Genetic insights into therapeutic targets for aortic aneurysms: A Mendelian randomization study

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Summary

Background As aortic aneurysms (AAs) enlarge, they can become life-threatening if left undiagnosed or neglected. At present, there is a lack of radical treatments for preventing disease progression. Therefore, we aimed to identify effective drug targets that slow the progression of AAs.

Methods A Mendelian randomization (MR) analysis was conducted to identify therapeutic targets which are associated with AAs. Summary statistics for AAs were obtained from two datasets: the UK Biobank (2228 cases and 408,565 controls) and the FinnGen study (3658 cases and 244,907 controls). Cis-expression quantitative trait loci (cis-eQTL) for druggable genes were retrieved from the eQTLGen Consortium and used as genetic instrumental variables. Colocalization analysis was performed to determine the probability that single nucleotide polymorphisms (SNPs) associated with AAs and eQTL shared causal genetic variants.

Findings Four drug targets (*BTN3A1, FASN, PLAU*, and *PSMA4*) showed significant MR results in two independent datasets. Proteasome 20S subunit alpha 4 (PSMA4) and plasminogen activator, urokinase (PLAU) in particular, were found to have strong evidence for colocalization with AAs, and abdominal aortic aneurysm in particular. Additionally, except for the association between PSMA4 and intracranial aneurysms, no association between genetically proxied inhibition of PLAU and PSMA4 was detected in increasing the risk of other cardiometabolic risks and diseases.

Interpretation This study supports that drug-targeting PLAU and PSMA4 inhibition may reduce the risk of AAs.

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Keywords: Aortic aneurysm; Genetics; Drug; Mendelian randomization

Introduction

Aortic aneurysms (AAs) are the 15th most common cause of death in individuals aged 55 years and over and occur when the progressive weakening of the aortic wall causes the aorta to dilate.[1,2] Small aneurysms remain mostly asymptomatic and can be monitored using a Doppler ultrasound or computed tomography (CT). However, AAs progression is slow, and large aneurysms can lead to aortic rupture and sudden death.[3] Since a few pharmacological treatments have been found to be effective so far, surgical, and endovascular repair are essential treatments for AAs. It is essential to identify effective drugs for the prevention of AAs.

A large-scale randomized clinical trial (RCT) is an efficient way to estimate drug treatment strategies; however, it requires extensive planning, time for design and execution, and resources. In recent years, it has become the most cost-effective way to integrate human genetics studies into drug development programs and has been

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Research in context

Evidence before this study

Aortic aneurysms (AAs) are potentially lethal conditions that cause more than 10,000 deaths per year around the world. In the clinical setting, surgical and endovascular repair are essential treatments for AAs. Unfortunately, there is a lack of effective medications for preventing disease progression.

Added value of this study

Our study aimed to identify therapeutic targets relevant to AAs by performing a Mendelian randomization analysis. We identified the genetically predicted expression of PSMA4 and PLAU were associated with AAs risk in two independent cohorts, which also had strong evidence for colocalization with AAs. Further, these two proteins were significantly increased in abdominal aortic aneurysms human samples.

Implications of all the available evidence

This study supports that drug-targeting PLAU and PSMA4 inhibition may reduce the risk of AAs, which may provide insight into the underlying mechanisms and corresponding interventions. Further investigation is needed to validate in basic and even clinical research in this field.

used successfully to assess the effects of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) inhibitors and proprotein convertase subtilisin-like/kexin type 9 (PCSK9) inhibitors on lowering coronary artery disease risks.[4,5]

Mendelian randomization (MR) is an approach that uses common variants as unconfounded unbiased proxies to investigate causal associations.[6] In drug target MR analysis, cis-expression quantitative trait loci (cis-eQTL) located in the genomic region of the drug target gene are often considered proxies, which function as regulators that influence gene expression. Such MR analyses have been applied to several diseases, such as COVID-19, and Parkinson's disease.[7,8] Interestingly, using genetic markers to identify drug targets for AAs, studies have demonstrated targeting low-density lipoprotein cholesterol might be an effective treatment strategy for preventing and managing abdominal aortic aneurysms,[9] which are now proven to benefit abdominal aortic aneurysm patients.[10] Additionally, some potential targets also were found.[11–13] However, genomic evidence for a range of potential drug targets for AAs has not yet been explored.

In this study, we aimed to identify potential drug targets to slow down AAs progression, and we performed MR analyses by combining eQTL found in the blood with two independent AAs genome-wide association study (GWAS) datasets. The association between genetically proxied druggable genes and AAs risks was investigated, as well as between the genes and 18 additional cardiometabolic risk factors and 13 common disease traits.

Methods

Ethics

Our study was a secondary analysis of publicly available data. Informed consent was obtained from all participants as per the original GWAS protocols, and all ethical approvals for the GWAS were obtained by the original GWAS authors. The human study was approved by the Institutional Review Board of the Tongji Hospital (Tongji Medical College, Wuhan, China) and was conducted following the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice.

Identifying cis-eQTL data linked to druggable genes

A total of 4302 druggable genes located on the autosomal chromosomes with HGNC names were identified.[14] These included 1375 protein therapeutic targets in clinical development, 646 proteins related to drug targets and compounds, and 2281 proteins associated with members of key drug target families.

Considering that cis-eQTL were more proximal to the gene of interest in the drug development studies, we obtained fully statistically significant cis-eQTL (false discovery rate <0.05, ±1 Mb from each probe) from the eQTLGen Consortium and eQTL meta-analysis of the peripheral blood of 31,684 individuals.[15] To generate genetic instruments to proxy 4302 druggable targets, we selected cis-eQTL within ±100 kb from each gene's genome position, and eQTLs were available for 2664 druggable genes in the final.

Three independent sources of protein QTL (pQTL) datasets (Ferkingstad's N = 35,559 Icelanders; Sun's: N = 3301 Europans, and Emilsson's N = 5457 Icelanders) were downloaded for two druggable proteins of interest, proteasome 20S subunit alpha 4 (PSMA4) and plasminogen activator urokinase (PLAU).[16–18] All significant cis-pQTL could be available in the original supplementary tables.

Outcome data

Aortic aneurysm. The UK Biobank is a large-scale biomedical database and research resource containing in-depth genetic and health information from over 500,000 participants. We defined AAs in the UK Biobank according to electronic health recodes (ICD-9 or ICD-10 diagnosis and hospital procedure codes, Table

S1) from hospital episode statistics and death certificates, which consisted of 2228 cases and 408,565 controls. GWAS summary statistics for AAs were obtained from Pan-UK Biobank (https://pan.ukbb.broadinstitute.org/), and GWAS analysis was adjusted for the following covariates: age, sex, age * sex, age2, age2 * sex, and the first 10 principal components. We used summary statistics from the FinnGen Consortium for external replication in an independent sample cohort (Table S1).[19] This study included 3658 patients with AAs and 244,907 controls, the GWAS was adjusted for age, sex, 10 principal components, and genotyping batch.

Cardiometabolic traits and diseases. We selected 18 cardiometabolic risk factors, including lipid traits (total cholesterol, triglycerides, high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), apolipoprotein A1 and B (ApoA1, ApoB), and lipoprotein α (Lp (a))),[20,21] blood pressure traits (systolic blood pressure, diastolic blood pressure, and pulse pressure),[22] glycemic traits (fasting glucose, fasting insulin, 2-hour glucose, and HbA1c),[23] and anthropometric traits (body mass index, waist circumference, hip circumference, and waist-to-hip ratio).[24–26] Additionally, 13 cardiometabolic diseases were included in the analysis, such as four kinds of stroke,[27] atrial fibrillation,[28] coronary artery disease,[29] heart failure,[30] type 1 diabetes,[31] type 2 diabetes,[32] chronic kidney disease,[33] intracranial aneurysms[34] and two subtypes of AAs[35] (Table S2).

Statistics

Mendelian randomization and colocalization. We conducted MR analyses using the TwoSampleMR R package. Before MR analysis, several rules were applied to filter low-quality genetic instruments. First, we excluded single nucleotide polymorphisms (SNPs) with weak strength (F-statistic <10). Then, after harmonizing the exposure and outcome summary data, we selected conditionally independent SNPs without linkage disequilibrium (r2<0.1, based on the 1000 Genomes European reference panel) as instrumental variables. We also removed genes that suggested greater variance than exposure in the AAs trait using Steiger filtering (Table S3).

For the main analysis, we used the Wald ratio method to compute the MR estimates for each SNP, and the SNP estimates were meta-analyzed using inverse variance weighted (IVW), MR-Egger, and weighted median models with multiple proposed instruments. For proposed instruments that contained more than two variants, MR-Egger regression was performed to account for potential pleiotropy in the association between the exposure of interest and outcomes. Bonferroni corrections were applied to establish multiple testing-adjusted significance thresholds for the sensitivity analyses. In the UK Biobank cohort, we defined p-values below 1.90E−5 (p = 0.05/2644) as significant, the significant targets were then replicated in the FinnGen cohort. Associations with p-values below 0.0045 (p = 0.05/11) in replication analyses, were regarded as significant.

For significant MR results in two dependent cohorts, we performed a colocalization analysis for AAs risk using the coloc R package with default priors. For the eQTL dataset, we used 1E−04 prior probability for cis-eQTL (H1) and AAs associations (H2) and set a prior probability that a single variant affects both traits (H4) at 1E−05. We set significant colocalization (posterior probability) at PPH4>0.80, and the genes strongly colocalized with AAs were regarded as potential targeted molecular.

For the potential safety aspects and alternative indications, the association between potential targeted molecular and 31 cardiovascular traits was explored by MR analysis and colocalization analysis. All significant traits were performed multi-trait colocalization analysis to distinguish causality from confounding by using the "Moloc" R package.

As many variants are pleiotropic, we conducted a multivariable MR analysis to explore the potential independent associations of genetically proxied PSMA4 and PLAU with AAs risk using the “MVMR” package in R. For PSMA4, a multivariable weighted regression analysis is performed with the AAs regressed on the genetic associations with other 4 risk factors (Body mass index, Hip circumference, HDH-C, and LDL-C) in a single regression model. For PLAU, we adjusted the potential effect of ApoA1, ApoB, and Lp(a).

Human subjects

A total of 70 subjects were recruited from Tongji Hospital, Tongji Medical College in Wuhan, China, between January 2018 and June 2020, including 35 abdominal aortic aneurysm (AAA) patients diagnosed with Doppler ultrasound or computed tomography (CT), and 35 matched healthy control subjects (Table S4). All blood samples were collected and plasma was separated by centrifugation immediately and stored at −80 °C until analysis.

Additionally, aortic tissue specimens were collected from patients with aortic aneurysm and dissection (n = 8). Normal control infrarenal aortic wall tissue specimens were also obtained from organ donors (n = 4).

Western blotting

Aortic tissues were washed with cold PBS and lysed in RIPA buffer to extract whole-cell protein, which was resolved by SDS-PAGE, transferred onto PVDF membrane, and blocked with 5% non-fat dry milk in TBS-T. The membrane was incubated with indicated primary

antibody overnight at 4 °C, followed by incubation with a peroxidase-conjugated secondary antibody for 2 h, and finally developed with the ECL system (Vazyme Biotech Co.,Ltd). The western blotting results were quantified by densitometry and processed with Image J software (National Institutes of Health software). The following antibodies were used: anti-uPA (1:1000, #17968-1-AP, Proteintech Group, Inc.) and anti-PSMA4 (1:1000, RK05687, ABclonal Technology (Wuhan, China))

Enzyme-linked immunosorbent assay (ELISA) analysis of u-PA

The levels of u-PA in human plasma were measured using the Human urokinase-type plasminogen activator (uPA) ELISA Kit (Jiangsu Meibiao Biotechnology Co., Ltd) according to the manufacturer's protocol.

Evaluation of druggability and clinical development activity

To evaluate the druggability of candidate target genes, we systematic searched DrugBank and ChEMBL Database to get information of potential small molecule compounds. We also complemented clinical development activity by searching through ClinicalTrials.gov website.

Role of funders

In the present study, none of the funding sources played a role in the study design, data collection, data analyses, interpretation, or writing the manuscript.

Results

Study design

Our study aimed to identify therapeutic targets relevant to AAs. A flow diagram summarizing the methodology is shown in Figure 1, and Table S2 provides the sources of the data used. First, we distinguished 4302 unique human protein-coding genes as drugged or druggable.[14] Next, we selected conditionally independent cis-eQTL variants robustly linked with concentrations and investigated the biological relevance of mRNA expression of therapeutic targets on AAs risk using a two-sample MR approach. For MR results that reached the significance threshold after adjusting for multiple testing and validated in a second cohort, we conducted colocalization to examine whether MR results were influenced by distinct causal variants that were in linkage disequilibrium with each other. The final therapeutic targets were further tested for MR assumptions, and the potential safety aspects and alternative indications were explored. Finally, the protein levels of targets in plasma and tissues were detected in a prospective cohort if possible.

Discovery analysis

Using cis-eQTL data available from the eQTLGen Consortium,[15] we identified 2644 druggable genes after clumping and performed a two-sample MR analysis on European summary statistics for patients with AAs. In the discovery cohort, which included 2228 patients and 408,565 controls from the UK Biobank, we used IVW meta-analysis to combine effect estimates from each genetic instrument. Genetically predicted expression of 11 genes was found to be associated with AAs risk after accounting for multiple testing ($p<1.90E-5$ [IVW], 0.05 Bonferroni-corrected for 2644 drug targets, Table S5-6).

Replication analysis

We attempted to replicate the effect estimates for the top 11 genes identified in the discovery stage using data from the FinnGen cohort (N = 248,565). Four drug targets (BTN3A1, FASN, PLAU, and PSMA4) were replicated beyond a stringent Bonferroni threshold ($p<0.0045$ [IVW], 0.05/11 genes, Table 1, Table S7-8), and there was a 100% consistency in the direction of effect. One other gene (*FBN1*) reached nominal significance ($p<0.05$ [IVW]).

Colocalization analysis

We conducted a colocalization analysis to determine further the probability that SNPs associated with AAs and eQTL shared causal genetic variants. The results suggested that *PSMA4* and AA likely share a causal variant within the *PSMA4* locus (PP.H4 = 0.97, Figure 2a-b), and PLAU in the blood was highlighted as a candidate for AAs risk (PP.H4 = 0.93, Figure 2c-d). Therefore, two potentially druggable genes with evidence of a shared genetic effect between the eQTL and AAs risk were identified from MR and colocalization analyses (Table S9).

PSMA4

PSMA4 showed a positive estimate effect in the MR results, indicating a relationship between increased *PSMA4* expression and increased AAs risk (OR = 1.93, 95% CI: 1.54−2.41). Therefore, PSMA4 antagonists may be a novel strategy to reduce the risk of AAs. However, it is important to consider side effects and other alternative indications in drug development studies. Hence, we assessed the causal relationships of genetically proxied inhibition of PSMA4 on 18 potentially modifiable risk factors and 13 additional diseases.

We did not observe clear evidence of an association between the genetically proxied antagonistic effect of PSMA4 and a range of lipid subclasses, blood pressure, and glycaemic outcomes ($p<0.0016$ [IVW], 0.05/31 outcomes, Figure 3a). However, genetically proxied PSMA4

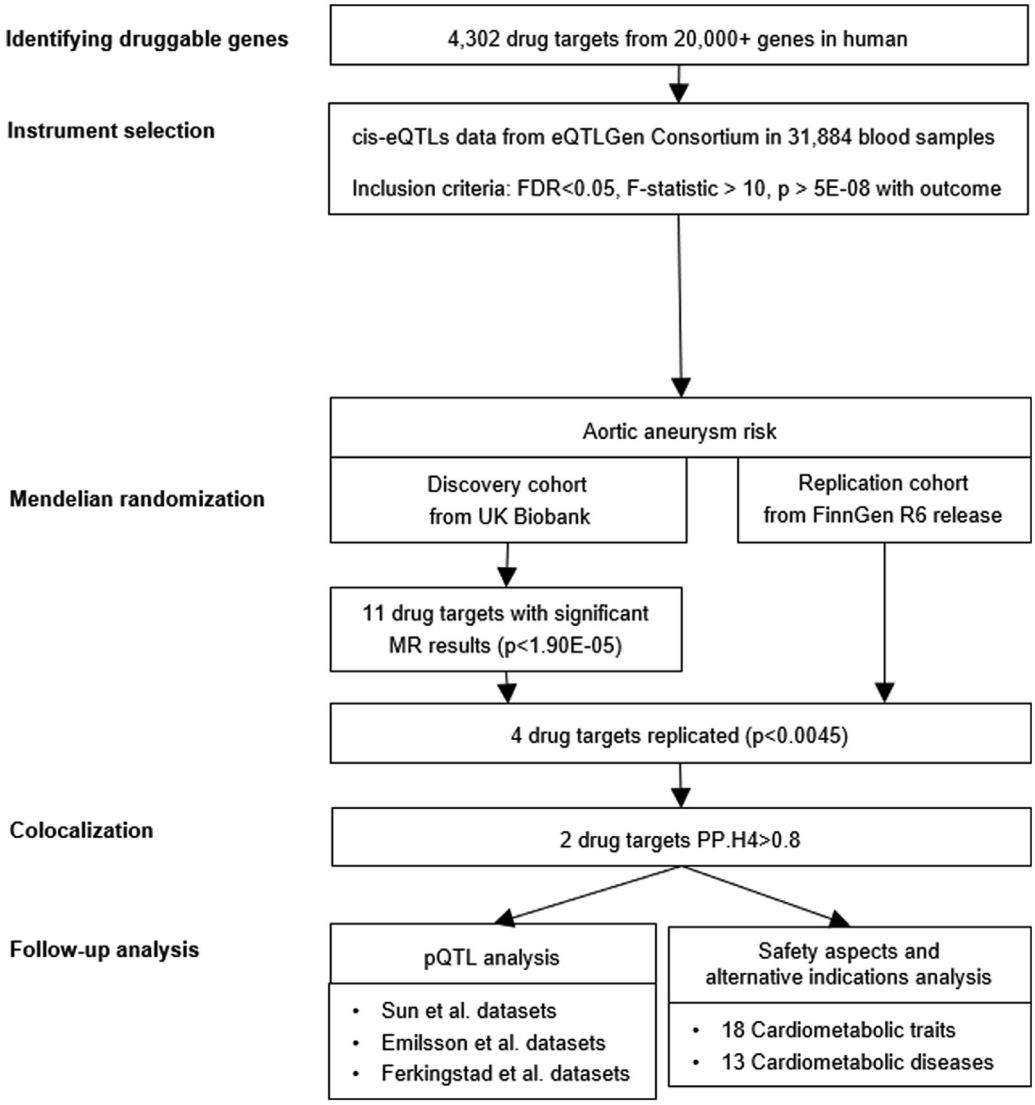

Figure 1. Overview of the study design in our Mendelian randomization study.

Genes	UK Biobank cohort					FINNGEN cohort				
	SNPs	OR (95% CI)	IVW *p*-value	MR-Egger intercept	Egger intercept *p*-value	SNPs	OR (95% CI)	IVW *p*-value	MR-Egger intercept	Egger intercept *p*-value
BTN3A1	17	0.75 (0.66−0.85)	5.51E−06	0.001	0.96	17	0.87 (0.80−0.95)	1.78E−03	0.002	0.93
FASN	20	1.24 (1.12−1.36)	1.40E−05	0.04	0.22	20	0.88 (0.82−0.95)	9.01E−04	−0.007	0.77
PLAU	10	1.73 (1.42−2.11)	6.29E−08	0.06	0.11	11	1.42 (1.23−1.63)	8.91E−07	0.017	0.51
PSMA4	8	1.93 (1.54−2.41)	8.53E−09	0.08	0.18	7	1.33 (1.12−1.59)	1.36E−03	0.05	0.46

***Table 1*: Mendelian randomization results.**

inhibition was weakly associated with body mass index (p = 0.003 [IVW]), HDL-C (p = 0.03 [IVW]), LDL (p = 0.04 [IVW]) and Hip circumference (p = 0.03 [IVW]). To gain the potential independent associations of PSMA4 with AAs risk, we performed multivariable MR analysis and the result show that there appear to be independent associations between PSMA4 and AAs risk (p = 6.6E−3 [IVW]).

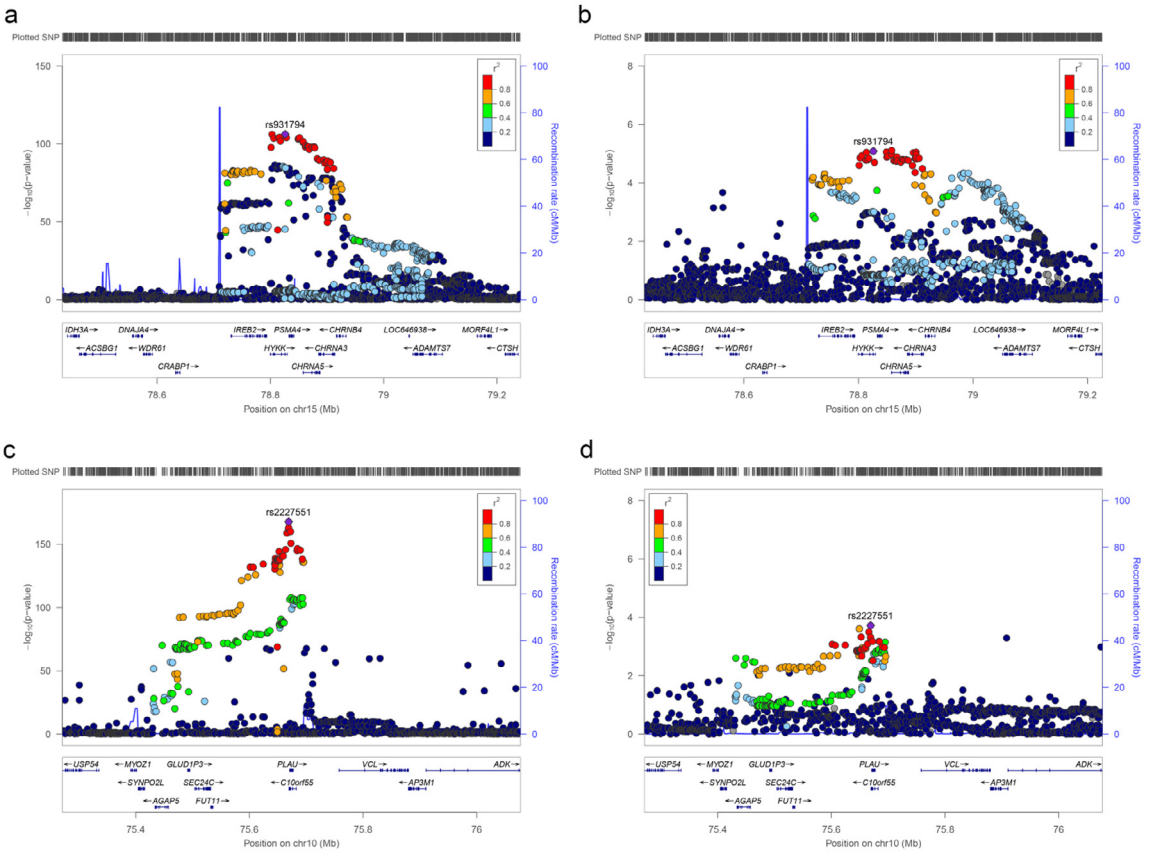

Figure 2. Regional Manhattan plot of associations of SNPs with PSMA4 and PLAU locus. (a) rs931794 was used to proxy serum PSMA4 expression. (b) rs931794 and its flanking 400 kb region to either side in aortic aneurysms. (c) rs2227551 was used to proxy serum PLAU expression. (d) rs2227551 and its flanking 400 kb region to either side in aortic aneurysm.

For cardiometabolic diseases, genetically predicted PSMA4 inhibition was significantly negatively associated with heart failure (OR = 0.89, 95% CI: 0.84−0.94, p = 3.92E−05 [IVW]), abdominal aortic aneurysm (OR = 0.53, 95% CI: 0.4−0.7, p = 9.26E−06 [IVW]), and intracranial aneurysm (OR: 0.53, 95% CI: 0.43−0.65, p = 3.57E−09 [IVW]) (Figure 3b). Furthermore, the association between PSMA4 and abdominal aortic aneurysm and intracranial aneurysm was confirmed by colocalization (PPH4>0.80, Table S9). We also use multi-trait colocalization analysis to distinguish causality from eQTL for PSMA4, abdominal aortic aneurysms, and intracranial aneurysms. The results show there was strong evidence (PPA = 0.999, Table S10) at eQTL for PSMA4, aortic aneurysms, and intracranial aneurysms.

To explore the possible relationship between PSMA4 and AAs risk, we detected the protein level in aortic tissues in AAD patients and found the expression of PSMA4 was significantly upregulated in patients (Figure 3c). At the single-cell level, PSMA4 is widely expressed in the blood (like monocytes, B cells, T cells, and Natural killer cells, https://atlas.fredhutch.org/nygc/multimodal-pbmc/) and aortic tissue (macrophage, smooth muscle cells, endothelial cells, and fibroblast, https://singlecell.broadinstitute.org/single_cell/study/SCP1265/deep-learning-enables-genetic-analysis-of-the-human-thoracic-aorta) (Figure S1a-d).

Additionally, many approved drugs that targeted the 20S proteosome had been widely used in clinical, such as carfilzomib and Bortezomib, but the number of molecule compounds that targeted PSMA4 is small (Table S11).

PLAU

PLAU was another druggable gene that passed the significance threshold in the colocalization analysis, and we further investigated plasma protein levels using pQTL data. Two cis-pQTL related to plasma u-PA (rs2227564[17] and rs2227551[16,18]) were identified (Table S12). Using cis-pQTL we found that u-PA levels were consistently positively associated with AAs risk (Figure 4a), which was in line with the eQTL results.

Additionally, no significant association between genetically proxied inhibition of PLAU and cardiometabolic disorder risk was detected (Figure 4b), but

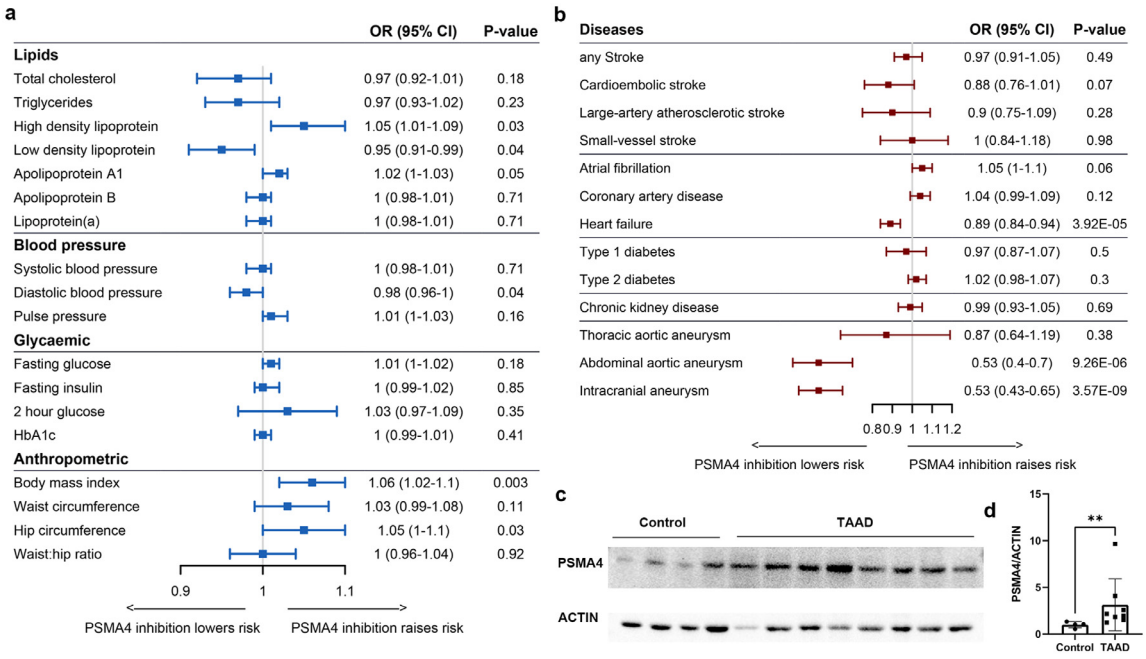

Figure 3. Associations between genetically predicted PSMA4 and other cardiovascular conditions. Forest plot mendelian randomization effect estimates and 95% confidence intervals for the genetic proxied antagonistic effect of PSMA4 and 18 cardiometabolic disorders (a) and 13 diseases (b) analysed. OR: odds ratio. 95% CI 95% confidence interval. (c) Representative Western blot analysis and quantification (d) of PSMA4 in aortic tissues, Mann-Whitney test, $^{**}p<0.01$.

genetically proxied PLAU inhibition was weakly associated with ApoA1 (p = 0.005), ApoB (p = 0.01), and Lp(a) (p = 0.01) (Figure 4b). We also conducted multivariable Mendelian randomization to adjust the potential confounders (ApoA1, ApoB, and Lp(a)) and found a significant association between PLAU and the risk of AAs (p = 1.3E-04) after adjusting the potential confounders.

Based on SNPs, we did not identify any associations between the plasma concentrations of PLAU and common diseases, including stroke, cardiovascular diseases, diabetes, chronic kidney disease, and intracranial aneurysms, except for abdominal aortic aneurysm (OR = 0.46, 95% CI: 0.36-0.59, p = 1.19E-09 [IVW]) (Figure 4c), the causality of which was strengthened by colocalization analysis (PP.H4 = 0.98, Table S9).

Furthermore, we also detected the protein level of u-PA in aortic tissue and human plasma, and found a higher expression in patients, indicating the level of u-PA is associated with AAs risk (Figure 4d-f). In the peripheral blood mononuclear cell, PLAU is mainly expressed in CD14 positive monocytes, but in aortic tissue, we find macrophage could express PLAU mainly, and so does smooth muscle cell, endothelial cell, and fibroblast (Figure S1d-e).

Currently, many molecule compounds targeted at PLAU were in experimental, and uPA Inhibitor WX-UK1 is being evaluated in clinical trials for advanced malignancies (Table S11). Amiloride, a potential inhibitor of PLAU, has already been approved for hypertension (Table S13).

Discussion

To explore putative druggable genes that might protect against AA, we performed a large-scale Mendelian randomization analysis by integrating GWAS datasets, the drug genome, and gene expression data (eQTL and pQTL). This analysis used 5886 patients with AA and 653,472 control individuals. Our results indicate that lifelong, naturally randomized, genetically proxied inhibition of PSMA4 and PLAU was significantly associated with a lower risk of AA. However, few associations support genetically proxied PSMA4 and PLAU inhibition with enhanced risks of other metabolic disorders and cardiovascular diseases.

PLAU, a urokinase-type plasminogen activator, encodes a secreted serine protease, u-PA, that mediates the conversion of plasminogen to plasmin. Active plasmin is critical for cleaving fibrin into soluble peptides and clearing fibrin overlay.[36] In our study, rs2227551, which was associated with PLAU gene expression, was also associated with AAs risks (UK Biobank: OR = 1.14, p = 1.9E−04; FinnGen: OR = 1.09, P = 3.3E−04). On the other hand, there was strong evidence of colocalization between rs2227551 and AAs (PPH4 = 0.93), and this cis-pQTL is likely to alter plasma u-PA levels. We also performed a phenome-wide scan of GWAS for

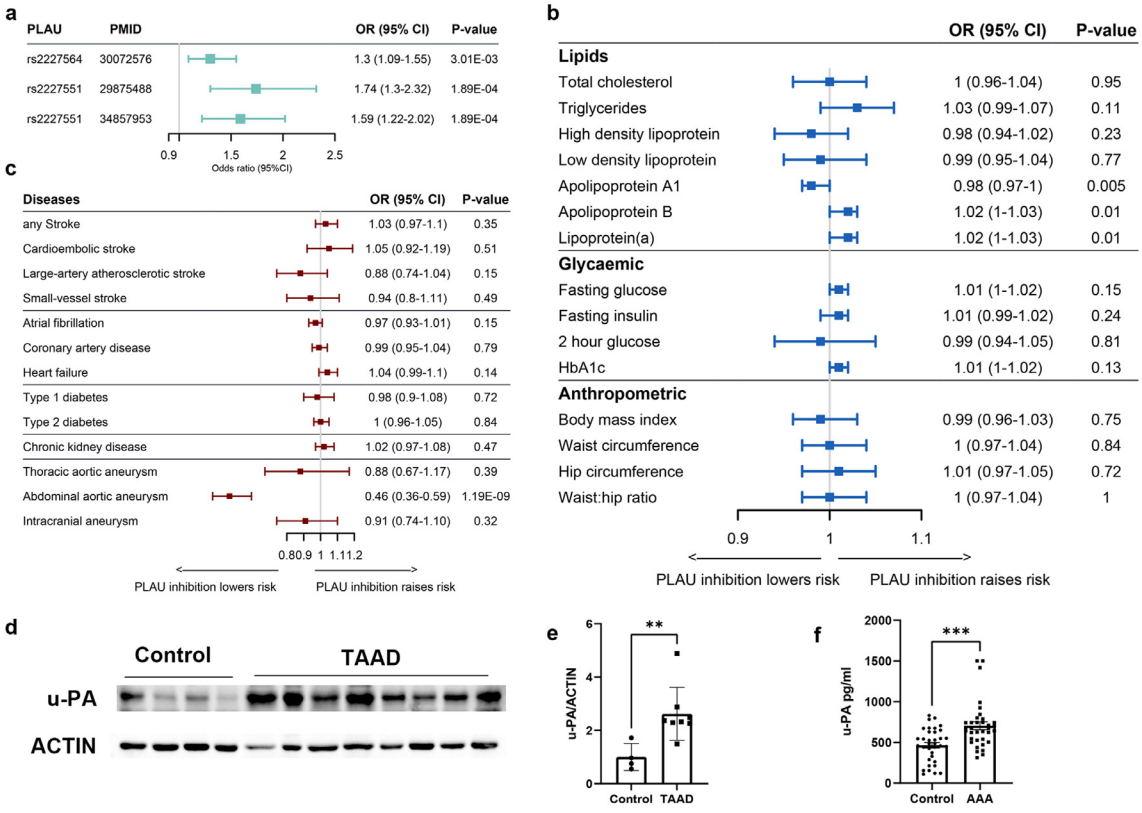

Figure 4. Associations between genetically predicted PLAU and other cardiovascular conditions. (a) forest plot showed genetically-predicted protein expression of PLAU is associated with aortic aneurysms risk. (b-c) Forest plot mendelian randomization effect estimates and 95% confidence intervals for the genetic proxied antagonistic effect of PLAU and 18 cardiometabolic disorders (b) and 13 diseases (c) analysed. OR: odds ratio. 95% CI 95% confidence interval. (d) Representative Western blot analysis and quantification (e) of u-PA in aortic tissues, Mann-Whitney test, $^{**}p<0.01$ (e) Quantification of u-PA in human plasma, Mann-Whitney test, $^{***}p<0.001$.

rs2227551, and the variant was not strongly associated with other risks that could affect the risk of AAs, indicating that this variant is unlikely to exhibit widespread horizontal pleiotropy.

Additionally, plasmin plays a crucial role in extracellular matrix (ECM) degradation (such as collagen type IV and fibronectin), matrix metalloprotease (MMP) zymogen activation (MMP-9 and MMP-12), inflammation regulation, and various growth factors (TGF-β and VEGF),[37,38] which have also been implicated in the pathogenesis of AA. In our study, we found that PLAU played a vital role in AA formation. Previous studies have demonstrated that the level of u-PA is elevated in the aneurysmal segment of the abdominal aorta of angiotensin (Ang) II-induced *ApoE*$^{-/-}$ mice and increased expression of u-PA is also observed in human abdominal AA.[39–41] Additionally, *ApoE*$^{-/-}$ *Plau*$^{-/-}$ knockout mice protect against aneurysm formation in Ang II-induced aneurysms with or without pre-existing hyperlipidemia and atherosclerosis.[42,43] Inflammatory cells, particularly macrophages, were the major source of increased u-PA in the aneurysmal tissue. It has been reported that u-PA plays an important role in promoting vascular inflammation by activating cytokines and MMPs (MMP-2 and MMP-9), which might degrade elastin directly or other ECM components indirectly.[43] In PLAU-deficient mice, cell migration, including macrophages and foam cells, was also reduced in the injured vessel walls.[39]

Therefore, it has been hypothesized that inhibition of PLAU (or u-PA) could be an effective treatment for AA. Plasminogen activator inhibitor-1 (PAI-1) is a primary endogenous inhibitor of uPA. Both male and female *Pai-1*$^{-/-}$ mice had significantly larger aneurysms. However, local delivery of the *Pai-1* gene completely prevented aneurysm formation and expansion in the early stage by decreasing inflammation and MMPs activity in Ang II-induced abdominal AA in *ApoE*$^{-/-}$ mice.[44,45]

PSMA4 encodes a proteasome subunit that plays a central role in regulating inflammation, signal pathway transduction, and stress response. Proteasome dysfunction leads to many cardiovascular diseases, including cardiomyopathies, heart failure, and atherosclerosis.[46,47] In

aneurysms, proteasome peptidase activity was increased in both human and mouse abdominal AA tissue, which could be inhibited by bortezomib, a potent, selective inhibitor of the chymotrypsin-like activity of the 20S proteasome. Additionally, studies have demonstrated that low-dose bortezomib injection appeared to reduce MMP activity, smooth muscle cell phenotypic switching, and elastin degradation, resulting in the attenuation of aneurysm formation.[48] Our MR analysis suggested that PSMA4, a mediator of cell proliferation and apoptosis,[49] was consistently associated with AAs ($p<1.90E-5$).

Proteasome inhibitors are widely used to treat malignancies but are also known to have unavoidable side effects, especially cardiovascular toxicity. Carfilzomib is associated with congestive heart failure and myocardial ischemia, but bortezomib shows better safety in the heart.[50] Our findings did not show an association between genetically predicted inhibition of PSMA4 and cardiovascular risk. However, contrary to expectations, it was inversely associated with heart failure. Further studies are required to assess any moderating effects.

As they enlarge, AAs is life-threatening if undiagnosed or neglected. With considerable progress in the molecular mechanisms of AAs, pharmacological treatments, such as beta-blockers, losartan, statins, antiplatelet agents, and metformin, have made progress in recent years.[51–53] Owing to side effects and inconsistent clinical trial results, it is important to investigate other potentially effective and safe therapeutic targets. To ensure credible results obtained from MR analysis, Bonferroni correction for multiple testing was applied to reduce the risk of false-positive results. We used several pleiotropy-robust MR methods and outlier detection to rigorously decrease the possibility that the findings were not biased due to pleiotropy. Additionally, pQTL was used as a proposed instrument to validate our results. Due to limited genetic studies on protein levels, we only found pQTL for PLAU and validated that PLAU protein levels were consistently positively associated with AAs risk.

Individuals of European ancestry are always considered homogenous, but in our study, of the 11 drug targets identified in the UK Biobank cohort, only 4 are successfully replicated in the FinnGen cohort, suggesting poor portability within individuals of European ancestry. Previous ancient human genome studies have reported that present-day Europeans could drive from three mainly differentiated sub-population, including 'hunter-gatherer-related' ancestry, 'northwestern-Anatolian-Neolithic-related' ancestry, and 'steppe-related' ancestry. Although they share a common genome of European ancestry, we recommend the role of different sub-population should be paid more attention to in the future.

This study had several strengths. Firstly, it is well-known that the process of novel drug development always takes a very long time, is extremely expensive, and considers a high failure rate. We focused our research on druggable genes to improve the efficacy, safety, and drug development success in AAs and found two drug targets (PLAU and PSMA4) associated with AAs. Further, we listed some targeted small molecule inhibitors under development currently, which at least pointed the way forward for the future drug development of these targets. Secondly, we also conducted a wide-angled MR analysis to identify the potential safety aspects and alternative indications, which was important if they might be used clinically someday. Thirdly, those two proteins were found associated with the risk of aortic aneurysms in our population-based studies as well.

This study had several limitations. First, it is difficult to completely exclude the potential influence of directional pleiotropy. The diagnosis of AAs was a little different between the UK Biobank cohort and the FinnGen cohort, which could result in poor portability of our study. Second, the examination of side effects in our study is confined to cardiovascular outcomes, more particular attention should be paid to systemic adverse reactions in the future. Third, clinical trials are needed to evaluate their efficacy and safety for the early management of AAs. Fourth, syndromic aneurysms have different etiologies for AAs, and we evaluated the outcomes of PLAU and PSMA in these patients. Fifth, PSMA4 has a strong association with smoking, which is a risk factor for AAs. Further special attention should be paid in non-smoking patients to minimize the potential pleiotropic effect. Finally, the study population was restricted to individuals of European ancestry; therefore, the insights gained cannot be extended to other ethnicities.

In conclusion, this study supports that targeting PLAU and PSMA4 can reduce the risk of AAs. However, randomized trials need to be conducted to evaluate the efficacy and safety of the prevention of AAs.

Contributors

All authors read and approved the final version of the manuscript. YHC, YW, and DWW conceptualized, and designed the study. Formal analyses were performed by YHC and YS. YHC, XX, LLW, KL, MH, LX, and JQD were involved in collecting and interpreting the data. YHC drafted the initial paper. Supervision, funding, manuscript reviewing, and editing was performed by DWW. YHC, YS, and DWW verified the underlying data.

Data sharing statement

The GWAS datasets generated and/or analyzed during the current study are publicly available.

Declaration of interests

There are no conflicts of interest to declare.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.104199.

References

1 Bossone E, Eagle KA. Epidemiology and management of aortic disease: aortic aneurysms and acute aortic syndromes. *Nat Rev Cardiol*. 2021;18(5):331–348.
2 Shen YH, LeMaire SA, Webb NR, Cassis LA, Daugherty A, Lu HS. Aortic aneurysms and dissections series. *Arterioscler Thromb Vasc Biol*. 2020;40(3):e37–e46.
3 Sharples L, Sastry P, Freeman C, et al. Aneurysm growth, survival, and quality of life in untreated thoracic aortic aneurysms: the effective treatments for thoracic aortic aneurysms study. *Eur Heart J*. 2021;43(25):2356–2369.
4 Ference BA, Robinson JG, Brook RD, et al. Variation in PCSK9 and HMGCR and risk of cardiovascular disease and diabetes. *N Engl J Med*. 2016;375(22):2144–2153.
5 Cohen JC, Boerwinkle E, Mosley Jr. TH, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med*. 2006;354(12):1264–1272.
6 Hingorani A, Humphries S. Nature's randomised trials. *Lancet*. 2005;366(9501):1906–1908.
7 Gaziano L, Giambartolomei C, Pereira AC, et al. Actionable druggable genome-wide Mendelian randomization identifies repurposing opportunities for COVID-19. *Nat Med*. 2021;27(4):668–676.
8 Storm CS, Kia DA, Almramhi MM, et al. Finding genetically-supported drug targets for Parkinson's disease using Mendelian randomization of the druggable genome. *Nat Commun*. 2021;12(1):7342.
9 Harrison SC, Holmes MV, Burgess S, et al. Genetic association of lipids and lipid drug targets with abdominal aortic aneurysm: a meta-analysis. *JAMA Cardiol*. 2018;3(1):26–33.
10 Nastasi DR, Norman R, Moxon JV, et al. The potential benefits and costs of an intensified approach to low density lipoprotein cholesterol lowering in people with abdominal aortic aneurysm. *Eur J Vasc Endovasc Surg*. 2021;62(4):643–650.
11 Das D, Gawdzik J, Dellefave-Castillo L, et al. S100A12 expression in thoracic aortic aneurysm is associated with increased risk of dissection and perioperative complications. *J Am Coll Cardiol*. 2012;60(8):775–785.
12 Bradley DT, Hughes AE, Badger SA, et al. A variant in LDLR is associated with abdominal aortic aneurysm. *Circ Cardiovasc Genet*. 2013;6(5):498–504.
13 Rykaczewska U, Suur BE, Rohl S, et al. PCSK6 is a key protease in the control of smooth muscle cell function in vascular remodeling. *Circul Res*. 2020;126(5):571–585.
14 Finan C, Gaulton A, Kruger FA, et al. The druggable genome and support for target identification and validation in drug development. *Sci Transl Med*. 2017;9(383):eaag1166. https://doi.org/10.1126/scitranslmed.aag1166.
15 Vosa U, Claringbould A, Westra HJ, et al. Large-scale cis- and trans-eQTL analyses identify thousands of genetic loci and polygenic scores that regulate blood gene expression. *Nat Genet*. 2021;53(9):1300–1310.
16 Sun BB, Maranville JC, Peters JE, et al. Genomic atlas of the human plasma proteome. *Nature*. 2018;558(7708):73–79.
17 Emilsson V, Ilkov M, Lamb JR, et al. Co-regulatory networks of human serum proteins link genetics to disease. *Science*. 2018;361(6404):769–773.
18 Ferkingstad E, Sulem P, Atlason BA, et al. Large-scale integration of the plasma proteome with genetics and disease. *Nat Genet*. 2021;53(12):1712–1721.
19 Kurki MI, Karjalainen J, Palta P, et al. FinnGen: unique genetic insights from combining isolated population and national health register data. 2022:2022.03.03.22271360.
20 Willer CJ, Schmidt EM, Sengupta S, et al. Discovery and refinement of loci associated with lipid levels. *Nat Genet*. 2013;45(11):1274–1283.
21 Barton AR, Sherman MA, Mukamel RE, Loh PR. Whole-exome imputation within UK Biobank powers rare coding variant association and fine-mapping analyses. *Nat Genet*. 2021.
22 Surendran P, Feofanova EV, Lahrouchi N, et al. Discovery of rare variants associated with blood pressure regulation through meta-analysis of 1.3 million individuals. *Nat Genet*. 2020;52(12):1314–1332.
23 Chen J, Spracklen CN, Marenne G, et al. The trans-ancestral genomic architecture of glycemic traits. *Nat Genet*. 2021;53(6):840–860.
24 Locke AE, Kahali B, Berndt SI, et al. Genetic studies of body mass index yield new insights for obesity biology. *Nature*. 2015;518(7538):197–206.
25 Wood AR, Esko T, Yang J, et al. Defining the role of common variation in the genomic and biological architecture of adult human height. *Nat Genet*. 2014;46(11):1173–1186.
26 Shungin D, Winkler TW, Croteau-Chonka DC, et al. New genetic loci link adipose and insulin biology to body fat distribution. *Nature*. 2015;518(7538):187–196.
27 Malik R, Chauhan G, Traylor M, et al. Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. *Nat Genet*. 2018;50(4):524–537.
28 Nielsen JB, Thorolfsdottir RB, Fritsche LG, et al. Biobank-driven genomic discovery yields new insight into atrial fibrillation biology. *Nat Genet*. 2018;50(9):1234–1239.
29 van der Harst P, Verweij N. Identification of 64 novel genetic loci provides an expanded view on the genetic architecture of coronary artery disease. *Circul Res*. 2018;122(3):433–443.
30 Shah S, Henry A, Roselli C, et al. Genome-wide association and Mendelian randomisation analysis provide insights into the pathogenesis of heart failure. *Nat Commun*. 2020;11(1):163.
31 Chiou J, Geusz RJ, Okino ML, et al. Interpreting type 1 diabetes risk with genetics and single-cell epigenomics. *Nature*. 2021;594(7863):398–402.
32 Mahajan A, Taliun D, Thurner M, et al. Fine-mapping type 2 diabetes loci to single-variant resolution using high-density imputation and islet-specific epigenome maps. *Nat Genet*. 2018;50(11):1505–1513.
33 Wuttke M, Li Y, Li M, et al. A catalog of genetic loci associated with kidney function from analyses of a million individuals. *Nat Genet*. 2019;51(6):957–972.
34 Bakker MK, van der Spek RAA, van Rheenen W, et al. Genome-wide association study of intracranial aneurysms identifies 17 risk loci and genetic overlap with clinical risk factors. *Nat Genet*. 2020;52(12):1303–1313.
35 Roychowdhury T, Lu H, Hornsby WE, et al. Regulatory variants in TCF7L2 are associated with thoracic aortic aneurysm. *Am J Hum Genet*. 2021.
36 Urano T, Castellino FJ, Suzuki Y. Regulation of plasminogen activation on cell surfaces and fibrin. *J Thromb Haemost*. 2018;16(8):1487–1497.
37 Carmeliet P, Collen D. Gene targeting and gene transfer studies of the plasminogen/plasmin system: implications in thrombosis, hemostasis, neointima formation, and atherosclerosis. *FASEB J*. 1995;9(10):934–938.
38 Rifkin DB, Mazzieri R, Munger JS, Noguera I, Sung J. Proteolytic control of growth factor availability. *APMIS*. 1999;107(1):80–85.
39 Wang YX, Martin-McNulty B, Freay AD, et al. Angiotensin II increases urokinase-type plasminogen activator expression and induces aneurysm in the abdominal aorta of apolipoprotein E-deficient mice. *Am J Pathol*. 2001;159(4):1455–1464.
40 Falkenberg M, Holmdahl L, Tjarnstrom J, Risberg B. Abnormal levels of urokinase plasminogen activator protein and tissue plasminogen activator activity in human aortic aneurysms. *Eur J Surg*. 2001;167(1):10–14.
41 Shireman PK, McCarthy WJ, Pearce WH, Shively VP, Cipollone M, Kwaan HC. Elevations of tissue-type plasminogen activator and differential expression of urokinase-type plasminogen activator in diseased aorta. *J Vasc Surg*. 1997;25(1):157–164.
42 Deng GG, Martin-McNulty B, Sukovich DA, et al. Urokinase-type plasminogen activator plays a critical role in angiotensin II-induced abdominal aortic aneurysm. *Circul Res*. 2003;92(5):510–517.
43 Carmeliet P, Moons L, Lijnen R, et al. Urokinase-generated plasmin activates matrix metalloproteinases during aneurysm formation. *Nat Genet*. 1997;17(4):439–444.
44 Qian HS, Gu JM, Liu P, et al. Overexpression of PAI-1 prevents the development of abdominal aortic aneurysm in mice. *Gene Ther*. 2008;15(3):224–232.

45 DiMusto PD, Lu G, Ghosh A, et al. Increased PAI-1 in females compared with males is protective for abdominal aortic aneurysm formation in a rodent model. *Am J Physiol Heart Circ Physiol*. 2012;302(7):H1378–H1386.

46 Chiao CC, Liu YH, Phan NN, et al. Prognostic and Genomic Analysis of Proteasome 20S Subunit Alpha (PSMA) family members in breast cancer. *Diagnostics (Basel)*. 2021;11(12).

47 Wang F, Lerman A, Herrmann J. Dysfunction of the ubiquitin-proteasome system in atherosclerotic cardiovascular disease. *Am J Cardiovasc Dis*. 2015;5(1):83–100.

48 Ren H, Li F, Tian C. Inhibition of proteasome activity by low-dose bortezomib attenuates angiotensin II-induced abdominal aortic aneurysm in Apo E(-/-) Mice. *Sci Rep*. 2015;5:15730.

49 Hansen HM, Xiao Y, Rice T, et al. Fine mapping of chromosome 15q25.1 lung cancer susceptibility in African-Americans. *Hum Mol Genet*. 2010;19(18):3652–3661.

50 Hahn VS, Zhang KW, Sun L, Narayan V, Lenihan DJ, Ky B. Heart failure with targeted cancer therapies: mechanisms and cardioprotection. *Circul Res*. 2021;128(10):1576–1593.

51 Lindeman JH, Matsumura JS. Pharmacologic management of aneurysms. *Circul Res*. 2019;124(4):631–646.

52 Quintana RA, Taylor WR. Cellular mechanisms of aortic aneurysm formation. *Circul Res*. 2019;124(4):607–618.

53 Anagnostakos J, Lal BK. Abdominal aortic aneurysms. *Prog Cardiovasc Dis*. 2021;65:34–43.