

Polygenic Architecture and Molecular Pathways Underlying Methadone Treatment Response in Opioid Use Disorder: Evidence from an Iranian Cohort

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Abstract

Background

Response to methadone maintenance therapy (MMT) varies widely, but its genetic basis remains poorly understood, particularly in non-European populations. We hypothesized that shared polygenic liability across psychiatric and substance use disorders contributes to earlier opioid use disorder (OUD) onset through specific pathways and predicts MMT response.

Methods

We analyzed 769 Iranian individuals with OUD receiving MMT. After quality control, subsets were used to: (i) test polygenic risk scores (PRS) for psychiatric, substance-use, and antidepressant response traits; and (ii) perform a genome-wide association study (GWAS) for both age of onset and methadone response, integrating locus-specific fine-mapping and MAGMA gene-based and pathway enrichment analyses.

Results

Higher MDD polygenic risk was associated with reduced odds of methadone treatment response and remained significant in a model jointly including bipolar disorder and schizophrenia PRSs; the latter PRSs were not independently significant. The age-of-onset of OUD GWAS identified a suggestive non-coding locus on chromosome 4 ($\beta = -2.74$, $P = 1.69 \times 10^{-6}$) and suggestive signals ($P = 1 \times 10^{-5}$ to 5×10^{-5}) near *GLP1R*, *MAPK10*, *PDE4B*, *TIA1* and *SNRPG*. The methadone response GWAS identified suggestive loci in *EPHA5* (OR = 1.81, $P = 9.9 \times 10^{-6}$) and *HOMER1* (OR = 0.54, $P = 1.6 \times 10^{-5}$).

Pathway analysis implicated branched-chain amino acid and fatty acid metabolism in age at onset (FDR<0.05), and TGF- β and JAK–STAT signaling in methadone response ($P = 9.9 \times 10^{-7}$ – 1.1×10^{-3} ; FDR<0.05).

Conclusions

Distinct but convergent polygenic and biological mechanisms shape opioid-use onset and methadone response, implicating psychiatric liability, metabolic regulation, and neuroimmune signaling as candidate mechanisms of clinical heterogeneity in OUD.

1. INTRODUCTION

Opioid use disorder (OUD) is a major global public health challenge, with Iran reporting one of the highest prevalences of opioid dependence worldwide, estimated at 2.1% of the adult population according to national surveys, resulting in substantial morbidity, mortality, and socioeconomic burden^{1–4}. Methadone maintenance treatment (MMT) remains one of the most effective pharmacological interventions for OUD, however, clinical response is highly heterogeneous, with a substantial proportion

of patients experiencing inadequate response, relapse, or adverse effects⁵. Identifying biological factors that contribute to this variability is critical for advancing personalized treatment strategies, particularly as real-world harm and the public health impact of the opioid epidemic remain high in the Iranian population^{2,4,6,7} and worldwide.

While scientific attention toward the genomic roots of substance uses and recovery outcomes is expanding, research remains disproportionately focused on European-descended groups, which narrows the clinical utility of the data for the rest of the world. By shifting the focus to diverse and underrepresented populations, such as cohorts from the Middle East, researchers may uncover unique biological drivers thus helping to ensure that the benefits of precision medicine are distributed more equitably. This approach directly addresses the technical and ethical gaps created by the current over-reliance on European-weighted genetic resources, which often suffer from portability limitations when applied to other global ancestries^{8,9}.

Accumulating evidence indicates that OUD shares genetic liability with other psychiatric and substance use disorders, including major depressive disorder (MDD), bipolar disorder (BD), schizophrenia (SCZ), and alcohol-related traits^{7,10}. Polygenic risk scores (PRS), which aggregate the effects of multiple genetic variants across the genome, provide a framework for quantifying this shared genetic architecture and evaluating its relevance to clinical outcomes¹¹. In the context of OUD, PRS derived from related psychiatric and behavioral phenotypes may capture underlying neurobiological vulnerabilities that influence both disease susceptibility and treatment response. However, the extent to which cross-disorder genetic risk contributes to variability in methadone response remains incompletely understood, particularly in non-European populations^{12,13}.

Genome-wide association studies (GWAS) offer a hypothesis-free approach to identifying genetic variants associated with complex traits, including treatment outcomes and age at onset^{14,15}. Given the polygenic nature of OUD and related phenotypes, integrating GWAS findings with gene-based and pathway-level analyses can enhance biological interpretability by highlighting convergent molecular mechanisms rather than isolated variants^{16,17}. Such approaches are particularly valuable in studies with moderate sample sizes, where individual variant signals may not reach genome-wide significance but can still inform relevant biological pathways.

In this study, we investigated the genetic architecture of methadone treatment response and age at onset of OUD in an Iranian cohort using a comprehensive approach that integrates GWAS, polygenic risk scoring, gene-based, and pathway enrichment analyses. Specifically, we aimed to (i) evaluate the association between PRS derived from psychiatric and substance use-related traits and methadone treatment response, and (ii) perform genome-wide association analyses of age at onset and treatment outcomes, incorporating downstream fine-mapping and pathway-level characterization to identify convergent molecular mechanisms underlying these phenotypes. Through this comprehensive framework, we sought to advance understanding of the genetic factors influencing treatment response in OUD and to contribute to the development of more personalized therapeutic strategies.

2. METHODS

2.1 Cohort and Phenotype Definition

Approval for the study was obtained from the Shahid Beheshti University of Medical Sciences Research Ethics Committee (Ethic No. IR.SBMU.RETECH. REC. 1398.213). Each participant was enrolled after completing the written consent form to conduct this research. All participants had the experimental procedure explained to them prior to the study onset. All study procedures were conducted in accordance with the principles of the Declaration of Helsinki and applicable institutional and national ethical guidelines¹⁸.

The study cohort comprised 769 Iranian individuals diagnosed with OUD who were receiving MMT at the same clinic. Inclusion required a confirmed diagnosis of OUD and active enrollment in a supervised MMT program, while exclusion was based on sample- and phenotype-level quality control, which removed individuals with excessive heterozygosity, low call rates (< 95%), relatedness, or being population outliers based on principal component analysis.

Following these filters, a total of 751 participants was retained for demographic characterization, of whom 738 (98.3%) were male and 13 (1.7%) were female.

Methadone treatment outcomes were assessed prospectively by the treating psychiatrists over a six-month observation period using a multidimensional clinical evaluation. The assessment incorporated retention in methadone maintenance treatment, adherence to the prescribed treatment regimen, adequacy and stability of the methadone dose, control of opioid withdrawal symptoms and craving, changes in non-prescribed opioid use, clinical and psychosocial stability, adverse effects, and serial urine toxicology results^{19,20}.

Participants classified as treatment responders (n = 513) remained engaged in treatment throughout the six-month observation period and demonstrated a clinically meaningful improvement, including stable methadone dosing, satisfactory control of withdrawal symptoms and craving, and a substantial reduction in non-prescribed opioid use, supported by predominantly opioid-negative urine toxicology results. Participants classified as non-responders (n = 256) demonstrated persistent clinically significant opioid use, inadequately controlled withdrawal symptoms or craving, or insufficient overall clinical improvement despite documented adherence and receipt of an individually optimized and clinically adequate methadone dose. Adherence and treatment response were evaluated using blood methadone levels, urine toxicology findings, and clinical assessment²¹.

For genetic analyses, subsets of participants with complete genotype and phenotype data were used as appropriate for each analysis. In particular, 627 (12 female) individuals were included in the methadone treatment response analyses, and 579 (11 female) individuals contributed to genome-wide association analyses of age at onset. Methadone treatment response was coded as a binary phenotype (2 = responder, 1 = non-responder). Age at onset of opioid use was defined as the participant's age at first

regular opioid use and was treated as a continuous phenotype. This information was obtained through a structured clinical interview conducted at enrolment and was based primarily on participant self-report. Where available, the reported age at onset was cross-checked against information documented in medical or addiction-treatment records and, when appropriate, corroborated by a family member^{22–24}.

All phenotype files were harmonized prior to analysis, duplicate records were resolved, and categorical variables (including sex, marital status, and treatment response) were consistently recoded to ensure compatibility with downstream logistic and linear regression models.

1. Genotype Quality Control

Genotyping was performed using the Illumina Global Screening Array (GSA) v1.0. Quality control procedures were conducted using PLINK v1.9 and v2.0²⁵ following the parameters described below.

Variants were filtered based on minor allele frequency ($MAF > 0.01$), Hardy–Weinberg equilibrium ($p > 1 \times 10^{-15}$), and call rate ($> 90\%$). Samples with call rate $< 95\%$ or excessive heterozygosity were excluded. Related individuals were identified using identity-by-descent estimates, and one individual from each related pair was removed ($PI_HAT > 0.125$)²⁶.

Population structure was assessed using principal component analysis (PCA) on linkage disequilibrium–pruned autosomal SNPs (Supplementary Figs. 1). Population outliers ($> \pm 6$ SD on any of the first 15 PCs) were excluded ($n = 8$) (Supplementary Fig. 2).

Variants were harmonized to rsID using genomic coordinates (CHR: BP) and reference databases, including the Haplotype Reference Consortium (HRC) r1.1 reference panel²⁷ and the hg19 (GRCh37) genomic assembly²⁸, and ambiguous or unresolved variants ($n = 205$) were excluded.

Genotype imputation was performed using the Michigan Imputation Server following pre-phasing with SHAPEIT. Imputation was conducted using the Haplotype Reference Consortium (HRC) r1.1 reference panel.

Post-imputation quality control included filtering variants based on imputation quality ($INFO > 0.8$), $MAF (> 0.01)$, and missingness ($CR > 95\%$). The first 15 PCs were retained as covariates based on inspection of the scree plot and their cumulative variance among the first 20 PCs examined (76.19%; Supplementary Fig. 3 and Supplementary Table 2). After quality control, 2,313,175 SNPs remained for downstream analyses.

1. Polygenic Risk Score (PRS) Computation

PRSs were computed using PRSice-2²⁹ with clumping ($r^2 = 0.1$, 250 kb window) across p-value thresholds ranging from 5×10^{-8} to 1 using the default PRSice-2 thresholding procedure. The threshold that yielded the strongest association with the outcome was selected for each PRS (see Supplementary

Table 1). Summary statistics were harmonized and subjected to standard QC procedures, including removal of ambiguous variants and filtering based on allele frequency and imputation quality³⁰.

PRSs were derived from publicly available GWAS summary statistics of major depressive disorder^{31,32}, bipolar disorder³³, schizophrenia³⁴, alcohol-related traits³⁵, and antidepressant treatment response³⁶ (Supplementary Table 2).

1. Genome Wide Association Study

Genome-wide association analysis of age at onset was performed using linear regression in PLINK2. Models included sex, age, treatment response, and the first 15 principal components as covariates.

GWAS of methadone treatment response was conducted using logistic regression in PLINK2, adjusting for sex and principal components. Benjamini–Hochberg False Discovery Rate (FDR), with FDR < 0.05 (5%) was accepted as the threshold for statistical significance.

1. *Locus-Specific Analysis*

Genomic regions with suggestive evidence of association ($P < 5 \times 10^{-5}$) were further examined using regional association plots and linkage disequilibrium patterns. Lead variants were annotated to nearby genes using ANNOVAR and functional effect determined using Ensembl Variant Effect Predictor^{37,38}. Additionally, lead variants were queried for potential regulatory effects using the Genotype-Tissue Expression (GTEx) portal to identify significant expression quantitative trait loci (eQTLs) in relevant tissues, including the brain³⁹.

2.2 Gene-Based and Pathway Analysis

Gene-based and pathway analyses were conducted using MAGMA v1.10⁴⁰. GWAS summary statistics were mapped to genes using hg19 annotations with a ± 5 kb window around gene boundaries. Gene-set enrichment analyses were performed using curated pathways from KEGG and Gene Ontology (biological processes) obtained from MSigDB using MAGMA. Multiple testing correction was performed using the FDR, with adjusted p-values used to determine significance.

2.2.1 Statistical analysis

1. *PRS Analysis and Multivariable Modeling*

Associations between PRS and binary methadone treatment response were evaluated using binomial logistic regression models. All PRSs were standardized ($\mu = 0$, $\sigma = 1$) prior to analysis to facilitate comparison of effect sizes. To ensure robust results, empirical p-values were obtained via permutation testing (10,000 permutations). Multiple testing correction was performed using the Benjamini-Hochberg FDR⁴¹, applied within each phenotype family (e.g., affective/psychotic disorders, substance use, antidepressant response) consistent with the disjunction testing approach^{42,43}. To build a more nuanced

model of genetic liability, we also employed multivariable regression where PRSs for MDD, bipolar disorder, and schizophrenia were simultaneously included in a single model.

1. *Genome-Wide Association Studies*

Genome-wide summary statistics were generated using PLINK2. For the continuous trait of age at onset, a linear regression model was used, adjusting for sex, age, treatment response, and the first 15 principal components (PCs). For methadone treatment response, a logistic regression model was employed, adjusting for sex and the first 15 PCs; age-related covariates were excluded from the response model to prevent over-adjustment. Suggestive significance for individual variants was defined at a nominal threshold of $P < 5 \times 10^{-5}$ for follow-up annotation.

1. *Gene-Based and Pathway Enrichment*

To move beyond single-variant signals, MAGMA v1.10 was used to perform gene-based and competitive pathway enrichment analyses. Genes were defined using a ± 5 kb window around their boundaries. In the absence of an Iranian-specific reference panel, linkage disequilibrium was estimated using the European ancestry reference panel from the 1000 Genomes Project, which was selected as the closest available ancestry proxy. Statistical significance was determined using an FDR threshold of $q < 0.05$.

1. *Covariate Selection and Population Stratification*

Covariates were selected based on both biological relevance (sex, age) and the need to control for technical and ancestral confounding. Specifically, ancestry principal components (first 15) were included in all models to robustly account for population stratification. The decision to retain 15 PCs was based on an inspection of the scree (elbow) plot and the fact that these components collectively explained 76.34% of the total genetic variance in the cohort.

3. RESULTS

3.1 Sample Characteristics

The cohort included 769 individuals with OUD receiving methadone maintenance therapy, of whom 66.7% were classified as responders. After genotype quality control, 627 individuals were included in treatment response analyses and 579 in age-of-onset GWASs (Table 1).

Table 1
Demographic and clinical characteristics of the opioid use disorder cohort (N = 769).

Characteristic	N (Summary)
Sex	751
Male	738 (98.3%)
Female	13 (1.7%)
Age (years)	724
Mean (SD)	45.1 (10.9)
Median (IQR)	43.0 (38.0–53.0)
Range	20–85
Marital status	769
Married	555 (72.2%)
Not married	214(27.8%)
Methadone	769
Responder	513(66.7%)
Non-responder	256 (33.3%)
Age at onset of opioid use (years)	705
Mean (SD)	26.4 (9.3)
Median (IQR)	25.0 (20.0–30.0)
Range	10–72

3.2 Shared Genetic Liability Between Psychiatric Disorders and Methadone Response

PRS for psychiatric and substance use traits were evaluated for association with methadone treatment outcome. Genetic liability to MDD was significantly associated with reduced odds of treatment response ($\beta = -0.31$, empirical $p = 5.5 \times 10^{-3}$, adjusted empirical p (FDR) = 0.015; Fig. 3). This effect remained significant when BD and SCZ PRSs were added to the model. However, BD and SCZ PRSs were not independently significantly associated with methadone response. PRSs for alcohol-related traits and antidepressant response showed nominal but non-significant associations after multiple testing correction (Fig. 1).

3.3 Genetic Architecture of Age of Onset

3.3.1 Genome-Wide Association and Suggestive Loci

No variants reached genome-wide significance (Fig. 5). One of the strongest signals was observed at rs12650576 on chromosome 4 ($\beta = -2.74$ years, $p = 1.69 \times 10^{-6}$), mapping to a noncoding region proximal to long intergenic RNA (LINC02498).

Additional suggestive associations were observed near *GLP1R*, *MAPK10*, *PDE4B*, *TIA1*, and *SNRPG*, further implicating metabolic and intracellular signaling pathways in the early initiation of opioid use.

3.3.2 Gene-Based and Pathway Enrichment Analyses

MAGMA Gene-based GWAS did not identify any genes reaching Bonferroni-corrected significance for age at onset ($FDR < 0.05$). However, competitive pathway analyses revealed enrichment in metabolic and intracellular signaling processes (Supplementary Fig. 4). The strongest signals included branched-chain amino acid (BCAA) metabolism (valine, leucine, and isoleucine degradation; KEGG $q < 1 \times 10^{-4}$) and fatty acid metabolism (KEGG $q = 2 \times 10^{-3}$). Gene Ontology (GO) analyses further supported enrichment in amino acid catabolic processes, particularly BCAA pathways (GO $q < 0.001$). Suggestive enrichment was also noted for MAPK signaling and actin cytoskeleton regulation ($q < 0.25$), suggesting that earlier onset is associated with coordinated metabolic dysregulation

3.4 Genetic Architecture of Methadone Treatment Response

3.4.1 Genome-Wide Association and Fine-Mapping

While no genome-wide significant association was identified, suggestive associations were observed within ephrin receptor A5 (*EPHA5*) and homer scaffold protein 1 (*HOMER1*) genes. For *EPHA5*, the lead SNP rs11737314 was associated with increased odds of response (OR = 1.81, $P = 9.9 \times 10^{-6}$). Fine-mapping of this locus identified rs11737314 with a Posterior Inclusion Probability (PIP) of ~ 0.135 ; notably, this variant is a significant cis-eQTL for *EPHA5* in the brain frontal cortex (GTEx), providing functional support for its regulatory involvement

A second signal mapped to *HOMER1*, where multiple correlated intronic variants, including rs12110078, exhibited consistent protective effects (OR = 0.54, $P = 1.6 \times 10^{-5}$). Fine-mapping for *HOMER1* yielded a 95% credible set of 755 variants ($\sim 65\%$), indicating a diffuse signal consistent with high linkage disequilibrium and modest power.

3.4.2 Gene-Based and Pathway-Level Characterization

Gene-based MAGMA results identified several top-ranked, though non-significant, candidates involved in immune and stress processes: Major Histocompatibility Complex, Class I, B (*HLA-B*), Cathepsin S (*CTSS*), Aryl Hydrocarbon Receptor Nuclear Translocator (*ARNT*), and WAS/WASL Interacting Protein Family Member 1 (*WIPF1*).

Pathway analyses highlighted a distinct biological profile centered on cell adhesion and neuroimmune signaling. Significant pathways (FDR-corrected $q < 0.05$), included adherens junctions, glycosaminoglycan biosynthesis (chondroitin sulfate), JAK-STAT signaling, and TGF- β signaling. These results suggest that methadone response may be influenced by a convergent biological axis involving synaptic structure and cytokine signaling.

4. DISCUSSION

This study employed polygenic risk scoring, GWAS, locus-level fine-mapping, and pathway analysis to interrogate two clinically consequential dimensions of OUD: (i) methadone maintenance therapy (MMT) response and (ii) age at onset of opioid use in a non-European cohort. While no single locus reached genome-wide significance, likely due to the polygenic architecture of addiction and our modest sample size, three convergent signals emerge translational relevance for risk stratification and target prioritization: increased liability for MDD was associated with poorer response to MMT; metabolic biology is enriched for earlier onset; and methadone response maps to a coordinated synaptic-neuroimmune axis.

4.1 Depression PRS as a Driver of "MMT Resistance"

A central finding is that, among the PRSs tested, genetic liability for MDD is the strongest independent predictor of methadone non-response. This association aligns with evidence that common variant liability is shared across psychiatric and substance-use phenotypes. In practical terms, this result supports a model where depressive genetic liability contributes to an "MMT resistance" profile, potentially mediated by stress responsivity^{44,45}, anhedonia-related reward dysfunction⁴⁶, and a higher burden of comorbid symptoms² that complicate stabilization.

In settings where MMT is scaled for harm reduction, this depression-linked signal suggests that precision addiction medicine should integrate psychiatric optimization as a core component of pharmacological response. Inherited liability may modulate the biological threshold for stabilization and craving control under real-world stressors.

4.2 Metabolic and Intracellular Signaling Programs in Early Vulnerability

Age of onset was characterized by robust enrichment in metabolic processes, most prominently BCAA and fatty-acid metabolism^{47–51}. These results reflect a conceptual shift in addiction biology: vulnerability reflects not only "synaptic" variation but also metabolic state and energy homeostasis. BCAAs and lipid pathways are tightly linked to mitochondrial function; metabolic perturbations can influence neurotransmission indirectly through redox balance, inflammatory tone, and gut–brain signaling^{52,53}.

Our genome-wide analysis further identified an intracellular signaling axis involving suggestive signals near *MAPK10*, *PDE4B*, *TIA1*, and *SNRPG*. The inclusion of *PDE4B* (phosphodiesterase 4B) is particularly

relevant as it regulates cAMP-mediated signaling and neuroinflammation, pathways central to the neuroadaptation seen in chronic opioid exposure^{54–56}. Furthermore, the signals at *TIA1* and *SNRPG* provide a plausible "psychiatric-addiction bridge"^{56–59}. *TIA1* is involved in the formation of stress granules, while *SNRPG* is a core component of the RNA splicing machinery; their involvement suggests that the biological threshold for early-onset OUD may be governed by the cellular response to environmental and physiological stress, as well as fundamental RNA processing^{57,58}. This aligns with the shared liability between addiction and major depression, where cellular stress responses are often compromised. Together with *MAPK10*, these signals indicate that early initiation is driven by a coordinated dysregulation of intracellular homeostasis and regulatory stress responses⁶⁰.

The suggestive signal near *GLP1R* is particularly notable as a "translational bridge". GLP-1 receptor signaling intersects directly with mesolimbic reward circuitry, where its activation has been shown to dampen dopaminergic reinforcement and attenuate opioid-seeking behaviors in preclinical models^{33,61}. This biological mechanism is increasingly validated by emerging clinical evidence⁴⁵. For instance, recent randomized clinical trials demonstrate that the GLP-1 analog semaglutide significantly reduces alcohol consumption in individuals with alcohol use disorder⁵³. Furthermore, large-scale observational data indicate that semaglutide treatment is associated with a reduced risk of opioid overdose among patients with comorbid type 2 diabetes and OUD⁶¹. With ongoing clinical trials now directly evaluating semaglutide's efficacy in promoting abstinence specifically within treatment-refractory OUD populations, this pathway represents a highly actionable therapeutic node⁶². Because GLP-1-based agents are already clinically deployable for metabolic indications, these findings prioritize the GLP-1 system as a high-value target for personalizing OUD treatment strategies⁴⁵.

4.3 Methadone Response Biology Converges on Synaptic Development, ECM Architecture, and Neuroimmune Signaling

The genetic architecture of methadone response in our cohort mapped to coordinated biological programs spanning synaptic plasticity, extracellular matrix (ECM) remodeling, and neuroimmune signaling, specifically involving the JAK–STAT and TGF- β pathways. Suggestive loci in ephrin receptor A5 (*EPHA5*) and homer scaffold protein 1 (*HOMER1*) provide mechanistically coherent candidates for these processes. Eph receptors, such as *EPHA5*, are integral to axon guidance, synapse formation, and experience-dependent remodeling, processes essential for the stabilization of reward circuitry during agonist treatment^{63–65}. Simultaneously, *HOMER1* encodes a postsynaptic scaffolding protein that regulates metabotropic glutamate receptor (mGluR) signaling and synaptic plasticity, which are core mechanisms underlying cue reactivity, reinforcement learning, and relapse vulnerability⁶⁶. Beyond these synaptic markers, the enrichment of glycosaminoglycan and neuroimmune pathways (JAK–STAT) dovetails with evidence that chronic opioid misuse involves broad glial and immune transcriptional

shifts⁴⁴. These complex signals suggest that persistent cue-reactivity and relapse pressure are rooted in biology that is not sufficiently addressed by μ -opioid receptor agonism alone.

4.4 Ancestry, Equity, and Portability in Non-European Cohorts

Most addiction genetics and PRS resources remain heavily weighted toward European ancestry, which creates significant portability limitations and potential health inequities if these tools are translated uncritically into clinical practice. This study addresses this critical gap by testing cross-trait psychiatric PRSs in an underrepresented Iranian population where the real-world harm of opioid use is high. By nominating convergent molecular axes, such as metabolism and neuroimmune signaling, we identify biological pathways that may offer greater cross-ancestry stability than individual single-nucleotide polymorphisms (SNPs), which are frequently limited by population-specific linkage disequilibrium and allele frequency differences^{16,67}. These findings align with recent ethical and technical calls to manage differential PRS performance across diverse groups and to improve multi-ancestry generalizability⁶⁸.

4.5 Limitations

Our findings are constrained by several factors that must be considered during interpretation. First, the cohort demographics showed a mostly male (98.3%) sample, which will limit the generalizability of these results to sex-balanced populations. Second, the modest sample size constrained our statistical power for genome-wide discovery and limited the resolution of our fine-mapping analyses, resulting in diffuse credible sets for our top loci. Third, our phenotypic definitions were necessarily pragmatic; methadone response was treated as a binary variable and age at onset relied on retrospective self-reporting, which may be subject to recall bias²². Finally, the portability of our polygenic risk scores is impacted by the fact that the discovery datasets were derived largely from European-ancestry GWAS, which may have reduced predictive accuracy in this Iranian cohort due to differences in allele frequencies and linkage disequilibrium structure⁶⁸.

4.6 Clinical Implications and Conclusions

In conclusion, these findings provide molecular reinforcement for the longstanding clinical observation that psychiatric distress and shared genetic liability for depression significantly impair the stability and adherence of methadone maintenance therapy. Importantly, our results offer a specific path toward precision pharmacology in OUD. The identification of suggestive loci in *EPHA5* and *HOMER1* points to glutamatergic signaling and synaptic plasticity as prioritized pharmacological targets for optimizing patient stabilization.

Furthermore, the enrichment of metabolic programs near *GLP1R* and *PDE4B* provides a hypothesis-generating translational links for preventive and adjunctive therapies. Given that GLP-1 receptor agonists (e.g., semaglutide) and PDE4 inhibitors are already clinically deployable or under investigation for other indications, they represent promising candidates for reducing opioid craving and preventing relapse in individuals with early-onset vulnerability or treatment resistance. Ultimately, this work provides a multi-

layered genomic framework for personalizing methadone therapy in underrepresented populations by integrating psychiatric liability and molecular signals to predict and optimize treatment trajectories.

DECLARATIONS

Conflict of Interest statement

James L. Kennedy serves on the Scientific Advisory Board of Myriad Neuroscience. James L. Kennedy, Clement C. Zai, Arun K. Tiwari, and Vanessa F. Gonçalves are named inventors on patents related to pharmacogenetic testing for psychiatric medications. James L. Kennedy and Clement C. Zai are also named inventors on a patent related to genetic biomarkers of suicide risk. Clement C. Zai has received an honorarium from Medscape for a review on bipolar disorder genetics. Sara Sadat Afjeh, Yuhan Huo, Fernanda C. Dos Santos, and Mir Davood Omrani declare no competing interests.

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Figures

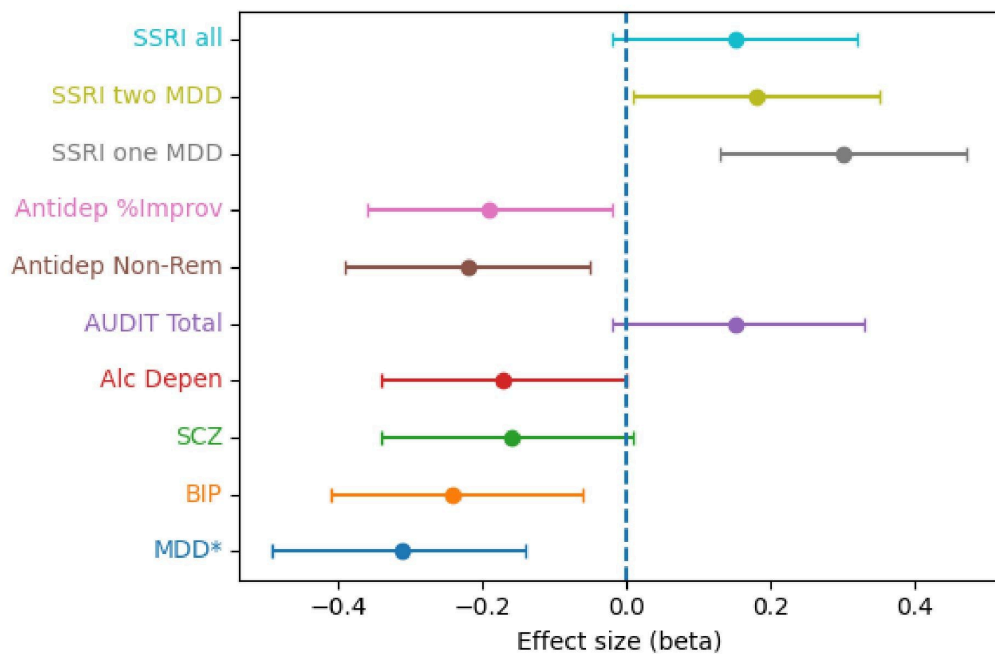


Figure 1 Associations between psychiatric, substance use, and antidepressant response polygenic risk scores and methadone treatment outcome. Forest plot showing standardized regression coefficients (β) and 95% confidence intervals for the association between each PRS and methadone response (responder vs. non-responder). Models were adjusted for sex and 15 ancestry principal components. Positive β values indicate higher genetic liability associated with increased likelihood of methadone response, whereas negative β values indicate increased risk of non-response. The dashed vertical line denotes the null effect ($\beta = 0$). Among all tested PRSs, MDD PRS shows the strongest and most robust negative association with treatment outcome, remaining significant after correction for multiple testing.

Abbreviations: SSRI, selective serotonin reuptake inhibitor; MDD, major depressive disorder; Antidep %Improv, percentage improvement following antidepressant treatment; Antidep Non-Rem, antidepressant non-remission; AUDIT, Alcohol Use Disorders Identification Test; Alc Depen, alcohol dependence; SCZ, schizophrenia; BIP, bipolar disorder; PRS, polygenic risk score; CI, confidence interval.

Figure 1

See image above for figure legend.

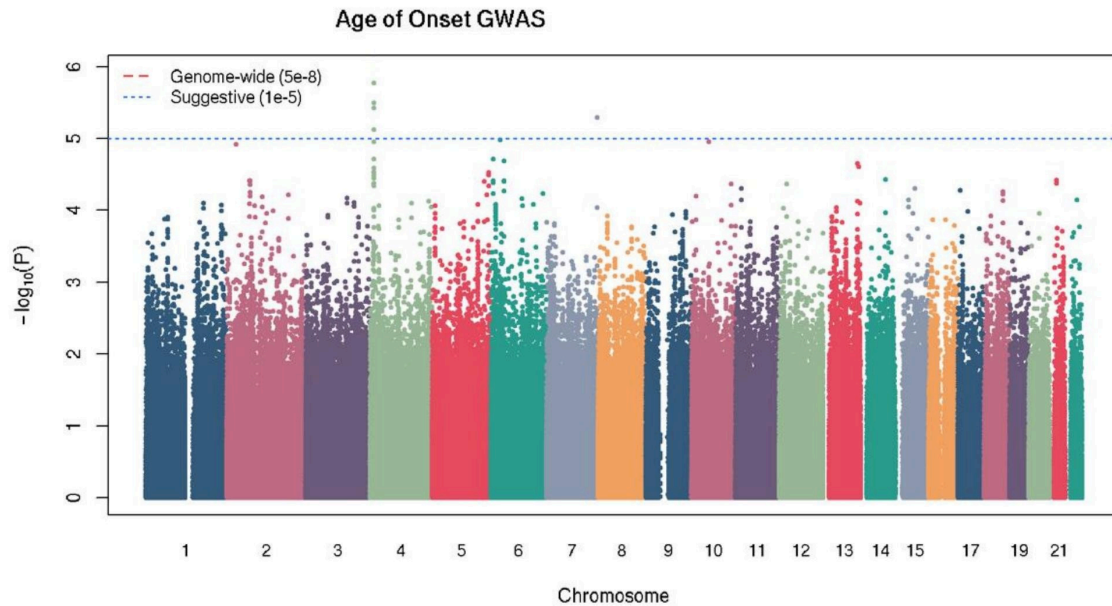


Figure 2 Manhattan plot of the age of onset GWAS highlighting a suggestive noncoding locus on chromosome 4 near *RP11-61G19.1*. Genome-wide association results for age of onset of opioid use ($N = 579$) are shown as $-\log_{10}(p)$ values plotted by chromosomal position (hg19). The blue dashed line indicates the suggestive significance threshold ($p < 5 \times 10^{-5}$). No variants reached genome-wide significance; however, a cluster of suggestive association signals is observed on chromosome 4 ($\approx 10.7\text{--}10.9\text{ Mb}$), with the lead SNP *rs12650576*. Variants in this region map to a region proximal to the long non-coding RNA, locus *LINC02498*.

Figure 2

See image above for figure legend.

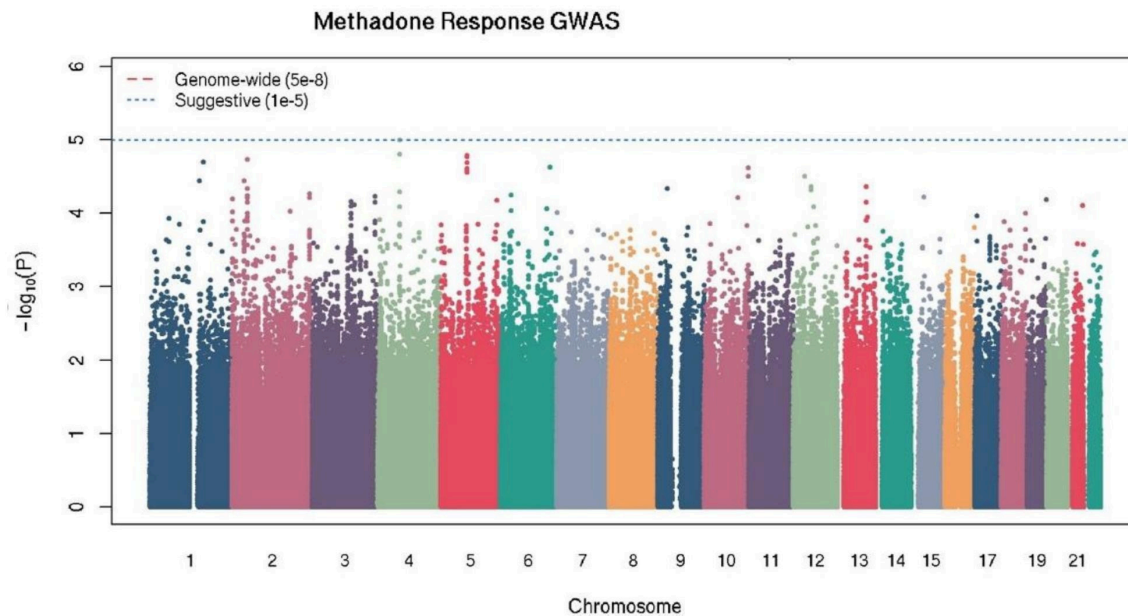


Figure 3 Methadone response GWAS identifies suggestive association signals in synaptic and immune-related loci.

Manhattan plot showing genome-wide association results for methadone treatment response in 627 individuals analyzed under a logistic regression model excluding age and age of onset, with sex and ancestry principal components included as covariates. The x-axis denotes chromosomal position and the y-axis shows $-\log_{10}(p)$ values for 2,312,988 variants. The genome-wide significance threshold ($p = 5 \times 10^{-8}$) and the blue dashed line denotes the suggestive significance threshold ($p < 5 \times 10^{-5}$). The single most significant variant is highlighted in blue and labeled, representing the top suggestive signal in the analysis. No variant reached genome-wide significance.

Figure 3

See image above for figure legend.

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