

Integrative Genome-wide Association Meta-analysis of Aortic Aneurysm and Dissection Identifies Five Novel Genes

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Abstract

Aortic aneurysm and dissection (AAD) is a multifaceted condition characterized by significant genetic predisposition and a considerable contribution to cardiovascular-related mortality. Previous studies have suggested that AAD subtypes share similar genetic mechanisms; however, these studies investigated the subtypes separately. Here, we performed a large genome-wide association study (GWAS) meta-analysis for AAD by combining its subtypes, including 11,148 cases and 708,468 controls of European ancestry. We identified 24 susceptibility loci, including four novel loci at 1p21.2 (*PALMD*), 2p22.2 (*CRIM1*), 6q22.1 (*FRK*), and 12q14.3 (*HMGA2*), which were partially validated in both internal and external populations. Cell type-specific analysis highlighted the artery as the most relevant tissue where the susceptibility variants may exert their effects in a tissue-specific manner. By using four approaches, we prioritized 53 genes, reinforcing the importance of elastic fiber formation and transforming growth factor-beta (TGF- β) signaling in the formation of AAD, and suggested potential target drugs for the treatment. Additionally, various cardiovascular diseases were genetically correlated with AAD, and several cardiovascular risk factors [e.g., body mass index (BMI), lipid levels, and pulse pressure] showed causal associations with AAD, underscoring their shared genetic structures and mechanisms underlying the comorbidity. Moreover, five prioritized genes (*PALMD*, *CRIM1*, *FRK*, *HMGA2*, and *NT5DC1*) at the novel loci were supported as regulators of smooth muscle and endothelial cell functions through *ex vivo* and *in vitro* experiments. Together, these findings enhance our understanding of the genetic architecture of AAD and provide novel insights into future biological mechanism studies and therapeutic strategies.

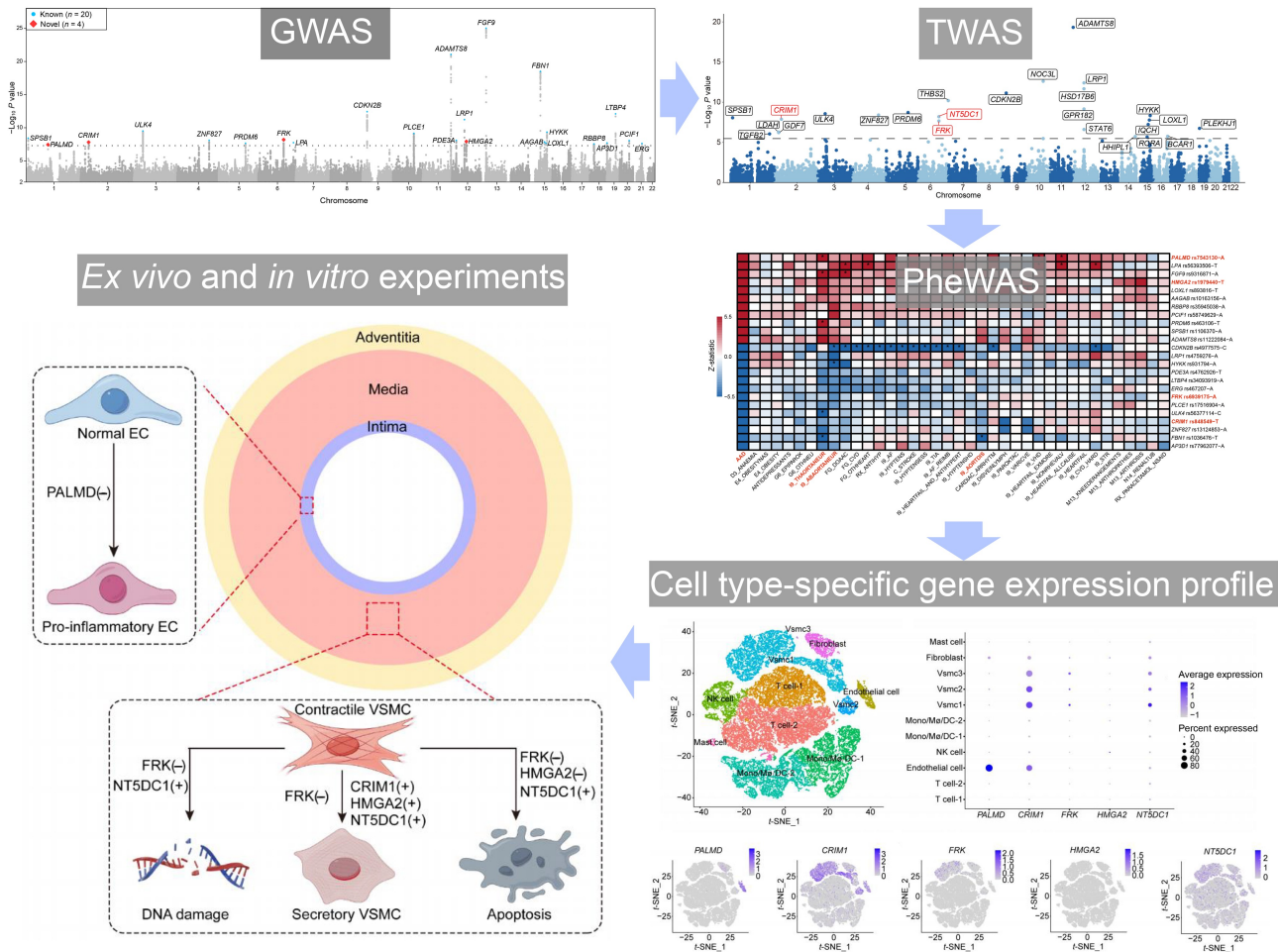
Key words: Genome-wide association study; Aortic aneurysm; Aortic dissection; Susceptibility locus; Integrative study.

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Graphical abstract



Introduction

Aortic aneurysm and dissection (AAD) is a common and lethal disease with no effective cure. Based on the aortic region involved, AAD can be classified as either thoracic AAD (TAAD) or abdominal AAD (AAAD). AAD causes more than 10,000 deaths per year in the United States [1] and 150,000–200,000 deaths per year worldwide [2]. Although no medications are currently effective in preventing AAD progression, these deaths can be reduced by identifying high-risk individuals and applying appropriate management strategies to avoid aneurysm ruptures [3–6]. The development of AAD is influenced by multifactorial etiology, encompassing both genetic predispositions and environmental factors, including smoking, atherosclerosis, and hypertension [6–8]. A family history of AAD could also increase the individual's risk of AAD [7,9]. The shared genetic determinants of AAD across subtypes remain largely unknown.

The origins of smooth muscle cells (SMCs) in the thoracic and abdominal aortas are different, resulting in distinct pathogenesis mechanisms of TAAD and AAAD [6]. Despite these differences, TAAD and AAAD share several common pathological features, including extracellular matrix (ECM) remodeling and SMC loss [7,10]. Additionally, the transforming growth factor-beta (TGF- β) signaling pathway and LRP1

pathway have been identified as potential biological pathways shared by both TAAD and AAAD [7,11]. Nevertheless, each subtype also demonstrates unique pathological characteristics. For instance, lipid metabolism and inflammatory responses are major contributors to AAAD pathogenesis but have minimal impact on TAAD [10–12]. Previous genome-wide association studies (GWASs) have been conducted for AAD mainly in populations of European ancestry; however, TAAD and AAAD were often analyzed separately [11–18]. The latest TAAD GWAS has identified 21 genes for TAAD, of which 7 genes (*TCF7L2*, *LRP1*, *SPSB1*, *ZNF827*, *PRDM6*, *PLCE1*, and *ADAMTS8*) are also associated with the genetic risk of AAAD [11]. Moreover, the largest AAAD GWAS has revealed that 24 AAAD index variants are also associated with TAAD, highlighting a moderate genetic correlation between these two subtypes [11].

To increase sample size and boost the power of GWASs, several studies have combined different histological subtypes. This strategy has enabled the identification of additional susceptibility loci and provided novel insights into the genetic architecture of complex diseases. For example, GWASs on inflammatory bowel disease often include two subtypes, Crohn's disease and ulcerative colitis [19,20]; those on osteoarthritis and venous thromboembolism incorporate subtypes

from different body sites [21–24]; GWAS on stroke includes subtypes of ischemic stroke and hemorrhagic stroke [25]; and pan-cancer GWASs encompass multiple cancer subtypes [26,27]. Furthermore, an increasing amount of tissue-specific and cell type-specific omics data, along with biobank-scale GWAS summary statistics, have become publicly accessible [28–33]. Moreover, lots of state-of-the-art computational approaches have been developed for post-GWAS integrative analyses [34,35]. Previous studies have performed post-GWAS analysis of cell type-specific enrichment and genetic correlation estimation for TAAD and AAAD separately. For instance, risk variants associated with AAAD are enriched in the functional regions of endothelial cells (ECs) and vascular SMCs of abdominal aortic tissue [11], whereas TAAD risk variants are enriched in vascular SMCs and fibroblasts of thoracic aortic tissue [12].

In our study, we performed a comprehensive GWAS for AAD by combining TAAD, AAAD, and aortic aneurysm of unspecified sites to uncover additional novel mechanistic candidate loci. We then integrated multiple omics datasets and phenotypic traits to identify relevant cell types, prioritized putative genes, and explored the genetic relationships

between AAD and other traits. We further validated the prioritized genes at the novel loci by *ex vivo* and *in vitro* experiments.

Results

GWAS meta-analysis identifies four novel loci

To enhance the understanding of AAD's genetic architecture, we performed a GWAS meta-analysis involving 11,148 cases and 708,468 controls, utilizing European-ancestry data from the UK Biobank (UKB) [32] and the FinnGen study [36] (Figure S1). Comprehensive details on GWAS quality control, sample size, and phenotype definitions are provided in the “Materials and methods” section. The liability heritability was 0.102 [standard error of the mean (SEM) = 0.010] when setting the population prevalence to the sample prevalence of 1.5%.

By using the clumping approach and stepwise conditional analysis, we identified independent signals ($P < 5 \times 10^{-8}$) at 24 loci (Figure 1A; Table 1), including 20 previously identified loci for AAD, AAD histological subtypes of AAAD and TAAD, coronary artery aneurysm and dissection, and brain aneurysm.

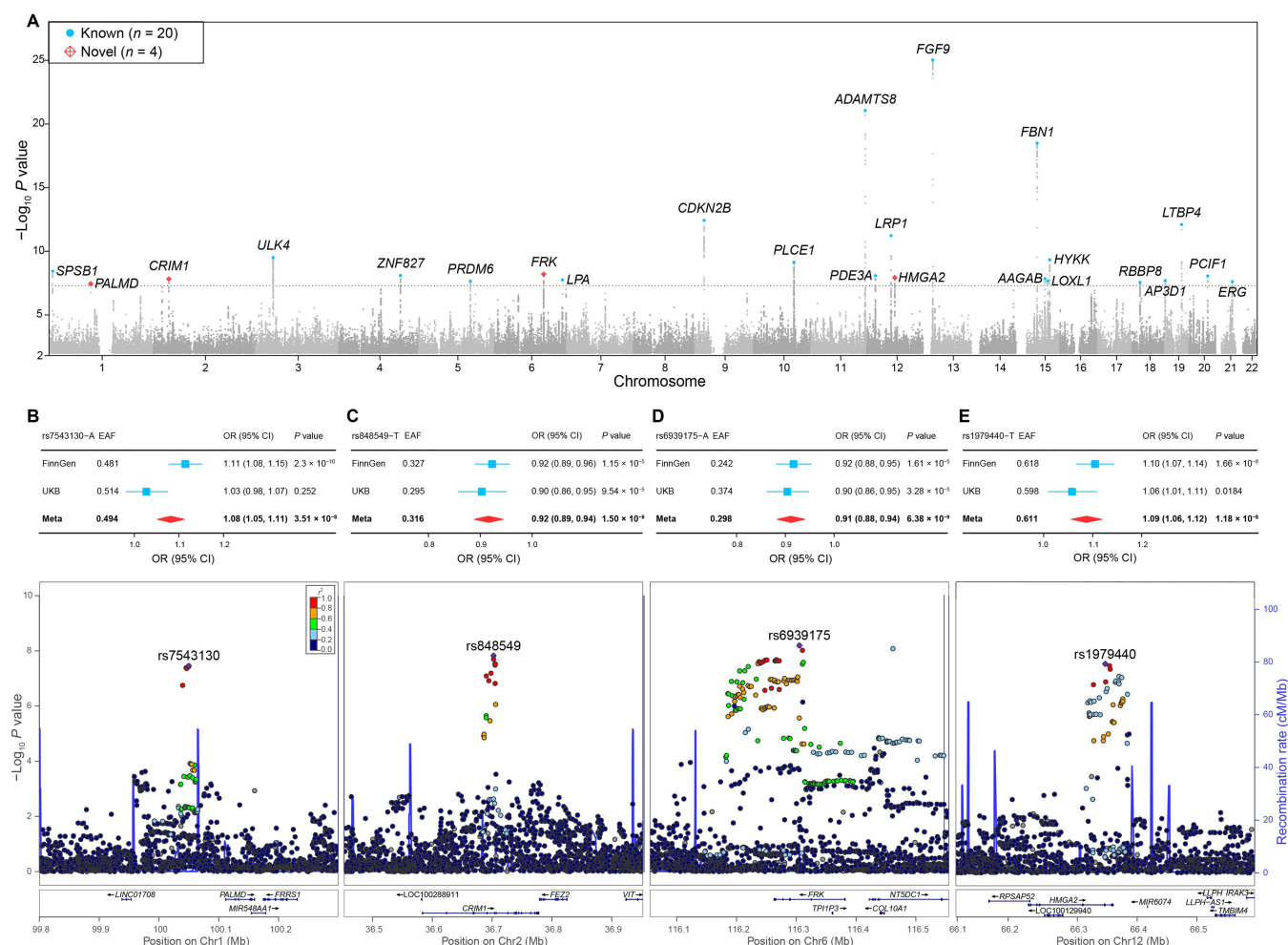


Figure 1 GWAS meta-analysis of AAD in 719,616 individuals

A. Manhattan plot displaying the P values of variants. The gray dashed line marks the genome-wide significance threshold of 5×10^{-8} . Top variants in the known loci are shown in blue, while those in novel loci are highlighted in red. **B–E.** Forest plots and regional plots for the novel loci *PALMD* at 1p21.2 (B), *CRIM1* at 2p22.2 (C), *FRK* at 6q22.1 (D), and *HMGA2* at 12q14.3 (E). In the forest plots, blue square represents the effect estimation in each study, and red diamond represents the effect estimation in the meta-analysis. GWAS, genome-wide association study; OR, odds ratio; CI, confidence interval; Chr, chromosome; EAF, effect allele frequency; AAD, aortic aneurysm and dissection; UKB, UK Biobank.

Table 1 Twenty-four loci identified in the genome-wide meta-analysis of AAD

Locus	SNP	Position	Gene	EA/NEA EAF		UK Biobank		FinnGen study		Meta	
				EA/NEA	EAF	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
1	rs1106370	1:9443340	SPSB1	A/G	0.49	1.08 (1.03–1.13)	8.52×10^{-4}	1.09 (1.06–1.13)	3.01×10^{-7}	1.09 (1.06–1.12)	3.70×10^{-9}
2	rs7543130	1:100049785	PALMD	A/C	0.49	1.03 (0.98–1.07)	2.52×10^{-1}	1.11 (1.08–1.15)	2.30×10^{-10}	1.08 (1.05–1.11)	3.51×10^{-8}
3	rs848549	2:36702389	CRIM1	T/C	0.32	0.9 (0.86–0.95)	9.54×10^{-5}	0.92 (0.89–0.96)	1.15×10^{-5}	0.92 (0.89–0.94)	1.50×10^{-8}
4	rs56377114	3:41869475	ULK4	C/G	0.81	0.89 (0.84–0.95)	2.00×10^{-4}	0.89 (0.86–0.93)	9.23×10^{-8}	0.89 (0.86–0.93)	3.07×10^{-10}
5	rs13124853	4:146784774	ZNF827	A/G	0.59	0.92 (0.88–0.96)	4.64×10^{-4}	0.92 (0.89–0.95)	1.40×10^{-6}	0.92 (0.89–0.95)	7.98×10^{-9}
6	rs463106	5:122502567	PRDM6	T/C	0.53	1.06 (1.01–1.11)	1.67×10^{-2}	1.10 (1.06–1.14)	4.68×10^{-8}	1.08 (1.05–1.11)	2.17×10^{-8}
7	rs6939175	6:116305183	FRK	A/G	0.30	0.9 (0.86–0.95)	3.28×10^{-5}	0.92 (0.88–0.95)	1.61×10^{-5}	0.91 (0.88–0.94)	6.38×10^{-9}
8	rs56393506	6:161089307	LPA	T/C	0.18	1.15 (1.08–1.22)	6.16×10^{-6}	1.09 (1.04–1.14)	1.16×10^{-4}	1.11 (1.07–1.15)	1.77×10^{-8}
	rs140570886	6:161013013		T/C	0.99	0.74 (0.63–0.87)	3.05×10^{-4}	0.72 (0.61–0.85)	8.43×10^{-5}	0.73 (0.65–0.82)	$2.19 \times 10^{-7*}$
9	rs4977575	9:22124744	CDKN2B	C/G	0.55	0.89 (0.85–0.93)	2.80×10^{-7}	0.91 (0.88–0.94)	3.83×10^{-8}	0.90 (0.88–0.93)	3.75×10^{-13}
10	rs17516904	10:96067242	PLCE1	A/G	0.16	0.89 (0.83–0.95)	4.76×10^{-4}	0.88 (0.84–0.92)	9.50×10^{-8}	0.89 (0.85–0.92)	7.28×10^{-10}
	rs10736085	10:95893052		A/T	0.54	0.94 (0.9–0.98)	5.99×10^{-3}	0.91 (0.88–0.94)	9.20×10^{-8}	0.92 (0.90–0.95)	$1.10 \times 10^{-8*}$
11	rs11222084	11:130273230	ADAMTS8	A/T	0.65	1.18 (1.13–1.24)	4.23×10^{-12}	1.14 (1.10–1.18)	9.75×10^{-13}	1.16 (1.12–1.19)	8.55×10^{-22}
12	rs4762926	12:20244126	PDE3A	T/G	0.25	0.91 (0.86–0.96)	2.00×10^{-4}	0.91 (0.87–0.95)	3.44×10^{-6}	0.91 (0.88–0.94)	8.11×10^{-9}
13	rs4759276	12:57526646	LRP1	A/G	0.38	0.89 (0.85–0.94)	3.89×10^{-6}	0.91 (0.88–0.94)	6.38×10^{-8}	0.90 (0.88–0.93)	5.82×10^{-12}
14	rs1979440	12:66346624	HMG2	T/C	0.61	1.06 (1.01–1.11)	1.84×10^{-2}	1.10 (1.07–1.14)	1.66×10^{-8}	1.09 (1.06–1.12)	1.18×10^{-8}
15	rs9316871	13:22861921	FGF9	A/G	0.79	1.22 (1.15–1.29)	1.84×10^{-11}	1.2 (1.15–1.25)	1.93×10^{-17}	1.21 (1.17–1.25)	9.57×10^{-26}
16	rs1036476	15:48914775	FBN1	T/C	0.92	0.79 (0.74–0.85)	4.31×10^{-11}	0.81 (0.76–0.86)	1.12×10^{-10}	0.80 (0.76–0.84)	3.15×10^{-19}
17	rs10163156	15:67503637	AAGAB	A/G	0.42	1.09 (1.04–1.14)	3.32×10^{-4}	1.08 (1.05–1.12)	3.72×10^{-6}	1.08 (1.05–1.12)	1.41×10^{-8}
18	rs893816	15:74228464	LOXLI	T/C	0.33	1.07 (1.02–1.12)	6.91×10^{-3}	1.10 (1.06–1.14)	1.63×10^{-7}	1.09 (1.06–1.12)	2.15×10^{-8}
19	rs931794	15:78826180	HYKK	A/G	0.66	0.92 (0.87–0.96)	2.99×10^{-4}	0.91 (0.88–0.94)	9.26×10^{-8}	0.91 (0.89–0.94)	4.62×10^{-10}
20	rs35945038	18:20245248	RBP8	A/G	0.32	1.10 (1.05–1.16)	5.23×10^{-5}	1.08 (1.04–1.12)	4.02×10^{-5}	1.09 (1.06–1.12)	2.83×10^{-8}
21	rs77962077	19:2109480	AP3D1	A/G	0.07	0.87 (0.77–1.00)	4.38×10^{-2}	0.83 (0.78–0.89)	3.33×10^{-8}	0.84 (0.79–0.89)	2.01×10^{-8}
22	rs34093919	19:41117300	LTBP4	A/G	0.02	0.62 (0.48–0.80)	2.22×10^{-4}	0.64 (0.56–0.74)	1.15×10^{-10}	0.64 (0.56–0.72)	7.96×10^{-13}
23	rs58749629	20:44571317	PCIF1	A/G	0.14	1.18 (1.11–1.26)	5.04×10^{-8}	1.08 (1.03–1.13)	1.64×10^{-3}	1.12 (1.08–1.17)	8.80×10^{-9}
24	rs467207	21:399996082	ERG	A/G	0.25	0.93 (0.88–0.99)	1.54×10^{-2}	0.90 (0.87–0.93)	7.86×10^{-8}	0.91 (0.88–0.94)	2.40×10^{-8}

Note: The lead SNP with the lowest *P* value and any conditionally significant SNPs in each locus are presented. The gene corresponds to the nearest protein-coding gene to the lead SNP. *, the conditional *P* values are 3.25×10^{-8} for rs140570886 and 1.69×10^{-11} for rs10736085. The novel loci are shown in bold. SNP, single nucleotide polymorphism; EA, effect allele; NEA, non-effect allele; EAF, effect allele frequency; OR, odds ratio; CI, confidence interval; AAD, aortic aneurysm and dissection.

Four novel loci were *PALMD* at 1p21.2 ($P = 3.51 \times 10^{-8}$ for rs7543130), *CRIM1* at 2p22.2 ($P = 1.5 \times 10^{-8}$ for rs848549), *FRK* at 6q22.1 ($P = 6.38 \times 10^{-9}$ for rs6939175), and *HMGA2* at 12q14.3 ($P = 1.8 \times 10^{-8}$ for rs1979440) (Figure 1B–E). The internal validation study for the novel loci in UKB and the FinnGen study showed that all lead variants had consistent effect directions and met the nominal significance threshold ($P < 0.05$), with the exception of rs7543130 at *PALMD* ($P = 0.252$ in UKB) (Figure 1B–E). For external validation study, we evaluated these loci in the BioBank of Japan (BBJ) and Michigan Genomics Initiative (MGI). All lead variants exhibited consistent effect directions except for rs1979440 at *HMGA2* (File S1; Figure S2).

Relevant tissues for AAD by partitioning the heritability

We used stratified linkage disequilibrium (LD) score regression (LDSC) to pinpoint the relevant tissues or cell types based on their specific gene expression and histone modification marks. The specific gene expression profiles from the aorta artery showed the most significant enrichment for the GWAS risk variants ($P = 9.29 \times 10^{-8}$) (Figure 2A). Other arteries, including the tibial and coronary arteries, also exhibited significant enrichment ($P < 0.0002$). Interestingly, we observed enrichment signals in the myometrium, uterus, and cartilage. In addition, the specific histone marks in four cell types (lung, aorta artery, osteoblast, and adipose nuclei) were significantly associated with AAD ($P < 0.0002$) (Figure 2B). Actually, osteoblasts are nearly indistinguishable from fibroblasts during cell culture [37]. The main components of myometrium, uterus, cartilage, and lung are SMCs or fibroblasts, both of which play critical roles in AAD development. Adipose can also contribute to the progression of AAD.

Gene prioritization for AAD

For the identification of AAD candidate genes, we employed four approaches and prioritized 53 distinct genes, including 24 genes based on their proximity to the lead variants (Table 1), 7 genes identified through significant nonsynonymous variants (Table S1), 29 genes from artery-specific expression quantitative trait locus (eQTL) mapping (Table S2), and 25 genes from artery-specific transcriptome-wide association study (TWAS) (Table S3). Among these genes, *ULK4*, *ADAMTS8*, and *LOXL1* were identified by four approaches, and *CRIM1*, *ZNF827*, *PRDM6*, *FRK*, *LRP1*, and *NOC3L* were prioritized by three approaches (Table S4). Although the artery-specific TWAS prioritized seven genes (*TGFB2*, *GDF7*, *LDAH*, *THBS2*, *HHLPL1*, *RORA*, and *BCAR1*) outside the susceptibility loci identified in our GWAS-meta analysis (Figure 2C), these genes were located at the known loci for AAD, AAAD, or brain aneurysm in the previous studies [11,16,17,38,39].

At the novel loci, both the artery-specific TWAS and eQTL mapping approaches prioritized another gene, *NT5DC1* at 6q22.1, where the gene nearest to the lead variant is *FRK*. At the novel locus 2p22.2, *CRIM1* was the unique prioritized gene by approaches of position mapping, artery-specific TWAS, and eQTL mapping. Notably, the GWAS signals and eQTLs for *CRIM1*, *FRK*, and *NT5DC1* were colocalized with a posterior probability of colocalization $H_4 > 0.8$ (Table S3). In addition, *PALMD* and *HMGA2* were unique genes only prioritized by position mapping at 1p21.2 and 12q14.3, respectively. To sum up, five genes (*PALMD*, *CRIM1*, *FRK*, *HMGA2*, and *NT5DC1*) were prioritized at

these four novel loci. These genes were moderately or highly expressed in bulk tissues, such as artery, fibroblast, and adipose (Figure S3). In the high-resolution cell types identified by single-cell RNA sequencing (scRNA-seq) analysis of thoracic aorta specimens, *PALMD* was highly expressed in the ECs; *CRIM1* and *NT5DC1* were highly expressed in the SMCs; *FRK* was moderately expressed in SMCs (Figure 2D–J). Meanwhile, in human abdominal aorta specimens (GSE224587), we observed similar expression patterns for these novel genes (Figure S4). Additionally, the functional annotations and drug targets for these prioritized genes are detailed in File S1, Figure S5, and Tables S5 and S6.

Functional characterization of five genes at the novel loci

We evaluated the protein localization and expression of five prioritized genes at the novel loci in the aorta of AAD patients and healthy controls. *PALMD* was predominantly expressed in the intima of healthy controls. *FRK*, *HMGA2*, and *CRIM1* were highly expressed in the SMCs of AAD patients, whereas *NT5DC1* was highly expressed in the SMCs of healthy controls (Figure 3).

Human umbilical vein endothelial cells (HUVECs) were transfected with a small interfering RNA (siRNA) targeting *PALMD* or a negative control siRNA (Figure 4A). Endothelial dysfunction initiates vascular remodeling and inflammation, attracting immune cells to the medial layer, which is associated with the p38 and p65 signaling pathways and endothelial adhesion molecules (VCAM-1 and ICAM-1) [40]. Activation of p38 MAPK and p65 NF- κ B leads to increased downstream inflammatory cytokine synthesis and plays a critical role in AAD formation [41,42]. *PALMD* knockdown in HUVECs exacerbated angiotensin II (Ang II)-induced increases in phosphorylated p65 (p-p65) and phosphorylated p38 (p-p38) levels (Figure 4A). Since downregulation of endothelial adhesion molecules (VCAM-1 and ICAM-1) has been found to attenuate Ang II-induced AAD progression [40,43], we also assessed their expression. Immunofluorescence staining revealed elevated levels of ICAM-1 and VCAM-1 in Ang II-challenged HUVECs with *PALMD* knockdown (Figure 4B, Figure S6).

Phenotypic switching and loss are significant characteristics of SMCs in AAD. Additionally, DNA damage is also commonly observed in AAD. In our *in vitro* experiments, we transfected human/mouse aortic SMCs with siRNAs targeting *CRIM1*, *Frk*, *Hmga2*, or *Nt5dc1*, followed by H_2O_2 challenge, and assessed the outcomes using Western blot (WB; for SM22- α , calponin, γ -H2AX, cleaved-caspase-3) and flow cytometry (FC). The results showed that contractile markers (SM22- α and calponin) were downregulated in challenged SMCs with *Frk* knockdown, but upregulated in challenged SMCs with *Hmga2*, *Nt5dc1*, or *CRIM1* knockdown. γ -H2AX levels were increased in challenged SMCs with *Frk* knockdown, but decreased with *Nt5dc1* knockdown. No remarkable change of γ -H2AX occurred in challenged SMCs with *CRIM1* or *Hmga2* knockdown. Moreover, apoptosis in H_2O_2 -challenged SMCs was aggravated by *Frk* or *Hmga2* knockdown, alleviated by *Nt5dc1* knockdown, and unaffected by *CRIM1* silencing (Figure 4C–I, Figure S6).

Genetic correlations with other diseases

Phenome-wide genetic correlations were assessed to investigate the genetic overlap and similarities between AAD and

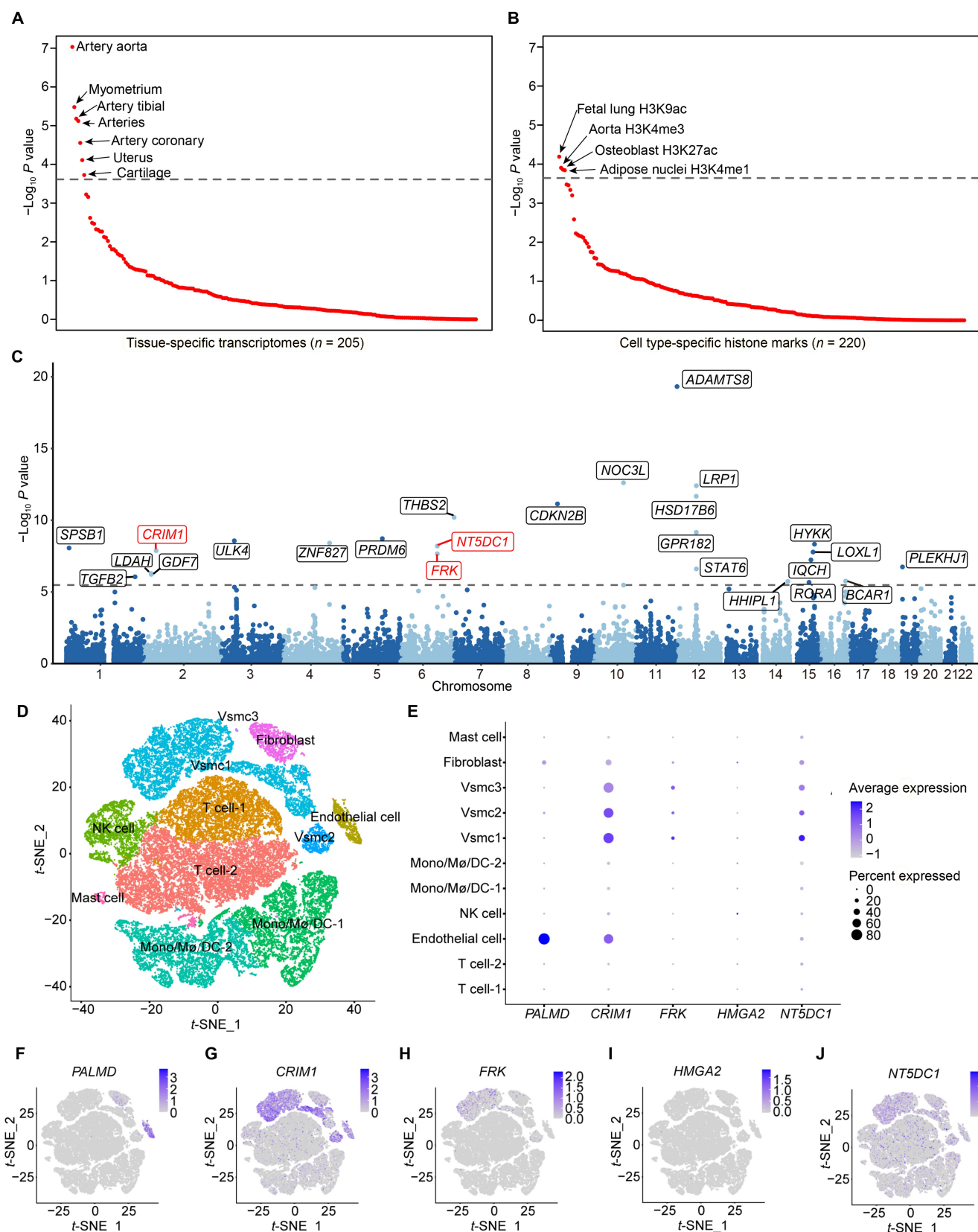


Figure 2 Relevant tissues and cell types for AAD

A. Enrichment analysis of relevant tissues based on gene expression in 205 tissues from human, mouse, and rat. The dashed line indicates the Bonferroni-corrected significance level at 0.0002. **B.** Enrichment analysis of relevant cell types based on histone marks in 220 cell types from human. The dashed line indicates the Bonferroni-corrected significance level at 0.0002. **C.** Manhattan plot for the P values of genes in artery-specific TWAS. The dashed line indicates the transcriptome-wide significance level of 3.32×10^{-6} . The genes in red mean those near the newly discovered loci. **D.** Cell type landscape in human sporadic type A aortic dissection based on scRNA-seq. **E.** Dot plot for five prioritized genes at the novel loci based on gene expression in different cell types. **F–J.** Relative expression of *PALMD* (F), *CRIM1* (G), *FRK* (H), *HMGA2* (I), and *NT5DC1* (J) in different cell types. Vsmc, vascular smooth muscle cell; Mono/Mφ/DC, monocyte/macrophage/dendritic cell; NK, natural killer; scRNA-seq, single-cell RNA sequencing; TWAS, transcriptome-wide association study; t -SNE, t -distributed stochastic neighbor embedding.

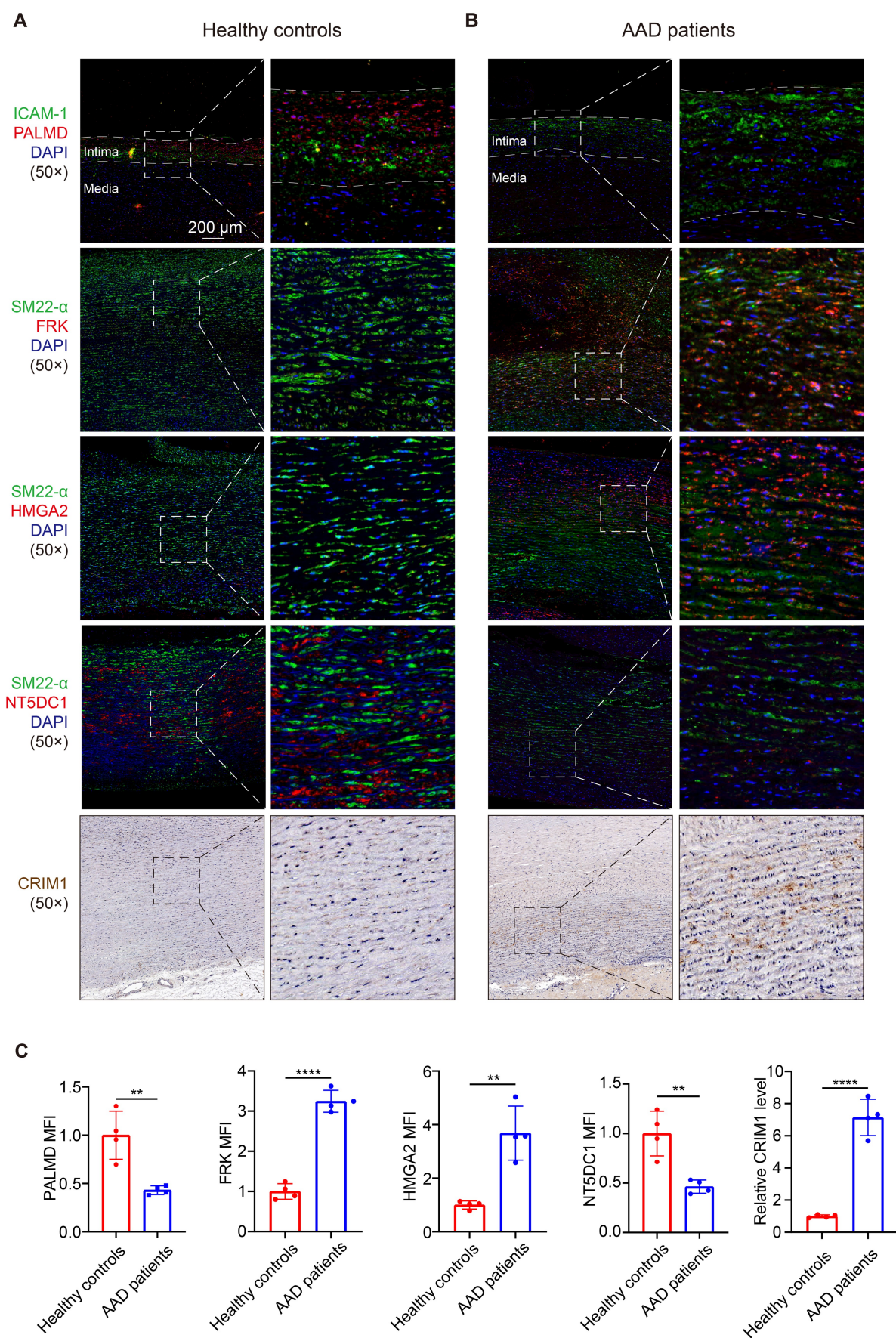
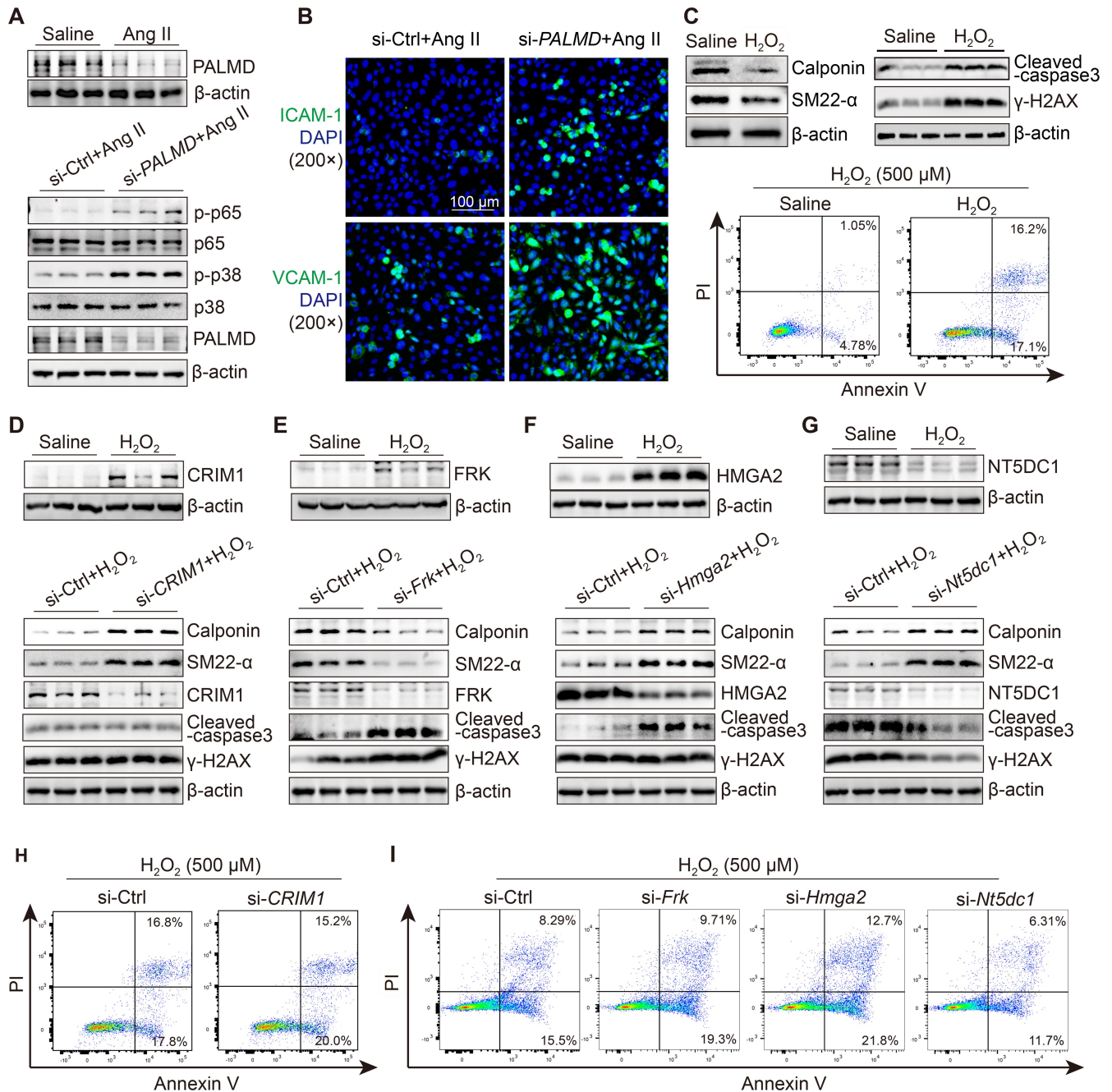


Figure 3 Localization and expression of prioritized genes in the aorta

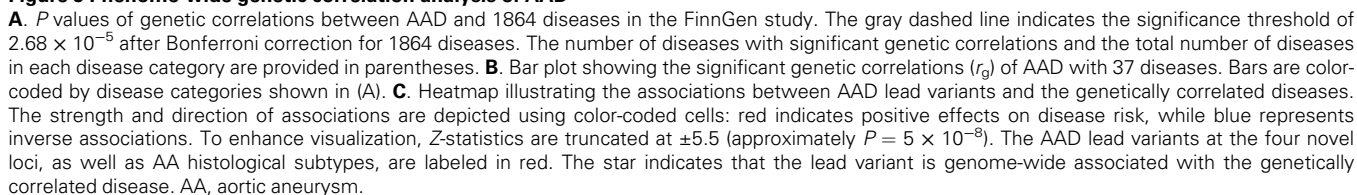
Representative images of aortic sections from healthy controls (**A**) and AAD patients (**B**) showing the localization of PALMD, FRK, HMGA2, NT5DC1, and CRIM1. Scale bar, 200 μ m. **C**. Quantitative analysis of protein levels in healthy controls and AAD patients. Data are represented as mean $\pm$ SE ($n = 4$). Statistical significance was determined by Student's *t*-test (**, $P < 0.01$; ****, $P < 0.0001$). MFI, mean fluorescence intensity; DAPI, 4',6-diamidino-2-phenylindole; SE, standard error.



1864 diseases in the FinnGen study. After Bonferroni correction ($P < 2.68 \times 10^{-5}$), ADD showed significant genetic correlations with 37 diseases, ranging from 0.18 to 0.93 (Figure 5A and B; Table S7). Multiple cardiovascular diseases exhibited significant genetic correlations with AAD, and several cardiovascular risk factors [e.g., body mass index (BMI), lipid levels, and pulse pressure] showed causal associations with AAD (File S1; Figures S7 and S8). AAD showed strong genetic correlations with its histological subtypes: thoracic aortic aneurysm (I9_THAORTANEUR, $r_g = 0.93$), dissection of

aorta (I9_AORTDIS, $r_g = 0.93$), and abdominal aortic aneurysm (I9_ABAORTANEUR, $r_g = 0.85$).

The lead variants associated with AAD exhibited consistent effect directions at 635 loci (71.5%) across the genetically correlated diseases (Figure 5C; Table S8). For the four novel loci, the effect directions of the lead variants in *PALMD*, *HMGA2*, *FRK*, and *CRIM1* for AAD subtypes (i.e., TAAD, AAAD, and dissection of aorta) were “+++”, “---”, “--+”, and “+++”, respectively. In addition, in 57 of the 60 sets of results (95%), the lead variants for



significantly associated with thoracic aortic aneurysm (I9_THAORTANEUR, $P = 2 \times 10^{-10}$), valvular heart disease excluding rheumatic fever (I9_VHD, $P = 1 \times 10^{-20}$), and non-rheumatic valve diseases (I9_NONRHEVALV, $P = 8.20 \times 10^{-21}$) in the FinnGen study. Moreover, the lead

variant rs4977575 at *CDKN2B* showed the greatest number ($n = 14$) of genome-wide significant associations with these genetically correlated diseases (Figure 5C).

Discussion

We performed a comprehensive GWAS meta-analysis for AAD and identified 24 susceptibility loci, including four novel ones. For the four novel loci, rs7543130 is an intergenic variant located near the *PALMD* gene, whereas rs848549, rs6939175, and rs1979440 are intronic variants in *CRIM1*, *FRK*, and *HMG2A*, respectively. Cell type-specific analysis highlighted artery as the most relevant tissue where AAD-associated genes likely exert their effects. Our integrative analysis prioritized 53 genes, including five genes located at the novel loci. In addition, various cardiovascular diseases were genetically correlated with AAD, and several cardiovascular risk factors showed causal associations with AAD.

Our discovery GWAS meta-analysis included two large populations of European ancestry from UKB and the FinnGen study. Different from the previous GWASs that separately studied TAAD and AAAD [11–18], we combined all histological subtypes of AAD as cases, a strategy that has been proven effective in identifying more loci and generating novel insights into other diseases [19–27]. Most of the lead variants within the identified susceptibility loci exhibited consistent effect directions across cardiovascular diseases, particularly among AAD histological subtypes, suggesting shared mechanisms. We validated the novel loci in both internal and external populations, with the primary focus on the effect directions of the lead variants. The signals at two novel loci, *PALMD* and *CRIM1*, were relatively robust in the internal and external populations, and consistent across AAD histological subtypes. However, in the meta-analysis of AAD susceptibility loci based on the external populations from BBJ and MGI, the lead variant rs1979440 at the novel loci *HMG2A* was the unique variant showing an opposite effect direction. Similarly, variants at *FRK* displayed consistent effects in thoracic and abdominal aortic aneurysms, but exhibited an opposite effect direction in aortic dissection. In summary, our GWAS meta-analysis identified four novel loci that were partially validated across populations and correlated diseases, though further replication in larger and more diverse populations is required.

As expected, AAD signals exhibited enrichment in functional regions and genes highly expressed in arteries, as well as in tissues abundant with SMCs, fibroblasts, or adipose tissue — all of which are relevant to the histological subtypes of AAD, including TAAD and AAAD [11,12]. Based on position mapping and tissue-specific annotation, five genes (*PALMD*, *CRIM1*, *FRK*, *HMG2A*, and *NT5DC1*) were prioritized at the novel loci. *PALMD* is highly expressed in the ECs of the cardiovascular system [44]. *PALMD* deficiency may promote the development of calcific aortic valve disease by activating NF- κ B signaling in valvular ECs [45]. Additionally, *PALMD* variants have been associated with aortic root size and aortic valve stenosis [46–48]. We showed that the knockdown of *PALMD* resulted in significantly elevated pro-inflammatory signals in ECs. Based on these findings, we speculate that *PALMD* plays a crucial role in maintaining EC homeostasis and may be involved in AAD pathogenesis. *CRIM1* encodes a transmembrane protein that anchors growth factors, such as vascular endothelial growth

factor A, to the cell surface [49]. Furthermore, *CRIM1* is involved in maintaining endothelial integrity and has a critical role in cardiovascular system development. *Crim1* deficiency causes primary defects in the extraglomerular vascular system in mice [50]. We demonstrated that knockdown of *CRIM1* in SMCs could increase the expression of SM22- α and calponin, promoting the contractile phenotype of SMCs, which could protect against the formation of AAD. However, *CRIM1* appears to have opposing effects in SMCs and ECs, suggesting a complex role in AAD progression. Further *in vivo* studies are necessary to determine its overall effect. *FRK* encodes a member of the SRC family of kinases that stabilizes PTEN and BRCA1 via tyrosine phosphorylation [51]. Additionally, it has been shown to inhibit the proliferation of human glioblastoma cells by reducing cyclin D1 nuclear accumulation [52]. Therefore, *FRK* may also regulate arterial SMCs proliferation. The variants in *FRK* are associated with apolipoprotein B and C-reactive protein, both of which are linked to coronary heart disease [53,54]. Our study found that *Frk* knockdown inhibited the contractile phenotype of SMCs and activated SMC apoptosis and DNA damage. Thus, *FRK* may act as a protective factor against aortic degeneration. *HMG2A* is an architectural transcription factor involved in DNA damage repair and stem cell self-renewal [55,56], potentially mitigating SMC loss and associated local inflammation — both essential for stabilizing aortic structure. The variants in *HMG2A* also showed associations with aortic root size [57] and cardiovascular risk factors of blood pressure and BMI [58,59]. Our experiment indicated that the knockdown of *Hmga2* could simultaneously promote SMC contractile phenotype and apoptosis, indicating its complex role in AAD pathophysiology. The function of *NT5DC1* is less well studied. It has been implicated in susceptibility to chronic obstructive pulmonary disease and as a marker for pancreatic adenocarcinoma [60,61]. In our study, *Nt5dc1* knockdown in SMCs significantly increased SM22- α and calponin expression, while downregulating proteins associated with DNA damage and apoptosis, suggesting that *NT5DC1* may aggravate AAD formation through its effects on SMCs.

In the phenome-wide genetic correlation analysis, AAD demonstrated significant positive genetic correlations with 37 other diseases, predominantly cardiovascular diseases. The moderate-to-strong genetic correlations with cardiovascular diseases emphasize their shared genetic architecture and mechanisms underlying comorbidities. Traditional cardiovascular risk factors, including low-density lipoprotein (LDL), total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), pulse pressure (PP), and BMI, were found to be causally associated with AAD. In any case, as the first study to comprehensively investigate the causal cardiovascular risk factors for AAD, we have identified several causal exposures that may either serve as common risk factors for various AAD subtypes or act as the predominant risk factors for specific AAD subtypes.

Several limitations warrant mention. First, although we tried to validate the novel loci in the internal and external populations, as well as AAD histological subtypes, we mainly focused on the sign of effect allele in the replication studies. These novel loci could not all be fully validated under a strict Bonferroni-corrected P value threshold. To provide more evidence to support the novel loci, we prioritized five genes by multi-omics integrative study and further conducted *ex vivo* and *in vitro* experiments to explore their potential genetic

mechanisms at the novel loci. Second, while we proposed a list of potential drug targets for AAD treatment, we did not perform drug intervention experiments to validate the effects of these potential drugs due to limited experimental technology. As a proxy approach, we performed drug–target Mendelian randomization (MR) analysis, and successfully highlighted the significant potential of LPA as a treatment target for AAD. However, we failed to identify instrumental variables for other proteins, which prevented us from evaluating their potential as AAD targets. Third, we found that several common cardiovascular risk factors, namely BMI, lipids, and pulse pressure, showed causal associations with AAD. However, these cardiovascular risk factors are correlated, and their causal relationships may be mediated. Conducting mediation analyses across these risk factors and multiple AAD subtypes will comprehensively clarify the regulatory network.

In conclusion, this large-scale integrative genome-wide analysis of various AAD subtypes has provided novel insights into the genetic architecture and mechanism for AAD. Our study has developed a comprehensive atlas of candidate genes, relevant tissues and cell types, and pathways implicated in AAD formation. Furthermore, we have identified a list of potential drug targets for AAD and investigated its associations with other diseases and traits.

Materials and methods

UKB genotyping and quality control

The UKB cohort is a large population-based cohort comprising approximately 500,000 individuals with extensive phenotypic and genetic data. Detailed protocols of sample processing, genotyping, and quality control have been described elsewhere [32]. In this study, we used the imputed genotypes of 15,456,785 variants in 378,474 European-ancestry individuals, obtained from our previous work [62]. The UKB Application No. for this study is 88159.

GWAS on AAD in UKB

The AAD phenotype in UKB was determined based on the first occurrence of any code mapped to the 3-character International Classification of Diseases 10th revision (ICD-10; category ID 1712 in UKB), which was achieved by integrating death register records, primary care data, hospital inpatient records, and self-reported medical conditions collected during the baseline assessments or subsequent visits to the UKB assessment center. AAD cases were defined as individuals diagnosed with AAD, corresponding to ICD-10 code I71, and the remaining participants were controls after further removing those with several circulatory system diseases [*i.e.*, atherosclerosis (I70), other aneurysm (I72), other peripheral vascular diseases (I73), arterial embolism and thrombosis (I74), other disorders of arteries and arterioles (I77), diseases of capillaries and disorders of arteries (I78), and arterioles and capillaries in diseases classified elsewhere (I79)], following the approach used in the FinnGen study. After quality control, as of July 2022, 3753 UKB participants of European ancestry were classified as cases, while 358,929 individuals of the same ancestry served as controls. Logistic regression implemented in PLINK [63] was applied to detect the AAD-associated variants with adjustments for sex, array batch, and the top 10 principal components (PCs) of genetic ancestry.

GWAS summary statistics for AAD from the FinnGen study

Summary statistics for AAD in the FinnGen study (data freeze 9, April 2022, code I9_AORTANEUR) are publicly available at <https://r9.finnngen.fi/>. In the FinnGen study, AAD cases were defined by ICD-10 code I71, with the exclusion of dissection of unspecified site of aorta (I71.0), resulting in 7395 cases. The remaining samples were controls ($n = 349,539$) after further removing those with atherosclerosis (I70), other aneurysm (I72), other peripheral vascular diseases (I73), arterial embolism and thrombosis (I74), other disorders of arteries and arterioles (I77), diseases of capillaries and disorders of arteries (I78), and arterioles and capillaries in diseases classified elsewhere (I79). The GWASs in the FinnGen study were conducted using Regenie [64], adjusting for sex, age, the top 10 PCs, and genotyping batch.

GWAS meta-analysis

The inverse-variance weighted meta-analysis using the fixed effect model in METAL [65] was performed, with genomic control correction applied to each input study. A total of 9,797,427 variants common to both UKB and FinnGen were included for the subsequent analysis. Variants reaching genome-wide significance ($P < 5 \times 10^{-8}$) were identified. To define the independent loci for the associated variants, we first performed clumping to select the independent lead variant at each locus by setting LD r^2 less than 0.1 within a 10-Mb region in PLINK [63]. The LD was calculated based on 1000 randomly selected European samples from UKB. Second, we merged adjacent lead variants that were located within 1000 kb into one locus. A total of 24 independent loci were identified, and the corresponding nearest genes were mapped. Furthermore, stepwise conditional analysis was conducted with genome-wide complex trait analysis (GCTA) [66] based on the aforementioned LD reference panel to detect additional significant signals within a locus.

Previously reported loci of AAA, TAAD, coronary artery aneurysm and dissection, and brain aneurysm in the GWAS Catalog (as of September 2023) [33] and the latest studies [11,12,67] were downloaded. Loci that were significant in our GWAS meta-analysis were designated as novel only if they met both of the following criteria: (1) they did not pass the genome-wide significance threshold in the GWAS Catalog and latest publications, and (2) their lead variant was located over 1000 kb away from any previously reported index variant in the GWAS Catalog and latest publications.

Tissue and cell type identification

LDSC [68] was used to assess whether AAD heritability was enriched in tissue-specific or cell type-specific functional annotations. These included 220 cell type-specific genomic functional regions defined by histone marks [69] and 205 tissue-specific gene expression sets [68]. A total of 53 non-tissue-specific baseline annotations [*e.g.*, coding, 3' untranslated region (UTR), 5' UTR, promoter, and intron] [68,69] were included in the LDSC model to minimize confounding factors and enhance cell type-specific signals. LD scores were calculated based on the genotypes of Europeans from the 1000 Genomes Project [69] and downloaded from <https://alkesgroup.broadinstitute.org/LDSCORE/>. For cell type-specific and subsequent phenome-wide genetic correlation analyses, only HapMap 3 single nucleotide polymorphisms

(SNPs) were used, with SNPs in the major histocompatibility complex (MHC) region excluded. Bonferroni correction was applied to account for multiple testing.

Candidate gene mapping and prioritization

Four approaches were used to map variants to protein-coding genes, including position mapping by the lead variant, nonsynonymous variant mapping, artery-specific eQTL mapping based on Functional Mapping and Annotation (FUMA; <https://fuma.ctglab.nl>) [70], and artery-specific TWAS and colocalization analysis with the functional summary-based imputation (FUSION) pipeline [71] (more details in File S1). Previous studies reported that the heritability of TAAD and AAAD was mainly enriched in aortic tissue, and accordingly performed TWAS and eQTL mapping on TAAD and AAAD using aortic data, respectively [11,12]. To identify more candidate genes, we conducted eQTL mapping and TWAS using data from aorta artery, coronary artery, and tibial artery from the Genotype-Tissue Expression (GTEx) project (v8), respectively. The results from these three arteries were then combined to boost the statistical power.

Cell type prioritization for genes at novel loci based on scRNA-seq data

To pinpoint cell types where the five prioritized genes (*PALMD*, *CRIM1*, *FRK*, *HMGA2*, and *NT5DC1*) at the novel loci might influence AAD risk, we utilized scRNA-seq datasets of thoracic aorta specimens (GSE155468) and abdominal aorta specimens (GSE224587) from TAAD/AAAD cases and controls. We reanalyzed these scRNA-seq datasets using Seurat (v4) [72]. Briefly, dimensionality reduction was conducted using *t*-distributed stochastic neighbor embedding (*t*-SNE), and cluster-defining genes were identified with the FindAllMarkers function. Feature plots, dot plots, and heatmaps were generated via Seurat (v4) [72].

Immunofluorescence and immunohistochemistry analysis of aorta arteries

We obtained freshly isolated aortic tissues from the consenting heart transplant recipients or AAD patients. The aorta samples were fixed in 4% paraformaldehyde and paraffin-embedded. Sections were cut, dewaxed, antigen-retrieved, blocked with donkey serum, and incubated overnight with primary antibodies. The slides were washed with phosphate-buffered saline with Tween 20 (PBST) and incubated with suitable secondary antibodies. Antibodies used are listed in File S1.

EC inflammation assay for *PALMD*

Confluent HUVECs were transfected with si-*PALMD* at a final concentration of 37.5 nM for 24 h. Meanwhile, the control group was transfected with the negative control siRNA. The medium was then changed, and HUVECs were stimulated with Ang II (1 μ M) for 24 h. After treatments, the protein levels of p-p65, p-p38, and *PALMD* were detected by WB and immunofluorescence staining. Detailed siRNA sequences are provided in Table S9, and antibodies used are listed in File S1.

SMC functional assays for *CRIM1*, *Frk*, *Hmga2*, and *Nt5dc1*

Human aortic SMCs were transfected with si-*CRIM1*, while mouse aortic SMCs were transfected with si-*Frk*, si-*Hmga2*, or si-*Nt5dc1*, at a final concentration of 37.5 nM for 24 h. Meanwhile, the control group was transfected with the negative control siRNA. The medium was then changed, and SMCs were stimulated with H₂O₂ (500 μ M) for 24 h. After treatments, the protein levels of SM22- α , calponin, cleaved-caspase3, γ -H2AX, *CRIM1*, *FRK*, *HMGA2*, and *NT5DC1* were detected by WB. The apoptotic proportions of challenged SMCs were detected using FC. Detailed siRNA sequences are provided in Table S9, and antibodies used are listed in File S1.

Phenome-wide genetic correlation analysis

We obtained the GWAS summary statistics for 2269 binary and 3 quantitative endpoints from FinnGen (data freeze 9, April 2022). Genetic correlations between AAD and these endpoints were calculated using cross-trait LDSC [73]. This method employs a weighted linear model to regress the product of Z-statistics for disease pairs on the LD scores of SNPs across the genome, providing unbiased estimates of genetic correlations for disease pairs, even when sample overlap exists between studies. We successfully estimated genetic correlations for 1864 pairs, and determined the significant correlations using a Bonferroni-correlated *P* value threshold of 2.68×10^{-5} . Additionally, the Z-statistics of lead variants at the AAD-associated loci were extracted from the correlated diseases for further comparison.

Ethical statement

The UKB study protocols were granted by the North West Multi-center Research Ethics Committee (Approval No. 11/NW/0382). This project was approved by the Ethics Committee of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, China (Approval No. UHCT-IEC-SOP-016-03-01).

Data availability

The GWAS meta-analysis summary statistics for AAD have been deposited in the Open Archive for Miscellaneous Data [74] at the National Genomics Data Center (NGDC), China National Center for Bioinformation (CNCB) (OMIX: OMIX007191), and are publicly accessible at <https://ngdc.cncb.ac.cn/omix>.

CRedit author statement

Yifan Du: Conceptualization, Data curation, Investigation, Software, Writing – original draft. **Yunlong Guan:** Investigation, Software, Writing – original draft. **Zhonghe Shao:** Data curation, Investigation, Software, Writing – original draft. **Minghui Jiang:** Data curation, Investigation, Software. **Minghan Qu:** Investigation, Methodology. **Yifan Kong:** Investigation, Methodology. **Hongji Wu:** Investigation, Validation. **Da Luo:** Investigation, Validation. **Shu Peng:** Investigation, Validation. **Si Li:** Investigation, Visualization. **Xi Cao:** Investigation, Visualization. **Jing Chen:** Conceptualization, Resources, Writing – review & editing. **Ping Ye:** Conceptualization, Resources, Writing – review & editing.

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Competing interests

The authors have declared no competing interests.

Supplementary material

Supplementary material is available at *Genomics, Proteomics & Bioinformatics* online (<https://doi.org/10.1093/gpbjnl/qzaf039>).

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