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Uncovering novel drug targets for bipolar disorder: a Mendelian randomization analysis of brain, cerebrospinal fluid, and plasma proteomes

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Abstract

Background. There is a clear demand for innovative therapeutics for bipolar disorder (BD).
Methods. We integrated the largest BD genome-wide association study (GWAS) dataset (N_{Case} = 41 917, $N_{Control}$ = 371 549) with protein quantitative trait loci from brain, cerebrospinal fluid, and plasma. Using a range of integrative analyses, including Mendelian randomization (MR), Steiger filter analysis, Bayesian colocalization, and phenome-wide MR analysis, we prioritized novel drug targets for BD. Additionally, we incorporated data from the UK Biobank (N_{Case} = 1064, $N_{Control}$ = 365 476) and the FinnGen study (N_{Case} = 7006, $N_{Control}$ = 329 192) for robust biological validation.
Results. Through MR analysis, we found that in the brain, downregulation of *DNM3*, *MCTP1*, *ABCB8* and elevation of *DFNA5* and *PDF* were risk factors for BD. In cerebrospinal fluid, increased BD risk was associated with increased levels of *FRZB*, *AGRP*, and *IL36A* and decreased *CTSF* and *LRP8*. Plasma analysis revealed that decreased *LMAN2L*, *CX3CL1*, *PI3*, *NCAM1*, and *TIMP4* correlated with increased BD risk, but *ITIH1* did not. All these proteins passed Steiger filtering, and Bayesian colocalization confirmed that 12 proteins were colocalized with BD. Phenome-wide MR analysis revealed no significant side effects for potential drug targets, except for *LRP8*. External validation further underscored the concordance between the primary and validation cohorts, confirming *MCTP1*, *DNM3*, *PDF*, *CTSF*, *AGRP*, *FRZB*, *LMAN2L*, *NCAM1*, and *TIMP4* are intriguing targets for BD.
Conclusions. Our study identified druggable proteins for BD, including *MCTP1*, *DNM3*, and *PDF* in the brain; *CTSF*, *AGRP*, and *FRZB* in cerebrospinal fluid; and *LMAN2L*, *NCAM1*, and *TIMP4* in plasma, delineating promising avenues to development of novel therapies.

Introduction

Bipolar disorder (BD) has a heritability of up to 70% and is associated with psychosocial impairment (Costanza et al., 2022), with a relapse rate of up to 90% and a suicide rate that is 20 times greater than that of the general population. Although current pharmacological interventions, such as mood stabilizers (e.g. lithium) and antipsychotics, provide symptomatic relief, they have limited efficacy, especially in modulating the hypothalamic–pituitary–adrenal (HPA) axis (Berardelli et al., 2020), and more pronounced side effects, according to the most recent summary of FDA approved BD medications (Goes, 2023) and the DRUGBANK database (https://go.drugbank.com/) (online Supplementary Table S1). Therefore, there is an urgent need for innovative treatments that can strike a balance between efficacy and tolerability. To address these issues, extensive research has explored the genetic and molecular basis of BD through genome-wide association studies (GWASs) and transcriptome-wide association studies. Several genetic loci associated with BD have been identified, such as *NCAM1* (Patel et al., 2010), *ITIH1* (Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011), and *TIMP4* (Lu, Forgetta, Greenwood, Zhou, & Richards, 2023). However, the use of only genomic data and a single tissue source limits the interpretability of these findings. Furthermore, due to the complexity of the disease, a variety of these loci are located in non-coding genomic regions, which makes it difficult to pinpoint the exact biological mechanisms involved and the potential drug targets.

Mendelian randomization (MR), which incorporates genetic instrumental variables, has proven beneficial in determining the causal links between genetic loci and diseases. Compared with other quantitative trait loci (QTLs), protein QTLs (pQTLs) are potential therapeutic targets for BD because they are the end products of gene expression and important participants in biological processes. Recent advances have been made in identifying new drug targets for multiple diseases, including multiple sclerosis (Lin, Zhou, & Xu, 2023), ischemic stroke (Chong et al., 2019), and Alzheimer's disease (Wingo et al., 2021), by combining GWAS and pQTL data. However, the application of this strategy in BD has not yet been fully explored.

Traditional pQTL studies have typically been limited to single tissue sources. In our research, we examined samples from the brain, cerebrospinal fluid (CSF), and plasma of patients with BD. The dorsolateral prefrontal cortex (DLPFC) is a crucial region associated with cognitive and executive impairments in patients with BD (Alonso-Lana et al., 2019). Investigating DLPFC pQTLs is crucial for exploring abnormal brain changes in BD patients and identifying potential drug targets. CSF contains pivotal proteins for information transmission between brain regions, directly reflecting changes in circulating substances within the central nervous system (Tumani, Huss, & Bachhuber, 2017). Recent proteomic analyses of CSF have highlighted proteins related to BD, including *SPOCK1*, *CLEC1B*, *DRAXIN*, and *TNFSF21* (Göteson et al., 2021). Additionally, peripheral fluid, such as plasma, is an important biological indicator with diagnostic and prognostic value in BD, as it can be collected easily with minimal risk (Kim et al., 2021). Therefore, we aimed to integrate the strengths of different studies focusing on the DLPFC, CSF, and plasma to identify tissue-specific druggable proteins associated with BD.

A five-step strategy was adopted to explore the relationship between protein profiles and BD. First, we performed two-sample MR by individually integrating pQTL datasets from the three tissues (brain (Wingo et al., 2021), plasma (Zheng et al., 2020), and CSF (Yang et al., 2021)) with the largest BD GWAS data from Mullins et al. (Mullins et al. 2021) to identify potential candidate proteins associated with BD. Second, sensitivity analysis was conducted to determine the causal direction between the candidate proteins and BD. Third, we applied Bayesian colocalization analysis to examine whether the two related signals (protein profile and BD phenotype) were influenced by a common causal single nucleotide polymorphism (SNP). Then, we conducted phenome-wide MR analysis to screen for possible side effects of the therapeutic targets for BD. To confirm our findings, we performed MR analysis with GWAS data from the UK Biobank and FinnGen cohorts for external validation. Figure 1 illustrates a graphical representation of the research design.

Methods

GWAS summary statistics

Summary statistics of BD were derived from the GWAS study conducted by Mullins et al. (Mullins et al. 2021) for our preliminary analysis. The GWAS meta-analysis included 57 BD cohorts comprising a total of 413 466 individuals (N_{Case} = 41 917, $N_{Control}$ = 371 549). In addition, to confirm our findings, we used two other datasets obtained from the UK Biobank (N_{Case} = 1064, $N_{Control}$ = 365 476) generated by Zhou et al. (Zhou et al. 2018) and the FinnGen study (N_{Case} = 7006, $N_{Control}$ = 329 192) generated by Kurki et al. (Kurki et al. 2023) for external validation.

Human brain, CSF and plasma pQTL data

The pQTL datasets for the brain, CSF and plasma were derived from studies conducted by Wingo et al. (Wingo et al. 2021), Yang et al. (Yang et al. 2021), and Zheng et al. (Zheng et al. 2020), respectively. Specifically, 616 cis-acting brain pQTLs linked to 608 proteins were chosen for our integrative analysis if they met the following criteria: (1) had a statistically significant genome-wide association ($p < 5 \times 10^{-8}$); (2) had linkage disequilibrium clumping ($r^2 < 0.001$); (3) were cis-acting pQTLs; and (4) contained robust SNPs with F-statistics >10. Similarly, the CSF pQTL data contained 233 cis-acting SNPs linked with 214 proteins, and the plasma pQTL data generated 616 cis-acting SNPs linked to 612 proteins. More detailed information on the pQTL and GWAS datasets is summarized in online Supplementary Table S2.

MR analysis

In this study, the SNPs linked to pQTLs in the brain, CSF, and plasma acted as exposure variables, and the BD GWAS data as the outcome variable. We performed MR analysis using the R package 'TwoSampleMR' (https://github.com/MRCIEU/TwoSampleMR) (Hemani et al., 2018). The Wald ratio approach was employed when a protein had only one pQTL; otherwise, the inverse variance weighted (IVW) analysis method was used. Bonferroni correction was used to adjust the p value (0.05/608 for brain, 0.05/214 for CSF, and 0.05/612 for plasma).

Sensitivity analysis

Due to the limited number of instrumental variables (IVs), post-MR analysis methods such as Cochran's Q test, MR-Egger intercept test, MRPRESSO test, and I^2GX test were unable to perform (Gleason, Yang, & Chen, 2021; Hemani et al., 2018). Therefore, we utilized Steiger filtering to explore the causal direction between proteins and BD. A p value less than 0.05 indicated statistical significance.

Bayesian colocalization analysis

We performed Bayesian colocalization analysis using the R package 'COLOC' to determine whether the risk of BD and the changes in protein levels were attributed to the same single-nucleotide variations. Bayesian colocalization analysis calculated posterior probabilities for the following five crucial hypotheses: H0, neither associated with GWAS nor pQTL; H1, association with GWAS, not with pQTL; H2, association with pQTL, not with GWAS; H3, association with GWAS and pQTL, two independent SNPs; and H4, association with GWAS and pQTL, one shared SNP. A threshold of 0.80 for the probability of hypothesis H4 indicated strong evidence for colocalization (Zheng et al., 2020).

Phenome-wide MR analysis

To elucidate the potential side effects (beneficial or adverse) of prior drug targets identified by aforementioned analyses, we performed an agnostic phenome-wide MR analysis of 783 disease traits. The SNPs linked to proteins were set as exposures (online Supplementary Table S3), and summary statistics of diseases from

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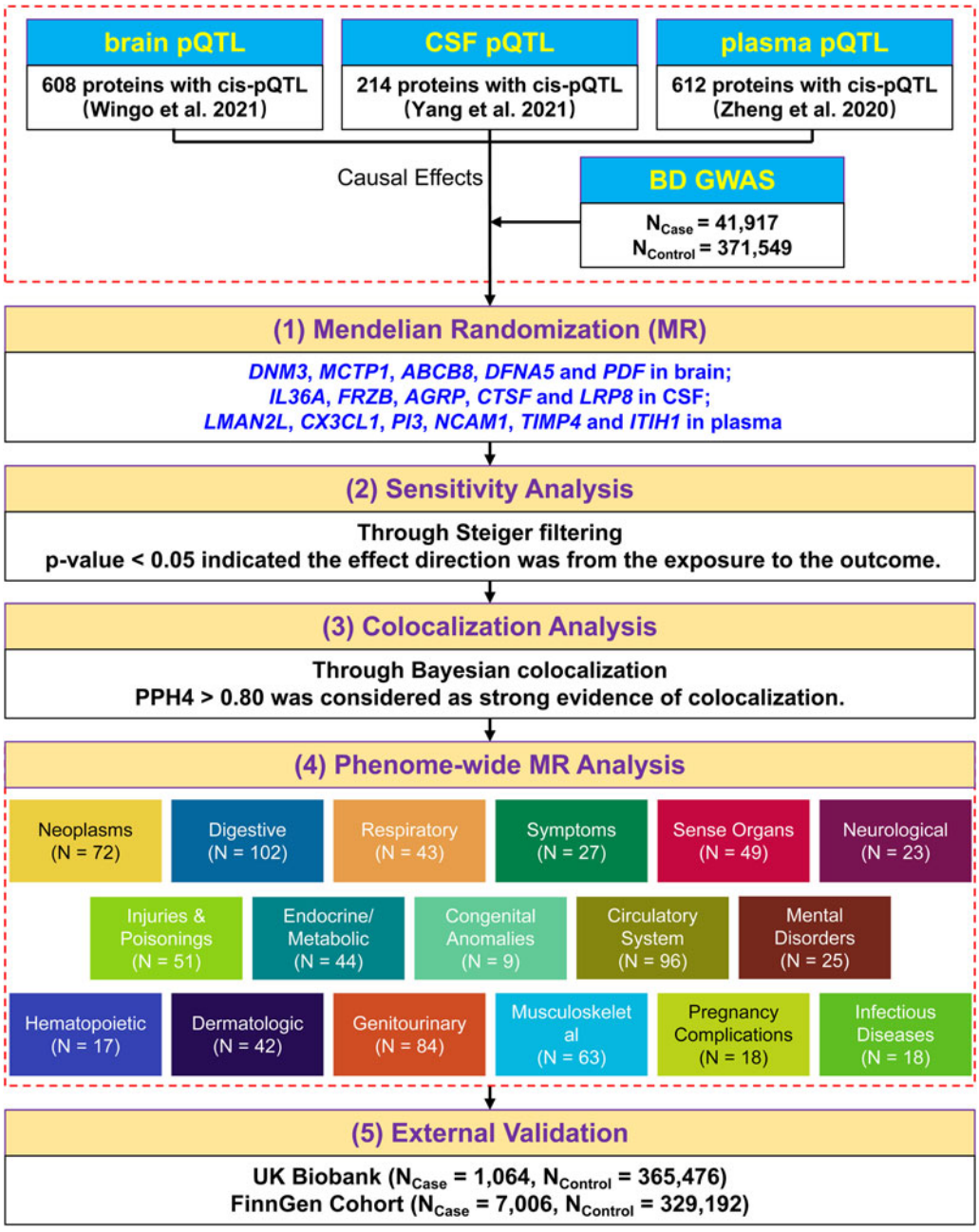

Figure 1. A schematic overview of the study design.

the UK Biobank cohort were set as outcomes. The Scalable and Accurate Implementation of Generalised Mixed Model (SAIGE V.0.29) method was applied to address the unbalanced case–control ratio (Zhou et al., 2018). To improve the interpretability of the results, we systematically selected 783 representative phenotypes for phenome-wide MR analysis, with more than 500 disease cases. Phenome-wide MR analysis was conducted using the same parameters as those used in the MR analysis. Bonferroni correction was applied ($0.05/(4 \times 783) = 1.60 \times 10^{-5}$).

External validation

MR analysis was repeated only on the preliminarily identified proteins with the same-variant and significant-variant strategies and GWAS data from the UK Biobank and FinnGen datasets, respectively. The concordance of the odds ratios (ORs) between the primary and validation cohorts was considered successful, and therefore, a p value less than 0.05 was considered strong evidence of replication.

Results

Screening the three proteomes for BD causal proteins

At different Bonferroni significance levels ($p < 8.22 \times 10^{-5}$, 0.05/608 for brain; $p < 2.34 \times 10^{-4}$, 0.05/214 for CSF; $p < 8.17 \times 10^{-5}$, 0.05/612 for plasma), MR analysis revealed that the altered protein abundances of 16 genes were associated with BD

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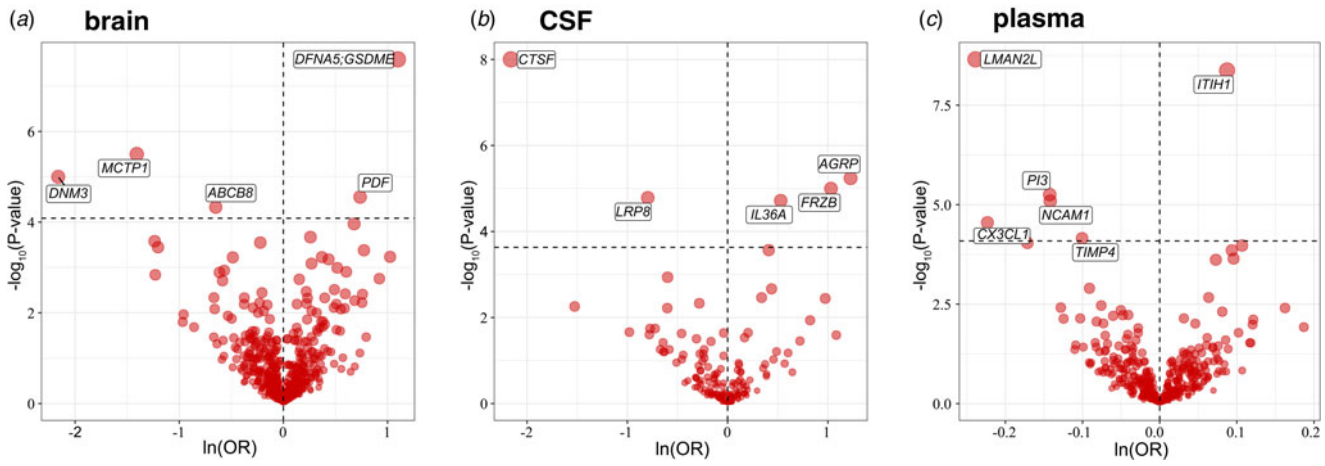

Figure 2. Volcano plot of the MR analysis results for proteins of three tissues. The horizontal dashed line indicates the diverse Bonferroni significance threshold ($p < 8.22 \times 10^{-5}$ in the brain, $p < 2.34 \times 10^{-4}$ in CSF, and $p < 8.17 \times 10^{-5}$ in plasma). The vertical dashed lines distinguish the effect direction. The proteins that passed the Bonferroni significance threshold are labeled with their names.

(Fig. 2a–2c), including *DNM3*, *MCTP1*, *ABCB8*, *DFNA5,* and *PDF* in the brain; *IL36A*, *FRZB*, *AGRP*, *CTSF,* and *LRP8* in CSF; and *LMAN2L*, *CX3CL1*, *PI3*, *NCAM1*, *TIMP4,* and *ITIH1* in plasma. In summary, decreased *DNM3*, *MCTP1*, *ABCB8*, *CTSF*, *LRP8*, *LMAN2L*, *CX3CL1*, *PI3*, *NCAM1,* and *TIMP4* were risk factors for BD, whereas elevated *DFNA5*, *PDF*, *IL36A*, *FRZB*, *AGRP,* and *ITIH1* increased the risk of BD (Fig. 2 and Table 1). We also observed that in plasma, *CTSF* (OR 0.84, 95% CI 0.77–0.92; $p = 9.14 \times 10^{-5}$) and *AGRP* (OR 1.11, 95% CI 1.05–1.17; $p = 1.06 \times 10^{-4}$) reached marginal significance for causal effects, and their effect directions were consistent with those in CSF (not shown in the figures and tables).

Sensitivity analysis for causal proteins

Although the paucity of IVs constrained the possible sensitivity analyses, we verified the directionality of the causal effects via Steiger filtering. All the proteins detected by MR satisfied the Steiger filtering criterion (Table 1), implying that the genetic variants had stronger associations with the exposure than with the outcome and were suitable for instrumental variables to avoid reverse causality.

Bayesian colocalization analysis of BD causal proteins

To confirm whether the associations between BD and pQTLs discovered by MR analysis were driven by a shared causal SNP, we performed Bayesian colocalization analysis, and the results are shown in Table 1. In the brain, *DFNA5*, *MCTP1*, *DNM3,* and *PDF* had the same genetic variants associated with both BD and protein abundance. In CSF, *CTSF*, *AGRP*, *FRZB,* and *LRP8* passed the Bayesian colocalization analysis, while *LMAN2L*, *NCAM1*, *CX3CL1,* and *TIMP4* were successfully validated in plasma.

Phenome-wide MR analysis of BD prior druggable genes

We conducted phenome-wide MR analysis of 783 non-BD diseases or traits in the UK Biobank to comprehensively characterize the side effect profiles of drug targets. Using the Wald ratio method, we detected no significant association for any potential drug target except for *LRP8* ($p < 1.60 \times 10^{-5}$, $0.05/(4 \times 783)$) (Fig. 3). Decreased *LRP8* expression was a risk factor for schizophrenia and other psychotic disorders (OR 0.013, 95% CI 0.002–0.091; $p = 9.28 \times 10^{-6}$).

External validation of BD causal proteins

We conducted a replication two-sample MR analysis utilizing the same-variant and significant-variant strategies for the UK Biobank and FinnGen cohorts, which included sufficient data to perform two-sample MR analysis for the 16 proteins we identified. For all 16 candidate proteins, the directional effects of 13 (81.25%) proteins were concordant between the primary and validation cohorts. Furthermore, the results for 5 proteins were replicated at $p < 0.05$ (Fig. 4a and 4b), among which *CTSF* was confirmed in both datasets. The results for other proteins were replicated in one dataset, such as *AGRP*, *PI3,* and *ITIH1* in UK Biobank and *LRP8* in FinnGen. From the perspective of tissues, *LRP8*, *AGRP*, *CTSF* in CSF; *PI3,* and *ITIH1* in plasma showed strong evidence of replication (Table 2 and Fig. 4).

Discussion

In the present study, we employed a five-step strategy that leveraged the strengths of various analyses to integrate proteomic data with BD GWAS and identified a total of 9 proteins that confer BD risk, including upregulated *PDF*, downregulated *MCTP1* and *DNM3* in the brain; upregulated *AGRP* and *FRZB*, downregulated *CTSF* in CSF; and downregulated *LMAN2L*, *NCAM1*, *TIMP4* in plasma. Risk proteins in plasma and CSF may be able to aid in the diagnosis of BD and the development of drugs targeting peripheral body fluid due to the availability of samples during the clinical process, while brain-specific risk proteins may be used to determine the molecular mechanisms of BD because of their presence in *in situ* lesions. Phenome-wide MR analysis was used to examine the safety and tolerability of the identified protein targets. Moreover, to explore their potential as clinical drug targets, we collected data on current medications targeting these causal proteins from the DRUGBANK database (https://go.drugbank.com/) (online Supplementary Table S4).

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Table 1. MR, Steiger filtering analysis and Bayesian colocalization analysis results for proteins significantly associated with BD

Tissue	Exposure	Chr	Uniprot ID	Method	Beta	Se	*p* value	OR	OR(95% CI)	Colocalization PPH4	Steiger filtering analysis (*p*)
brain	*DFNA5*	7	O60443	Wald ratio	1.10	0.20	2.58×10^{-8}	3.00	2.04–4.42	0.995[a]	1.81×10^{-12}
brain	*MCTP1*	5	Q6DN14	Wald ratio	−1.41	0.30	3.17×10^{-6}	0.24	0.14–0.44	0.950[a]	9.86×10^{-9}
brain	*DNM3*	1	Q9UQ16	Wald ratio	−2.16	0.49	1.00×10^{-5}	0.12	0.04–0.30	0.928[a]	9.57×10^{-8}
brain	*PDF*	16	Q9HBH1	Wald ratio	0.74	0.18	2.80×10^{-5}	2.09	1.48–2.95	0.815[a]	9.87×10^{-9}
brain	*ABCB8*	7	Q9NUT2	Wald ratio	−0.65	0.16	4.68×10^{-5}	0.52	0.38–0.71	0.767	6.28×10^{-10}
CSF	*CTSF*	11	Q9UBX1	Wald ratio	−2.16	0.38	9.95×10^{-9}	0.12	0.06–0.24	0.962[a]	3.04×10^{-7}
CSF	*AGRP*	16	O00253	Wald ratio	1.23	0.27	5.75×10^{-6}	3.41	2.01–5.79	0.830[a]	3.20×10^{-9}
CSF	*FRZB*	2	Q92765	Wald ratio	1.03	0.23	1.01×10^{-5}	2.80	1.77–4.43	0.966[a]	3.50×10^{-13}
CSF	*LRP8*	1	Q14114	Wald ratio	−0.80	0.18	1.64×10^{-5}	0.45	0.31–0.65	0.905[a]	2.68×10^{-17}
CSF	*IL36A*	2	Q9UHA7	Wald ratio	0.53	0.12	1.92×10^{-5}	1.70	1.33–2.17	0.000362	5.92×10^{-79}
plasma	*LMAN2L*	2	Q9H0V9	Wald ratio	−0.24	0.04	2.22×10^{-9}	0.79	0.73–0.85	0.924[a]	1.25×10^{-21}
plasma	*ITIH1*	3	P19827	Wald ratio	0.09	0.01	4.23×10^{-9}	1.09	1.06–1.12	0.012	7.15×10^{-170}
plasma	*PI3*	20	P19957	Wald ratio	−0.14	0.03	5.57×10^{-6}	0.87	0.82–0.92	0.004	2.95×10^{-33}
plasma	*NCAM1*	11	P13591	Wald ratio	−0.14	0.03	8.14×10^{-6}	0.87	0.82–0.92	0.872[a]	1.18×10^{-51}
plasma	*CX3CL1*	16	P78423	Wald ratio	−0.22	0.05	2.82×10^{-5}	0.80	0.72–0.89	0.969[a]	3.71×10^{-11}
plasma	*TIMP4*	3	Q99727	Wald ratio	−0.10	0.03	7.00×10^{-5}	0.90	0.86–0.95	0.840[a]	3.31×10^{-51}

All SNPs used were cis-acting. Abbreviation: MR, Mendelian randomization; CSF, cerebrospinal fluid; BD, bipolar disorder; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval.
[a]The results of PPH4 are over 0.80, which indicates that a genetic variant is shared by both traits of the protein level and BD.

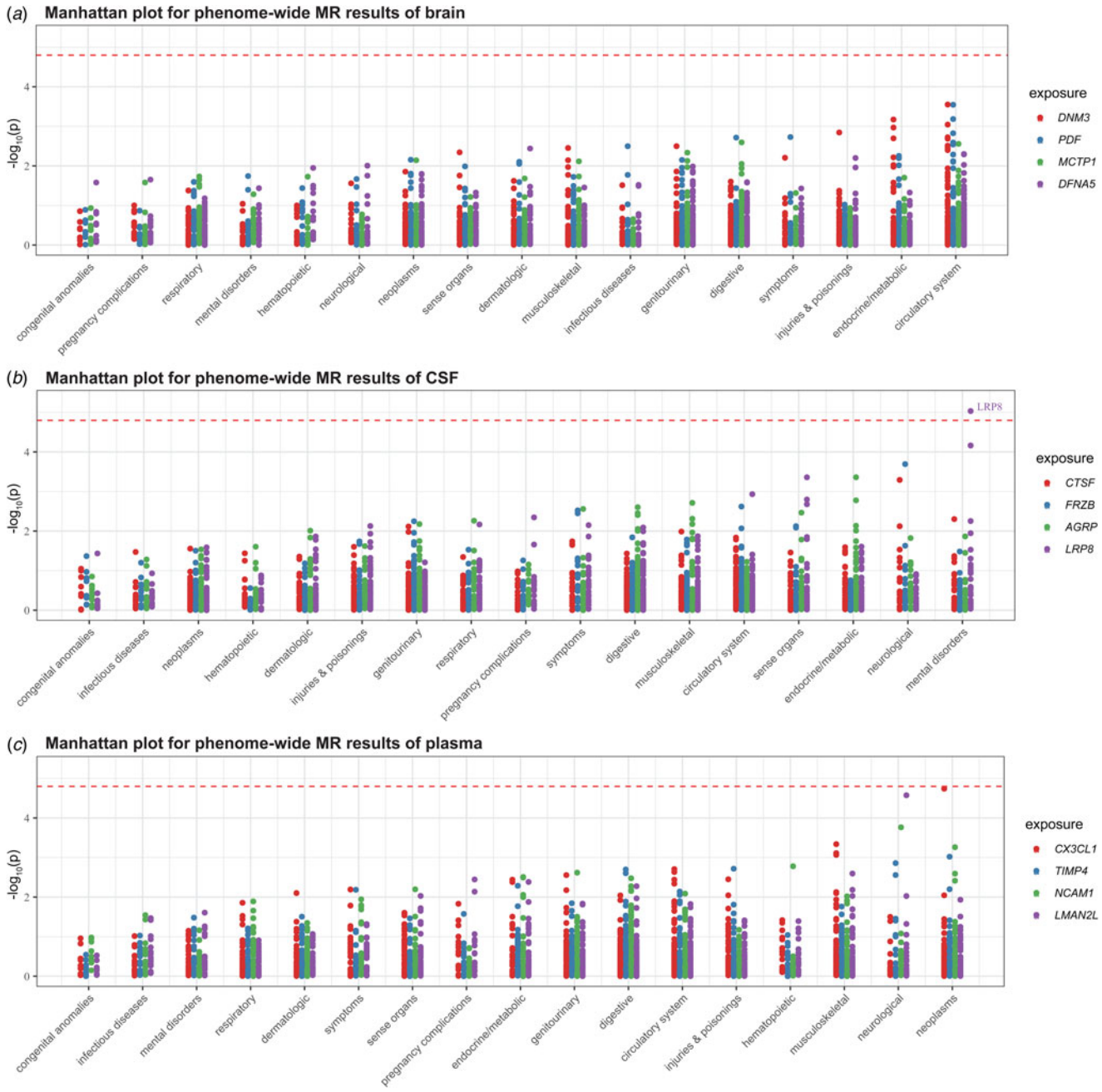

Figure 3. Manhattan plot for phenome-wide MR results of three tissues. To enhance visualization, the y-axis shows $-\log_{10}(p)$. A dot represents a disease trait, and different colors represent the MR results of different proteins. Only Bonferroni-significant disease associations are illustrated ($p < 0.05/(4 \times 783) = 1.60 \times 10^{-5}$).

In brain tissue, we identified upregulated *PDF* and downregulated *MCTP1* and *DNM3* as risk factors for BD, among which *PDF* and *DNM3* were reported for the first time in this disease. *PDF* is a peptide release factor that regulates mitochondrial protein synthesis by removing the N-terminal formyl group (Bögeholz, Mercier, Wintermeyer, & Rodnina, 2021). Impaired mitochondrial pathways are an important hallmark of BD patients (Cuperfain, Zhang, Kennedy, & Goncalves, 2018). However, the association between mental disorders and *PDF* has not been investigated, and further studies are warranted to elucidate its mechanism. *DNM3* encodes a dynamin protein involved in synaptic vesicle endocytosis (Raimondi et al., 2011). A recent exon-focused GWAS reported the association of *DNM3* with obsessive-compulsive disorder and schizophrenia (Costas et al., 2016), suggesting that this gene may cause cross-disorder risk. The BD risk gene *MCTP1* (Scott et al., 2009) is a transmembrane protein that regulates calcium ion binding activity. Basic research has shown that dysregulation of *MCTP1* might cause altered synaptic vesicle recycling and oxidative stress resulting from glutamate toxicity (Qiu, Yu, & Liang, 2015). This evidence supports its connection with BD risk. Interestingly, both *MCTP1* and *DNM3* are involved in the regulation of synaptic vesicles, and the dysregulation of synaptic proteins in the DLPFC has been extensively discussed in BD patients (Aryal et al., 2023). Therefore, mitochondrial pathways and synaptic vesicle-related pathways are important directions for future research on the molecular mechanisms of BD pathogenesis.

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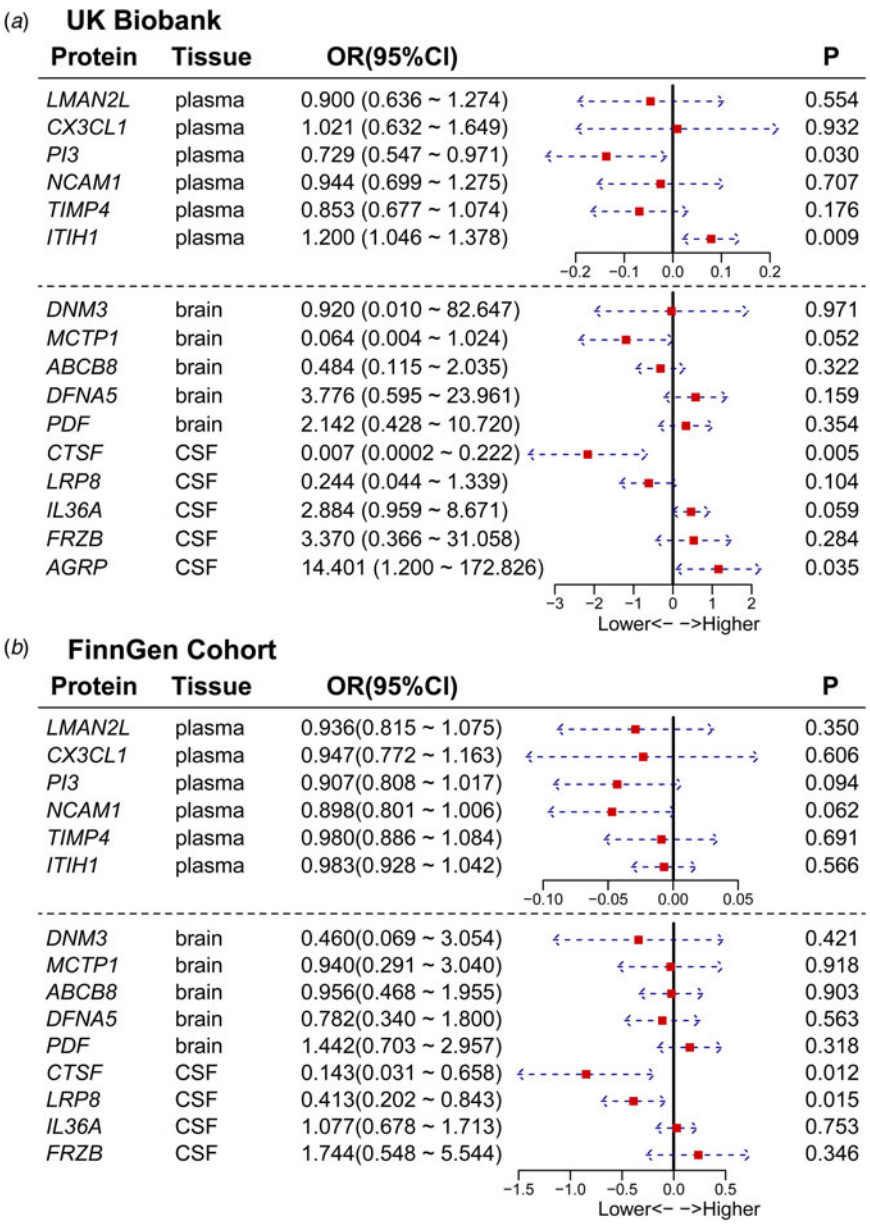

Figure 4. Forest plot of the results of external validation. The figure shows the effect size and 95% confidence interval of the MR analysis for proteins on BD risk. To facilitate the visualization of the OR forest plot results, we log10-transformed the OR values.

For cerebrospinal fluid, we found that upregulated *AGRP* and *FRZB*, downregulated *CTSF* increased BD risk. *AGRP*, a biased agonist of melanocortin receptors coexpressed with neuropeptide Y and gamma-aminobutyric acid (GABA) in the hypothalamus, mediates neuroendocrine responses to immune (Boutagouga Boudjadja et al., 2022) and inflammatory regulation (Klima et al., 2023; Xiao, Xia-Zhang, Vulliemoz, Ferin, & Wardlaw, 2003) by *AGRP* neurons. A previous case–control study reported that AGRP levels were higher in euthymic bipolar disorder patients than in healthy controls (Özkorumak Karagüzel et al., 2018), which is consistent with our findings. Currently, there are no drugs targeting *AGRP* (online Supplementary Table S4). Hence, drug development targeting *AGRP* may be promising, and future studies are needed to clarify the specific pathogenic mechanism and the potential of using *AGRP* as a clinical drug target. *FRZB* is a known secreted Wnt antagonist (Leyns, Bouwmeester, Kim, Piccolo, & De Robertis, 1997), and the Wnt signaling pathway is closely involved in the microenvironment of the central nervous system by regulating blood-brain barrier homeostasis (Liebner, Dijkhuizen, Reiss, Plate, & Agalliu, 2018). *CTSF* is a cysteine protease that is involved in the lysosomal protein degradation system (Turk, Turk, & Turk, 2000). A lack of *CTSF* causes lysosomal substrate degradation disorders, leading to neurological lysosomal storage diseases (Berkovic et al., 2019; Tang et al., 2006). A previous GWAS reported that *CTSF* was located in the linkage disequilibrium region of the BD risk locus rs10896135, with an OR of 0.89 (Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011), which is consistent with our findings that downregulated *CTSF* may confer BD risk. Notably, strong evidence of *CTSF* replication was obtained in two different independent validation cohorts, suggesting that *CTSF* is a high-confidence risk gene for BD. We found that proteins dysregulated in CSF mainly affect the homeostasis of the nervous system and neuroendocrine system, which not only suggests the importance of body fluid to internal homeostasis but also reveals the value of these proteins as biomarkers of disease.

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Table 2. Summary results of five-step stragety of BD associated genes

Tissue	Gene	Mendelian randomization	Sensitivity analysis	Bayesian colocalization analysis[a]	Phenome-wide MR analysis	External validation[c]
brain	*DFNA5*	Yes	Yes	Yes	Yes	No
brain	*MCTP1*	Yes	Yes	Yes	Yes	Yes
brain	*DNM3*	Yes	Yes	Yes	Yes	Yes
brain	*PDF*	Yes	Yes	Yes	Yes	Yes
brain	*ABCB8*	Yes	Yes	No	–[e]	Yes
CSF	*CTSF*	Yes	Yes	Yes	Yes	Yes[d]
CSF	*AGRP*	Yes	Yes	Yes	Yes	Yes[d]
CSF	*FRZB*	Yes	Yes	Yes	Yes	Yes
CSF	*LRP8*	Yes	Yes	Yes	No[b]	Yes[d]
CSF	*IL36A*	Yes	Yes	No	–[e]	Yes
plasma	*LMAN2L*	Yes	Yes	Yes	Yes	Yes
plasma	*ITIH1*	Yes	Yes	No	–[e]	No
plasma	*PI3*	Yes	Yes	No	–[e]	Yes
plasma	*NCAM1*	Yes	Yes	Yes	Yes	Yes
plasma	*CX3CL1*	Yes	Yes	Yes	Yes	No
plasma	*TIMP4*	Yes	Yes	Yes	Yes	Yes

Abbreviation: MR, Mendelian randomization; BD, bipolar disorder.
[a]Values above 0.80 were considered as strong evidence of colocalization.
[b]Potential drugs targeting *LRP8* showed a potential curable effect on schizophrenia and other psychotic disorders.
[c]The consistent directionality of OR values between primary and validation cohorts were considered as successful replication.
[d]These proteins passed Bayesian colocalization analysis and were not only consistent in the directionality of OR values between primary and both validation cohorts, but also had *p* values less than 0.05 in at least one dataset, which were considered as strong evidence of replication.
[e]The phenome-wide MR analysis was limited to proteins exhibiting strong colocalization.

In plasma, downregulation of BD risk genes *LMAN2L* (Chen et al., 2013; Lim et al., 2014; Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011), *NCAM1* (Arai et al., 2004; Atz, Rollins, & Vawter, 2007; Ayalew et al., 2012), and *TIMP4* (Lu et al., 2023) results in increased disease risk. *NCAM1*, also known as *CD56*, is a neural cell adhesion molecule involved in synaptic plasticity, neurodevelopment, and neurogenesis (Kiss & Muller, 2001). It regulates inflammatory cascades mediated by mitogen-activated protein kinase (*MAPK*) (Krushel, Tai, Cunningham, Edelman, & Crossin, 1998) and nuclear factor-κB ($NF-\kappa B$) (Krushel, Cunningham, Edelman, & Crossin, 1999) that are activated by neuroinflammatory environments. A clinical study revealed that the level of *NCAM1* in whole blood is related to the severity of BD (Jesudas, Nandeesha, Menon, & Allimuthu, 2020). *NCAM1* is also considered a common causative gene of schizophrenia and BD (Ayalew et al., 2012), and a recent genome-wide pleiotropic analysis identified *NCAM1* as a shared potential pleiotropic locus in gastrointestinal diseases and psychiatric diseases, including BD (Gong et al., 2023). These findings highlight the outstanding potential of *NCAM1* as a disease marker. *TIMP4* is an important inhibitor of matrix metalloproteinases, and the latter is closely related to blood-brain barrier damage caused by neuroinflammation (Han & Jiang, 2021). A previous MR study proposed that an increase in the genetically predicted circulating *TIMP4* level was associated with a reduced risk of BD (minimum OR 0.88, 95% CI 0.82–0.94) (Lu et al., 2023) *LMAN2L* encodes a lectin mannose-binding protein that mediates the trafficking and secretion of glycoproteins in the endoplasmic reticulum. *LMAN2L* may promote the trafficking of neuroreceptors under endoplasmic reticulum stress conditions (Fu, Zhang, & Mu, 2019; Qin et al., 2012). Similar to those in CSF, dysregulated proteins in plasma are mostly related to homeostasis imbalances caused by inflammatory stimuli and endoplasmic reticulum stress. These studies corroborated our conclusion that circulating protein levels have the potential to be an auxiliary diagnostic instrument for disease.

We acknowledge some limitations of our study. First, the proteins we identified in different tissues were distinct, which may be attributed to the following reasons: (1) pQTLs are specific to different tissues. (2) Due to the restriction of sample sources in the original studies, the MR analysis included limited number of pQTLs. However, we still found that *CTSF* and *AGRP*, which were identified in CSF, showed a marginal causal effect in plasma, and their effect directions in both tissues were concordant. Second, our analysis was mainly based on data from European populations, due to the lack of ethnic diversity in the proteomic samples and may not be applicable to other ethnic groups. In addition, post-MR analysis methods such as Cochran's *Q* test and MR-Egger intercept test were not performed due to the limitation of IVs. Additional relevant analyses are still needed to validate the associations between risk genes and BD, and other post-GWAS analyses, such as proteome-wide association studies (Brandes, Linial, & Linial, 2020), are also good methodologies. Finally, the causal mechanisms of most of these candidate proteins in the pathogenesis of BD are still unclear, and biological validation is required to confirm our findings.

This study has several strengths. Firstly, we focused on pQTLs from 3 different tissue sources, the advantages of which could

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complement each other and have varied clinical significance (Luykx et al., 2015; Yang et al., 2021). Risk genes found in the brain may be related to disease pathogenesis, while risk genes in CSF and plasma may serve as diagnostic and prognostic markers. Secondly, the phenome-wide MR analysis results showed that most of the risk genes we identified were promising for drug development and did not affect other vital systems or organs, increasing the application value of our findings. Interestingly, several studies have identified *LRP8* as a common risk gene for schizophrenia and BD (Li et al., 2016; Xiao et al., 2020). The effect direction of the risk gene *LRP8* on schizophrenia found in our phenome-wide MR analyses was consistent with that for BD, suggesting that drugs developed against this target may have potential curative effects in both diseases. Finally, the introduction of external validation cohorts confirmed the consistency of the effect directions of most risk genes, which increased the reliability of our conclusions. Intervention targeting these genes to increase (or decrease) their protein abundance is expected to become an important method for the treatment of BD in the future.

Conclusions

In conclusion, we identified nine candidate druggable proteins for BD, including *MCTP1*, *DNM3*, and *PDF* in the brain; *CTSF*, *AGRP*, and *FRZB* in CSF; and *LMAN2L*, *NCAM1*, and *TIMP4* in plasma. Our study provides novel insights into the molecular mechanisms of BD and highlights promising candidate proteins for therapeutic interventions.

Abbreviations

BD: bipolar disorder; CSF: cerebrospinal fluid; GWAS: genome-wide association studies; pQTLs: protein quantitative trait loci; CI: confidence interval; IVW: inverse variance weighted; LD: linkage disequilibrium; MR: Mendelian randomization; OR: odds ratio; SNP: single nucleotide polymorphism; *DNM3*: dynamin 3; *MCTP1*: multiple C2 and transmembrane domain containing 1; *ABCB8*: ATP binding cassette subfamily B member 8; *DFNA5*: deafness, autosomal dominant 5; *PDF*: peptide deformylase; *CTSF*: cathepsin F; *LRP8*: LDL receptor related protein 8; *IL36A*: interleukin 36 alpha; *FRZB*: frizzled related protein; *AGRP*: agouti related neuropeptide; *LMAN2L*: lectin, mannose binding 2 like; *CX3CL1*: C-X3-C motif chemokine ligand 1; *PI3*: peptidase inhibitor 3; *NCAM1*: neural cell adhesion molecule 1; *TIMP4*: TIMP metallopeptidase inhibitor 4; *ITIH1*: inter-alpha-trypsin inhibitor heavy chain H1

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0033291724001077.

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Ethical standards. The present study is a secondary analysis of publicly available data. Ethical approval was granted for each of the original GWASs. In addition, no individual-level data were used in this study. Therefore, no new ethical review board approval was needed.

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