Covariates for the measures of continued opioid use and relapse that were accounted for in the statistical models included: sex, age in years, methadone dose in milligrams, duration on MMT in months, and 5 principal components accounting for differences in ethnicity. Covariates accounted for in the measure of methadone dose were sex, age, duration on MMT, weight in kilograms, and the principal components. For the sex stratified analyses, the same variables as above were included in the additive models.

Genetic variants tested were identified from literature reviews, systematic reviews, candidate gene studies and genome-wide association studies as those related to \textit{OPRM1} or \textit{CYP2B6} and associated with altered methadone metabolism, methadone plasma concentrations, methadone dose, opioid use, or other treatment outcomes. Details about each selected SNP are shown in Table 1.

Data handling

DNA was extracted from blood samples and the genotyping was performed by the Genome Quebec Innovation Centre (Montreal, Canada) [30], using the Illumina Global Screening Array-24 v1.0. Standard genetic association study quality control checks were applied using PLINK v1.09 and the RStudio interface for R i386 3.5.1 [31–33]. Genotype imputation in participants of European ancestry (as confirmed by PCA, n = 1,226) was performed using the Haplotype Reference Consortium’s data as a reference panel via Michigan Imputation Server, using EAGLE2 and Minimac4 [34–36]. Post-imputation variant filtering was conducted, excluding SNPs with Rsq quality metrics of less than 0.3 and/or minor allele frequencies lower than 0.05.

SNPs reported in high linkage disequilibrium ($r^2 > 0.2$) were pruned, keeping the SNP with the most reported clinical significance and published associations, as seen on NCBI’s SNP database [37]. As such, \textit{OPRM1} rs7451325, and \textit{CYP2B6} rs2279343, rs10403955, and rs8192719 were excluded. HaploView was used to visualize SNPs in linkage disequilibrium and calculate r-squared coefficients [38].

A detailed description as well as a flowchart outlining the different steps conducted to reach the final sample size are available in S2 File.

Bias

Measures were taken in this study to identify areas of bias and address them. However, there remained potential sources of bias that could not be avoided, and thus are reported here. Outcomes of continued opioid use and relapse were defined through UTSs as opposed to relying on patient self-reports to remain as objective and unbiased as possible. However, measures such as methadone dose and duration on MMT were self-reported, allowing for a potential of social desirability bias, where participants might provide false information in lieu of more accurate responses that might be viewed as less desirable. Social desirability bias could also have elicited differing responses within males and females as behaviours might seem more desirable in one sex but not the other [39]. In addition, the findings might be affected by volunteer bias, wherein the sample recruited could not have been representative of the entire OUD population receiving treatment. Furthermore, only participants of European ethnicities were included in the analyses conducted. This might result in data that are not generalizable or lack replicability in other ethnic populations. Lastly, since the nature of this study is observational, it is not possible to control for all variables present, and as such undetected biases could have contributed to the findings reported.
Statistical methods

Descriptive statistical analyses were conducted on the total samples and stratified by sex to describe the demographic and clinical characteristics of the sample. Continuous variables were expressed as means with standard deviations, while categorical variables were expressed as counts. Chi square tests were conducted for categorical variables and t-tests for continuous variables to measure differences between the sexes.

Separate regression analyses were performed to test the association between each set of gene SNPs and the outcomes of continued opioid use, relapse, and methadone dose. An additive genetic model was used for all variants and all tests. Logistic regressions were conducted.
to test the associations of continued opioid use and relapse, with the analyses testing for the association of having the minor allele and the outcomes as specified earlier. A linear regression model was used to test the association of having the minor allele and the outcome of methadone dose. All covariates were adjusted for, measuring their associations with the outcomes of interest. Furthermore, identical but separate regression analyses were conducted for male and female subsets, respectively. For analyzing sex differences, interaction analyses were performed with SNP x Sex as the interaction term in the regression models.

Samples with missing outcome values were excluded from the analysis. For the logistic regression analyses, missing values for the covariates of methadone dose and duration on MMT were imputed via mean substitution, from the averages of the values calculated per analysis. The same method was used to impute for missing weight and duration on MMT values for the linear regression.

Bonferroni corrected p-values of $P < 0.017$ for OPRM1 SNPs and $P < 0.05$ for CYP2B6 SNPs were used as thresholds for significance. All statistical analyses were performed on PLINK v1.09 and the RStudio interface for R i386 3.5.1 [31, 32].

**Results**

**Participants**

Samples from 1,226 participants and 5,563,682 SNPs passed quality control checks and filtering after imputation. After sample data cleanup and applying eligibility criteria for each outcome of interest, 1,129 samples were analyzed for continued opioid use, 944 samples for relapse, and 1,165 samples for methadone dose (S2 File).

**Descriptive data**

Participant demographics and clinical characteristics can be seen in Table 2. Of the 1,226 ethnically European participants, 57% were male and 43% were female. The majority of participants were never married, unemployed, on methadone, and not prescribed opioid medications. The mean duration on MMT, age of first opioid use, and total number of positive opioid urine screens, as well as continued opioid use and relapse outcomes, did not differ significantly between the sexes. The weight and dose of methadone administered were lower in females than males, as would have been expected, as individuals of lower weight tend to be prescribed lower doses of MMT. In addition, the ratio of employed to unemployed males (0.70) was significantly higher than that of females (0.37).

**Main results**

Results of the sex-stratified association analyses between the OPRM1 SNPs (rs73568641, rs1799971, rs10485058) and continued opioid use, relapse, and methadone dose are shown in Table 3. No associations reached the Bonferroni adjusted significance threshold of $P < 0.017$. However, some near-significant associations were observed within females but not within males, notably regarding rs73568641. Allele C expressed a potential of decreased odds of continued opioid use within females [$OR = 0.71, 95\% CI = 0.47, 1.07, P = 0.098$]. Its presence also signified a potentially more pronounced decrease in methadone dose in females [$\beta = -7.99, SE = 3.73, P = 0.033$] than in males [$\beta = -2.36, SE = 3.33, P = 0.48$].

Results of the sex-stratified association analyses between the CYP2B6 SNP rs3745274 and continued opioid use, relapse, and methadone dose are shown in Table 4. No associations were found to be significant ($P < 0.05$). Nonetheless, a near-significant association between the
Table 2. Characteristics of participants of European ancestry with available genotype data in GENOA.

| Characteristic                                      | Total   | Male     | Female   | p-value  |
|-----------------------------------------------------|---------|----------|----------|----------|
| N (%)                                               | 1226    | 699 (57) | 527 (43) |          |
| Age in years*, Mean (SD)                            | 39 (11) | 40 (11)  | 38 (11)  | 9.25E-03*|
| Weight in kg*, Mean (SD)                            | 80 (21) | 86 (20)  | 72 (19)  | 2.2E-16* |
| Marital status†, N (%)                              |         |          |          | 3.51E-03*|
| Common law                                          | 236 (19)| 118 (17) | 118 (22) |          |
| Divorced                                            | 125 (10)| 77 (11)  | 48 (9)   |          |
| Currently married                                   | 144 (12)| 95 (14)  | 48 (9)   |          |
| Never married                                       | 555 (45)| 328 (47) | 227 (43) |          |
| Separated                                           | 134 (11)| 64 (9)   | 70 (13)  |          |
| Widowed                                             | 31 (3)  | 15 (2)   | 16 (3)   |          |
| Employment‡, N (%)                                  |         |          |          | 4.86E-07*|
| Employed                                            | 430 (35)| 287 (41) | 143 (27) |          |
| Unemployed                                          | 793 (65)| 411 (59) | 382 (73) |          |
| Methadone dose in mg*, Mean (SD), [Range]           | 75 (45), [1–400] | 78 (47), [2–400] | 71 (43), [1–280] | 6.28E-03* |
| MAT§, N (%)                                         |         |          |          | 0.69     |
| Methadone                                           | 1172 (96)| 666 (96)| 506 (96) |          |
| Suboxone                                            | 52 (4)  | 31 (4)   | 21 (4)   |          |
| Duration on MMT in months§, Mean (SD)               | 45 (48) | 45 (48)  | 44 (49)  | 0.74     |
| Age of first opioid use§, Mean (SD)                 | 25 (9)  | 25 (9)   | 25 (9)   | 0.93     |
| Participant taking opioid prescription‡, N (%)       |         |          |          | 0.83     |
| Prescribed opioids                                  | 34 (3)  | 20 (3)   | 14 (3)   |          |
| Not prescribed opioids                              | 1192 (97)| 679 (97)| 513 (97) |          |
| Total number of opioid screens†, Mean (SD)          | 74 (35) | 74 (34)  | 75 (35)  | 0.57     |
| Total number of positive opioid screens‡, Mean (SD)  | 13 (21)| 13 (20)  | 13 (22)  | 0.70     |
| Continued opioid use outcome‡, N (%)                |         |          |          | 0.32     |
| Continued opioid use                                | 893 (79)| 513 (80)| 380 (78) |          |
| No continued opioid use                             | 236 (21)| 127 (20)| 109 (22) |          |
| Relapse outcome§, N (%)                             |         |          |          | 0.30     |
| Relapse                                             | 433 (46)| 251 (47)| 182 (44) |          |
| No relapse                                          | 511 (54)| 279 (53)| 232 (56) |          |

*260 of reported total included participants screened only for opiates.
†Significant difference between males and females.
All means were calculated excluding missing values.

*aData available for nTotal = 1226, nMale = 699, nFemale = 527.

bData available for nTotal = 1216, nMale = 693, nFemale = 523.

*cData available for nTotal = 1224, nMale = 697, nFemale = 527.

†Data available for nTotal = 1223, nMale = 698, nFemale = 525.

‡Data available for nTotal = 1166, nMale = 664, nFemale = 502.
§Data available for nTotal = 1224, nMale = 697, nFemale = 527.

| Data available for nTotal = 1162, nMale = 661, nFemale = 501.

Data available for nTotal = 1197, nMale = 685, nFemale = 512.

Data available for nTotal = 1226, nMale = 699, nFemale = 527.

Data available for nTotal = 1226, nMale = 699, nFemale = 527.

Data available for nTotal = 1218, nMale = 692, nFemale = 526.

Data available for nTotal = 1129, nMale = 640, nFemale = 489.

Data available for nTotal = 944, nMale = 530, nFemale = 414.

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T allele of rs3745274 and continued opioid use within males \( \text{OR} = 0.73, 95\% \text{CI} = 0.52, 1.014, P = 0.06 \) was observed.

Exploratory analyses showcasing differences in associations between males and females were conducted. No significant results are reported. For detailed results see Tables G and H in S2 File.

### Discussion

#### Key results

This study did not observe any associations that reached the significance threshold set. However, differences in the levels of significance within males and females were detected. Females with the C allele of \textit{OPRM1} rs73568641 showed higher significance levels and stronger protective properties towards continued opioid use than males, as well as a potentially decreased

| Outcome                  | SNP        | N    | Minor Allele | OR/BETA | 95% CI/SE | P     |
|--------------------------|------------|------|--------------|---------|-----------|-------|
| Continued opioid use     | rs73568641 | 1129 | C            | 0.84    | 0.63, 1.10 | 0.21  |
|                          | Male       | 640  | 0.99         | 0.67, 1.45 | 0.95      |
|                          | Female     | 489  | 0.71         | 0.47, 1.07 | 0.098*    |
| rs1799971                | 1129       | G    | 0.97         | 0.70, 1.36 | 0.88      |
|                          | Male       | 640  | 1.11         | 0.72, 1.72 | 0.64      |
|                          | Female     | 489  | 0.87         | 0.51, 1.48 | 0.61      |
| rs10485058               | 1129       | G    | 0.96         | 0.71, 1.30 | 0.78      |
|                          | Male       | 640  | 0.89         | 0.59, 1.36 | 0.60      |
|                          | Female     | 489  | 1.00         | 0.64, 1.57 | 0.99      |
| Relapse                  | rs73568641 | 944  | C            | 0.98    | 0.76, 1.25 | 0.85  |
|                          | Male       | 530  | 0.97         | 0.69, 1.34 | 0.82      |
|                          | Female     | 414  | 1.04         | 0.70, 1.54 | 0.86      |
| rs1799971                | 944        | G    | 0.82         | 0.61, 1.90 | 0.17      |
|                          | Male       | 530  | 0.76         | 0.52, 1.09 | 0.14      |
|                          | Female     | 414  | 0.94         | 0.58, 1.52 | 0.80      |
| rs10485058               | 944        | G    | 1.10         | 0.83, 1.44 | 0.51      |
|                          | Male       | 530  | 1.02         | 0.70, 1.49 | 0.91      |
|                          | Female     | 414  | 1.15         | 0.77, 1.73 | 0.50      |
| Methadone dose           | rs73568641 | 1165 | C            | -4.24   | 2.49      | 0.089* |
|                          | Male       | 664  | -2.36        | 3.33    | 0.48      |
|                          | Female     | 501  | -7.99        | 3.73    | 0.033**   |
| rs1799971                | 1165       | G    | 0.20         | 2.90    | 0.95      |
|                          | Male       | 664  | 2.59         | 3.76    | 0.49      |
|                          | Female     | 501  | -4.92        | 4.63    | 0.29      |
| rs10485058               | 1165       | G    | -0.45        | 2.72    | 0.87      |
|                          | Male       | 664  | -0.50        | 3.69    | 0.89      |
|                          | Female     | 501  | 0.24         | 4.00    | 0.95      |

The minor alleles are also the reference and tested alleles. OR is odds ratio and BETA is the beta coefficient for the regression. 95% CI is the 95% confidence interval levels (lower, upper) and SE is the standard error. All results reported are odds ratios and 95% confidence intervals, except for the methadone dose outcomes, which are BETA coefficients and standard errors. P is the p-value for the t-statistic. The significance threshold is $P < 0.017$.

*P < 0.1.

**P < 0.05.

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Table 3. \textit{OPRM1} SNPs and associated outcomes.
methadone dose. However, the T allele of CYP2B6 rs3745274 in males showed potential for being more protective and significant when it came to continued opioid use.

**Interpretation**

The possible involvement of the C allele of OPRM1’s rs73568641 in a decreased chance of opioid use and/or decreased methadone dose in females suggests the involvement of OPRM1 gene in not only opioid use disorder, but also treatment outcomes. The similar direction of association observed with respect to continued opioid use and methadone dose is interesting given that previous research has reported that higher methadone doses are more effective at decreasing opioid use while on MMT [40]. However, since the variable of methadone dose was accounted for in the analysis model of continued opioid use, the results of the associations can be viewed as independent. When compared to the literature, these associations conflict with the only other published findings. OPRM1 rs73568641 (allele C) seems to have an opposite effect in an African American population [28]. In a genome-wide association study subset (n = 383), it was found to slightly increase daily methadone dose [$β = 0.681, P = 2.81E-08$]. Unfortunately, no conclusions could be drawn due to the possibility that the differences observed between these findings could be a result of the ethnic contribution to the genetic makeup. This highlights the importance of ethnically diverse research and how interindividual differences of patients of different ethnic backgrounds could play a role in patient treatment outcomes.

While the role of the CYP2B6 rs3745274 SNP was not determined in this study with regards to an MMT outcome, other studies have reported evidence of association across different haplotypes of CYP2B6, especially those where this SNP is found, and plasma methadone concentrations. In a pharmacokinetics study, CYP2B6*6 carriers were observed to have higher S-methadone plasma concentrations than non-carriers [41]. It was also determined that CYP2B6 inhibition reduces methadone clearance and increases plasma methadone concentrations [41]. This was further supported by other studies where CYP2B6*6 was shown to have slower S-methadone intravenous clearance, slower R- and S-methadone oral clearance, higher plasma concentrations, and lower methadone dose requirements in carriers [20, 21, 42]. However, given methadone’s racemic mixture and findings supporting R-methadone’s heavier contribution to opioid effects, more evidence on the genetic effects on R-methadone metabolism is available.

| Outcome                  | SNP       | N   | Minor Allele | OR/BETA | 95% CI/SE | P     |
|--------------------------|-----------|-----|--------------|---------|-----------|-------|
| Continued opioid use     | rs3745274 | 1129| T            | 0.82    | 0.64, 1.05| 0.11  |
|                          |           |     | Male         | 0.73    | 0.52, 1.01| 0.06* |
|                          |           | 640 | Female       | 0.95    | 0.66, 1.37| 0.80  |
| Relapse                  | rs3745274 | 944 | T            | 0.91    | 0.73, 1.14| 0.42  |
|                          |           | 530 | Male         | 0.86    | 0.64, 1.16| 0.32  |
|                          |           | 414 | Female       | 1.07    | 0.76, 1.49| 0.71  |
| Methadone dose           | rs3745274 | 1165| T            | 1.26    | 2.17      | 0.56  |
|                          |           | 664 | Male         | -1.17   | 2.99      | 0.70  |
|                          |           | 501 | Female       | 4.19    | 3.18      | 0.19  |

The minor alleles are also the reference and tested alleles. OR is odds ratio and BETA is the beta coefficient for the regression. 95% CI is the 95% confidence interval levels (lower, upper) and SE is the standard error. All results reported are odds ratios and 95% confidence intervals, except for the methadone dose outcomes, which are BETA coefficients and standard errors. P is the p-value for the t-statistic. The significance threshold is P < 0.05.

*P < 0.1.
required [5]. When comparing CYP2B6 rs3745274 to literature findings on other treatment outcomes, the T allele seems to be associated with an increased frequency in methadone fatalities ($P = 1.2E-03$) in a sample of European ethnicity ($n = 125$) [21]. Though these fatality findings support the discussed literature, as a higher plasma concentration of methadone could also have negative effects and risks, such as death, the differing sample sizes of control and methadone-only groups ($n = 255$ and $n = 125$, respectively) could have contributed to such results.

This study was unique in stratifying analyses by sex and observing differential findings for each sex. The sex-based differences observed in the strengths of the associations could not be fully attributed to sample size, as seen in the strength of OPRM1 rs73568641’s associations in females despite having a smaller sample size than their male subset’s counterpart. This could be indicative of larger biology-based differences within the sexes, which could have influenced the results. Examples could be the differing CYP enzyme activities between the sexes that could affect drug metabolism, or neuroanatomical differences in the dopaminergic pathway that can influence the effects of a drug on the system [43, 44]. It is also possible that gender construct and its implications can affect the results, even if indirectly. Women are more likely to become dependent on prescribed opioids than males, experience faster dependence progression rates, and have higher relapse rates [23, 24]. Men, on the other hand, report higher prevalence cannabis use and are more likely to be employed and financially secure [22, 45]. These are only a few examples of how the behavioural and social functioning implications associated with gender can influence phenotypes measures, such as continued opioid use and relapse.

**Limitations and generalizability**

Aside from the sources of bias discussed earlier, some limitations in this study were faced and need to be addressed. Firstly, the findings are specific to a sample of European ethnic descent, making them not generalizable to samples of other ethnicities. Similarly, the sex-specific results may not be comparable to other study findings that do not conduct sex-stratified analyses. Another limitation is that there was a high degree of missingness within the data with respect to the measure of relapse, resulting in a smaller sample size for that set of analyses. Though a power analysis was conducted for the original GENOA project, it is not applicable due to the different SNPs analyzed in this specific study. Additionally, due to a lack of a reported and reliable effect size in the literature and the disputably misleading results of a post-hoc power analysis, an informative power calculation could not have been conducted [46]. Further data missingness was observed in the UTS results reported across the sample population. As the duration of UTS result collection ranged from 3 to 15 months, the outcomes of continued opioid use and relapse were not consistently measured. However, given that and the inevitable variability in how long participants had been on MMT, the duration on MMT was accounted for in all statistical models. An additional data-related limitation includes the inability to accurately use methadone dose as an indicator of treatment response in MMT patients. This is mostly due to the fact that patients on MMT could be at any of the induction, treatment, stabilization, or tapering stages, each of which characterized by a variable pattern of methadone dose administration. This participant variability also plays a role in the measurement of the relapse outcome, posing a challenge in accounting for all participants including those with some breakthrough opioid use while on treatment. Finally, since the exploratory between-sex analyses were insignificant, the interpretation of the sex-stratified results are made with caution. Though an insignificant interaction term could be interpreted as an absence of a difference between males and females, it could also be highly indicative of an under-powered study.
Conclusion
Given that the study had a larger sample size than most similar published research within this field, it was able to address a gap in the genetics of MMT research. Though none of the results were significant, this study identified a need for ethnically diverse research, and uncovered the important contribution sex measures have towards outcomes of continued opioid use and methadone dose in MMT patients. Future recommendations towards more powered studies including sex in the analysis models are made.

Supporting information
S1 File. STREGA checklist.
(DOCX)
S2 File. Appendix.
(DOCX)

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References
1. Joseph H, Stancliff S, Langrod J. Methadone maintenance treatment (MMT): A review of historical and clinical issues. In: Mount Sinai Journal of Medicine [Internet]. 2000 [cited 2020 Aug 25]. p. 347–64. Available from: https://europepmc.org/article/med/11064485 PMID: 11064485
2. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 5th Edition. Arlington, VA; 2013.
3. Methadone | CAMH [Internet]. [cited 2020 Aug 25]. Available from: https://www.camh.ca/en/health-info/mental-illness-and-addiction-index/methadone
4. Marsch LA. The efficacy of methadone maintenance interventions in reducing illicit opiate use, HIV risk behavior and criminality: a meta-analysis. Addiction [Internet]. 1998 Apr; 93(4):515–32. Available from: http://doi.wiley.com/https://doi.org/10.1046/j.1360-0443.1998.9345157.x PMID: 9684390
5. Eap CB, Buclin T, Baumann P. Interindividual Variability of the Clinical Pharmacokinetics of Methadone. Clin Pharmacokinet [Internet]. 2002; 41(14):1153–93. Available from: http://link.springer.com/https://doi.org/10.2165/00003088-200214140-00003 PMID: 12405865
6. Amato L, Davoli M, Minozzi S, Ferroni E, Ali R, Ferri M. Methadone at tapered doses for the management of opioid withdrawal. Cochrane Database Syst Rev [Internet]. 2013 Feb 28;(2):CD003409. Available from: http://doi.wiley.com/10.1002/14651858.CD003409.pub4 PMID: 23450540
7. Joe GW, Simpson DD, Sells SB. Treatment Process and Relapse to Opioid Use During Methadone Maintenance. Am J Drug Alcohol Abuse [Internet]. 1994 Jan 7; 20(2):173–97. Available from: http://www.tandfonline.com/doi/full/10.3109/00952999409106781 PMID: 8042602
8. Centers for Disease Control and Prevention. Calculating Total Daily Dose of Opioids for Safer Dosage. Centers Dis Control Prev. 2017;
9. Crettol S, Monnat M, Eap CB. Could pharmacogenetic data explain part of the interindividual sensitivity to methadone-induced respiratory depression? [Internet]. Vol. 11, Critical Care. BioMed Central Ltd.; 2007 [cited 2020 Aug 25], p. 119. Available from: /pmc/articles/PMC2151888/?report=abstract https://doi.org/10.1186/cc5699 PMID: 17338832
10. Li Y, Kantelip J-P, Schieven PG, Davani S. Interindividual Variability of Methadone Response. Mol Diagn Ther [Internet]. 2008 Mar 16 [cited 2020 Aug 25]; 12(2):109–24. Available from: http://link.springer.com/10.1007/BF03256276 PMID: 18422375
11. Berrettini W. A brief review of the genetics and pharmacogenetics of opioid use disorders. Dialogues Clin Neurosci [Internet]. 2017 Sep 1 [cited 2020 Aug 25]; 19(3):229–36. Available from: /pmc/articles/PMC5741106/?report=abstract https://doi.org/10.31887/DCNS.2017.19.3/wberrettini PMID: 29302220
12. Dennis BB, Bawor M, Thabane L, Sohani Z, Samaan Z. Impact of ABCB1 and CYP2B6 Genetic Polymorphisms on Methadone Metabolism, Dose and Treatment Response in Patients with Opioid Addiction: A Systematic Review and Meta-Analysis. Zhang H, editor. PLoS One [Internet]. 2014 Jan 29 [cited 2020 Jul 7]; 9(1):e86114. Available from: /pmc/articles/PMC3906028/?report=abstract https://doi.org/10.1371/journal.pone.0086114 PMID: 24486983
13. Crist RC, Berrettini WH. Pharmacogenetics of OPRM1 [Internet]. Vol. 123, Pharmacology Biochemistry and Behavior. Elsevier Inc.; 2014 [cited 2020 Aug 25], p. 25–33. Available from: /pmc/articles/PMC4010567/?report=abstract
14. Pasternak GW, Pan YX. Mu opioids and their receptors: Evolution of a concept [Internet]. Vol. 65, Pharmacological Reviews. American Society for Pharmacology and Experimental Therapeutics; 2013 [cited 2020 Aug 25], p. 1257–317. Available from: /pmc/articles/PMC3792296/?report=abstract https://doi.org/10.1124/pr.112.0107138 PMID: 24076545
15. Mistry C, Bawor M, Desai D, Marsh D, Samaan Z. Genetics of Opioid Dependence: A Review of the Genetic Contribution to Opioid Dependence. Curr Psychiatry Rev [Internet]. 2014 Jan 8 [cited 2020 Aug 25]; 10(2):156–67. Available from: /pmc/articles/PMC4155832/?report=abstract https://doi.org/10.2174/1573405010666140320000928 PMID: 25242908
16. Crist RC, Clarke TK, Berrettini WH. Pharmacogenetics of Opioid Use Disorder Treatment. CNS Drugs [Internet], 2018 Apr 1 [cited 2020 Jul 7]; 32(4):305–20. Available from: /pmc/articles/PMC5935553/?report=abstract https://doi.org/10.1007/s40263-018-0513-9 PMID: 29623639
17. Hedrich WD, Hassan HE, Wang H. Insights into CYP2B6-mediated drug-drug interactions [Internet]. Vol. 6, Acta Pharmaceutica Sinica B. Chinese Academy of Medical Sciences; 2016 [cited 2020 Aug 25], p. 413–25. Available from: /pmc/articles/PMC5045548/?report=abstract https://doi.org/10.1016/j.apsb.2016.07.016 PMID: 27709010
18. Ahmad T, Valentinovic MA, Rankin GO. Effects of cytochrome P450 single nucleotide polymorphisms on methadone metabolism and pharmacodynamics. Biochem Pharmacol [Internet]. 2018 Jul 1 [cited 2020 Jul 7]; 153:196–204. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0006295218300777 PMID: 29458047
19. Wang S-C, Ho I-K, Tsou H-H, Tian J-N, Hsiao C-F, Chen C-H, et al. CYP2B6 Polymorphisms Influence the Plasma Concentration and Clearance of the Methadone S-Enantiomer. J Clin Pharmacol [Internet]. 2011 Aug [cited 2020 Jul 7]; 31(4):463–9. Available from: http://journals.lww.com/00004714-201108000-00010 PMID: 21694616
20. Levrano O, Peles E, Hamon S, Randesi M, Adelson M, Kreek MJ. CYP2B6 SNPs are associated with methadone dose required for effective treatment of opioid addiction. Addict Biol. 2013; 18(4):709–16. doi:https://doi.org/10.1111/j.1369-1600.2011.00349.x PMID: 21790905
21. Ahmad T, Sabet S, Primerano DA, Richards-Waugh LL, Rankin GO. Tell-Tale SNPs: The Role of CYP2B6 in Methadone Fatalities. J Anal Toxicol [Internet]. 2017 May 1 [cited 2020 Jul 7]; 41(4):325–33. Available from: http://useast.ensembl.org/Tools/VEP https://doi.org/10.1093/jat/bkw135 PMID: 28184434

22. Bawor M, Dennis BB, Varenbut M, Dailer J, Marsh DC, Plater C, et al. Sex differences in substance use, health, and social functioning among opioid users receiving methadone treatment: a multicenter cohort study. Biol Sex Differ [Internet]. 2015 Dec 10; 6(1):21. Available from: http://www.bsd-journal.com/content/6/1/21

23. Bawor M, Dennis BB, Bhalerao A, Plater C, Worster A, Varenbut M, et al. Sex differences in outcomes of methadone maintenance treatment for opioid use disorder: a systematic review and meta-analysis. C Open [Internet]. 2015 Sep 25 [cited 2020 Aug 25]; 3(3):E344–51. Available from: /pmc/articles/PMC4596116/?report=abstract

24. Ngun TC, Ghahramani N, Sánchez FJ, Boeklandt S, Vilain E. The genetics of sex differences in brain and behavior. Vol. 32, Frontiers in Neuroendocrinology. Academic Press; 2011. p. 227–46. https://doi.org/10.1016/j.yfrne.2010.10.001 PMID: 20951723

25. Gilks WP, Abbott JK, Morrow EH. Sex differences in disease genetics: Evidence, evolution, and detection [Internet]. Vol. 30, Trends in Genetics. Elsevier Ltd; 2014 [cited 2020 Aug 25]. p. 453–63. Available from: http://www.cell.com/article/S016895251400136X/fulltext https://doi.org/10.1016/j.tig.2014.08.006 PMID: 25239223

26. Little J, Higgins JPT, Ioannidis JPA, Moher D, Gagnon F, von Elm E, et al. Strengthening the Reporting of Genetic Association Studies (STREGA)—An Extension of the STROBE Statement. PLoS Med [Internet]. 2009 Feb 3; 6(2):e1000022. Available from: https://dx.plos.org/10.1371/journal.pmed.1000022

27. Samaan Z, Bawor M, Dennis BB, Plater C, Varenbut M, Dailer J, et al. Genetic influence on methadone treatment outcomes in patients undergoing methadone maintenance treatment for opioid addiction: a pilot study. Neuropsychiatr Dis Treat [Internet]. 2014 Aug;1503. Available from: http://www.dovepress.com/genetic-influence-on-methadone-outcomes-in-patients-undergoing-peer-reviewed-article-NDT https://doi.org/10.2147/NDT.S66234 PMID: 25187714

28. Smith AH, Jensen KP, Li J, Nunez Y, Farrer LA, Hakonarson H, et al. Genome-wide association study of therapeutic opioid dosing identifies a novel locus upstream of OPRM1. Mol Psychiatry [Internet]. 2017 Mar 1 [cited 2020 Jul 6]; 22(3):346–52. Available from: www.nature.com/mp https://doi.org/10.1038/mp.2016.257 PMID: 28115739

29. Zhou H, Rentsch CT, Cheng Z, Kember RL, Nunez YZ, Sherva RM, et al. Association of OPRM1 Functional Coding Variant With Opioid Use Disorder. JAMA Psychiatry [Internet]. 2020 Oct 1 [cited 2020 Jul 6]; 77(10):1072. Available from: https://jamanetwork.com/doi/10.1001/jamapsychiatry.2020.1206 PMID: 32492095

30. Centre d’expertise et de services Génomique Québec [Internet]. [cited 2020 Aug 25]. Available from: https://cesgq.com/

31. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, et al. PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. Am J Hum Genet [Internet]. 2007 Sep; 81(3):559–75. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0002929707613524 https://doi.org/10.1086/519795 PMID: 17701901

32. R Core Team. R: A language and environment for statistical computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2020. Available from: https://www.r-project.org/

33. Anderson CA, Pettersson FH, Clarke GM, Cardon LR, Morris AP, Zondervan KT. Data quality control in genetic case-control association studies. Nat Protoc [Internet]. 2010 Sep 26; 5(9):1564–73. Available from: http://www.nature.com/articles/nprot.2010.116 https://doi.org/10.1038/nprot.2010.116 PMID: 201085122

34. Loh PR, Danecek P, Palamara PF, Fuchsberger C, Reshef YA, Finucane HK, et al. Reference-based phasing using the Haplotype Reference Consortium panel. Nat Genet [Internet]. 2016 Nov 1 [cited 2020 Aug 21]; 48(11):1443–8. Available from: /pmc/articles/PMC596458/?report=abstract https://doi.org/10.1038/ng.3679 PMID: 27694958

35. Das S. Next Generation of Genotype Imputation Methods [Internet]. University of Michigan; 2017 [cited 2020 Aug 21]. Available from: https://deepblue.lib.umich.edu/bitstream/handle/2027.42/138466/sayantani_1.pdf?sequence=1

36. Das S, Forer L, Schönher S, Sidore C, Locke AE, Kwong A, et al. Next-generation genotype imputation service and methods. Nat Genet [Internet]. 2016 Oct 28; 48(10):1284–7. Available from: http://www.nature.com/articles/ng.3656 https://doi.org/10.1038/ng.3656 PMID: 27571263

37. Sherry ST. dbSNP: the NCBI database of genetic variation. Nucleic Acids Res [Internet]. 2001 Jan 1; 29(1):308–11. Available from: https://academic.oup.com/nar/article-lookup/doi/https://doi.org/10.1093/nar/29.1.308 PMID: 1125122
38. Barrett JC, Fry B, Maller J, Daly MJ. Haplovew: analysis and visualization of LD and haplotype maps. Bioinformatics [Internet]. 2005 Jan 15; 21(2):263–5. Available from: https://academic.oup.com/bioinformatics/article-lookup/https://doi.org/10.1093/bioinformatics/bth457 PMID: 15297300

39. Paunonen SV. Sex Differences in Judgments of Social Desirability. J Pers [Internet]. 2016 Aug 1 [cited 2020 Sep 1]; 84(4):423–32. Available from: https://pubmed.ncbi.nlm.gov.libaccess.library.mcmaster.ca/25728192/ https://doi.org/10.1111/jopy.12169 PMID: 25728192

40. Farré M, Mas A, Torrens M, Moreno V, Camí J. Retention rate and illicit opioid use during methadone maintenance interventions: a meta-analysis. Drug Alcohol Depend [Internet]. 2002 Feb; 65(3):283–90. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0376871601001715 https://doi.org/10.1016/s0376-8716(01)00171-5 PMID: 11841899

41. Kharasch ED, Stubbert K. Role of Cytochrome P4502B6 in Methadone Metabolism and Clearance. J Clin Pharmacol [Internet]. 2013 Mar; 53(3):305–13. Available from: http://doi.wiley.com/https://doi.org/10.1002/jcph.1 PMID: 23361846

42. Kharasch ED, Regina KJ, Blood J, Friedel C. Methadone pharmacogenetics: CYP2B6 polymorphisms determine plasma concentrations, clearance, and metabolism. Anesthesiology. 2015 Nov 1; 123(5):1142–53. https://doi.org/10.1097/ALN.0000000000000867 PMID: 26389554

43. Bobzean SAM, DeNobrega AK, Perrotti LI. Sex differences in the neurobiology of drug addiction. Exp Neurol [Internet]. 2014 Sep; 259:64–74. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0014488614000351 https://doi.org/10.1016/j.expneurol.2014.01.022 PMID: 24508560

44. Scandlyn MJ, Stuart EC, Rosengren RJ. Sex-specific differences in CYP450 isoforms in humans, Expert Opin Drug Metab Toxicol [Internet]. 2008 Apr 23 [cited 2020 Aug 26]; 4(4):413–24. Available from: http://www.tandfonline.com/doi/full/10.1517/17425255.4.4.413 PMID: 18524030

45. Zahnow R, Winstock AR, Maier LJ, Levy J, Ferris J. Injecting drug use: Gendered risk. Int J Drug Policy [Internet]. 2018 Jun 1 [cited 2020 Aug 26]; 56:81–91. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0955395918300884 https://doi.org/10.1016/j.drugpo.2018.03.018 PMID: 29614392

46. Zhang Y, Hedo R, Rivera A, Rull R, Richardson S, Tu XM. Post hoc power analysis: is it an informative and meaningful analysis? Gen Psychiatry [Internet]. 2019 Aug 8; 32(4):e100069. Available from: http://gpsych.bmj.com/lookup/doi/https://doi.org/10.1136/gpsych-2019-100069 PMID: 31552383