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TRANSLATIONAL RESEARCH
Genetics

Dyslipidemia, inflammation, calcification, and adiposity in aortic stenosis: a genome-wide study

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Abstract

Aims Although highly heritable, the genetic etiology of calcific aortic stenosis (AS) remains incompletely understood. The aim of this study was to discover novel genetic contributors to AS and to integrate functional, expression, and cross-phenotype data to identify mechanisms of AS.

Methods and results A genome-wide meta-analysis of 11.6 million variants in 10 cohorts involving 653 867 European ancestry participants (13 765 cases) was performed. Seventeen loci were associated with AS at $P \leq 5 \times 10^{-8}$, of which 15 replicated in an independent cohort of 90 828 participants (7111 cases), including *CELSR2–SORT1*, *NLRP6*, and *SMC2*. A genetic risk score comprised of the index variants was associated with AS [odds ratio (OR) per standard deviation, 1.31; 95% confidence interval (CI), 1.26–1.35; $P = 2.7 \times 10^{-51}$] and aortic valve calcium (OR per standard deviation, 1.22; 95% CI, 1.08–1.37; $P = 1.4 \times 10^{-3}$), after adjustment for known risk factors. A phenome-wide association study indicated multiple associations with coronary artery disease, apolipoprotein B, and triglycerides. Mendelian randomization supported a causal role for apolipoprotein B-containing lipoprotein particles in AS (OR per g/L of apolipoprotein B, 3.85; 95% CI, 2.90–5.12; $P = 2.1 \times 10^{-20}$) and replicated previous findings of causality for lipoprotein(a) (OR per natural logarithm, 1.20; 95% CI, 1.17–1.23; $P = 4.8 \times 10^{-73}$) and body mass index (OR per kg/m^2, 1.07; 95% CI, 1.05–1.9; $P = 1.9 \times 10^{-12}$). Colocalization analyses using the GTEx database identified a role for differential expression of the genes *LPA*, *SORT1*, *ACTR2*, *NOTCH4*, *IL6R*, and *FADS*.

Conclusion Dyslipidemia, inflammation, calcification, and adiposity play important roles in the etiology of AS, implicating novel treatments and prevention strategies.

Structured Graphical Abstract

Key Question

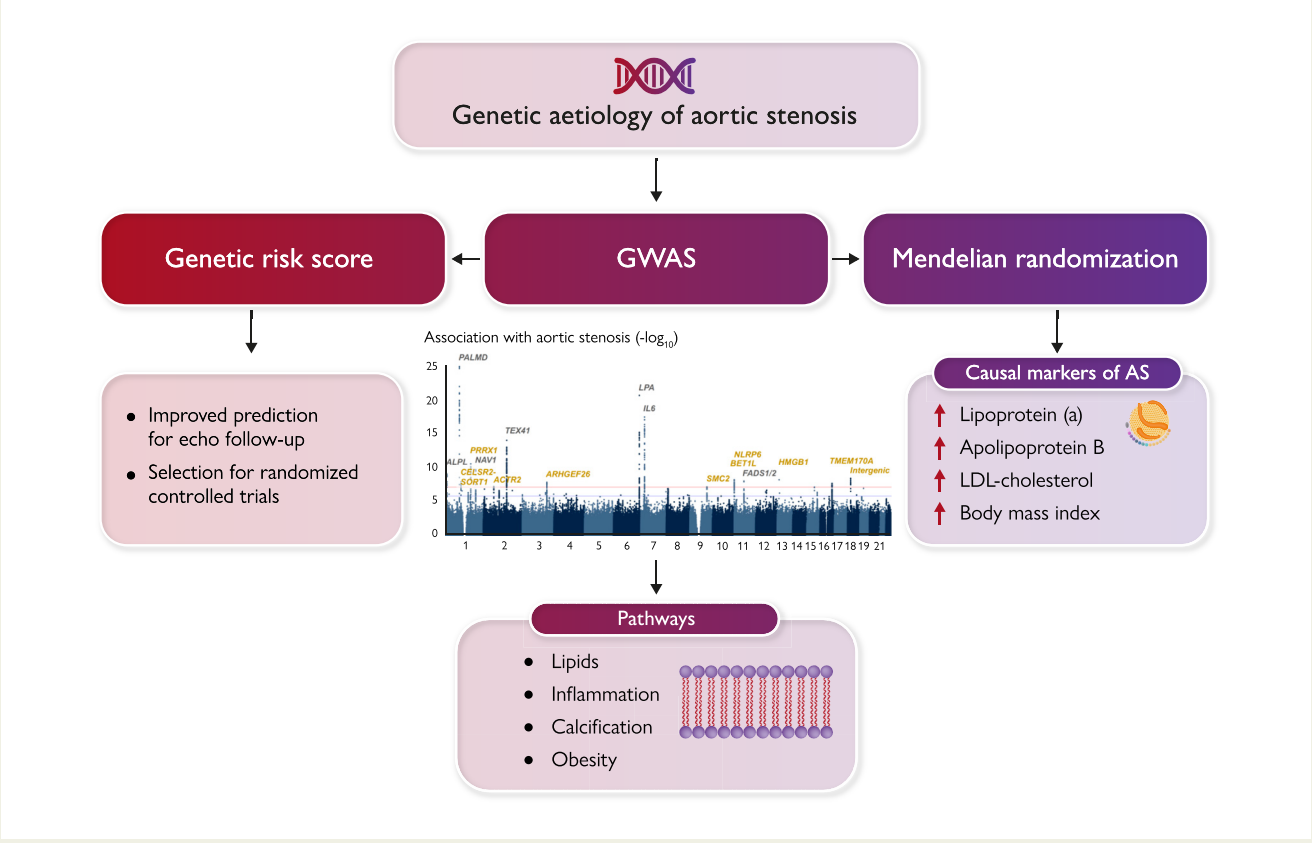
What genes and biological pathways play key roles in the aetiology of aortic stenosis (AS)?

Key Finding

Genetic variation that contributes to aortic stenosis was found to impact lipids, inflammation, calcification, and obesity.

Take Home Message

The identification of specific genes and pathways increases our understanding of the disease processes that result in aortic stenosis, and may eventually lead to the identification of high-risk patients and novel therapeutic approaches.



Genetic etiology of aortic stenosis. This study meta-analyzed 13 765 AS cases vs. 640 102 controls and confirmed 15 genetic loci associated with AS. Downstream analyses implicated additional candidate genes involved in dyslipidemia, inflammation, calcification, and adiposity. The Manhattan plot shows variants with $P \geq 1 \times 10^{-25}$, for improved visualization. Genetic loci in grey were previously identified, and those in gold are new discoveries. Abbreviations: AS, aortic stenosis; GWAS, genome-wide association study; LDL, low-density lipoprotein.

Keywords Aortic stenosis • Genome-wide association study • Mendelian randomization • Phenome-wide association study • Gene expression • Genetic risk score

Translational perspective

This large-scale genetic study of calcific aortic stenosis (AS) in 653 867 European ancestry participants (13 765 cases) identified 15 robustly replicated genetic loci, including *SORT1–CELSR2*, involved in lipid metabolism, and *NLRP6*, involved in the inflammatory response. We provided evidence in favor of a causal association for apolipoprotein B, lipoprotein(a), body mass index, and low-density lipoprotein cholesterol. A genetic risk score including all identified loci was associated with both AS and aortic valve calcium and improved the classification of AS when added to risk factors. The differential expression of several genes in relevant tissues highlights their role in the etiology of AS. Together, these findings provide candidates for therapeutic targeting and highlight the use of a genetic risk score to improve clinical risk assessment.

Introduction

Calcific aortic stenosis (AS) is the leading form of incident valvular heart disease in high-income populations.[1] In individuals over 75 years, the prevalence of AS is 10%–15% but is expected to more than double by 2040.[2] Although a replacement of the aortic valve is effective for severe AS cases,[3,4] there are no treatments to prevent progression to valve replacement. Furthermore, it remains unclear which patients are at high risk of a severe prognosis.

A genetic component contributes to the etiology of AS, as siblings of AS patients have more than four-fold the risk of AS.[5] Several families have also been identified with many affected members, including 1 extended family with 48 cases of severe AS.[6] Previous genome- and transcriptome-wide association efforts have identified seven genetic loci associated with AS,[7–11] including *LPA*[7] [which codes for the apolipoprotein(a) moiety of lipoprotein(a)], *IL6*[10] (which codes for interleukin-6), and the *FADS1/2* gene cluster[11] (which codes for desaturases involved in fatty acid biosynthesis). Additionally, Mendelian randomization studies supported a causal contribution of low-density lipoprotein cholesterol (LDL-C),[12] non-high-density lipoprotein cholesterol,[9] lipoprotein(a),[13] arachidonic acid[11], and body mass index (BMI)[14] to AS, suggesting susceptibility to AS is partially mediated by lipid metabolism and inflammation.

Identifying additional genetic loci for AS could provide novel targets for therapeutic intervention and improve risk stratification. Accordingly, we combined genome-wide association study (GWAS) results from 10 cohorts to identify novel loci. We conducted functional analyses for significant variants, examined their association with biomarkers and other diseases, assessed cross-ancestry transferability of variants, and developed an AS genetic risk score to assess its association with diagnosed AS. Finally, we investigated whether individual genes or gene sets were predicted to be differentially expressed in AS cases.

Methods

Cohorts and case definition

The cohorts included in the meta-analysis are listed in *Table 1*. A full description of the cohorts and their case definition for identifying AS is in the Supplementary material online.

Genome-wide meta-analysis for aortic stenosis

We performed centralized, cohort-specific quality control of genome-wide summary statistics for prevalent AS from 10 cohorts totaling 653 867 European ancestry participants (13 765 cases) (*Table 1*). With the exception of the Malmö Diet and Cancer Study, which excluded variants with >5% genotype missingness, minor allele frequency ≤1%, and Hardy–Weinberg equilibrium test $P \geq 1 \times 10^{-4}$, and deCODE, which excluded variants not found in the Haplotype Reference Consortium version r1.1 panel, quality control used unified criteria. For all cohorts, we included bi-allelic variants with non-ambiguous strands (i.e. no C/G or A/T allele pairs), imputation quality score ≥0.3, and minor allele frequency ≥0.001 and whose associations with AS had standard errors less than or equal to the median standard error plus five times the interquartile range, calculated from all summary statistics from each cohort. Using PLINK version 1.9, we performed inverse variance-weighted, fixed-effects meta-analysis for 11 591 806 variants with summary statistics that passed quality control and the allele frequency threshold in at least two cohorts. For independent ($r^2 \leq 0.01$) and genome-wide significant ($P \leq 5 \times 10^{-8}$) variants, i.e. index variants and variants in high linkage disequilibrium (LD) ($r^2 \geq 0.95$) in European ancestry individuals of the Genetic Epidemiology Research on Adult Health and Aging (GERA) cohort or the 1000 Genomes Project Phase 3,[15] we used the University of California Santa Cruz[16] GRCh37 assembly for genomic location.

Among 90 828 participants (7111 AS cases) from the Copenhagen Hospital Biobank or the Danish Blood Donor Study, we evaluated replication of the association with AS for all index variants. Associations were modeled using logistic regression adjusted for age, sex, and 10 principal components, with a $P \leq 0.05$ considered as replication. For all index variants, we also performed inverse variance-weighted, fixed-effects meta-analysis of summary statistics from the discovery and replication cohorts. For additional cohort details, see the Supplementary material online.

Region, gene-based, and functional analysis of variants

We used Annotate Variation (ANNOVAR)[17] to extract predicted function and pathogenicity of variants, including scores generated by Combined Annotation Dependent Depletion (CADD),[18] Deleterious Annotation of genetic variants using Neural Networks (DANN),[19] Linear INSIGHT (LINSIGHT),[20] Eigen-Principal Component (EIGEN-PC),[21] and Functional Analysis Through Hidden Markov Models-Multiple Kernel Learning (FATHMM-MKL) non-coding.[22] From Genotype-Tissue Expression Project (GTEx) version 8,[23] we identified significant expression quantitative trait loci in the aorta, left ventricle, liver, and whole blood. To identify gene regions associated with AS, we employed the single-nucleotide polymorphism (SNP)-wise mean approach of Multi-marker Analysis of GenoMic Annotation (MAGMA)[24] to test for association. We employed MetaXcan[25] and coloc[26] to identify variants whose effects on AS may be mediated by gene expression. We also applied the Genotype Imputed Gene Set Enrichment Analysis (GIGSEA)[27] approach, to assess whether sets of genes with shared function or regulation demonstrate differential predicted expression. We examined gene sets defined by the Kyoto Encyclopedia of Genes and Genomes (KEGG),[28] genes in the same pathway; Gene Ontology (GO),[29] genes with related functions; Functional ANnoTation Of the Mammalian genome version 5 (FANTOM5),[30] genes with shared transcription factor binding sites; and miRBase,[31] genes with the same microRNA seed sequence in their 3' untranslated region.

Genetic risk scores

We constructed three separate genetic risk scores. The first two used PLINK version 2.0[32] with weights estimated from a meta-analysis excluding the UK Biobank: a GRS_{18}, using all 18 genome-wide significant index variants from the discovery analysis, as well as a GRS_{559} including the 559 variants at $P < 1 \times 10^{-4}$. We assessed the association of GRS_{18} and GRS_{559} with AS in 220 159 unrelated White British participants in the UK Biobank aged 55 years or older (3091 cases) (see Supplementary material online) using a logistic regression model adjusted for age[2] and sex and then further adjusted for diabetes, LDL-C, systolic blood pressure, smoking (ever/never), BMI, and coronary artery disease (CAD). The UK Biobank participants in these and subsequent analyses differed slightly from the discovery analysis as an updated version of the data became available. In addition, we performed a polygenic risk score (PRS) analysis with LDpred2[33] in 244 641 UK Biobank participants (3410 cases). We also assessed the association of all the risk scores with the presence of aortic valve calcium (AVC) in the Multi-Ethnic Study of Atherosclerosis (MESA), using logistic regression adjusted for age and sex and then fully adjusted for fasting glucose, LDL-C, systolic blood pressure, smoking (ever/never), BMI, and coronary artery calcium. The PLINK-derived risk scores used 17 and 550 variants as neither variants nor proxies were available for some SNPs in this dataset. For the GRS_{17} and GRS_{550} risk scores, 2440 unrelated European participants (381 cases of AVC >0) were analyzed. The PRS analysis with LDpred2 included 2205 unrelated European participants (355 cases with AVC >0) of MESA. The area under the receiver operating characteristic curve (AUC) for the null hypothesis of no risk score was compared to other models using DeLong's test for two correlated ROC curves from the pROC R package.

Table 1 **Cohorts in the genome-wide meta-analysis**

Cohort	Country	Aortic stenosis definition	No. of cases	No. controls	Median age (Quartile 1, Quartile 3)	Genotyping array	Imputation reference panel	No. of variants
Vanderbilt University Biobank	USA	her	759	7555	68 (62, 75)	Illumina Multi-Ethnic Genotyping Array	HRC version r1.1	10 689 407
CAVS-France1	France	Echocardiography	1261	1305	75 (7079)[a]	Affymetrix Axiom Genome-Wide CEU-1 Array	HRC version r1.1	10 395 306
CAVS-France2	France	Echocardiography	1495	2707	77 (70, 83)[a]	Affymetrix Axiom Genome-Wide Precision Medicine Research Array	HRC version r1.1	10 031 533
CAVS-France3	France	Echocardiography	367	2519	74 (67,79)[a]	Affymetrix Axiom Genome-Wide Precision Medicine Research Array	HRC version r1.1	9 884 426
deCODE	Iceland	her	2464	351 068	77 (68, 83)[a]	Illumina Chips	Icelandic reference panel by deCODE Genetics	9 812 907
GERA	USA	EHR	3469	51 723	66 (61, 74)	Affymetrix Axiom Genome-Wide EUR	HRC version r1.1	10 578 354
Malmö Diet and Cancer Study	Sweden	her	464	4878	58 (53, 63)	Illumina Human Omni Express Exome BeadChip	HRC version r1.1	6 130 056
Penn Medicine Biobank	USA	EHR	1593	4550	71 (62, 79)	Illumina Quad Omni Genotyping Chip	HRC version r.1.1	11 016 108
UK Biobank[b]	UK	her	1675	213 361	63 (59, 66)	Affymetrix UK Biobank Axiom Array	HRC version r1.1 and UK10K + 1000 Genomes Project Phase 3	9 927 329
Umeå University	Sweden	Surgery	218	436	60 (60, 66)	Affymetrix UK Biobank Axiom Array r3	HRC version r1.1	8 810 694

[a]In cases only.
[b]Provided are the number of cases and controls in the genome-wide association study for aortic stenosis. For analyses performed using UK Biobank data for the polygenic risk scores, phenome-wide association study, and Mendelian randomization, an updated dataset consisting of 2213 cases and 255 018 controls was used. See the UK Biobank cohort description for more details.
Abbreviations: CAVS, calcific aortic valve stenosis; EHR, electronic health records; EUR, European; GERA, Genetic Epidemiology Research on Adult Health and Aging; HRC, Haplotype Reference Consortium.

Cross-ancestry and cross-phenotype associations

In the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium, we extracted summary statistics[7] for the association of the index variants with prevalent AVC in an inverse variance-weighted fixed-effects meta-analysis of three independent cohorts totaling 6942 European participants (2245 cases), adjusting for age and sex. Variants that were not available were replaced with one in high LD ($r^2 \geq 0.8$) when available.

To examine the association of index variants with AS in other ancestries, we analyzed 1917 African American participants (86 cases) and 3482 Latin American participants (159 cases) of the GERA cohort, adjusted for age[2] and sex.

We performed a phenome-wide association study of the index variants with 58 diseases, serum biomarkers, and physiological measurements, among 257 231 unrelated White British UK Biobank participants aged 55 years or older. Disease cases were identified using hospital diagnosis codes, procedure codes, and causes of death. Levels of alkaline phosphatase, C-reactive protein, gamma-glutamyl transferase, lipoprotein(a), and triglycerides were natural logarithm transformed. Logistic and linear regression models were adjusted for age[2] and sex, except for breast cancer (analyzed only in women). A false discovery rate correction of 5% was applied across phenotypes for each variant tested.

For six traits associated with multiple variants in the phenome-wide association study, we performed two-sample Mendelian randomization to assess the causal contribution to AS. We performed a GWAS for each trait in unrelated UK Biobank White British participants (up to 383 533 participants). We constructed a genetic instrument for each biomarker using genome-wide significant variants ($331 \leq n \leq 702$) which were independent ($r^2 \leq 0.01$) with imputation quality ≥ 0.3 and minor allele frequency ≥ 0.001. We used the R package MendelianRandomization version 0.4.2[34] to estimate the inverse variance-weighted association with AS, using summary statistics from our discovery meta-analysis. The summary statistics for AS were generated with meta-analysis results that did not include UK Biobank so that instruments and outcomes were from non-overlapping cohorts. In secondary analyses, we also applied the contamination mixture, penalized weighted median, and Egger approaches.

Correlation and conditional analyses

We estimated the genetic correlation of AS with 157 cardiovascular biomarkers, risk factors, and diseases using the LD score regression method[35] as implemented on LD Hub.[36] We selected GWAS or meta-analyses which had been performed in European populations and applied a 5% false discovery rate correction.

To identify additional variants associated with AS, we used the conditional and joint analysis method[37] from the Genome-wide Complex Trait Analysis (GCTA) software[38] to re-estimate the summary statistics from our genome-wide meta-analysis conditioned upon the index variants. Variants not genome-wide significant in the original meta-analysis but which (i) became genome-wide significant in the conditional analysis and (ii) were independent ($r^2 \leq 0.01$) would be deemed to be novel associations.

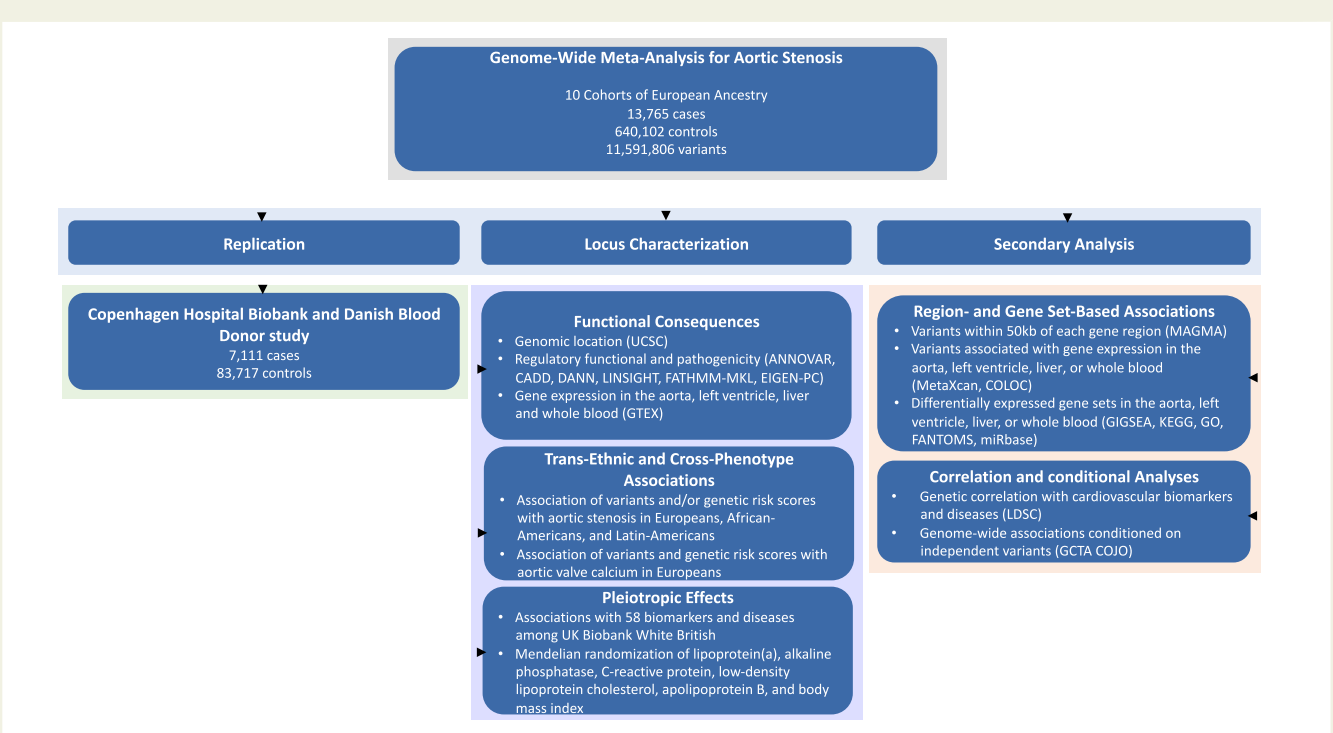


Figure 1 Design of the genome-wide meta-analysis and follow-up analyses. Abbreviations: ANNOVAR, Annotate Variation; CADD, Combined Annotation Dependent Depletion; DANN, Deleterious Annotation of genetic variants using Neural Networks; EIGEN-PC, Eigen-Principal Component; FANTOM5, Functional Annotation of the Mammalian Genome version 5; FATHMM-MKL, Functional Analysis Through Hidden Markov Models-Multiple Kernel Learning; GCTA COJO, Genome-wide Complex Trait Analysis Conditional and Joint Association Analysis; GIGSEA, Genotype Imputed Gene Set Enrichment Analysis; GO, Gene Ontology; GTEx, Genotype-Tissue Expression project; KEGG, Kyoto Encyclopedia of Genes and Genomics; LDSC, Linkage Disequilibrium Score Regression; LINSIGHT, Linear INSIGHT; MAGMA, Multi-marker Analysis of Genomic Annotation; UCSC, University of California Santa Cruz Genome Browser.

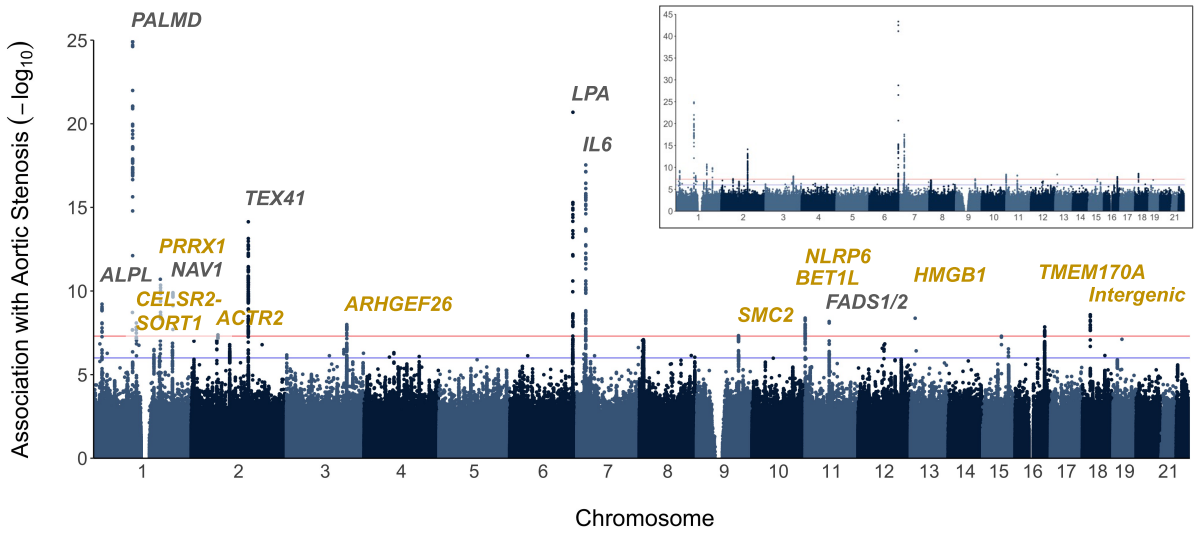


Figure 2 *P* for the association of 11 591 806 variants with aortic stenosis in the meta-analysis. The inset shows all associations, while the main plot shows variants with $P \geq 1 \times 10^{-25}$, for improved visualization. Genetic loci in grey were previously identified and those in gold are new discoveries.

Results

Genome-wide meta-analysis identifies 10 novel loci for aortic stenosis

We performed a genome-wide meta-analysis for AS using summary statistics from 10 European ancestry cohorts totaling 653 867 participants (13 765 cases) (*Table 1*). Estimates for each of 11 591 806 variants were combined in an inverse variance-weighted, fixed-effects model (see *Figure 1* for study design overview). We observed no evidence of strongly inflated test statistics in the meta-analysis (genomic inflation factor [λ] = 1.04 and LD score regression intercept = 1.020; Supplementary material online, *Figure S1*).

We identified 17 genetic loci containing one or more independent variants ($r^2 \leq 0.01$) which exceeded a genome-wide significance threshold ($P \leq 5 \times 10^{-8}$) for association with AS (*Figure 2*). We confirmed all 7 known loci and identified 10 loci not previously reported to be genome-wide significant. After pruning for variants in LD, we identified 18 independent variants ($r^2 \leq 0.01$) (see meta-analysis results in *Table 2* and forest plots in Supplementary material online, *Figure S2*). The association with AS of variants surrounding the index variants is provided in Supplementary material online, *Figure S3*).

Subsequently, we performed a replication study of the association for each of the 10 previously unreported loci with AS in 90 828 individuals (7111 AS cases) from the Danish Blood Donor Study and Copenhagen Hospital Biobank, and variants at 8 of these 10 loci were nominally associated with AS with concordant direction of effect ($P \leq 0.05$) (*Table 2*). The two least common variants, *BET1L* rs73386631 and *HMGB1* rs181753401, were not associated with AS in this cohort, and meta-analysis of the discovery and replication cohorts did not achieve genome-wide significance (*Table 2*).

When we re-estimated the association of variants with AS in the genome-wide meta-analysis, conditioned upon the 18 index variants, the *PLG* variant rs191108153 became genome-wide significant [odds ratio (OR) per T allele, 1.57; 95% CI, 1.34–1.83; $P = 9.6 \times 10^{-9}$]. Given the proximity of this variant to *LPA*, we tested the association of this variant with AS conditioned on genetically predicted levels of lipoprotein(a) and observed substantial attenuation (OR per T allele, 1.20; 95% CI, 1.03–1.40; $P = 0.019$).

We examined publicly available databases for functional effects of the index variants and their proxies in high LD ($r^2 \geq 0.95$) (see Supplementary material online, *Tables S1 and S2*). *ARGHEF26* rs6794263 was in high LD ($r^2 = 0.99$)[39] with the missense variant rs13096373 (p.Phe203Ser). This substitution was predicted by the CADD software[40] to be in the 5% most deleterious substitutions (scaled C-score = 14.5). Variants in the *CELSR2–SORT1*, *PRRX1*, *NLRP6*, *PALMD*, and *IL6* loci and the intergenic region at 18q11.2 had high CADD scores, and variants at the *ACTR2* and *NLRP6* loci were in the 5% most pathogenic variants according to DANN[19] (ranked score ≥ 0.95). Variants at the *CELSR2–SORT1*, *PRRX1 PALMD*, *IL6*, *FADS1/2*, and 18q11.2 regions were classified as deleterious using the FATHMM-MKL non-coding approach ($P \geq 0.5$).[22]

Identified variants are also associated with aortic valve calcium

From a previous meta-analysis for AVC involving 6942 European participants from three cohorts in the CHARGE consortium (2245 participants with AVC >0),[7] we identified fixed-effects associations with prevalent AVC for the index variants. For unavailable variants, a proxy in high LD ($r^2 \geq 0.8$) was used, but no proxies were found for *LPA* rs140570886 and *HMGB1* rs181753401. Five variants were nominally associated with the presence of AVC in the same direction of effect as for AS ($P \leq 0.05$): *PRRX1* rs61817383 (OR per minor allele, 1.10; 95% CI, 1.01–1.21; $P = 0.035$), *ACTR2* rs62139062 (OR per minor allele, 1.11; 95% CI, 1.01–1.21; $P = 0.029$), *LPA* rs10455872 (OR per minor allele, 2.05; 95% CI, 1.63 to 2.57; $P = 9.0 \times 10^{-10}$), *NLRP6* rs17156153 (OR per minor allele, 1.17; 95% CI, 1.02–1.35; $P = 0.028$),

Table 2 Association with aortic stenosis of new and previously identified genetic loci for aortic stenosis in the discovery and replication cohorts

Chr.	Locus	Variant	Minor allele	Discovery					Replication			Discovery and replication	
				MAF	Odds ratio per minor allele (95% CI)	*P*	I^2% (95% CI)	Imputation quality score range	MAF	Odds ratio per minor allele (95% CI)	*P*	odds ratio per minor allele (95% CI)	*P*
New loci													
1	*CELSR2–SORT1*	rs12740374	T	0.21	0.90 (0.87, 0.94)	8.4×10^{-09}	0 (0, 0)	0.77 to 1.00	0.23	0.95 (0.91, 0.99)	0.016	0.92 (0.90, 0.95)	2.4×10^{-09}
1	*PRRX1*	rs61817383	T	0.26	1.11 (1.08, 1.14)	2.0×10^{-11}	10 (0, 66)	0.94 to 1.00	0.26	1.06 (1.02, 1.10)	8.1×10^{-03}	1.09 (1.06, 1.12)	3.7×10^{-12}
2	*ACTR2*	rs62139062	T	0.28	1.09 (1.06, 1.12)	4.2×10^{-08}	32 (0, 68)	0.92 to 1.00	0.26	1.08 (1.04, 1.12)	2.3×10^{-04}	1.08 (1.06, 1.11)	4.4×10^{-11}
3	*ARHGEF26*	rs6794263	C	0.11	0.88 (0.84, 0.92)	1.0×10^{-08}	39 (0, 71)	0.98 to 1.00	0.11	0.93 (0.88, 0.99)	0.013	0.90 (0.87, 0.93)	1.5×10^{-09}
9	*SMC2*	rs55909255	C	0.39	1.08 (1.05, 1.11)	4.7×10^{-08}	53 (3, 77)	0.99 to 1.00	0.36	1.05 (1.01, 1.09)	0.014	1.07 (1.04, 1.09)	5.1×10^{-09}
11	*BET1L*	rs73386631	T	0.043	1.22 (1.14, 1.31)	2.8×10^{-08}	20 (0, 62)	0.37 to 1.00	0.038	1.06 (0.97, 1.17)	0.19	1.16 (1.10, 1.23)	1.9×10^{-07}
11	*NLRP6*	rs17156153	T	0.085	1.16 (1.10, 1.22)	4.2×10^{-09}	0 (0, 56)	0.73 to 1.00	0.075	1.16 (1.09, 1.24)	1.3×10^{-05}	1.16 (1.11, 1.21)	2.6×10^{-13}
13	*HMGB1*	rs181753401	A	2.4×10^{-03}	2.29 (1.74, 3.02)	4.2×10^{-09}	36 (0, 73)	0.40 to 1.00	3.9×10^{-03}	1.23 (0.92, 1.63)	0.16	1.69 (1.38, 2.06)	2.1×10^{-07}
16	*TMEM170A*	rs11643207	C	0.38	0.92 (0.89, 0.95)	1.4×10^{-08}	50 (0, 76)	0.98 to 1.00	0.39	0.92 (0.89, 0.96)	8.6×10^{-06}	0.92 (0.90, 0.94)	5.6×10^{-13}
18	*Intergenic* (18q11.2)	rs551520	T	0.23	0.91 (0.88, 0.94)	2.6×10^{-09}	27 (0, 65)	0.99 to 1.01	0.24	0.94 (0.90, 0.98)	2.8×10^{-03}	0.92 (0.90, 0.94)	6.8×10^{-11}
Previously identified loci													
1	*ALPL*	rs6696066	A	0.48	0.92 (0.89, 0.94)	6.1×10^{-10}	0 (0, 6.3)	0.99 to 1.00	0.47	0.93 (0.89, 0.96)	2.3×10^{-05}	0.92 (0.90, 0.94)	7.1×10^{-14}
1	*PALMD*	rs6702619	G	0.49	1.16 (1.13, 1.19)	1.2×10^{-25}	59 (18, 80)	0.97 to 1.00	0.51	1.17 (1.13, 1.21)	7.1×10^{-19}	1.16 (1.14, 1.19)	8.7×10^{-43}
1	*NAV1*	rs631556	A	0.41	1.10 (1.07, 1.13)	1.4×10^{-10}	0 (0, 44)	0.98 to 1.00	0.38	1.08 (1.04, 1.12)	5.3×10^{-05}	1.09 (1.07, 1.12)	4.1×10^{-14}
2	*TEX41*	rs7593336	G	0.38	1.12 (1.09, 1.15)	7.3×10^{-15}	59 (17, 79)	0.95 to 1.00	0.41	1.14 (1.10, 1.18)	2.7×10^{-12}	1.12 (1.10, 1.15)	1.7×10^{-25}
6	*LPA*	rs10455872	G	0.069	1.42 (1.35, 1.49)	4.6×10^{-44}	28 (0, 65)	0.76 to 1.00	0.081	1.50 (1.41, 1.60)	1.1×10^{-33}	1.45 (1.39, 1.51)	1.4×10^{-75}
		rs140570886	C	0.014	1.55 (1.40, 1.73)	5.1×10^{-16}	24 (0, 64)	0.83 to 1.00	0.010	1.31 (1.10, 1.56)	3.0×10^{-03}	1.48 (1.35, 1.62)	2.3×10^{-17}
7	*IL6*	rs1800797	A	0.45	1.13 (1.10, 1.16)	2.9×10^{-18}	45 (0, 73)	0.88 to 1.00	0.46	1.12 (1.08, 1.16)	9.3×10^{-11}	1.13 (1.10, 1.15)	1.9×10^{-27}
11	*FADS1/2*	rs174533	A	0.34	0.91 (0.88, 0.94)	6.7×10^{-09}	0 (0, 54)	0.93 to 1.00	0.34	0.93 (0.89, 0.96)	4.8×10^{-05}	0.92 (0.89, 0.94)	2.0×10^{-12}

Abbreviation: MAF, minor allele frequency.

and *FADS1/2* rs174533 (OR per minor allele, 0.91; 95% CI, 0.83–0.99; $P = 0.024$) (see Supplementary material online, *Table S3*).

Genetic risk scores are associated with aortic stenosis and aortic valve calcium

The prevalence of AS increased across GRS_{18} risk score tertiles, ranging from 0.97% in the lowest tertile to 1.92% in the highest tertile (see Supplementary material online, *Figure S4*). Each 1 standard deviation (SD) higher GRS_{18} was associated with 37% higher odds of AS (OR per SD, 1.37; 95% CI, 1.32–1.41; $P = 3.0 \times 10^{-72}$) with an AUC of 0.697, when adjusted for age[2] and sex, with a similar OR after additional adjustment for diabetes, LDL-C, systolic blood pressure, smoking, BMI, and CAD (OR per SD, 1.31; 95% CI, 1.26–1.35; $P = 2.6 \times 10^{-51}$) (see Supplementary material online, *Table S4*). The AUC for this full model was 0.824, the addition of the genetic risk score modestly improving the AUC compared to a model restricted to non-genetic cardiovascular risk factors only (AUC = 0.818; $P_{AUC\ difference} = 5.9 \times 10^{-6}$). The GRS_{559} demonstrated stronger effect sizes and higher discrimination in the fully adjusted model (OR 1.53 per 1-SD GRS559, 95% CI 1.48–1.58; $P = 1.54 \times 10^{-141}$; AUC = 0.829; $P_{AUC\ difference} = 3.2 \times 10^{-9}$). In an additional sensitivity analysis, we dropped the six *LPA* region SNPs from the GRS_{559}, and this resulted in an OR of 1.50 per 1-SD for AS (CI 1.45–1.55; $P = 7.21 \times 10^{-130}$). Using the LDpred2 approach, we observed similar results with an OR of 1.45 per 1-SD (95% CI 1.41, 1.50; $P = 7.44 \times 10^{-122}$) and an AUC of 0.706 when adjusting only for age[2] and sex. In the model also adjusting for cardiovascular risk factors, we observed an OR of 1.40 (95% CI 1.36, 1.45; $P = 1.19 \times 10^{-84}$) and an AUC of 0.827 (see Supplementary material online, *Table S4*).

When we applied the GRS_{17} to MESA, the prevalence of AVC was 15.2%, 13.9%, and 17.7% in the first, second, and third tertiles, respectively (see Supplementary material online, *Figure S4*). After adjustment for age and sex, each 1 SD higher genetic risk score was associated with 22% higher odds of AVC (OR per SD, 1.22; 95% CI, 1.08–1.37; $P = 1.44 \times 10^{-3}$; AUC = 0.796), and this association persisted after additional adjustment for fasting glucose, LDL-C, systolic blood pressure, smoking, BMI, and coronary artery calcium (OR per SD, 1.23; 95% CI, 1.09–1.39; $P = 1.10 \times 10^{-3}$; AUC = 0.815). The GRS_{550} demonstrated weaker effects and worse discrimination with AVC when adjusted for age and sex (OR 1.15, 95% CI 1.02–1.29; $P = 0.022$; AUC = 0.794) as well as in the fully adjusted model (OR 1.15, 95% CI 1.02–1.30; $P = 0.023$; AUC = 0.814). Without the six *LPA* region SNPs, the OR was 1.11 per 1-SD for AVC (CI 0.98–1.25; $P = 0.093$). However, we observed stronger effects and better discrimination with LDpred2, an OR of 1.27 (1.12, 1.44; $P = 1.34 \times 10^{-9}$) and an AUC of 0.799 when adjusted for age and sex. In the fully adjusted model, we observed an OR of 1.32 (1.16, 1.50; $P = 2.24 \times 10^{-5}$) and an AUC of 0.823 (see Supplementary material online, *Table S4*).

A subset of the identified variants replicates in African and Latin Americans

When we examined the index variants among 1917 African American participants (86 cases) and 3482 Latin American participants (159 cases) from the GERA cohort, we observed replication ($P \leq 0.05$ for AS with concordant direction of effects) for *CELSR2–SORT1* rs12740374 in both ancestries, for *ALPL* rs6696066 and *NLRP6* rs17156153 in Latin Americans, and for *LPA* rs10455872 in African Americans (see Supplementary material online, *Table S5*).

Region-based analysis identifies additional associations with aortic stenosis

For each of 18 539 protein-coding genes, we used MAGMA[24] to examine the joint contribution of all variants in a gene region. We tested the mean association with AS of variants within 50 kb of each gene, correcting for LD between variants. We identified 95 regions associated with AS after Bonferroni correction (see Supplementary material online, *Table S6*), including regions spanning 11 of the 17 loci identified with single variant analysis (*ALPL*, *CELSR2*, *PRRX1*, *NAV1*, *ARHGEF26*, *LPA*, *IL6*, *BET1L*, *NLRP6*, *FADS1/2*, and *TMEM170A*). The *TMEM170A* gene region had a level of significance similar to an overlapping region defined by CFDP1 ($P = 2.9 \times 10^{-10}$ for *CFDP1* vs. $P = 4.5 \times 10^{-10}$ for *TMEM170A*). The three most significant regions not identified in the single variant-based approaches were *LDLR* ($P = 2.3 \times 10^{-10}$), *AGO2* ($P = 5.9 \times 10^{-10}$), and *XKR6* ($P = 9.8 \times 10^{-10}$). Although this approach did not account for LD between regions, these three regions were >100 Mb away from another association.

Expression-based analyses

We used MetaXcan[25] to analyze gene expression and expression quantitative trait loci (eQTL) extracted from GTEx project data.[23] We examined four tissues which may be involved in the AS disease process: aorta, left ventricle, liver, and whole blood. With a false discovery rate of 5%, we identified 42 genes with predicted expression that differed between cases and controls (see Supplementary material online, *Figure S5*). Increased *LPA* expression in the liver was predicted for AS cases (Z score = 5.25; $P = 1.5 \times 10^{-7}$). Expression of *IL6R* was inferred to be increased in the blood of cases (Z score = 4.66; $P = 3.1 \times 10^{-6}$). *NOTCH4* mRNA in the aorta was predicted to be decreased in cases (Z score = −4.07; $P = 4.7 \times 10^{-5}$). In contrast to these single tissue effects, inferred *RPS23* expression was lower in the blood, left ventricle, and aorta in AS cases ($P \leq 3.3 \times 10^{-5}$). The most significant, predicted differential expression was decreased *ZEB2* expression in the aorta of individuals with AS (Z score = −7.14; $P = 9.2 \times 10^{-13}$). Additional GIGSEA[27] were performed to infer the differential expression of genes (see Supplementary material online, *Table S7*).

As identified in GTEx,[23] the index variants at the *ACTR2* and *BET1L* loci were in high LD ($r^2 \geq 0.98$) with the most significant eQTL for their respective genes in the aorta. The index variant at the *ARHGEF26* locus was in high LD ($r^2 = 0.96$) with the most significant eQTL for *ARHGEF26* in the left ventricle. In addition, rs55909255 was associated with the expression of *SMC2* in the left ventricle, and rs140570886 was associated with the expression of *LPA* in the liver. Index variants and proxies at the *CELSR2–SORT1*, *IL6*, and *FADS1/2* loci were all eQTL for multiple genes in three to four tissues (see Supplementary material online, *Table S2*). Notably, the index variant at the *CELSR2–SORT1* locus was the most significant hepatic eQTL for *CELSR2* and *SORT1*, and the index variant at the *FADS1/2* locus was in high LD ($r^2 \geq 0.96$) with the most significant eQTL for *FADS1* in the aorta and liver. Neither the index variant at the *IL6* locus nor any variants in high LD ($r^2 \geq 0.95$) were associated with *IL6* expression in the tissues examined. However, the index variant was in high LD with the most significant eQTL for IL6 antisense RNA 1 (*IL6-AS1*) in the blood ($r^2 = 0.96$) (see Supplementary material online, *Table S2*). Several of these results were confirmed by additional colocalization analyses using MetaXcan and coloc (see Supplementary material online, *Figure S5*, and Supplementary material online, *Table S8*). Notably,

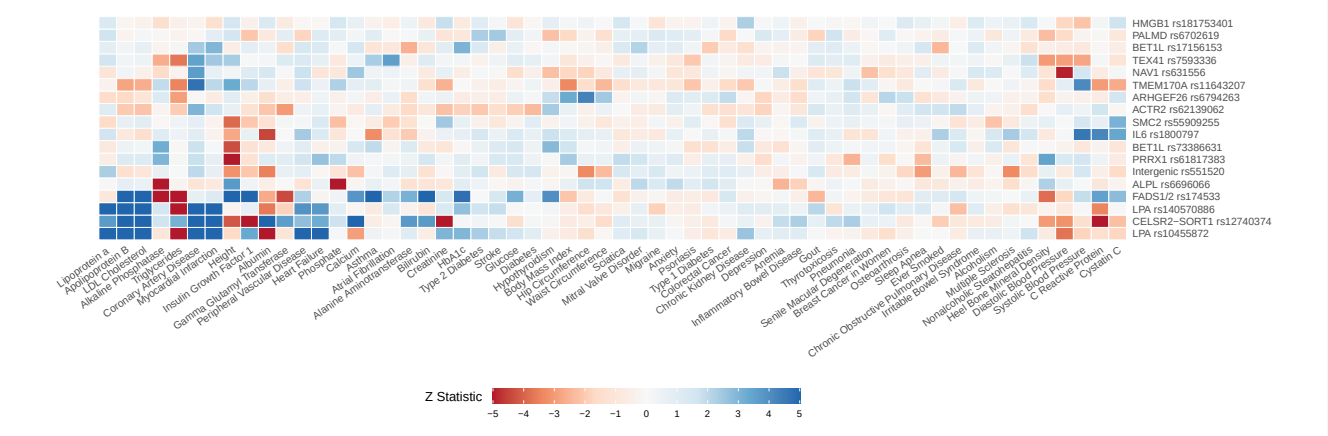


Figure 3 Association of aortic stenosis variants with biomarkers, physiological measurements, and diseases. Variants were ordered to reflect similarity in their associations with traits and vice versa. The strength and direction of associations are represented by cells of different colors, with blue cells indicating positive effects on the odds of aortic stenosis and red cells indicating inverse associations. For ease of visualization, *Z* statistics >5 or less than −5 were rounded to 5 and −5, respectively.

we identified high posterior probabilities for colocalization for SORT1 expression in the liver for rs6702619, as well as ACTR2 expression in the aorta for rs7556894 and FADS1 in whole blood and left ventricle for rs174535.

AS is genetically correlated with adiposity

Using LD score regression[35] as implemented on LD Hub,[36] we computed the genetic correlation of AS with 157 traits related to cardiovascular risk factors, metabolites, and immunological diseases using GWAS summary statistics. With a false discovery rate of 5%, we observed 32 (18.5%) traits that were genetically correlated with AS, most of which involved adiposity, glycemia, or lipids (see Supplementary material online, *Table S9*). Measures of adiposity were positively correlated with AS and represented seven of the 10 most significant correlations. The only inverse correlations were with high-density lipoprotein particles. The total lipid content of chylomicrons and large very low-density lipoprotein particles shared the highest absolute genetic correlation with AS (r_g, 0.31; 95% CI, 0.13 to 0.48; $P = 6.0 \times 10^{-4}$).

Pleiotropic effects of variants associated with aortic stenosis

We assessed the association of the index variants with 58 biomarkers, physiological measurements, and diseases among 257 231 UK Biobank White British participants. Traits were selected to reflect cardiovascular, cerebrovascular, immune, hepatic, and adiposity phenotypes. We observed 98 associations with false discovery rate-adjusted $P \leq 0.05$ and the five most significant associations involved apolipoprotein B, lipoprotein(a), LDL-C, or triglycerides (see Supplementary material online, *Table S10*). The traits with the greatest number of associations were height (eight variants); CAD, albumin, and triglycerides (six variants each); C-reactive protein and apolipoprotein B (five variants each); and heel bone mineral density, diastolic blood pressure, and LDL-C cholesterol (four variants each) (*Figure 3*; Supplementary material online, *Table S10*).

Blood-based biomarkers are causal contributors to aortic stenosis

We performed Mendelian randomization for six traits associated with several variants in the phenome-wide association study. We constructed genetic instruments using summary statistics from GWAS performed on UK Biobank (White British) and assessed the association of these instruments with AS. Using the IVW method, genetically elevated levels of apolipoprotein B, lipoprotein(a), LDL-C, and BMI were associated with increased odds of AS [OR per g/L of apolipoprotein B, 3.85; 95% CI, 2.90–5.12; $P = 2.1 \times 10^{-20}$; OR per natural logarithm of lipoprotein(a), 1.20; 95% CI, 1.17–1.23; $P = 3.0 \times 10^{-53}$; OR per mmol/L of LDL-C, 1.57; 95% CI, 1.44–1.71; $P = 1.5 \times 10^{-25}$; OR per kg/m^2 of BMI, 1.07; 95% CI, 1.05, 1.09; $P = 1.9 \times 10^{-12}$, respectively], with no evidence of pleiotropy (all Egger intercepts $P > 0.05$) (see Supplementary material online, *Table S11*). The associations between AS and higher genetically predicted levels of both C-reactive protein and alkaline phosphatase were pleiotropic (Egger intercept *P*-values of 0.024 and 0.003), and MR results were not consistent across methods limiting causal inference (see Supplementary material online, *Table S11*).

Discussion

In this genome-wide meta-analysis combining summary statistics from 10 cohorts of European ancestry, we confirmed association for seven previously identified loci and identified 10 loci not previously reported to be genome-wide significant for AS. For newly discovered variants, we observed modest changes in AS odds (9%–55% increased odds per risk allele). In independent replication, 8 of the 10 previously unreported variants were associated with AS, with the exception of *BET1L* rs73386631 and *HMGB1* rs181753401 which had low allele frequencies. Our work brings the total number of loci robustly associated with AS to 15. Several of these variants were associated with AS in other ancestries, and a risk score composed of all identified variants was a predictor of AS independent of other risk factors. Further analysis using region- and gene-based methods identified additional gene regions associated with AS, including *LDLR* and *NOTCH4*, as well as

differential expression of co-regulated groups of genes. Finally, Mendelian randomization supported a causal contribution of lipoprotein(a), apolipoprotein B, LDL-C, and BMI to AS.

Our findings support four key etiological mechanisms for AS: calcification, lipid metabolism, adiposity, and inflammation (*Structured Graphical Abstract*). The variants at the *PRRX1*, *ACTR2*, *LPA*, *NLRP6*, and *FADS1/2* loci were also associated with aortic valve calcification assessed by computed tomography. We observed associations of the index variants at the *CELSR2–SORT1*, *PRRX1*, *TEX41*, and *FADS1/2* loci with heel bone mineral density, suggesting systemic effects on calcification. The paired-related homeobox protein 1, the product of the locus *PRRX1*, is a transcription factor required for osteoblast differentiation by TNF-α[41] and is an inducer of the epithelial–mesenchymal transition,[42] a key process in early calcification. Interestingly, zinc finger e-box binding homeobox 2, coded by *ZEB2*, is a repressor of this process.[43] Predicted *ZEB2* expression in the aorta was the most significant differentially expressed gene. Consistent with a potential role in calcification, earlier work demonstrated that *TEX41* variants may be associated with AS through long-range chromatin interactions with the *ZEB2* promoter region, including in the aorta[9] implicating *TEX41*, *ZEB2*, and *PRRX1* in inducing early calcification and subsequent valve stenosis.

Located in the 3' untranslated region of *CELSR2*, rs12740374 affects expression of *SORT1*,[44] which has been reported to decrease hepatic excretion of apolipoprotein B and increase catabolism of LDL-C.[45] Studies have reported the association of lower LDL-C[46,47] and lower odds of CAD,[48,49] and in a study involving two cohorts participating in the current study, a variant in perfect LD with rs12740374 was associated with AS after Bonferroni correction ($P = 3.4 \times 10^{-4}$).[9] The present analysis identified genome-wide significance in the association of the *CELSR2–SORT1* variant with AS. Consistent with its effects on LDL-C and apolipoprotein B, the minor allele conferred a reduction in the odds of AS. Additional evidence that lipid metabolism is a causal mechanism for AS was provided by Mendelian randomization, which confirmed the role of LDL-C[12] and identified a contribution of apolipoprotein B, which extends this association to all apolipoprotein B-containing lipoprotein particles. The findings were consistent with work demonstrating an association of a non-high-density lipoprotein cholesterol genetic risk score with AS.[9] In addition to these lipoprotein-mediated effects, sortilin, coded by *SORT1*, is a regulator of vascular calcification and is associated with increased aortic calcification in mice.[50] Furthermore, sortilin expression is associated with increased expression of *ALPL*,[50] a known AS locus replicated in this study.

Both overall and abdominal obesity have been previously associated with AS,[51] and a Mendelian randomization study provided support for the causality of BMI.[14] Our Mendelian randomization analyses replicate and extend these findings by observing genetic correlations between AS and multiple measures of adiposity, including BMI, waist and hip circumferences, and obesity. However, only one of our genome-wide significant variants, *ARHGEF26* rs6794263, which was in high LD with the missense variant rs13096373 (p.Phe203Ser), was associated with BMI and hip circumference in our phenome-wide analysis. Another missense variant rs12493885 (p.Val29Leu) in *ARHGEF26* was previously associated with CAD,[52] mediated by a gain of function for ARHGEF26 that may lead to increased transendothelial migration, greater adhesion of leukocytes, and proliferation of vascular smooth muscle cells.[52] However, this variant was independent of *ARHGEF26* rs6794263 ($r^2 = 0.018$)[39] and was not associated with AS in our meta-analysis (OR per T allele, 1.02; 95% CI, 0.98–1.06; $P = 0.44$ for rs1713812, which is in perfect LD with rs12493885[39]). Conversely, the allele of rs6794263 that confers a lower odds of AS has also been associated with lower odds of CAD, but not at genome-wide significance (OR per C allele, 0.96; 95% CI, 0.92–1.00; $P = 0.037$).[53] The presence of two independent, missense mutations in *ARHGEF26* with discordant effects on AS and CAD suggests pleiotropic effects of this locus.

The accumulation of inflammatory cells in the aortic valve is associated with remodeling and fibrosis,[54] highlighting the role for inflammation in disease progression. We confirmed that the *FADS1/2* locus was associated with AS.[11] Both *FADS1* and *FADS2* encode key enzymes in the conversion of dietary *n*-6 fatty acids to arachidonic acid, a precursor of pro-inflammatory leukotrienes and prostaglandins.[55] In addition, we also identified a novel variant at the locus for NLRP6, which assembles an inflammasome that plays a role in immunity to bacterial infection as well as proinflammatory responses to other stimuli, including fatty acids.[56,57] We replicated that the association previously reported between an *IL6* variant and AS[10] and the *IL6* variant rs2069832 ($r^2 = 0.95$ with our index variant) colocalizes with *IL6-AS1* expression.[10] Notably, the risk-increasing alleles are associated with increased expression of both *IL6* and *IL6-AS1* in fibroblasts in GTEx.[23] Our analyses therefore indicate a pro-inflammatory association with AS in the *IL6* region but also higher predicted *IL6R* expression in the blood cells of AS cases. Thus, several orthogonal signals support the association of pro-inflammatory pathways with AS.

The present study represents the largest genome-wide meta-analysis of AS, a common condition with no available medical treatment, and included 653 867 participants (13 765 cases) in which we applied variant-, gene-, and gene set-based analyses to identify additional risk loci and disease mechanisms. Our work highlights novel mechanisms and pathways which may have important clinical and future research implications. First, our analyses point to several possible therapeutic interventions using both lifestyle and novel drugs. Our findings of lipoproteins and adiposity as key drivers of aortic stenosis suggest that maintenance of healthy lifestyle and adherence to lipid-lowering recommendations may reduce the incidence of aortic stenosis. Furthermore, lipoprotein(a)-lowering therapies currently in development[58,59] may provide a new avenue for prevention of disease progression. Whether novel hypoglycemics, which also lead to marked weight loss and metabolic improvements,[60] and anti-inflammatory agents (e.g. that inhibit IL-6 signaling[61]) could reduce AS requires randomized trials. Finally, our observation of a robust association between a genetic risk score and AS may allow identification of patients at risk for rapid disease progression in clinical practice, who may require echocardiographic follow-up, as well as permit targeted enrolment of such at-risk patients in future randomized trials.

Despite the strengths of the study, there are several limitations. Since our discovery cohorts were of European ancestry, the transferability of our results to other ancestries may be limited.[62–64] Although we attempted cross-ancestry replication of genome-wide significant variants, the observed limited reproducibility may be due to the low numbers of African and Latin American participants. Future studies should focus on non-Europeans. Our analyses also made exclusive use of bioinformatic methods to identify genetic loci. These results require confirmation using complementary approaches. Lastly, cases were selected using various criteria (international classification of diseases (ICD) codes, surgical AVR, etc.) and not solely by echocardiography. Therefore we could not entirely exclude bicuspid aortic valves; however, these likely represent a small proportion of the cases.

In conclusion, our results identify novel genetic contributors to AS and identify specific contributions to disease etiology that are characterized by the effects of calcification, altered lipid metabolism, adiposity, and inflammation. An AS genetic risk score was an independent predictor of both clinical and subclinical disease, providing additional discrimination when added to clinical risk factors. Established and novel

genetic loci warrant investigation as potential therapeutic targets to prevent the initiation of aortic calcification and progression to stenosis.

Author contributions

Concept and design: Chen, Engert, and Thanassoulis. *Acquisition, analysis, or interpretation of data:* Chen, Dina, Small, Shaffer, Levinson, Helgadóttir, Capoulade, Munter, Martinsson, Cairns, Trudsø, Hoekstra, Burr, Marsh, Dufresne, Messika-Zeitoun, Le Scouarnec, Ghouse, Olesen, Christensen, Mikkelsen, Jacobsen, Dowsett, Pedersen, Erikstrup, Ostrowski, Budoff, Post, Rotter, Bundgaard, Le Tourneau, Smith, Hólm, Söderberg, Schott, Engert, and Thanassoulis. *Drafted the manuscript:* Chen. *Critical revision of the manuscript for important intellectual content:* Helgadóttir, Capoulade, Martinsson, Cairns, Trudsø, Hoekstra, Marsh, Dufresne, Hólm, Christensen, Mikkelsen, Erikstrup, Ostrowski, Post, Rotter, Bundgaard, Johansson, Ljungberg, Näslund, Smith, Söderberg, Schott, Clarke, Engert, and Thanassoulis. *Obtained funding:* Gudnason, Näslund, Post, Rotter, Lathrop, Le Tourneau, Messika-Zeitoun, Smith, Söderberg, Schott, Engert, and Thanassoulis. *Administrative, technical, or material support:* Ranatunga, Whitmer, Bonnefond, Lathrop, Ljungberg, Näslund, Le Tourneau, Smith, Söderberg, Schott, Engert, and Thanassoulis. *Supervision:* Damrauer, Budoff, Gudnason, Rotter, Johansson, Smith, Rader, Clarke, Engert, and Thanassoulis.

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Supplementary data

Supplementary data is available at *European Heart Journal* online.

Data availability

The summary level statistics from the meta-GWAS that support the findings of this study are available online @ Zenodo.org (https://doi.org/10.5281/zenodo.7505361).

Conflict of interest

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