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ORIGINAL RESEARCH

Proteome-Wide Mendelian Randomization Identifies Therapeutic Targets for Abdominal Aortic Aneurysm

Ka Zhang, PhD*; Yuan Liu, MD*; Aiqin Mao, PhD; Changzhu Li, PhD; Li Geng, PhD; Hao Kan, PhD

BACKGROUND: The proteome is a key source of therapeutic targets. We conducted a comprehensive Mendelian randomization analysis across the proteome to identify potential protein markers and therapeutic targets for abdominal aortic aneurysm (AAA).

METHODS AND RESULTS: Our study used plasma proteomics data from the UK Biobank, comprising 2923 proteins from 54 219 individuals, and from deCODE Genetics, which measured 4907 proteins across 35 559 individuals. Significant proteomic quantitative trait loci were used as instruments for Mendelian randomization. Genetic associations with AAA were sourced from the AAAgen consortium, a large-scale genome-wide association study meta-analysis involving 37 214 cases and 1 086 107 controls, and the FinnGen study, which included 3869 cases and 381 977 controls. Sequential analyses of colocalization and summary-data-based Mendelian randomization were performed to verify the causal roles of candidate proteins. Additionally, single-cell expression analysis, protein–protein interaction network analysis, pathway enrichment analysis, and druggability assessments were conducted to identify cell types with enriched expression and prioritize potential therapeutic targets. The proteome-wide Mendelian randomization analysis identified 34 proteins associated with AAA risk. Among them, 2 proteins, COL6A3 and PRKD2, were highlighted by colocalization analysis, summary-data-based Mendelian randomization, and the heterogeneity in an independent instrument test, providing the most convincing evidence. These protein-coding genes are primarily expressed in macrophages, smooth muscle cells, and mast cells within abdominal aortic aneurysm tissue. Several causal proteins are involved in pathways regulating lipid metabolism, immune responses, and extracellular matrix organization. Nine proteins have already been targeted for drug development in diabetes and other cardiovascular diseases, presenting opportunities for repurposing as AAA therapeutic targets.

CONCLUSIONS: This study identifies causal proteins for AAA, enhancing our understanding of its molecular cause and advancing the development of therapeutics.

Key Words: abdominal aortic aneurysm ■ biomarker ■ drug target ■ plasma protein ■ proteome-wide Mendelian randomization

Abdominal aortic aneurysm (AAA) is a severe vascular disease characterized by a permanent dilation of the arterial wall, measuring 50% or more compared with the normal diameter.[1,2] As the disease progresses, it can lead to aortic rupture, which has a mortality rate >80%.[1] Despite significant advancements in surgical and endovascular treatments for AAAs over the past decade, no proven pharmacological interventions exist to slow AAA growth or prevent aortic rupture.[3]

Population-based and clinical studies have identified obesity, smoking, unhealthy diets, and sedentary lifestyles as major modifiable risk factors for AAA.[4–6] To enhance genetic understanding, several large-scale

Correspondence to: Hao Kan, PhD, Wuxi School of Medicine, Jiangnan University, 1800 Li Hu Road, Wuxi 214122, China. Email: kanhao@jiangnan.edu.cn

*K. Zhang and Y. Liu contributed equally.

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CLINICAL PERSPECTIVE

What Is New?

- The current study identified 34 circulating plasma proteins that were causally linked to abdominal aortic aneurysms.
- COL6A3 and PRKD2 were supported by Mendelian randomization, colocalization analysis, and summary-data-based Mendelian randomization analysis as plasma circulating proteins strongly associated with the risk of abdominal aortic aneurysm.

What Are the Clinical Implications?

- These findings further support the evaluation of COL6A3 and PRKD2 as potential pharmacological targets for abdominal aortic aneurysm treatment.

Nonstandard Abbreviations and Acronyms

HEIDI	heterogeneity in independent instrument
MR	Mendelian randomization
pQTL	protein quantitative trait loci
SMR	summary-data-based Mendelian randomization
STRING	Search Tool for the Retrieval of Interacting Genes
UKB-PPP	UK Biobank Pharma Proteomics Project

genome-wide association studies (GWASs) have been conducted, revealing >140 loci associated with AAA, thereby increasing the usefulness of genetic prediction.[4,6,7] With the advent of high-throughput techniques for serum protein detection and quantification, numerous studies have explored protein associations with AAA risk to elucidate the molecular pathological basis.[4,8] A population-based study identified 118 plasma proteins linked to AAA in the discovery phase, but only 2 proteins demonstrated a causal relationship with AAA.[8] Another larger study found causal relationships between 23 circulating protein levels and genetic susceptibility to AAA.[4] However, these studies often had limitations, such as focusing on a limited number of candidate proteins, using observational designs, or having small sample sizes, thereby constraining the understanding of the causal role of protein markers in AAA risk.

The increasing use of high-throughput proteomics platforms in large-scale genotyped biobanks presents new opportunities for deriving biological insights from GWAS data.[9,10] Notably, the Mendelian randomization (MR) approach facilitates the identification of causal relationships between exposures and diseases, effectively mitigating the potentially confounding effects of environmental factors.[11,12]

In this study, we conducted the largest investigation to date using MR to identify potential causal effects of circulating proteins on AAA phenotypes. We used blood-based proteomics data from 2 large, independent cohorts (UK Biobank and deCODE Genetics) and well-powered GWASs for AAA. Recognizing that MR alone may be insufficient for pinpointing credible proteins in causal pathways, we also used colocalization, summary-data-based MR (SMR), and the heterogeneity in independent instrument (HEIDI) test. Furthermore, single-cell-type expression analysis was used to detect the enrichment of these proteins in specific cell types within AAA tissue. Finally, we performed druggability evaluations to explore their potential as therapeutic targets for AAA.

METHODS

Data Availability

The GWAS summary statistics for AAA based on the AAAgen Consortium can be accessed at https://csg.sph.umich.edu/willer/public/AAAgen2023/. The data of FinnGen can be accessed at https://www.finngen.fi/en. The protein quantitative trait loci (pQTL) data can be accessed at https://registry.opendata.aws/ukbppp/ and https://www.decode.com/summarydata/. The single-cell RNA sequencing data of AAA tissue can be accessed at https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE166676.

Proteomic Data Source and the Processing of Genetic Instruments

Two large-scaled proteomic studies, UKB-PPP (UK Biobank Pharma Proteomics Project)[13] and deCODE Genetics,[14] were used to extract summary statistics of genetic associations with plasma proteins. The UKB-PPP involves profiling plasma pQTL data for 2923 proteins using the Olink Explore 3072 platform from a cohort of 54 219 UK Biobank participants. For deCODE Genetics, 4907 proteins in 35 559 individuals were measured to obtain their plasma pQTL data using the SOMAscan version 4 assay (Table S1).

We used pQTL from circulating plasma from the 2 studies above as genetic instruments for analysis. The platform identification for each protein from each study was mapped to the gene symbol and unified based on annotations provided by the original studies and manual review.[15] To obtain pQTLs for MR analysis, all summary statistics were subjected to a quality control (QC) filtering process that excluded insertion–deletion variants,

variants with a minor allele frequency <0.001, palindromic variants with minor allele frequency >0.42, and variants in trans-pQTL, including the extended major histocompatibility complex region (Chr6: 25–34 Mb). The inclusion of trans-pQTL helps to enhance statistical capacity and evaluate and control potential pleiotropic effects through sensitivity analysis. We selected variants that achieved genome-wide significance ($P>5\times10^{-8}$) for linkage disequilibrium pruning and then divided genome-wide significant variants into cis-pQTL and trans-pQTL, where cis-pQTLs are variants within 1 Mb upstream and downstream of the associated protein-coding genes (ensemble 108 annotations). QC-filtered genome-wide significant variants outside of the cis region are considered trans-pQTLs.[16,17] The single-nucleotide polymorphisms (SNPs) corresponding to each pQTL variant were unified according to the human genome Build 37 (National Center for Biotechnology Information GRCh37) genome coordinates, based on annotations provided by the original study and manual reviews (https://github.com/HaobinZhou/Get_MR/blob/main/2.0/Get_MR2.0.r). Linkage disequilibrium clumping was then conducted to identify independent pQTLs for each protein ($r^2<0.001$). The R^2 and F statistic ($R^2=2\times$Expected average frequency (EAF)$\times(1-\text{EAF})\times\beta$; $F=R^2\times(N-2)/(1-R^2)$) were used to estimate the strength of genetic instruments, where R^2 was the proportion of the variability of the protein levels explained by each genetic instrument.[18]

Data Sources for AAA

Data on the associations of protein-associated SNPs with AAA were obtained from the AAAgen Consortium[4] and the FinnGen study (https://r10.finngen.fi/). The AAAgen Consortium included 14 studies with a total of 37 214 cases and 1 086 107 controls of European descent. We used the latest release data on AAA from the FinnGen study R10 in this analysis, which comprised 3869 cases and 381 977 controls. There were no sample overlaps between the 2 outcome data sets. In MR analysis, we treated the AAAgen Consortium as the discovery study and the FinnGen R10 study as the replication. The basic information for these data sets is shown in Table S2.

Statistical Analysis

Proteome-Wide MR Analysis

We performed MR analyses using the TwoSampleMR package[19,20] within the R environment. When only 1 instrument was available for a particular protein, we applied the Wald ratio method. The inverse-variance weighted method was used to obtain the MR effects estimates for proteins with >1 instrument. The heterogeneity test was performed to assess the heterogeneity of the genetic instruments based on the Q statistic.[21] To estimate horizontal pleiotropy, the MR-Egger intercept test was used. Bonferroni correction was used for multiple testing correction, with $P<2.07\times10^{-5}$ (0.05/2410) as the significance level for cis-pQTLs and with $P<1.70\times10^{-5}$ (0.05/2938) as the significance level for trans-pQTLs. Replication MR analysis was further performed for the identified proteins based on AAA GWAS summary data from the FinnGen study. A false discovery rate (FDR) <0.05 was defined as the significance level for replication. The Benjamini-Hochberg procedure was applied for multiple comparisons to deal with type I error.

Colocalization Analysis

Colocalization analysis was used to test whether the identified associations of proteins with AAA were driven by linkage disequilibrium, providing further validation of the MR results. We used summary statistics of proteins and AAAgen meta-GWASs to perform Bayesian colocalization analysis using the coloc package.[22] For each protein, SNPs within ±500 kb upstream and downstream of their corresponding gene were examined for colocalization with AAA. The analysis considered 5 hypotheses: (1) no causal variant for either protein or AAA in the locus (H0); (2) association with protein only (H1); (3) association with AAA only (H2); (4) both protein and AAA associated, but with distinct causal variants (H3); and (5) both protein and AAA associated, sharing the same causal variant (H4).[23] A posterior probability for H4 >80% under different priors and windows was considered strong evidence of colocalization. The LocusCompareR package was used to visualize colocalization results.[24]

SMR Analysis

SMR analysis was conducted as complementary evidence to verify the causal associations between proteins and AAA.[25,26] The HEIDI test, using multiple SNPs (up to 20 SNPs) in a region, was used to distinguish proteins associated with AAA risk due to a shared genetic variant rather than genetic linkage.[25,26] The SMR and HEIDI tests were performed using SMR software (SMR version 1.3.1). A P value $<1.47\times10^{-3}$ (0.05/34) was defined as the significance level for SMR. A P value of the HEIDI test >0.05 indicated that the association of protein and AAA was not driven by linkage disequilibrium.

Single-Cell-Type Expression Analysis

The cell-type-specific expression of target genes with evidence for a potential causal effect on AAA at the plasma protein levels was further evaluated using single-cell RNA sequencing data of human AAA profiled from the Gene Expression Omnibus database.[27] The raw single-cell RNA-seq data were analyzed using the Seurat package. Cells meeting these criteria were removed: (1) <500 genes (low quality) or >5000 genes

detected (potential doublets) and (2) >10% of unique molecular identifiers originating from the mitochondrial genome. Data normalization and batch effect removal were performed using the Seurat package. Cell clusters were annotated based on the SingleR package and references to relevant articles.[28–30] To examine whether the identified AAA causal protein-coding genes were highly expressed in a particular cell type in AA tissue, differential expression analysis based on the Wilcoxon rank sum test was performed. Genes with an average $\log_2$ fold change >0.5 and an FDR-adjusted P value <0.05 were identified as enrichment genes in a cell type.

Protein–Protein Interaction, Pathway Enrichment Analysis, and Druggability Evaluation

To explore the potential interactions between identified proteins, a protein–protein interaction network was constructed using the STRING (Search Tool for the Retrieval of Interacting Genes) database (https://string-db.org/).[31] Pathway enrichment analysis was performed through the ClusterProfiler package to analyze the biological functions enriched by the genes of the identified protein targets.[32] To assess whether the identified proteins could serve as potential therapeutic targets, we identified approved compounds, immunotherapies, and known compounds that may interact with these proteins through the DGIdb (Drug–Gene Interaction Database),[33] ChEMBL (Chemical Database of bioactive molecules with drug-like properties),[34] and DrugBank[35] databases.

RESULTS

An overview of the study design is shown in Figure 1. Our MR instruments included a total of 10 187 cis-pQTLs linked to 1660 unique proteins assessed within the UKB-PPP cohort and 13 229 cis-pQTLs related to 1385 unique proteins measured in the deCODE cohort (Table S3). Although 635 proteins were assayed on both platforms, yielding a total of 2410 unique proteins examined in MR using cis-pQTL instruments, we maintained 2410 analyses for our Bonferroni correction to conservatively account for the lowest available P value. Separately, we used (as instruments for MR analysis) 8544 trans-pQTL instruments (excluding the extended major histocompatibility complex region) corresponding to 1737 unique proteins assessed within the UKB-PPP cohort, and 28 916 trans-pQTLs related to 1899 unique proteins measured in the deCODE cohort (Table S3). Among these, 698 unique proteins were assayed by both platforms. Despite this overlap, we conservatively controlled for 2938 tests in our Bonferroni corrections of the trans-pQTL MR results.

Proteome-Wide MR Analysis Identified 34 Circulating Proteins for AAA

The F statistics of all genetic instruments were >10, indicating a good strength (Table S3). Using cis-pQTLs as instrumental variables, 21 proteins were significantly associated with AAA risk after Bonferroni correction ($P<2.07\times10^{-5}$) based on Wald ratio or inverse-variance weighted method (Figure 2A). In the replication stage, 12 proteins (AGT, APOC3, APOE, CELSR2, COL6A3, DPP4, INHBC, LPL, MMP12, PLTP, PRKD2, and ZPR1) were successfully validated in the FinnGen data set (FDR <0.05) based on the Wald ratio or inverse-variance weighted method (Table 1). No protein showed significant horizontal pleiotropy ($P_{pleiotropy}>0.05$) or heterogeneity ($P_{heterogeneity}>0.05$) (Table S4). Genetically predicted higher levels of AGT, APOC3, INHBC, MMP12, PLTP, PRKD2, and ZPR1 were associated with an increased risk of AAA, whereas the other 5 proteins (APOE, CELSR2, COL6A3, DPP4, and LPL) were negatively associated with AAA risk, suggesting that lower levels of these proteins were associated with a higher risk of AAA (Figure 3 and Table 1). These associations were consistent in additional analyses, including weighted mode, weighted median, and MR-Egger, except for simple mode (Table S4).

The separate MR analysis using trans-pQTLs as instrument variables identified 48 proteins meeting the Bonferroni correction threshold ($P<1.70\times10^{-5}$) that may be causally associated with the susceptibility to AAA (Figure 2B). Finally, 22 proteins were validated in the FinnGen data set (FDR<0.05) and tested for heterogeneity ($P_{heterogeneity}>0.05$) and horizontal pleiotropy ($P_{pleiotropy}>0.05$) (Table 1). Among these, 13 proteins (APOB, BPIFA2, C1ORF56, CD70, DDAH1, DLD, GRN, LBP, NPL, NR3C2, PTGES2, SNCA, and ULBP2) were associated with an increased risk of AAA, whereas the other 9 proteins (ANGPTL3, ANTXR1, ASGR1, CA11, COL3A1, HPGDS, HS3ST3B1, NAP1L2, and PSAPL1) were negatively associated with AAA risk (Figure 3 and Table S5). These associations results were consistent across all 5 analysis methods (Table S4).

Colocalization Analysis Supported the Causality of 8 Proteins With AAA

Among 34 MR-identified proteins in relation to AAA, 8 proteins had high support from colocalization analysis (posterior probability for H4 ≥0.8), including ANTXR1, APOE, C1ORF56, CA11, CELSR2, COL6A3, GRN, and PRKD2 (Table 1). This suggests that there is likely a common causal variation between these protein levels and AAA risk. Figures S1 through S8 show the regional association for colocalization results.

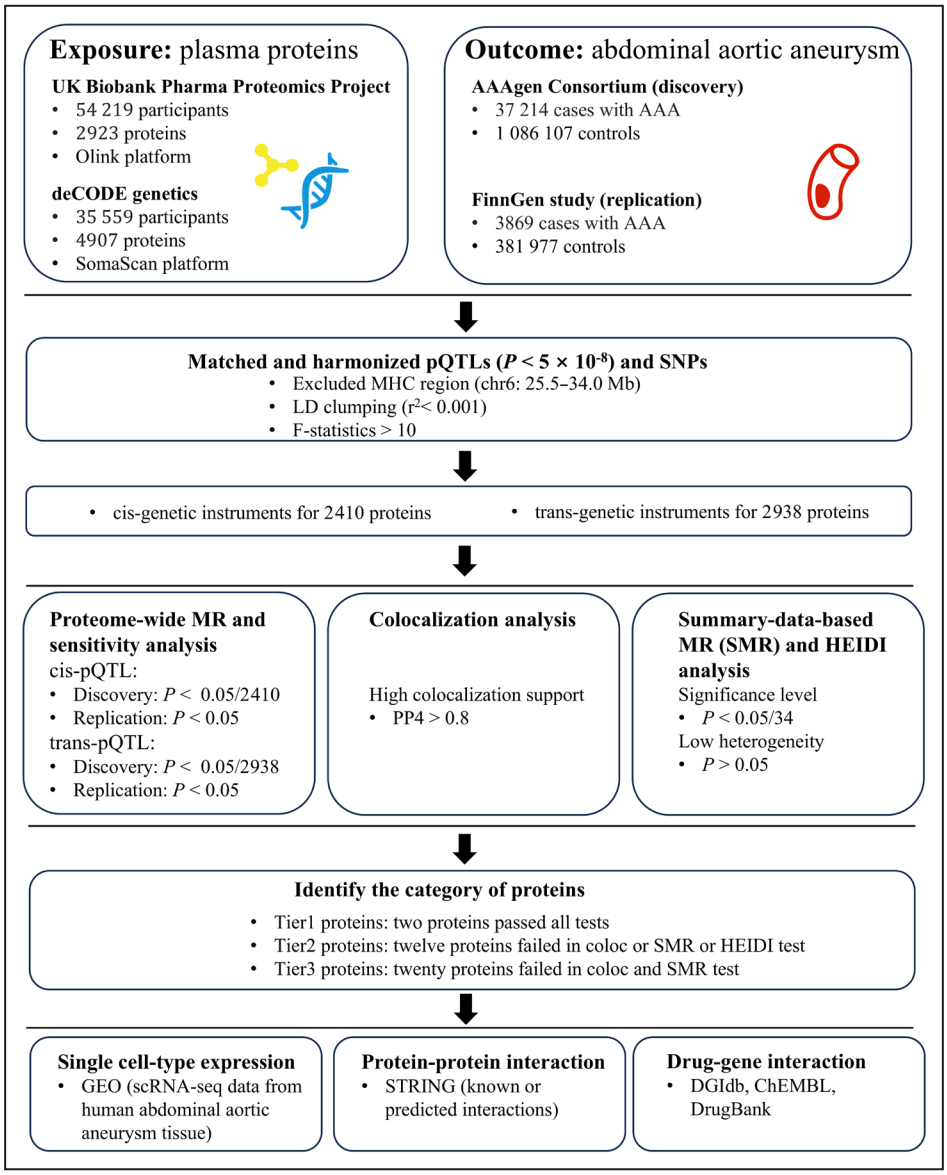

Figure 1. Research flowchart of the study.
AAA indicates abdominal aortic aneurysm; ChEMBL, Chemical Database of Bioactive Molecules with Drug-Like Properties; DGIdb, Drug-Gene Interaction Database; GEO, Gene Expression Omnibus; HEIDI, heterogeneity in independent instrument; LD, linkage disequilibrium; MHC, major histocompatibility complex; MR, Mendelian randomization; PP4, posterior probability for both protein and AAA associated, sharing the same causal variant; pQTLs, protein quantitative trait loci; scRNA-seq, single-cell RNA sequencing; SMR, summary-data-based Mendelian randomization; SNPs, single-nucleotide polymorphisms; and STRING, Search Tool for the Retrieval of Interacting Genes.

SMR and HEIDI Tests Verified 6 Causal Proteins

To further verify the findings, we performed SMR and HEIDI tests for 34 proteins with full summary-level data. Twelve of 34 proteins passed the SMR test ($P<1.47\times10^{-3}$), and 6 of them passed the HEIDI test ($P>0.05$) (Table 1). The SMR locus plot and effect plots of 7 proteins are shown in Figures S9 through S14. Based on this evidence, we classified these proteins into 3 tiers. Two proteins (COL6A3 and PRKD2) passed all tests and were classified into tier 1 (Table 1). Twelve proteins that failed colocalization analysis, the SMR test, or the HEIDI test (ANTXR1, APOC3, APOE, C1ORF56, CA11, CELSR2, DDAH1, DPP4, GRN, INHBC, MMP12, and ZPR1) were classified into tier 2. Twenty proteins (AGT, ANGPTL3, APOB, ASGR1, BPIFA2, CD70, COL3A1, DLD, HPGDS, HS3ST3B1, LBP, LPL, NAP1L2, NPL, NR3C2, PLTP, PSAPL1, PTGES2, SNCA, and ULBP2)

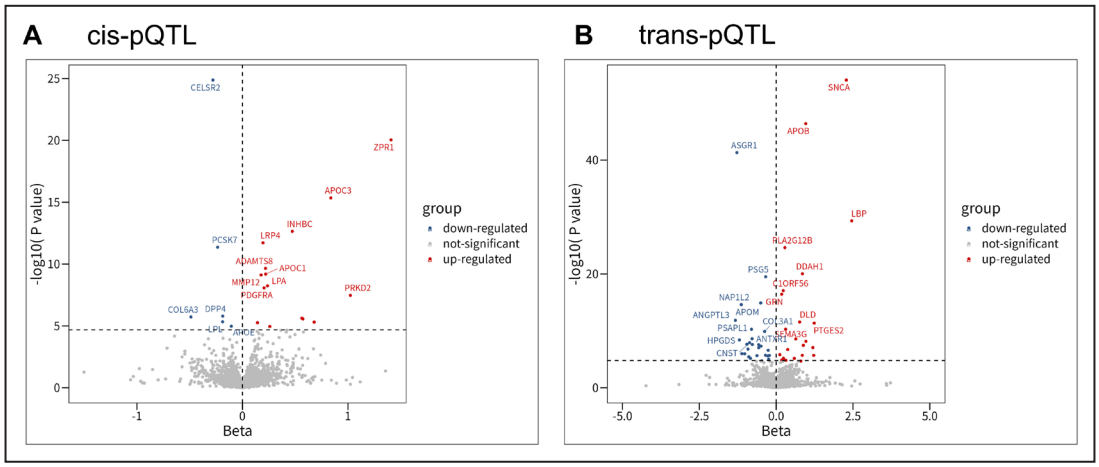

Figure 2. Volcano plot showing results of proteome-wide MR analysis.
MR using cis-pQTLs (**A**) and trans-pQTLs (**B**) in the discovery stage of AAA. The *P* value was calculated by the inverse-variance weighted or Wald ratio method. N=2410 proteins for cis-pQTLs and n=2938 proteins for trans-pQTLs. Beta is the effect value calculated by MR analysis. AAA indicates abdominal aortic aneurysm; AGT, Angiotensin; ANGPTL3, Angiopoietin-related protein 3; ANTXR1, Anthrax toxin receptor 1; APOB, Apolipoprotein B; APOC3, Apolipoprotein C-III; APOE, Apolipoprotein E; ASGR1, Asialoglycoprotein receptor 1; BPIFA2, BPI fold-containing family A member 2; C1ORF56, Protein MENT; CA11, Carbonic anhydrase-related protein 11; CD70, CD70 antigen; CELSR2, Cadherin EGF LAG seven-pass G-type receptor 2; COL3A1, Collagen type III alpha 1 chain; COL6A3, Collagen type VI alpha 3 chain; DDAH1, dimethylarginine dimethylaminohydrolase 1; DLD, Dihydrolipoyl dehydrogenase; DPP4, Dipeptidyl peptidase 4; GRN, Granulin; HPGDS, Hematopoietic prostaglandin D synthase; HS3ST3B1, Heparan sulfate glucosamine 3-O-sulfotransferase 3B1; INHBC, Inhibin beta C chain; LBP, Lipopolysaccharide-binding protein; LPL, Lipoprotein lipase; MMP12, Macrophage metalloelastase; MR, Mendelian randomization; NAP1L2, Nucleosome assembly protein 1-like 2; NPL, N-acetylneuraminate lyase; NR3C2, Nuclear Receptor Subfamily 3 Group C Member 2; PLTP, Phospholipid transfer protein; pQTL, protein quantitative trait loci; PRKD2, Protein Kinase D2; PSAPL1, Proactivator polypeptide-like 1; PTGES2, Prostaglandin E synthase 2; SNCA, Alpha-synuclein; ULBP2, UL16-binding protein 2; and ZPR1, Zinc finger protein.

that failed in both colocalization analysis and HEIDI test were classified into tier 3.

Cell-Type Specificity Expression in the Human Abdominal Aortic Aneurysm Tissue

To explore whether the coding genes of 34 circulating proteins had any cell type-specific enrichment in human AAA tissue, we performed single-cell-type expression analysis using single-cell RNA-seq data from Gene Expression Omnibus. Cells were clustered into 14 cell types (endothelial cells, smooth muscle cells, T cells, natural killer cells, fibroblasts cells, mast cells, erythrocytes, dendritic cells, macrophage, B cell, neutrophils, plasma cells, epithelial cells, and basal cells) (Figure 4A). Thirty-two of 34 protein-coding genes had expression data in AAA tissue, whereas BPIFA2 and INHBC expression was undetected; Figures 4B and 4C show the single-cell expression of these 32 coding genes in every cluster. Among them, 8 protein-coding genes had cell type-specific enrichment in AAA tissue at average log2 fold change >0.5 and FDR <0.05 (Figure 4D). Specifically, APOE, GRN, and PLTL were mainly enriched in macrophages, whereas COL3A1 and COL6A3 were enriched in smooth muscle cells, and HPGDS was enriched in mast cells.

Protein–Protein Interaction, Pathway Enrichment Analysis, and Druggability Evaluation of Potential Therapeutic Targets

Protein–protein interaction analysis identified interactions between the potential causal proteins APOE, APOB, APOC3, PLTP, LPL, ANGPTL3, and ASGR1, which form the central hub of the protein–protein interaction network in AAA (Figure S15). Pathway enrichment analyses revealed that these proteins are significantly associated with the regulation of lipid metabolic processes and inflammatory responses (Table S6). Additionally, several potential causal proteins (COL3A1, ANTXR1, DPP4, AGT, MMP12) were enriched in signaling pathways related to extracellular matrix organization, which plays a critical role in vascular wall damage and plaque formation in aortic aneurysms.

We identified 9 actionable drug targets (AGT, APOC3, APOE, COL6A3, DPP4, LPL, MMP12, PLTP, and PRKD2) for AAA using proteins with strictly significant associations obtained from MR analysis using

Table 1. Summary Results From MR, Colocalization, and SMR for 13 Proteome-Wide MR-Identified Proteins

Protein	Protein full name	MR			Colocalization	SMR			Cis/trans	Category
		β	$P_{discovery}$	$FDR_{replication}$	PP4 >0.8	β	*P* value	P_{HEIDI} value		
AGT	Angiotensin	0.56	2.36E-06	6.27E-03	No	0.03	5.71E-02	1.64E-01	Cis	Tier 3
ANGPTL3	Angiopoietin-related protein 3	−1.33	1.40E-12	2.37E-04	No	−0.06	3.38E-02	8.76E-01	Trans	Tier 3
ANTXR1	Anthrax toxin receptor 1	−0.79	2.41E-09	8.52E-03	Yes	−0.44	2.27E-02	2.07E-01	Trans	Tier 2
APOB	Apolipoprotein B	0.96	3.88E-47	3.23E-07	No	…	…	…	Trans	Tier 3
APOC3	Apolipoprotein C-III	0.84	4.47E-16	6.27E-03	No	0.42	1.53E-05	1.20E-01	Cis	Tier 2
APOE	Apolipoprotein E	−0.11	1.06E-05	3.89E-02	Yes	−0.14	1.91E-38	3.60E-06	Cis	Tier 2
ASGR1	Asialoglycoprotein receptor 1	−1.28	4.74E-42	3.44E-02	No	−0.16	4.01E-03	9.21E-02	Trans	Tier 3
BPIFA2	BPI fold-containing family A member 2	0.97	6.79E-09	3.85E-02	No	0.05	3.62E-01	4.58E-01	Trans	Tier 3
C1ORF56	Protein MENT	0.22	2.05E-17	3.44E-02	Yes	0.30	1.43E-02	5.39E-01	Trans	Tier 2
CA11	Carbonic anhydrase-related protein 11	−0.89	3.55E-06	6.60E-03	Yes	−0.11	3.96E-01	5.37E-01	Trans	Tier 2
CD70	CD70 antigen	0.38	1.90E-07	1.86E-02	No	0.01	7.15E-01	3.84E-01	Trans	Tier 3
CELSR2	Cadherin EGF LAG seven-pass G-type receptor 2	−0.28	1.34E-25	1.67E-03	Yes	−0.29	1.87E-39	2.90E-02	Cis	Tier 2
COL3A1	Collagen α-1(III) chain	−0.38	1.25E-10	6.27E-03	No	−0.39	2.90E-02	…	Trans	Tier 3
COL6A3	Collagen α-3(VI) chain	−0.49	1.88E-06	3.89E-02	Yes	−0.49	9.38E-06	8.52E-02	Cis	Tier 1
DDAH1	Dimethylarginine dimethylaminohydrolase 1	0.85	9.09E-21	5.90E-03	No	0.49	9.38E-06	8.52E-02	Trans	Tier 2
DLD	Dihydrolipoyl dehydrogenase	0.76	2.74E-12	8.91E-03	No	0.05	4.11E-01	9.86E-01	Trans	Tier 3
DPP4	Dipeptidyl peptidase 4	−0.19	1.60E-06	1.73E-02	No	−0.19	2.14E-06	1.91E-01	Cis	Tier 2
GRN	Granulin	0.18	3.68E-17	5.44E-03	Yes	0.03	4.31E-01	3.76E-01	Trans	Tier 2
HPGDS	Hematopoietic prostaglandin D synthase	−1.20	3.83E-09	3.44E-02	No	−0.03	2.07E-01	8.61E-06	Trans	Tier 3
HS3ST3B1	Heparan sulfate glucosamine 3-O-sulfotransferase 3B1	−0.27	2.40E-06	6.27E-03	No	0.01	6.33E-01	3.12E-01	Trans	Tier 3
INHBC	Inhibin β C chain	0.47	2.24E-13	2.00E-02	No	0.07	5.43E-06	7.63E-02	Cis	Tier 2
LBP	Lipopolysaccharide-binding protein	2.46	4.68E-30	3.23E-07	No	0.01	9.94E-01	6.63E-01	Trans	Tier 3
LPL	Lipoprotein lipase	−0.19	4.59E-06	3.69E-02	No	−0.07	1.98E-04	5.78E-05	Cis	Tier 3
MMP12	Macrophage metalloelastase	0.18	7.57E-10	6.27E-03	No	0.14	5.16E-16	9.11E-02	Cis	Tier 2
NAP1L2	Nucleosome assembly protein 1-like 2	−1.13	2.41E-15	5.90E-03	No	…	…	…	Trans	Tier 3
NPL	N-acetylneuraminate lyase	0.31	2.06E-06	1.05E-02	No	0.01	9.36E-01	6.26E-01	Trans	Tier 3
NR3C2	Nuclear receptor subfamily 3 group C member 2	1.19	8.58E-08	3.85E-02	No	…	…	…	Trans	Tier 3
PLTP	Phospholipid transfer protein	0.14	5.44E-06	2.04E-02	No	0.14	6.95E-13	2.59E-16	Cis	Tier 3
PRKD2	Protein kinase D2	1.02	3.35E-08	6.72E-03	Yes	1.02	7.68E-05	4.34E-01	Cis	Tier 1
PSAPL1	Proactivator polypeptide-like 1	−0.80	5.21E-11	1.05E-02	No	−0.01	5.89E-01	6.45E-01	Trans	Tier 3
PTGES2	Prostaglandin E synthase 2	1.24	4.33E-12	7.96E-04	No	0.06	5.83E-01	6.10E-01	Trans	Tier 3
SNCA	α-synuclein	2.28	8.59E-55	1.00E-03	No	0.13	3.99E-01	4.99E-01	Trans	Tier 3
ULBP2	UL16-binding protein 2	0.64	2.61E-09	1.55E-04	No	0.01	7.90E-01	5.31E-01	Trans	Tier 3
ZPR1	Zinc finger protein	1.41	9.09E-21	5.90E-03	No	1.41	7.70E-08	4.83E-01	Cis	Tier 2

PP4 >0.8 means 2 signals were considered to have a strong support of colocalization. β indicates effect value calculated by MR analysis; HEIDI, heterogeneity in independent instrument; MR, Mendelian randomization; $P_{discovery}$, MR analysis *P* value in discovery cohort; PP4, posterior probability for both protein and AAA associated, sharing the same causal variant; $FDR_{replication}$, MR analysis adjust *P* value in replication cohort; P_{HEIDI}, *P* value of the HEIDI test; and SMR, summary-data-based MR.

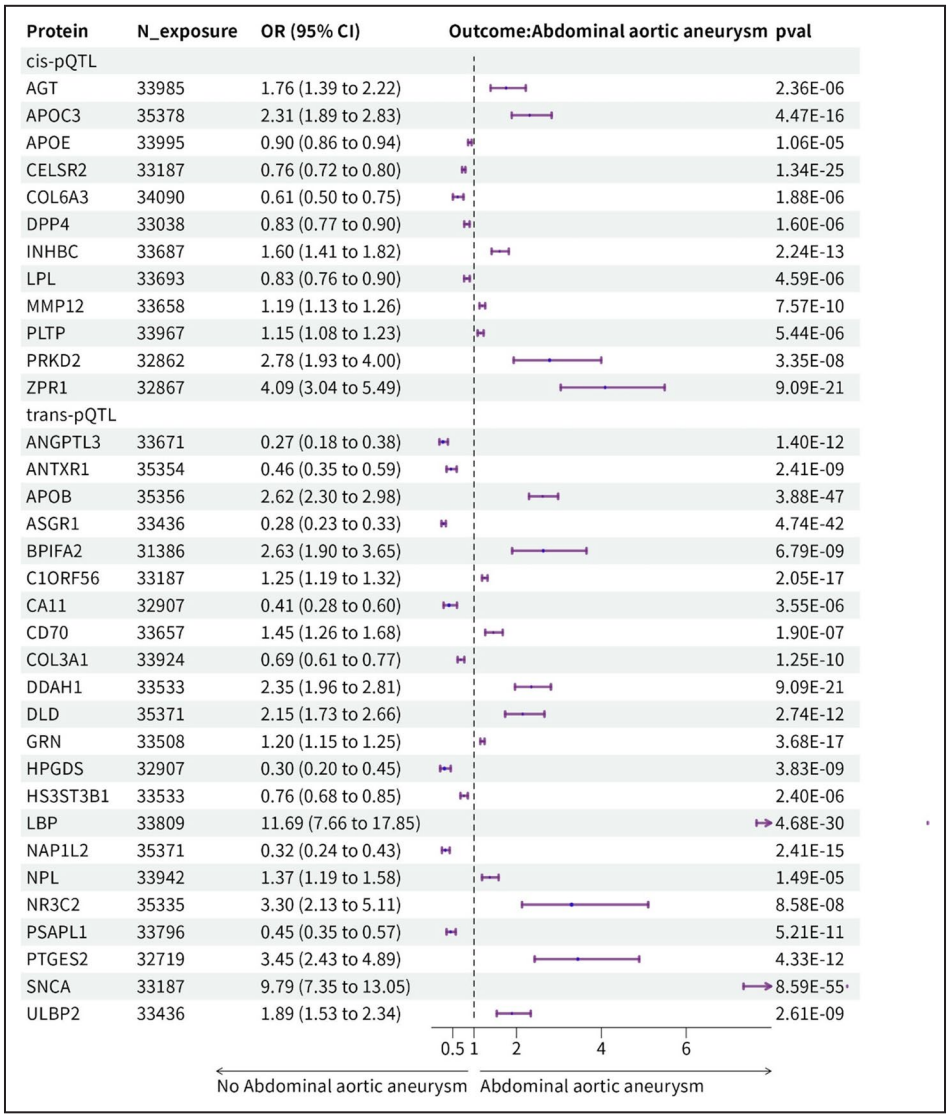

Figure 3. MR analyses show causal effect of 34 proteome-wide identified proteins on AAA.

The *P* value was calculated by the inverse-variance weighted or Wald ratio method. AAA indicates abdominal aortic aneurysm; AGT indicates Angiotensin; ANGPTL3, Angiopoietin-related protein 3; ANTXR1, Anthrax toxin receptor 1; APOB, Apolipoprotein B; APOC3, Apolipoprotein C-III; APOE, Apolipoprotein E; ASGR1, Asialoglycoprotein receptor 1; BPIFA2, BPI fold-containing family A member 2; C1ORF56, Protein MENT; CA11, Carbonic anhydrase-related protein 11; CD70, CD70 antigen; CELSR2, Cadherin EGF LAG seven-pass G-type receptor 2; COL3A1, Collagen type III alpha 1 chain; COL6A3, Collagen type VI alpha 3 chain; DDAH1, dimethylarginine dimethylaminohydrolase 1; DLD, Dihydrolipoyl dehydrogenase; DPP4, Dipeptidyl peptidase 4; GRN, Granulin; HPGDS, Hematopoietic prostaglandin D synthase; HS3ST3B1, Heparan sulfate glucosamine 3-O-sulfotransferase 3B1; INHBC, Inhibin beta C chain; LBP, Lipopolysaccharide-binding protein; LPL, Lipoprotein lipase; MMP12, Macrophage metalloelastase; MR, Mendelian randomization; NAP1L2, Nucleosome assembly protein 1-like 2; N_exposure, number of individuals; NPL, N-acetylneuraminate lyase; NR3C2, Nuclear Receptor Subfamily 3 Group C Member 2; OR, odds ratio; PLTP, Phospholipid transfer protein; pQTL, protein quantitative trait loci; PRKD2, Protein Kinase D2; PSAPL1, Proactivator polypeptide-like 1; PTGES2, Prostaglandin E synthase 2; pval; *P* value; SNCA, Alpha-synuclein; ULBP2, UL16-binding protein 2; and ZPR1, Zinc finger protein.

cis-pQTLs as genetic instruments (Table S7). Several of these proteins are targets of drugs already approved for cardiovascular disease indications. For instance, drugs targeting AGT, such as aspirin, hydrochlorothiazide, and benazepril, have been approved by the Food and Drug Administration for the treatment of

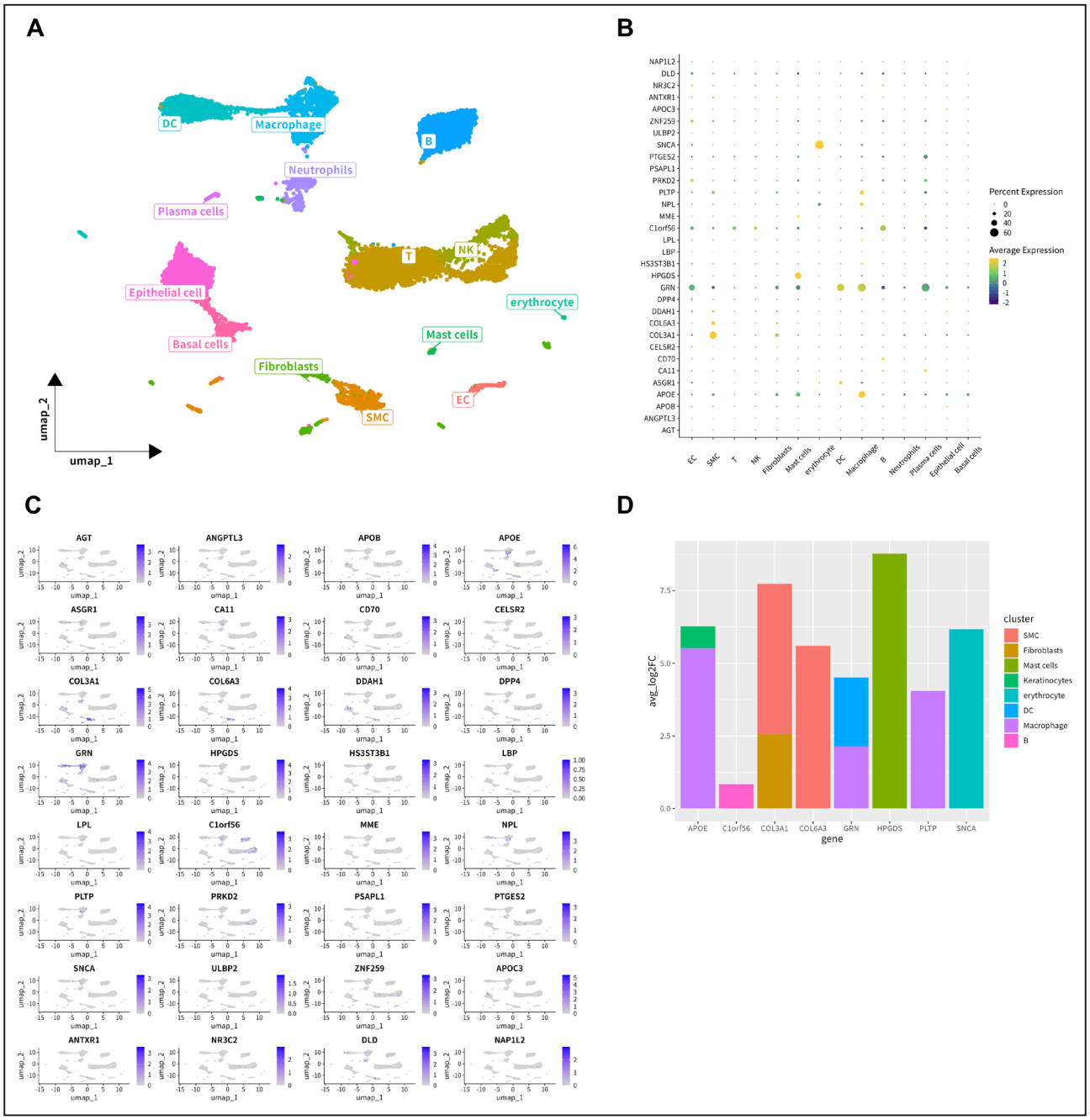

Figure 4. Single-cell type expression in abdominal aortic tissue for the coding genes of proteins identified by proteome-wide MR.

A, A total of 14 cell types were identified. **B** and **C**, Expression of protein-coding genes in each cluster. **D**, Eight protein-coding genes had evidence of enrichment in a cell type at average $\log_2$FC >0.5, and false discovery rate <0.05 was calculated by the Benjamini-Hochberg method. N=6. ; DC indicates Dendritic Cell; B ,B Cell; T, T Cell; NK, Natural Killer Cell; SMC, Smooth Muscle Cell; EC, Endothelial Cell. AGT, Angiotensin; ANGPTL3, Angiopoietin-related protein 3; ANTXR1, Anthrax toxin receptor 1; APOB, Apolipoprotein B; APOC3, Apolipoprotein C-III; APOE, Apolipoprotein E; ASGR1, Asialoglycoprotein receptor 1; C1orf56, Protein MENT; CA11, Carbonic anhydrase-related protein 11; CD70, CD70 antigen; CELSR2, Cadherin EGF LAG seven-pass G-type receptor 2; COL3A1, Collagen alpha-1(III) chain; COL6A3, Collagen alpha-3(VI) chain; DDAH1, dimethylarginine dimethylaminohydrolase 1; DLD, Dihydrolipoyl dehydrogenase; DPP4, Dipeptidyl peptidase 4; GRN, Granulin; HPGDS, Hematopoietic prostaglandin D synthase; HS3ST3B1, Heparan sulfate glucosamine 3-O-sulfotransferase 3B1; LBP, Lipopolysaccharide-binding protein; $\log_2$FC, $\log_2$ fold change; LPL, Lipoprotein lipase; MME, Membrane metalloendopeptidase; MR, mendelian randomization; NAP1L2, Nucleosome assembly protein 1-like 2; NPL, N-acetylneuraminate lyase; NR3C2, Nuclear Receptor Subfamily 3 Group C Member 2; PLTP, Phospholipid transfer protein; PRKD2, Protein Kinase D2; PSAPL1, Proactivator polypeptide-like 1; PTGES2, Prostaglandin E synthase 2; SNCA, Alpha-synuclein; ULBP2, UL16-binding protein 2; and ZPR1, Zinc finger protein.

hypertension. Similarly, drugs targeting APOE (fluvastatin, fenofibrate, pravastatin sodium), LPL (lovastatin, clofibrate, pravastatin sodium), and PLTP (simvastatin) have been approved for the treatment of hyperlipidemia. Furthermore, drugs targeting DPP4, including enalapril maleate and captopril for hypertension, and saxagliptin anhydrous, alogliptin, vildagliptin, and sitagliptin for diabetes, are currently in use. Drugs targeting MMP12 have been used to treat convulsions (acetazolamide), inflammation (zileuton), and hypertension (captopril).

In the analysis of drug potential for 2 proteins identified as tier 1, it was found that collagenase clostridium histolyticum-aaes, which targets COL6A3, is approved for the treatment of Dupuytren contracture. Additionally, 2 drugs targeting PRKD2, gefitinib and erlotinib, are used in cancer therapy. These findings suggest significant potential for developing new therapeutic strategies targeting these proteins for the treatment of AAAs.

DISCUSSION

In this study, we conducted a comprehensive analysis of the causal relationships between 4907 plasma proteins and the risk of AAA. We identified 34 plasma proteins with potential causal links to AAA; elevated levels of 20 proteins and reduced levels of 14 proteins were associated with increased susceptibility to the condition. Bayesian colocalization analysis highlighted 8 protein biomarkers with causal effects, and the SMR and HEIDI tests confirmed the significance of these proteins. Among these, 2 proteins (COL6A3 and PRKD2) showed the strongest evidence (tier 1), 12 proteins exhibited convincing evidence (tier 2), and 20 proteins demonstrated moderate evidence (tier 3). Notably, 20 proteins (ANTXR1, ASGR1, BPIFA2, C1ORF56, CA11, CD70, DDAH1, DLD, GRN, HPGDS, HS3ST3B1, INHBC, NAP1L2, NPL, NR3C2, PRKD2, PSAPL1, PTGES2, SNCA, and ULBP2) were identified as novel plasma protein markers associated with AAA. Furthermore, we confirmed the differential expression of these protein-coding genes in macrophages, smooth muscle cells, and mast cells. Druggability evaluations prioritized 2 tier 1 protein biomarkers for potential repurposing as therapeutic targets for AAA, originally developed for Dupuytren contracture and cancer.

Our analysis corroborates findings from previous studies, which reported evidence of associations between certain circulating proteins and AAA. For example, AGT, ANGPTL3, APOB, APOC3, APOE, CELSR2, COL3A1, COL6A3, DPP4, LBP, LPL, MMP12, PLTP, and ZPR1 have been previously implicated in AAA through genetic polymorphisms, mRNA levels, or protein levels. Among these, APOB, APOC3, APOE, LBP, and LPL are involved in lipid metabolism and have been extensively studied in the context of AAA.[4,36–40] AGT, part of the renin-angiotensin-aldosterone system, and ANGPTL3, an inhibitor of lipoprotein lipase, have emerged as therapeutic targets for AAA prevention.[41,42] Moreover, risk variants of CELSR2 and ZPR1 are strongly linked to AAA incidence.[8] Collagen proteins COL3A1 and COL6A3 are associated with aortic and arterial aneurysms, with COL6A3 showing the most convincing evidence.[43,44] Mechanistic studies have shown that DPP4 is upregulated in AAA and contributes to its pathophysiology.[45,46] Our findings further support the causal role of elevated DPP4 protein levels in AAA risk. MMP12 has a controversial role in AAA, with studies showing both detrimental and protective effects, yet our findings suggest high plasma levels of MMP12 increase AAA risk.[47,48] PLTP regulates inflammation and immune response, promoting AAA development.[49]

Among the newly identified proteins, PRKD2 (tier 1) stands out with strong evidence of association with AAA. PRKD2 is part of the serine–threonine kinase family, playing a crucial role in tumor cell survival, proliferation, migration, and angiogenesis.[50] Although direct evidence linking PRKD2 to AAA is lacking, recent studies have suggested a positive correlation between PRKD2 mutations and cardiovascular diseases.[51] Further research is needed to confirm these findings, considering that antitumor agents targeting PRKD2 (gefitinib and erlotinib) may have therapeutic potential for AAA.

This study has several strengths, including the large number of plasma proteins analyzed, the significant number of AAA cases, and validation across 2 independent cohorts. The consistency of results across multiple rigorous analyses confirms the robustness of our findings. Additional insights from single-cell-type expression analysis, protein–protein interaction, pathway enrichment analysis, and druggability evaluation highlight the potential pathogenic roles of candidate proteins in AAA and identify druggable targets. Although there is a lack of drug information for several proteins (eg, CELSR2 and INHBC), these proteins still represent promising new therapeutic targets for AAA. In particular, PRKD2 has been found to be useful in antitumor therapy[52] and may have the potential to treat cardiovascular disease.[51]

Several limitations must be considered when interpreting our results. First, the current analysis was restricted to European populations, which limits the generalizability of our findings to other populations. Second, we assessed the role of plasma proteins in AAA but could not estimate the levels of relevant proteins in other tissues. Evaluating protein levels in other tissues, particularly aortic tissue, could provide more insight into AAA pathogenesis. Third, the strict

significance threshold and evidence grading criteria may lead to underestimating the associations of certain proteins, such as ANTXR1, which did not meet the stringent significance threshold for the *P* value in the SMR test, despite having $P<0.05$. Furthermore, the current statistical analyses and strict significance threshold might exclude plasma proteins that are downstream of the driver proteins. Fourth, 65% of protein markers had only trans-pQTLs. Although trans-pQTLs can expand understanding of the relationships between proteins and diseases,[15] interpreting the current findings is challenging. Nonetheless, some proteins with trans-pQTLs (eg, ANTXR1, GRN) had robust colocalization evidence, indicating potential vertical pleiotropy. Fifth, out of the 34 significant proteins, 20 proteins were assayed in both the deCODE and UKB-PPP cohort, with concordant results in both. The relatively high concordance between cis-pQTL MR findings in the 2 data sets indicates that technical differences may not hinder meta-analytic studies across the SomaScan and Olink platforms. Last, this hypothesis is supported by studies showing that the MR results derived from trans-pQTL instruments had poor correlations when measured on SomaScan and Olink platforms,[53] suggesting that caution should be applied in interpreting these results.

In summary, our study identifies numerous plasma proteins with strong causal associations with AAA risk, providing new insights into the cause of AAA and highlighting promising targets for screening biomarkers and therapeutic drugs. Our findings also suggest that PRKD2 inhibitors or antagonists may represent promising therapeutic targets for AAA.

ARTICLE INFORMATION

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Affiliations

Wuxi School of Medicine (K.Z., Y.L., A.M., C.L., L.G., H.K.) and School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, China (H.K.).

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Ethical Approval and Consent to Participate

This study is based on publicly available data, and individual studies within each GWAS obtained approval from the relevant institutional review board and conformed to the principles outlined in the Declaration of Helsinki. Informed consent was secured from participants, caregivers, legal guardians, or proxies as applicable.

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Disclosures

None.

Supplemental Material

Tables S1–S7
Figures S1–S15

REFERENCES

1. Nordon IM, Hinchliffe RJ, Loftus IM, Thompson MM. Pathophysiology and epidemiology of abdominal aortic aneurysms. *Nat Rev Cardiol*. 2011;8:92–102. doi: 10.1038/nrcardio.2010.180
2. Davis FM, Daugherty A, Lu HS. Updates of recent aortic aneurysm research. *Arterioscler Thromb Vasc Biol*. 2019;39:e83–e90. doi: 10.1161/ATVBAHA.119.312000
3. Baxter BT, Terrin MC, Dalman RL. Medical management of small abdominal aortic aneurysms. *Circulation*. 2008;117:1883–1889. doi: 10.1161/CIRCULATIONAHA.107.735274
4. Roychowdhury T, Klarin D, Levin MG, Spin JM, Rhee YH, Deng A, Headley CA, Tsao NL, Gellatly C, Zuber V, et al. Genome-wide association meta-analysis identifies risk loci for abdominal aortic aneurysm and highlights PCSK9 as a therapeutic target. *Nat Genet*. 2023;55:1831–1842. doi: 10.1038/s41588-023-01510-y
5. Golledge J, Thanigaimani S, Powell JT, Tsao PS. Pathogenesis and management of abdominal aortic aneurysm. *Eur Heart J*. 2023;44:2682–2697. doi: 10.1093/eurheartj/ehad386
6. Klarin D, Verma SS, Judy R, Dikilitas O, Wolford BN, Paranjpe I, Levin MG, Pan C, Tcheandjieu C, Spin JM, et al. Genetic architecture of abdominal aortic aneurysm in the million veteran program. *Circulation*. 2020;142:1633–1646. doi: 10.1161/CIRCULATIONAHA.120.047544
7. Larsson SC, Mason AM, Bäck M, Klarin D, Damrauer SM, Michaëlsson K, Burgess S. Genetic predisposition to smoking in relation to 14 cardiovascular diseases. *Eur Heart J*. 2020;41:3304–3310. doi: 10.1093/eurheartj/ehaa193
8. Steffen BT, Pankow JS, Norby FL, Lutsey PL, Demmer RT, Guan W, Pankratz N, Li A, Liu G, Matsushita K, et al. Proteomics analysis of genetic liability of abdominal aortic aneurysm identifies plasma neogenin and kit ligand: the ARIC study. *Arterioscler Thromb Vasc Biol*. 2023;43:367–378. doi: 10.1161/ATVBAHA.122.317984
9. Kopylov AT, Stepanov AA, Butkova TV, Malsagova KA, Zakharova NV, Kostyuk GP, Elmuratov AU, Kaysheva AL. Consolidation of metabolomic, proteomic, and GWAS data in connective model of schizophrenia. *Sci Rep*. 2023;13:2139. doi: 10.1038/s41598-023-29117-7
10. Yang C, Fagan AM, Perrin RJ, Rhinn H, Harari O, Cruchaga C. Mendelian randomization and genetic colocalization infer the effects of the multitissue proteome on 211 complex disease-related phenotypes. *Genome Med*. 2022;14:140. doi: 10.1186/s13073-022-01140-9
11. Smith GD, Ebrahim S. Mendelian randomization: prospects, potentials, and limitations. *Int J Epidemiol*. 2004;33:30–42. doi: 10.1093/ije/dyh132
12. Davies NM, Holmes MV, Davey SG. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. *BMJ*. 2018;362:k601. doi: 10.1136/bmj.k601
13. Sun BB, Chiou J, Traylor M, Benner C, Hsu YH, Richardson TG, Surendran P, Mahajan A, Robins C, Vasquez-Grinnell SG, et al. Plasma proteomic associations with genetics and health in the UK biobank. *Nature*. 2023;622:329–338. doi: 10.1038/s41586-023-06592-6
14. Pietzner M, Wheeler E, Carrasco-Zanini J, Cortes A, Koprulu M, Wörheide MA, Oerton E, Cook J, Stewart ID, Kerrison ND, et al. Mapping the proteo-genomic convergence of human diseases. *Science*. 2021;374:eabj1541. doi: 10.1126/science.abj1541
15. Sun J, Zhao J, Jiang F, Wang L, Xiao Q, Han F, Chen J, Yuan S, Wei J, Larsson SC, et al. Identification of novel protein biomarkers and drug targets for colorectal cancer by integrating human plasma proteome with genome. *Genome Med*. 2023;15:75. doi: 10.1186/s13073-023-01229-9

16. Fauman EB, Hyde C. An optimal variant to gene distance window derived from an empirical definition of cis and trans protein QTLs. *BMC Bioinformatics*. 2022;23:169. doi: 10.1186/s12859-022-04706-x
17. Sun BB, Maranville JC, Peters JE, Stacey D, Staley JR, Blackshaw J, Burgess S, Jiang T, Paige E, Surendran P, et al. Genomic atlas of the human plasma proteome. *Nature*. 2018;558:73–79. doi: 10.1038/s41586-018-0175-2
18. Papadimitriou N, Dimou N, Tsilidis KK, Banbury B, Martin RM, Lewis SJ, Kazmi N, Robinson TM, Albanes D, Aleksandrova K, et al. Physical activity and risks of breast and colorectal cancer: a Mendelian randomisation analysis. *Nat Commun*. 2020;11:597. doi: 10.1038/s41467-020-14389-8
19. Burgess S, Davey Smith G, Davies NM, Dudbridge F, Gill D, Glymour MM, Hartwig FP, Kutalik Z, Holmes MV, Minelli C, et al. Guidelines for performing Mendelian randomization investigations: update for summer 2023. *Wellcome Open Res*. 2019;4:186. doi: 10.12688/wellcomeopenres.15555.1
20. Yavorska OO, Burgess S. MendelianRandomization: an R package for performing Mendelian randomization analyses using summarized data. *Int J Epidemiol*. 2017;46:1734–1739. doi: 10.1093/ije/dyx034
21. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol*. 2016;40:304–314. doi: 10.1002/gepi.21965
22. Giambartolomei C, Vukcevic D, Schadt EE, Franke L, Hingorani AD, Wallace C, Plagnol V. Bayesian test for colocalisation between pairs of genetic association studies using summary statistics. *PLoS Genet*. 2014;10:e1004383. doi: 10.1371/journal.pgen.1004383
23. Foley CN, Staley JR, Breen PG, Sun BB, Kirk PDW, Burgess S, Howson JMM. A fast and efficient colocalization algorithm for identifying shared genetic risk factors across multiple traits. *Nat Commun*. 2021;12:764. doi: 10.1038/s41467-020-20885-8
24. Liu B, Gloudemans MJ, Rao AS, Ingelsson E, Montgomery SB. Abundant associations with gene expression complicate GWAS follow-up. *Nat Genet*. 2019;51:768–769. doi: 10.1038/s41588-019-0404-0
25. Wu Y, Zeng J, Zhang F, Zhu Z, Qi T, Zheng Z, Lloyd-Jones LR, Marioni RE, Martin NG, Montgomery GW, et al. Integrative analysis of omics summary data reveals putative mechanisms underlying complex traits. *Nat Commun*. 2018;9:918. doi: 10.1038/s41467-018-03371-0
26. Zhu Z, Zhang F, Hu H, Bakshi A, Robinson MR, Powell JE, Montgomery GW, Goddard ME, Wray NR, Visscher PM, et al. Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets. *Nat Genet*. 2016;48:481–487. doi: 10.1038/ng.3538
27. Davis FM, Tsoi LC, Melvin WJ, denDekker A, Wasikowski R, Joshi AD, Wolf S, Obi AT, Billi AC, Xing X, et al. Inhibition of macrophage histone demethylase JMJD3 protects against abdominal aortic aneurysms. *J Exp Med*. 2021;218:e20201839. doi: 10.1084/jem.20201839
28. Butler A, Hoffman P, Smibert P, Papalexi E, Satija R. Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nat Biotechnol*. 2018;36:411–420. doi: 10.1038/nbt.4096
29. Davis FM, Tsoi LC, Ma F, Wasikowski R, Moore BB, Kunkel SL, Gudjonsson JE, Gallagher KA. Single-cell transcriptomics reveals dynamic role of smooth muscle cells and enrichment of immune cell subsets in human abdominal aortic aneurysms. *Ann Surg*. 2022;276:511–521. doi: 10.1097/SLA.0000000000005551
30. Chen J, Zhang X, DeLaughter DM, Trembley MA, Saifee S, Xiao F, Chen J, Zhou P, Seidman CE, Seidman JG, et al. Molecular and spatial signatures of mouse embryonic endothelial cells at single-cell resolution. *Circ Res*. 2024;134:529–546. doi: 10.1161/CIRCRESAHA.123.323956
31. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, Doncheva NT, Legeay M, Fang T, Bork P, et al. The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res*. 2021;49:D605–D612. doi: 10.1093/nar/gkaa1074
32. Wu T, Hu E, Xu S, Chen M, Guo P, Dai Z, Feng T, Zhou L, Tang W, Zhan L, et al. ClusterProfiler 4.0: a universal enrichment tool for interpreting omics data. *Innovation (Camb)*. 2021;2:100141.
33. Freshour SL, Kiwala S, Cotto KC, Coffman AC, McMichael JF, Song JJ, Griffith M, Griffith OL, Wagner AH. Integration of the drug-gene interaction database (DGIdb 4.0) with open crowdsource efforts. *Nucleic Acids Res*. 2021;49:D1144–D1151. doi: 10.1093/nar/gkaa1084
34. Mendez D, Gaulton A, Bento AP, Chambers J, de Veij M, Félix E, Magariños MP, Mosquera JF, Mutowo P, Nowotka M, et al. ChEMBL: towards direct deposition of bioassay data. *Nucleic Acids Res*. 2019;47:D930–D940. doi: 10.1093/nar/gky1075
35. Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, Sajed T, Johnson D, Li C, Sayeeda Z, et al. DrugBank 5.0: a major update to the DrugBank database for 2018. *Nucleic Acids Res*. 2018;46:D1074–d1082. doi: 10.1093/nar/gkx1037
36. Martínez-López D, Cedó L, Metso J, Burillo E, García-León A, Canyelles M, Lindholt JS, Torres-Fonseca M, Blanco-Colio LM, Vázquez J, et al. Impaired HDL (high-density lipoprotein)-mediated macrophage cholesterol efflux in patients with abdominal aortic aneurysm-brief report. *Arterioscler Thromb Vasc Biol*. 2018;38:2750–2754. doi: 10.1161/ATVBAHA.118.311704
37. McConathy WJ, Alaupovic P, Woolcock N, Laing SP, Powell J, Greenhalgh R. Lipids and apolipoprotein profiles in men with aneurysmal and stenosing aorto-iliac atherosclerosis. *Eur J Vasc Surg*. 1989;3:511–514. doi: 10.1016/S0950-821X(89)80125-2
38. Zhang J, Cao Y, Ren R, Sui W, Zhang Y, Zhang M, Zhang C. Medium-dose formoterol attenuated abdominal aortic aneurysm induced by EPO via β2AR/cAMP/SIRT1 pathway. *Adv Sci (Weinh)*. 2024;11:e2306232. doi: 10.1002/advs.202306232
39. Wortmann M, Xiao X, Wabnitz G, Samstag Y, Hakimi M, Böckler D, Dihlmann S. AIM2 levels and DNA-triggered inflammasome response are increased in peripheral leukocytes of patients with abdominal aortic aneurysm. *Inflamm Res*. 2019;68:337–345. doi: 10.1007/s00011-019-01212-4
40. Wang S, Subramanian V, Lu H, Howatt DA, Moorleghen JJ, Charnigo R, Cassis LA, Daugherty A. Deficiency of receptor-associated protein attenuates angiotensin II-induced atherosclerosis in hypercholesterolemic mice without influencing abdominal aortic aneurysms. *Atherosclerosis*. 2012;220:375–380. doi: 10.1016/j.atherosclerosis.2011.11.013
41. Chen Y, Huang M, Xuan Y, Li K, Xu X, Wang L, Sun Y, Xiao L, Xu P, Kong W, et al. Association between lipid levels and risk for different types of aneurysms: a mendelian randomization study. *J Pers Med*. 2021;11:11. doi: 10.3390/jpm11111171
42. Jones GT, Thompson AR, van Bockxmeer FM, Hafez H, Cooper JA, Golledge J, Humphries SE, Norman PE, van Rij AM. Angiotensin II type 1 receptor 1166C polymorphism is associated with abdominal aortic aneurysm in three independent cohorts. *Arterioscler Thromb Vasc Biol*. 2008;28:764–770. doi: 10.1161/ATVBAHA.107.155564
43. Maegdefessel L, Azuma J, Toh R, Merk DR, Deng A, Chin JT, Raaz U, Schoelmerich AM, Raiesdana A, Leeper NJ, et al. Inhibition of microRNA-29b reduces murine abdominal aortic aneurysm development. *J Clin Invest*. 2012;122:497–506. doi: 10.1172/JCI61598
44. Urbonavicius S, Lindholt JS, Delbosc S, Urbonaviciene G, Henneberg EW, Vorum H, Meilhac O, Honoré B. Proteins associated with the size and expansion rate of the abdominal aortic aneurysm wall as identified by proteomic analysis. *Interact Cardiovasc Thorac Surg*. 2010;11:433–441. doi: 10.1510/icvts.2010.238139
45. Lindquist Liljeqvist M, Eriksson L, Villard C, Lengquist M, Kronqvist M, Hultgren R, Roy J. Dipeptidyl peptidase-4 is increased in the abdominal aortic aneurysm vessel wall and is associated with aneurysm disease processes. *PLoS One*. 2020;15:e0227889. doi: 10.1371/journal.pone.0227889
46. Lu HY, Huang CY, Shih CM, Lin YW, Tsai CS, Lin FY, Shih CC. A potential contribution of dipeptidyl peptidase-4 by the mediation of monocyte differentiation in the development and progression of abdominal aortic aneurysms. *J Vasc Surg*. 2017;66:1217–1226.e1211. doi: 10.1016/j.jvs.2016.05.093
47. Boytard L, Hadi T, Silvestro M, Qu H, Kumpfbeck A, Sleiman R, Fils KH, Alebrahim D, Boccalatte F, Kugler M, et al. Lung-derived HMGB1 is detrimental for vascular remodeling of metabolically imbalanced arterial macrophages. *Nat Commun*. 2020;11:4311. doi: 10.1038/s41467-020-18088-2
48. Salarian M, Ghim M, Toczek J, Han J, Weiss D, Spronck B, Ramachandra AB, Jung JJ, Kukreja G, Zhang J, et al. Homeostatic, non-canonical role of macrophage elastase in vascular integrity. *Circ Res*. 2023;132:432–448. doi: 10.1161/CIRCRESAHA.122.322096
49. Deckert V, Kretz B, Habbout A, Raghay K, Labbé J, Abello N, Desrumaux C, Gautier T, Lemaire-Ewing S, Maquart G, et al. Development of abdominal aortic aneurysm is decreased in mice with plasma phospholipid transfer protein deficiency. *Am J Pathol*. 2013;183:975–986. doi: 10.1016/j.ajpath.2013.05.018
50. Azoitei N, Fröhling S, Scholl C, Seufferlein T. PRKD2: a two-pronged kinase crucial for the tumor-supporting activity of HSP90. *Mol Cell Oncol*. 2015;2:e981444. doi: 10.4161/23723556.2014.981444

51. Giardoglou P, Bournele D, Park M, Kanoni S, Dedoussis GV, Steinberg SF, Deloukas P, Beis D. A zebrafish forward genetic screen identifies an indispensable threonine residue in the kinase domain of PRKD2. *Biol Open*. 2021;10:10. doi: 10.1242/bio.058542
52. Liu YQ, Xu YW, Zheng ZT, Li D, Hong CQ, Dai HQ, Wang JH, Chu LY, Liao LD, Zou HY, et al. Serine/threonine-protein kinase D2-mediated phosphorylation of DSG2 threonine 730 promotes esophageal squamous cell carcinoma progression. *J Pathol*. 2024;263:99–112. doi: 10.1002/path.6264
53. Yuan S, Xu F, Li X, Chen J, Zheng J, Mantzoros CS, Larsson SC. Plasma proteins and onset of type 2 diabetes and diabetic complications: proteome-wide Mendelian randomization and colocalization analyses. *Cell Rep Med*. 2023;4:101174. doi: 10.1016/j.xcrm.2023.101174