Association of Circulating Cathepsin B Levels With Blood Pressure and Aortic Dilation

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Hypertension is a key risk factor for spontaneous coronary artery dissection (SCAD) and aortic dilation. Circulating proteins play key roles in a range of biological processes and represent a major source of druggable targets. The aim of this study was to identify circulating proteins that were associated with blood pressure (BP), SCAD and aortic dilation. We identified shared genetic variants of BP and SCAD in genome-wide association studies, searched for circulating protein affected by these variants and examined the association of circulating protein levels with BP, aortic aneurysm and dissection (AAD) and aortic diameters by integrating data from circulating protein quantitative trait loci (pQTL) studies and genome wide association study (GWAS) in individuals from the UK Biobank using two-sample Mendelian randomization analysis methods. Single nucleotide polymorphisms (SNPs) in *JAG1*, *ERI1*, *ULK4*, *THSD4*, *CMIP*, *COL4A2*, *FBN1*, *FAM76B*, *FGGY*, *NUS1*, and *HNF4G*, which were related to extracellular matrix components, were associated with both BP and SCAD. We found 49 significant pQTL signals among these SNPs. The regulated proteins were encoded by *MMP10*, *IL6R*, *FIGF*, *MMP1*, *CTSB*, *IGHG1*, *DSG2*, *TTC17*, *RETN*, *POMC*, *SCARF2*, *RELT*, and *GALNT16*, which were enriched in biological processes such as collagen metabolic process and multicellular organism metabolic process. Causal associations between BP and AAD and aortic diameters were detected. Significant associations between circulating levels of cathepsin B, a well-known prorenin processing enzyme, and BP and aortic diameters were identified by using several Mendelian randomization analysis methods and were validated by independent data.

Conclusion: The present study identified the association between circulating cathepsin B and BP and aortic diameters. The findings indicated that BP-associated genetic variants may influence aortic dilation risk by circulating proteins that regulate BP.

Keywords: blood pressure, genome-wide association study, circulating proteins, cathepsin, spontaneous coronary artery dissection (SCAD), aortic dissection (AD)

INTRODUCTION

Aortic dissection (AD) is the major diseases affecting the aorta and a leading cause of morbidity and mortality. Recently, the Global Burden Disease 2010 project demonstrated that the overall global death rate from aortic aneurysms and AD increased from 2.49 per 100,000 to 2.78 per 100,000 inhabitants between 1990 and 2010 (1, 2). The burden increases with age, and men are more often affected than women (1). Spontaneous coronary artery dissection (SCAD) is one of the most dangerous forms of vascular disease and is characterized by endometrial rupture and intramural hematoma formation. Until now, the occurrence and development of SCAD and AD have been unpredictable.

Aortic disease in Marfan syndrome is the result of defects in the fibrillin-1 (*FBN1*) gene that localizes to chromosome 15q15-31 (3). Genetic studies have demonstrated familial aggregation of AD and have resulted in the mapping of several novel genetic loci for the condition (4–6). Genes associated with AD include *FBN1*, *TGFBR1/2*, *TGFB1*, *EFEMP2*, *MYH11*, *ACTA2*, *COL3A1*, *SLC2A10*, *SMAD3*, *LOX*, *FOXE3*, *MFAP5*, *MAT2A*, *PRKG1*, and so on (7). Genetic variants in *LRP1* and *ULK4* are associated with sporadic thoracic AD (5).

The common occurrence of SCAD in young patients without risk factors for atherosclerosis and the identification of cases with familial association have led to the hypothesis of possible genetically mediated pathophysiologic mechanisms. Pathogenic variants were found in *COL3A1* (8), *SMAD3* (8, 9), *TGFBR1/2*, *TGFB 2/3* (10), *FBN1*, *PHACTR1/EDN1* (11), *TSR1* (12, 13), *TLN1* (14), and others (14–16). A recent genome wide association study (GWAS) has identified associations at chromosome 1q21.2 in *ADAMTSL4*, chromosome 6p24.1 in *PHACTR1* and chromosome 12q13.3 in *LRP1* and identified associations of non-coding variants in the *LRP1* gene at chromosome 12q13.3 and near *MRP6/KCNE3* at chromosome 21q22.11 for SCAD (15). The findings of these studies support a complex genetic basis of SCAD. However, although some of the identified variants have been shown to affect gene expression, most of the genetic associations have remained unexplained.

Environmental factors such as hypertension (HTN) that cause increased vascular pressure and lead to activation of stress- and stretch-induced pathways in aortic smooth muscle cells contribute to increased risk and progression of SCAD. Therefore, factors associated with increased blood pressure (BP) were risk factors for SCAD and AD. GWAS have identified many genetic loci for BP (17), and there were overlapping associated genes between BP, SCAD and AD. Genetic factors may directly influence the structure of the arterial wall or act indirectly through risk factors such as BP, ultimately resulting in an increase in arterial stiffness. The indirect factors involved in the regulatory pathway by which genetic variants influence SCAD and AD risk *via* BP may play roles in SCAD and AD prevention by controlling BP. However, these factors were largely unknown. Identification of indirect factors can help to explain genetic associations.

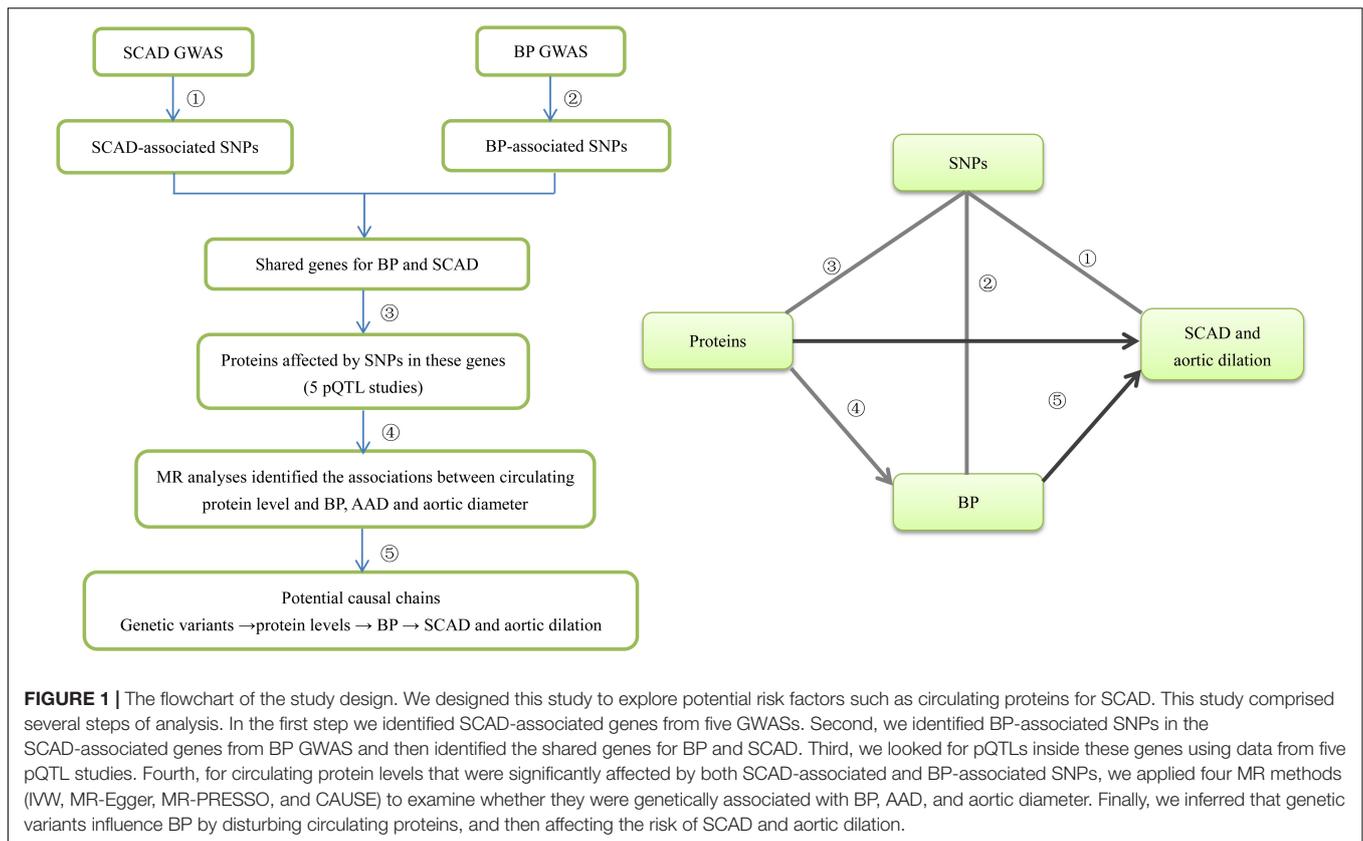Despite increasing investment in research and development in the pharmaceutical industry, the rate of success for novel drugs continues to fall. Lower success rates make new therapeutics more expensive, reducing the availability of effective medicines and increasing healthcare costs. Systematically evaluating the genetic evidence in support of potential target-indication pairs is a potential strategy to prioritize development programs. Circulating proteins play key roles in a range of biological processes and represent a major source of druggable targets (18, 19). Recently, published GWAS of circulating proteins have identified a large number of single nucleotide polymorphisms (SNPs) associated with circulating proteins (protein quantitative trait loci, or pQTLs) (20–24). These genetic associations offered the opportunity to systematically test the causal effects of a large number of proteins on diseases. Indeed, the GWAS identified loci containing genes that encode proteins or regulate the expression of proteins involved in arterial wall structure and/or function. Circulating proteins such as TGF β receptor, collagen α1 chain, fibrillin, endotelin-1, ribosome maturation factor, and talin 1 were implicated in the development of SCAD (25). Currently, multiple cytokines, including interleukins, interferon, the tumor necrosis factor superfamily, colony stimulating factor, chemotactic factor, growth factor and so on, have all been demonstrated to play a critical role in SCAD.

Identification of functional variants in these genetic loci for BP, SCAD and AD will increase our understanding of the pathological mechanisms. The recently developed two-sample Mendelian randomization (MR) methods based on summary statistics provide feasible ways to integrate omics data from independent GWAS, including pQTL studies (26–28). This study represents an effort to identify potential proteins or biological pathways implicated in the development of SCAD and AD through BP regulation based on their shared genetic basis.

MATERIALS AND METHODS

Determination of Spontaneous Coronary Artery Dissection and Blood Pressure Associated SNPs

In this study, we determined genetic loci for SCAD from five GWASs (5, 15, 16, 29, 30). Genes and top SNPs in the identified loci were collected from the GWAS papers and related supplementary online files (**Figure 1**). Specifically, genome-wide summary statistics were obtained from a recent GWAS (15). This GWAS comprised 270 SCAD cases and 5,263 controls of European descent. The raw data used in the present analysis were the downloaded summary results from the initial GWAS, which included association *P*-values of 607,778 genotyped variants with SCAD (15). The SCAD GWAS summary datasets were publicly available at the NHGRI-EBI GWAS Catalog under accession number GCST90000582. SNPs with *P*-values less than 1.0×10^{-5} were selected and the SCAD loci were determined. The genome-wide significance level was set to 5.0×10^{-8} in this study. The BP loci were identified in a GWAS examined associations between more than 10 million SNPs and systolic BP (SBP) and diastolic BP (DBP) in 317,756 individuals enrolled in the UK Biobank (17). SNPs associated with both BP and SCAD were identified.

FIGURE 1 | The flowchart of the study design. We designed this study to explore potential risk factors such as circulating proteins for SCAD. This study comprised several steps of analysis. In the first step we identified SCAD-associated genes from five GWASs. Second, we identified BP-associated SNPs in the SCAD-associated genes from BP GWAS and then identified the shared genes for BP and SCAD. Third, we looked for pQTLs inside these genes using data from five pQTL studies. Fourth, for circulating protein levels that were significantly affected by both SCAD-associated and BP-associated SNPs, we applied four MR methods (IVW, MR-Egger, MR-PRESSO, and CAUSE) to examine whether they were genetically associated with BP, AAD, and aortic diameter. Finally, we inferred that genetic variants influence BP by disturbing circulating proteins, and then affecting the risk of SCAD and aortic dilation.

Identification of Circulating Proteins

We carried out pQTL analysis to obtain relevant evidence for the identified SNPs shared by BP and SCAD. The associations between SNPs and circulating protein levels were searched in five pQTL studies. First, the pQTL data of the KORA study which contained associations between 509,946 SNPs and 1,124 proteins were downloaded from the pGWAS Server (21).[1] The second pQTL study performed genome-wide testing of 10.6 million imputed autosomal variants against levels of 2,994 circulating proteins in 3,301 individuals of European descent from the INTERVAL study (23). The summary data are available at http://www.phpc.cam.ac.uk/ceu/proteins/. The third pQTL study analyzed 83 proteins measured in 3,394 individuals (20).[2] The fourth pQTL study provided the results of GWAS of 71 high-value cardiovascular disease proteins in 6,861 Framingham Heart Study participants (24). In the fifth pQTL study, 4,137 proteins covering most predicted extracellular proteins were measured in the serum of 5,457 Icelanders over 65 years of age (22). pQTL signals with P-values less than 1.0×10^{-5} were considered in this study.

Functional Enrichment Analysis

The purpose of gene ontology (GO) is to unify biological factors while integrating specific definitions, clear structures,

and controlled vocabulary into a database (31). GO analysis was performed to explore the biological properties of the genes coding the identified proteins. The Database for Annotation, Visualization and Integrated Discovery (DAVID) database,[3] an online bioinformatics tool, was used to illustrate the functional enrichment analysis (32). The advantages of DAVID include the detailed analysis and classification of gene and protein functions. A false positive rate less than 0.1 was considered significant.

MR Analysis on Proteins

To obtain supporting evidence for proteins identified in pQTL analysis, we performed MR analysis to examine the associations between protein levels and BP. The protein data used in these MR analyses were from the pQTL studies described above. The BP GWAS dataset comprised the summary statistics for the association between more than 10 million SNPs and SBP and DBP, which were evaluated in 317,756 individuals enrolled in the UK Biobank (17). This dataset can be downloaded at https://data.broadinstitute.org/alkesgroup/UKBB/. Summary data necessary to MR analysis were unavailable for SCAD, which kept us from examining the associations between protein levels and SCAD.

Instead, we explored the role of the identified proteins in aortic remodeling, which were highly related to BP. GWAS summary data of aortic aneurysm and dissection (AAD) (ICD-10 code: I71), which contains 452,264 individuals from the UK Biobank (30), was obtained. These GWAS included 1,470 AAD

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[1]http://metabolomics.helmholtz-muenchen.de/pgwas/index.php?task=download
[2]https://zenodo.org/record/264128#.X3u5ga95uUk

[3]https://david.ncifcrf.gov/

patients and 450,794 controls. The genome-wide association analysis of the UK Biobank GWAS comprised 62,394 genotyped variants and 9,113,133 imputed variants that passed quality control. The summary data for AAD can be found by searching "Aortic aneurysm and dissection" at http://geneatlas.roslin.ed. ac.uk/downloads/. In addition, summary data from GWAS of ascending aortic (AA) and descending aortic (DA) diameters were obtained. AA and DA diameters were evaluated in cardiac magnetic resonance images from the UK Biobank by a deep learning model (33).

We employed the weighted median, inverse-variance weighted (IVW) MR (34), MR-Egger (35), MR pleiotropy residual sum and outlier (MR-PRESSO) (36) and the Causal Analysis Using Summary Effect estimates (CAUSE) (37) methods to test for potential causal relationships between circulating protein levels and BP and SCAD. The inverse-variance weighted

method combines the ratio estimates from each IV in a meta-analysis model (34). If the associations with circulating protein levels were to lead to horizontal pleiotropy, the intercept from MR-Egger would be expected to differ from zero (35). In such cases, we interpreted the coefficient from MR-Egger as being the more valid causal estimate. Conversely, in the absence of statistical evidence for horizontal pleiotropy from the intercept on MR-Egger, we used the IVW MR analysis as it retains greater power. The IVW MR and MR-Egger analyses were performed by using the MendelianRandomization R package (38). We also detected horizontal pleiotropy and outlier-corrected causal estimation by using MR-PRESSO tests (36). The outlier test in MR-PRESSO is the procedure to test for the MR assumption of no pleiotropy. The source code and documents for MR-PRESSO are available at https://github.com/rondolab/MR-PRESSO. The default parameters were used for the MR-PRESSO analysis.

TABLE 1 | The SCAD loci collected from GWAS.

Study	Loci	Number of SNPs with P-value < 1.0E-5	Number of SNPs with P-value < 5.0E-8	Top SNP	
				rs ID	P-value
(15)	1p32.1	7	0	rs11207415	3.23E-06
	1q21.2	291	149	rs12740679	2.19E-12
	2p23.2	1	0	rs4535004	8.32E-06
	2q33.2	3	0	rs78377252	2.43E-06
	4q34.3	1	0	rs2715408	7.64E-06
	5q23.2	1	0	rs17839701	9.94E-06
	6p24.1	5	1	rs9349379	4.36E-08
	6q22.1	3	0	rs7775726	6.94E-06
	6q25.3	11	0	rs78349783	1.03E-06
	12q13.3	14	5	rs11172113	2.63E-08
	12q21.33	1	0	rs2722224	6.71E-06
	12q23.2	3	0	rs1014675	5.45E-06
	21q22.11	3	0	rs28451064	1.19E-07
(16)	1q21.3	–	1	rs4970935	3.26E-16
	1q24.2	1	–	rs6700122	1.40E-06
	3q22.3	1	–	rs189056	7.53E-06
	4q34.3	1	–	rs79603310	7.32E-05
	6p24.1	–	1	rs9349379	4.59E-14
	8q24.3	1	–	rs10095937	5.74E-05
	12q13.3	–	1	rs11172113	1.42E-13
	15q21.1	–	1	rs2015637	2.12E-09
	16q24.1	1	–	rs67049921	5.40E-05
	21q22.11	–	1	rs28451064	1.09E-09
(39)	1p32.1	1	–	rs12402265	2.30E-07
	2q32.1	1	–	rs6741522	2.29E-06
	4q12	–	1	rs6820391	2.36E-08
	6p24.1	–	1	rs9349379	1.00E-11
	12q13.3	2	–	rs11172113	1.90E-07
(5)	3p22.1	–	1	rs2272007	1.15E-09
	12q13.3	–	1	rs11172113	2.74E-08
	15q21.1	–	1	rs1042078	1.08E-10
(4)	15q21.1	–	5	rs2118181	5.90E-12
	Total	353	170	–	–

In the pQTL summary data, SNPs with a *P*-value less than 1.0×10^{-5} were selected as potential instrumental variables. The selection criterion was set to 1.0×10^{-5} for the two pQTL studies because 5.0×10^{-8} would lead to too few instrumental variables. We clumped SNPs (LD $r^2 < 0.01$ within 10,000 kb) based on data from Europeans from the 1000 Genomes project using the "clump_data" function in the R package TwoSampleMR to select independent instrumental variables. The effect allele of each SNP in the BP GWAS and pQTL studies was manually checked for consistency.

For proteins that passed the three MR tests, we applied the CAUSE method to account for horizontal pleiotropic effects by pathways other than those considered in the multivariable approach. CAUSE is a Mendelian randomization accounting for correlated and uncorrelated horizontal pleiotropic effects using genome-wide summary statistics (37). Data used in the CAUSE analysis were from pQTL studies and GWAS described above. We used 1,000,000 genetic variants to estimate the nuisance parameters. Other parameters were left as their defaults in the CAUSE analysis.

RESULTS

The Spontaneous Coronary Artery Dissection-Associated Loci

Based on published GWAS papers, we collected up to 162 SCAD-associated genes (**Supplementary Table 1**). Genes such as *FBN1* and *PHACTR1* were confirmed in several GWAS. The most recent GWAS published at Nature Communication identified many novel genes and confirmed previously identified genes. In this GWAS, we selected SNPs in 13 SCAD-associated loci (**Table 1**). The top signals were located in 1q21.2, in which 149 SNPs showed significant associations with SCAD ($P < 5.0 \times 10^{-8}$). The top SNP associated with SCAD in 1q21.2 was rs12740679 ($P = 2.19 \times 10^{-12}$). There were fewer genome-wide significant SNPs in other selected loci. Five SNPs in 12q13.3 and one SNP in 6p24.1 were significantly associated with SCAD. For the remaining ten selected loci, no SNPs reached the genome-wide significance level. For 2p23.2, 4q34.3, 5q23.2, and 12q21.33, only one SNP reached 1.0×10^{-5} in each locus. In 6q25.3, 11 SNPs passed 1.0×10^{-5}. The top SNP in 6q25.3 was rs78349783 (1.03×10^{-6}).

Spontaneous Coronary Artery Dissection-Associated Genes Shared by Blood Pressure

We selected 230 thousand SNPs inside the 162 SCAD-associated genes according to the UCSC database. Then we looked up associations between these SNPs and BP in GWAS summary data. We found 204 and 228 SNPs that were significantly associated with SBP and DBP ($P < 5.0 \times 10^{-8}$), respectively. These SNPs were located in 11 genes, including *JAG1*, *ERI1*, *ULK4*, *THSD4*, *CMIP*, *COL4A2*, *FBN1*, *FAM76B*, *FGGY*, *NUS1*, and *HNF4G*. A total of 111 and 114 SNPs in the *ULK4* gene were associated with SBP and DBP ($P < 5.0 \times 10^{-8}$), respectively. SNPs in

JAG1 and *ERI1* both associated with SBP and DBP. SNPs in *FGGY*, *HNF4G* and *NUS1* were associated with SBP; SNPs in *CMIP*, *COL4A2*, *FAM76B*, *FBN1*, and *THSD4* were associated with DBP. These SNPs were located in SCAD-associated genes, and therefore were considered to be shared SNPs for SCAD and BP. We next searched whether these SNPs were associated with circulating proteins.

Potential Proteins Related to Blood Pressure and Spontaneous Coronary Artery Dissection

We looked for pQTL signals in five studies. We found significant signals in three studies (20, 21, 23) for the shared SNPs (**Table 2**). A total of 49 pQTL signals were identified. The SNPs were located in *FAM76B*, *COL4A2*, *JAG1*, *ITSN1*, *ERI1*, and *CMIP*. The regulated proteins were encoded by 13 genes, including *MMP10*, *IL6R*, *FIGF*, *MMP1*, *CTSB*, *IGHG1*, *DSG2*, *TTC17*, *RETN*, *POMC*, *SCARF2*, *RELT*, and *GALNT16*. BP-associated SNPs in *ERI1* were strongly associated with circulating cathepsin B (*CTSB*), and evidence was found in KORA and INTERVAL studies (21, 23).

Functional Enrichment Analysis

To examine the potential biological functions of the proteins affected by SCAD and BP-associated SNPs, we performed GO analysis in the DAVID database. Among the 11 shared genes, *COL4A2*, *THSD4*, and *FBN1* were related to extracellular matrix components ($P = 1.10 \times 10^{-3}$) and proteinaceous extracellular matrix ($P = 8.60 \times 10^{-7}$). The 13 BP- and SCAD-associated proteins were enriched in specific GO biological process, cellular component and molecular function terms (false discovery rate < 0.1), including cellular components such as extracellular space ($P = 2.70 \times 10^{-3}$); biological process such as collagen metabolic process ($P = 5.70 \times 10^{-7}$), multicellular organism metabolic process ($P = 6.90 \times 10^{-7}$); molecular function such as serine-type peptidase activity ($P = 6.50 \times 10^{-4}$), serine hydrolase activity ($P = 6.70 \times 10^{-4}$) and endopeptidase activity ($P = 3.30 \times 10^{-3}$) (**Table 3**). Proteins encoded by *IGHG1*, *MMP1*, *MMP10*, *CTSB*, *POMC*, *RETN*, and *IL6R* may have functional roles in BP regulation and the development of SCAD.

Proteins Causally Associated With Blood Pressure and Aortic Remodeling

We tested whether the seven proteins were genetically associated with BP using several MR methods (**Table 4**). We found that the association between circulating cathepsin B level and DBP was significant in MR-Egger analyses using data from KORA and INTERVAL studies. Significant association between circulating cathepsin B level and DBP was also found in the weighted median analysis using data from INTERVAL study. After adjusting for outliers in MR-PRESSO analysis, the associations between circulating cathepsin B level and DBP was marginal significant ($P = 0.0580$). Significant association between circulating cathepsin B level and SBP was found in the weighted median analysis using data from INTERVAL study. Furthermore, we corrected for correlated and uncorrelated horizontal pleiotropy using the CAUSE method and retained

TABLE 2 | Proteins related to BP-associated SNPs.

SNP	Gene	CHR	Position (hg19)	GWAS		pQTL		
				P-value	Trait	*P*-value	Protein	Study
rs11776838	*ERI1*	8	8794801	1.22E-08	SBP	5.23E-05	*CTSB*	(21)
rs11776838	*ERI1*	8	8794801	1.33E-12	DBP	5.23E-05	*CTSB*	(21)
rs2186739	*FAM76B*	11	95327374	8.55E-11	DBP	2.48E-05	*IGHG1*	(21)
rs2186739	*FAM76B*	11	95327374	8.55E-11	DBP	7.98E-05	*DSG2*	(21)
rs2156468	*FAM76B*	11	95327412	4.18E-08	DBP	2.41E-05	*IGHG1*	(21)
rs2508895	*FAM76B*	11	95321747	5.04E-11	DBP	2.34E-04	*MMP10*	(20)
rs2254626	*FAM76B*	11	95327169	8.78E-11	DBP	8.21E-04	*MMP10*	(20)
rs2186739	*FAM76B*	11	95327374	8.55E-11	DBP	8.45E-04	*MMP10*	(20)
rs12323265	*COL4A2*	13	111014716	1.50E-08	DBP	4.55E-04	*IL6R*	(20)
rs6077929	*JAG1*	20	10819210	6.67E-12	DBP	6.27E-04	*FIGF*	(20)
rs2251854	*ITSN1*	21	35056247	3.99E-06	DBP	8.58E-04	*MMP1*	(20)
rs7281625	*ITSN1*	21	35066836	4.89E-06	DBP	9.74E-04	*MMP1*	(20)
rs475259	*ERI1*	8	8610267	2.68E-09	SBP	4.68E-05	*TTC17*	(23)
rs475259	*ERI1*	8	8610267	6.46E-10	DBP	4.68E-05	*TTC17*	(23)
rs28446104	*ERI1*	8	8795901	1.10E-11	DBP	2.95E-05	*RETN*	(23)
rs28446104	*ERI1*	8	8795901	1.10E-11	DBP	2.75E-05	*CTSB*	(23)
rs7819827	*ERI1*	8	8797055	4.74E-08	SBP	4.90E-05	*CTSB*	(23)
rs7819827	*ERI1*	8	8797055	3.34E-12	DBP	4.90E-05	*CTSB*	(23)
rs7844374	*ERI1*	8	8798684	4.25E-08	SBP	3.80E-05	*CTSB*	(23)
rs7844374	*ERI1*	8	8798684	2.26E-12	DBP	3.80E-05	*CTSB*	(23)
rs6601274	*ERI1*	8	8799059	2.03E-08	SBP	2.88E-05	*RETN*	(23)
rs6601274	*ERI1*	8	8799059	2.03E-08	SBP	3.89E-05	*CTSB*	(23)
rs6601274	*ERI1*	8	8799059	3.31E-12	DBP	3.89E-05	*CTSB*	(23)
rs6601274	*ERI1*	8	8799059	3.31E-12	DBP	2.88E-05	*RETN*	(23)
rs7837026	*ERI1*	8	8801692	5.80E-09	SBP	3.80E-05	*CTSB*	(23)
rs7837026	*ERI1*	8	8801692	5.80E-09	SBP	3.55E-05	*RETN*	(23)
rs7837026	*ERI1*	8	8801692	3.79E-13	DBP	3.55E-05	*RETN*	(23)
rs7837026	*ERI1*	8	8801692	3.79E-13	DBP	3.80E-05	*CTSB*	(23)
rs7823898	*ERI1*	8	8803487	5.25E-11	DBP	7.59E-06	*RETN*	(23)
rs12930850	*CMIP*	16	81602212	7.24E-09	DBP	3.24E-06	*POMC*	(23)
rs12929303	*CMIP*	16	81602264	3.47E-09	DBP	3.24E-06	*POMC*	(23)
rs11649004	*CMIP*	16	81602499	9.12E-09	DBP	1.66E-06	*POMC*	(23)
rs11644375	*CMIP*	16	81602681	8.88E-09	DBP	5.50E-06	*POMC*	(23)
rs8045875	*CMIP*	16	81603235	6.24E-09	DBP	5.25E-06	*POMC*	(23)
rs8045100	*CMIP*	16	81603400	5.80E-09	DBP	3.09E-06	*POMC*	(23)
rs11640346	*CMIP*	16	81603848	6.83E-09	DBP	6.03E-06	*POMC*	(23)
rs12931242	*CMIP*	16	81604778	6.11E-09	DBP	2.09E-06	*POMC*	(23)
rs7198940	*CMIP*	16	81605559	2.40E-08	DBP	4.27E-06	*POMC*	(23)
rs12918645	*CMIP*	16	81606569	2.55E-08	DBP	4.07E-06	*POMC*	(23)
rs12924701	*CMIP*	16	81607756	4.18E-09	DBP	3.31E-06	*POMC*	(23)
rs4483835	*CMIP*	16	81607833	4.81E-09	DBP	2.51E-06	*POMC*	(23)
rs7196548	*CMIP*	16	81609774	6.20E-09	DBP	1.91E-06	*POMC*	(23)
rs7199293	*CMIP*	16	81614892	4.29E-08	DBP	1.78E-05	*POMC*	(23)
rs6134025	*JAG1*	20	10743782	4.64E-10	DBP	2.29E-05	*SCARF2*	(23)
rs75612301	*JAG1*	20	10744573	5.36E-10	DBP	4.07E-05	*RELT*	(23)
rs75612301	*JAG1*	20	10744573	5.36E-10	DBP	2.00E-05	*SCARF2*	(23)
rs6134028	*JAG1*	20	10744714	1.76E-09	DBP	3.09E-05	*SCARF2*	(23)
rs1009757	*JAG1*	20	10828640	2.67E-14	DBP	3.72E-05	*GALNT16*	(23)
rs6074192	*JAG1*	20	10829248	3.28E-14	DBP	4.17E-05	*GALNT16*	(23)

CHR, Chromosome; GWAS, Genome-wide association study; QTL, Quantitative trait locus; SNP, Single nucleotide polymorphism.

TABLE 3 | Potential biological functions of proteins regulated by BP and SCAD-associated SNPs.

Catalog	Term	Gene	P-value	Benjamini
Molecular function	Serine-type endopeptidase activity	IGHG1, MMP1, MMP10, CTSB	4.90E-04	1.50E-02
Molecular function	Serine-type peptidase activity	IGHG1, MMP1, MMP10, CTSB	6.50E-04	1.50E-02
Molecular function	Serine hydrolase activity	IGHG1, MMP1, MMP10, CTSB	6.70E-04	1.50E-02
Molecular function	Endopeptidase activity	IGHG1, MMP1, MMP10, CTSB	3.30E-03	5.60E-02
Biological process	Collagen metabolic process	MMP1, RETN, IL6R, MMP10, CTSB	5.70E-07	1.80E-04
Biological process	Multicellular organismal macromolecule metabolic process	MMP1, RETN, IL6R, MMP10, CTSB	6.90E-07	1.80E-04
Biological process	Multicellular organism metabolic process	MMP1, RETN, IL6R, MMP10, CTSB	1.20E-06	2.20E-04
Cellular component	Extracellular space	POMC, IGHG1, RETN, IL6R, MMP10, CTSB	2.70E-03	6.20E-02

TABLE 4 | The causation between circulating cathepsin B level and BP and aortic diameter.

Trait	pQTL study	Weighted median			IVW			MR-Egger			MR-PRESSO		
		Beta	SE	P-value	Beta	SE	P-value	Beta	SE	P-value	Beta	SE	P-value
DBP	KORA	−0.0901	0.0405	0.0263	−0.0829	0.0720	0.2496	−0.3668	0.0822	8.15×10^{-6}	−0.0883	0.0757	0.3636
	INTERVAL	−0.1499	0.0453	9.25×10^{-4}	−0.0688	0.0520	0.1864	−0.3057	0.0630	1.23×10^{-6}	−0.0937	0.0451	0.0580
SBP	KORA	−0.0131	0.0057	0.0213	−0.0053	0.0098	0.5909	−0.0437	0.0196	0.0257	−0.0053	0.0098	0.6194
	INTERVAL	−0.0214	0.0064	8.56×10^{-4}	−0.0120	0.0077	0.1186	−0.0197	0.0186	0.2908	−0.0120	0.0077	0.1381
AA	KORA	−0.0247	0.0135	0.0676	−0.0239	0.0148	0.1058	−0.0950	0.0280	6.81×10^{-4}	−0.0238	0.0147	0.1811
	INTERVAL	−0.0596	0.0152	8.34×10^{-5}	−0.0397	0.0136	3.63×10^{-3}	−0.0234	0.0324	0.4712	−0.0397	0.0136	9.38×10^{-3}
DA	KORA	−0.0232	0.0105	0.0282	−0.0221	0.0091	0.0153	−0.0340	0.0248	0.1706	−0.0221	0.0040	5.18×10^{-3}
	INTERVAL	−0.0249	0.0134	0.0629	−0.0106	0.0134	0.4293	−0.0521	0.0303	0.0852	−0.0106	0.0134	0.4397

BP, Blood pressure; IVW, inverse-variance weighted; MR, Mendelian randomization; MR-PRESSO, MR pleiotropy residual sum and outlier; SE, Standard error.

an indication for the causal effect of cathepsin B on BP (**Figure 2**). By using INTERVAL data, we found that the causal model was significantly better than the null and sharing models for SBP ($P = 0.020$ and 0.024, respectively). The instrumental variables used in the MR analyses were presented in **Supplementary Table 2**.

For aortic remodeling, we first examined the association between BP and AAD and aortic diameters by MR analysis (**Table 5**). Significant associations between DBP and AAD, AA and DA diameters were found. For SBP, significant association between SBP and AA diameter were found in weighted median analysis and MR-PRESSO analysis. Marginal significant association between DBP and AAD was found in the CAUSE analysis ($P = 0.049$). Significant association between DBP and AA diameter was found in the CAUSE analysis ($P = 7.48 \times 10^{-4}$) (**Figure 3**). The instrumental variables used in the MR analyses were presented in **Supplementary Table 3**.

We then examined the association between circulating cathepsin B level and AAD and aortic diameters by MR analysis (**Table 4**). Significant association between circulating cathepsin B level and AAD was not found. We found that significant associations between circulating cathepsin B level and AA diameter in weighted median ($P = 8.34 \times 10^{-5}$), IVW ($P = 3.63 \times 10^{-3}$) and MR-PRESSO ($P = 9.38 \times 10^{-3}$) analyses by using the INTERVAL data. We found that nominal associations between circulating cathepsin B level and AA ($P = 0.038$) and DA ($P = 0.067$) diameters in the CAUSE analysis by using the INTERVAL data. The instrumental variables used in the MR analyses were presented in **Supplementary Table 4**.

DISCUSSION

This study searched for potential SCAD-related proteins that may act through BP regulation by integrative analysis of data from AAD, aortic diameters and BP GWAS and pQTL studies. We identified shared genetic variants and circulating proteins for BP and SCAD. Additionally, causal associations between BP and AAD and aortic diameters were detected. Moreover, we found proteins associated with BP and aortic diameters and highlighted circulating cathepsin B that was causally associated with BP and aortic diameters.

Genetic factors play roles in the etiology of SCAD. The findings of genetic association studies provided new biological leads for further mechanistic investigation of arterial pathobiology. In this study, we collected SCAD susceptibility genes in GWAS-identified loci and identified BP-associated SNPs in *JAG1*, *ERI1*, *ULK4*, *THSD4*, *CMIP*, *COL4A2*, *FBN1*, *FAM76B*, *FGGY*, *NUS1*, and *HNF4G*. The shared genetic variants in these genes both disturbed BP regulation and affected SCAD risk. As HTN is a causal risk factor for SCAD, the shared genetic variants may first regulate BP and then affect SCAD risk. The shared genes were related to extracellular matrix components, indicating that the effect of the genetic variants on BP regulation and their effects on SCAD risk may act through extracellular matrix components. The regulatory effects of genetic variants on BP should also be indirect. Therefore, identification of the indirect factors in the regulatory pathway was the key to elucidating the genetic association. Circulating proteins are critical factors implicated in the development of SCAD (25). We found that the shared
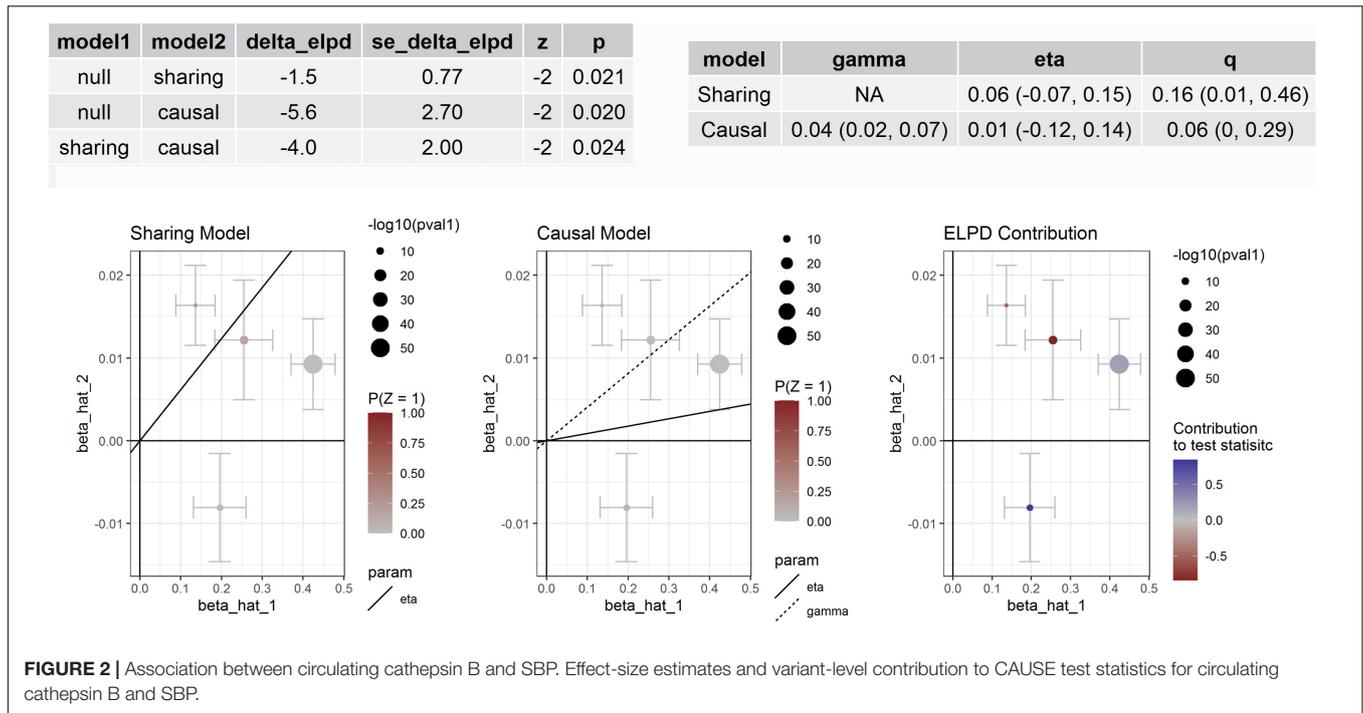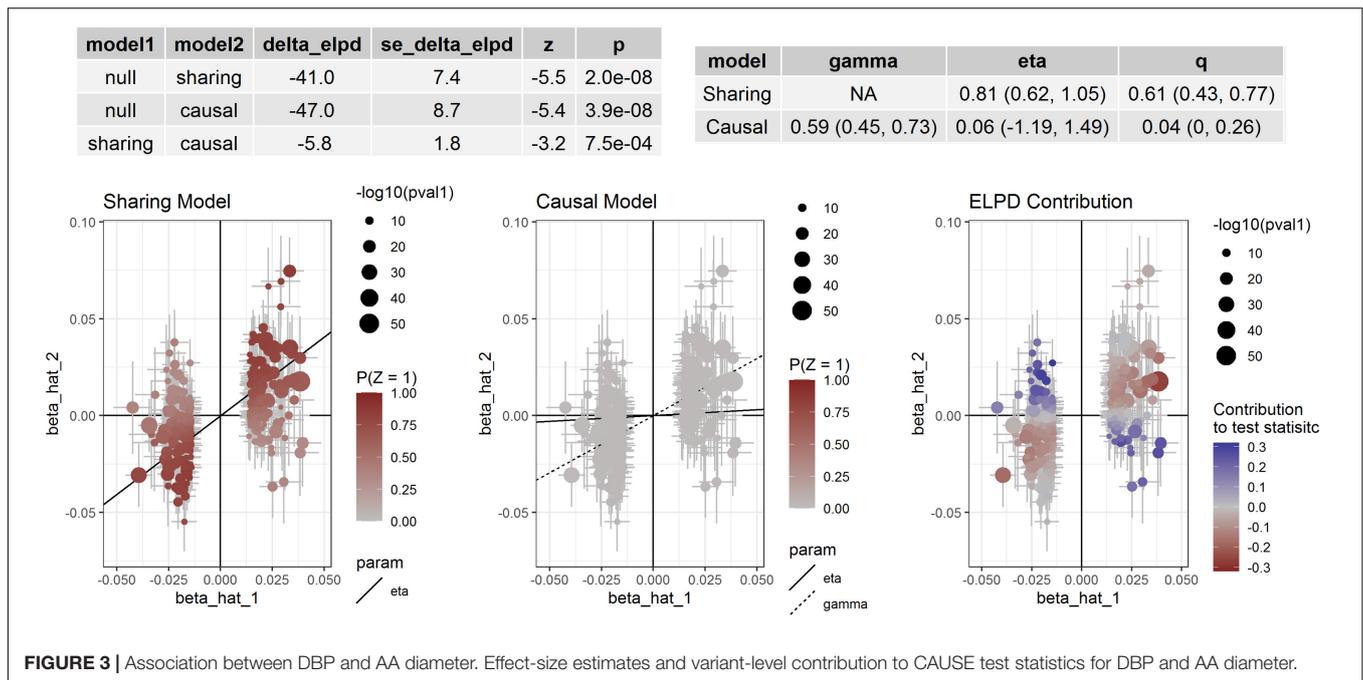
model1	model2	delta_elpd	se_delta_elpd	z	p
null	sharing	-1.5	0.77	-2	0.021
null	causal	-5.6	2.70	-2	0.020
sharing	causal	-4.0	2.00	-2	0.024

model	gamma	eta	q
Sharing	NA	0.06 (-0.07, 0.15)	0.16 (0.01, 0.46)
Causal	0.04 (0.02, 0.07)	0.01 (-0.12, 0.14)	0.06 (0, 0.29)

FIGURE 2 | Association between circulating cathepsin B and SBP. Effect-size estimates and variant-level contribution to CAUSE test statistics for circulating cathepsin B and SBP.

TABLE 5 | The causation between BP and AAD and aortic diameter.

X	Y	Method	Beta	SE	*P*-value
DBP	AAD	Weighted median	0.0028	0.0008	3.83E-04
DBP	AAD	IVW	0.0025	0.0006	1.26E-05
DBP	AAD	MR-Egger	0.0019	0.0020	0.3538
DBP	AAD	MR-PRESSO	0.0023	0.0005	4.99E-05
DBP	AA diameter	Weighted median	0.4463	0.0511	2.41E-18
DBP	AA diameter	IVW	0.4841	0.0560	5.73E-18
DBP	AA diameter	MR-Egger	0.5877	0.1965	2.78E-03
DBP	AA diameter	MR-PRESSO	0.4477	0.0446	8.81E-19
DBP	DA diameter	Weighted median	0.1732	0.0351	8.08E-07
DBP	DA diameter	IVW	0.1413	0.0355	7.03E-05
DBP	DA diameter	MR-Egger	0.0829	0.1143	0.4681
DBP	DA diameter	MR-PRESSO	0.1599	0.0299	1.78E-07
SBP	AAD	Weighted median	0.0010	0.0008	0.1987
SBP	AAD	IVW	0.0005	0.0006	0.4545
SBP	AAD	MR-Egger	0.0028	0.0021	0.1851
SBP	AAD	MR-PRESSO	0.0006	0.0006	0.3399
SBP	AA diameter	Weighted median	0.1634	0.0554	3.16E-03
SBP	AA diameter	IVW	0.0729	0.0734	0.3204
SBP	AA diameter	MR-Egger	0.0480	0.2403	0.8416
SBP	AA diameter	MR-PRESSO	0.1969	0.0498	1.19E-04
SBP	DA diameter	Weighted median	0.0454	0.0473	0.3374
SBP	DA diameter	IVW	−0.0425	0.0574	0.4592
SBP	DA diameter	MR-Egger	−0.1035	0.1878	0.5815
SBP	DA diameter	MR-PRESSO	0.0230	0.0440	0.6018

genetic variants were strongly associated with many circulating proteins and that the associated proteins have specific biological functions. We found *IGHG1, MMP1, MMP10, CTSB, POMC, RETN,* and *IL6R,* which were related to endopeptidase activity, collagen metabolic process and extracellular space. Among these associated protein-coding genes, *MMP1, CTSB, POMC, RETN,* and *IL6R* are known to be associated with SCAD (40, 41) or other cardiovascular diseases (42–44). As circulating proteins are

model1	model2	delta_elpd	se_delta_elpd	z	p
null	sharing	-41.0	7.4	-5.5	2.0e-08
null	causal	-47.0	8.7	-5.4	3.9e-08
sharing	causal	-5.8	1.8	-3.2	7.5e-04

model	gamma	eta	q
Sharing	NA	0.81 (0.62, 1.05)	0.61 (0.43, 0.77)
Causal	0.59 (0.45, 0.73)	0.06 (-1.19, 1.49)	0.04 (0, 0.26)

FIGURE 3 | Association between DBP and AA diameter. Effect-size estimates and variant-level contribution to CAUSE test statistics for DBP and AA diameter.

druggable targets, identification of the regulated proteins in the pathway was helpful for understanding the relationship between BP and SCAD and valuable for SCAD prevention.

CTSB encodes cathepsin B, which is related to the activated TLR4 signaling and degradation of the extracellular matrix pathway and participates in intracellular degradation and turnover of proteins (45). Cathepsin B could be involved in the intracellular processing of prorenin that is locally synthesized or taken up from the extracellular compartment (46). The circulating level of cathepsin B was associated with cardiovascular events and mortality (42). However, the association between circulating levels of cathepsin B and BP or HTN has not been reported. Renin is the key enzyme of the renin-angiotensin system, which is involved in BP regulation. Cathepsin B has been proposed as a prorenin processing enzyme because of its colocalization with renin and its ability to activate prorenin (47–49). In this study, we found that BP-associated SNPs were strongly associated with cathepsin B levels in two independent studies. The association between cathepsin B level and BP, AAD and aortic diameters was identified by several MR analysis methods and was validated by independent data. Therefore, cathepsin B was associated with BP and may be involved in aortic remodeling.

The present study has some potential limitations. First, although we found significant associations between circulating protein levels and BP and aortic diameters, the direct relationships between circulating protein levels and SCAD were not detected. This was because that necessary summary data (e.g., beta and se) of the SCAD GWAS were unavailable and the sample size of the AAD GWAS was small ($n = 1,470$), in which the necessary summary data were available. Second, most of the associations between the identified SNPs and proteins have not been validated in another independent sample because

of lack of data. Finally, the functional relationships have not been validated by technical and biological experiments. Further *in vitro* studies are needed to determine their functions in related cell lines such as vascular smooth muscle cells.

CONCLUSION

In summary, the present study identified genetic variants shared by BP and SCAD and found that the associated SNPs may have strong effects on circulating protein levels. Therefore, this study found circulating proteins that were pleiotropically associated with BP and SCAD. Circulating cathepsin B was associated with BP and aortic diameters and therefore may play important roles in BP regulation and aortic remodeling. The findings suggested that genetic variants of BP genes may have regulatory potential to affect circulating proteins involved in BP regulation and ultimately affect risk of cardiovascular disease.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**.

AUTHOR CONTRIBUTIONS

TC and LC designed this study. TC, XY, and ZQ collected the data. TC was responsible for the statistical analysis. MT and TC wrote the draft. MT, XL, and LC revised this draft. LC

finalized this manuscript. All authors read and approved the final manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcvm.2022.762468/full#supplementary-material

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