Uncovering bi-directional causal relationships between plasma proteins and psychiatric disorders: A proteome-wide study leveraging GWAS summary data

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Abstract

Psychiatric disorders as a whole represents a major public health burden yet their etiologies remain poorly understood, and treatment advances have been limited. In addition, there are no reliable biomarkers for diagnosis or progress monitoring of psychiatric disorders.

Here we performed a 'proteome-wide' causal association study covering 1569 plasma proteins and 23 psychiatric traits or disorders. We conducted Mendelian randomization (MR) analysis leveraging GWAS summary data of plasma protein measurements and psychiatric disorders. In total ~90000 MR tests were conducted. The analysis is bi-directional: we studied how proteins may affect psychiatric disorder risks, but also looked into how psychiatric disorders may be causal risk factors for changes in protein levels. We also performed a variety of additional analysis to prioritize protein-disease associations, including HEIDI test for distinguishing functional association from linkage, analysis restricted to *cis*- acting variants and replications in independent datasets. Identifying proteins causal to disease development have potential implications on drug discovery or repurposing. Findings from this study may also guide the development of blood-based biomarkers for the prediction or diagnosis of psychiatric disorders, as well as those for monitoring disease progression and recovery.

Introduction

Psychiatric disorders as a whole represent a major public health burden and are leading causes of disability worldwide[1]. Nevertheless, there remain significant challenges in the diagnosis and treatment of psychiatric disorders. The etiologies of most psychiatric disorders are not fully understood yet, and we still have limited knowledge about the factors leading to disease progression or recovery. There have been limited advances on the treatment of psychiatric disorders, especially on the discovery of drugs with novel mechanisms of actions.

Another longstanding challenge is that unlike many other medical diseases, there are yet *no* reliable biomarkers for diagnosis, prognosis or monitoring progress of psychiatric disorders[2]. The biomarker should preferably easily accessible and measured, and blood/plasma proteins represent one type of biomarkers fulfilling such requirement. A number of studies have investigated into differences in protein levels in various psychiatric disorders, many of which are hypothesis-driven[2,3]. The rise of high-throughput proteomic platform now enables interrogation of a large panel of proteins at the same time. However, a recent review pointed out several important limitations of studies to date such as inadequate sample sizes, methodological inhomogeneity, and risk of confounding[4].

An important question underlying many protein marker (or proteomics) study in observational samples is that it is often difficult to assess causality. Is the change in the level of a protein the cause or consequence of the disorder? Ideally one may follow-up a group of subjects at risk for the disorder and regularly monitor the protein level to ascertain the temporal relationship between disease onset and rise or fall in protein levels. However, this is often impractical due to low incidence of the disease, the cost and difficulty in identifying at-risk subjects, and loss to follow-up of subjects. Another important concern is confounding bias. For example, patients are often medicated at the time of recruitment, and numerous other known or unknown confounders (e.g. age, gender, stressors, comorbid medical conditions) can result in spurious associations between a protein and the psychiatric disorder.

The recent decade has witnessed a rapid growth in genomic research in psychiatric disorders; in particular, high-throughput technologies such as genome-wide association studies (GWAS) have been highly successful in unraveling genetic variants associated with many neuropsychiatric traits. With the advent of GWAS, a new approach to causal inference, known as Mendelian randomization (MR), is gaining increased attention. An MR study can be considered a "naturalistic" randomized controlled trial (RCT)[5,6]. The basic principle is to use genetic variants as "instruments" to represent the actual risk factor, and analyzed its relationship with the outcome. For example, a person who has inherited a low-density lipoprotein (LDL) lowering allele (or allelic score) at a relevant genetic locus is analogous to receiving a LDL-lowering drug, and vice versa. To estimate the causal effects of reduced LDL, we can then test whether people who have inherited the lipid-lowering genetic variants are less likely to develop cardiovascular diseases. The random allocation of alleles at conception is analogous to randomization in clinical trials; as such, MR studies are less vulnerable to confounding bias than observational studies[5]. And since a person's genetic profile is fixed, this approach also avoids problems of reversed causality.

In this work we aim to investigate potential casual relationships between plasma protein levels and a range of psychiatric disorders/traits, based on the Mendelian randomization approach. As discussed above, a major advantage is that MR is less susceptible to confounder bias and reversed causality. MR also enables us to test direction of causal effects in both directions (protein-to-disease as well as disease-to-protein). In addition, MR can be performed using GWAS summary statistics, which are usually based on very large samples (ususally >10000). On the contrary, the sample sizes of previous proteomics studies are small (N mostly <100; see[4]). The MR approach also provides a cost-effective way to explore a wide panel of proteins against a variety of psychiatric traits, which will be prohibitively costly to carry out in a conventional observational study. Here we made use of recently published results of proteomic quantitative trait loci (pQTLs) and large-scale GWAS data from the Psychiatric Genomics Consortium (PGC), UK Biobank and other sources to perform MR analysis.

While many of the MR studies were hypothesis-driven and focused on testing causal associations of known risk factors, MR can also be extended to screen for a larger number of risk factors to uncover novel causal associations. In the field of psychiatric genetics, hypothesis-driven candidate gene studies have now turned out to be largely irreproducible[7,8], suggesting that candidate-based approaches to 'omics' studies have important limitations. Here we adopted a systematic and unbiased 'hypothesis-generating' or 'hypothesis-free' approach and scans all available proteomic markers for casual relationships with psychiatric disorders.

By exploring proteins causal to the development of psychiatric disorders, we may have a better understanding in the pathogenesis of psychiatric disorders, providing important mechanistic insight. From a more clinical point of view, drugs that target the causal proteins may be effective for disease prevention and treatment. If such drugs are already in use, they can be repositioned for the disorders, saving the high cost and time for new drug development. In addition, proteins with causal relationship with the disease may also serve as predictive biomarkers for at-risk patients before the full development of disease.

On the other hand, the study of proteins whose levels are affected as a consequence of disease liability has important implications. Such proteins may help us understand the molecular mechanisms underlying disease progression and complications. From a clinical perspective, such proteins may be candidate biomarkers for monitoring and assessing disease progress or recovery. If a disease is a causal risk factor for protein level change (let's say increase), then when the disease is cured, the protein level will change (decrease) accordingly. The reverse (i.e. rise in protein level) may be observed when the disease becomes more severe or during relapses. This may be particularly relevant in psychiatry, as patient progress is usually monitored by clinical observations and self-reported symptoms, with no objective biomarkers available. In addition to the above, sometimes a protein whose level changes as a result of a disease may yet be a risk factor for another condition. For example, depression is associated with increased risk of cardiometabolic disorders[9], and some have postulated inflammatory pathways and proteins as mediating factors[10,11].

In this work, we first employed two-sample MR to identify proteins causally associated with a total of 23 GWAS datasets of psychiatric disorders/traits, with replication in the UK Biobank sample. Next we also conducted MR analysis with psychiatric disorders/traits as exposure and protein levels as outcome. The full analysis results are made publicly available, which we believe will serve as a useful resource for further clinical or experimental studies.

Methods

Blood Plasma Protein pQTL Datasets

Blood plasma protein pQTL data were taken from the recently published KORA[1] and INTERVAL[2] studies [12,13]. The KORA is a German cohort with GWAS performed on levels of 1124 protein levels from 1000 subjects from a population-based cohort. Proteins were quantified by a aptamer-based affinity proteomics platform known as SOMAscan. While the original paper by Suhre et al.[12] also included a sample from the QMDiab study, that sample was recruited from Arab and South Asian countries, and was excluded from our analysis since most other GWAS datasets we analyzed were from European subjects. The INTERVAL study is a similar proteomics study from a UK cohort, investigating the association of 3622 plasma proteins in 3301 healthy subjects[13]. Again the SOMAscan platform was employed for protein quantification. For details of each dataset, please refer to the original papers[12,13].

We have included these two studies based on their relatively large sample sizes, the use of same platform for protein quantification, and the fact that both studies have full GWAS summary statistics available for download. Full proteomics GWAS data are required for our MR analysis to identify which proteins may be affected as a consequence of liability to psychiatric disorders and for HEIDI analysis.

Both studies classified pQTL-plasma-protein association as *cis* or *trans*, and in this work we would perform analysis both *cis & trans* pQTL combined and with *cis*-pQTLs only. *Cis*-pQTLs have stronger prior biological evidence and are less susceptible (but not entirely immune) to horizontal pleiotropy in MR as they are close to the proteins studied. Here horizontal pleiotropy refers to instrument SNPs having effect on the outcome through pathways other than the exposure. However, both proteomic GWAS also identified a substantial number of *trans*-pQTLs passing genome-wide significance, and that modeling more SNPs will improve the power of MR analysis and may enable one to deal with horizontal pleiotropy. For this reason, we conducted analysis with *cis & trans* pQTLs, as well as *cis*-pQTLs only.

Psychiatric GWAS datasets

We extracted psychiatric GWAS summary datasets mainly from the Psychiatric Genomic Consortium (PGC) and UK Biobank (UKBB). We included in total 23 GWAS datasets, which encompass a

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[1] "Download data - pGWAS server." https://metabolomics.helmholtz-muenchen.de/pgwas/index.php?task=download.

[2] "Proteins - Cardiovascular Epidemiology Unit." https://www.phpc.cam.ac.uk/ceu/proteins/.

comprehensive range of psychiatric disorders (and traits closely linked to the disorders). They include attention deficit and hyperactivity disorder (ADHD)[14], anorexia nervosa (AN)[15], Alzheimer's disease (ALZ)[16], autistic spectrum disorders (ASD)[17], anxiety disorders (including generalized anxiety disorder, phobic disorder, social phobia, agoraphobia, and specific phobias; results from both case-control study and derived factor score included), depressive symptoms (DS)[18], major depressive disorder (MDD)[19,20] (two sources, one sample[20] comprised female patients enriched for severe melancholic depression), longest period of depressed mood, number of episodes of depressed mood, neuroticism (NEU)[18], obsessive compulsive disorder (OCD)[21], post-traumatic stress disorder (PTSD)[22], schizophrenia (SCZ)[23] and history of deliberate self-harm (DSH) or suicide. We also include GWAS on antidepressant response (as both binary and quantitative outcome)[24], and a GWAS on SCZ versus bipolar disorder (BD) patients[25]. For ADHD and PTSD, we also performed separate analysis on male-only, female-only and combined samples, as the corresponding summary statistics has been made available and there is good evidence for gender differences in these disorders[26,27].

For part of the psychiatric disorders/traits, a large-scale ('primary') study is available from PGC or a related source, while a corresponding trait is also recorded in UKBB. The details are given in Table S3a. For these disorders/traits, we also included the UKBB association dataset as a 'replication' set when we study protein effects on disease. The complete list of psychiatric disorders/traits is given in Table S1j and S3a.

MR analysis: protein as exposure and psychiatric disorder/trait as outcome

We first treated plasma protein levels as exposure and psychiatric disorders/traits as outcome (we sometimes refer it as 'forward' analysis for simplicity). As discussed above, for some of the disorders/traits, a large-scale study dedicated to the study of the specific disorder is available in PGC (or a similar source such as IGAP[16]), while a similar corresponding trait was also recorded in the UKBB sample. In such cases, we would conduct a 'primary' analysis based on the PGC data, then extract the top results (FDR<0.1, based on combined *cis & trans* pQTLs) for replication analyses using the UKBB data.

We extracted pQTL according to those provided in the original papers (Supplementary data 1 in Suhre et al.[12] and Supplementary Table 4 in Sun et al.[13]). Exposure instrument SNPs were harmonized with psychiatric GWAS summary data with the harmonise_data function in the R package "TwoSampleMR". LD-clumping was performed with the clump_data function in "TwoSampleMR" at an r-squared threshold of 0.1 (within 10000 kb). We set a more liberal r-squared threshold than the default (0.001) to allow possibly more instruments to be included. The threshold was set to a relatively low level such that only weak LD is allowed, in order to prevent instability in MR estimates. Residual SNP correlations were properly taken into account of in our inverse-variance weighted (IVW) and MR-Egger analyses (see Supp. Text)[28,29].

Statistical analysis methodology

The method of MR analysis varies according to the number of SNPs (Table 1). If there is evidence of significant pleiotropy as revealed by significant intercept term in Egger regression, we would use the MR-Egger approach, which enables valid causal estimates to be given in the presence of imbalanced horizontal pleiotropy. Otherwise, the standard Wald ratio or inverse-variance weighted (IVW) approach was used. The R packages "MendelianRandomization" was used, which also allowed correlations between instrument SNP to be accounted for.

To control for multiple testing, we employed the false discovery rate (FDR) approach, which controls the expected *proportion* of false positives within the rejected hypotheses. FDR was computed by the Benjamini-Hochberg method[30] and a modified approach by Storey[31]. The latter approach (implemented in the R package "qvalue") also estimates the proportion of null which could lead to improvement in power (this is the default method used in this study). Note that the FDR approach is also valid under positive (regression) dependency of hypothesis tests[32]. The FDR-adjusted *p*-value, or *q*-value, refers to the lowest FDR incurred when calling a feature significant[31].

Other sensitivity tests

<u>*Cis*-acting pQTLs</u>

We primarily present results from analysis of combined *cis*- and *trans*- pQTLs, as this would allow more instruments to be included, improving the power and reliability of results, and would enable methods such as MR-Egger to be performed to correct for imbalanced pleiotropy. We noted that a substantial proportion of pQTLs identified by Suhre and Sun et al. and were in fact *trans*- pQTLs (~27% and 70% respectively), hence discarding *trans*-acting variants will result in substantial waste of available data and statistical power.

Nevertheless, c*is*-acting pQTL have strong prior probability of association and may be less susceptible to horizontal pleiotropy, although they can also affect the outcome via other pathways. Here we also performed additional analysis by sub-setting the original data to produce 2 extra exposure datasets (named cis-KORA, cis-INTERVAL), and the abovementioned MR analysis was repeated with *cis*-pQTLs only.

<u>HEIDI test</u>

Sometimes the instrument variant can be in LD with another SNP, which is causally associated with the outcome. This scenario is known as 'linkage' by Zhu et al.[33], which may be distinguished with functional associations (same SNP affecting both protein level and outcome) by the SMR-HEIDI test[33]. The test was designed especially for single-SNP MR analysis with the assumption of a single causal variant in the region. We employed the SMR program

(https://cnsgenomics.com/software/smr/#SMR) written by the authors. Default settings were used, in which up to the top 20 pQTLs in the cis region were extracted for the HEIDI test. The test requires summary statistics of the proteomics GWAS, and is relatively computationally time-consuming to perform. We conducted HEIDI to single-SNP MR results with FDR<0.2 in the joint analysis to keep the total number of tests to a few hundreds. An HEIDI *p*-value >0.05 is regarding as passing the test, but due to issue of multiple testing of a large number of hypotheses, we also presented FDR-adjusted *p*-values.

In practice, we note that sometimes HEIDI would fail to produce results due to presumably insufficient number of SNPs in the *cis* region of the target pQTL. Another important limitation is that currently HEIDI or other co-localization methods cannot produce reliable results with the number of causal variants >=2. For these reasons we included HEIDI as a sensitivity test, but we do not exclude results failing this test from subsequent analyses. If multiple testing is taken into account of by the FDR approach, we did not observe any HEIDI results with FDR<0.05 (see results).

Replication Analysis

As mentioned above, we sought replication for the results from our primary analysis (mainly PGC studies) in an independent sample from the UK Biobank. Replication was performed for three sets of MR results (each result represents a protein-disorder pair): (1) MR results based on KORA sample with FDR<0.1; (2) Results based on INTERVAL sample with FDR<0.1; (3) Results from combined KORA + INTERVAL meta-analysis with estimated FDR<0.1, which only include intersecting proteins in the two samples. Strictly speaking the statistical significance from the above combined analysis of exposure data may be inflated due to dependency between effect estimates; however our aim is just to prioritize a subset of protein-disorder pairs that showed strong associations in the two pQTL datasets, so this is not a major concern. We also computed the maximum *p*-value (max_*p*) as a test of the hypothesis of non-null associations in *both* samples, as described by Nichols et al[34]. In addition, the Simes test[35] was conducted to combine the *p*-values to test the global null hypothesis (i.e. association in *any* sample). The max_p and Simes test procedures are both robust to (positive) dependencies of hypothesis tests[34,35]. We found the max *p*-value and Simes *p*-value are highly correlated with that from IVW meta-analysis (Spearman rho = 0.79 and 0.87 respectively, *p*<2.2e-16), so prioritization of candidate proteins using whatever criteria will not likely result in much differences.

We adopted a one-sided test for computing p-value in the replication sample. As is common in omics studies such as GWAS, we performed a meta-analysis with “metagen” in R to obtain an aggregate estimate of causal effects from both PGC studies and UKBB.

We also attempted a more novel analysis known as replicability FDR (repFDR), which assesses the expected proportion of false replications[36]. This is a far more stringent test which tests the “union null hypothesis”, i.e. H_0 is that the result is null in *any* one sample. H_1 represents non-null results or

true associations in *every* testing sample, taking into account the number of hypothesis tested. Intuitively, when a large number of hypothesis is tested, to achieve low repFDR the hypothesis needs to be highly significant (having low FDR) in every sample. The authors proposed the "r value", which is defined as "the lowest FDR level at which we can say that the finding is among the replicated ones"[36]. The analysis was carried out with default settings using an online program provided by the authors (http://www.math.tau.ac.il/~ruheller/App.html).

MR in the other direction: Psychiatric disorders/traits as exposure and proteins as outcome

We then performed MR analysis in the other direction. The 23 psychiatric disorders/traits were treated as exposure, while the plasma protein levels were considered as outcome.

SNPs in psychiatric GWAS datasets were first selected based on the genome-wide significance threshold ($p < 5E-08$), then clumped at $r^2=0.1$ (within 10000 kb). The rest of the analysis follows what was described in the previous sections. The HEIDI test was performed for single-SNP analyses. In this case we have two independent outcome datasets (KORA and INTERVAL), so we also performed 'replication' of the results from the first dataset on the second one. We conducted meta-analysis on the intersecting proteins and repFDR was computed as described above.

Results

MR analysis: Proteins as exposure and psychiatric disorders as outcome

The pQTLs of KORA and INTERVAL studies are listed in Tables S1a and S1b. In total 539 pQTLs were extracted from the KORA sample, of which 391 are *cis* variants. In total 1980 pQTLs were extracted from the INTERVAL data, of which 589 are *cis* variants. The KORA and INTERVAL datasets covered 284 and 1478 proteins respectively.

We have conducted totally 6214 and 25963 MR tests based on the KORA and INTERVAL samples respectively. Each test examined the effect of a protein on the psychiatric disorder/trait under study. We also repeated the analysis restricted to *cis* variants only, with 4389 and 8620 MR tests performed on KORA and INTERVAL respectively. A summary of results is shown in Table S1c.

The main MR analysis results are presented in Table 2 and Supplementary Tables S1d to S1e. For the analysis based on KORA pQTLs, 12 protein-disease pairs were significant at an FDR level of 0.1 and 18 at FDR<0.2. For the analysis using pQTLs from INTERVAL sample, we found 377 associations at FDR<0.1 and 437 at FDR<0.2.

We also performed HEIDI test as a sensitivity test to detect associations that may be due to linkage (Table S1f, S1g). Sometimes the program may fail to produce an output, presumably due to inadequate number of SNPs in the *cis* region that pass the preset p-value threshold (1.57E-3,

equivalent to chi-square statistic of 10), or some variants may be filtered away due to too low or too high LD. The original authors used the conventional p=0.05 as the threshold for passing or failing the test; however, we also note there is a high chance of false positives in view of the large number of tests performed. We computed FDR with the Benjamini-Hochberg method and overall did not observe strong evidence for linkage (all FDR>0.05) among the protein-disease associations tested by the HEIDI algorithm. In addition, failing the HEIDI does not always mean the result is invalid. For example, there can be other true causal variant(s) affecting the protein and outcome at the same time, which is/are also in LD with the target pQTL. However, the test is still useful in prioritizing proteins for further studies.

We also performed a combined analysis on the intersecting proteins between the two pQTL datasets (Table 2 and Table S1h), in order to obtain an estimate of the aggregate causal effect and to identify strong protein-disease associations across different datasets. The results of IVW meta-analysis, maximum p (a test for non-null association in every sample) and Simes test (a test for non-null association in at least one sample) are given in Table S1h. Top 25 results sorted by Simes p-value are presented in Table 2.

We also performed ***cis*-only** analysis and the results are summarized in Table S2. Results of KORA- or INTERVAL-only analysis are given in Table S2a and S2b, while joint analysis is presented in Table S2c. HEIDI results are shown is Tables S2d and S2e.In total 4 and 6 associations achieved FDR<0.1 and <0.2 in the KORA sample respectively; 15 and 25 associations achieved FDR<0.1 and <0.2 in the INTERVAL sample respectively. There is significant overlap in the results between the *cis*- only and combined *cis & trans* pQTL analyses. For example, 63 and 42 protein-disease associations showed Simes $p < 0.01$ in the *cis & trans* and *cis*-only analysis respectively, among which 39 of them overlapped. Results that are significant in cis-only analysis are likely to be affected by horizontal pleiotropy biologically, but the number of instruments is usually very small so the power is reduced and some tests such as MR-Egger or heterogeneity test cannot be performed.

Replication analysis

Replication analysis was performed for MR results having FDR<0.1 in KORA sample, INTERVAL sample or in joint analysis (Table S3). For the KORA dataset, altogether 7 associations were replicated with one-sided p-value <0.05 in the UKBB dataset (spanning three unique protein-disease pairs) (Table S3b). Note that sometimes more than one UKBB dataset may correspond to the original phenotype and we included all of them in the replications set. The use of parental history of Alzheimer disease as a proxy of the disease phenotype has been successfully adopted in previous works[37]. In the INTERVAL sample, 92 associations achieved replication p (one-sided test) <0.05 in UKBB (Table S3c). We observed that many of the top results were from Alz-IGAP or SCZ; this is likely due to relatively large sample size and power of these two datasets. However, some of the top

results for Alzheimer disease showed heterogeneity of instrument effects. This can be due to various reasons[38], but horizontal pleiotropy could be one, therefore such results may be viewed with caution. For comprehensiveness, we also extracted a set of intersecting proteins between pQTL datasets (with estimated FDR<0.1 in joint analysis), and carried out meta-analysis combining PGC results with UKBB, using either KORA (Table S3d) or INTERVAL (Table S3e) pQTL data.

If a protein is causal to a psychiatric disorder, then a drug which targets the protein as an antagonist or inhibitor may be able to treat the disease if increased protein levels are linked to the disease. On the contrary, the psychiatric disorder may be a side-effect of that drug if reduced protein levels are associated with disease. The reverse is true for the drugs acting as agonists. To facilitate the discovery of candidates for repositioning and exploration of drug side-effects, we also searched ChEMBL database for existing drugs for top proteins in joint analysis (Tabkles S1i and S3f).

MR analysis: Psychiatric disorders/traits as exposure and proteins as outcome

We then performed MR in the other direction, with psychiatric disorders/traits as exposure and proteins as outcome. The results are presented in Table S4. In total 15 psychiatric disorders have at least one genetic variant having p<5e-8 (Table S4a). However, some of these genome-wide significant SNPs cannot map to the SNP panels of KORA and INTERVAL. Finally 10 and 14 psychiatric traits remain to be analyzed with KORA and INTERVAL, with protein levels as outcome.

Totally 11239 and 36109 MR tests were performed based on the KORA and INTERVAL datasets respectively. Note that since KORA and INTERVAL are independent outcome samples, we may consider the analyses as 'replications' of one another. Based on the KORA sample alone, 8 disease-protein associations achieved FDR<0.1 while 25 had FDR<0.2 (Table S4b). Based on the INTERVAL sample, 526 had FDR<0.1 and 1040 had FDR<0.2 (Table S4c), owing to the larger sample size. Finally we conducted meta-analysis on the pairs of associations analyzed in both pQTL samples (Table S4d). In total 129 and 293 meta-analysis results achieved FDR <0.1 and <0.2 respectively. In occasional cases single-SNP MR has to be performed; HEIDI results are presented in Tables S4e to S4f.

Discussion

Here we have conducted a proteome-wide association study of over 1500 plasma proteins with 23 GWAS datasets of psychiatric traits/disorders. We leveraged GWAS summary statistics of plasma protein measurements and psychiatric disorders, and perform Mendelian randomization analysis to uncover casual relationships between the two entities. Importantly, we have conducted *bi*-directional analyses to investigate not only how proteins affect psychiatric disorder risks, but also how psychiatric traits/disorders may be causal risk factors for changes in protein levels. We have performed more than 90,000 MR tests in total. We also performed several additional analyses such as

HEIDI test, analysis in *cis*- variants only as well as replication in independent datasets (for both protein-to-disease and disease-to-proteins analyses). We believe this work will shed light on the pathophysiology in the development and progression of psychiatric disorders. It may also facilitate the development of novel biomarkers for prediction, diagnosis and monitoring of disease progress or recovery. By targeting proteins that may be causal to the disease, it will also help drug development or repurposing for psychiatric disorders.

We briefly highlight a few proteins that may be of interest. For example, for protein-to-disease associations that were replicated in UKBB (Table S3), immune-related proteins such as protease C1 inhibitor and Tumor necrosis factor receptor superfamily member 19L are among the top-listed associations for SCZ. Inflammation and immune pathways have been strongly implicated in the etiology of SCZ[39], and further experimental work may reveal the mechanisms involved. Reduction in Complement decay accelerating factor (DAF) (Table S3d/e) was observed to contribute to Alzhiemer disease risk and ASD, which was replication in UKBB regardless of whether KORA or INTERVAL was used as the source of pQTL data. DAF prevents complement activation by inhibiting C3 and C5 covertases, and has been found to reduce neuronal cell death and apoptosis in a chemical-induced hypoxia model[40]. However the exact mechanisms underlying its contribution to ALZ or ASD remain to be elucidated. Myeloid cell surface antigen (CD33) is another replicated causal association with Alzheimer disease (Table S3d/e), which has been reported to be associated with amyloid pathology and increased microglial activation[41], and has been suggested as a promising therapeutic target[42].

We also studied how the psychiatric disorder (or the underlying quantitative trait reflecting the disease) can be a causal risk factor for changes in plasma proteins. This is a unique feature of the current study, as previous works on 'omics'-based MR usually focused on, for example, how expression or methylation changes contribute to diseases[33]. Causal relationships in the reverse direction are far less explored. Such analyses can shed light on the mechanism underlying disease progression and complications. In addition, by definition of causality, if an intervention is applied to the cause (disease), then the outcome (protein) should change. Therefore such biomarkers are theoretically justified to evaluate the disease progression, treatment response or resolution from disease symptoms.

In this work, we have found a number of putative disease-to-protein associations which may be worthy of further investigations (Table 3 and Table S4). For example, we found SCZ is causally linked to increase in complement C4 levels (Table 3 and S4d). The complement system, especially C4, has been shown to be implicated in SCZ[43,44]. Interestingly, a recent clinical study reported that C4 was significantly increased in chronic SCZ patient and was associated with positive and negative symptom severity[45], suggesting the potential of C4 as a disease activity biomarker. Another interesting finding was that SCZ was found to be a causal risk factor for rise in thyroid-stimulating hormone (TSH) [Table S4d]. In a clinical study involving 40,483 patients with hypothyroidism and similar number of controls, Sharif et al. observed a higher prevalence of schizophrenia (2.01% vs.

1.25%, respectively, $p < 0.0001$)[46]. There is likely a complex link between neurotransmission and thyroid dysregulation[47]. Our findings suggest that the disease of SCZ itself may contribute to increased TSH (and hence hypothyroidism), amongst other possible risk factors such as medications. We also observed SCZ is a causal risk factor for decreased Interleukin (IL)-17 receptor C levels. The soluble secreted IL-17 receptors present in the blood may serve as extracellular antagonists to cytokine signaling[48] (i.e. reduced soluble receptors lead to rise in cytokine levels). Interestingly, IL-17 was observed to be positively correlated with total PANSS scores in schizophrenia. After risperidone treatment, the proportion of Th17 cells decreased and the change in proportion was correlated with the change rate of PANSS score[49]. This is consistent with our present finding, and suggests proteins in the IL-17 signaling pathway may be useful biomarkers to evaluate disease progress or treatment response.

As a technical note, when the MR exposure is a binary variable (disease or not), it is preferable to conceptualize it as a (possibly latent) continuous risk factor[50]. For example, if the studied exposure is hypertension, then blood pressure would be the underlying continuous risk factor or trait[50]. For most psychiatric disorders, we may also conceptualize a continuous factor underlying the disorder. For instance, depressive symptoms can be the latent factor underlying MDD. Similarly, one can conceptualize a continuous scale of (or tendency to) anxiety, psychosis, OCD etc. as the latent risk factor or trait. The principles of MR causal inference still apply.

In addition to the potential clinical applications described above, we also wish to high two other aspects of translational potential from our study. It is worth noting that besides diagnosis-based phenotypes, we also included in our analysis a GWAS on antidepressant response (as both binary and continuous outcome), and a GWAS on SCZ *versus* bipolar disorder (BD) patients. The former analysis aims to reveal proteins causally associated with response to antidepressants, and may help discover candidate protein markers to predict treatment response. MR analysis of the latter dataset (SCZ vs BD) could help to explore differences in proteomic profiles between the two highly related disorders. The results may ultimately help to prioritize protein biomarkers that may contribute to differential diagnosis (DDx) between BD and SCZ, given that response to medications such as mood stabilizers can differ between the disorders. We believe the presented analytic framework also points to a new direction for finding biomarkers to predict drug response and for DDx in general.

There are several limitations and points that are worth noting. The MR approach is less vulnerable to reverse causality and confounding compared to observational studies, however in some scenarios it is still not easy to establish causality. In the study of causal effects of proteins to psychiatric disorders, the number of instrument SNPs is usually quite small, and it is quite common to rely on only one instrument (i.e. single-SNP MR). There are no methods to distinguish between causality and pleiotropy in single-SNP MR, although the HEIDI test can help to identify 'linkage' from the same SNP being associated with protein levels and disease at the same time[33]. When the number of instruments is larger, more tools are available to address the assumptions of MR, especially

pleiotropy[38]. However, the number of instruments is still typically small when proteins are treated as exposure; the power of methods like Egger regression to detect pleiotropy is relatively weak under such scenarios[29]. Methods based on outlier removal (e.g. MR-PRESSO[51]) are also not applicable in the presence of few instruments. On the whole, we may still prioritize proteins that more likely causal to diseases, but fully establishing causality remains difficult. Of course, at least the current efforts could show which proteins may be *associated with* various psychiatric disorders, which helps to limit the search space for suitable protein biomarkers or target proteins for drug development. For MR analysis with psychiatric disorders as exposure, the above problem of inadequate instruments is less severe, especially for disorders like schizophrenia and Alzheimer's disease with large GWAS sample sizes. Nevertheless, for some other disorders such anorexia, the number of available instruments is small so the same problem can occur.

The MR approach itself also has limitations. As MR is based on genetic instruments to represent the exposure, the results reflect a chronic exposure of (genetically predicted) higher/lower protein levels to the psychiatric disorder. The effects of short-term exposure, such as taking an inhibitor targeting the protein, may not be the same as effects from long-term exposure as predicted by MR.

With regards to the study samples, many psychiatric disorders are highly heterogeneous with regards to their presentations, course of disease, prognosis etc[52]. A protein may be associated with a subgroup of patients with certain clinical characteristics only (e.g. female patients with early onset) but not with the other groups. A future direction is to conduct the analysis in stratified or more deeply-phenotyped samples (e.g. male or female-only; early or late-onset only; with a particular clinical presentation etc.). Also, for some of the samples (especially those from UKBB), the disease diagnosis or symptoms are self-reported instead of being assessed by a clinical professional. The latter is probably not practical in a biobank due to high cost of performing a detailed professional assessment. Nevertheless, further replications in more carefully assessed and phenotyped samples are warranted.

Also related to sample characteristics, we have tried to employ the latest and largest GWAS samples. Nevertheless, for some of the psychiatric disorders (e.g. OCD or PTSD), the sample size may still be relatively small. Also we have performed replication analysis in the UKBB, however the UKBB in general does not contain a lot of patients with psychiatric disorders (except for depression and anxiety). As a result, the power of the replication study is relatively limited and absence of significant findings does not exclude true associations.

One potential translational aspect is the development of blood-based biomarkers for psychiatric disorders. While this work we mainly study the protein candidates one by one, in practice and future studies, we may combine different biomarkers and even other modalities (e.g. imaging) to improve the performance of the test. This will be left as a direction for future investigations.

In summary, we have conducted a proteome-wide association study to decipher the causal relationship between plasma proteins and psychiatric disorders. We believe this work sheds light on the pathophysiology of psychiatric disorders, and will help facilitate the development of protein biomarkers as well as new or repurposed drugs for treatment.

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Author contributions

Conceived and designed the study: HCS. Study supervision: HCS. Main data analysis: CKLC. Data interpretation: HCS, CKLC, PCS. Data analysis methodology: HCS, PCS. Literature review, assistance in data analysis: ALCL. Drafted the manuscript: HCS.

Conflicts of interest

The author declares no conflict of interest.

Supplementary Text

MR analysis methods

The IVW framework is very widely used in MR studies. Here we used an IVW approach that is able to account for SNP correlations as described in Burgess et al.[28]. Briefly, assume $\hat{\beta}_{YG}$ to be the vector of estimated regression coefficients when the outcome is regressed on genetic instruments and σ_{YG} to be the corresponding standard errors (SE), and $\hat{\beta}_{XG}$ to be the estimated coefficients when the risk factor is regressed on the genetic instruments with SE σ_{XG}. We also assume the correlation between two genetic variants G_1 and G_2 to be ρ_{G1G2}, and $\Sigma_{G_1G_2} = \rho_{G_1G_2}\sigma_{YG_1}\sigma_{YG_2}$.

The estimate from a weighted generalized linear regression can be formulated by

$$\hat{\beta} = \left(\hat{\beta}'_{XG}\Sigma\hat{\beta}_{XG}\right)^{-1}\hat{\beta}'_{XG}\Sigma^{-1}\hat{\beta}_{YG}$$

with SE

$$SE(\hat{\beta}) = \sqrt{\left(\hat{\beta}'_{XG}\Sigma\hat{\beta}_{XG}\right)^{-1}}$$

A similar approach may be used for MR-Egger, which allows an intercept term in the weighted regression. Please also refer to ref. [29,53] for details. The presence of imbalanced horizontal pleiotropy could be assessed by the significance of the intercept term (testing whether intercept=0). We employed the R package "MendelianRandomization" for the above analyses.

As remarked by Burgess et al.[28], inclusion of a larger number of variants in partial LD may improve the power of MR, as the variance explained by the instruments might go up. Including "redundant" SNPs in addition to the causal variant(s) will not improve power; on the other hand it will not invalidate the results. However, including too many variants with high correlations may also result in instability of MR estimates[54]. In this study, we performed LD-clumping of genetic instruments at an r^2 threshold of 0.1, balancing the benefits and risks of using a too high or too low threshold.

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Table 1 MR approach adopted according to the number of instrument SNPs

Number of SNP in Harmonized Data (*n*)	**2MR Test Applied**
$n = 1$	Wald Ratio
$n = 2$	Inverse variance weighted (IVW)

$n \geq 3$	If (pleiotropy *p*-value < 0.05)
	Egger's Regression
	else
	Inverse variance weighted

Table 2 MR results with plasma proteins as exposure and psychiatric disorders/traits as outcome (joint analysis of KORA and INTERVAL)

		KORA						INTERVAL					HEIDI Test		Joint analysis		
Protein TargetFullName (Exposure)	**Psy Disorder (Outcome)**	**nsnp**	**Pleio_p**	**Hetero-geneity. pval**	**Method**	**Beta**	**P**	**nsnp**	**Hetero-geneity. pval**	**Method**	**Beta**	**P**	**KORA**	**INTERVAL**	**Simes_P**	**MAX_P**	**TE.fixed**
Endothelial cell-selective adhesion molecule	SCZ2	1	NA	NA	Wald	0.204	1.50E-08	1	NA	Wald	0.285	1.50E-08	PASS	PASS	1.50E-08	1.50E-08	0.232
Myeloid cell surface antigen CD33	Alz-IGAP	3	0.758	0.226	IVW	0.083	3.67E-08	1	NA	Wald	0.101	6.66E-08	NA	FAIL	6.66E-08	6.66E-08	0.090
Plasma protease C1 inhibitor	SCZ2	3	0.935	0.875	IVW	-0.052	3.03E-05	1	NA	Wald	-0.084	2.02E-05	NA	FAIL	3.03E-05	3.03E-05	-0.061
Cathepsin B	NeuroticFull	1	NA	NA	Wald	0.029	1.77E-04	1	NA	Wald	0.040	2.14E-05	FAIL	PASS	4.28E-05	1.77E-04	0.034
Cathepsin S	SCZ2	2	NA	0.071	IVW	0.072	1.28E-04	1	NA	Wald	0.105	2.67E-05	NA	FAIL	5.34E-05	1.28E-04	0.084
Contactin-5	NeuroticFull	1	NA	NA	Wald	-0.016	8.65E-02	2	0.961	IVW	-0.030	8.37E-05	PASS	PASS	1.67E-04	8.65E-02	-0.025
Cathepsin B	ASD	1	NA	NA	Wald	0.118	1.05E-04	1	NA	Wald	0.139	2.34E-04	NA	PASS	2.10E-04	2.34E-04	0.127
Lysozyme C	ASD	2	NA	0.436	IVW	0.058	1.20E-04	1	NA	Wald	0.051	8.30E-02	NA	PASS	2.39E-04	8.30E-02	0.057
Ectonucleoside triphosphate diphosphohydrolase 5	OCD	2	NA	0.461	IVW	0.180	2.80E-04	1	NA	Wald	0.234	9.80E-04	NA	PASS	5.59E-04	9.80E-04	0.198
Death-associated protein kinase 2	NumOfDepressEpisodes	1	NA	NA	Wald	0.058	2.81E-04	1	NA	Wald	0.007	5.38E-01	PASS	PASS	5.62E-04	5.38E-01	0.025
Interleukin-6 receptor subunit alpha	SCZ2	3	0.925	0.108	IVW	0.029	3.19E-04	1	NA	Wald	0.023	1.73E-02	NA	FAIL	6.39E-04	1.73E-02	0.026
Hemojuvelin	SelfHarmSuicide-logistic	1	NA	NA	Wald	-0.855	4.33E-04	1	NA	Wald	0.262	5.49E-01	PASS	PASS	8.66E-04	5.49E-01	-0.592
Neutral ceramidase	ADHD-2017-Euro	2	NA	0.059	IVW	-0.078	2.28E-03	1	NA	Wald	-0.092	4.36E-04	NA	PASS	8.72E-04	2.28E-03	-0.085
Haptoglobin	ASD	7	0.682	0.015	IVW	-0.021	2.58E-01	1	NA	Wald	-0.070	4.36E-04	NA	NA	8.72E-04	2.58E-01	-0.045

Interleukin-12 receptor subunit beta-1	NeuroticFull	1	NA	NA	Wald	0.007	2.11E-01	1	NA	Wald	0.064	4.65E-04	NA	FAIL	9.31E-04	2.11E-01	0.011
MAP kinase-activated protein kinase 2	NeuroticFull	1	NA	NA	Wald	0.006	6.17E-01	1	NA	Wald	-0.036	4.65E-04	NA	NA	9.31E-04	6.17E-01	-0.019
Interleukin-7 receptor subunit alpha	NeuroticFull	1	NA	NA	Wald	-0.002	7.39E-01	1	NA	Wald	0.058	4.65E-04	PASS	NA	9.31E-04	7.39E-01	0.007
Low molecular weight phosphotyrosine protein phosphatase	Alz-IGAP	3	0.822	0.644	IVW	-0.043	1.49E-03	1	NA	Wald	-0.049	5.10E-04	PASS	PASS	1.02E-03	1.49E-03	-0.046
Endothelial cell-selective adhesion molecule	ASD	1	NA	NA	Wald	0.145	1.92E-03	1	NA	Wald	0.203	1.92E-03	NA	PASS	1.92E-03	1.92E-03	0.165
SPARC-like protein 1	AnxCaseCtrl	2	NA	0.058	IVW	0.122	5.71E-03	2	0.704	IVW	0.180	1.09E-03	NA	PASS	2.18E-03	5.71E-03	0.144
Serine/threonine-protein kinase 17B	NeuroticFull	1	NA	NA	Wald	-0.028	1.96E-02	1	NA	Wald	-0.021	1.15E-03	FAIL	PASS	2.31E-03	1.96E-02	-0.023
Annexin A1	NeuroticFull	1	NA	NA	Wald	-0.013	3.17E-01	1	NA	Wald	-0.021	1.15E-03	(no result)	PASS	2.31E-03	3.17E-01	-0.020
Integrin alpha-I: beta-1 complex	NeuroticFull	1	NA	NA	Wald	0.014	3.17E-01	1	NA	Wald	-0.035	1.15E-03	FAIL	FAIL	2.31E-03	3.17E-01	-0.017
Annexin A2	NeuroticFull	2	NA	0.888	IVW	0.001	7.91E-01	1	NA	Wald	-0.015	1.15E-03	PASS	PASS	2.31E-03	7.91E-01	-0.007
Caspase-3	NeuroticFull	1	NA	NA	Wald	-0.003	8.41E-01	1	NA	Wald	-0.046	1.15E-03	NA	PASS	2.31E-03	8.41E-01	-0.022

Pleio_p, pleiotropy p-value; beta, MR causal estimate; Simes_p, p-value from Simes' test; MAX_p, maximum p-value across two samples (test whether the hypothesis is non-null in every sample); TE.fixed, aggregate effect based on fixed-effects model. Please refer to Table S1j for abbreviations of each disorder.

Table 3 MR results with psychiatric disorders/traits as exposure and proteins as outcome (joint analysis of KORA and INTERVAL)

		KORA						INTERVAL						Meta-analysis			
Psy disorder	**Protein (outcome)**	**nsnp**	**Pleiotropy.pval**	**Method**	**b**	**se**	**pval**	**nsnp**	**Pleiotropy.pval**	**Method**	**b**	**se**	**pval**	**Effect**	**SE**	**pval**	**qval.Storey**
SCZ2	MHC class I polypeptide-related sequence B	86	0.001	Egger	1.265	0.311	4.75E-05	132	0.001	Egger	0.918	0.170	6.91E-08	0.998	0.149	2.33E-11	1.44E-07
Alz_IGAP	FACT complex subunit SSRP1	18	0.420	IVW	-0.037	0.065	5.71E-01	44	0.000	Egger	-0.117	0.020	2.51E-09	-0.111	0.019	4.35E-09	1.35E-05
SCZ2	cAMP-specific 3',5'-cyclic phosphodiesterase 4D	86	0.742	IVW	0.153	0.082	6.30E-02	132	0.271	IVW	0.434	0.073	2.23E-09	0.311	0.054	1.09E-08	1.72E-05
SCZ2	Interferon lambda-2	86	0.700	IVW	0.120	0.067	7.25E-02	132	0.703	IVW	0.168	0.031	4.66E-08	0.160	0.028	1.11E-08	1.72E-05
Alz_IGAP	Apolipoprotein E (isoform E2)	18	0.442	IVW	0.306	0.083	2.30E-04	44	0.092	IVW	0.126	0.029	9.48E-06	0.145	0.027	7.29E-08	9.03E-05
Alz_IGAP	Apolipoprotein B	18	0.262	IVW	0.137	0.075	6.94E-02	44	0.245	IVW	0.166	0.033	6.64E-07	0.161	0.031	1.29E-07	1.33E-04
Alz_IGAP	C-reactive protein	18	0.022	Egger	-0.167	0.064	9.26E-03	44	0.364	IVW	-0.107	0.023	3.66E-06	-0.114	0.022	1.63E-07	1.44E-04
SCZ2	Trypsin-3	86	0.597	IVW	-0.218	0.091	1.65E-02	132	0.456	IVW	-0.335	0.072	3.00E-06	-0.290	0.056	2.58E-07	1.99E-04
MDD2018	MHC class I polypeptide-related sequence B	3	0.086	IVW	2.468	0.562	1.13E-05	5	0.002	Egger	7.958	2.230	3.59E-04	2.796	0.545	2.89E-07	1.99E-04
Alz_IGAP	Chordin-like protein 1	18	0.316	IVW	-0.120	0.046	8.68E-03	44	0.008	Egger	-0.080	0.019	2.62E-05	-0.086	0.018	9.97E-07	6.17E-04
Alz_IGAP	Low-density lipoprotein receptor-related protein 8	18	0.501	IVW	-0.059	0.056	2.94E-01	44	0.025	Egger	-0.093	0.020	3.29E-06	-0.089	0.019	2.21E-06	1.14E-03
Alz_IGAP	Interleukin-22 receptor subunit alpha-1	18	0.805	IVW	-0.038	0.054	4.90E-01	44	0.117	IVW	0.146	0.026	2.48E-08	0.112	0.024	2.33E-06	1.14E-03
Alz_IGAP	N-acylethanolamine-hydrolyzing acid amidase	18	0.354	IVW	-0.109	0.054	4.34E-02	44	0.040	Egger	-0.088	0.021	1.87E-05	-0.091	0.019	2.39E-06	1.14E-03
Alz_IGAP	Granulocyte colony-stimulating factor receptor	18	0.714	IVW	0.097	0.061	1.10E-01	44	0.026	Egger	0.085	0.019	1.00E-05	0.086	0.018	2.67E-06	1.15E-03
SCZ2	Interleukin-5 receptor subunit alpha	86	0.558	IVW	0.073	0.091	4.24E-01	132	0.528	IVW	0.149	0.032	2.81E-06	0.141	0.030	2.79E-06	1.15E-03
SCZ2	Fibroblast growth factor 20	86	0.671	IVW	0.078	0.071	2.74E-01	132	0.969	IVW	0.143	0.032	7.85E-06	0.132	0.029	6.04E-06	2.34E-03

Alz_IGAP	Diablo homolog, mitochondrial	18	0.816	IVW	-0.085	0.053	1.10E-01	44	0.031	Egger	-0.079	0.019	2.86E-05	-0.079	0.018	7.55E-06	2.49E-03
Alz_IGAP	C-C motif chemokine 22	18	0.203	IVW	0.016	0.066	8.06E-01	44	0.007	Egger	-0.098	0.021	2.00E-06	-0.088	0.020	8.03E-06	2.49E-03
SCZ2	Interleukin-19	86	0.548	IVW	0.110	0.074	1.39E-01	132	0.260	IVW	0.167	0.039	2.00E-05	0.155	0.035	8.13E-06	2.49E-03
SCZ2	Secreted frizzled-related protein 1	86	0.053	IVW	0.228	0.069	9.81E-04	132	0.416	IVW	0.108	0.032	6.83E-04	0.129	0.029	8.14E-06	2.49E-03
SCZ2	Thyroid Stimulating Hormone	86	0.075	IVW	0.088	0.068	1.93E-01	132	0.538	IVW	0.156	0.036	1.37E-05	0.141	0.032	8.47E-06	2.49E-03
SCZ2	WAP, Kazal, immunoglobulin, Kunitz and NTR domain-containing protein 2	86	0.162	IVW	0.029	0.063	6.52E-01	132	0.095	IVW	0.161	0.034	1.65E-06	0.132	0.030	8.83E-06	2.49E-03
SCZ2	Bone morphogenetic protein 7	86	0.258	IVW	0.028	0.080	7.26E-01	132	0.301	IVW	0.125	0.028	6.84E-06	0.115	0.026	1.27E-05	3.42E-03
Alz_IGAP	Pleiotrophin	18	0.384	IVW	-0.068	0.048	1.58E-01	44	0.037	Egger	-0.082	0.020	4.04E-05	-0.080	0.018	1.48E-05	3.82E-03
SCZ2	Complement C4	86	0.003	Egger	0.765	0.309	1.32E-02	132	0.016	Egger	0.528	0.147	3.27E-04	0.572	0.133	1.64E-05	3.83E-03

Please also refer to legends of Table 2. Please refer to Table S1j for abbreviations of each disorder. qval.Stroey: q-value based on the Storey method.