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# Association of intestinal anti-inflammatory drug target genes with psychiatric Disorders: A Mendelian randomization study

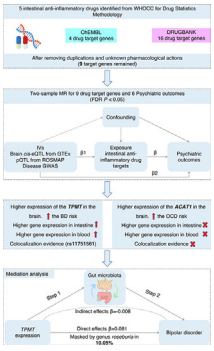
Guorui Zhao [a,1], Zhe Lu [a,1], Yundan Liao [a], Yaoyao Sun [a], Yuyanan Zhang [a], Zhewei Kang [a], Xiaoyang Feng [a], Junyuan Sun [a], Weihua Yue [a,b,c,*]

[a] Peking University Sixth Hospital, Peking University Institute of Mental Health, NHC Key Laboratory of Mental Health (Peking University), National Clinical Research Center for Mental Disorders (Peking University Sixth Hospital), Beijing 100191, China
[b] PKU-IDG/McGovern Institute for Brain Research, Peking University, Beijing 100871, China
[c] Chinese Institute for Brain Research, Beijing 102206, China

## HIGHLIGHTS

- There are comorbidities between inflammatory bowel disease and psychiatric disorders.
- Mendelian randomization explores new targets for treating psychiatric disorders.
- High multi-tissue expression of *TPMT* is associated with an elevated risk of bipolar disorder.
- Mendelian randomization effect supported by colocalization evidence.
- Olsalazine may be a potential drug for the treatment of bipolar disorder.

## GRAPHICAL ABSTRACT



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## ABSTRACT

*Introduction:* Psychiatric disorders present a substantial global public health burden with limited drug options. The gut-brain axis connects inflammatory bowel diseases and psychiatric disorders, which often have comorbidities. While some evidence hints at anti-inflammatory drugs aiding in treating psychiatric conditions, the specific effects of intestinal anti-inflammatory drugs remain unclear.
*Objectives:* This study investigates the causal effect of intestinal anti-inflammatory drug targets on psychiatric disorders. We hypothesize that these drug targets may offer new insights into the treatment and prevention of such disorders. Additionally, we explore gut microbiota's mediating role between drug target genes and psychiatric disorders.
*Methods:* We performed two-sample Mendelian randomization (MR) using summary data from existing expression quantitative trait loci (eQTL) and protein QTL in the brain, along with public genome-wide association studies of disease. We also explored gut microbiota's mediating effect. The statistics encompassed six psychiatric disorders involving 9,725–500,199 individuals. Colocalization analysis enhanced the MR evidence.

*Abbreviations:* ADHD, Attention deficit hyperactivity disorder; ASD, autism spectrum disorder; BD, bipolar disorder; MDD, major depressive disorder; OCD, obsessive-compulsive disorder; SZ, schizophrenia; IBD, inflammatory bowel disease; MR, Mendelian randomization; QTL, gene quantitative trait loci; IVs, instrumental variables; GWAS, genome-wide association study; LD, linkage disequilibrium; GTEx, Genotype-Tissue Expression; SNP, single nucleotide polymorphism; pQTL, protein abundant quantitative trait loci; ROSMAP, Religious Orders Study and Rush Memory and Aging Project; PGC, Psychiatric Genomics Consortium; IVW, inverse-variance weighted; FDR, false-discovery rate; ORs, odds ratios; CIs, confidence intervals; PP.H4, posterior probability for hypothesis 4; RNA-seq, RNA sequencing; mbQTL, quantitative microbiome trait loci; *ACAT1*, acetyl-CoA acetyltransferase; *TPMT*, Thiopurine S-Methyltransferase; *PPAGR*, peroxisome proliferator-activated receptor gamma; *PTGS2*, prostaglandin G/H synthase 2; *ALOX5*, arachidonate 5-Lipoxygenase; *IKBKB*, inhibitor of nuclear factor kappa-B kinase subunit beta; TNF, tumor necrosis factor.

* Corresponding author at: Institute of Mental Health, Peking University Sixth Hospital, No. 51 Hua Yuan Bei Road, Beijing 100191, P.R. China.
*E-mail address:* dryue@bjmu.edu.cn (W. Yue).
[1] These authors contributed equally to this work.

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*Results:* We uncovered a causal link between *TPMT* (a target of olsalazine) expression in the amygdala and bipolar disorder (BD) risk (odds ratio [OR] = 1.08; $P = 4.29 \times 10^{-4}$). This association was observed even when the sigmoid colon and whole blood eQTL were considered as exposures. Colocalization analysis revealed a shared genetic variant (rs11751561) between *TPMT* expression and BD, with a posterior probability of 61.6 %. Interestingly, this causal effect was influenced by a decrease in the gut microbiota abundance of the genus *Roseburia* (effect proportion = 10.05 %). Moreover, elevated *ACAT1* expression was associated with higher obsessive–compulsive disorder risk (OR = 1.62; $P = 3.64 \times 10^{-4}$; posterior probability = 3.1 %).

*Conclusion:* These findings provide novel targets for the treatment of psychiatric disorders, underscore the potential of repurposing olsalazine, and emphasize the importance of *TPMT* and *ACAT1* in future drug development.



## Introduction

Psychiatric disorders such as attention deficit hyperactivity disorder (ADHD), autism spectrum disorder (ASD), bipolar disorder (BD), major depressive disorder (MDD), obsessive–compulsive disorder (OCD) and schizophrenia (SZ) seriously burden global public health [1]. However, the current drug options are limited and often ineffective for many patients [2]. New insights into the treatment and prevention are urgently needed.

Researches have indicated comorbidities between inflammatory bowel disease (IBD) and psychiatric disorders [3,4]. A cross-sectional study found that patients with IBD were more prone to BD than matched comparison patients without IBD [5]. Another population-based study identified MDD as a clinically significant comorbidity of IBD [6]. The presence of the gut-brain axis connects IBD and psychiatric disorders and plays an important role in their mutual influence. Inflammatory immune response is pivotal in the pathogenesis of psychiatric disorders and IBD [7,8]. Limited evidence suggests certain anti-inflammatory drugs may assist in treating psychiatric disorders [9]. However, whether intestinal anti-inflammatory drugs affect psychiatric disorders remains unclear.

The standard approach for determining drug treatment effects involves expensive randomized clinical trials, which may not always be feasible. Two-sample Mendelian randomization (MR) of drug targets offers a cost-effective solution. It aims to establish a causal relationship between the exposure and outcomes, using drug target gene quantitative trait loci (QTL) as instrumental variables (IVs) for exposure and genome-wide association study (GWAS) data on psychiatric disorders as the outcome [10]. The causal inference could be strengthened using colocalization techniques that mitigate the influence of linkage disequilibrium (LD) [11]. Exploring the relationship between intestinal anti-inflammatory drug target genes and psychiatric disorders may reveal potential treatment and prevention targets for these disorders.

Many drugs modulate target proteins' expression, indicating their therapeutic effects [2]. Reduced expression of drug target genes can mirror the inhibitory effect of certain drugs on these proteins [12]. This understanding helps assess their potential impact on psychiatric disorder risk [13], aiding clinicians in informed prescribing decisions, particularly for patients with comorbid IBD.

The gut-brain axis, crucial in neurological and intestinal inflammatory immune responses, involves the gut microbiota [14]. It may mediate between the intestinal anti-inflammatory drugs targets and psychiatric disorders [15]. Analyzing this aspect will help mitigate psychiatric disorder risk and clarify the gene expression-psychiatric disorders mechanism.

In this study, we examined the causal effects of intestinal anti-inflammatory drugs on psychiatric disorders to assess the impact of intestinal anti-inflammatory drug exposure on these disorders. Our hypothesis: "Intestinal anti-inflammatory drug targets may offer new treatment avenues for psychiatric disorders." We also explored gut microbiota's mediating role between drug target genes and psychiatric disorders.

## Methods and materials

The two-sample MR analysis relied on publicly accessible GWAS summary statistics. Supplementary Table S1 summarizes the data sources used in this study. The study design is illustrated in Fig. 1.

### *Ethics statement*

Only publicly available GWAS data were used in this study. Ethical approval and consent to participate were available in the original GWAS study.

### *Selecting intestinal anti-inflammatory drug targets*

We used the Anatomical Therapeutic Chemical Classification System of the World Health Organization Collaborating Center for Drug Statistics Methodology to identify five non-steroidal intestinal anti-inflammatory drugs (Table 1). Subsequently, we identified the target genes of these drugs in DrugBank and ChEMBL databases based on their active ingredients [16,17]. Target genes with unknown pharmacological action in DrugBank were excluded from subsequent analyses (Supplementary Table S2).

### *Gene expression datasets used for genetic instrument selection*

When discussing psychiatric disorders, gene expression in the brain becomes a major focus. Brain eQTL data from all Genotype-Tissue Expression (GTEx version 8) results were retrieved (139–255 subjects with genotypes, Supplementary Table S1) [18]. The GTEx dataset mostly originated from European ancestral individuals (~85 %) [18]. To minimize the potential influence of horizontal pleiotropy, which is more likely to occur during *trans*-effects [19], we exclusively utilized *cis*-eQTLs as IVs. Genetic instruments associated with the expression of intestinal anti-inflammatory drug targets were selected ($P < 1 \times 10^{-04}$). Nine genes that showed significant expression in the brain were identified, and the top single nucleotide polymorphism (SNP) of each gene was used as an instrument.

We also extracted protein-abundant quantitative trait loci (pQTL) summary data from recent studies as exposure (n = 376) [20], generated from the dorsolateral prefrontal cortex of post-mortem brain samples donated by people of European descent who participated in the Religious Orders Study and Rush Memory and Aging Project (ROSMAP) [21]. Variants significantly associated

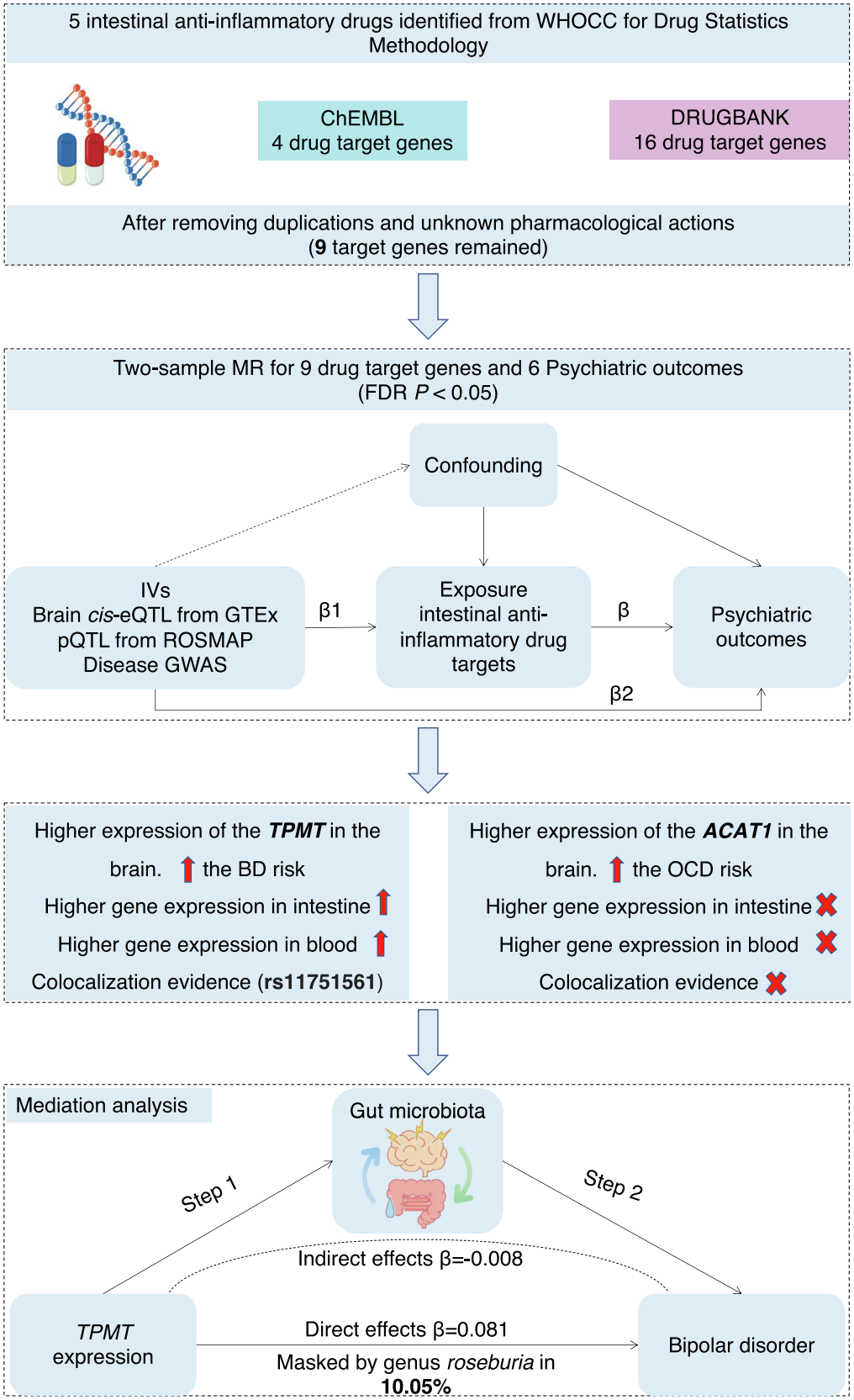


**Fig. 1. Study design and summary results**. β1and β2 denote to the gene–exposure and gene–outcome association, respectively; β represents the causal association between exposure and outcome, where β = β2/β1. BD, bipolar disorder; eQTL, expression quantitative trait loci. GTEx, Genotype-Tissue Expression; GWAS, genome-wide association study; FDR, false-discovery rate; MR, mendelian randomization; IVs, instrumental variables; OCD, obsessive–compulsive disorder; WHOCC, World Health Organization Collaborating Centre.

**Table 1**
Target genes for anti-inflammatory drugs identified using the DrugBank and ChEMBL databases.

| Drug class | Medication Subclass | ATC Code | Drug name | Target Gene(s) | |
|---|---|---|---|---|---|
| | | | | ChEMBL | DrugBank |
| Intestinal anti-inflammatory agents | Antiallergic agents, excl. corticosteroids | A07EB01 | cromoglicic acid | NA | *S100P** |
| | Aminosalicylic acid and similar agents | A07EC01 | sulfasalazine | *PTGS1, PTGS2, ALOX5* | *ALOX5, PTGS2, PTGS1, ACAT1, PLA2G1B, IKBKB, SLC7A11*, NFKB1*, NFKB2*, CHUK*, PPARG** |
| | | A07EC02 | mesalazine | *PTGS1, PTGS2, ALOX5, PPARG* | *PTGS2, PTGS1, ALOX5, PPARG, CHUK*, IKBKB*, NOS2** |
| | | A07EC03 | olsalazine | *PTGS1, PTGS2, ALOX5, PPARG* | *PTGS1, PTGS2, TPMT, IFNG, XDH** |
| | | A07EC04 | balsalazide | *PTGS1, PTGS2, ALOX5, PPARG* | *PPARG, PTGS2, PTGS1, ALOX5* |

Note: ACT code indicates the code of Anatomical Therapeutic Chemical (ATC) classification system. * indicates the unknown pharmacological action. NA, not available.

with the expression of drug target proteins were excluded as genetic instruments ($P < 0.05$).

Furthermore, to avoid weak IV bias, we also calculated the *F*-statistic for each genetic instrument using the SNP-exposure association ($\hat{\gamma}_j$) and the standard error of the SNP-exposure association ($\sigma X_j$) for SNP *j* by the formula Fj = $\hat{\gamma}_j^2$ / $\sigma X_j^2$ [22].

### *Outcome data*

Genetic predictors for psychiatric disorders were obtained from the publicly available GWAS datasets, including ADHD (20,183 cases and 35,191 controls), ASD (18,381 cases and 27,969 controls), OCD (2,688 cases and 7,037 controls), BD (41,917 cases and 371,549 controls) [23], SZ (53,386 cases and 77,258 controls) from the Psychiatric Genomics Consortium (PGC) [24], and MDD (170,756 cases and 329,443 controls) from PGC and UK Biobank (excluding 23andme) [25]. All individuals were of European descent.

### *Mendelian randomization*

Genetic variants encoding protein targets of intestinal anti-inflammatory drugs (*cis*-variants) were identified as genetic IVs for two-sample MR analysis. For genetic instruments with one top SNP and for those with more than one SNP, the Wald ratio analysis method was used, and the inverse-variance weighted (IVW) regression method was used, respectively [26]. All analyses were performed using the *TwoSampleMR* R package [27]. In this analysis, to control for false-positive findings, we applied false discovery rate (FDR) adjustments using the Benjamini-Hochberg method to calculate the adjusted P-values in our main analyses. A significance level of FDR < 5 % was used to determine the statistical significance of the MR effect estimates. Results with a nominal *P* value of less than 0.05, failing the FDR adjustment, were considered suggestive of potential causality [28]. The MR findings were presented as odds ratios (ORs) and their corresponding 95 % confidence intervals (CIs). These ORs reflected the risk of outcomes associated with unit changes in gene expression.

### *Sensitivity analyses*

Based on evidence from MR (FDR < 0.05), we conducted a series of sensitivity analyses: 1) using GTEx pooled data only from the European population; 2) using the gene expression in intestine from GTEx as exposure (sample size from 187 to 406) [18]; 3) using full significant *cis*-eQTL results (FDR < 0.05) in whole blood from the eQTLGen consortium as exposure (N = 31,684) [29]; 4) performing Bayesian colocalization analyses to confirm the results of MR. Associations with a posterior probability for hypothesis 4 (PP.H4) greater than 0.5 were considered indicative of likely colocalization, as this assigns the highest probability to hypothesis 4 being accurate (for detail, see Supplementary methods) [30].

We used single-cell RNA sequencing (RNA-seq) to investigate gene expression levels in specific brain cell clusters [31]. Velmeshev et al. detected gene expression patterns in single cells of the human brain using post-mortem tissues from 16 patients with ASD and 16 healthy controls. By annotating cell clusters based on the expression of known cell-type marker genes, they identified 11 types of neurons and 6 types of glial cells [31].

### *Two-step Mendelian randomization for mediation analysis*

Two-step MR was conducted to detect the potential mediating effects of the gut microbiota using summary data from a GWAS of the gut microbiota in 14,306 individuals of European ancestry [32]. When the expression of the target gene influenced the gut microbiota, which in turn influenced disease risk, we used the "product of coefficients" method [33]. The analysis included 211 taxa (131 genera, 35 families, 20 orders, 16 classes, and 9 phyla) used for quantitative microbiome trait loci (mbQTL) mapping in the two-step MR analyses [32]. Gut microbiota where both steps of MR were significant ($P < 0.05$) and overlapping was regarded as a mediator. A methodological description is provided in the Supplementary methods section.

## Results

### *Causal effects of drug targets on psychiatric outcomes*

MR analyses show that increased Thiopurine S-Methyltransferase (*TPMT*) gene expression in the amygdala is associated with a higher risk of BD (OR = 1.08; 95 % CI, 1.03 to 1.12; nominal $P = 4.29 \times 10^{-4}$; $P_{FDR} = 4.76 \times 10^{-2}$) (Fig. 2 and Supplementary Table S5). Additionally, suggestive associations are observed between higher peroxisome proliferator-activated receptor gamma (*PPARG*) levels in the hypothalamus, higher acetyl-CoA acetyltransferase (*ACAT1*) levels in the cervical spinal cord, lower *PPARG* levels in the cervical spinal cord, and an increased risk of BD.

Higher *ACAT1* levels in the cervical spinal cord were significantly associated with a risk-increasing effect of OCD after adjustment for FDR (OR = 1.62; 95 % CI, 1.24 to 2.10; nominal $P = 3.64 \times 10^{-4}$; $P_{FDR} = 4.76 \times 10^{-2}$). We also found suggestive causal relationships between higher inhibitor of nuclear factor kappa-B kinase subunit beta (*IKBKB*) levels in the caudate basal ganglia, lower *ACAT1* levels in the hippocampus, lower *TPMT* levels in the cervical spinal cord, and decreased risk of OCD (Supplementary Table S7).

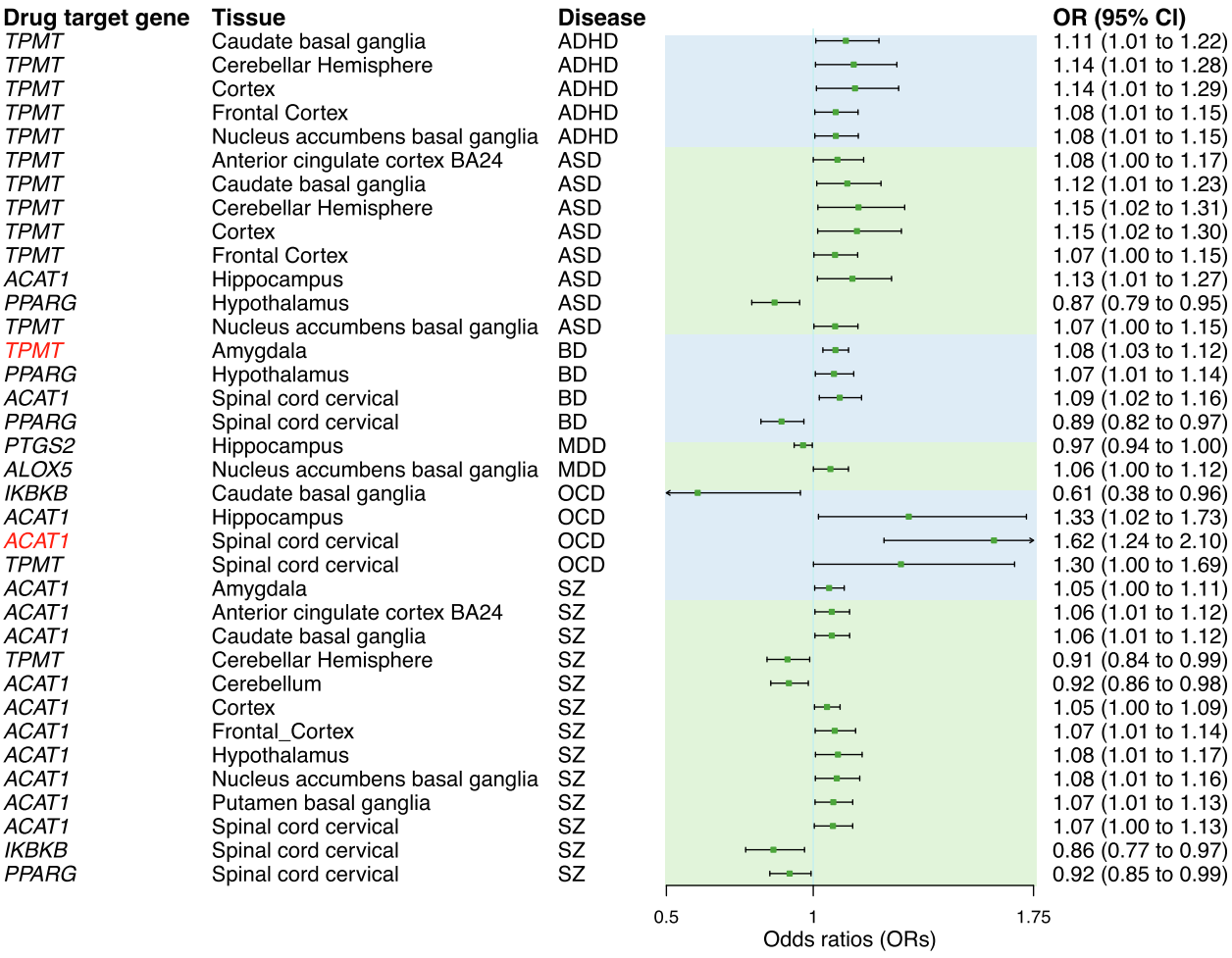


**Fig. 2. Summary suggestively significant results of MR from drug target genes expression and psychiatric disorders.** The highlighted red indicates that FDR correction has been passed. ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; BD, bipolar disorder; CI, confidence interval; MDD, major depressive disorder; MR, Mendelian Randomization; OCD, obsessive–compulsive disorder; SZ, schizophrenia; OR, odds ratio; SNP, single nucleotide polymorphism. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Furthermore, the association of protein abundance from the ROSMAP pQTL with psychiatric disorders did not meet the FDR adjustment criteria (Supplementary Table S9). However, the effects of *TPMT* and *ACAT1* expression in other psychiatric disorders have been widely suggested to be significant (Supplementary Results).

### *Sensitivity analyses*

For significant results with FDR correction, the GTEx sample containing only the European population was used for repeated MR analyses. These results did not differ from those of the main analysis (Supplementary Table S10). As no suitable genetic instruments associated with the expression of *TPMT* or *ACAT1* were found in the transverse colon and small intestine terminal ileum ($P > 1 \times 10^{-04}$), we used gene expression of *TPMT* and *ACAT1* in the sigmoid colon from GTEx as exposure. We found the potential causal effect from *TPMT* expression in sigmoid to BD (OR = 1.08; 95 % CI, 1.02 to 1.15; nominal $P = 0.01$), but the effect of *ACAT1* expression in sigmoid on OCD was not significant (Supplementary Table S11). Moreover, the high expression of *TPMT* in blood similarly indicates a heightened causal risk effect for BD (IVW OR = 1.09; 95 % CI, 1.03 to 1.16; nominal $P = 1.75 \times 10^{-03}$; Supplementary Table S12).

### *Colocalization results for TPMT and ACAT1*

To further ensure the stability of the MR results, we conducted a colocalization analysis between *TPMT* expressed in the amygdala and BD and *ACAT1* expressed in the cervical spinal cord and OCD, respectively. Specifically, we observed an indication of likely colocalization with a posterior probability of 61.6 % of a shared genetic variant (rs11751561) between *TPMT* gene expression and BD, which assigned the highest likelihood of Hypothesis 4 being accurate (Fig. 3A and Supplementary Table S13). Sensitivity analysis of colocalization ensured the robustness of the result (Fig. 3B). However, the posterior probability did not reach "highly likely to colocalize" (PP.H4 > 0.8), probably due to the lack of sufficient samples in GTEx. Additionally, there was a low posterior probability for a common variant in the association between *ACAT1* and OCD (PP.H4 = 3.11 %) (Fig. 3C and 3D).

### *Gene expression analysis by single-cell clusters*

Interestingly, single-cell RNA-seq data showed a similar expression pattern for both *TPMT* and *ACAT1* in the brain, with both genes mainly expressed in excitatory neurons (including upper-layer excitatory neurons, deep-layer corticocortical excitatory projection neurons, and layer 4 excitatory neurons), SV2C expressing

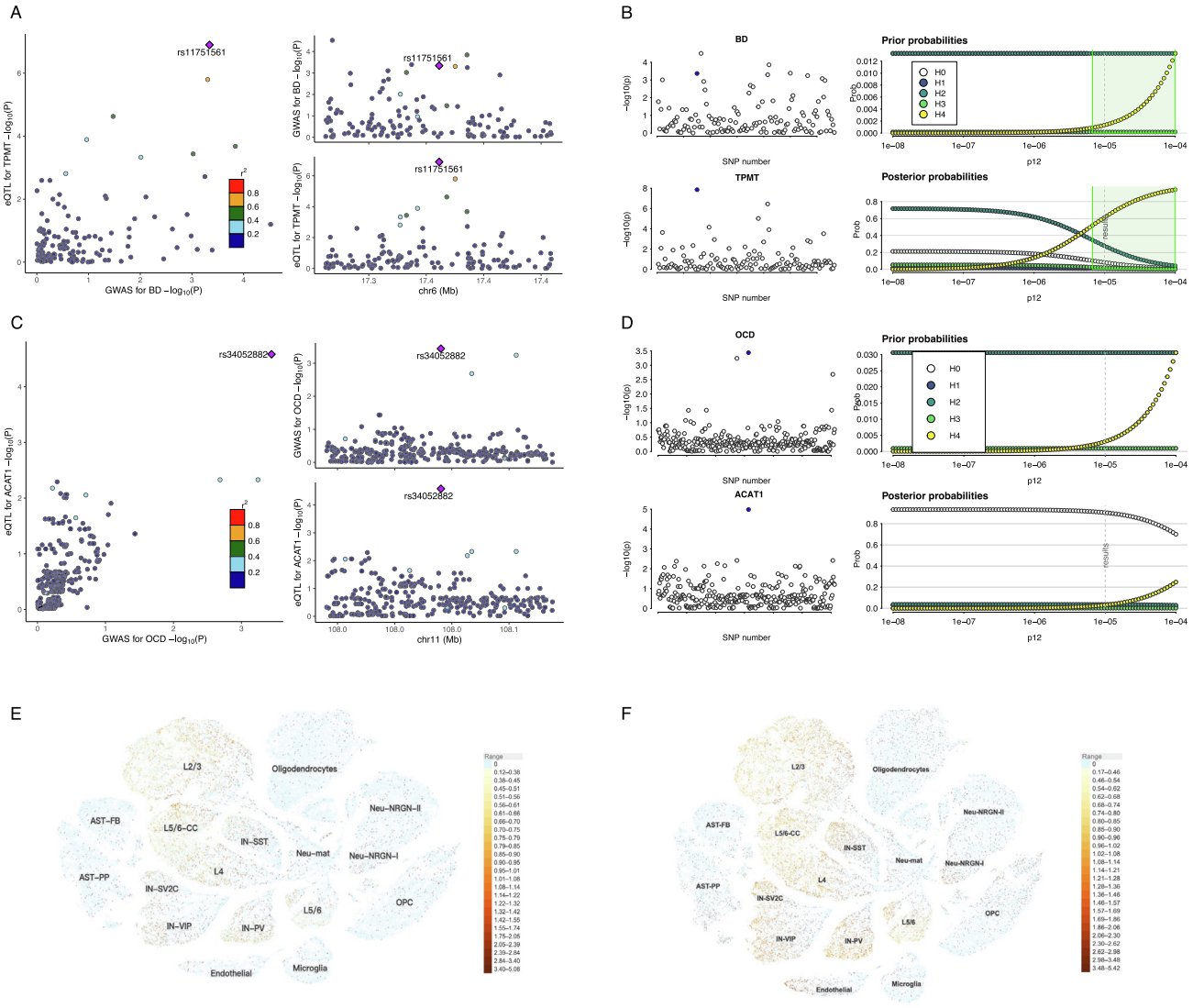


**Fig. 3. A) LocusCompare plots comparing eQTL results for *TPMT* and GWAS for BD.** The plots show as lead SNP that with the highest posterior probability according to coloc and other SNPs are colored according to their LD $r^2$ with the lead SNP. **B) Sensitivity analysis for colocalization of *TPMT* and BD in the rule of H4 > 0.5.** The panels on the left displays local Manhattan plots for the two traits, while the panels on the right illustrate prior and posterior probabilities for H0-H4 as a function of $p_{12}$. The dashed vertical line represents the value of $p_{12}$ used in the initial analysis (the value about which sensitivity is to be checked). In the green region, the conclusion of colocalization looks quite robust. SNP, single nucleotide polymorphism. **C) LocusCompare plots comparing eQTL results for *ACAT1* and GWAS for OCD. D) Sensitivity analysis for colocalization of *ACAT1* and OCD in the rule of H4 > 0.5. E and F) *TPMT* and *ACAT1* expression patterns from single-cell RNA-seq data.** Cell types abbreviations: L2/3 Upper-layer excitatory neurons, AST-FB Fibrous astrocytes, AST-PP Protoplasmic astrocytes, L5/6-CC Deep-layer cortico-cortical excitatory projection neurons, IN-SV2C SV2C expressing interneurons, IN-VIP VIP interneurons, Oligodendrocytes Oligodendrocytes, IN-SST Somatostatin interneurons, L4 Layer 4 excitatory neurons, Neu-mat Immature neurons, L5/6 Deep-layer cortico-subcortical excitatory projection neurons, IN-PV Parvalbumin interneurons, Endothelial endothelial neurons, Microglia microglia, Neu-NRGN-II NRGN expressing neurons, Neu-NRGN-I NRGN expressing neurons, OPC oligodendrocyte precursor cells. BD, bipolar disorder; chr, chromosome; GWAS, genome-wide association study; eQTL, expression quantitative trait loci; OCD, obsessive–compulsive disorder; SNP, single nucleotide polymorphism. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

interneurons, VIP interneurons, and parvalbumin interneurons (Fig. 3E and 3F).

### *Mediation analysis*

To further assess whether the association between drug targets and psychiatric disorders affects gut microbiota abundance, we performed a two-step MR analysis. First, genetic instruments for *TPMT* were used to estimate the causal effect of gene expression on the mediators. Information on the *TPMT* gene instrument was extracted from 207 mediation outcomes, and five significant MR results were found (Supplementary Table S14). These results indicated the higher *TPMT* expression levels in the amygdala may increase the abundance of the genus *Ruminococcus Gnavus* group ($\beta$ = 0.10, $P$ = 0.01), genus *Parasutterella* ($\beta$ = 0.07, $P$ = 0.02), and genus *Eisenbergiella* ($\beta$ = 0.10, $P$ = 0.03), while decreasing the abundance of genus *Roseburia* ($\beta$ = -0.05, $P$ = 0.03) and genus *Allisonella* ($\beta$ = -0.11, $P$ = 0.04) (Fig. 4A). In the second step, gut microbiota with significant MR in the first step were extracted as exposures to assess the causal effect of the mediators on BD risk. We found the increased abundance of genus *Roseburia* was associated with higher BD risk (IVW OR = 1.18; 95 % CI, 1.01 to 1.37; $P$ = 0.04) (Fig. 4B and Supplementary Table S15). To address potential reverse causation, we supplemented the reverse MR analysis with BD as the exposure and found a non-significant reverse effect (Supplementary Table S16). In summary, the mediation analysis revealed a masking effect, wherein the indirect effect (β = -0.008) of genus *Roseburia* showed an opposite sign to the direct effect

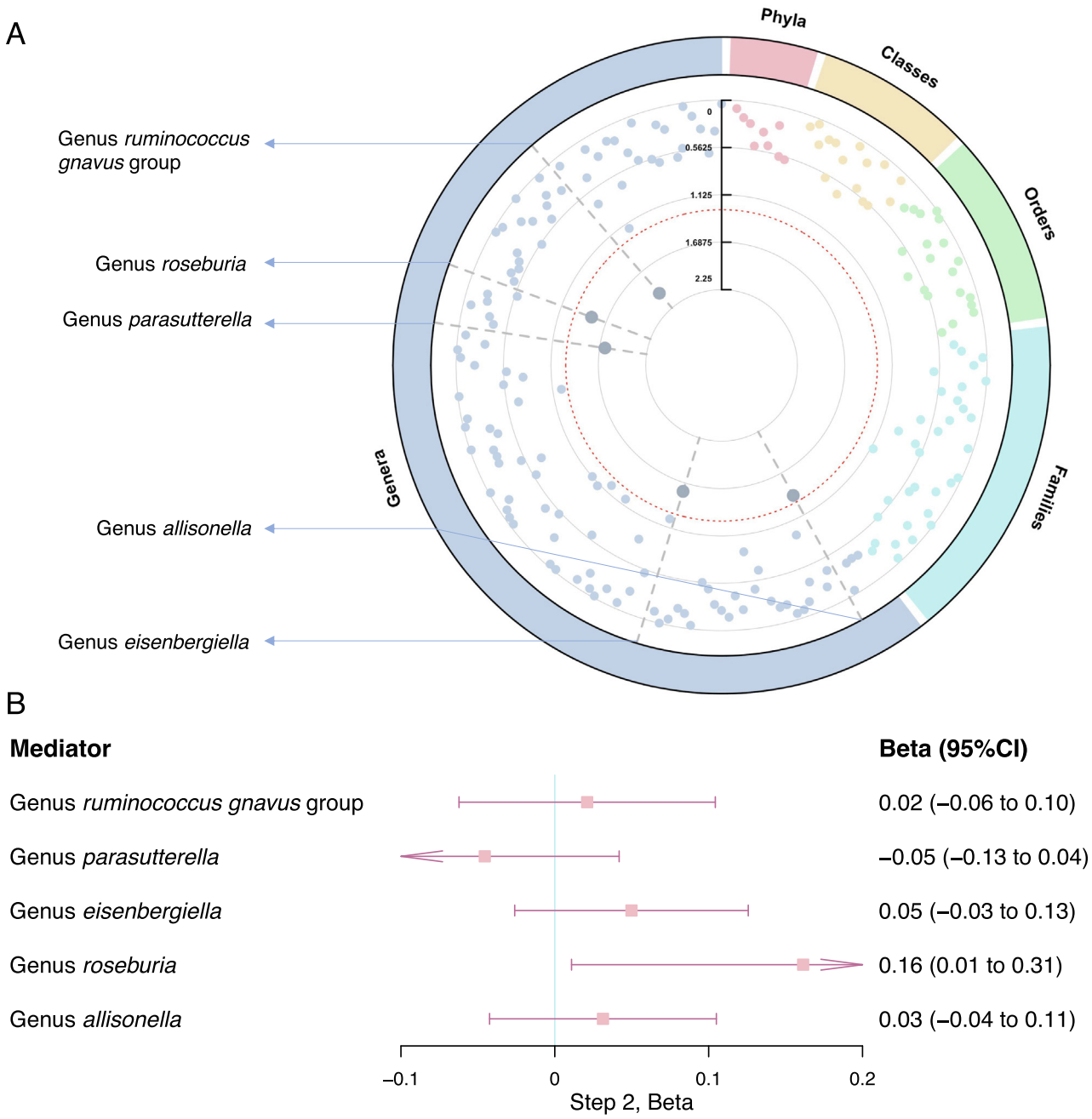


**Fig. 4. A) Circular manhattan plot MR results of *TPMT* expression and 207 taxa gut microbiota.** The red circular dotted line represents the 0.05 threshold. **B) The MR effects of 5 taxa gut microbiota and BD risk.** BD, bipolar disorder; CI, confidence interval; MR, Mendelian Randomization. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(β = 0.081) [34]. However, the masking effect accounted for only 10.05 %, and *TPMT* still exhibited a risk effect on BD (total effect, β = 0.073).

## Discussion

We investigated the effects of genetic variations in intestinal anti-inflammatory drug targets on psychiatric disorder risk using public QTL datasets and GWAS summary data. Evidence indicated that higher *TPMT* expression (target gene of olsalazine) in the amygdala may increase BD risk, with mediation analysis suggesting a masking effect by reduced genus *Roseburia*. At an FDR of < 0.05, we also found that higher *ACAT1* expression in the cervical spinal cord was associated with a higher OCD risk. Our results were robust after sensitivity analyses, and colocalization analysis supported the MR results of *TPMT* gene expression in BD.

Our results indicate that higher *TPMT* expression in the amygdala is causally linked to an increased BD risk. The amygdala, crucial for mood regulation and BD pathophysiology [35], is related to inflammation and immune responses [36]. Previous studies have suggested a negative correlation between tumor necrosis factor (TNF)-α and amygdala functional connectivity [37]. Animal studies

show anti-inflammatory drugs reversing neuroimmune inflammatory responses in BD amygdala mice [38]. Furthermore, the gut-brain axis links the intestine and brain, and *TPMT* expression levels across tissues consistently relate to BD risk. Thus, TPMT emerges as a potential drug target for BD treatment. Additionally, olsalazine, a typical intestinal anti-inflammatory drug, inhibits TPMT [39], making low *TPMT* gene expression levels a potential surrogate indicator of olsalazine's effect [12]. Therefore, we propose olsalazine as a promising therapeutic candidate for BD, especially in cases of comorbid IBD and BD, where it may be more suitable. These findings provide strong genetic evidence for olsalazine repositioning.

ACAT1 is a mitochondria-localized enzyme involved in ketogenesis and ketolysis.[40] Its elevation has been observed in various human cancer cell lines, making it a potential novel target for anticancer therapies [40]. Similarly, psychiatric disorders are often considered metabolic disorders [41]. In our study, high *ACAT1* expression in the cervical spinal cord was associated with an increased OCD risk, though sensitivity analysis results were insignificant. However, notable findings include *TPMT* and *ACAT1* expression levels in different brain regions, suggesting potential causal effects on different psychiatric disorder risks, with most showing a risk effect (OR > 1), though many did not pass the adjustment. This indicates that high expression levels of *TPMT* and *ACAT1* in the brain may be risk factors for various psychiatric disorders.

Single-cell sequencing data analysis suggested the potential roles of *TPMT* and *ACAT1* in the brain. Excitatory neurons across brain layers play a crucial role in the pathogenesis of psychiatric disorders [42]. Loss or dysfunction of parvalbumin interneurons contributes to BD development [43]. VIP interneurons have also been associated with psychiatric disorders [44]. Additionally, these single-cell expression results will help guide cell type selection for subsequent functional experiments.

The microbiota-gut-brain axis regulates interactions between the intestinal and nervous systems [45]. The mediation analysis revealed a masking effect of the genus *Roseburia* between *TPMT* expression levels and BD risk, suggesting a decrease in *Roseburia* abundance attenuates *TPMT*'s impact on BD risk when *TPMT* is highly expressed. However, small-sample observational studies have indicated reduced *Roseburia* in BD [46], conflicting with our second-step MR results. Cross-sectional observational studies may lack evidence of reverse causality [47], and BD onset is not solely mediated by the gut microbiota. One plausible explanation is that following BD onset induced by other factors, there is a decrease in the abundance of *Roseburia*. To address this, we conducted a reverse MR analysis, yielding insignificant but consistent results across all MR methods, which may have decreased the abundance of the *Roseburia* genus. Future studies should focus on long-term longitudinal research to further elucidate the temporal sequence and causal relationship between changes in *Roseburia* abundance and BD onset.

Our study has some limitations. First, we only included a few representative non-steroidal intestinal anti-inflammatory drugs. Second, our results can only predict the on-target effects of drugs; the effects of drugs not passing through the target cannot be inferred. Third, the eQTL cohort sample size was relatively smaller than a typical GWAS, which may limit the confidence in the findings. Fourth, we used only the top SNP as a genetic instrument, potentially reducing the efficiency of the MR Assessment. Therefore, we used stronger IVs ($F > 10$), sensitivity analyses, and colocalization validations. Finally, the genetic variation used for MR has a lifelong effect that does not fully represent short-term drug exposure. Inferring the true effects of drug exposure from MR results is challenging due to factors such as drug dose, duration of exposure, individual metabolic differences, and the capacity to reach relevant tissues (e.g., crossing the blood–brain barrier) [13]. Therefore, further studies are required to determine the effects of intestinal anti-inflammatory drugs on psychiatric disorders.

## Conclusions

Our study identified a causal relationship between elevated *TPMT* expression in the amygdala and a higher risk of BD, supported by multi-tissue MR and colocalization analysis. Higher expression of the *ACAT1* was associated with a higher risk of developing OCD. Mediation analysis of the genus *Roseburia* provided additional insight into the mechanism of *TPMT* in BD. Our findings provide new targets for the treatment of psychiatric disorders and suggest olsalazine as a potential therapeutic candidate for BD. Future research should delve deeper into the mechanisms, efficacy, and safety of olsalazine in BD treatment. Additionally, It is important to consider the roles of *TPMT* and *ACAT1* when developing psychiatric drugs.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jare.2024.05.002.

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