SVN Stroke & Vascular Neurology

# PRCP is a promising drug target for intracranial aneurysm rupture supported via multi-omics analysis

Jinghao Wu,[1] Yunyun Mei,[2] XinYu Li,[3] Wen-Kai Yu,[4] Zi Han Zhou,[5] Yinghao Yang,[1] Pengpeng Niu,[1] Yunchao Wang ,[1] Chang-He Shi ,[4] Hanghang Zhu,[1] Wenjun He,[3] Yuan Gao,[1] Yuming Xu ,[1,6,7] Yusheng Li [1,6,7]

**To cite:** Wu J, Mei Y, Li X, *et al*. PRCP is a promising drug target for intracranial aneurysm rupture supported via multi-omics analysis. *Stroke & Vascular Neurology* 2024;**0**. doi:10.1136/svn-2023-003076

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/svn-2023-003076).

JW, YM and XL contributed equally.

Received 28 December 2023
Accepted 30 July 2024



For numbered affiliations see end of article.

**Correspondence to**
Professor Yusheng Li;
fccliyusheng@zzu.edu.cn

## ABSTRACT

**Background** Cerebral aneurysms are life-threatening cerebrovascular disorders. Currently, there are no effective treatments for preventing disease progression. Mendelian randomisation (MR) is widely used to repurify licensed drugs and identify new therapeutic targets. Therefore, this study aims to investigate effective drug targets for preventing the formation and rupture of cerebral aneurysms and analyse their potential mechanisms.

**Methods** We performed a comprehensive study integrating two-sample MR analysis, colocalisation analysis and summary data-based Mendelian randomisation (SMR) to assess the causal effects of blood and brain druggable cis-expression quantitative trait loci (cis-eQTLs) on intracranial aneurysm (IA), unruptured intracranial aneurysm (UIA) and subarachnoid haemorrhage of IA rupture (SAH). Druggable genes were obtained from the study by Chris Finan *et al*, cis-eQTLs from the eQTLGen and PsychENCODE consortia. Results were validated using proteomic and transcriptomic data. Single-gene functional analyses probed potential mechanisms, culminating in the construction of a drug-gene regulation network.

**Results** Through the MR analysis, we identified four potential drug targets in the blood, including prolylcarboxypeptidase (PRCP), proteasome 20S subunit alpha 4 (PSMA4), LTBP4 and GPR160 for SAH. Furthermore, two potential drug targets (PSMA4 and SLC22A4) were identified for IA and one potential drug target (KL) for UIA after accounting for multiple testing (P(inverse-variance weighted)<8.28e-6). Strong evidence of colocalisation and SMR analysis confirmed the relevance of PSMA4 and PRCP in outcomes. Elevated PRCP circulating proteins correlated with a lower SAH risk. PRCP gene expression was significantly downregulated in the disease cohort.

**Conclusions** This study supports that elevated PRCP gene expression in blood is causally associated with the decreased risk of IA rupture. Conversely, increased PSMA4 expression in the blood is causally related to an increased risk of IA rupture and formation.

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Previous studies identified genes related to cerebral aneurysms, but causal relationships, especially regarding proteasome 20S subunit alpha 4 (PSMA4) and prolylcarboxypeptidase (PRCP) and their potential as drug targets, were unclear. Our study aimed to fill this gap using a comprehensive multi-omics approach and diverse consortia data to explore causal associations between gene expression, cerebral aneurysm outcomes and potential therapeutic targets.

### WHAT THIS STUDY ADDS

⇒ This study establishes PSMA4 and PRCP as promising and previously unrecognised drug targets for cerebral aneurysms, shedding light on their causal relationships with outcomes and providing valuable insights for future therapeutic strategies.

### HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Identifying PSMA4 and PRCP as potential drug targets in cerebral aneurysms may pave the way for targeted therapies, influencing future research and clinical practices in preventing or treating intracranial aneurysm rupture.

## INTRODUCTION

A cerebral aneurysm is a life-threatening cerebrovascular disorder characterised by balloon-shaped dilatation of the intracranial artery.[1] The prevalence of cerebral aneurysms is 3% in the adult general population.[2] Small unruptured cerebral aneurysms remain asymptomatic and are often detected using neuroimaging.[3] However, ruptured cerebral aneurysms may lead to adverse outcomes, including subarachnoid haemorrhage (SAH) and sudden death.[3 4] Hence, the development of effective interventions and treatments for cerebral aneurysms is significant.

The treatment of cerebral aneurysms poses significant challenges. Despite extensive efforts over the past few decades to explore disease-modifying treatments, progress has been limited. Currently, essential treatments for cerebral aneurysms include surgical clipping and endovascular modalities; however, they still entail certain risks.[5] Therefore, it is advisable to prioritise drug treatment for cerebral aneurysms.

Randomised controlled clinical trials (RCTs) are generally considered the gold

standard for evaluating the safety and efficacy of drugs. However, extrapolating study results to clinical practice may face challenges. Additionally, the substantial time and cost associated with performing control trials make their implementation difficult.

In recent years, Mendelian randomisation (MR), a genetic instrumental variable analysis, has gained widespread use in developing drug targets for various diseases. This method integrates summary data from disease genome-wide association studies (GWASs) and cis-expression quantitative trait loci (cis-eQTL) located in the genomic region of the drug target gene. MR uses single-nucleotide polymorphisms as unconfounded proxies for exposures to explore causality between exposures and the outcomes of interest. Unlike RCTs, MR serves as a valuable strategy for causal inference. The allocation of genotypes from parents to offspring is random, and genetic variation is less likely to be influenced by environmental factors. Previous studies have shown that using human genetic data to select drug targets is more likely to be successful in clinical trials.[6]

Therefore, this study aims to investigate therapeutic targets for cerebral aneurysms. We performed a systematic exploration of the druggable genome-wide MR by integrating eQTL with independent cerebral aneurysm GWAS datasets to identify potential interventions.

## METHODS

### Study design

(1) Selection of genetic variants as proxies for cis-eQTL data associated with druggable genes. (2) Selection of three outcomes summary-level data: intracranial aneurysm (IA), SAH of IA rupture and unruptured intracranial aneurysm (UIA). (3) Performing a two-sample MR analysis to estimate the causal effects of the chosen druggable eQTLs on each of the three outcomes. (4) Using the genetic colocalisation approach to assess the reliability of the expression instrumental variables. (5) Performing a summary-data-based MR (SMR) procedure to evaluate the relationship between the expression levels of the identified genes (prolylcarboxypeptidase (PRCP) and proteasome 20S subunit alpha 4 (PSMA4)) and outcomes. (6) Validating the targets using protein quantitative trait loci (pQTL) data. (7) Detecting the differential expression of PRCP and PSMA4 between the control and disease groups using transcriptomic data. (8) Conducting a single-gene function analysis of PRCP. (9) Constructing drug-gene regulatory networks. Figure 1 depicts the drug target screening process. In this study, we performed MR using the TwoSampleMR R package (V.0.5.6) and the 'coloc' R package (V.5.2.2) for colocalisation analysis. SMR was conducted using the SMR software, V.1.03 (https://cnsgenomics.com/software/smr/#Overview).

### Data sources

#### Identifying druggable genes

Overall, 4302 druggable genes were obtained from a study by Finan *et al*.[7] In their investigation, they identified 4479 druggable genes among 20 300 protein-coding genes. After excluding genes located on the sex chromosomes, 4302 genes with HGNC names were confirmed. These included 1375 protein therapeutic targets for clinical development, 646 proteins related to drug targets and compounds and 2281 proteins associated with key drug-target families.

#### Identifying cis-eQTL data related to druggable genes

cis-eQTL in close proximity to the drug target gene is frequently used as proxies. Blood and brain eQTL data were obtained from eQTLGen (https://eqtlgen.org/) and PsychENCODE consortia (http://resource.psychencode.org), respectively. These datasets included fully statistically significant cis-eQTL (false discovery rate <0.05), identified within a 1 Mb range from each probe in the eQTLGen Consortium and PsychENCODE consortia single nucleotide polymorphisms (SNPs) within 100 kb windows from 4302 druggable genes were selected as genetic instruments proxying 4302 druggable targets.

#### Outcome data

We sourced GWAS summary-level data for cerebral aneurysm outcomes (IA, SAH and UIA) from a comprehensive meta-analysis. This meta-analysis included individuals of European ancestry and incorporated individual-level genotypes from 23 separate cohorts, which were then integrated into nine European ancestry strata based on the genotyping platform and country of origin. All cases SAH of IA rupture and UIAs were confirmed using imaging; however, all controls were unselected controls.[4] Specifically, the summary data for IA comprised 7495 cases and 71 934 controls. For SAH, it included 5140 cases and 71 952 controls, and for UIA, it involved 2070 cases and 71 952 controls. Online supplemental table 1 provides detailed information about these data.

#### Transcriptomic data collection

The transcriptomic data for this study were exclusively obtained from the GEO database, specifically GSE15629. GSE15629, based on GPL6244, includes eight ruptured IAs, six UIA samples and five normal control samples.

### Statistics

#### MR analysis

In our study, we used cis-eQTL data associated with 4302 druggable genes as the exposure and three types of GWAS summary-level data for cerebral aneurysms (IA, SAH and UIA) as the outcomes to perform MR using the TwoSampleMR R package. Initially, we identified significant SNPs (p<5e-8) and chose robust SNPs (F-statistic >10, calculated as $F=\beta^{\wedge}/se^{\wedge}$) as instruments. Subsequently, we removed outcome-related SNPS. We then clumped SNPs in each eQTL at a threshold of linkage disequilibrium $r^2<0.1$ and a physical distance of 10 000 kb, with the 1000 Genomes Project serving as the reference panel to ensure independence between SNPs included in genetic instruments. We harmonised the selected SNPs and outcome data. For eQTL instruments with one SNP,

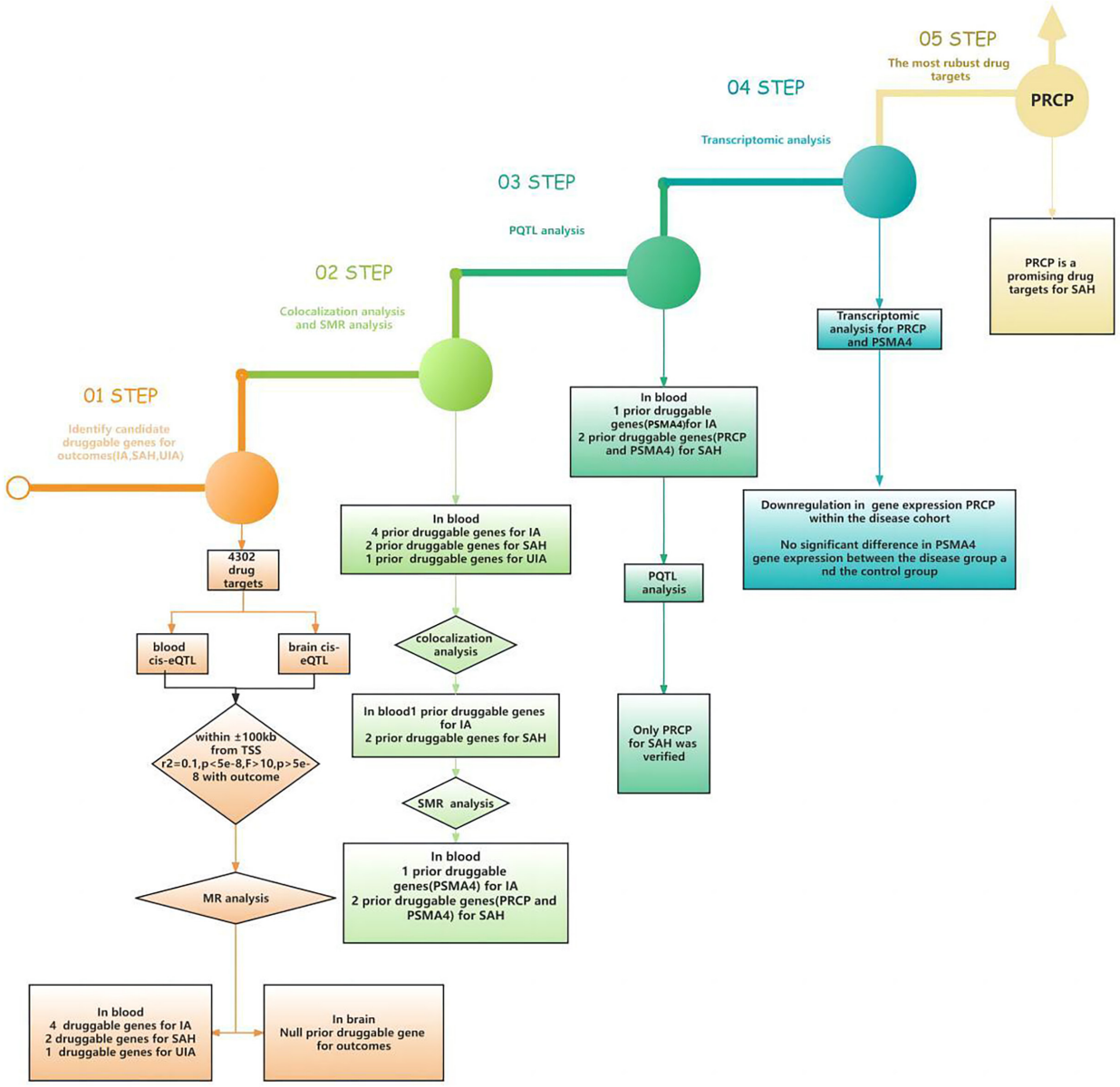


**Figure 1** The diagram of drug target screening. eQTL, expression quantitative trait locus; IA, intracranial aneurysm; pQTL, protein quantitative trait loci; SAH, subarachnoid haemorrhage (the rupture of intracranial aneurysms); SMR, summary-data-based Mendelian randomisation; TSS, transcription start sites; UIA, unruptured of intracranial aneurysm.

we used the Wald ratio method to calculate MR estimates, whereas for instruments with two SNP, we used the inverse-variance weighted (IVW) analysis technique. For eQTL instruments incorporating more than two SNPs, our primary analysis method was the IVW analysis technique, supplemented by weighted median, MR-Egger, simple mode and weighted mode as additional analysis methods. The IVW approach provides an accurate estimate if the assumption that each included SNP can serve as an effective instrument variant (IV) is satisfied.[8] While MR-Egger regression can detect and adjust for pleiotropy, it is important to note that the estimation accuracy of this method is relatively poor.[9] Estimates obtained through the weighted median are considered accurate when at least 50% of the IVs are valid.[10] While the simple mode provides robustness against pleiotropy, it is not as powerful as the IVW method.[11] The weighted mode, although highly sensitive, poses challenges in selecting bandwidth for mode estimation.[12] For data derived from eQTLGen, we defined p values of IVW below 8.28e- 6 (p=0.05/2013×3) as significant potential drug targets. For the data derived from the PsychENCODE consortia, we defined IVW p values below 1.87e-5 (p=0.05/891×3) as significant potential drug targets.

## Sensitivity analysis

Several sensitivity analyses were performed to identify the potential drug targets. MR-Egger regression analysis was used to assess the potential horizontal pleiotropy of the SNPs used as IVs. In MR-Egger regression, the intercept

term serves as a valuable indicator of directional horizontal pleiotropy, with p<0.05 indicating the presence of horizontal pleiotropy.[9] Additionally, the MR-PRESSO global test was used to detect outliers. If significant outliers (p<0.05) were identified, they were excluded from the analysis to ensure an unbiased causal estimate. For IVW analysis, Cochran's Q statistic was adopted, and we assessed heterogeneity using this method, where p<0.05 indicates the presence of heterogeneity.[13]

### Reverse causality detection

To enhance the probability that MR accurately identifies the causal direction between exposure and outcome, we performed Steiger filtering and directionality tests to identify reverse causation. Steiger filtering is used to assess whether the directionality of a single SNP is correct, operating under the assumption that a genuine genetic variant should account for more variance in exposure than the outcome. Genetic variants indicating a reversed causal direction, failing to meet this criterion, were subsequently removed.[14] Additionally, Steiger directionality tests were performed to ensure the directionality of the association between the exposures and outcomes. Results with p<0.05 were considered statistically significant.

### Colocalisation analysis

Moreover, we focused on the SNPs located within 100 kilobases (kb) of the candidate druggable genes. This subset of SNPs was subjected to colocalisation analysis to determine the probability that SNPs associated with outcomes (IA, SAH and UIA) and eQTL shared causal genetic variants. Specifically, P1 indicates the probability of a specific SNP being associated with the outcome, P2 signifies the probability of a specific SNP being a significant eQTL and P12 represents the probability of the SNP being associated with both traits. We evaluated all hypotheses, denoted as PPH0–PPH4, using the posterior probability (PP) to determine their level of support. PPH0 suggests no association between either trait; PPH1 indicates an association with gene expression but not the outcome; PPH2 indicates an association with the outcome but not with gene expression; PPH3 is associated with the outcome and expression of genes with distinct causal variants; PPH4 is associated with both the outcome and expression of a gene with a shared causal variant. We considered significant colocalisation (PP) to be PPH4>0.80. Genes that strongly colocalised with the outcomes (IA, SAH and UIA) were considered potential target genes.

### SMR analysis

We performed SMR to determine the relationships between the expression levels of the identified genes and outcomes, using summary data from GWAS and eQTL studies. Furthermore, we used the heterogeneity in dependent instruments (HEIDI) test to evaluate the relationship in the observed association. A HEIDI test results with p<0.05 was considered as supporting evidence suggesting that the observed association might be influenced by significant levels of linkage disequilibrium between two distinct genetic variants.[15]

### pQTL validation

We obtained summary-level data on the genetic associations between circulating proteins PRCP and PSMA4 from a large-scale pQTL study involving 35 559 Icelanders.[16] For the PRCP, we extracted SNPs using a significance threshold of p<5e-8. However, for PSMA4, owing to limited data, we used a significance threshold of p<5e-6. Subsequently, we performed clumping of SNPs in each pQTL, setting a threshold of linkage disequilibrium $r^2$<0.001 and a physical distance of 10 000 kb with the 1000 Genomes Project as a reference panel. Finally, we performed a two-sample MR analysis using pQTL and the outcome data to validate the targets.

### Transcriptomic analysis

The Wilcoxon rank-sum test was used to detect differential expression of PRCP and PSMA4 between the control and experimental groups.

### Single-gene function analysis of PRCP

The detailed methods are shown in online supplemental additional file 1.

### Drug-gene interactions

Developing a new drug is typically a time-consuming process. To expedite the discovery of potential treatments, we searched the DGIdb database to identify drugs already in clinical use for regulating target genes. Subsequently, we constructed a drug-gene regulation network. Leveraging existing drug-gene regulatory networks allows for quicker identification of new treatments and helps reduce the time and cost associated with new drug development.

## RESULTS

### Candidate druggable genes for cerebral aneurysms

Using data from the study by Finan *et al*, we identified 4302 genes as potentially druggable genes (online supplemental table 2). Subsequently, we acquired druggable eQTLs from the blood using cis-eQTL data from the eQTLGen Consortium. Simultaneously, we obtained druggable eQTLs from the brain by using cis-eQTL data from the PsychENCODE consortia.

We used cis-eQTL data from the eQTLGen Consortium, resulting in final gene counts of 2013 for SAH, 2013 for IA, 1998 for UIA in our analysis. Subsequently, we performed a two-sample MR analysis using European summary statistics for patients with SAH, IA and UIA. Consequently, we identified four potential drug targets in the blood (PRCP (Pivw=1.20e-08, OR=0.59, 95% CI 0.49–0.70), PSMA4 (Pivw=7.52e-08, OR=2.00, 95% CI 1.53–2.50), LTBP4 (Pivw=2.45e-06, OR=0.48, 95% CI 0.35–0.65) and GPR160 (Pivw=7.57e-08, OR=0.83, 95% CI 0.77–0.90)) for SAH, two potential drug targets

| EXPOSURE | OUTCOME | METHOD | nSNP | OR(95%CI) | P-VALUE |
|---|---|---|---|---|---|
| PRCP | SAH | Inverse variance weighted | 7 | 0.59(0.49-0.70) | 1.200000e-08 |
| | | Weighted median | 7 | 0.55(0.44-0.68) | 4.970000e-08 |
| | | Weighted mode | 7 | 0.55(0.43-0.69) | 2.317016e-03 |
| | | Simple mode | 7 | 0.55(0.39-0.78) | 1.224077e-02 |
| | | MR Egger | 7 | 0.43(0.27-0.69) | 1.772329e-02 |
| PSMA4 | SAH | Inverse variance weighted | 2 | 1.96(1.53-2.50) | 7.520000e-08 |
| LTBP4 | SAH | Inverse variance weighted | 6 | 0.48(0.35-0.65) | 2.450000e-06 |
| | | Weighted median | 6 | 0.41(0.28-0.62) | 7.460000e-06 |
| | | Weighted mode | 6 | 0.38(0.22-0.64) | 1.417775e-02 |
| | | Simple mode | 6 | 0.38(0.21-0.68) | 2.782357e-02 |
| | | MR Egger | 6 | 1.07(0.41-2.80) | 9.025140e-01 |
| GPR160 | SAH | Inverse variance weighted | 3 | 0.83(0.77-0.90) | 7.570000e-06 |
| | | Weighted median | 3 | 0.84(0.77-0.91) | 1.610000e-05 |
| | | Weighted mode | 3 | 0.84(0.77-0.91) | 5.106901e-02 |
| | | Simple mode | 3 | 0.83(0.76-0.91) | 5.715426e-02 |
| | | MR Egger | 3 | 0.91(0.59-1.41) | 7.540743e-01 |
| PSMA4 | IA | Inverse variance weighted | 2 | 1.90(1.54-2.35) | 3.570216e-09 |
| SLC22A4 | IA | Inverse variance weighted | 6 | 1.41(1.26-1.58) | 3.699583e-09 |
| | | Weighted median | 6 | 1.40(1.22-1.60) | 1.436834e-06 |
| | | Weighted mode | 6 | 1.39(1.21-1.60) | 5.215431e-03 |
| | | Simple mode | 6 | 1.37(1.14-1.65) | 2.101001e-02 |
| | | MR Egger | 6 | 1.12(0.82-1.54) | 5.063622e-01 |
| KL | UIA | Inverse variance weighted | 12 | 0.51(0.39-0.68) | 3.885816e-06 |
| | | Weighted median | 12 | 0.57(0.41-0.79) | 8.683655e-04 |
| | | Weighted mode | 12 | 0.58(0.41-0.83) | 1.157771e-02 |
| | | Simple mode | 12 | 0.59(0.34-1.02) | 8.509464e-02 |
| | | MR Egger | 12 | 1.13(0.61-2.11) | 7.041095e-01 |

The MR results of exposures(genes) on outcomes

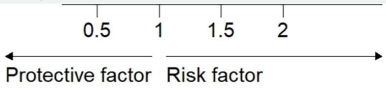


**Figure 2** Forest plot for MR results between blood eQTL and outcomes (IA, SAH and UIA). eQTL, expression quantitative trait locus; IA, intracranial aneurysm; MR, Mendelian randomisation; SAH, subarachnoid haemorrhage (the rupture of intracranial aneurysms); UIA, unruptured of intracranial aneurysm.

(PSMA4 (Pivw=3.57e-09, OR=1.90, 95% CI 1.54–2.35) and SLC22A4 (Pivw=3.70e-09, OR=1.41, 95% CI 1.26–1.58)) for IA and one potential drug targets (KL (Pivw=3.89e-06, OR=0.51, 95% CI 0.39–0.68)) for UIA after adjusting for multiple testing (P(IVW)<8.28e-6) (figure 2).

Using the cis-eQTL data from the PsychENCODE consortia, the analysis included a final count of 892 for SAH, 892 for IA and 891 for UIA. Subsequently, we performed a two-sample MR analysis using European summary statistics for patients with IA, UIA and SAH. Unfortunately, no significant potential drug targets were identified for the outcomes (IA, UIA and SAH) after multiple tests (p<1.87 e-5 (IVW)).

## Sensitivity analysis

Cochran's Q test for IVW-MR analysis showed no evidence of heterogeneity in any of the presented results (table 1). The intercept term in the MR-Egger regression indicated no overall horizontal pleiotropy, except for KL (all p>0.05, except for KL (p=0.021) (table 1)). MR-PRESSO analysis revealed no significant outliers.

## Reverse causality detection

The Steiger directionality test confirmed directionality, except for LTBP4 (p=0.31) (table 1). Moreover, the MR-Steiger filtering method detected no invalid SNPs (those with a reversed causal direction) (online supplemental tables 3–9).

## Colocalisation analysis

We performed a colocalisation analysis to assess the probability that SNPs associated with outcomes (IA, SAH and UIA) and eQTL shared causal genetic variants. This analysis focused on SNP within ±100 kb from the potentially druggable genes. Specifically, for IA, we identified one gene in the blood, PSMA4, with strong evidence of colocalisation. For SAH, we identified two genes in the blood, PSMA4 and PRCP, with strong evidence of colocalisation. The results suggest that PSMA4 and IA likely share a causal variant within the PSMA4 locus (PP.H4=0.97), PSMA4 and SAH likely share a causal variant within the PSMA4 locus (PP.H4=0.97), and PRCP and SAH likely share a causal variant within the PRCP locus (PP.H4=0.93).

Table 1 The results of MR analysis, sensitivity analysis and Steiger directionality test

| Exposure | Tissue | Outcome | Method | Nsnp | Pval | OR | Lci95 | Uci95 | Q_pval | Pleiotropy_pval | correct_causal_direction | steiger_pval |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PSMA4 | Blood | SAH | IVW | 2 | 7.52e-08 | 2.00 | 1.53 | 2.50 | 0.44 | NA | TRUE | 0.02 |
| PRCP | Blood | SAH | IVW | 7 | 1.20e-08 | 0.59 | 0.49 | 0.70 | 0.64 | 0.23 | TRUE | 0.001 |
| LTBP4 | Blood | SAH | IVW | 6 | 2.45e-06 | 0.48 | 0.35 | 0.65 | 0.39 | 0.16 | TRUE | 0.31 |
| GPR160 | Blood | SAH | IVW | 3 | 7.57e-08 | 0.83 | 0.77 | 0.90 | 0.37 | 0.74 | TRUE | 1.01e-54 |
| KL | Blood | UIA | IVW | 12 | 3.89e-06 | 0.51 | 0.39 | 0.68 | 0.29 | 0.02 | TRUE | 0.005 |
| PSMA4 | Blood | IA | IVW | 2 | 3.57e-09 | 1.90 | 1.54 | 2.35 | 0.66 | NA | TRUE | 0.02 |
| SLC22A4 | Blood | IA | IVW | 6 | 3.70e-09 | 1.41 | 1.26 | 1.58 | 0.51 | 0.20 | TRUE | 2.55e-12 |

IA, intracranial aneurysm; Lci, lower CI; MR, Mendelian randomisation; SAH, subarachnoid haemorrhage (the rupture of intracranial aneurysms); Uci, upper CI; UIA, unruptured of intracranial aneurysm.

Consequently, two potentially druggable genes showing evidence of a shared genetic effect between eQTL and outcomes (IA and SAH) were identified through the MR and colocalisation analyses. Table 2 presents the results.

### SMR analysis

We performed SMR analyses focusing on potential drug targets identified as significant in the colocalisation analysis. The SMR study results suggest a correlation between an increased risk of IA and SAH and elevated PSMA4 expression in the blood, implying that PSMA4 inhibitors may potentially decrease the likelihood of IA formation and rupture. Additionally, our results indicate a correlation between a decreased risk of SAH and elevated PRCP gene expression in the blood, suggesting that PRCP promoters may reduce the likelihood of IA rupture (Figure 3). The HEIDI test for the SMR analysis indicated that none of the observed associations were linked (p>0.05). Table 2 presents all the results.

### pQTL analysis

The results indicate a correlation suggesting a lower risk of SAH with elevated levels of circulating PRCP proteins (P(IVW)=0.014, OR=0.641, 95% CI 0.45–0.91). This supports our results at the protein level. However, our results showed no correlation between circulating PSMA4 proteins and IA or SAH (online supplemental tables 10–12).

### Transcriptomic analysis

This study analysed the expression profiles of PRCP and PSMA4 in the GSE15629 dataset. This investigation revealed a significant downregulation of PRCP gene expression within the disease cohort when using two distinct grouping methodologies. In contrast, PSMA4 gene expression demonstrated minimal variability (additional file 2; online supplemental figure S7A).

### Single-gene function analysis of PRCP

Detailed results are shown in online supplemental additional file 1.

### Drug-gene interactions

We identified that CARFILZOMIB, BORTEZOMIB, OPROZOMIB, IXAZOMIB CITRATE, MARIZOMIB and COTININE can regulate the gene expression of PSMA4. Regarding the PRCP gene, BENAZEPRIL was the only identified regulator of its expression (additional file 2; online supplemental figure S10).

## DISCUSSION

This groundbreaking study uses a multi-omics approach, using eQTL data from the PsychENCODE consortia and eQTLGen Consortium to explore potential drug targets for cerebral aneurysms through two-sample MR. Identifying four potential drug targets for SAH, two for IA and one for UIA, the study accounts for multiple tests. Colocalisation analysis suggests that PSMA4 likely shares the

Table 2 The results of colocalisation analysis, SMR analysis and HEDI test

| Exposure | Tissue | Outcome | Colocalisation analysis PPH4 | PPH4>0.80 | SMR beta | SMR P | SMR P(HEDI) |
|---|---|---|---|---|---|---|---|
| PSMA4 | Blood | SAH | 0.99 | YES | 0.63 | 6.44e-06 | 0.998 |
| PRCP | Blood | SAH | 0.97 | YES | −0.62 | 4.81e-06 | 0.366 |
| SLC22A4 | Blood | SAH | 0.57 | NO | – | – | – |
| LTBP4 | Blood | SAH | 0.55 | NO | – | – | – |
| GPR160 | Blood | SAH | 0.27 | NO | – | – | – |
| PSMA4 | Blood | IA | 0.98 | YES | 0.62 | 3.80e-07 | 0.93 |
| SLC22A4 | Blood | IA | 0.57 | NO | – | – | – |
| KL | Blood | UIA | 0.01 | NO | – | – | – |

HEDI, heterogeneity in dependent instruments; IA, intracranial aneurysm; SAH, subarachnoid haemorrhage (the rupture of intracranial aneurysms); SMR, summary-data-based Mendelian randomisation; UIA, unruptured of intracranial aneurysm.

same variant in IA and SAH, marking the first comprehensive exploration of cerebral aneurysm drug targets using a multi-omics strategy. Colocalisation analysis suggests that PRCP likely shares the same variant as SAH. SMR analysis provides evidence that the PSMA4 gene is causally associated with IA and SAH, while the PRCP gene is causally associated with SAH. These results indicate that PSMA4 and PRCP are the most promising drug targets for the treatment of cerebral aneurysms. Further investigation using pQTL data reveals that elevated plasma protein levels of PRCP are associated with a reduced risk of SAH, whereas the association of PSMA4 with IA formation and rupture was not validated at the protein level. Transcriptomic analysis identified significant downregulation of PRCP gene expression in the disease cohort, while the PSMA4 expression remained relatively stable.

## PROTEASOME 20S SUBUNIT ALPHA 4

PSMA4 encodes the core alpha subunit of the 20S proteasome, which is essential for controlling signal transduction, stress response, and inflammation.[17] Previous studies have shown that elevated PSMA4 levels are associated with aortic aneurysms (AA) and IA[18]; however, its relationship with SAH remained unclear. Our study not only validated the relationship between PSMA4 and IA but also revealed its relationship with SAH. Confirming the involvement of PSMA4 in IA and discovering its role in SAH enhances our understanding of its contribution to these conditions, emphasising its potential role in their development or progression. Transcriptome analysis revealed minimal variability in PSMA4 gene expression between the control and disease groups, limiting its discriminatory potential.

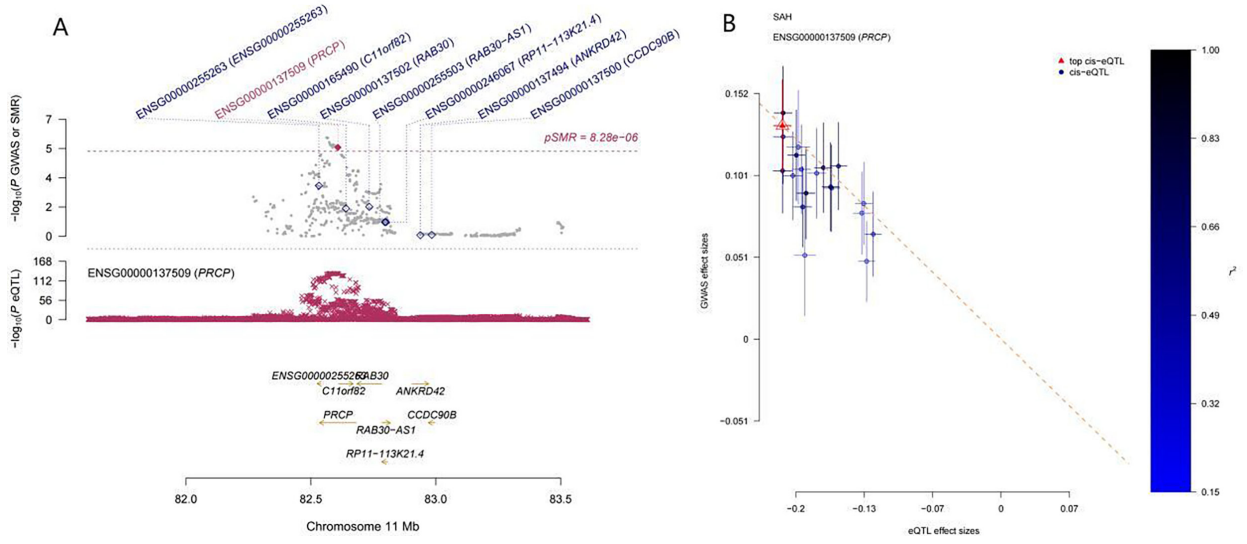


Figure 3 The summary-data-based Mendelian randomisation (SMR) result of PRCP with SAH risk. (A) The SMR locusplot. Top plot, gray dots represent the p values for SNPs from the SAH genome-wide association studies, diamond represents the p value for PRCP from the SMR test and the dashed line indicates the Bonferroni-corrected p value threshold. Bottom plot represent the eQTL p values of SNPs associated with expression PRCP in the blood. (B) The SMR effect plot. The horizontal axis represents the effect sizes of SNPs on PRCP, While the vertical axis represents the effect sizes of SNPs on SAH risk. eQTL, expression quantitative trait locus; PRCP, prolylcarboxypeptidase; SAH, subarachnoid haemorrhage (the rupture of intracranial aneurysms); SNP, single nucleotide polymorphism.

### PROLYLCARBOXYPEPTIDASE

PRCP emerges as a robust target, well-supported by diverse omics data. The gene encoding for PRCP is a serine protease involved in degrading angiotensin II and a-melanocyte-stimulating hormone.[19] With implications in blood pressure regulation, pain perception, and metabolism, PRCP associations with diseases such as hypertension, obesity, and diabetes further underscore its significance.[20]

Preventing ruptures in patients with IAs is crucial, yet no specific drugs currently exist for this purpose. Encouragingly, our two-sample MR analysis demonstrated that elevated PRCP gene expression in the blood is causally associated with a lower risk of SAH (IA rupture). This genetic evidence supports the development of targeted drugs to prevent IA ruptures, addressing a critical gap in current medical interventions. Additionally, pQTL served as a proposed instrument to validate our findings, consistently showing a negative association between PRCP protein levels and IA rupture. Transcriptomic analysis of PRCP expression in the disease and control groups revealed significantly lower PRCP levels in the disease group. This finding strengthens the association between PRCP downregulation and IA rupture.

In our analysis of the PRCP gene function, we discovered its crucial role in immune responses, metabolic processes and signal transduction pathways. Significantly, our study revealed a positive correlation between PRCP gene expression and endothelial cell scores, aligning with previous findings indicating that PRCP deficiency leads to reduced endothelial cell growth.[21] Frösen *et al* considered the loss of endothelial cells and smooth muscle cells as a marker of aneurysm rupture based on the results from a human IA sample investigation.[22] This suggests that PRCP prevents IA rupture by fostering endothelial cell proliferation for vessel repair. Gene ontology analysis suggests that PRCP may be associated with reactive oxygen species processes, consistent with previous reports of its antioxidant role in the endothelium.[23 24] Considering oxidative stress as a potential mechanism for aneurysm rupture,[25] it is reasonable to speculate that PRCP may prevent IA rupture through its antioxidant effects. Additionally, PRCP plays an important role in hypertension development, with benazepril regulating its expression. This suggests prioritising benazepril for blood pressure control in patients with IAs. Enhancing PRCP expression through research offers new therapeutic strategies, aiding in the development of more effective drugs to reduce adverse events. Further in-depth exploration is warranted in this research area.

## STRENGTHS AND LIMITATIONS

The strengths of our study are as follows: first, we performed MR and colocalisation analyses to estimate the causal effects of genes on outcomes (IA, UIA and SAH). Second, we performed extensive sensitivity analyses, excluding pleiotropy, heterogeneity and reverse causation. Third, validation of our results was achieved through the SMR method, supported by proteomic and transcriptomic data, enhancing the reliability of the study. Furthermore, we used single-gene functional analyses to explore the potential mechanisms between the target gene and the disease. Finally, we identified approved drugs capable of regulating target gene expression, making it possible to explore new applications for existing medications.

Our study had some limitations. First, we employed a relatively relaxed linkage disequilibrium removal threshold ($r^2$=0.1). Second, owing to data constraints, our outcome data relied solely on one study and lacked validation from other sources. Third, our GWAS data originated from European populations, potentially restricting the generalisability of our findings to other ethnic groups. Then, due to limitations in data availability and methodological constraints, we were compelled to remove sex chromosome targets from our analysis. This exclusion may hinder a comprehensive understanding of the disease state. Additionally, at present, there is no stratified outcome data by gender; consequently, we are unable to grasp the influence of gender on the disease. Finally, our control group for outcome data did not undergo imaging screening, which may introduce certain biases to our final results.

## CONCLUSION

In conclusion, our study underscores the potential of targeting PSMA4 to mitigate the risk of IA and SAH. Additionally, our findings highlight PRCP as a robust target for preventing IA rupture, supported by diverse omics data. PRCP may prevent IA rupture through its antihypertensive, anti-inflammatory, antioxidant and endothelial cell growth-promoting effects. However, further studies are required to confirm these findings.

**Author affiliations**
[1]Department of Neurology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China
[2]Department of Neurosurgery, Fudan University Shanghai Cancer Center, Shanghai, Shanghai, China
[3]The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China
[4]Department of Neurology, Zhengzhou University, Zhengzhou, Henan, China
[5]Reproductive Medicine Center, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China
[6]NHC Key Laboratory of Prevention and treatment of Cerebrovascular Disease, zhengzhou, henan, People's Republic of China
[7]Henan Key Laboratory of Cerebrovascular Diseases, zhengzhou, henan, People's Republic of China

**Acknowledgements** We appreciated all participants and investigators for this research.

**Contributors** YL specialise in the diagnosis, treatment, basic and clinical research of difficult neurological diseases such as cerebrovascular disease, cerebral small vessel disease and sleep disorders.

**Funding** This research was supported by a grant from the Henan Province medical science and technology research major project (SBGJ202101016).

**Competing interests** None declared.

**Patient consent for publication** Not applicable.

**Ethics approval** Not applicable.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** All data relevant to the study are included in the article or uploaded as supplementary information.



**ORCID iDs**

Yunchao Wang http://orcid.org/0000-0002-3702-3826
Chang-He Shi http://orcid.org/0000-0002-4843-0072
Yuming Xu http://orcid.org/0000-0003-2689-9897
Yusheng Li http://orcid.org/0000-0003-2437-0295

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