# PHARMACOGENETICS OF METHADONE MAINTENANCE TREATMENT OUTCOMES

# PHARMACOGENETICS OF METHADONE MAINTENANCE TREATMENT OUTCOMES IN OPIOID USE DISORDER PATIENTS

**By CAROUL CHAWAR, B.Sc.**

A Thesis Submitted to the School of Graduate Studies in Partial Fulfilment of the Requirements for the Degree Master of Science

## Descriptive Note

MASTER OF SCIENCE (2020) McMaster University
(Neuroscience Graduate Program) Hamilton, Ontario

TITLE: Pharmacogenetics of Methadone Maintenance Treatment Outcomes in Opioid Use Disorder Patients

AUTHOR: Caroul Chawar, H.B.Sc. (McMaster University)

SUPERVISOR: Dr. Zainab Samaan

COMMITTEE MEMBERS: Dr. Lehana Thabane and Dr. Flavio Kapczinski

NUMBER OF PAGES: xii, 102

## Lay Abstract

Recently, opioid use disorder (OUD) has been declared a national crisis in Canada. OUD treatments are helpful in reducing opioid use and adverse events. However, their dosing and metabolism in patients can impact continued opioid use, relapse, or treatment dose changes. Due to the variability in response between individuals, there might be a genetic basis to treatment outcomes. This thesis explores which genetic variants reported in previous studies are involved in OUD treatment outcomes. Then, it tests select genetic variants in *OPRM1* and *CYP2B6* genes to see if they are linked to specific outcomes in an Ontario population and tries to identify if these associations differ by sex. No significant associations were found, though associations in males and females had near-significant results in one sex but not the other. Despite suggesting sex's possible involvement in treatment outcomes, more research is necessary to confirm these findings.

## Abstract

**Background:** Opioid use disorder (OUD) has been an increasing concern in Canada as mortality rates continue to rise. Though OUD treatments, such as methadone maintenance treatment (MMT), reduce its burden, they could potentially cause harm due to OUD's variance in severity and presentation across individuals. It is hypothesized that genetic variants such as single nucleotide polymorphisms (SNPs) could predispose patients to respond differently to MMT. In addition, sex differences have been observed in opioid use patterns, treatment outcomes, and genetic make-up. As such, this thesis aims to identify significant SNPs associated with treatment outcomes in genome-wide association studies, and test biologically relevant SNPs with MMT outcomes of interest, while highlighting sex differences. This is achieved through a systematic review protocol, a systematic review, and a candidate gene study.

**Methods:** A protocol was prepared for the planning of the first ever systematic review of genome-wide significant findings of medication-assisted treatment outcomes for OUD patients. The systematic review assessed the literature findings and study qualities, narratively summarizing significant associations. Next, a candidate gene study analyzed the association between SNPs in *OPRM1* and *CYP2B6* genes, and continued opioid use, relapse, and methadone dose within an ancestrally European sample (n=1226). Sex-stratified and sex-interaction analyses were also conducted.

**Results:** The systematic review included 5 studies and qualitatively assessed 43 unique genetic variants. The candidate gene study showed no significant associations between the selected *OPRM1* and *CYP2B6* SNPs and outcomes of interest. While no significant differences between the sexes were observed, rs73568641 and rs3745274 showed near significance associations in only one sex, females, and males, respectively.

**Discussion:** Through the study of genetic variants associated with treatment outcomes in the literature and our sample of ancestrally European individuals on MMT, we were able to highlight gaps in pharmacogenetics research and identify areas of focus for future studies.

## Acknowledgements

I would like to sincerely thank Dr. Samaan, my supervisor, teacher and mentor, for giving me the opportunity to be a part of her lab and providing me with endless opportunities to grow. You have challenged me to push and stretch myself outside of what I thought possible to achieve. Thank you for supporting and encouraging me from day one in reaching my goals and going after the path I want. Your guidance and advice every step of the way was wholly appreciated. I would not be the student I am today if it were not for your influence.

To Drs. Thabane, Kapczinski, and Pare, thank you for your continued guidance and help. Your expertise has been instrumental in helping me form a healthy foundation in statistics and study design. Thank you for continually supporting my work, being the sound of reason, and helping me grow over the past two years.

Dr. Amel Lamri, I want to extend a huge thank you for being a supportive and patient mentor throughout the past two years. You have taught me everything I know about genetic analysis and GWAS data clean-up. Thank you for the countless meetings, and for being the first-line support for my genetic research crises. Thank you for taking the time to build my knowledge in PLINK and R, and for always making it fun.

To my research colleague and dear friend Alannah Hillmer, thank you for being along for the ride. I could not have accomplished what I have without your help, support, and encouragement. You were there for every step of the way, the analyzing, writing, editing, presenting, and most importantly decision-making. I could not have asked for a better teammate; for all that and more, I am eternally thankful.

To Ms. Jackie Hudson, thank you for being an amazing mentor, friend, and lab mom. Your contagious laugh and funny stories have made the past two years more fun than they would have been. Thank you for making our lab more than a workspace and for creating a welcoming and accepting environment. I cannot deny how your hard work, compassion, and work ethic has influenced me, so thank you for that.

To the MEND research team, it has been an honour working with you. Nitika Sanger, Alessia D'Elia, and Balpreet Panesar, you have made this experience an unforgettable one with lots of laughs. Thank you for all the recruitment road trips and for being my ongoing and on-call support system. I cannot wait to see what exciting things you have ahead.

A huge thank you to the GENOA team, past and present, for helping with this research project. The time and commitment you have put in in designing this

project, making it happen, and collecting data has been instrumental for my work. Without your efforts, this thesis would be null, so thank you.

To my beloved friends and family, I cannot thank you enough for standing by me and encouraging me throughout this journey. Thank you for enduring me at my worst and for pretending to understand what my research is about. Your unwavering love and faith have inspired me. I am excited to start a new chapter with you all by my side.

Lastly, I would like to thank the Neuroscience Graduate Program at McMaster University, the Canadian Institutes of Health Research (Institute Community Support Program), and Dr. Zainab Samaan for their generous financial support.

## Table of Contents

## List of Figures



## List of Tables

## List of Abbreviations and Symbols

OUD – Opioid use disorder
DSM-5 – Diagnostic and Statistical Manual of Mental Disorders, 5th edition
MAT – Medication-assisted treatment
MMT – Methadone maintenance treatment
SNP – Single nucleotide polymorphism
PROSPERO – International Prospective Register of Systematic Reviews
WHO – World Health Organization
HHS – U.S. Department of Health and Human Services
GWAS – Genome-wide association study
PRISMA-P – Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols
PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-Analyses
Q-Genie – Quality of Genetic Association Studies
GRADE – Grading of Recommendations Assessment, Development, and Evaluation
HuGENet – Human Genome Epidemiology Network
*OPRM1* – Mu-opioid receptor 1
*CYP2B6* – Cytochrome P450 2B6
STREGA – Strengthening the Reporting of Genetic Association studies
STROBE – Strengthening the Reporting of Observational Studies in Epidemiology
CATC – Canadian Addiction Treatment Centre
UTS – Urine toxicology screen
OSAT – Opioid substitution or antagonist therapy
HWE – Hardy-Weinberg Equilibrium
MAF – Minor allele frequency
LD – Linkage disequilibrium

## Declaration of Academic Achievement

I, Caroul Chawar, am the primary author of all presented studies and manuscripts. I have made significant contributions to the studies by determining the study questions, conducting the analyses, and writing the manuscripts. Co-authors respective to each study are listed on the first page of each manuscript, with their contributions detailed at the end of the manuscript.

# 1 CHAPTER 1: Introduction

## 1.1 Background and Significance

The prevalence of opioid use disorder (OUD) is growing with the rise of the opioid crisis and the increase in opioid use in Canada. Opioid-related deaths in Ontario were the second highest in Canada, totalling 867 deaths in 2016(1). A more recent report from the Government of Canada shows that in 2019 opioid-related deaths in Ontario have increased to 1535, making it the province with the highest opioid mortality(1). These increasing trends in the burden of opioid use have also been observed to be variant by sex. The Canadian Institute for Health Information reported an increasing trajectory of opioid poisoning hospitalizations in Canada over the span of five years, from 2013 to 2017, with females showing a 10% increase while males showing a 48% increase, with a substantial amount of that change seen from 2016 to 2017(2). In addition, Public Health Ontario has reported more cases of opioid-related emergency department visits in Ontario for males than females in 2018(3). These data collectively indicate a need for effective interventions to reduce opioid use and its burden on patients as well as the healthcare system in Ontario.

OUD has been characterized by the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) as a series of psychological and behavioural symptoms that lead to compulsive opioid seeking and intake behaviours(4). However, the severity and presentation of OUD is very variable and dependent on the number of symptoms present, where more symptoms signify an increased severity(4). Despite that variability, treatments offered to OUD patients, falling under the umbrella term of Medication Assisted Treatments (MATs), generally consist of the same behavioural and pharmacological interventions, with differing doses and modes of administration(5). While the most common MAT, methadone maintenance treatment (MMT), has been seen to decrease the number of patients with OUD, the 12-month retention rate of the therapy has been observed to be around 40.5%, with lower compliance to the methadone dose upon initiation(6). Further studies have shown the initiation period of the treatment to be associated with higher mortality rates, as patients tend to continue illicit opioid use to satisfy their cravings and avoid withdrawal symptoms(7).

The emergence of a more personalized medical approach and the need for it in this population has directed a lot of research to focus on whether a genetic basis for OUD exists(8,9). Though genetic risk factors and predispositions for OUD have been identified, limited research currently exists on the genetic factors affecting treatment outcomes in patients. Few of the relevant candidate gene studies published have shown significant associations(10). In addition, with the increased popularity and means of genome-wide analysis, there has been a surge in the publication of relevant studies within this field(11–13). The results of

which, however, have not been systematically summarized yet, leaving the research community unsure on what gap needs to be addressed and which results require replication.

Research presented in this thesis aims to address the need for a comprehensive and current literature search for significant results, to inform clinical applications and the direction of future research. Through a large sample size of comparable male to female ratios, this research also aims to confirm findings in the literature within this sample and conduct robust genetic association analyses, while accounting for differences in sex.

## 1.2 Objectives

The specific objectives of this thesis addressed through the three included manuscripts are the following:

1. To systematically and methodologically search the literature on genetic study findings regarding treatment outcomes for OUD;
2. To summarize these findings and assess the quality of the published literature;
3. To determine if any associations are found between pre-identified biologically relevant single nucleotide polymorphisms (SNPs) and the outcomes of continued opioid use, relapse, and methadone dose in patients undergoing MMT in Ontario; and
4. To determine if there are differences within the sexes in the SNP-outcome associations measured (outlined in objective 3), through sex-stratified analyses.

## 1.3 Coherence of Thesis Chapters

The peer-reviewed systematic review protocol (Chapter 2) outlines the detailed study design and search strategy for the systematic review to follow. It ensures methodological transparency and specifies the basis of the systematic review and its significance. The systematic review (Chapter 3) applies the search strategy outlined in the protocol and discusses the findings of the recently screened and included articles. It provides a summary of relevant significant genome-wide association study results, highlighting potential SNPs of interest and identifying gaps within the literature. This acts as a basis for the third paper, the candidate gene study (Chapter 4), that tests if there is an association between SNPs identified through the systematic review as well as the literature and negative MMT outcomes. The analyses performed in this final paper allow for the testing of these associations within the contextual population of Ontario OUD patients, while remaining mindful of the sex-based differences in this population.

This primary article provides room for conclusions to be drawn, clinical implications to be outlined, and further gaps in research to be identified.

It should be noted that since there are many common threads across the three independent chapters, including but not limited to the population of interest, treatment of interest, and genetic nature of the studies, the chapter-specific backgrounds and literature reviews might contain some overlapping information and concepts. Nonetheless, each study discussed in these chapters is unique and serves a specific purpose.

# 2 CHAPTER 2

## 2.1 Introduction

This chapter consists of a systematic review protocol, “GWAS-identified genetic variants associated with medication-assisted treatment outcomes in patients with opioid use disorder: A systematic review and meta-analysis protocol”, published in the journal of Systematic Reviews on September 1st, 2020. It includes a brief literature review on genetic studies of MAT outcomes in OUD patients and outlines the rationale as well as objectives of the systematic review to follow. It serves as a methodology and study design paper that readers can reference for further information regarding the review. It not only reports the intention of the review to the research community, but also holds the review accountable to the preset standards outlined in the protocol, thus decreasing the risk of bias. This protocol has been published at a peer-reviewed journal to maintain scientific transparency. Additionally, the systematic review has been registered with the International Prospective Register of Systematic Reviews (PROSPERO).

## 2.2 Copyright Statement

## 2.3 GWAS-identified genetic variants associated with medication-assisted treatment outcomes in patients with opioid use disorder: A systematic review and meta-analysis protocol

**Authors**

Caroul Chawar [1], Alannah Hillmer [2], Stephanie Sanger [3], Alessia D'Elia [4], Balpreet Panesar [5], Lucy Guan [6], Dave Xiaofei Xie [7], Nandini Bansal [8], Aamna Abdullah [9], Flavio Kapczinski [10], Guillaume Pare [11], Lehana Thabane [12], Zainab Samaan[13]*

1. Neuroscience Graduate Program, McMaster University, Hamilton, ON, Canada; Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; chawarc@mcmaster.ca
2. Neuroscience Graduate Program, McMaster University, Hamilton, ON, Canada; Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; hillmea@mcmaster.ca
3. Health Sciences Library, McMaster University, Hamilton, ON, Canada; sangers@mcmaster.ca
4. Neuroscience Graduate Program, McMaster University, Hamilton, ON, Canada; Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; deliaa@mcmaster.ca
5. Neuroscience Graduate Program, McMaster University, Hamilton, ON, Canada; Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; panesabk@mcmaster.ca
6. Health Sciences Program, McMaster University, Hamilton, ON, Canada; Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; guanx1@mcmaster.ca
7. Health Sciences Program, McMaster University, Hamilton, ON, Canada; Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; xiex11@mcmaster.ca
8. Health Sciences Program, McMaster University, Hamilton, ON, Canada; Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; bansan1@mcmaster.ca
9. Health Sciences Program, McMaster University, Hamilton, ON, Canada; Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; abdula19@mcmaster.ca
10. Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; kapczinf@mcmaster.ca
11. Population Health Research Institute, Hamilton, ON, Canada; Department of Health Research Method, Evidence, and Impact, McMaster University, Hamilton, ON, Canada; pareg@mcmaster.ca
12. Department of Health Research Method, Evidence, and Impact, McMaster University, Hamilton, ON, Canada; Population Health Research Institute,

Hamilton, ON, Canada; Father Sean O'Sullivan Research Centre, St. Joseph's Healthcare Hamilton; thabanl@mcmaster.ca
13. Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; samaanz@mcmaster.ca

*Corresponding Author:
Dr. Zainab Samaan
100 West 5th St., Hamilton, ON L8N3K7, Canada
Telephone: 905-522-1155 ext. 35448
Fax: 905-381-5629

### 2.3.1 Abstract

**Background:** The burden of opioid use disorder (OUD) has been increasing in North America. Administration of medication-assisted treatments (MATs) for OUD on an individual-dose basis has been shown to affect patient responses to treatment, proving to be, on occasion, dangerous. A genetic basis has been identified for some MAT responses in a candidate gene context, but consensus has not been reached for any genome-wide significant associations. This systematic review aims to identify and assess any genetic variants associated with MAT patient outcomes at genome-wide significance.
**Methods:** The databases searched by the authors will be: MEDLINE, Web of Science, EMBASE, CINAHL and Pre-CINAHL, GWAS Catalog, GWAS Central, and NIH Database of Genotypes and Phenotypes. A title and abstract screening, full-text screening, data extraction, and quality assessment will be completed in duplicate for each study via Covidence. Treatment outcomes of interest include continued opioid use or abstinence during treatment or at follow-up, time to relapse, treatment retention rates, opioid overdose, other substance use, comorbid psychiatric disorders, risk taking behaviours, MAT plasma concentrations, and mortality rates. Analysis methods applied, if appropriate, will include random effects meta-analysis with pooled odds ratios for all outcomes. Sub-group analyses will also be implemented, when possible.
**Discussion:** This systematic review can hopefully inform the direction of future research, aiding in the development of a safer and more patient-centred treatment. It will be able to highlight genome-wide significant variants that are replicable and associated with MAT patient outcomes.
**Systematic Review Registration:** This systematic review protocol has been registered with the International Prospective Register of Systematic Reviews (PROSPERO) (registration ID CRD42020169121).

**Keywords**
Genome-wide association, medication-assisted treatment, opioid use, treatment response, SNP, pharmacogenetics, systematic review, protocol

### 2.3.2 Background

Opioid use disorder (OUD) is characterized by the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) as a series of physical and psychological symptoms that promote compulsive opioid-seeking behaviours and hinder the constraint of opioid consumption,(1). The World Health Organization (WHO) reports that roughly 27 million people suffered from OUD in 2016, and about 118 thousand died due to OUD-related drug use in 2015,(2). The continual increase of opioid-related deaths in North America has called the U.S. Department of Health and Human Services (HHS) and the Ministry of Health in Canada to declare an opioid crisis and take appropriate federal action, in 2017 and 2016, respectively,(3,4).

The most prevalent OUD treatments are a combination of pharmacological and behavioural therapies, commonly known as medication-assisted treatments (MATs),(5). The medications act as either agonists or antagonists to endogenous opioid receptors, regulating the inhibition or stimulation of the opioid reward system,(6,7). FDA-approved MATs include methadone, buprenorphine, buprenorphine in combination with naloxone, and naltrexone,(5). In addition to those listed, Health Canada has also recently approved the use of injectable heroin-assisted treatment for severe OUD cases,(8).

The regulated administration of these MATs at an individual-based dose is essential in ensuring the effectiveness of the treatment and safety of the patients, as well as averting overdose or mortality cases,(9). Methadone dosing, for example, has been shown to be a key factor in predicting treatment outcomes. Very low doses of this agonist put patients at a higher risk of relapse,(10,11), while too high doses and the induction of methadone have been associated with a higher risk of cardiac arrhythmia and mortality, respectively,(9,12).

MAT efficacy in keeping patients from illicitly using opioids has been variable,(10,11,13), calling into question whether a genetic basis for how patients respond to treatment exists. Several genetic studies have identified variants associated with a higher risk of developing OUD and MAT metabolism or clearance,(14,15). However, no clear consensus has been formed regarding genes that contribute to treatment outcomes, including negative ones, in OUD patients seeking treatment. Furthermore, literature has not been systematically reviewed for genetic variants of genome-wide significance in this area, to date.

#### 2.3.2.1 Objectives

This systematic review aims to assess all the identified genetic variants from genome-wide association studies (GWASs) significantly associated with treatment outcomes for OUD patients receiving MAT.
The specific objectives of this study include:

1. Summarize the genome-wide significant variants associated with MAT outcomes within the current literature.
2. Compare and meta-analyze significant GWAS findings relevant to treatment outcomes, applying sub-group analyses based on ethnicity, sex and other variables, if possible.
3. Critically review the literature to identify gaps that need to be addressed within the pharmacogenomics of MAT research.

### 2.3.3 Methods

This protocol has been reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P) reporting guidelines,(16). An accompanying checklist could be found in Additional file 1.

#### 2.3.3.1 Eligibility Criteria

Studies included in this review will be limited to GWASs. Other types of genetic studies, such as candidate-gene, twin, linkage-analysis, segregation-analysis, and familial aggregation, will not be included. Studies included will also investigate a MAT in an OUD population. For the purposes of this review, study populations with opioid/heroin/fentanyl dependence, use, abuse, or addiction will be included. Examples of MATs included are methadone, suboxone, buprenorphine, naltrexone, naloxone, heroin-assisted, levacetylmethadol, and fentanyl. Studies whose participants are solely on clonidine, lofexidine, or any other opioid withdrawal medication not administered with a MAT will be excluded as these measures are for short term management of acute withdrawal and not maintenance treatments. The inclusion of studies will not be restricted based on MAT treatment administration setting, such as community, residential, or institutional, or population characteristics, such as age, ethnicity, sex, or gender.

#### 2.3.3.2 Information Sources and Search Strategy

A librarian from the Health Sciences Library at McMaster University with expertise in systematic reviews will be consulted in developing the search strategy. A unique and pre-determined search strategy will be developed for

exporting publications from each of the select databases and GWAS data-sharing sites. These include MEDLINE, Web of Science (All Databases), EMBASE, CINAHL and Pre-CINAHL, GWAS Catalog, GWAS Central, and NIH Database of Genotypes and Phenotypes. Studies will not be restricted by language or date of publication but will be limited to human participants if limiting by species is made possible through the database. Databases will be searched from inception until present. All sources of literature, including gray literature, will be searched. Handsearching techniques will also be applied to identify articles of interest that are not detected by the databases systematically searched. A detailed search strategy is presented in Table 1. The start date of the study is March 1st, 2020.

#### 2.3.3.3 Study Records

Data Management

All studies will be exported from the previously mentioned databases using the search strategy in Table 1 and imported into Zotero,(17), a citation management software, where they will be screened for duplicates. We will then import studies into Covidence,(18), for another round of duplicate screening and removal, title and abstract screening, full text screening, and data extraction. Each study will be screened and reviewed in duplicate through a team of 8 reviewers. In the case of any disagreements, the conflict will be resolved by a senior reviewer (CC or AH). As per the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines,(19), a flow chart detailing the stepwise screening process will be provided.

Selection Process

Studies will be screened twice in pairs; once assessing the title and abstract, and another time at the full text phase. All articles will be screened for the same inclusion criteria previously mentioned, during both screening processes. All reviewers will partake in a calibration phase to ensure that the purpose of this review and the inclusion criteria are understood by all, and that no discrepancies exist across the reviewers. Since the screening of studies will occur via Covidence, reviewers are blinded to their colleagues' votes until after they have inputted their own votes, reducing the potential for bias.

Data Collection Process

Data extraction will be completed in pairs for any articles that pass the screening process. A full text extraction form will be constructed on excel and then uploaded onto Covidence. The data extraction form will be pilot tested independently in duplicate to ensure its feasibility in this systematic review. For any missing data from studies during the data extraction phase, contact will be made with the study authors to supplement the missing data. All records of communication and contact with the authors will be documented.

#### 2.3.3.4 Data Items

Information collected on this form will include: author(s), year of publication, country, cohort population, number of participants (separated by MAT), ethnicity of participants, mean age, sex ratio, type and dose of MAT, MAT outcomes (as outlined under “Outcome Measures”), any genetic variants found to be significantly associated with the outcomes, method of statistical measures, and p-values. The traditional genome-wide significance threshold reported in the literature is $p \leq 5x10\text{-}8$. However, since a considerable number of studies with a borderline genome-wide significance have been shown to be replicable and showcase genuine associations, $p \leq 1x10\text{-}7$ will be used as the significance threshold for this review,(20).

#### 2.3.3.5 Outcomes and Prioritization

The main focus of this systematic review will be to assess GWAS-identified genetic variants significantly associated with MAT outcomes.

The primary MAT outcome of interest is illicit (unprescribed) opioid use throughout the duration of the MAT and at follow-up periods, the duration of which are to be determined based on the different studies reviewed. Continued illicit opioid use and abstinence from opioids will be assessed from urine toxicological screens and/or self-reported data.

Secondary outcomes of MAT to be considered in this review are:

1. Time to relapse, defined as the duration to the first use of illicit opioids after achieving abstinence.
2. Treatment retention, defined as the length of time a participant remains on MAT, and reasons for stopping MAT or dropping out.
3. Opioid overdose incidence, measured by self report, adjudication of medical records, emergency admissions, opioid-related hospitalization, or use of naloxone.
4. Non-opioid substance use, self-reported or identified through urine toxicology screens.
5. Comorbid psychiatric disorders, self-reported or diagnosed.
6. Risk-taking behaviours related to drug use (i.e. injection, needle sharing), criminal activities, and social adversities, as reported in the original studies.
7. MAT and metabolite plasma concentrations and clearance, obtained through blood plasma analysis.
8. MAT doses, measured throughout the administration of MAT and at follow-up periods, as reported in the original studies
9. All-cause mortality, including opioid-related mortality.

#### 2.3.3.6 Risk of Bias in Individual Studies

Quality assessment and risk of bias scores of included studies will be provided independently by each reviewer. The Quality of Genetic Association Studies (Q-Genie) tool [Version 1.1] developed by McMaster University will be used to assess both the qualitative and quantitative aspects of each study,(21). It is tailored to assess the validity and reliability of genetic association studies. Through Q-Genie, a quality score that corresponds to 'low', 'moderate', or 'high' quality would be calculated for each study. The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) tool will be used to assess the risk of bias, strength of evidence, and consistency of included studies,(22). Disagreements occurring between two reviewers regarding the risk of bias score will be resolved through discussion. If a unanimous decision is not reached, then a third senior author will be consulted.

#### 2.3.3.7 Data Synthesis

If appropriate, quantitative methods of synthesis will be applied. Heterogeneity between the studies will be assessed through the $I^2$ statistic and 95% confidence interval. If low heterogeneity levels are observed, quantitative methods of synthesis applied will include a random effects meta-analysis with pooled odds ratios for main and secondary outcomes previously mentioned. If a large number of studies is identified in this systematic review, subgroup analyses will be used, where the studies will be separated based on the ethnicities of their respective populations and analyzed accordingly, as genetic associations might be more predominant in certain ethnic groups than others. Other subgroup analyses to be considered are based on variables observed to influence MAT outcomes. These include sex, type of MAT, type of illicit opioid used (for example, heroin versus prescription opioids), and alcohol use comorbidity, if discussed in the original studies. All statistical analysis will be conducted via the RStudio [1.1.456] interface of R statistical software,(23).

#### 2.3.3.8 Meta-bias

To address the potential publication bias that might be encountered, PROSPERO and ClinicalTrials.gov databases will be searched for relevant clinical trial protocols that might not have been followed by a publication of results,(24,25).

#### 2.3.3.9 Confidence in Cumulative Evidence

To assess the risk of bias within and across studies in the systematic review proposed, GRADE will be used,(22). It will be implemented to evaluate the study

limitations and biases that contribute to each outcome of interest reported. The GRADE approach will assess the effect of the limitations on the results, effects being ‘not serious’, ‘serious’, or ‘very serious’. Downgrading of the quality of the study will take place depending on the assessed effect level.

#### 2.3.3.10 Presenting and Reporting of Results

Results will be reported according to PRISMA guidelines, with special considerations to Human Genome Epidemiology Network (HuGENet) guidelines when applicable to GWAS data presentation,(19,26). Though HuGENet guidelines are more pertinent to systematic reviews and meta-analyses of candidate gene studies with foci on single or multiple related genes, they will be used to uphold a standard when presenting genetic association data, when feasible. Tables will be used to present information on each genetic variant-phenotype association reported, including the study details, population, findings, and source of data. Forest plots will be used to display meta-analysis results, should a meta-analysis be appropriate to conduct. The overall quality of each published result will be discussed, taking into account the risk of bias scores.

### 2.3.4 Discussion

This systematic review will be able to identify GWASs that have been conducted regarding MATs for OUD. Having a clear list of relevant studies will enable easier access to published results by the public and researchers alike. Results of the meta-analysis will be informative in determining if any genetic markers have been identified to have an impact on MAT outcomes in patients. This will help direct which genes are of interest for future candidate gene studies or GWASs. It will also allow for a consensus to be made regarding whether genetics affect treatment outcomes in the OUD population. Furthermore, if performed, stratified meta-analyses based on population ethnicities will contribute to the breadth of knowledge of genetic differences between ethnic groups. In addition, this review will allow for more informed treatment plans for individuals with differing ethnicities and genetic makeup. A potential limitation that could arise would be the inability to conduct sub-group meta-analyses due to high calculated heterogeneity between studies or small study numbers. In that case, the studies will be qualitatively reviewed and critically assessed according to their risk of bias scores. Another limitation of the proposed review is the exclusion of results obtained from candidate gene studies. Although some relevant SNP-outcome associations will not be reported on, the level of those reported will be of genome-wide significance, highlighting associations that can be expected and replicated in GWASs.

### 2.3.5 List of Abbreviations

OUD – Opioid use disorder
MAT – Medication-assisted treatment
PROSPERO – International Prospective Register of Systematic Reviews
DSM-5 – Diagnostic and Statistical Manual of Mental Disorders, 5th edition
WHO – World Health Organization
HHS – U.S. Department of Health and Human Services
GWAS – Genome-wide association study
PRISMA-P – Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols
PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-Analyses
Q-Genie – Quality of Genetic Association Studies
GRADE – Grading of Recommendations Assessment, Development, and Evaluation
HuGENet – Human Genome Epidemiology Network

### 2.3.6 Declarations

#### 2.3.6.1 Ethics Approval and Consent to Participate

Not applicable.

#### 2.3.6.2 Consent for Publication

Not applicable.

#### 2.3.6.3 Availability of Data and Materials

Not applicable. No data were generated, analyzed, or reported in this manuscript.

#### 2.3.6.4 Competing Interests

The authors declare that they have no competing interests.

#### 2.3.6.5 Funding

This work was partially supported by CIHR (grant number PJT-156306), which has no role in the study design, analysis, reporting or publication of the results.

#### 2.3.6.6 Authors' Contributions

ZS is the guarantor. CC and ZS conceptualized the systematic review protocol. CC implemented the design of the protocol with the aid of AH and SS (health sciences librarian). CC prepared the first draft. AH, SS, AD, BP, LG, DX, NB, AA,

FK, GP, LT, and ZS reviewed and revised the protocol draft. All authors read and approved the final manuscript.

#### 2.3.6.7 Acknowledgements

Not applicable.

#### 2.3.6.8 Amendments

If amendments to this protocol are made, they will be documented and communicated to the journal. A date of amendment, description, and rationale will accompany each amendment.

### 2.3.7 References

1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders [Internet]. Fifth Edition. American Psychiatric Association; 2013 [cited 2020 Mar 24]. Available from: https://psychiatryonline.org/doi/book/10.1176/appi.books.9780890425596
2. WHO. WHO | Information sheet on opioid overdose [Internet]. 2018 [cited 2020 Feb 6]. Available from: https://www.who.int/substance_abuse/information-sheet/en/
3. Government of Canada. Federal Action on Opioids - Canada.ca [Internet]. 2020 [cited 2020 Feb 6]. Available from: https://www.canada.ca/en/health-canada/services/substance-use/problematic-prescription-drug-use/opioids/federal-actions.html
4. HHS. HHS Acting Secretary Declares Public Health Emergency to Address National Opioid Crisis | HHS.gov [Internet]. 2017 [cited 2020 Feb 6]. Available from: https://www.hhs.gov/about/news/2017/10/26/hhs-acting-secretary-declares-public-health-emergency-address-national-opioid-crisis.html
5. FDA. Information about Medication-Assisted Treatment (MAT) | FDA [Internet]. 2019 [cited 2020 Feb 6]. Available from: https://www.fda.gov/drugs/information-drug-class/information-about-medication-assisted-treatment-mat
6. O'Brien C, Kampman KM. Antagonists of Opioids. In: Galanter M, Kleber H, editors. Textbook of Substance Abuse and Treatment. 4th ed. Arlington, VA: American Psychiatric Publishing; 2008.
7. Stotts AL, Dodrill CL, Kosten TR. Opioid Dependence Treatment: Options In Pharmacotherapy. Expert Opin Pharmacother. 2009 Aug;10(11):1727–40.
8. Government of Canada. Government of Canada approves new treatment options for opioid use disorder and supports research,

treatment and harm reduction projects in Ontario - Canada.ca [Internet]. Health Canada. 2019 [cited 2020 Feb 6]. Available from: https://www.canada.ca/en/health-canada/news/2019/05/government-of-canada-approves-new-treatment-options-for-opioid-use-disorder-and-supports-research-treatment-and-harm-reduction-projects-in-ontario.html

9. Sordo L, Barrio G, Bravo MJ, Indave BI, Degenhardt L, Wiessing L, et al. Mortality risk during and after opioid substitution treatment: systematic review and meta-analysis of cohort studies. BMJ. 2017 Apr 26;357:j1550.
10. Joe GW, Simpson DD, Sells SB. Treatment Process and Relapse to Opioid Use During Methadone Maintenance. Am J Drug Alcohol Abuse. 1994;20(2):173–97.
11. Amato L, Davoli M, Minozzi S, Ferroni E, Ali R. Methadone at tapered doses for the management of opioid withdrawal. [Internet]. 2013 [cited 2018 Aug 15]. Available from: http://publichealthwell.ie/node/69404?source=relatedblock&content=resource&member=6841&catalogue=none&collection=none&tokens_complete=true
12. Modesto-Lowe V, Brooks D, Petry N. Methadone Deaths: Risk Factors in Pain and Addicted Populations. J Gen Intern Med. 2010;25(4):305–9.
13. Nosyk B, Marsh DC, Sun H, Schechter MT, Anis AH. Trends in methadone maintenance treatment participation, retention, and compliance to dosing guidelines in British Columbia, Canada: 1996-2006. J Subst Abuse Treat. 2010 Jul;39(1):22–31.
14. 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. PLoS One. 2014;9(1):e86114.
15. J Mistry C, Bawor M, Desai D, C Marsh D, Samaan Z. Genetics of opioid dependence: a review of the genetic contribution to opioid dependence. Curr Psychiatry Rev. 2014;10(2):156–167.
16. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev. 2015 Jan 1;4(1):1.
17. Center for History New Media, George Mason University. Zotero: The Next-Generation Research Tool [Internet]. 2009. Available from: http://www.zotero.org/
18. Veritas Health Innovation. Covidence systematic review software [Internet]. Melbourne, Australia; Available from: www.covidence.org

19. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009 Jul 21;6(7):e1000097.
20. Panagiotou OA, Ioannidis JPA. What should the genome-wide significance threshold be? Empirical replication of borderline genetic associations. Int J Epidemiol. 2012 Feb 1;41(1):273–86.
21. Sohani ZN, Meyre D, de Souza RJ, Joseph PG, Gandhi M, Dennis BB, et al. Assessing the quality of published genetic association studies in meta-analyses: the quality of genetic studies (Q-Genie) tool. BMC Genet [Internet]. 2015 May 15 [cited 2019 Dec 17];16. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4431044/
22. Guyatt GH, Oxman AD, Schünemann HJ, Tugwell P, Knottnerus A. GRADE guidelines: a new series of articles in the Journal of Clinical Epidemiology. J Clin Epidemiol. 2011 Apr;64(4):380–2.
23. RStudio Team. RStudio: Integrated Development for R. RStudio, Inc. [Internet]. Boston, MA; 2015. Available from: http://www.rstudio.com/
24. U.S. National Library of Medicine. Home - ClinicalTrials.gov [Internet]. [cited 2020 Mar 30]. Available from: https://clinicaltrials.gov/ct2/home
25. Search | Cochrane Library [Internet]. [cited 2020 Apr 7]. Available from: https://www.cochranelibrary.com/central
26. Little J, Higgins J. The HuGENetTM HuGE Review Handbook, version 1.0. 2006;59.

### 2.3.8 Tables

**Table 1. Search Strategy**

Medline (Ovid):

1. Genome-Wide Association Study/
2. Genotyping Techniques/
3. Genome, Human/
4. Genetic Variation/
5. genetics/ or exp human genetics/
6. (human* adj2 (genotyp* or genome* or genetic*)).ti,ab,kw,kf.
7. (GWS or GWAS or GWA).mp.
8. genome wide.ti,ab,kw,kf.
9. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8
10. exp Opioid-Related Disorders/
11. ((opiate* or opioid* or heroin* or codeine* or dilaudid* or fentanyl* or narcotic* or drug* or substance*) adj2 (overdose* or use* or using or misuse* or abus* or dependence* or addict*)).ti,ab,kw,kf.
12. Opiate Substitution Treatment/
13. ((opiate* or opioid*) adj2 (treatment* or therap*)).ti,ab,kw,kf.

14. exp buprenorphine/ or exp naloxone/
15. exp Methadone/
16. (suboxone or methadone or buprenorphine or naloxone).ti,ab,kw,kf.
17. 10 or 11 or 12 or 13 or 14 or 15 or 16
18. 9 and 17
19. *Limit 18 to humans*

Web of Science – All Databases:

1. TS=(genome-wide association study or genome-wide association or GWAS or GWA or genome wide or genome)
2. TS=((opiate* or opioid* or heroin* or fentanyl* or narcotic* or drug* or substance*) NEAR/2 (overdose* or use* or using or misus* or abus* or dependence* or addict*))
3. TS=((treatment* or therap*) NEAR/2 (opiate* or opioid* or heroin* or fentanyl* or narcotic* or drug* or substance*))
4. TS=(methadone or buprenorphine or naloxone or naltrexone or heroin-assisted or suboxone)
5. #3 or #4
6. #1 and #2 and #5

EMBASE (Ovid):

1. Genome-Wide Association Study/
2. Genotyping Techniques/
3. Genome, Human/
4. Genetic Variation/
5. genetics/ or exp human genetics/
6. (human* adj2 (genotyp* or genome* or genetic*)).ti,ab,kw.
7. (GWS or GWAS or GWA).mp.
8. genome wide.ti,ab,kw.
9. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8
10. exp Opioid-Related Disorders/
11. ((opiate* or opioid* or heroin* or codeine* or dilaudid* or fentanyl* or narcotic* or drug* or substance*) adj2 (overdose* or use* or using or misuse* or abus* or dependence* or addict*)).ti,ab,kw.
12. Opiate Substitution Treatment/
13. ((opiate* or opioid*) adj2 (treatment* or therap*)).ti,ab,kw.
14. exp buprenorphine/ or exp naloxone/
15. exp Methadone/
16. (suboxone or methadone or buprenorphine or naloxone).ti,ab,kw.
17. 10 or 11 or 12 or 13 or 14 or 15 or 16
18. 9 and 17
19. *Limit 18 to human*

CINAHL and Pre-CINAHL:

1. genome-wide association study or genome-wide association or GWAS or GWA or genome wide or genome
2. opiate* or opioid* or heroin* or fentanyl* or narcotic* or drug* or substance*
3. overdose* or use* or using or misus* or abus* or dependence* or addict*
4. S2 and S3
5. treatment* or therap*
6. S5 and S2
7. methadone or buprenorphine or naloxone or naltrexone or heroin-assisted or suboxone
8. S6 or S7
9. S1 and S4 and S8
10. *Limit to Human*

GWAS Catalog - publications:
- methadone
- opioid
- heroin
- drug abuse

GWAS Central – studies list:
- methadone
- heroin
- opioid
- opiate
- addiction
- drug abuse
- opioid dependence
- opioid addiction
- fentanyl

NIH Database of Genotypes and Phenotypes:
- Search (opioid)
- Search (heroin)

### 2.3.9 Additional Files

Additional file 1 – PRISMA-P 2015 Checklist.
This file is submitted in .pdf format and shows adherence to the PRISMA-P guidelines.

# 3 CHAPTER 3

## 3.1 Introduction

This chapter consists of an unpublished systematic review manuscript, “A systematic review of GWAS-identified SNPs associated with medication-assisted treatment outcomes in patients with opioid use disorder”. In its literature review, it explores genes and genetic variants that have been found to be associated with MAT outcomes in OUD patients globally. It follows a rigorous design in identifying studies, screening studies and extracting significant findings from GWASs. It highlights biologically relevant SNPs that have been recurrently associated with outcomes of interest in the literature, as well as novel ones within different ethnic contexts. This review also critically analyzes the findings and their respective studies, assessing the quality of evidence and risk of bias using tools specific for genetic studies. Therefore, it is the first in its field to create a summary of relevant and significant data that the research community can use for future directions and clinicians and public health officials can use as a guide in implementing individual-based therapies.

## 3.2 A systematic review of GWAS-identified SNPs associated with medication-assisted treatment outcomes in patients with opioid use disorder

**Authors & Affiliations**
Caroul Chawar 1, Alannah Hillmer 2, Stephanie Sanger 3, Alessia D'Elia 4, Balpreet Panesar 5, Lucy Guan 6, Dave Xiaofei Xie 7, Nandini Bansal 8, Aamna Abdullah 9, Flavio Kapczinski 10, Guillaume Pare 11, Lehana Thabane 12, Zainab Samaan13*

1. Neuroscience Graduate Program, McMaster University, Hamilton, ON, Canada; Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; chawarc@mcmaster.ca
2. Neuroscience Graduate Program, McMaster University, Hamilton, ON, Canada; Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; hillmea@mcmaster.ca
3. Health Sciences Library, McMaster University, Hamilton, ON, Canada; sangers@mcmaster.ca
4. Neuroscience Graduate Program, McMaster University, Hamilton, ON, Canada; Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; deliaa@mcmaster.ca
5. Neuroscience Graduate Program, McMaster University, Hamilton, ON, Canada; Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; panesabk@mcmaster.ca
6. Health Sciences Program, McMaster University, Hamilton, ON, Canada; Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; guanx1@mcmaster.ca
7. Health Sciences Program, McMaster University, Hamilton, ON, Canada; Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; xiex11@mcmaster.ca
8. Health Sciences Program, McMaster University, Hamilton, ON, Canada; Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; bansan1@mcmaster.ca
9. Health Sciences Program, McMaster University, Hamilton, ON, Canada; Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; abdula19@mcmaster.ca
10. Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; kapczinf@mcmaster.ca

11. Population Health Research Institute, Hamilton, ON, Canada; Department of Health Research Method, Evidence, and Impact, McMaster University, Hamilton, ON, Canada; pareg@mcmaster.ca
12. Department of Health Research Method, Evidence, and Impact, McMaster University, Hamilton, ON, Canada; Population Health Research Institute, Hamilton, ON, Canada; Father Sean O'Sullivan Research Centre, St. Joseph's Healthcare Hamilton; thabanl@mcmaster.ca
13. Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; samaanz@mcmaster.ca

*Corresponding Author:
Dr. Zainab Samaan
100 West 5th St., Hamilton, ON L8N3K7, Canada
Telephone: 905-522-1155 ext. 35448
Fax: 905-381-5629

### 3.2.1 Abstract

**Background:** Patients with opioid use disorder (OUD) respond differently to medication-assisted treatment (MAT). A genetic basis may explain the variability in this response, where a genetic variant can attribute to a change in treatment outcome, such as continued illicit opioid use or abstinence. However, no consensus has been reached regarding which genetic variants significantly contribute to MAT outcomes.

**Objectives:** This systematic review aims to summarize genome-wide significant findings on MAT outcomes and critically appraise the quality of the studies involved.

**Methods:** Databases searched from inception until August 21st, 2020 include: MEDLINE, Web of Science, EMBASE, CINAHL and Pre-CINAHL, GWAS Catalog and GWAS Central. The included studies had to be GWASs that assessed MAT in an OUD population. All studies were screened in duplicate. The quality of the included studies was scored and assessed using the Q-Genie tool. Quantitative analysis, as planned in the protocol, was not feasible, so the studies were analyzed qualitatively.

**Results:** Our search identified 7292 studies. Five studies meeting the eligibility criteria were included. However, only three of which reported results that met our significance threshold of $p \leq 1.0 \times 10^{-7}$. In total, 43 genetic variants were identified. Variants corresponding to *CNIH3* were reported to be associated with daily heroin injection in Europeans, *OPRM1*, *TRIB2*, and *ZNF146* with methadone dose in African Americans, *EYS* with methadone dose in European Americans,

and *SPON1* and intergenic regions in chromosomes 9 and 3 with plasma concentrations of S-methadone, R-methadone, and R-EDDP, respectively, in Han Chinese.

**Limitations:** The limitations of this study include not being able to synthesize the data in a quantitative way and a conservative eligibility and data collection model.

**Conclusion:** The results from this systematic review will aid in highlighting significant genetic variants that can be replicated in future OUD pharmacogenetics research to ascertain their role in patient-specific MAT outcomes.

**SR registration number:** CRD42020169121

**Keywords:** opioid, pharmacogenetic, MAT, GWAS, epidemiology, systematic review

## 3.2.2 Introduction

### 3.2.2.1 Rationale

Opioid use has been on the rise over the past decade, causing the United States and Canada, amongst other countries, to declare an opioid crisis and epidemic(1,2). In a 2019 report, the United Nations estimated about 53 million past-year users of opioids for 2017 worldwide(3). That same year, 110,000 deaths were attributed to opioid use(3).

Treatments for opioid use disorder (OUD) have become more available and accessible under the term medication-assisted treatments (MATs). MATs include the controlled administration of an opioid agonist or antagonist along with behavioural therapy or counselling with the objective of full recovery from opioid use(4). Pharmacological agents of MAT include the commonly used methadone, buprenorphine, buprenorphine/naloxone combination, naltrexone, and heroin-assisted treatment.

The introduction of MAT has been shown by evidence-based research to decrease the risk of overdose and mortality within individuals with OUD. A recent systematic review has reported the pooled overdose crude mortality rates for individuals being treated with MAT compared to after the cessation of MAT and during untreated periods being 0.24, 0.68, and 2.43, respectively(5). Another review summarizing MAT effectiveness in randomized controlled trials reported that the administration of MAT medication at least doubles the rates of opioid abstinence when compared to placebo medications or no medications(6).

Though MAT has established its effectiveness within the OUD population, its administration has been observed to have negative effects on patients under certain circumstances. As mentioned earlier, mortality risks tend to spike shortly after MAT cessation(5). Additionally, induction of methadone has shown an increased risk of overdose in multiple studies (7,8). Methadone dosing can affect electrocardiographic QTc interval prolongation, inducing respiratory depression amongst patients and increasing the risk for overdose mortality(9). This is indicative that perhaps dosing of MAT and its metabolism in patients are important factors in determining patient outcomes.

Given the individual basis of the treatment administration, a genetic predisposition to MAT responses may be involved. OUD is a complex polygenic disorder with not one genetic variant attributing to a large risk or effect. Genetic association studies researching genetic variants or single-nucleotide polymorphisms (SNPs) associated with OUD or its treatment outcomes require large sample sizes to generate enough power to identify such variants(10).

Currently, the most common SNPs reported to be associated with MAT outcomes correspond to *OPRM1*, *OPRD1*, *ABCB1*, and *CYP2B6* genes(11,12). *OPRM1*, *ABCB1*, and *CYP2B6* variants have been associated with altered methadone doses in previous studies(12). *ABCB1* along with *CYP2B6* variants have also been linked to variable methadone plasma concentrations. Other studies showed variants in *OPRD1* to be associated with opioid-positive urine screens and therapeutic responses in patients administered methadone versus buprenorphine(11,12).

Though there seem to be numerous studies assessing the pharmacogenetics of MAT, many of which are candidate gene studies with small samples sizes. To produce replicable results and discover new significantly associated SNPs, robust genome-wide association studies (GWASs) need to be performed and assessed. As there is no current review of published GWAS findings with respect to MAT outcomes, it is difficult to identify the gap and address it. This systematic review is the first to summarize the current literature, assess the quality of the findings, and report on the areas that need to be addressed within this field.

#### 3.2.2.2 Objectives

The aims of this systematic review are to highlight any significant GWAS genetic variants that are associated with MAT outcomes for patients with OUD.

The specific objectives are to:

1. Summarize the genome-wide significant SNP outcome associations reported in the literature and highlight novel ones.

2. Critically examine and assess the quality of the findings extracted within the relevant studies using the Q-Genie tool.
3. Identify gaps within the literature that need to be addressed with respect to pharmacogenetic research of MAT outcomes.

### 3.2.3 Methods

This systematic review is reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines(13). A supplementary PRISMA checklist could be found in Supplementary File 1. Since the focus of this review is on GWASs, it does not conform with the Human Genome Epidemiology Network (HuGENet) guideline expectations of reporting on candidate gene study findings(14). However, the HuGENet guideline is used to supplement the PRISMA guidelines, to provide a more informed review, upholding a standard of reporting specific to genetic association studies.

#### 3.2.3.1 Protocol and registration

This systematic review has been registered with the International Prospective Register of Systematic Reviews (PROSPERO)(15); registration ID CRD42020169121. A systematic review protocol has been published in the journal of Systematic Reviews(16). The detailed methods of this systematic review are specified and documented in the registration and protocol.

#### 3.2.3.2 Eligibility criteria and Search Strategy

The eligibility for inclusion in this systematic review is three-fold. The study design of included studies is limited to GWASs specific to genetic variants of interest reported as SNPs. The included studies have to look at an OUD population. Lastly, included studies have to investigate a MAT, such as methadone, Suboxone, buprenorphine, naltrexone, naloxone, or heroin-assisted treatment. Studies are not restricted by language, patient demographics, or MAT administration setting.

A search strategy was developed with help from a Health Sciences Librarian (SS). Table 1 outlines the databases searched and the search terms used. All databases were searched from inception to August 21st, 2020. Handsearching was used to identify relevant studies that were not detected by the search strategy.

#### 3.2.3.3 Data collection and Outcomes

Title and abstract screening, full-text screening, and data extraction of studies are all completed in duplicate via Covidence(17). The voting of reviewers remains

blinded and conflict resolution for the screening stages is performed by a senior reviewer (AH or CC), keeping the process unbiased. The data extraction form was pilot tested in duplicate prior to proceeding with data collection.

Data extracted include study information, baseline participant characteristics, relevant and significant measured outcomes, statistical measures, and reported study limitations and conflicts. For the purposes of this review, the significance threshold of SNP outcome associations extracted is $p \leq 1x10^{-7}$, as some GWAS results with this significance level have been shown to be replicable within the literature(18).

The outcomes of interest in this review pertain to genetic variants significantly associated with MAT outcomes observed in OUD patients. The primary MAT outcome considered is illicit opioid use or abstinence during or following MAT. The secondary MAT outcomes include time to relapse, treatment retention, opioid overdose, non-opioid substance use, comorbid psychiatric disorders, drug-related risk-taking behaviours, MAT and metabolite plasma concentration, MAT dose, and mortality.

#### 3.2.3.4 Quality Assessment and Data Analysis

Quality assessment of each included study is done using the Quality of Genetic Association Studies (Q-Genie) tool [Version 1.1], assessing the study validity, reliability, and risk of bias(19). Quality assessments are completed in duplicate, and conflicts regarding the scoring are resolved by the reviewers.

A heterogeneity test and random effects meta-analysis through pooled odds ratios were planned to quantitatively assess the data, as outlined in the protocol. However, these measures were not appropriate as data extracted from each study was unique and could not be synthesized.

For the aforementioned reasons, subgroup meta-analyses and risk of bias assessments across studies also could not be completed.

### 3.2.4 Results

#### 3.2.4.1 Study selection

A total of 5 studies were eligible for inclusion in this systematic review(20–24). The search strategy along with handsearching techniques identified 7292 studies, with 5809 advancing to the title and abstract screening after the removal of duplicates by both the Zotero reference manager and Covidence(17,25). Of the 38 full-text studies assessed for eligibility, 5 GWASs (3 prospective, 1 cross-sectional, and 1 case-control) underwent data extraction and qualitative assessment. See flow diagram in Figure 1.

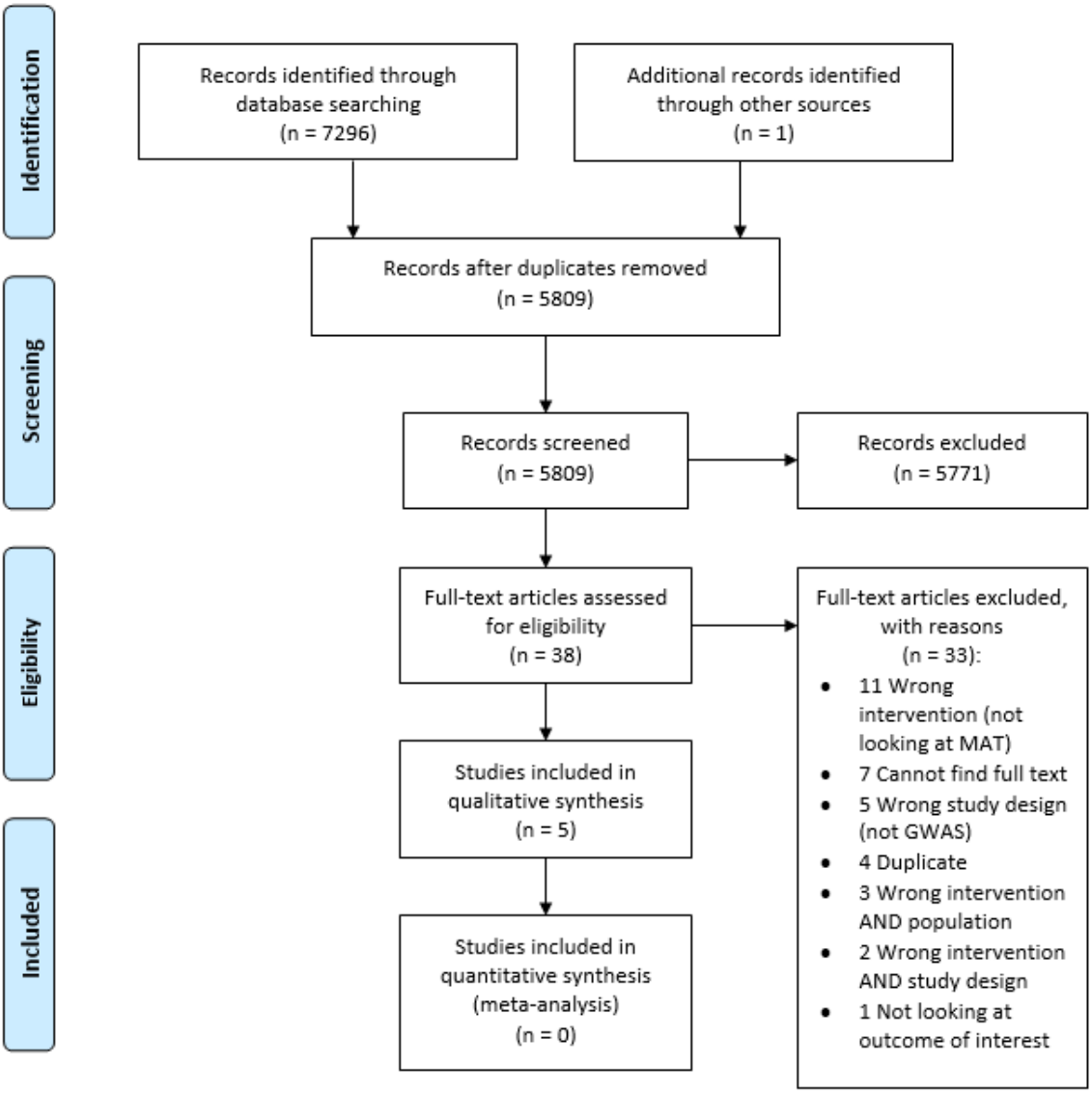



**Figure 1. PRISMA Flow Diagram of Study Inclusion.**

### 3.2.4.2 Study characteristics

Table 2 provides a summary of the included study characteristics. All five studies were published in English. Three were prospective studies, one case-control, and one cross-sectional. The sample size studied varied from a few hundreds to thousands of participants, the smallest being 344 and largest 4049. All studies had a majority male study population, varying from 59.72% to 81.6% males. The mean age per studied population varied from 33.03 (5.45) to 45.6 (8.4). Ancestries of the participants included in these GWASs were European, African

American, and/or Han Chinese, with Europeans constituting the largest sample. Two of the studies identified used the same sample population of Han Chinese individuals for their analyses, though performed different statistical measures(23,24). Three of the studies reported that participants were administered methadone as their MAT(22–24), and two did not specify(20,21). The outcomes of interest that were reported to be associated with genetic variants were opioid cessation, daily heroin injection while on MAT, methadone dose, and plasma concentrations of methadone and its metabolite EDDP. No study assessed relapse, treatment retention, opioid overdose, non-opioid substance use, psychiatric disorders, risk-taking behaviours, or mortality as outcomes associated with genetic variation.

#### 3.2.4.3 Risk of bias within studies

The quality and validity of each study was assessed using the Q-Genie tool on a scale of 1 to 7(19). Studies with a control group and with overall scores of greater than or equal to 45, as well as studies with no control group with overall scores of greater than 40 were considered of good quality. All but one study were assessed to be of good quality, while Nelson et al. was deemed to be of moderate quality(21). It should be noted that the primary objectives of Nelson et al.'s study might not have been to assess an MAT outcome per se, but rather opioid dependence end points amongst opioid-dependent daily injectors (cases) versus nondaily injecting opioid misusers (controls). However, due to satisfying the eligibility criteria and analyzing an outcome of interest to us in only the cases, this study was included. Three of the included studies report insufficient sample sizes that might result in not detecting genome-wide significant SNPs(22–24). The three studies also disclose conflicts of interest that are reported to not be interferent with the research conducted(20–22). See Table 3 for a summary of the reported limitations and conflicts of interest, as well as the quality assessments.

#### 3.2.4.4 Results of individual studies

Of the five studies included, only three reported outcomes that reached the threshold of significance set for this systematic review (Table 4)(21,22,24).

Nelson et al. identified three SNPs associated with opioid dependence end point in the gene *CNIH3* (chromosome 1). The participants were daily heroin-injecting patients on methadone or buprenorphine of European ethnicity. The three SNPs reported are in moderate to high linkage disequilibrium, with the odds of the risk alleles being found in the daily heroin injecting group approximately 50% lower than in the control group(21).

Smith et al. identified thirty-seven SNPs associated with methadone dose in varying genes across methadone-treated African American and European

American populations. Amongst participants of African American ethnicity, the SNPs correlated to the following genes: *OPRM1* (chromosome 6), *TRIB2* (chromosome 2), and *ZNF146* (chromosome 19). On the other hand, the SNPs identified in European Americans correlated to only one gene, *EYS* (chromosome 6). The leading SNP nearest to the *OPRM1* gene (rs73568641) was reported to be in mid to high linkage disequilibrium with neighbouring SNPs identified. Linkage disequilibrium amongst SNPs of other genes was not reported as they were not genome-wide significant. The presence of the risk alleles in the *OPRM1*, *TRIB2*, and *ZNF146* genes is observed to be associated with an increase in the usual daily methadone dose in African American patients. In contrast, the presence of the risk alleles in the *EYS* gene is observed to be associated with a decrease in the usual daily methadone dose in European Americans(22).

Lastly, Yang et al. identified three SNPs associated with methadone and EDDP plasma concentrations. The participants were methadone-administered patients in Taiwan of Han Chinese ancestry. One SNP was associated with plasma concentration of R-methadone, corresponding to an intergenic region (chromosome 9), one with plasma concentration of S-methadone, corresponding to the *SPON1* gene (chromosome 11), and the last one associated with plasma concentration of R-EDDP, corresponding to another intergenic region (chromosome 3). The measure and magnitude of association for these SNPs were not reported(24).

### 3.2.5 Discussion

#### 3.2.5.1 Summary of evidence

Advances in pharmacogenetic research within OUD populations have been on the rise. Yet, no attempt has been made in quantitatively and qualitatively analyzing the literature and critiquing the quality of evidence reported by GWASs. This systematic review was able to summarize findings from GWASs with borderline genome-wide significance and the potential of being replicable in future studies. We have identified five eligible studies, three of which with significant results that match our criteria. SNPs associated with outcomes of daily heroin injection, methadone dose, and methadone and EDDP plasma concentration were found to be significant. SNPs corresponding to genetic regions of *CNIH3* were reported to be more prevalent in daily heroin injecting patients. SNPs corresponding to or near *OPRM1*, *TRIB2*, *ZNF146*, and *EYS* were associated with methadone dose levels, depending on ethnicity. SNPs in an intergenic region on chromosome 9, *SPON1*, and an intergenic region on chromosome 3 were associated with differing plasma concentration of R-methadone, S-methadone, and R-EDDP, respectively. The quality of research and reporting of each study was assessed with the Q-Genie tool and no study was deemed to be of poor quality. Varying sample sizes were however observed,

with some being too small for what is considered acceptable for GWAS analysis. With sample sizes of thousands required to produce adequately powered results in GWASs (26), sample sizes from Yang et al. (n=344) and the African American population of Smith et al. (n=383) fell short.

One gene related to the SNPs identified has been reported previously within candidate gene studies and has an established biological relevance within the genetics and pharmacogenetics of OUD research. The *OPRM1* gene encodes the mu-opioid receptor, which binds endogenous and exogenous (licit and illicit) opioids(27). As such, it has been reported to be highly influential in opioid dependency, and, by some findings, OUD treatment outcomes, such as methadone dose and plasma concentrations, in European patients(28). Therefore, it is not a surprise for SNPs in this gene to be associated with methadone dose at a GWAS significance level. Though, Smith et al.'s results are interesting because they found an *OPRM1* association in patients of African American ethnicity but not of European ethnicity, as was expected. This incongruity calls for additional powered research in both ethnic populations to be conducted for a consensus.

Another gene identified has not been published with associations relevant to OUD or MAT outcomes but does illustrate the potential biological relevance. The *CNIH3* gene encodes the protein cornichon homolog 3, which regulates AMPA receptor trafficking(27). This gene has been prevalently expressed in brain tissue(29). It has also been identified in schizophrenia studies by NCBI's Gene database(29). Therefore, it is possible that *CNIH3* could be associated with the regulation of opioid use.

Most of the genes involving an identified SNP summarized in this systematic review do not seem to have been relevant to OUD or MAT outcomes, nor could a biological relevance be identified for them. These genes include *TRIB2*, *ZNF146*, *EYS*, *SPON1*, as well as the intergenic regions for the SNPs located on chromosomes 3 and 9. The *TRIB2* gene encodes the tribbles homolog 2 protein that regulates MAP kinase proteins' activation(27). This gene is evident in many tissues, most prominently in the ovaries, spleen, and nymph node tissues(29). It has also been reported in the NCBI Gene Database to be identified in studies researching schizophrenia, neuropsychiatric disorders, autism, and aging(29). *ZNF146* encodes the zinc finger protein OZF, the primary function of which is to regulate DNA binding and transcription(27). As such, it is present in a lot of tissues, including the brain, but is more prominent in the endometrium and thyroid(29). In humans, *EYS* encodes the protein eyes shut homolog, which as deduced from the name, is involved in vision, more specifically, in maintaining the morphological integrity of photoreceptor cells through the possible involvement in channel regulations(27). *EYS* is most prevalently expressed in fat and testis tissue(29), which shows no direct relation to methadone dose or metabolism as

identified in Smith et al. Lastly, *SPON1* encodes spondin-1, which is a cell adhesion protein within the nervous system(27). *SPON1* is mostly expressed in the gall bladder tissue(29), which does not provide a clear biological link to its function nor the outcome of methadone plasma concentration reported by Yang et al.(29). Further research is required to make any conclusive statements concerning the biological relevance of SNPs in these genes to the observed MAT outcomes.

In general, the results of this systematic review are able to inform future candidate gene studies and GWASs of key SNPs that require further research in larger cohorts as well as replications to solidify their associations to MAT outcomes in OUD patients. The findings from such studies are able to inform the clinical and pharmacological response to patient doses and drug outcomes for administered MATs.

#### 3.2.5.2 Limitations

Though rigorous, this systematic review has some limitations associated with the strict eligibility criteria predetermined in the protocol. It is important to note that in the process of including studies that were primary GWASs, GWAS meta-analyses were excluded. This could have affected the number, quality, and significance of the findings. An example is the exclusion of the GWAS meta-analysis findings from Nelson et al. that replicated original findings in a larger meta-analyzed sample, highlighting new SNPs that achieved significance (rs10799590, rs12130499, and rs298733) and SNPs that fell below our significance threshold in the process (rs1436175)(21). Another limitation could be the exclusion of studies that reported genetic variance in the form of haplotypes. Though their inclusion might have made a meta-analysis possible, they did not satisfy the eligibility criteria of a SNP identified by a GWAS and would, therefore, not be very informative within the scope of our systematic review.

As stated previously, a meta-analysis was not feasible with the heterogeneity of the reported findings. This makes consensus more difficult to reach and the findings less generalizable, especially when considering differing ethnicities.

In addition, since we have considered to highlight SNPs of near genome-wide significance, there is the possibility that we have not included findings due to publication bias. It is conceivable that if a study does not achieve genome-wide significance in their findings, then they are less likely to publish said results. We are in turn unable to include such results, even if they do meet the $1x10^{-7}$ significance threshold we have set.

### 3.2.6 Conclusions

Through this systematic review, we were able to summarize GWAS significant findings in the field of OUD pharmacogenetics. We were able to inform the availability of data by highlighting what has been done within this research field, and what gap exists and needs to be addressed. Recommendations of further powered research are made, with close attention to the ethnicities of participating cohorts to test whether SNP outcome associations within one ethnicity hold competing levels of validity in another.

### 3.2.7 Funding

This systematic review was partially funded by CIHR (grant number PJT-156306). The funding agency had no role in the study design, interpretation of data, or dissemination of results.

### 3.2.8 Authors' Contributions

ZS is the guarantor. CC and ZS conceptualized the systematic review. CC implemented the design of the review and search strategy with the aid of AH and SS. CC, AH, AD, BP, LG, DX, NB, and AA screened studies, extracted data, and assessed the quality of the studies. CC prepared the first draft. AH, SS, AD, BP, LG, DX, NB, AA, FK, GP, LT, and ZS reviewed and revised the protocol draft. All authors read and approved the final manuscript.

### 3.2.8 References

1. Government of Canada. Federal Action on Opioids - Canada.ca [Internet]. 2020 [cited 2020 Feb 6]. Available from: https://www.canada.ca/en/health-canada/services/substance-use/problematic-prescription-drug-use/opioids/federal-actions.html
2. HHS. HHS Acting Secretary Declares Public Health Emergency to Address National Opioid Crisis | HHS.gov [Internet]. 2017 [cited 2020 Feb 6]. Available from: https://www.hhs.gov/about/news/2017/10/26/hhs-acting-secretary-declares-public-health-emergency-address-national-opioid-crisis.html
3. United Nations Office on Drug and Crimes. World drug report 2019. 2019.
4. SAMHSA. Medication and Counseling Treatment [Internet]. [cited 2020 Jun 2]. Available from: https://www.samhsa.gov/
5. Ma J, Bao Y-P, Wang R-J, Su M-F, Liu M-X, Li J-Q, et al. Effects of medication-assisted treatment on mortality among opioids users: a systematic review and meta-analysis. Mol Psychiatry. 2019;24(12):1868–83.

6. Connery HS. Medication-Assisted Treatment of Opioid Use Disorder: Review of the Evidence and Future Directions. Harv Rev Psychiatry. 2015 Apr;23(2):63–75.
7. Sordo L, Barrio G, Bravo MJ, Indave BI, Degenhardt L, Wiessing L, et al. Mortality risk during and after opioid substitution treatment: systematic review and meta-analysis of cohort studies. BMJ [Internet]. 2017 Apr 26 [cited 2020 Jun 2];357. Available from: http://www.bmj.com/content/357/bmj.j1550
8. Buster MCA, van Brussel GHA, van den Brink W. An increase in overdose mortality during the first 2 weeks after entering or re-entering methadone treatment in Amsterdam [Internet]. 2002 [cited 2020 Jun 2]. Available from: https://onlinelibrary-wiley-com.libaccess.lib.mcmaster.ca/doi/full/10.1046/j.1360-0443.2002.00179.x
9. Chou R, Cruciani RA, Fiellin DA, Compton P, Farrar JT, Haigney MC, et al. Methadone Safety: A Clinical Practice Guideline From the American Pain Society and College on Problems of Drug Dependence, in Collaboration With the Heart Rhythm Society. J Pain. 2014 Apr 1;15(4):321–37.
10. Hong EP, Park JW. Sample size and statistical power calculation in genetic association studies. Genomics Inform. 2012 Jun;10(2):117–22.
11. Berrettini W. A brief review of the genetics and pharmacogenetics of opioid use disorders. Dialogues Clin Neurosci. 2017 Sep;19(3):229–36.
12. Fonseca F, Torrens M. Pharmacogenetics of Methadone Response. Mol Diagn Ther. 2018 Feb 1;22(1):57–78.
13. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009 Jul 21;6(7):e1000097.
14. Little J, Higgins J. The HuGENetTM HuGE Review Handbook, version 1.0. 2006;59.
15. PROSPERO: International prospective register of systematic reviews. CRD42020169121 [Internet]. 2020. Available from: https://www.crd.york.ac.uk/PROSPERO/
16. Chawar C, Hillmer A, Sanger S, D'Elia A, Panesar B, Guan L, et al. GWAS-identified genetic variants associated with medication-assisted treatment outcomes in patients with opioid use disorder: a systematic review and meta-analysis protocol. Syst Rev. 2020 Sep 1;9(1):200.
17. Veritas Health Innovation. Covidence systematic review software [Internet]. Melbourne, Australia; Available from: www.covidence.org
18. Panagiotou OA, Ioannidis JPA. What should the genome-wide significance threshold be? Empirical replication of borderline genetic associations. Int J Epidemiol. 2012 Feb 1;41(1):273–86.
19. Sohani ZN, Meyre D, de Souza RJ, Joseph PG, Gandhi M, Dennis BB, et al. Assessing the quality of published genetic association studies in meta-analyses: the quality of genetic studies (Q-Genie) tool. BMC Genet

[Internet]. 2015 May 15 [cited 2019 Dec 17];16. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4431044/
20. Cox JW, Sherva RM, Lunetta KL, Johnson EC, Martin NG, Degenhardt L, et al. Genome-Wide Association Study of Opioid Cessation. J Clin Med. 2020 Jan 9;9(1):180.
21. Nelson EC, Agrawal A, Heath AC, Bogdan R, Sherva R, Zhang B, et al. Evidence of CNIH3 involvement in opioid dependence. Mol Psychiatry. 2016 May;21(5):608–14.
22. 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. 2017 Mar;22(3):346–52.
23. Wang S-C, Chung R-H, Kuo H-W, Liu T-H, Fang C-P, Liu SC, et al. GRK5 Is Associated with the Regulation of Methadone Dosage in Heroin Dependence. Int J Neuropsychopharmacol. 2018 Oct 1;21(10):910–7.
24. Yang H-C, Chu S-K, Huang C-L, Kuo H-W, Wang S-C, Liu S-W, et al. Genome-Wide Pharmacogenomic Study on Methadone Maintenance Treatment Identifies SNP rs17180299 and Multiple Haplotypes on CYP2B6, SPON1, and GSG1L Associated with Plasma Concentrations of Methadone R- and S-enantiomers in Heroin-Dependent Patients. Barsh GS, editor. PLOS Genet. 2016 Mar 24;12(3):e1005910.
25. Center for History New Media, George Mason University. Zotero: The Next-Generation Research Tool [Internet]. 2009. Available from: http://www.zotero.org/
26. Ziyatdinov A, Kim J, Prokopenko D, Privé F, Laporte F, Loh P-R, et al. Estimating the effective sample size in association studies of quantitative traits. bioRxiv. 2020 Jan 4;2019.12.15.877217.
27. UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res. 2019 Jan 8;47(D1):D506–15.
28. Crist RC, Clarke T-K, Berrettini WH. Pharmacogenetics of Opioid Use Disorder Treatment. CNS Drugs. 2018 Apr;32(4):305–20.
29. National Library of Medicine (US), National Center for Biotechnology Information. Gene - NCBI [Internet]. [cited 2020 Jun 5]. Available from: https://www-ncbi-nlm-nih-gov.libaccess.lib.mcmaster.ca/gene/

### 3.2.9 Tables

**Table 1. Search Strategy**

Medline (Ovid):

1. Genome-Wide Association Study/
2. Genotyping Techniques/
3. Genome, Human/
4. Genetic Variation/
5. genetics/ or exp human genetics/
6. (human* adj2 (genotyp* or genome* or genetic*)).ti,ab,kw,kf.
7. (GWS or GWAS or GWA).mp.
8. genome wide.ti,ab,kw,kf.
9. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8
10. exp Opioid-Related Disorders/
11. ((opiate* or opioid* or heroin* or codeine* or dilaudid* or fentanyl* or narcotic* or drug* or substance*) adj2 (overdose* or use* or using or misuse* or abus* or dependence* or addict*)).ti,ab,kw,kf.
12. Opiate Substitution Treatment/
13. ((opiate* or opioid*) adj2 (treatment* or therap*)).ti,ab,kw,kf.
14. exp buprenorphine/ or exp naloxone/
15. exp Methadone/
16. (suboxone or methadone or buprenorphine or naloxone).ti,ab,kw,kf.
17. 10 or 11 or 12 or 13 or 14 or 15 or 16
18. 9 and 17
19. *Limit 18 to humans*

Web of Science – All Databases:

1. TS=(genome-wide association study or genome-wide association or GWAS or GWA or genome wide or

genome)
2. TS=((opiate* or opioid* or heroin* or fentanyl* or narcotic* or drug* or substance*) NEAR/2 (overdose* or use* or using or misus* or abus* or dependence* or addict*))
3. TS=((treatment* or therap*) NEAR/2 (opiate* or opioid* or heroin* or fentanyl* or narcotic* or drug* or substance*))
4. TS=(methadone or buprenorphine or naloxone or naltrexone or heroin-assisted or suboxone)
5. #3 or #4
6. #1 and #2 and #5

EMBASE (Ovid):
1. Genome-Wide Association Study/
2. Genotyping Techniques/
3. Genome, Human/
4. Genetic Variation/
5. genetics/ or exp human genetics/
6. (human* adj2 (genotyp* or genome* or genetic*)).ti,ab,kw.
7. (GWS or GWAS or GWA).mp.
8. genome wide.ti,ab,kw.
9. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8
10. exp Opioid-Related Disorders/
11. ((opiate* or opioid* or heroin* or codeine* or dilaudid* or fentanyl* or narcotic* or drug* or substance*) adj2 (overdose* or use* or using or misuse* or abus* or dependence* or addict*)).ti,ab,kw.
12. Opiate Substitution Treatment/
13. ((opiate* or opioid*) adj2 (treatment* or therap*)).ti,ab,kw.
14. exp buprenorphine/ or exp naloxone/
15. exp Methadone/
16. (suboxone or methadone or buprenorphine or naloxone).ti,ab,kw.

17. 10 or 11 or 12 or 13 or 14 or 15 or 16
18. 9 and 17
19. *Limit 18 to human*

CINAHL and Pre-CINAHL:

1. genome-wide association study or genome-wide association or GWAS or GWA or genome wide or genome
2. opiate* or opioid* or heroin* or fentanyl* or narcotic* or drug* or substance*
3. overdose* or use* or using or misus* or abus* or dependence* or addict*
4. S2 and S3
5. treatment* or therap*
6. S5 and S2
7. methadone or buprenorphine or naloxone or naltrexone or heroin-assisted or suboxone
8. S6 or S7
9. S1 and S4 and S8
10. *Limit to Human*

GWAS Catalog - publications:

- methadone
- opioid
- heroin
- drug abuse

GWAS Central – studies list:

- methadone
- heroin
- opioid

- opiate
- addiction
- drug abuse
- opioid dependence
- opioid addiction
- fentanyl

NIH Database of Genotypes and Phenotypes:
- Search (opioid)
- Search (heroin)

**Table 2. Summary of Included Studies**

| First Author Last Name, Year of Publication | Journal of Publication | Title of Publication | N | % Male | Mean Age (SD) | Ethnicity | Type of MAT | Study Design | Relevant Outcomes Measured |
|---|---|---|---|---|---|---|---|---|---|
| Cox, 2020(19) | Journal of Clinical Medicine | Genome-Wide Association Study of Opioid Cessation | 4049 | 63.45 % | NA | African American =1130, European =2919 | Opioid Substitution Treatment (unspecified) | Prospective | Opioid cessation<br>- USA sample: defined as abstinence from illicit opioids for >1 year (ceased) or <6 months (not ceased) before the interview date.<br>- Australia sample: last |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | use of an opioid was at least one year before the age at the interview (ceased) or the age of last use of an opioid was the same as the age at the interview (not ceased). |
| Nelson, 2016(20) | Molecular Psychiatry | Evidence of *CNIH3* involvement in opioid dependence | 1167 cases, 161 controls | 60.1% | 36.9 (8.4) | European | Methadone or Buprenorphine Opioid Replacement Therapy (cases) | Case-control | Continued opioid use ($OD_E$ - daily heroin injection while on treatment) |
| Smith, 2017(21) | Molecular Psychiatry | Genome-wide association study of therapeutic opioid dosing identifies a novel locus upstream of *OPRM1* | 1410 | 59.72 % | AA: Males: 45.6 (8.4); Females: 43.0 (7.2). EA: Males: 37.2 (10.1); Females: 37.5 (9.8) | African American =383, European-American =1027 | Methadone | Prospective | Usual daily methadone dose (self-reported) (mg) |
| Wang, 2018(22) | International Journal of Neuropsychophar | *GRK5* Is Associated with the Regulation of Methadone Dosage in Heroin | 344 | 81.68 % | 38.17 (7.69) | Han Chinese (Taiwan) | Methadone | Cross-sectional | Methadone dose (mg) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | macology | Dependence | | | | | | | |
| Yang, 2016(23) | PLOS Genetics | Genome-Wide Pharmacogenomic Study on Methadone Maintenance Treatment Identifies SNP rs17180299 and Multiple Haplotypes on *CYP2B6*, *SPON1*, and *GSG1L* Associated with Plasma Concentrations of Methadone R- and S-enantiomers in Heroin-Dependent Patients | 344 | 81.68 % | Males: 39.31 (7.66); Females: 33.03 (5.45) | Han Chinese (Taiwan) | Methadone | Prospective | Plasma concentrations of methadone and its metabolite EDDP R- and S-enantiomers (ng/ml/mg/dose) |

**Table 3. Quality Assessments and Reported Study Limitations and Conflicts**

| First Author Last Name, Year | Reported conflicts of interest | Reported study limitations | Q-Genie Score | Quality Assessment |
|---|---|---|---|---|
| Cox, 2020 | H.R.K. is a member of the American Society of Clinical Psychopharmacology's Alcohol Clinical Trials Initiative, which was supported for the last three years by AbbVie, Alkermes, Ethypharm, Indivior, Lilly, Lundbeck, Otsuka, Pfizer, Arbor, and Amygdala Neurosciences. Drs. Kranzler and Gelernter are named as inventors on PCT patent application #15/878,640 entitled: "Genotype-guided dosing of opioid agonists," filed January 24, 2018. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. | • Used cross-sectional data to study a phenotype that would require long-term follow-up to define cessation more accurately.<br>• Used a slightly different definition for cessation in the CATS dataset than in the Yale-Penn dataset.<br>• The opioid cessation GWAS sample had limited power to detect genome-wide significant association signals. | 63 | Good quality |
| Nelson, 2016 | Although unrelated to the current study, Dr Kranzler has been a consultant or advisory board member for Alkermes, Lilly, Lundbeck, Pfizer and Roche. He is also a member of the American Society of Clinical Psychopharmacology's Alcohol Clinical Trials Initiative, which is supported by Lilly, Lundbeck, Abbott and Pfizer. The remaining authors declare no conflict of interest. | • Small size of control group ($OU_{IP}$).<br>• A more detailed characterization of the opioid use in the $OU_{IP}$ group was not obtained. | 45 | Moderate quality |
| Smith, 2017 | Dr. Kranzler reports being a consultant, continuing medical education (CME) speaker, or advisory board member for Alkermes, Indivior, Lundbeck, and Otsuka, and a member of the American Society of Clinical Psychopharmacology's Alcohol Clinical Trials Initiative, which was supported in the last three years by AbbVie, Alkermes, Ethypharm, Indivior, Lilly, | • Small sample size compared to mega-GWASs with pooled data.<br>• Daily methadone dose was self-reported. | 47 | Good quality |

| | | | | |
|---|---|---|---|---|
| | Lundbeck, Otsuka, Pfizer, and XenoPort. | | | |
| Wang, 2018 | None | • No statistically significant GWAS findings that pass the threshold $p<3.2x10^{-6}$.<br>• Small sample size.<br>• Most subjects were male and 95% tested positive for HCV.<br>• Study was cross-sectional in design. | 44 | Good quality |
| Yang, 2016 | None | • Moderate sample size.<br>• Small replication sample - may not have detected significant associations (insufficient power). | 53 | Good quality |

**Table 4. Summary of SNP Outcome Associations**

| First Author Last Name, Year | SNP IDs | Chromosome:Position | Alleles | Minor Allele | Gene or Locus | MAF | N | Outcome associated with SNP | Measure of Association | Measure of Association value | Measure of Variability | Measure of Variability value | p-value | Ethnicity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cox, 2020(19) | NA | | | | | | | | | | | | | |
| Nelson, 2016(20) | rs1436171 | 1:224881828 | A/? | A | *CNIH3* | 0.44 | 1167 | opioid dependence end point (daily heroin injection) | OR | 0.54 | 95% CI | 0.42-0.68 | 6.26 E-07 | European |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | rs13698 46 | 1:22489409 5 | C/? | C | *CNIH 3* | 0.38 | 116 7 | opioid dependence end point (daily heroin injection) | OR | 0.52 | 95% CI | 0.41-0.66 | 9.42 E-08 | European |
| | rs14361 75 | 1:22490836 6 | T/? | T | *CNIH 3* | 0.37 | 116 7 | opioid dependence end point (daily heroin injection) | OR | 0.5 | 95% CI | 0.39-0.64 | 2.72 E-08 | European |
| Smith, 2017(21) | rs73568 641 | 6:15402513 9 | C/T | C | *OPR M1* | 0.1 | 383 | methadone dose | β | 0.680 8 | SE | 0.12 26 | 2.81 E-08 | African American |
| | rs74513 25 | 6:15401651 7 | C/T | C | *OPR M1* | 0.1 | 383 | methadone dose | β | 0.680 7 | SE | 0.12 26 | 2.83 E-08 | African American |
| | rs11155 9266 | 6:15399856 0 | A/G | A | *OPR M1* | 0.1 | 383 | methadone dose | β | 0.654 6 | SE | 0.12 52 | 1.72 E-07 | African American |
| | rs76499 485 | 6:15400436 4 | A/G | A | *OPR M1* | 0.1 | 383 | methadone dose | β | 0.648 7 | SE | 0.12 57 | 2.48 E-07 | African American |
| | rs75783 47 | 2:13121168 | T/C | T | *TRIB 2* | 0.43 | 383 | methadone dose | β | 0.392 6 | SE | 0.07 64 | 2.77 E-07 | African American |
| | rs75783 29 | 2:13121135 | T/C | T | *TRIB 2* | 0.43 | 383 | methadone dose | β | 0.392 4 | SE | 0.07 64 | 2.81 E-07 | African American |
| | rs13423 393 | 2:13120763 | T/C | T | *TRIB 2* | 0.43 | 383 | methadone dose | β | 0.392 2 | SE | 0.07 64 | 2.85 E-07 | African American |
| | rs67452 83 | 2:13120700 | A/T | A | *TRIB 2* | 0.43 | 383 | methadone dose | β | 0.392 3 | SE | 0.07 65 | 2.93 E-07 | African American |
| | rs73568 677 | 6:15404647 1 | T/C | T | *OPR M1* | 0.09 | 383 | methadone dose | β | 0.643 1 | SE | 0.12 61 | 3.42 E-07 | African American |
| | rs11677 7827 | 6:15408453 4 | T/C | T | *OPR M1* | 0.11 | 383 | methadone dose | β | 0.598 1 | SE | 0.11 76 | 3.64 E-07 | African American |
| | rs46698 99 | 2:13121465 | T/C | T | *TRIB 2* | 0.41 | 383 | methadone dose | β | 0.392 8 | SE | 0.07 79 | 4.62 E-07 | African American |
| | rs46699 00 | 2:13121525 | T/C | T | *TRIB 2* | 0.41 | 383 | methadone dose | β | 0.392 5 | SE | 0.07 8 | 4.87 E-07 | African American |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| rs4669901 | 2:13121591 | G/A | G | *TRIB2* | 0.41 | 383 | methadone dose | β | 0.393 | SE | 0.0781 | 4.88E-07 | African American |
| rs13397286 | 2:13120841 | A/G | A | *TRIB2* | 0.42 | 383 | methadone dose | β | 0.3926 | SE | 0.0781 | 5.01E-07 | African American |
| rs12664381 | 6:154054500 | T/C | T | *OPRM1* | 0.11 | 383 | methadone dose | β | 0.5873 | SE | 0.1171 | 5.26E-07 | African American |
| rs12527630 | 6:154064934 | G/A | G | *OPRM1* | 0.11 | 383 | methadone dose | β | 0.5868 | SE | 0.1172 | 5.50E-07 | African American |
| rs73570652 | 6:154070563 | T/C | T | *OPRM1* | 0.11 | 383 | methadone dose | β | 0.5867 | SE | 0.1172 | 5.52E-07 | African American |
| rs12663416 | 6:154057383 | T/C | T | *OPRM1* | 0.11 | 383 | methadone dose | β | 0.5856 | SE | 0.117 | 5.54E-07 | African American |
| rs12104412 | 19:36731058 | T/A | T | *ZNF146* | 0.15 | 383 | methadone dose | β | 0.4939 | SE | 0.099 | 6.00E-07 | African American |
| rs57072980 | 2:13122014 | T/C | T | *TRIB2* | 0.43 | 383 | methadone dose | β | 0.3823 | SE | 0.0772 | 7.36E-07 | African American |
| rs9360217 | 6:67338593 | G/T | G | *EYS* | 0.22 | 1027 | methadone dose | β | -0.2613 | SE | 0.0525 | 6.55E-07 | European American |
| rs9345875 | 6:67370087 | G/T | G | *EYS* | 0.21 | 1027 | methadone dose | β | -0.2602 | SE | 0.0524 | 6.95E-07 | European American |
| rs9342570 | 6:67368858 | A/T | A | *EYS* | 0.21 | 1027 | methadone dose | β | -0.2589 | SE | 0.0523 | 7.53E-07 | European American |
| rs9345867 | 6:67359694 | C/T | C | *EYS* | 0.21 | 1027 | methadone dose | β | -0.258 | SE | 0.0522 | 7.83E-07 | European American |
| rs2045196 | 6:67339443 | G/C | G | *EYS* | 0.21 | 1027 | methadone dose | β | -0.265 | SE | 0.0537 | 8.15E-07 | European American |
| rs1026388 | 6:67348220 | A/C | A | *EYS* | 0.21 | 1027 | methadone dose | β | -0.2576 | SE | 0.0523 | 8.55E-07 | European American |
| rs4142573 | 6:67388037 | T/C | T | *EYS* | 0.21 | 1027 | methadone dose | β | -0.2561 | SE | 0.052 | 8.57E-07 | European American |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | rs9363624 | 6:67387453 | C/T | C | *EYS* | 0.21 | 1027 | methadone dose | β | -0.2561 | SE | 0.052 | 8.57E-07 | European American |
| | rs9354462 | 6:67383719 | T/C | T | *EYS* | 0.21 | 1027 | methadone dose | β | -0.2565 | SE | 0.0521 | 8.64E-07 | European American |
| | rs9351587 | 6:67400119 | T/C | T | *EYS* | 0.21 | 1027 | methadone dose | β | -0.256 | SE | 0.052 | 8.65E-07 | European American |
| | rs4710324 | 6:67352212 | T/C | T | *EYS* | 0.21 | 1027 | methadone dose | β | -0.2574 | SE | 0.0523 | 8.72E-07 | European American |
| | rs9342572 | 6:67386966 | T/C | T | *EYS* | 0.21 | 1027 | methadone dose | β | -0.2574 | SE | 0.0523 | 8.72E-07 | European American |
| | rs4710621 | 6:67389232 | G/A | G | *EYS* | 0.21 | 1027 | methadone dose | β | -0.2559 | SE | 0.052 | 8.74E-07 | European American |
| | rs2124198 | 6:67366749 | C/T | C | *EYS* | 0.21 | 1027 | methadone dose | β | -0.2567 | SE | 0.0522 | 8.89E-07 | European American |
| | rs9345880 | 6:67391212 | C/T | C | *EYS* | 0.21 | 1027 | methadone dose | β | -0.2556 | SE | 0.052 | 9.00E-07 | European American |
| | rs9360224 | 6:67397651 | T/C | T | *EYS* | 0.21 | 1027 | methadone dose | β | -0.2553 | SE | 0.052 | 9.26E-07 | European American |
| | rs2124199 | 6:67391889 | A/T | A | *EYS* | 0.21 | 1027 | methadone dose | β | -0.2539 | SE | 0.0518 | 9.65E-07 | European American |
| Wang, 2018(22) | NA | | | | | | | | | | | | | |
| Yang, 2016(23) | rs17180299 | 9:NA | A/G | G | intergenic | 0.09 | 344 | plasma concentration of R-methadone | β | NA | | | 2.24E-08 | Han Chinese |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | AX-165344 52 | 11:NA | NA | *SPO N1* | NA | 344 | plasma concentration of S-methadone | β | NA | 4.83 E-07 | Han Chinese |
| | rs14483 32 | 3:NA | NA | interg enic | NA | 344 | plasma concentration of R-EDDP | β | NA | 8.18 E-07 | Han Chinese |

# 4 CHAPTER 4

## 4.1 Introduction

This chapter consists of an unpublished primary candidate gene study "Implications of *OPRM1* and *CYP2B6* variants on treatment outcomes in MMT patients in Ontario: Exploring sex differences". This study looks at a possible genetic basis for MMT outcomes in two specific genes, *OPRM1* and *CYP2B6*, through the examination of pre-selected SNPs. Following Chapter 3's systematic review, the *OPRM1* SNPs identified in an African American sample subset were found to be of most interest due to their biological relevance. Smith et al., as observed in the review, were also the first to report associations involving these SNPs. In what could be considered a replicative effort, we try to examine two of their most GWAS-significant SNPs (rs73568641 and rs7451325) in our candidate gene study, within a larger European cohort (n=1226). *OPRM1* SNPs studied were also supplemented by two from the literature that were shown to be biologically relevant as well but did not reach a clear consensus. In a more exploratory effort, select *CYP2B6* SNPs from the literature were studied separately to identify any associations that were significant and relevant to our outcomes of interest. Focusing on this gene for its known involvement in drug metabolism supplemented the gaps that would have existed if the focus were solely on the *OPRM1*, a neurobiologically-involved gene. Finally, looking at these findings from a sex-stratified lens challenges the existing models of all-sex or only-male analyses and includes females as a separate variable, with the same applied to males. That is especially made possible with the large, yet comparable sample sizes collected for each sex ($n_{Male}$=699, $n_{Female}$=527). As the first genetic study in this field showcasing how sex plays a role in genetic association studies and their findings, this study pushes researchers to consider sex within their analyses, given its proven involvement in opioid use disorder, opioid use patterns, and treatment outcomes.

## 4.2 Implications of *OPRM1* and *CYP2B6* variants on treatment outcomes in MMT patients in Ontario: Exploring sex differences

**Authors**
Caroul Chawar[1], Alannah Hillmer[2], Amel Lamri[3], Flavio Kapczinski[4], Lehana Thabane[5], Guillaume Pare[6], Zainab Samaan[7]*

**Affiliations**

1. Neuroscience Graduate Program, McMaster University, Hamilton, ON, Canada; Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; chawarc@mcmaster.ca
2. Neuroscience Graduate Program, McMaster University, Hamilton, ON, Canada; Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; hillmea@mcmaster.ca
3. Population Health Research Institute, Hamilton, ON, Canada; Department of Health Research Method, Evidence, and Impact, McMaster University, Hamilton, ON, Canada; Department of Medicine, McMaster University, Hamilton, ON, Canada; lamria@mcmaster.ca
4. Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; kapczinf@mcmaster.ca
5. Department of Health Research Method, Evidence, and Impact, McMaster University, Hamilton, ON, Canada; Population Health Research Institute, Hamilton, ON, Canada; Father Sean O'Sullivan Research Centre, St. Joseph's Healthcare Hamilton; thabanl@mcmaster.ca
6. Population Health Research Institute, Hamilton, ON, Canada; Department of Health Research Method, Evidence, and Impact, McMaster University, Hamilton, ON, Canada; pareg@mcmaster.ca
7. Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada; samaanz@mcmaster.ca

*Corresponding Author:
Dr. Zainab Samaan
100 West 5th St., Hamilton, ON L8N3K7, Canada
Telephone: 905-522-1155 ext. 35448
Fax: 905-381-5629

### 4.2.1 ABSTRACT

**Background:** Patient response to treatment in opioid use disorder has been seen to have a genetic contribution to its occurrence. Genetic variants in opioid

receptor *OPRM1* and drug and methadone metabolizer *CYP2B6* have been linked to negative treatment outcomes in patients treated with methadone of different ethnicities, with little consensus on their effect. Also, little to no research has been done on how sex differences play a role in these associations.

**Objectives:** This study aims to test the associations between SNPs of *OPRM1* (rs73568641, rs7451325, rs10485058, rs1799971) and *CYP2B6* (rs2279343, rs10403955, rs8192719, rs3745274) and outcomes of continued opioid use, relapse, and methadone dose. It also aims to test these associations within the sexes to observe any existing differences.

**Methods:** Patients treated with methadone, n=1226 ($n_{Male}$=699, $n_{Female}$=527), were included in this study, genotyped SNPs were quality checked, and imputed data were used to run regression analyses. Logistic regressions were conducted to assess the association between the SNPs and continued opioid use and relapse, while a linear regression was conducted for the outcome of methadone dose. Covariates adjusted for included age, sex, methadone dose, duration on MMT, weight, and population stratification, as appropriate to the regression. All analyses were re-run and stratified by sex, as well as tested for between-sex differences through an interaction term.

**Results:** SNPs rs73568641 and rs7451325 from *OPRM1* and all the tested *CYP2B6* SNPs were detected to be in high linkage disequilibrium. No SNP-outcome associations reached the Bonferroni-corrected significance levels. Though, rs73568641 was observed to be more significant in females (continued opioid use: OR=0.7062, 95%CI=0.4678,1.066, P=0.09776; methadone dose: β=-7.988, SE=3.727, P=0.03258), and rs3745274 in males (continued opioid use: OR=0.7263, 95%CI=0.5203, 1.014, P=0.06024), suggesting the contribution of sex in the outcomes of methadone dose and continued opioid use. However, no significant differences were observed between the sexes.

**Conclusion:** The genetic contribution of *OPRM1* and *CYP2B6* variants to continued opioid use, relapse, and methadone dose of MMT patients was not observed. A research gap has been identified, and a focus towards ancestry and sex-based analyses is recommended.

**Keywords:** genetic variant, methadone, opioid, SNP, pharmacogenetic, treatment outcome, *OPRM1*, *CYP2B6*

### 4.2.2 INTRODUCTION

**Background/Rationale**

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 opioid use disorder, 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 the methadone blood concentration for a given dose, as well as metabolism(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).

Two genes of interest within this field have been the mu-opioid receptor (*OPRM1*) and the cytochrome P450 2B6 (*CYP2B6*) genes. The *OPRM1*-encoded opioid receptor proteins bind endogenous and exogenous opioids, resulting in pain relief and feelings of euphoria(13). Single nucleotide polymorphisms (SNPs) in this gene can cause a change in 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 *CYP2B6*-encoded enzymes are involved in metabolizing 2 to 10% of clinically administered drugs, including methadone(17). SNPs in this gene can lead to no observable change, loss of function, or gain of function, 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).

Though much of this conducted research is beginning to be more ethnically diverse, studying samples of African American, East Asian, and Iranian descent in addition to European, leaps in sex-based analyses have not been observed. 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). Despite all this evidence, no sex-based analyses have been conducted with respect to the genetic predisposition to MMT outcomes.

Studying select *OPRM1* and *CYP2B6* SNPs in a European sample would allow us to not only confirm the results 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 been overlooked in past studies.

#### 4.2.2.1 Objectives

This study aims to report some new genetic associations that have not been tested previously, as well as replicate findings from the literature within a larger sample of European descent. The objectives of this study are to:

1. Determine if there is an 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 through an additive candidate gene model;
2. Determine if there are differences in associations within the sexes through sex stratification;
3. Determine through exploratory analysis if there are differences in associations between the sexes; and
4. Compare our findings to those previously reported in the literature.

### 4.2.3 METHODS

This candidate gene study is reported according to Strengthening the Reporting of Genetic Association studies (STREGA) guideline, an extension of Strengthening the Reporting of Observational studies in Epidemiology (STROBE) statement(26). An accompanying STREGA checklist could be found in Supplementary File 1.

#### 4.2.3.1 Study Design and Setting

This research reports data collected by the Genetics of Opioid Addiction (GENOA) study, which is an observational cohort study of 1536 participants recruited from Canadian Addiction Treatment Centres (CATCs) across

Ontario(27). Data collected at the baseline (enrollment in the study), 3 months prior to study enrollment, and up to a 12-month follow up 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. The GENOA study was approved by the Hamilton Integrated Research Ethics Board (#11056). All the participants enrolled in the study provided written informed consent.

#### 4.2.3.2 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 DSM-5 OUD diagnosis, undergo an opioid substitution or antagonist therapy (OSAT) 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 OSAT.

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. Additionally, any 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.

#### 4.2.3.3 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 principal components accounting for population stratification within a group of the same ancestry. 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 GWASs as those related to *OPRM1* or *CYP2B6* and associated with altered methadone metabolism, methadone plasma concentrations, methadone dose, opioid use, or other treatment outcomes. The SNP details could be seen in Table 1.

#### 4.2.3.4 Data Sources/Measurement

Blood samples collected were shipped to the Genome Quebec Innovation Centre in Montreal for DNA extraction and genotyping(28). The data were genotyped using Infinium technology on the Illumina Global Screening Array-24 v1.0 panel. GWAS quality control checks for all samples and SNPs were applied using PLINK v1.09 and the RStudio interface for R i386 3.5.1(29–31). Only data from samples of European descent (n=1226) were then submitted to Michigan Imputation Server for imputation, as none of the samples of other ethnicities were large enough to provide powered ethnically stratified analysis at later stages(32). EAGLE2 and Minimac4 with the HRC reference panel were used for phasing and SNP imputation, respectively(33,34). Post-imputation filtering was conducted, excluding SNPs with Rsq quality metrics of less than 0.3 and minor allele frequencies lower than 0.05, resulting in 5,563,682 variants. See Supplementary file 2 for a detailed description of the steps taken.

#### 4.2.3.5 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(35). 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.

#### 4.2.3.6 Study Size

A sample of 1226 participants was used for this study. See Figure 1 for a flowchart outlining the steps conducted to reach this final sample size, and the sample sizes specific for each analysis.

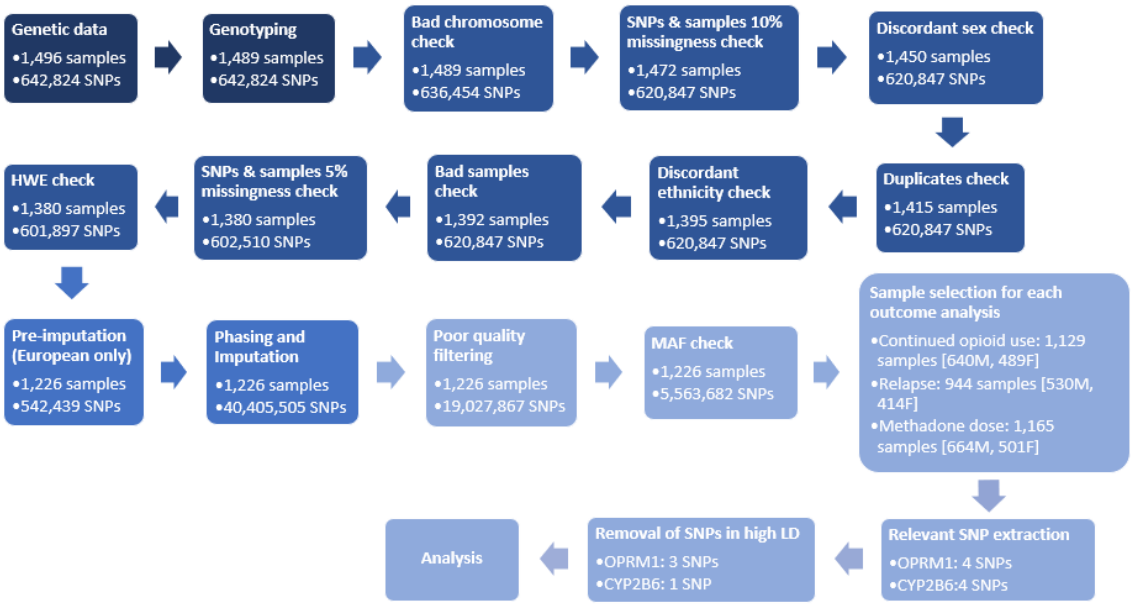



**Figure 1. Flowchart of sample and SNP count changes throughout genotyping, quality control checks, and imputation.** HWE refers to Hardy-Weinberg equilibrium, MAF to minor allele frequency, M to the male sample, F to the female sample, and LD to linkage disequilibrium.

#### 4.2.3.7 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.

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. Logistic regressions were conducted to measure associations of continued opioid use and relapse, while a linear regression model was used for methadone dose. All covariates were adjusted for by using additive models, testing for 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-Sex as the interaction term in the regression models.

For the logistic regression analyses, missing values for the covariates of methadone dose and duration on MMT were imputed 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. Samples with missing outcome values were excluded from the analysis.

SNPs reported in high linkage disequilibrium (LD) were pruned, keeping the SNP with the most reported clinical significance and published associations, as seen on NCBI's SNP database(36). As such, rs7451325, rs2279343, rs10403955, and rs8192719 were excluded from analysis. After accounting for LD, 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(29,30). HaploView was used to visualize SNPs in LD and calculate r-squared coefficients(37).

### 4.2.4 RESULTS

#### 4.2.4.1 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 (Figure 1).

#### 4.2.4.2 Descriptive data

Participant demographics and clinical characteristics can be seen in Table 2. Of the 1226 ancestrally 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 did not differ substantially within 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 higher than that of females (0.37).

#### 4.2.4.3 Main results

Results of the sex-stratified association analyses between the *OPRM1* SNPs (rs73568641, rs1799971, rs10485058) and continued opioid use, relapse, and methadone dose can be observed in Table 3. No associations reached the Bonferroni adjusted significance threshold. However, higher significance levels were observed for some associations within females than within males, notably regarding rs73568641. Allele C showed decreased odds of continued opioid use within females [OR=0.7062, 95%CI=0.4678,1.066, P=0.09776] than within males [OR=0.987, 95%CI=0.6713, 1.451, P=0.9469]. Its presence also signified a more pronounced decrease in methadone dose in females [β=-7.988, SE=3.727, P=0.03258] than in males [β=-2.359, SE=3.332, P=0.4792].

Results of the sex-stratified association analyses between the *CYP2B6* SNP rs3745274 and continued opioid use, relapse, and methadone dose can be observed in Table 4. No associations were significant. Nonetheless, a higher significance level was observed in the association between the T allele of rs3745274 and continued opioid use within males [OR=0.7263, 95%CI=0.5203, 1.014, P=0.06024] than females [OR=0.9544, 95%CI=0.6644, 1.371, P=0.8004].

Exploratory analyses showcasing differences in associations between males and females were conducted. No significant results are reported. For detailed results see Supplementary File 2.

### 4.2.5 DISCUSSION

#### 4.2.5.1 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 variant of rs73568641 showed higher significance levels and stronger protective properties towards continued opioid use and relapse than males. However, rs3745274 with variant T in males was shown to be more protective and significant when it came to continued opioid use.

#### 4.2.5.2 Interpretation

The possible involvement of 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(38). 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. rs73568641 (variant C) seems to have an opposite effect in an African American population(39). In a genome-wide association study subset (n=383), it was found to be associated with slightly increased daily methadone dose [β=0.6808, SE=0.1226, 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 ancestral contribution to the genetic makeup. This highlights the importance of ancestrally diverse research and how interindividual differences of patients of different ethnic backgrounds could play a role in patient treatment outcomes.

rs3745274 T variant's possible lowering effect on continued opioid use in MMT patients could be explained as a decrease in the *CYP2B6* gene activity, which could increase plasma methadone concentrations and subside the need for additional opioid intake. The risk variant of this SNP, as well as those of other SNPs in LD (rs2279343 and rs10403955), have been reported in a candidate gene study (n=366) to be associated with high S-methadone plasma concentrations and lower S-methadone clearance, supporting this hypothesis in a Taiwanese sample(19). When comparing rs3745274 to literature findings on other treatment outcomes, the T variant seems to be associated with an increased frequency in methadone fatalities in a sample of European ancestry (n=125)(21). The frequency of the T variant in rs3745274 was found to be more specifically enhanced in the methadone-only group [MAF=31.9%] compared to the control group with non-substance use related fatalities [MAF=22.4%]. Though this association with a negative outcome opposes the direction of our findings, this could support the previous hypothesis in suggesting that a higher plasma concentration of methadone could also have negative effects and risks, such as death.

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 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(40,41). 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(22,42). 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.

#### 4.2.5.3 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(43). 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 indicative of an under-powered study.

However, 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.

### 4.2.6 FUNDING

This work was partially supported by CIHR (grant number PJT-156306), which played no role in the study design, analysis, reporting, or publication of the results.

### 4.2.7 AUTHOR CONTRIBUTIONS

ZS is the guarantor. CC and ZS conceptualized the genetic study. CC implemented the design, quality control steps, and methodology with the aid of

AH and AL. CC prepared the first draft. LT, GP, FK, and ZS revised the study design, data analysis, and data interpretation.

### 4.2.8 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
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. 1998;
5. Eap CB, Buclin T, Baumann P. Interindividual variability of the clinical pharmacokinetics of methadone: Implications for the treatment of opioid dependence. Clinical Pharmacokinetics. 2002.
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 of Systematic Reviews. 2013.
7. Joe GW, Simpson DD, Sells SB. Treatment process and relapse to opioid use during methadone maintenance. Am J Drug Alcohol Abuse. 1994;
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
10. Li Y, Kantelip J-P, Gerritsen-Van Schieveen P, Davani S. Interindividual Variability of Methadone Response Impact of Genetic Polymorphism. Vol. 12, Mol Diag Ther. 2008.
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
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 [Internet]. Vol. 9, PLoS ONE. Public Library of Science;

2014 [cited 2020 Jul 7]. Available from: /pmc/articles/PMC3906028/?report=abstract
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/PMC3799236/?report=abstract
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 Jul 8 [cited 2020 Aug 25];10(2):156–67. Available from: /pmc/articles/PMC4155832/?report=abstract
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
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
18. Ahmad T, Valentovic 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
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 Psychopharmacol [Internet]. 2011 Aug [cited 2020 Jul 7];31(4):463–9. Available from: http://journals.lww.com/00004714-201108000-00010
20. Levran 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.
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
22. Bawor M, Dennis BB, Varenbut M, Daiter 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. 2015;

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 reviewand 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, Bocklandt S, Vilain E. The genetics of sex differences in brain and behavior. Vol. 32, Frontiers in Neuroendocrinology. Academic Press; 2011. p. 227–46.
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
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 Medicine. 2009.
27. Samaan Z, Bawor M, Dennis BB, Plater C, Varenbut M, Daiter J, et al. Genetic influence on methadone treatment outcomes in patients undergoing methadone maintenance treatment for opioid addiction: A pilot study. Neuropsychiatr Dis Treat. 2014;
28. Centre d'expertise et de services Génome Québec [Internet]. [cited 2020 Aug 25]. Available from: https://cesgq.com/
29. 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. 2007;
30. Allaire JJ. RStudio: Integrated development environment for R. J Wildl Manage. 2015;
31. R Development Core Team R. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. 2011.
32. Das S, Forer L, Schönherr S, Sidore C, Locke AE, Kwong A, et al. Next-generation genotype imputation service and methods. Nat Genet. 2016;
33. 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/PMC5096458/?report=abstract
34. 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/sayantan_1.pdf?sequence=1
35. Paunonen S V. 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-nih-gov.libaccess.lib.mcmaster.ca/25728192/
36. Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM, et al. DbSNP: The NCBI database of genetic variation. Nucleic Acids Res. 2001;

37. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: Analysis and visualization of LD and haplotype maps. Bioinformatics. 2005;
38. 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. 2002;
39. 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
40. Bobzean SAM, DeNobrega AK, Perrotti LI. Sex differences in the neurobiology of drug addiction. Experimental Neurology. 2014.
41. 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
42. Injecting drug use: Gendered risk. Int J Drug Policy. 2018 Jun 1;56:81–91.
43. 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. 2019;

### 4.2.9 Tables

**Table 1. Selected SNP details and genotype counts.**

| Gene | Chr:Position | SNP/Genotype | Genotype count | MAF | HWE p-value |
|---|---|---|---|---|---|
| *OPRM1* | 6:154025139 | rs73568641 | | 0.1525 | 0.1513 |
| | | CC | 35 | | |
| | | CT | 304 | | |
| | | TT | 887 | | |
| *OPRM1* | 6:154016517 | rs7451325 | | 0.1521 | 0.1494 |
| | | CC | 35 | | |
| | | CT | 303 | | |
| | | TT | 888 | | |
| *OPRM1* | 6:154445215 | rs10485058 | | 0.1317 | 0.9005 |
| | | GG | 20 | | |
| | | GA | 283 | | |
| | | AA | 923 | | |
| *OPRM1* | 6:154360797 | rs1799971 | | 0.1097 | 0.6622 |
| | | GG | 16 | | |
| | | GA | 237 | | |
| | | AA | 973 | | |
| *CYP2B6* | 19:41515263 | rs2279343 | | 0.2463 | 0.2817 |
| | | GG | 67 | | |
| | | GA | 470 | | |
| | | AA | 689 | | |
| *CYP2B6* | 19:41509438 | rs10403955 | | 0.2594 | 0.9408 |
| | | GG | 83 | | |
| | | GT | 470 | | |
| | | TT | 673 | | |
| *CYP2B6* | 19:41518773 | rs8192719 | | 0.2398 | 0.4334 |
| | | TT | 65 | | |
| | | TC | 458 | | |
| | | CC | 703 | | |
| *CYP2B6* | 19:41512841 | rs3745274 | | 0.2337 | 0.5769 |
| | | TT | 63 | | |
| | | TG | 447 | | |
| | | GG | 716 | | |

Sample is of European ancestry (N=1226). Build is GRCh37.
MAF = minor allele frequency. HWE = Hardy-Weinberg equilibrium.

**Table 2. Participant demographics.**

| | Total | Male | Female |
|---|---|---|---|
| **N (%)** | 1226 | 699 (57) | 527 (43) |
| **Age in years[a], Mean (SD)** | 38.5 (11.12) | 39.22 (11.44) | 37.55 (10.63) |
| **Weight in kg[b], Mean (SD)** | 80.11 (20.95) | 86.15 (20.38) | 72.12 (18.93) |
| **Marital status[c], N (%)** | | | |
| 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[d], N (%)** | | | |
| Employed | 430 (35) | 287 (41) | 143 (27) |
| Unemployed | 793 (65) | 411 (59) | 382 (73) |
| **Methadone dose in mg[e], Mean (SD)** | 74.75 (45.49) | 77.91 (47.16) | 70.57 (42.86) |
| **MAT[f], N (%)** | | | |
| Methadone | 1172 (96) | 666 (96) | 506 (96) |
| Suboxone | 52 (4) | 31 (4) | 21 (4) |
| **Duration on MMT in months[g], Mean (SD)** | 44.88 (48.27) | 45.29 (47.80) | 44.35 (48.92) |
| **Age of first opioid use[h], Mean (SD)** | 25.09 (9.20) | 25.11 (9.43) | 25.06 (8.88) |
| **Opioid prescription[i], N (%)** | | | |
| Prescribed opioids | 34 (3) | 20 (3) | 14 (3) |
| Not prescribed opioids | 1192 (97) | 679 (97) | 513 (97) |
| **Total number of opioid screens[j]*, Mean (SD)** | 74.34 (34.64) | 73.99 (34.01) | 74.80 (35.49) |
| **Total number of positive opioid screens[k], Mean (SD)** | 14.58 (22.25) | 14.58 (21.73) | 14.58 (22.93) |

*260 of reported total included participants screened only for opiates.
All means were calculated excluding missing values.
[a]Data available for $n_{Total}$=1226, $n_{Male}$=699, $n_{Female}$=527
[b]Data available for $n_{Total}$=1216, $n_{Male}$=693, $n_{Female}$=523
[c]Data available for $n_{Total}$=1224, $n_{Male}$=697, $n_{Female}$=527
[d]Data available for $n_{Total}$=1223, $n_{Male}$=698, $n_{Female}$=525
[e]Data available for $n_{Total}$=1166, $n_{Male}$=664, $n_{Female}$=502
[f]Data available for $n_{Total}$=1224, $n_{Male}$=697, $n_{Female}$=527
[g]Data available for $n_{Total}$=1162, $n_{Male}$=661, $n_{Female}$=501
[h]Data available for $n_{Total}$=1197, $n_{Male}$=685, $n_{Female}$=512
[i]Data available for $n_{Total}$=1226, $n_{Male}$=699, $n_{Female}$=527

[j]Data available for $n_{Total}$=1223, $n_{Male}$=696, $n_{Female}$=527
[k]Data available for $n_{Total}$=1218, $n_{Male}$=692, $n_{Female}$=526

**Table 3. *OPRM1* SNPs and associated outcomes.**

| Outcome | SNP | N | Minor Allele | OR/BETA | 95% CI/SE | P |
|---|---|---|---|---|---|---|
| **Continued opioid use** | **rs73568641** | 1129 | C | 0.8354 | 0.6326, 1.103 | 0.2049 |
| | *Male* | 640 | | 0.987 | 0.6713, 1.451 | 0.9469 |
| | *Female* | 489 | | 0.7062 | 0.4678, 1.066 | 0.09776* |
| | **rs1799971** | 1129 | G | 0.9737 | 0.6989, 1.357 | 0.875 |
| | *Male* | 640 | | 1.11 | 0.7165, 1.721 | 0.6394 |
| | *Female* | 489 | | 0.8701 | 0.5113, 1.481 | 0.6079 |
| | **rs10485058** | 1129 | G | 0.9569 | 0.7055, 1.298 | 0.777 |
| | *Male* | 640 | | 0.8938 | 0.5858, 1.364 | 0.6026 |
| | *Female* | 489 | | 1.002 | 0.6418, 1.565 | 0.9922 |
| **Relapse** | **rs73568641** | 944 | C | 0.9753 | 0.7591, 1.253 | 0.8447 |
| | *Male* | 530 | | 0.9659 | 0.6944, 1.343 | 0.8366 |
| | *Female* | 414 | | 1.036 | 0.697, 1.54 | 0.8614 |
| | **rs1799971** | 944 | G | 0.8151 | 0.6106, 1.088 | 0.1652 |
| | *Male* | 530 | | 0.7551 | 0.5211, 1.094 | 0.1376 |
| | *Female* | 414 | | 0.9404 | 0.5807, 1.523 | 0.8026 |
| | **rs10485058** | 944 | G | 1.097 | 0.8338, 1.443 | 0.5089 |
| | *Male* | 530 | | 1.021 | 0.6984, 1.493 | 0.9135 |
| | *Female* | 414 | | 1.151 | 0.7677, 1.725 | 0.4965 |
| **Methadone dose** | **rs73568641** | 1165 | C | -4.236 | 2.487 | 0.08876* |
| | *Male* | 664 | | -2.359 | 3.332 | 0.4792 |
| | *Female* | 501 | | -7.988 | 3.727 | 0.03258** |
| | **rs1799971** | 1165 | G | 0.2018 | 2.902 | 0.9446 |
| | *Male* | 664 | | 2.587 | 3.759 | 0.4915 |
| | *Female* | 501 | | -4.918 | 4.627 | 0.2884 |
| | **rs10485058** | 1165 | G | -0.4485 | 2.715 | 0.8688 |
| | *Male* | 664 | | -0.4994 | 3.694 | 0.8925 |
| | *Female* | 501 | | 0.2362 | 4.003 | 0.953 |

The minor alleles are also the reference and tested alleles. OR is odds ratio and BETA is the beta coefficient for the additive 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

**Table 4. *CYP2B6* SNPs and associated outcomes.**

| Outcome | SNP | N | Minor Allele | OR/BETA | 95% CI/SE | P |
|---|---|---|---|---|---|---|
| **Continued opioid use** | **rs3745274** | 1129 | T | 0.8186 | 0.6413, 1.045 | 0.1081 |
| | *Male* | 640 | | 0.7263 | 0.5203, 1.014 | 0.06024* |
| | *Female* | 489 | | 0.9544 | 0.6644, 1.371 | 0.8004 |
| **Relapse** | **rs3745274** | 944 | T | 0.9137 | 0.7337, 1.138 | 0.42 |
| | *Male* | 530 | | 0.8589 | 0.6382, 1.156 | 0.3156 |
| | *Female* | 414 | | 1.066 | 0.7614, 1.492 | 0.7101 |
| **Methadone dose** | **rs3745274** | 1165 | T | 1.257 | 2.172 | 0.5628 |
| | *Male* | 664 | | -1.169 | 2.987 | 0.6957 |
| | *Female* | 501 | | 4.19 | 3.177 | 0.1878 |

The minor alleles are also the reference and tested alleles. OR is odds ratio and BETA is the beta coefficient for the additive 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 $P<0.05$.
*$P<0.1$

# 5 CHAPTER 5: Conclusion

## 5.1 Overview

Each chapter presented in this thesis addresses the overall aims of robustly reviewing the literature and highlighting a genetic basis for MMT outcomes in the opioid use population. Methodologically planning a systematic review and meta-analysis of genetic studies (Chapter 2) and implementing it, while assessing the quality of the findings (Chapter 3), yielded an effective summary of genome-wide significant variants associated with outcomes of daily heroin injection, methadone dose, and plasma concentrations of methadone and methadone metabolite. The systematic review not only emphasizes potentially relevant SNPs in *CNIH3*, *OPRM1*, *TRIB2*, *ZNF146*, *EYS*, *SPON1*, and chromosomes 3 and 9 intergenic regions, but also highlighted the presence of significant associations within different ethnic groups. The findings of the systematic review were able to inform the SNPs and outcomes to-be-tested in the European sample of the candidate gene study (Chapter 4). SNPs of *OPRM1* and *CYP2B6* genes were selected for their biological relevance and recognition within genetic studies of opioid treatment outcomes. It was observed that none of the studies reviewed in Chapter 3 considered sex-based differences. Thus, along with a hypothesis pointing towards the involvement of sex in observing differing outcomes in MMT patients, the genetic study presented conducted sex-stratified and between-sex analyses in addition to total-sample analyses. None of the associations with the outcomes of continued opioid use, relapse, or methadone dose reached the significance threshold set. However, associations of higher significance were observed in females than males with respect to the *OPRM1* SNP rs73568641 and methadone dose and continued opioid use, despite the marginally smaller female sample size. *CYP2B6* SNP rs3745274 showed an opposite pattern, where the relationship was stronger in males than in females. Finally, there were no significant findings in the SNP-Sex interaction models conducted.

## 5.2 Overall Implications

The evidence provided in this thesis significantly contributes to the field of MAT pharmacogenetics. The systematic review protocol and manuscript were the first to comprehensively summarize GWAS findings of treatment response in an opioid-using population, while upholding complete transparency in reporting. The genetic study presented was also one of the first to introduce sex differences to the analysis of MAT pharmacogenetics and analyze same-sex groups independently.

This research aims to disseminate information to fellow researchers, addiction clinicians, and policymakers via a number of ways. Through the

systematic review findings, it informs researchers in this field of what has been done, as well identifies the genes and SNPs that might be of significance to MAT outcomes and might show potential for future focus. The protocol and review, in a combined effort, also promote transparency and high-quality research. They not only aim to be exemplary within their reporting and the proper guidelines followed, but in identifying areas of reduced research quality in reviewed studies, are able to highlight potential limitations that future researchers might face following suit.

By conducting research on a vulnerable population, this thesis is able to provide more information for opioid addiction clinicians on how to improve treatments for patients, especially given the current opioid crisis and the increased mortality risk the OUD population is consequently facing. Additionally, in informing policymakers of the genetic predisposition that might play a role in how patients respond to treatment, they are able to take more evidence-based actions when it comes to MAT administration policies and individual-based treatments. This evidence, with the support of further research, can become a tool to aid in the advancement of preventative medicine in a population that highly relies on it.

### 5.3 Future Directions

Given the observation of genome-wide analyses done on relatively small sample sizes and the lack of significant results in the genetic study reported, a recommendation is made for larger and more powered genetic-association studies within the OUD population. A natural subsequent step would be to conduct an exploratory GWAS to identify any novel SNPs associated with MAT outcomes. Due to the inability to quantitatively meta-analyze the results, a replication of published findings is advised. Doing so within and across ancestries will also allow for the validity of the findings to be verified, in addition to testing their generalizability.

In opioid addiction research, it is recommended to apply a stronger focus on sex and gender, especially in the pharmacogenetic field, where that is currently lacking. Though the presented research was able to highlight sex-based differences through sex-stratification and tried to assess whether a difference between the sexes was present, the insignificant results urge future more powered studies to introduce sex as an interaction term in analyses to clarify the role of sex as a measure in OUD treatment outcomes.

## 6 Reference List


1. Public Health Agency of Canada. Opioid-related harms in Canada [Internet]. Public Health Agency of Canada. 2020 [cited 2020 Aug 31]. Available from: https://health-infobase.canada.ca/substance-related-harms/opioids/graphs?index=441
2. Canadian Institute for Health Information. Opioid-Related Harms in Canada, December 2018 [Internet]. 2018 [cited 2020 Sep 8].
3. Public Health Ontario. Interactive Opioid Tool | Opioid-related morbidity and mortality in Ontario [Internet]. 2020 [cited 2020 Sep 2]. Available from: https://www.publichealthontario.ca/en/data-and-analysis/substance-use/interactive-opioid-tool#/ageSex
4. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 5th Edition. Arlington, VA; 2013.
5. Hill KP, Rice LS, Connery HS, Weiss RD. Diagnosing and treating opioid dependence | MDedge Family Medicine. J Fam Pract [Internet]. 2012 [cited 2020 Sep 2];61(10):588–97. Available from: https://www.mdedge.com/familymedicine/article/64873/addiction-medicine/diagnosing-and-treating-opioid-dependence
6. Nosyk B, Marsh DC, Sun H, Schechter MT, Anis AH. Trends in methadone maintenance treatment participation, retention, and compliance to dosing guidelines in British Columbia, Canada: 1996-2006. J Subst Abuse Treat. 2010;
7. Sordo L, Barrio G, Bravo MJ, Indave BI, Degenhardt L, Wiessing L, et al. Mortality risk during and after opioid substitution treatment: systematic review and meta-analysis of cohort studies. BMJ. 2017;
8. 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 Jul 8 [cited 2020 Aug 25];10(2):156–67. Available from: /pmc/articles/PMC4155832/?report=abstract
9. Crist RC, Reiner BC, Berrettini WH. A review of opioid addiction genetics. Current Opinion in Psychology. 2019.
10. 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 [Internet]. Vol. 9, PLoS ONE. Public Library of Science; 2014 [cited 2020 Jul 7]. Available from: /pmc/articles/PMC3906028/?report=abstract
11. Zhou H, Rentsch CT, Cheng Z, Kember RL, Nunez YZ, Tate JP, et al. GWAS including 82,707 subjects identifies functional coding variant in OPRM1 gene associated with opioid use disorder. medRxiv [Internet]. 2019 Sep 18 [cited 2020 Jul 6];19007039. Available from: http://medrxiv.org/content/early/2019/09/18/19007039.abstract
12. Cox JW, Sherva RM, Lunetta KL, Johnson EC, Martin NG, Degenhardt L,

et al. Genome-Wide Association Study of Opioid Cessation. J Clin Med. 2020;

13. 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

# 7 Appendix

## 7.1 CHAPTER 2 Additional File 1



### PRISMA-P 2015 Checklist

This checklist has been adapted for use with systematic review protocol submissions to BioMed Central journals from Table 3 in Moher D et al: Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews* 2015 4:1

An Editorial from the Editors-in-Chief of *Systematic Reviews* details why this checklist was adapted - Moher D, Stewart L & Shekelle P: Implementing PRISMA-P: recommendations for prospective authors. *Systematic Reviews* 2016 5:15

| Section/topic | # | Checklist item | Information reported | | Line number(s) |
|---|---|---|---|---|---|
| | | | Yes | No | |
| **ADMINISTRATIVE INFORMATION** | | | | | |
| Title | | | | | |
| Identification | 1a | Identify the report as a protocol of a systematic review | ☒ | ☐ | 1-3 |
| Update | 1b | If the protocol is for an update of a previous systematic review, identify as such | ☐ | ☒ | |
| Registration | 2 | If registered, provide the name of the registry (e.g., PROSPERO) and registration number in the Abstract | ☒ | ☐ | 84-86 |
| Authors | | | | | |
| Contact | 3a | Provide name, institutional affiliation, and e-mail address of all protocol authors; provide physical mailing address of corresponding author | ☒ | ☐ | 5-48 |
| Contributions | 3b | Describe contributions of protocol authors and identify the guarantor of the review | ☒ | ☐ | 322-326 |
| Amendments | 4 | If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments | ☒ | ☐ | 331-333 |
| Support | | | | | |
| Sources | 5a | Indicate sources of financial or other support for the review | ☒ | ☐ | 318-320 |
| Sponsor | 5b | Provide name for the review funder and/or sponsor | ☐ | ☒ | |
| Role of sponsor/funder | 5c | Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol | ☐ | ☒ | |
| **INTRODUCTION** | | | | | |
| Rationale | 6 | Describe the rationale for the review in the context of what is already known | ☒ | ☐ | 94-121 |
| Objectives | 7 | Provide an explicit statement of the question(s) the review will address with reference to | ☒ | ☐ | 122-131 |

BioMed Central
The Open Access Publisher

| Section/topic | # | Checklist item | Information reported | | Line number(s) |
|---|---|---|---|---|---|
| | | | Yes | No | |
| | | participants, interventions, comparators, and outcomes (PICO) | | | |
| **METHODS** | | | | | |
| **Eligibility criteria** | 8 | Specify the study characteristics (e.g., PICO, study design, setting, time frame) and report characteristics (e.g., years considered, language, publication status) to be used as criteria for eligibility for the review | ☒ | ☐ | 138-149 |
| **Information sources** | 9 | Describe all intended information sources (e.g., electronic databases, contact with study authors, trial registers, or other grey literature sources) with planned dates of coverage | ☒ | ☐ | 151-161 |
| **Search strategy** | 10 | Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated | ☒ | ☐ | 151-161 |
| ***STUDY RECORDS*** | | | | | |
| Data management | 11a | Describe the mechanism(s) that will be used to manage records and data throughout the review | ☒ | ☐ | 164-172 |
| Selection process | 11b | State the process that will be used for selecting studies (e.g., two independent reviewers) through each phase of the review (i.e., screening, eligibility, and inclusion in meta-analysis) | ☒ | ☐ | 174-180 |
| Data collection process | 11c | Describe planned method of extracting data from reports (e.g., piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators | ☒ | ☐ | 182-188 |
| **Data items** | 12 | List and define all variables for which data will be sought (e.g., PICO items, funding sources), any pre-planned data assumptions and simplifications | ☒ | ☐ | 190-198 |
| **Outcomes and prioritization** | 13 | List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale | ☒ | ☐ | 200-222 |
| **Risk of bias in individual studies** | 14 | Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis | ☒ | ☐ | 224-233 |
| ***DATA*** | | | | | |
| **Synthesis** | 15a | Describe criteria under which study data will be quantitatively synthesized | ☒ | ☐ | 235-246 |
| | 15b | If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data, and methods of combining data from studies, including any planned exploration of consistency (e.g., $I^2$, Kendall's tau) | ☒ | ☐ | 235-246 |
| | 15c | Describe any proposed additional analyses (e.g., sensitivity or subgroup analyses, meta-regression) | ☒ | ☐ | 235-246 |

| Section/topic | # | Checklist item | Information reported | | Line number(s) |
|---|---|---|---|---|---|
| | | | Yes | No | |
| | 15d | If quantitative synthesis is not appropriate, describe the type of summary planned | ☒ | ☐ | 235-246, 122-131 |
| **Meta-bias(es)** | 16 | Specify any planned assessment of meta-bias(es) (e.g., publication bias across studies, selective reporting within studies) | ☒ | ☐ | 248-251 |
| **Confidence in cumulative evidence** | 17 | Describe how the strength of the body of evidence will be assessed (e.g., GRADE) | ☒ | ☐ | 235-258 |

## 7.2 Published Protocol

Chawar *et al. Systematic Reviews* (2020) 9:200
https://doi.org/10.1186/s13643-020-01461-z

Systematic Reviews

PROTOCOL Open Access

# GWAS-identified genetic variants associated with medication-assisted treatment outcomes in patients with opioid use disorder: a systematic review and meta-analysis protocol

Caroul Chawar[1,2], Alannah Hillmer[1,2], Stephanie Sanger[3], Alessia D'Elia[1,2], Balpreet Panesar[1,2], Lucy Guan[2,4], Dave Xiaofei Xie[2,4], Nandini Bansal[2,4], Aamna Abdullah[2,4], Flavio Kapczinski[2], Guillaume Pare[5,6], Lehana Thabane[5,6,7] and Zainab Samaan[2*]

**Abstract**

**Background:** The burden of opioid use disorder (OUD) has been increasing in North America. Administration of medication-assisted treatments (MATs) for OUD on an individual-dose basis has been shown to affect patient responses to treatment, proving to be, on occasion, dangerous. A genetic basis has been identified for some MAT responses in a candidate gene context, but consensus has not been reached for any genome-wide significant associations. This systematic review aims to identify and assess any genetic variants associated with MAT patient outcomes at genome-wide significance.

**Methods:** The databases searched by the authors will be: MEDLINE, Web of Science, EMBASE, CINAHL and Pre-CINAHL, GWAS Catalog, GWAS Central, and NIH Database of Genotypes and Phenotypes. A title and abstract screening, full-text screening, data extraction, and quality assessment will be completed in duplicate for each study via Covidence. Treatment outcomes of interest include continued opioid use or abstinence during treatment or at follow-up, time to relapse, treatment retention rates, opioid overdose, other substance use, comorbid psychiatric disorders, risk taking behaviors, MAT plasma concentrations, and mortality rates. Analysis methods applied, if appropriate, will include random effects meta-analysis with pooled odds ratios for all outcomes. Subgroup analyses will also be implemented, when possible.

**Discussion:** This systematic review can hopefully inform the direction of future research, aiding in the development of a safer and more patient-centered treatment. It will be able to highlight genome-wide significant variants that are replicable and associated with MAT patient outcomes.

(Continued on next page)

* Correspondence: samaanz@mcmaster.ca
[2]Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada
Full list of author information is available at the end of the article

(Continued from previous page)

**Systematic review registration:** This systematic review protocol has been registered with the International Prospective Register of Systematic Reviews (PROSPERO) (registration ID CRD42020169121).

**Keywords:** Genome-wide association, Medication-assisted treatment, Opioid use, Treatment response, SNP, Pharmacogenetics, Systematic review, Protocol

## Background

Opioid use disorder (OUD) is characterized by the *Diagnostic and Statistical Manual of Mental Disorders, 5th edition* (DSM-5) as a series of physical and psychological symptoms that promote compulsive opioid-seeking behaviors and hinder the constraint of opioid consumption [1]. The World Health Organization (WHO) reports that roughly 27 million people suffered from OUD in 2016, and about 118 thousand died due to OUD-related drug use in 2015 [2]. The continual increase of opioid-related deaths in North America has called the U.S. Department of Health and Human Services (HHS) and the Ministry of Health in Canada to declare an opioid crisis and take appropriate federal action, in 2017 and 2016, respectively [3, 4].

The most prevalent OUD treatments are a combination of pharmacological and behavioral therapies, commonly known as medication-assisted treatments (MATs) [5]. The medications act as either agonists or antagonists to endogenous opioid receptors, regulating the inhibition or stimulation of the opioid reward system [6, 7]. FDA-approved MATs include methadone, buprenorphine, buprenorphine in combination with naloxone, and naltrexone [5]. In addition to those listed, Health Canada has also recently approved the use of injectable heroin-assisted treatment for severe OUD cases [8].

The regulated administration of these MATs at an individual-based dose is essential in ensuring the effectiveness of the treatment and safety of the patients, as well as averting overdose or mortality cases [9]. Methadone dosing, for example, has been shown to be a key factor in predicting treatment outcomes. Very low doses of this agonist put patients at a higher risk of relapse [10, 11], while too high doses and the induction of methadone have been associated with a higher risk of cardiac arrhythmia and mortality, respectively [9, 12].

MAT efficacy in keeping patients from illicitly using opioids has been variable [10, 11, 13], calling into question whether a genetic basis for how patients respond to treatment exists. Several genetic studies have identified variants associated with a higher risk of developing OUD and MAT metabolism or clearance [14, 15]. However, no clear consensus has been formed regarding genes that contribute to treatment outcomes, including negative ones, in OUD patients seeking treatment. Furthermore, literature has not been systematically reviewed for genetic variants of genome-wide significance in this area, to date.

## Objectives

This systematic review aims to assess all the identified genetic variants from genome-wide association studies (GWASs) significantly associated with treatment outcomes for OUD patients receiving MAT.

The specific objectives of this study include:

1. Summarizing the genome-wide significant variants associated with MAT outcomes within the current literature.
2. Comparing and meta-analyzing significant GWAS findings relevant to treatment outcomes, applying sub-group analyses based on ethnicity, sex and other variables, if possible.
3. Critically reviewing the literature to identify gaps that need to be addressed within the pharmacogenomics of MAT research.

## Methods

This protocol has been reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P) reporting guidelines [16]. An accompanying checklist could be found in Additional file 1.

### Eligibility criteria

Studies included in this review will be limited to GWASs. Other types of genetic studies, such as candidate-gene, twin, linkage-analysis, segregation-analysis, and familial aggregation, will not be included. Studies included will also investigate a MAT in an OUD population. For the purposes of this review, study populations with opioid/heroin/fentanyl dependence, use, abuse, or addiction will be included. Examples of MATs included are methadone, suboxone, buprenorphine, naltrexone, naloxone, heroin-assisted, levacetylmethadol, and fentanyl. Studies whose participants are solely on clonidine, lofexidine, or any other opioid withdrawal medication not administered with a MAT will be excluded as these measures are for short-term management of acute withdrawal and not maintenance

treatments. The inclusion of studies will not be restricted based on MAT treatment administration setting, such as community, residential, or institutional, or population characteristics, such as age, ethnicity, sex, or gender.

#### Information sources and search strategy

A librarian from the Health Sciences Library at McMaster University with expertise in systematic reviews will be consulted in developing the search strategy. A unique and predetermined search strategy will be developed for exporting publications from each of the select databases and GWAS data-sharing sites. These include MEDLINE, Web of Science (All Databases), EMBASE, CINAHL and Pre-CINAHL, GWAS Catalog, GWAS Central, and NIH Database of Genotypes and Phenotypes. Studies will not be restricted by language or date of publication but will be limited to human participants if limiting by species is made possible through the database. Databases will be searched from inception until present. All sources of literature, including gray literature, will be searched. Handsearching techniques will also be applied to identify articles of interest that are not detected by the databases systematically searched. A detailed search strategy is presented in Table 1. The start date of the study is March 1, 2020.

### Study records

#### Data management

All studies will be exported from the previously mentioned databases using the search strategy in Table 1 and imported into Zotero [17], a citation management software, where they will be screened for duplicates. We will then import studies into Covidence [18], for another round of duplicate screening and removal, title and abstract screening, full text screening, and data extraction. Each study will be screened and reviewed in duplicate through a team of 8 reviewers. In the case of any disagreements, the conflict will be resolved by a senior reviewer (CC or AH). As per the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [19], a flow chart detailing the stepwise screening process will be provided.

#### Selection process

Studies will be screened twice in pairs; once assessing the title and abstract, and another time at the full text phase. All articles will be screened for the same inclusion criteria previously mentioned, during both screening processes. All reviewers will partake in a calibration phase to ensure that the purpose of this review and the inclusion criteria are understood by all, and that no discrepancies exist across the reviewers. Since the screening of studies will occur via Covidence, reviewers are blinded to their colleagues' votes until after they have inputted their own votes, reducing the potential for bias.

#### Data collection process

Data extraction will be completed in pairs for any articles that pass the screening process. A full text extraction form will be constructed on excel and then uploaded onto Covidence. The data extraction form will be pilot tested independently in duplicate to ensure its feasibility in this systematic review. For any missing data from studies during the data extraction phase, contact will be made with the study authors to supplement the missing data. All records of communication and contact with the authors will be documented.

### Data items

Information collected on this form will include: author(s), year of publication, country, cohort population, number of participants (separated by MAT), ethnicity of participants, mean age, sex ratio, type and dose of MAT, MAT outcomes (as outlined under "Outcome Measures"), any genetic variants found to be significantly associated with the outcomes, method of statistical measures, and $p$ values. The traditional genome-wide significance threshold reported in the literature is $p \leq 5 \times 10^{-8}$. However, since a considerable number of studies with a borderline genome-wide significance have been shown to be replicable and showcase genuine associations, $p \leq 1 \times 10^{-7}$ will be used as the significance threshold for this review [20].

### Outcomes and prioritization

The main focus of this systematic review will be to assess GWAS-identified genetic variants significantly associated with MAT outcomes.

The primary MAT outcome of interest is illicit (unprescribed) opioid use throughout the duration of the MAT and at follow-up periods, the duration of which are to be determined based on the different studies reviewed. Continued illicit opioid use and abstinence from opioids will be assessed from urine toxicological screens and/or self-reported data.

Secondary outcomes of MAT to be considered in this review are:

1. Time to relapse, defined as the duration to the first use of illicit opioids after achieving abstinence.
2. Treatment retention, defined as the length of time a participant remains on MAT, and reasons for stopping MAT or dropping out.
3. Opioid overdose incidence, measured by self-report, adjudication of medical records, emergency admissions, opioid-related hospitalization, or use of naloxone.

**Table 1** Search strategy

Medline (Ovid):
1. Genome-Wide Association Study/
2. Genotyping Techniques/
3. Genome, Human/
4. Genetic Variation/
5. genetics/or exp human genetics/
6. (human* adj2 (genotyp* or genome* or genetic*)).ti,ab,kw,kf.
7. (GWS or GWAS or GWA).mp.
8. genome wide.ti,ab,kw,kf.
9. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8
10. exp Opioid-Related Disorders/
11. ((opiate* or opioid* or heroin* or codeine* or dilaudid* or fentanyl* or narcotic* or drug* or substance*) adj2 (overdose* or use* or using or misuse* or abus* or dependence* or addict*)).ti,ab,kw,kf.
12. Opiate Substitution Treatment/
13. ((opiate* or opioid*) adj2 (treatment* or therap*)).ti,ab,kw,kf.
14. exp buprenorphine/ or exp naloxone/
15. exp Methadone/
16. (suboxone or methadone or buprenorphine or naloxone).ti,ab,kw,kf.
17. 10 or 11 or 12 or 13 or 14 or 15 or 16
18. 9 and 17
19. *Limit 18 to humans*

Web of Science—All databases:
1. TS = (genome-wide association study or genome-wide association or GWAS or GWA or genome wide or genome)
2. TS = ((opiate* or opioid* or heroin* or fentanyl* or narcotic* or drug* or substance*) NEAR/2 (overdose* or use* or using or misus* or abus* or dependence* or addict*))
3. TS = ((treatment* or therap*) NEAR/2 (opiate* or opioid* or heroin* or fentanyl* or narcotic* or drug* or substance*))
4. TS = (methadone or buprenorphine or naloxone or naltrexone or heroin-assisted or suboxone)
5. #3 or #4
6. #1 and #2 and #5

EMBASE (Ovid):
1. Genome-Wide Association Study/
2. Genotyping Techniques/
3. Genome, Human/
4. Genetic Variation/
5. genetics/or exp human genetics/
6. (human* adj2 (genotyp* or genome* or genetic*)).ti,ab,kw.
7. (GWS or GWAS or GWA).mp.
8. genome wide.ti,ab,kw.
9. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8
10. exp Opioid-Related Disorders/
11. ((opiate* or opioid* or heroin* or codeine* or dilaudid* or fentanyl* or narcotic* or drug* or substance*) adj2 (overdose* or use* or using or misuse* or abus* or dependence* or addict*)).ti,ab,kw.
12. Opiate Substitution Treatment/
13. ((opiate* or opioid*) adj2 (treatment* or therap*)).ti,ab,kw.
14. exp buprenorphine/or exp naloxone/
15. exp Methadone/
16. (suboxone or methadone or buprenorphine or naloxone).ti,ab,kw.
17. 10 or 11 or 12 or 13 or 14 or 15 or 16
18. 9 and 17
19. *Limit 18 to human*

CINAHL and Pre-CINAHL:
1. genome-wide association study or genome-wide association or GWAS or GWA or genome wide or genome
2. opiate* or opioid* or heroin* or fentanyl* or narcotic* or drug* or substance*
3. overdose* or use* or using or misus* or abus* or dependence* or addict*
4. S2 and S3
5. treatment* or therap*
6. S5 and S2
7. methadone or buprenorphine or naloxone or naltrexone or heroin-assisted or suboxone
8. S6 or S7
9. S1 and S4 and S8
10. *Limit to Human*

GWAS Catalog—publications:
- methadone
- opioid
- heroin
- drug abuse

GWAS Central—studies list:
- methadone
- heroin
- opioid
- opiate
- addiction
- drug abuse
- opioid dependence
- opioid addiction
- fentanyl

NIH Database of Genotypes and Phenotypes:
- Search (opioid)
- Search (heroin)

4. Non-opioid substance use, self-reported or identified through urine toxicology screens.
5. Comorbid psychiatric disorders, self-reported or diagnosed.
6. Risk-taking behaviors related to drug use (i.e., injection, needle sharing), criminal activities, and social adversities, as reported in the original studies.
7. MAT and metabolite plasma concentrations and clearance, obtained through blood plasma analysis.
8. MAT doses, measured throughout the administration of MAT and at follow-up periods, as reported in the original studies
9. All-cause mortality, including opioid-related mortality.

### Risk of bias in individual studies

Quality assessment and risk of bias scores of included studies will be provided independently by each reviewer. The Quality of Genetic Association Studies (Q-Genie) tool [Version 1.1] developed by McMaster University will be used to assess both the qualitative and quantitative aspects of each study [21]. It is tailored to assess the validity and reliability of genetic association studies. Through Q-Genie, a quality score that corresponds to "low", "moderate", or "high" quality would be calculated for each study. The Grading of Recommendations Assessment, Development, and Evaluation (GRADE) tool will be used to assess the risk of bias, strength of evidence, and consistency of included studies [22]. Disagreements occurring between two reviewers regarding the risk of bias score will be resolved through discussion. If a unanimous decision is not reached, then, a third senior author will be consulted.

### Data synthesis

If appropriate, quantitative methods of synthesis will be applied. Heterogeneity between the studies will be assessed through the $I^2$ statistic and 95% confidence interval. If low heterogeneity levels are observed, quantitative methods of synthesis applied will include a random effects metaanalysis with pooled odds ratios for main and secondary outcomes previously mentioned. If a large number of studies are identified in this systematic review, subgroup analyses will be used, where the studies will be separated based on the ethnicities of their respective populations and analyzed accordingly, as genetic associations might be more predominant in certain ethnic groups than others. Other subgroup analyses to be considered are based on variables observed to influence MAT outcomes. These include sex, type of MAT, type of illicit opioid used (for example, heroin versus prescription opioids), and alcohol use comorbidity, if discussed in the original studies. All statistical analysis will be conducted via the RStudio [1.1.456] interface of R statistical software [23].

### Metabias

To address the potential publication bias that might be encountered, PROSPERO and ClinicalTrials.gov databases will be searched for relevant clinical trial protocols that might not have been followed by a publication of results [24, 25].

### Confidence in cumulative evidence

To assess the risk of bias within and across studies in the systematic review proposed, GRADE will be used [22]. It will be implemented to evaluate the study limitations and biases that contribute to each outcome of interest reported. The GRADE approach will assess the effect of the limitations on the results, effects being "not serious", "serious", or "very serious". Downgrading of the quality of the study will take place depending on the assessed effect level.

### Presenting and reporting of results

Results will be reported according to PRISMA guidelines, with special considerations to Human Genome Epidemiology Network (HuGENet) guidelines when applicable to GWAS data presentation [19, 26]. Though HuGENet guidelines are more pertinent to systematic reviews and meta-analyses of candidate gene studies with foci on single or multiple related genes, they will be used to uphold a standard when presenting genetic association data, when feasible. Tables will be used to present information on each genetic variant-phenotype association reported, including the study details, population, findings, and source of data. Forest plots will be used to display meta-analysis results, should a meta-analysis be appropriate to conduct. The overall quality of each published result will be discussed, taking into account the risk of bias scores.

## Discussion

This systematic review will be able to identify GWASs that have been conducted regarding MATs for OUD. Having a clear list of relevant studies will enable easier access to published results by the public and researchers alike. Results of the meta-analysis will be informative in determining if any genetic markers have been identified to have an impact on MAT outcomes in patients. This will help direct which genes are of interest for future candidate gene studies or GWASs. It will also allow for a consensus to be made regarding whether genetics affect treatment outcomes in the OUD population. Furthermore, if performed, stratified meta-analyses based on population ethnicities will contribute to the breadth of knowledge of genetic differences between ethnic groups. In addition, this review will allow for more informed treatment plans for individuals with differing ethnicities and genetic makeup. A potential limitation that could arise would be the inability to conduct subgroup meta-analyses due to high calculated heterogeneity between studies or small study numbers. In that case, the studies will be qualitatively reviewed and critically assessed according to their risk of bias scores. Another limitation of the proposed review is the exclusion of results obtained from candidate gene studies. Although some relevant SNP-outcome associations will not be reported on, the level of those reported will be of genome-wide significance, highlighting associations that can be expected and replicated in GWASs.

## Supplementary information

**Supplementary information** accompanies this paper at https://doi.org/10.1186/s13643-020-01461-z.

**Additional file 1.** PRISMA-P 2015 Checklist. This file is submitted in .pdf format and shows adherence to the PRISMA-P guidelines.

#### Abbreviations

OUD: Opioid use disorder; MAT: Medication-assisted treatment; PROSPERO: International Prospective Register of Systematic Reviews; DSM-5: Diagnostic and Statistical Manual of Mental Disorders, 5th edition; WHO: World Health Organization; HHS: U.S. Department of Health and Human Services; GWAS: Genome-wide association study; PRISMA-P: Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; Q-Genie: Quality of Genetic Association Studies; GRADE: Grading of Recommendations Assessment, Development, and Evaluation; HuGENet: Human Genome Epidemiology Network

#### Acknowledgements

Not applicable.

**Amendments**
If amendments to this protocol are made, they will be documented and communicated to the journal. A date of amendment, description, and rationale will accompany each amendment.

**Authors' contributions**
ZS is the guarantor. CC and ZS conceptualized the systematic review protocol. CC implemented the design of the protocol with the aid of AH and SS (health sciences librarian). CC prepared the first draft. AH, SS, AD, BP, LG, DX, NB, AA, FK, GP, LT, and ZS reviewed and revised the protocol draft. All authors read and approved the final manuscript.

**Funding**
This work was partially supported by CIHR (grant number PJT-156306), which has no role in the study design, analysis, reporting or publication of the results.

**Availability of data and materials**
Not applicable. No data were generated, analyzed, or reported in this manuscript.

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have no competing interests.

**Author details**
[1]Neuroscience Graduate Program, McMaster University, Hamilton, ON, Canada. [2]Department of Psychiatry and Behavioural Neurosciences, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada. [3]Health Sciences Library, McMaster University, Hamilton, ON, Canada. [4]Health Sciences Program, McMaster University, Hamilton, ON, Canada. [5]Population Health Research Institute, Hamilton, ON, Canada. [6]Department of Health Research Method, Evidence, and Impact, McMaster University, Hamilton, ON, Canada. [7]Father Sean O'Sullivan Research Centre, St. Joseph's Healthcare Hamilton, Hamilton, ON, Canada.

Received: 20 April 2020 Accepted: 21 August 2020
Published online: 01 September 2020

**References**

1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders [Internet]. Fifth Edition. American Psychiatric Association; 2013 [cited 2020 Mar 24]. Available from: https://doi.org/10.1176/appi.books.9780890425596.
2. WHO. WHO | Information sheet on opioid overdose [Internet]. 2018 [cited 2020 Feb 6]. Available from: https://www.who.int/substance_abuse/information-sheet/en/.
3. Government of Canada. Federal Action on Opioids - Canada.ca [Internet]. 2020 [cited 2020 Feb 6]. Available from: https://www.canada.ca/en/health-canada/services/substance-use/problematic-prescription-drug-use/opioids/federal-actions.html.
4. HHS. HHS Acting Secretary Declares Public Health Emergency to Address National Opioid Crisis | HHS.gov [Internet]. 2017 [cited 2020 Feb 6]. Available from: https://www.hhs.gov/about/news/2017/10/26/hhs-acting-secretary-declares-public-health-emergency-address-national-opioid-crisis.html.
5. FDA. Information about Medication-Assisted Treatment (MAT) | FDA [Internet]. 2019 [cited 2020 Feb 6]. Available from: https://www.fda.gov/drugs/information-drug-class/information-about-medication-assisted-treatment-mat.
6. O'Brien C, Kampman KM. Antagonists of Opioids. In: Galanter M, Kleber H, editors. Textbook of substance abuse and treatment. 4th ed. Arlington, VA: American Psychiatric Publishing; 2008.
7. Stotts AL, Dodrill CL, Kosten TR. Opioid dependence treatment: options in pharmacotherapy. Expert Opin Pharmacother. 2009 Aug;10(11):1727–40.
8. Government of Canada. Government of Canada approves new treatment options for opioid use disorder and supports research, treatment and harm reduction projects in Ontario - Canada.ca [Internet]. Health Canada. 2019 [cited 2020 Feb 6]. Available from: https://www.canada.ca/en/health-canada/news/2019/05/government-of-canada-approves-new-treatment-options-for-opioid-use-disorder-and-supports-research-treatment-and-harm-reduction-projects-in-ontario.html.
9. Sordo L, Barrio G, Bravo MJ, Indave BI, Degenhardt L, Wiessing L, et al. Mortality risk during and after opioid substitution treatment: systematic review and meta-analysis of cohort studies. BMJ. 2017;357:j1550.
10. Joe GW, Simpson DD, Sells SB. Treatment process and relapse to opioid use during methadone maintenance. Am J Drug Alcohol Abuse. 1994;20(2):173–97.
11. Amato L, Davoli M, Minozzi S, Ferroni E, Ali R. Methadone at tapered doses for the management of opioid withdrawal. [Internet]. 2013 [cited 2018 Aug 15]. Available from: http://publichealthwell.ie/node/69404?source = relatedblock&content = resource&member = 6841&catalogue = none&collection = none&tokens_complete = true.
12. Modesto-Lowe V, Brooks D, Petry N. Methadone deaths: risk factors in pain and addicted populations. J Gen Intern Med. 2010;25(4):305–9.
13. Nosyk B, Marsh DC, Sun H, Schechter MT, Anis AH. Trends in methadone maintenance treatment participation, retention, and compliance to dosing guidelines in British Columbia, Canada: 1996-2006. J Subst Abuse Treat. 2010 Jul;39(1):22–31.
14. 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. PLoS One. 2014;9(1):e86114.
15. J Mistry C, Bawor M, Desai D, C Marsh D, Samaan Z. Genetics of opioid dependence: a review of the genetic contribution to opioid dependence. Curr Psychiatry Rev. 2014;10(2):156–167.
16. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev. 2015;4(1):1.
17. Center for History New Media, George Mason University. Zotero: The next-generation research tool [Internet]. 2009. Available from: http://www.zotero.org/.
18. Veritas Health Innovation. Covidence systematic review software [Internet]. Melbourne, Australia; Available from: www.covidence.org.
19. Moher D, Liberati A, Tetzlaff J, Altman DG. PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med. 2009;6(7):e1000097.
20. Panagiotou OA, Ioannidis JPA. What should the genome-wide significance threshold be? Empirical replication of borderline genetic associations. Int J Epidemiol. 2012;41(1):273–86.
21. Sohani ZN, Meyre D, de Souza RJ, Joseph PG, Gandhi M, Dennis BB, et al. Assessing the quality of published genetic association studies in meta-analyses: the quality of genetic studies (Q-Genie) tool. BMC Genet [Internet]. 2015 15 [cited 2019 Dec 17];16. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4431044/.
22. Guyatt GH, Oxman AD, Schünemann HJ, Tugwell P, Knottnerus A. GRADE guidelines: a new series of articles in the Journal of Clinical Epidemiology. J Clin Epidemiol. 2011 Apr;64(4):380–2.
23. RStudio Team. RStudio: Integrated Development for R. RStudio, Inc. [Internet]. Boston, MA; 2015. Available from: http://www.rstudio.com/.
24. U.S. National Library of Medicine. Home - ClinicalTrials.gov [Internet]. [cited 2020 Mar 30]. Available from: https://clinicaltrials.gov/ct2/home.
25. Search | Cochrane Library [Internet]. [cited 2020 Apr 7]. Available from: https://www.cochranelibrary.com/central.
26. Little J, Higgins J. The HuGENet™ HuGE Review Handbook, version 1.0. 2006;59.

## Publisher's Note

## 7.3 CHAPTER 3 Supplementary File 1

PRISMA 2009 Checklist

| Section/topic | # | Checklist item | Reported on page # |
|---|---|---|---|
| **TITLE** | | | |
| Title | 1 | Identify the report as a systematic review, meta-analysis, or both. | 1 |
| **ABSTRACT** | | | |
| Structured summary | 2 | Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number. | 2-3 |
| **INTRODUCTION** | | | |
| Rationale | 3 | Describe the rationale for the review in the context of what is already known. | 3-4 |
| Objectives | 4 | Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS). | 4 |
| **METHODS** | | | |
| Protocol and registration | 5 | Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number. | 4 |
| Eligibility criteria | 6 | Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale. | 5 |
| Information sources | 7 | Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched. | 5 |
| Search | 8 | Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated. | 5 |
| Study selection | 9 | State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis). | 5 |
| Data collection process | 10 | Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators. | 5 |
| Data items | 11 | List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made. | 5 |
| Risk of bias in individual studies | 12 | Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis. | 5 |
| Summary measures | 13 | State the principal summary measures (e.g., risk ratio, difference in means). | 5 |
| Synthesis of results | 14 | Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., $I^2$) for each meta-analysis. | 5 |

Page 1 of 2

## PRISMA 2009 Checklist

| Section/topic | # | Checklist item | Reported on page # |
|---|---|---|---|
| Risk of bias across studies | 15 | Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies). | 5 |
| Additional analyses | 16 | Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified. | 5 |
| **RESULTS** | | | |
| Study selection | 17 | Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram. | 6 |
| Study characteristics | 18 | For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations. | 7 |
| Risk of bias within studies | 19 | Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12). | 7 |
| Results of individual studies | 20 | For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot. | 7-8 |
| Synthesis of results | 21 | Present results of each meta-analysis done, including confidence intervals and measures of consistency. | NA |
| Risk of bias across studies | 22 | Present results of any assessment of risk of bias across studies (see Item 15). | NA |
| Additional analysis | 23 | Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]). | NA |
| **DISCUSSION** | | | |
| Summary of evidence | 24 | Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers). | 8-9 |
| Limitations | 25 | Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias). | 9-10 |
| Conclusions | 26 | Provide a general interpretation of the results in the context of other evidence, and implications for future research. | 10 |
| **FUNDING** | | | |
| Funding | 27 | Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. | 10 |

*From:* Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: www.prisma-statement.org.

Page 2 of 2

### 7.4 CHAPTER 4 Supplementary File 1

# Reporting checklist for genetic association study.

Based on the STREGA guidelines.

## Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STREGAreporting guidelines, and cite them as:

Little J, Higgins JP, Ioannidis JP, Moher D, Gagnon F, von Elm E, Khoury MJ, Cohen B, Davey-Smith G, Grimshaw J, Scheet P, Gwinn M, Williamson RE, Zou GY, Hutchings K, Johnson CY, Tait V, Wiens M, Golding J, van Duijn C, McLaughlin J, Paterson A, Wells G, Fortier I, Freedman M, Zecevic M, King R, Infante-Rivard C, Stewart A, Birkett N; STrengthening the REporting of Genetic Association Studies. STrengthening the REporting of Genetic Association Studies (STREGA): An Extension of the STROBE Statement.

| | | Reporting Item | Page Number |
|---|---|---|---|
| **Title and abstract** | | | 1-2 |
| Title | #1a | Indicate the study's design with a commonly used term in the title or the abstract | |

| | | | |
|---|---|---|---|
| Abstract | #1b | Provide in the abstract an informative and balanced summary of what was done and what was found | |
| **Background/rationale** | | | 3-4 |
| | #2 | Explain the scientific background and rationale for the investigation being reported | |
| **Objectives** | | | 4 |
| | #3 | State specific objectives, including any prespecified hypotheses. State if the study is the first report of a genetic association, a replication effort, or both. | |
| **Study design** | | | 4 |
| | #4 | Present key elements of study design early in the paper | |
| **Setting** | | | 4 |
| | #5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | |
| **Eligibility criteria** | | | 4-5 |
| | #6a | Cohort study – Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. Case-control study – | |

| | | | |
|---|---|---|---|
| | | Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls. Cross-sectional study – Give the eligibility criteria, and the sources and methods of selection of participants. Give information on the criteria and methods for selection of subsets of participants from a larger study, when relevant. | |
| | #6b | Cohort study – For matched studies, give matching criteria and number of exposed and unexposed. Case-control study – For matched studies, give matching criteria and the number of controls per case. | |
| **Variables** | | | 5-6 |
| | #7a | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | |
| | #7b | Clearly define genetic exposures (genetic variants) using a widely-used nomenclature system. Identify variables likely to be associated with population stratification (confounding by ethnic origin). | |

| | | | |
|---|---|---|---|
| **Data sources/measurement** | | | 6 |
| | #8a | For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable. | |
| | #8b | Describe laboratory methods, including source and storage of DNA, genotyping methods and platforms (including the allele calling algorithm used, and its version), error rates and call rates. State the laboratory / centre where genotyping was done. Describe comparability of laboratory methods if there is more than one group. Specify whether genotypes were assigned using all of the data from the study simultaneously or in smaller batches. | |
| **Bias** | | | 6-7 |
| | #9a | Describe any efforts to address potential sources of bias | |
| | #9b | Describe any efforts to address potential sources of bias | |

| | | | |
|---|---|---|---|
| **Study size** | | | 7 |
| | #10 | Explain how the study size was arrived at | |
| **Quantitative variables** | | | 5 |
| | #11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why. If applicable, describe how effects of treatment were dealt with. | |
| **Statistical methods** | | | 7-8 |
| | #12a | Describe all statistical methods, including those used to control for confounding. State software version used and options (or settings) chosen. | |
| | #12b | Describe any methods used to examine subgroups and interactions | |
| | #12c | Explain how missing data were addressed | |
| | #12d | If applicable, explain how loss to follow-up was addressed | |
| | #12e | Describe any sensitivity analyses | |
| | #12f | State whether Hardy-Weinberg equilibrium was considered and, if so, how. | |

| | | | |
|---|---|---|---|
| | #12g | Describe any methods used for inferring genotypes or haplotypes | |
| | #12h | Describe any methods used to assess or address population stratification. | |
| | #12i | Describe any methods used to address multiple comparisons or to control risk of false positive findings. | |
| | #12j | Describe any methods used to address and correct for relatedness among subjects | |
| **Participants** | | | 8 |
| | #13a | Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable. Report numbers of individuals in whom genotyping was attempted and numbers of individuals in whom genotyping was successful. | |
| | #13b | Give reasons for non-participation at each stage | |
| | #13c | Consider use of a flow diagram | |

| | | | |
|---|---|---|---|
| **Descriptive data** | | | 8-9 |
| | #14a | Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable. Consider giving information by genotype | |
| | #14b | Indicate number of participants with missing data for each variable of interest | |
| | #14c | Cohort study – Summarize follow-up time, e.g. average and total amount. | |
| **Outcome data** | | | 5-6, 9-11 |
| | #15 | Cohort study Report numbers of outcome events or summary measures over time.Give information separately for exposed and unexposed groups if applicable. Report outcomes (phenotypes) for each genotype category over time Case-control study – Report numbers in each exposure category, or summary measures of exposure.Give information separately for cases | |

| | | | |
|---|---|---|---|
| | | and controls . Report numbers in each genotype category. Cross-sectional study – Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable. Report outcomes (phenotypes) for each genotype category | |
| **Main results** | | | 9-11 |
| | #16a | Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included | |
| | #16b | Report category boundaries when continuous variables were categorized | |
| | #16c | If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period | |
| | #16d | Report results of any adjustments for multiple comparisons | |
| **Other analyses** | | | 11 |
| | #17a | Report other analyses done—e.g., analyses of subgroups and | |

| | | | |
|---|---|---|---|
| | | interactions, and sensitivity analyses | |
| | #17b | Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses | |
| | #17c | Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses | |
| **Key results** | | | 11 |
| | #18 | Summarise key results with reference to study objectives | |
| **Limitations** | | | 12 |
| | #19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias. | |
| **Interpretation** | | | 11-12 |
| | #20 | Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence. | |
| **Generalisability** | | | 12 |

| | | | |
|---|---|---|---|
| | #21 | Discuss the generalisability (external validity) of the study results | |
| **Funding** | | | 12 |
| | #22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | |

## 7.5 CHAPTER 4 Supplementary File 2

*Implications of OPRM1 and CYP2B6 Variants on treatment outcomes in MMT patients in Ontario: Exploring sex differences*

SUPPLEMENTARY FILE 2.

**Data clean-up and quality control**
The following clean-up and quality control steps were conducted on the Pilot GENOA and GENOA data, which were merged into and analyzed as one dataset(1). All analyses were performed on PLINK 1.90 and the RStudio interface of R i386 3.5.1(2–4).

First the genotyped files were converted into .bed, .bim, and .fam files and merged into one dataset. Chromosomes that were marked as "bad" through genotyping were removed.

All samples that were genotyped were cross-referenced with the sample shipment documents to ensure that there were no missing samples.

To identify duplicates within the Pilot, within GENOA, and across Pilot and GENOA data, a preliminary list of all the duplicates was formed by combining kept records of duplications. To determine which genetic samples of the duplicates were to be excluded (duplicate vs original), an .imiss file was generated on PLINK to show the level of sample missingness and help determine the samples with the highest missingness rates. The duplicates and originals from the excel dataset were matched up with their respective level of missingness ("F_MISS") on R. A list with samples to be eliminated was made for reference for later sample duplicate quality control steps. However, 4 sets of duplicates with the same FIDs and varying IIDs were identified. The samples with the highest missingness rates were promptly removed.

Samples and SNPs with more than 10%, and later 5%, missingness were removed.

Samples with discordant sex information were identified. The samples were divided into two subsets based on sex, and their "F" value was graphed to visualize the X chromosome inbreeding coefficient distribution across each subset. Males with a coefficient equal to or greater than 0.8 were kept; females with a coefficient equal to or less than 0.4 were kept.

The sample heterozygosity rates were checked. The resultant values of the heterozygosity rate were calculated using the equation "(N(NM)-O(Hom))/N(NM)". A histogram was graphed of the heterozygosity rate, and the threshold was determined to be 0.22. Samples with a calculated rate of less than or equal to 0.17 were checked to be of Native American ancestry. One sample was removed.

Heterozygous haploids and nonmale Y chromosome genotype calls were set as missing on PLINK.

The samples were stratified on PLINK and a principal component analysis was conducted. Relationship and distance matrix calculations were completed, and a multidimensional scaling analysis was performed. Results from these analyses were used as part of the ancestry checks. The self-reported ethnicities of the samples were plotted against the genetically determined

ancestries (through principal component vectors) to visually highlight any outliers. Outliers were defined as samples whose genetically determined ancestries fall too far from the self-reported ethnicities (could be a result of contamination, sample switches, or other errors). Samples whose ancestries were corrected were those that were determined to possibly partially belong to the genetically determined ancestral group (ex. self-reported as 'European' but is 'mixed European and Native North American'). Samples that failed the ancestry check were removed.

Samples with high relatedness values (PI_HAT>=0.89) were identified. Along with samples that were believed to be duplicates, have failed the sex check, ancestry check, and/or genotyping, they were visualized on their respective plate positions to see if any unusual patterns could be observed. Any newly identified duplicates were checked against the case report forms to verify their duplicate status. All verified duplicates were then removed.

A threshold of p<1E-6 was used to remove SNPs that significantly deviated from Hardy-Weinberg equilibrium.

**Pre-imputation and imputation**

To prepare the data for imputation by the Michigan Imputation Server(5), the following steps were run on a Linux operating system.

The build was updated using resources and instructions outlined on Will Rayner's site (https://www.well.ox.ac.uk/~wrayner/strand/)(6).

The reference alleles for the European sample subset were set up to match those from HRC reference panel, and those of other ancestries to match 1000 Genomes reference panel. The frequency file used for the 1000 Genomes match was taken from the McCarthy Group tools (https://www.well.ox.ac.uk/~wrayner/tools/)(7). Non-European ancestry subsets were matched to those of 1000 Genomes, as labelled on the Mathgen site (https://mathgen.stats.ox.ac.uk/impute/1000GP_Phase3.html)(8). SNPs with high MAF that don't match the respective reference panel (HRC or 1000G) were removed.

Since at that stage only the European subset had a sample size sufficient for analysis (other ancestries of less than 100 samples would not be powered enough for ancestrally-stratified analysis), only samples of European descent were submitted for imputation and later analyzed.

Phasing was done using Eagle2 and imputation using Minimac4, with the HRC reference panel(9,10).

**Post-imputation filtering and quality control**

The following steps were performed using a virtual machine instance and cloud storage supported by the Google Cloud Platform (https://console.cloud.google.com/)(11). Imputed

individual chromosome files were recoded from .vcf to .ped/.map files, and then to .bed/.bim/.fam files before being merged into one file on PLINK for easy handling.

The Rsq values reported by Michigan Imputation Server matrix (Minimac 4) were used for filtering. SNPs with equal to or less than 0.3 rsq were identified to be of low quality and removed. Further, SNPs with MAF<0.05 were removed.

**References**

1. Samaan Z, Bawor M, Dennis BB, Plater C, Varenbut M, Daiter J, et al. Genetic influence on methadone treatment outcomes in patients undergoing methadone maintenance treatment for opioid addiction: A pilot study. Neuropsychiatr Dis Treat. 2014;

2. 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. 2007;

3. Allaire JJ. RStudio: Integrated development environment for R. J Wildl Manage. 2015;

4. R Development Core Team R. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. 2011.

5. Das S, Forer L, Schönherr S, Sidore C, Locke AE, Kwong A, et al. Next-generation genotype imputation service and methods. Nat Genet. 2016;

6. Rayner W. Strand Home [Internet]. [cited 2020 Aug 21]. Available from: https://www.well.ox.ac.uk/~wrayner/strand/

7. Rayner W. McCarthy Tools [Internet]. [cited 2020 Aug 21]. Available from: https://www.well.ox.ac.uk/~wrayner/tools/

8. 1,000 Genomes haplotypes -- Phase 3 integrated variant set release in NCBI build 37 (hg19) coordinates [Internet]. 2015 [cited 2020 Aug 21]. Available from: https://mathgen.stats.ox.ac.uk/impute/1000GP_Phase3.html

9. 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/PMC5096458/?report=abstract

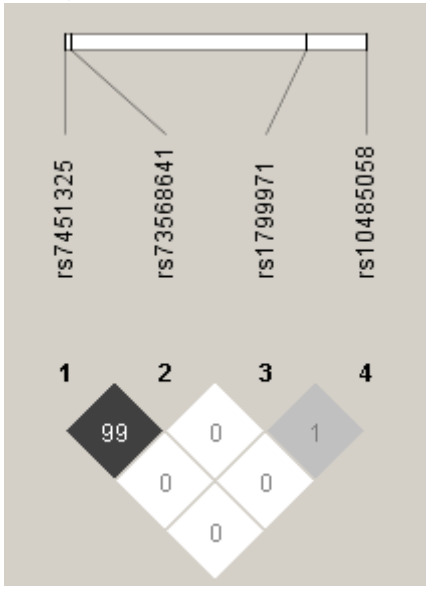
10. Das S. Next Generation of Genotype Imputation Methods [Internet]. University of Michigan; 2017 [cited 2020 Aug 21]. Available from: https://deepblue.lib.umich.edu/bitstream/handle/2027.42/138466/sayantan_1.pdf?sequence=1

11. Google Cloud. Google Cloud [Internet]. 2020 [cited 2020 Aug 21]. Available from: https://cloud.google.com/

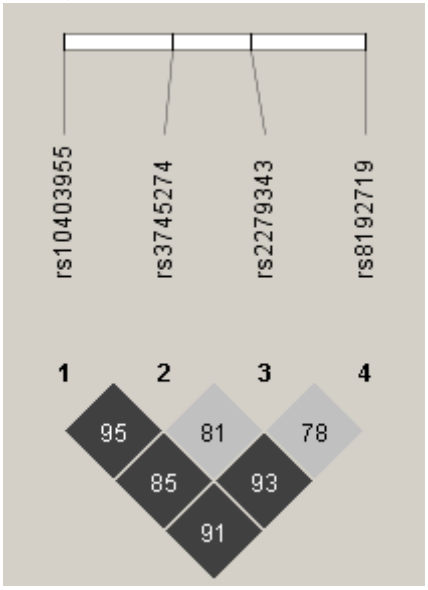
**Linkage disequilibrium plots**

**Continued opioid use**

***a) OPRM1***

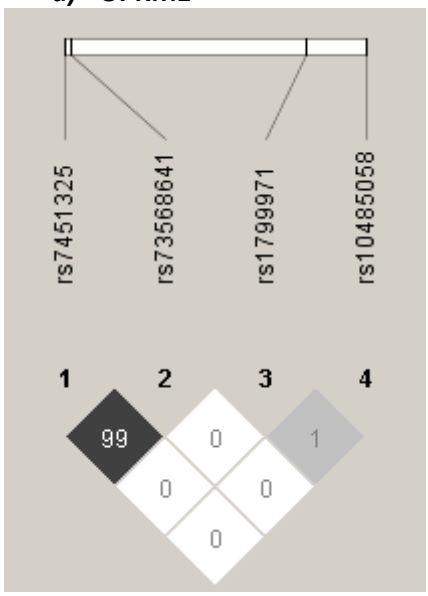



***b) CYP2B6***

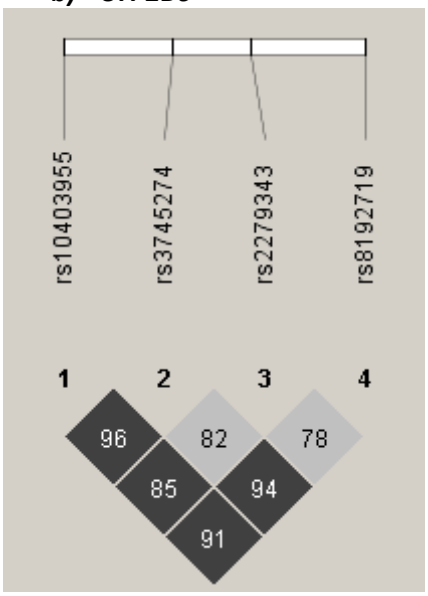



**Relapse**

***a) OPRM1***




***b) CYP2B6***



**Association tables**

For all the tables below: *P<0.05, **P<0.1. ADD is the additive regression test including the variables listed below it and principal component vectors. A1 represents the minor and tested allele. OR is odds ratio and BETA is the beta coefficient for the regression. STAT is the T-statistic and P is the p-value for it.

| **Continued opioid use – *OPRM1*** | | | | | | |
|---|---|---|---|---|---|---|
| **SNP** | **N** | **A1** | **TEST** | **OR** | **STAT** | **P** |
| **rs73568641** | 1129 | C | ADD | 0.8354 | -1.268 | 0.2049 |
| | | | Age | 0.985 | -2.036 | 0.04173 |
| | | | Gender | 0.8275 | -1.241 | 0.2144 |
| | | | Dose | 0.998 | -1.212 | 0.2253 |
| | | | Duration on MMT | 0.9942 | -3.672 | 0.000241 |
| *Male* | 640 | | ADD | 0.987 | -0.06662 | 0.9469 |
| | | | Age | 0.9784 | -2.221 | 0.02632 |
| | | | Dose | 0.9963 | -1.722 | 0.08513 |
| | | | Duration on MMT | 0.9942 | -2.647 | 0.008127 |
| *Female* | 489 | | ADD | 0.7062 | -1.656 | 0.09776** |
| | | | Age | 0.9928 | -0.6324 | 0.5271 |
| | | | Dose | 1.001 | 0.3166 | 0.7516 |
| | | | Duration on MMT | 0.9938 | -2.638 | 0.008339 |
| **rs1799971** | 1129 | G | ADD | 0.9737 | -0.1573 | 0.875 |
| | | | Age | 0.9851 | -2.032 | 0.04217 |
| | | | Gender | 0.8335 | -1.194 | 0.2326 |
| | | | Dose | 0.9981 | -1.137 | 0.2555 |
| | | | Duration on MMT | 0.9942 | -3.654 | 0.000258 |
| *Male* | 640 | | ADD | 1.11 | 0.4686 | 0.6394 |
| | | | Age | 0.9779 | -2.266 | 0.02347 |
| | | | Dose | 0.9963 | -1.738 | 0.08229 |
| | | | Duration on MMT | 0.9942 | -2.649 | 0.008084 |
| *Female* | 489 | | ADD | 0.8701 | -0.513 | 0.6079 |
| | | | Age | 0.9934 | -0.5753 | 0.5651 |
| | | | Dose | 1.001 | 0.4463 | 0.6554 |
| | | | Duration on MMT | 0.9939 | -2.604 | 0.009225 |
| **rs10485058** | 1129 | G | ADD | 0.9569 | -0.2833 | 0.777 |
| | | | Age | 0.985 | -2.045 | 0.04087 |
| | | | Gender | 0.8365 | -1.172 | 0.2411 |
| | | | Dose | 0.9981 | -1.142 | 0.2533 |

| | | | | | |
|---|---|---|---|---|---|
| | | Duration on MMT | 0.9943 | -3.629 | 0.000285 |
| *Male* | 640 | ADD | 0.8938 | -0.5206 | 0.6026 |
| | | Age | 0.9783 | -2.238 | 0.02524 |
| | | Dose | 0.9963 | -1.726 | 0.08439 |
| | | Duration on MMT | 0.9942 | -2.643 | 0.008222 |
| *Female* | 489 | ADD | 1.002 | 0.0098 | 0.9922 |
| | | Age | 0.9935 | -0.5654 | 0.5718 |
| | | Dose | 1.001 | 0.4775 | 0.633 |
| | | Duration on MMT | 0.9939 | -2.593 | 0.009508 |

| **Continued opioid use – *CYP2B6*** | | | | | | |
|---|---|---|---|---|---|---|
| **SNP** | **N** | **A1** | **TEST** | **OR** | **STAT** | **P** |
| **rs3745274** | 1129 | T | ADD | 0.8186 | -1.607 | 0.1081 |
| | | | Age | 0.9849 | -2.051 | 0.04029 |
| | | | Gender | 0.8435 | -1.116 | 0.2646 |
| | | | Dose | 0.9982 | -1.123 | 0.2614 |
| | | | Duration on MMT | 0.9942 | -3.646 | 0.000266 |
| *Male* | 640 | | ADD | 0.7263 | -1.879 | 0.06024** |
| | | | Age | 0.9792 | -2.146 | 0.03186 |
| | | | Dose | 0.9962 | -1.789 | 0.07366 |
| | | | Duration on MMT | 0.9943 | -2.601 | 0.009291 |
| *Female* | 489 | | ADD | 0.9544 | -0.2528 | 0.8004 |
| | | | Age | 0.9934 | -0.5798 | 0.5621 |
| | | | Dose | 1.001 | 0.4928 | 0.6222 |
| | | | Duration on MMT | 0.9939 | -2.609 | 0.009068 |

| **Relapse – *OPRM1*** | | | | | | |
|---|---|---|---|---|---|---|
| **SNP** | **N** | **A1** | **TEST** | **OR** | **STAT** | **P** |
| **rs73568641** | 944 | C | ADD | 0.9753 | -0.1958 | 0.8447 |
| | | | Age | 1.002 | 0.2456 | 0.806 |
| | | | Gender | 0.9282 | -0.5536 | 0.5798 |
| | | | Dose | 1.004 | 2.463 | 0.01376 |
| | | | Duration on MMT | 1.001 | 0.7126 | 0.4761 |
| *Male* | 530 | | ADD | 0.9659 | -0.2062 | 0.8366 |
| | | | Age | 0.9947 | -0.6175 | 0.5369 |
| | | | Dose | 1.005 | 2.362 | 0.01819 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | Duration on MMT | 0.9989 | -0.5279 | 0.5976 |
| *Female* | 414 | | ADD | 1.036 | 0.1746 | 0.8614 |
| | | | Age | 1.011 | 1.057 | 0.2905 |
| | | | Dose | 1.003 | 1.352 | 0.1765 |
| | | | Duration on MMT | 1.003 | 1.157 | 0.2473 |
| **rs1799971** | 944 | G | ADD | 0.8151 | -1.388 | 0.1652 |
| | | | Age | 1.002 | 0.2897 | 0.7721 |
| | | | Gender | 0.9201 | -0.6172 | 0.5371 |
| | | | Dose | 1.004 | 2.489 | 0.01281 |
| | | | Duration on MMT | 1.001 | 0.7206 | 0.4711 |
| *Male* | 530 | | ADD | 0.7551 | -1.485 | 0.1376 |
| | | | Age | 0.9954 | -0.5389 | 0.5899 |
| | | | Dose | 1.005 | 2.437 | 0.01482 |
| | | | Duration on MMT | 0.9989 | -0.5348 | 0.5928 |
| *Female* | 414 | | ADD | 0.9404 | -0.2499 | 0.8026 |
| | | | Age | 1.011 | 1.048 | 0.2944 |
| | | | Dose | 1.003 | 1.322 | 0.1861 |
| | | | Duration on MMT | 1.003 | 1.159 | 0.2466 |
| **rs10485058** | 944 | G | ADD | 1.097 | 0.6605 | 0.5089 |
| | | | Age | 1.002 | 0.2369 | 0.8127 |
| | | | Gender | 0.924 | -0.5864 | 0.5576 |
| | | | Dose | 1.004 | 2.484 | 0.013 |
| | | | Duration on MMT | 1.001 | 0.6689 | 0.5035 |
| *Male* | 530 | | ADD | 1.021 | 0.1087 | 0.9135 |
| | | | Age | 0.9946 | -0.6297 | 0.5289 |
| | | | Dose | 1.005 | 2.372 | 0.01768 |
| | | | Duration on MMT | 0.9989 | -0.5264 | 0.5986 |
| *Female* | 414 | | ADD | 1.151 | 0.68 | 0.4965 |
| | | | Age | 1.011 | 1.03 | 0.3032 |
| | | | Dose | 1.003 | 1.331 | 0.1832 |
| | | | Duration on MMT | 1.003 | 1.079 | 0.2806 |

| **Relapse – *CYP2B6*** | | | | | | |
|---|---|---|---|---|---|---|
| **SNP** | **N** | **A1** | **TEST** | **OR** | **STAT** | **P** |
| **rs3745274** | 944 | T | ADD | 0.9137 | -0.8065 | 0.42 |
| | | | Age | 1.002 | 0.2433 | 0.8078 |

| | | | | | |
|---|---|---|---|---|---|
| | | Gender | 0.9323 | -0.5207 | 0.6026 |
| | | Dose | 1.004 | 2.487 | 0.01289 |
| | | Duration on MMT | 1.001 | 0.7118 | 0.4766 |
| *Male* | 530 | ADD | 0.8589 | -1.004 | 0.3156 |
| | | Age | 0.9949 | -0.594 | 0.5525 |
| | | Dose | 1.005 | 2.374 | 0.01758 |
| | | Duration on MMT | 0.999 | -0.4975 | 0.6189 |
| *Female* | 414 | ADD | 1.066 | 0.3717 | 0.7101 |
| | | Age | 1.011 | 1.066 | 0.2863 |
| | | Dose | 1.003 | 1.336 | 0.1816 |
| | | Duration on MMT | 1.003 | 1.168 | 0.2427 |

| **Methadone dose – *OPRM1*** | | | | | | |
|---|---|---|---|---|---|---|
| **SNP** | **N** | **A1** | **TEST** | **BETA** | **STAT** | **P** |
| **rs73568641** | 1165 | C | ADD | -4.236 | -1.703 | 0.08876** |
| | | | Age | 0.412 | 3.258 | 0.001156 |
| | | | Gender | -5.453 | -2.041 | 0.04147 |
| | | | Duration on MMT | 0.1728 | 5.918 | 4.28E-09 |
| | | | Weight | 1.29E-01 | 2.856 | 0.004366 |
| *Male* | 664 | | ADD | -2.359 | -0.7079 | 0.4792 |
| | | | Age | 0.4889 | 2.932 | 0.003488 |
| | | | Duration on MMT | 0.2234 | 5.56 | 3.92E-08 |
| | | | Weight | 0.1316 | 1.529 | 0.1268 |
| *Female* | 501 | | ADD | -7.988 | -2.143 | 0.03258* |
| | | | Age | 0.2834 | 1.463 | 0.1441 |
| | | | Duration on MMT | 0.1187 | 2.797 | 5.36E-03 |
| | | | Weight | 0.1297 | 2.532 | 0.01166 |
| **rs1799971** | 1165 | G | ADD | 0.2018 | 0.06952 | 0.9446 |
| | | | Age | 0.4092 | 3.228 | 0.001283 |
| | | | Gender | -5.282 | -1.975 | 0.04855 |
| | | | Duration on MMT | 1.74E-01 | 5.933 | 3.94E-09 |
| | | | Weight | 0.1299 | 2.884 | 0.003999 |
| *Male* | 664 | | ADD | 2.587 | 0.6884 | 0.4915 |
| | | | Age | 0.4734 | 2.831 | 0.004785 |
| | | | Duration on MMT | 0.2237 | 5.566 | 3.81E-08 |
| | | | Weight | 0.1335 | 1.552 | 0.1211 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Female* | 501 | | ADD | -4.918 | -1.063 | 0.2884 |
| | | | Age | 0.2984 | 1.536 | 0.1252 |
| | | | Duration on MMT | 0.1204 | 2.829 | 4.87E-03 |
| | | | Weight | 0.1312 | 2.552 | 0.01101 |
| **rs10485058** | 1165 | G | ADD | -0.4485 | -0.1652 | 0.8688 |
| | | | Age | 0.4099 | 3.237 | 0.001244 |
| | | | Gender | -5.28E+00 | -1.976 | 0.04844 |
| | | | Duration on MMT | 0.1737 | 5.932 | 3.96E-09 |
| | | | Weight | 0.1295 | 2.87 | 0.004183 |
| *Male* | 664 | | ADD | -0.4994 | -0.1352 | 0.8925 |
| | | | Age | 0.4831 | 2.898 | 0.003879 |
| | | | Duration on MMT | 0.2236 | 5.56 | 3.93E-08 |
| | | | Weight | 0.133 | 1.542 | 0.1236 |
| *Female* | 501 | | ADD | 0.2362 | 0.05901 | 0.953 |
| | | | Age | 0.2998 | 1.54 | 0.1242 |
| | | | Duration on MMT | 0.1206 | 2.815 | 5.08E-03 |
| | | | Weight | 0.1303 | 2.528 | 0.01179 |

| **Methadone dose – *CYP2B6*** | | | | | | |
|---|---|---|---|---|---|---|
| **SNP** | **N** | **A1** | **TEST** | **BETA** | **STAT** | **P** |
| **rs3745274** | 1165 | T | ADD | 1.257 | 0.5789 | 0.5628 |
| | | | Age | 0.4101 | 3.239 | 0.001233 |
| | | | Gender | -5.368 | -2.006 | 0.04511 |
| | | | Duration on MMT | 0.1732 | 5.923 | 4.17E-09 |
| | | | Weight | 1.30E-01 | 2.881 | 0.004033 |
| *Male* | 664 | | ADD | -1.169 | -0.3912 | 0.6957 |
| | | | Age | 0.485 | 2.91 | 0.003738 |
| | | | Duration on MMT | 0.2238 | 5.566 | 3.81E-08 |
| | | | Weight | 0.1371 | 1.586 | 0.1131 |
| *Female* | 501 | | ADD | 4.19 | 1.319 | 0.1878 |
| | | | Age | 0.3129 | 1.61 | 0.1081 |
| | | | Duration on MMT | 0.12 | 2.82 | 5.00E-03 |
| | | | Weight | 0.134 | 2.603 | 0.009518 |

**Between-Sex Association Tables**

| ***OPRM1* Gene** | | | | | | |
|---|---|---|---|---|---|---|
| **Outcome** | **SNP** | **N** | **Minor Allele** | **OR/BETA** | **95% CI/SE** | **P** |
| **Continued opioid use** | **rs73568641** | 1129 | C | 1.48 | 0.6236, 3.512 | 0.374 |
| | *SNPxSex* | | | | | 0.1663 |
| | **rs1799971** | 1129 | G | 1.441 | 0.5259, 3.95 | 0.4773 |
| | *SNPxSex* | | | | | 0.4148 |
| | **rs10485058** | 1129 | G | 0.8245 | 0.3206, 2.121 | 0.6889 |
| | *SNPxSex* | | | | | 0.7449 |
| **Relapse** | **rs73568641** | 944 | C | 0.9207 | 0.3877, 0.4306 | 0.8311 |
| | *SNPxSex* | | | | | 0.8748 |
| | **rs1799971** | 944 | G | 0.6778 | 0.2841, 1.617 | 0.3807 |
| | *SNPxSex* | | | | | 0.6588 |
| | **rs10485058** | 944 | G | 0.9312 | 0.3979, 2.179 | 0.8695 |
| | *SNPxSex* | | | | | 0.6902 |
| **Methadone dose** | **rs73568641** | 1165 | C | 2.416 | 7.549 | 0.749 |
| | *SNPxSex* | | | | | 0.3509 |
| | **rs1799971** | 1165 | G | 9.653 | 8.72 | 0.2686 |
| | *SNPxSex* | | | | | 0.2507 |
| | **rs10485058** | 1165 | G | -1.062 | 8.271 | 0.8979 |
| | *SNPxSex* | | | | | 0.9375 |
| The minor alleles are also the reference and tested alleles. OR is odds ratio and BETA is the beta coefficient for the additive 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.<br>*P<0.1<br>**P<0.05 | | | | | | |

| ***CYP2B6* Gene** | | | | | | |
|---|---|---|---|---|---|---|
| **Outcome** | **SNP** | **N** | **Minor Allele** | **OR/BETA** | **95% CI/SE** | **P** |
| **Continued opioid use** | **rs73568641** | 1129 | C | 0.5673 | 0.2676, 1.203 | 0.1393 |
| | *SNPxSex* | | | | | 0.314 |
| **Relapse** | **rs73568641** | 944 | C | 0.7022 | 0.3576, 1.379 | 0.3045 |
| | *SNPxSex* | | | | | 0.4182 |
| **Methadone dose** | **rs73568641** | 1165 | C | -6.896 | 6.68 | 0.3021 |
| | *SNPxSex* | | | | | 0.1971 |
| The minor alleles are also the reference and tested alleles. OR is odds ratio and BETA is the beta coefficient for the additive 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