Genomics of Calcific Aortic Stenosis

Hao Yu Chen, MSc

Division of Experimental Medicine

McGill University, Montreal

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To my parents:

We did it.

This is the immigrant dream: to not just live but to flourish in a new land.

Your love and sacrifices have made this possible.

Abstract

Background: Left untreated, calcific aortic stenosis (AS) culminates in heart failure and death. Aortic valve replacement remains the only treatment option but is not indicated for mild or moderate cases. An improved understanding of the genetic aetiology of AS could inform prevention and treatment strategies.

Methods: I reviewed the literature to identify risk factors for valvular calcification, with an emphasis on aortic valve calcification (AVC) and stenosis. Among 44,703 European-ancestry participants (3,469 AS cases) of the Genetic Epidemiology Research on Adult Health and Aging (GERA) cohort, I estimated the association with AS of cardiovascular risk factors and performed Mendelian randomization to assess for causality. I also estimated the association of two *LPA* variants and an unweighted *LPA* risk score with AS. To identify novel loci for AS, I performed a genome-wide association study and tested a variant which had demonstrated suggestive evidence of association in seven replication cohorts totalling 256,926 participants (5,926 AS cases). Finally, I also performed a genome-wide meta-analysis involving 653,867 European-ancestry participants from 10 cohorts (13,765 AS cases).

Results: Cardiovascular risk factors have been associated with AS in multiple studies, as well as genetic variants in the *LPA*, *PALMD*, and *TEX41* loci. I confirmed these risk factors were associated with AS in the GERA cohort (all $p \leq 0.05$), with increased triglycerides being causally associated (odds ratio [OR] per SD, 1.27; 95% CI, 1.01 to 1.59; $p=0.01$). The *LPA* variants rs10455872 and rs3798220 and an *LPA* risk score conferred increases in AS odds (OR per risk allele, 1.34; 95% CI, 1.23 to 1.47; $p=1.7\times10^{-10}$, 1.31; 95% CI, 1.09 to 1.58; $p=3.6\times10^{-3}$, and 1.35;

95% CI, 1.24 to 1.46; $p = 1.3 \times 10^{-12}$, respectively). I demonstrated novel, genome-wide significant association with AS with 11 variants at 10 loci, including *FADS1/2* rs174547 and *PRRX1* rs61817383, with consistent effects for AVC.

Conclusions: Known and newly identified loci implicate calcification, lipid metabolism, and inflammation as key drivers of AS development. Genetic risk scores improve the classification of AS, offering an opportunity for risk stratification in primary prevention. Novel loci should be examined for their potential as therapeutic targets.

Résumé

Contexte : La sténose aortique calcifiante (SA) mène à l'insuffisance cardiaque et au décès. Le remplacement de la valve aortique reste le seul traitement mais n'est pas indiqué pour les cas légers ou modérés. Une meilleure compréhension de l'étiologie génétique de la SA pourrait permettre d'élaborer de nouveaux traitements.

Méthodes : J'ai passé en revue la littérature afin d'identifier les facteurs de risque de calcification valvulaire, y compris la calcification de la valve aortique (AVC) et sa sténose. Chez 44 703 participants d'ascendance européenne (3 469 cas) de la cohorte GERA (Genetic Epidemiology Research on Adult Health and Aging), j'ai estimé l'association entre les facteurs de risque cardiovasculaire et la SA et j'ai effectué une randomisation mendélienne pour évaluer la causalité. J'ai également estimé l'association de deux variants de *LPA* et d'un score de risque de *LPA* avec la SA. Pour identifier de nouveaux loci, j'ai réalisé une étude d'association à l'échelle du génome dans la cohorte GERA et une méta-analyse à l'échelle du génome comprenant 653 867 participants d'ascendance européenne issus de 10 cohortes (13 765 cas).

Résultats : Des facteurs de risque cardiovasculaire ont été associés à la SA dans de nombreuses études, ainsi que les loci *LPA*, *PALMD* et *TEX41*. Ces facteurs de risque étaient associés à la SA dans la cohorte GERA (tous à $p \leq 0{,}05$) et les triglycérides étaient causales (odds ratio [OR] par écart-type, 1,27; IC à 95 %, 1,01 à 1,59; $p=0{,}041$). Les variants *LPA* rs10455872 et rs3798220 et le score de risque *LPA* ont augmentés la probabilité de SA (OR par allèle à risque, 1,34; IC à 95%, 1,23 à 1,47; $p=1{,}7\times10^{-10}$, 1,31; IC à 95%, 1,09 à 1,58; $p=3{,}6\times10^{-3}$, et 1,35; IC à 95%, 1,24 à 1,46; $p=1{,}3\times10^{-12}$, respectivement). J'ai démontré une nouvelle association avec la SA avec 11 variants

sur 10 loci, y compris *FADS1/2* rs174547 et *PRRX1* rs61817383, avec des effets semblables pour l'AVC.

Conclusions : Les loci connus et nouvellement identifiés suggèrent que la calcification, le métabolisme lipidique et l'inflammation sont des facteurs clés du développement de la SA. De nouveaux loci devraient être examinés pour leur potentiel en tant que cibles thérapeutiques.

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List of Abbreviations

A	
AA	Arachidonic acid
ALA	α-linolenic acid
ANNOVAR	Annotate Variation
AS	Aortic stenosis
AUC	Area under the curve
AVC	Aortic valve calcium
AVR	Aortic valve replacement

B	
BioVU	Vanderbilt DNA Biobank
BMI	Body mass index

C	
CAD	Coronary artery disease
CADD	Combined Annotation Dependent Depletion
CAVD	Calcific aortic valve disease
CAVS	Calcific aortic valve stenosis
CHARGE	Cohorts for Heart and Aging Research in Genomic Epidemiology
CHS	Cardiovascular Health Study
CI	Confidence interval

D	
DANN	Deleterious Annotation of Genetic Variants Using Neural Networks
DOI	Digital object identifier

E	
ECG	Electrocardiogram
EHR	Electronic health record
EIGEN-PC	Eigen – Principal Component
EPA	Eicosapentaenoic acid
EPIC-NORFOLK	European Prospective Investigation of Cancer and Nutrition - Norfolk
EUR	European

F	
FADS	Fatty acid desaturase
FANTOM5	Functional Annotation of the Mammalian Genome Version Five
FATHMM-MKL	Functional Analysis Through Hidden Markov Models – Multiple Kernel Learning
FDR	False discovery rate

FH	Familial hypercholesterolemia
FOS	Framingham Offspring Study

G

GCTA-COJO	Genome-Wide Complex Trait Analysis Conditional and Joint Association Analysis
GERA	Genetic Epidemiology Research on Adult Health and Aging
GIGSEA	Genotype Imputed Gene Set Enrichment Analysis
GO	Gene Ontology
GRS	Genetic risk score
GTEx	Genotype-Tissue Expression Project
GWAS	Genome-wide association study

H

HR	Hazard ratio
HRC	Haplotype Reference Consortium

I

ICD	International Classification of Diseases

K

KEGG	Kyoto Encyclopedia of Genes and Genomics

L

LA	Linoleic acid
LD	Linkage disequilibrium
LDL-C	Low-density lipoprotein cholesterol
LDSC	Linkage Disequilibrium Score Regression
LINSIGHT	Linear INSIGHT
Lp(a)	Lipoprotein(a)

M

MAC	Mitral annular calcification
MAGMA	Multi-Marker Analysis of Genomic Annotation
MDCS	Malmö Diet and Cancer Study
MESA	Multi-Ethnic Study of Atherosclerosis
MI	Myocardial infarction
miR	Micro-RNA
MR	Mendelian randomization

O

OR	Odds ratio

P	
PMBB	Penn Medicine Biobank
PRS	Polygenic risk score
Q	
QUEBEC-CAVS	Quebec City Case-Control Calcific Aortic Valve Stenosis
R	
RR	Risk ratio
S	
SBP	Systolic blood pressure
SD	Standard deviation
SNP	Single-nucleotide polymorphism
U	
UCSC	University of California Santa Cruz Genome Browser

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Contributions to Original Knowledge

The five manuscripts in this dissertation are composed of one literature review (chapter two) and four original research studies (chapters three to six, inclusive). The literature review summarizes the published genetic and observational evidence for risk factors for valvular calcification, including aortic valve calcium (AVC) and aortic stenosis (AS). The four original research studies each represent original and distinct contributions to the field of AS genomics.

In chapter three, I estimated the odds for AS of several cardiovascular risk factors in the large-scale Genetic Epidemiology Research on Adult Health and Aging (GERA) cohort. I also performed Mendelian randomization (MR) analyses to assess which risk factors demonstrated evidence of a causal contribution to AS, highlighting factors which should be investigated further for their potential as therapeutic targets. Prior observational studies had assessed either a subset of these risk factors, or the analyses were performed in cohorts with small sample sizes, which may have yielded imprecise estimates. Prior MR studies likewise examined the causal contribution of only a subset of risk factors. My manuscript presented a more complete and unified assessment of the observational and causal contributions of risk factors to AS within a single cohort, which permitted an intra-study comparison of the relative contributions of these risk factors.

In chapter four, I examined the association of *LPA* variants rs10455872 and rs3798220 with AS in the GERA cohort. I modelled these associations 1) as single variants, 2) combined in an unweighted genetic risk score (GRS), and 3) in terms of specific risk allele combinations. I demonstrated that both *LPA* variants were associated with moderate increases in the per-allele odds for AS. When the variants were combined into an *LPA* GRS, I observed a moderate increase in

disease odds, consistent with the single-variant analyses. While the association of the rs10455872 variant with AS has been well studied, the rs3798220 variant is less well described because it is not as common in European-ancestry populations. Due to the large number of AS cases in the GERA cohort, there was adequate power to observe the association of *LPA* rs3798220 with AS, both as a single variant and as part of a GRS. Thus, the study confirmed the presence of a second variant at the *LPA* locus in linkage equilibrium with rs10455872 that was also associated with AS. Additionally, I observed that possessing two risk alleles in any combination across these two variants was associated with a doubling in the odds for AS. This observation provided novel evidence that compound heterozygous individuals, *i.e.* individuals with one rs10455872 risk allele and one rs3798220 risk allele, possessed elevated odds of disease similar to individuals who were homozygous for the risk allele of either variant.

In chapter five, I performed a genome-wide association study (GWAS) for AS in the GERA cohort and observed a variant which associated with the disease. Following replication of this variant in seven replication cohorts, I confirmed that variation at the *FADS1/2* locus was associated with AS. Given the role of this locus in n-6 and n-3 polyunsaturated fatty acid metabolism, I examined whether n-6 and n-3 fatty acids were associated with AVC in two US community cohorts, as this data was not available in the AS cohorts. I demonstrated that a higher ratio of linoleic acid to arachidonic acid, indicating a greater synthesis of long-chain n-6 fatty acids, was associated with greater odds of AVC. I did not observe an association between the synthesis of long-chain n-3 fatty acids and AVC. My MR analyses indicated that higher *FADS1* gene expression or plasma concentrations of arachidonic acid were causal contributors to AS and AVC. These findings identified a novel locus for AS, implicated elevated n-6 fatty acid levels in the pathogenesis of AS,

and highlighted pharmacological targeting of the *FADS1/2* locus and dietary modification as possible therapeutic options to be examined further.

In chapter six, I performed a genome-wide meta-analysis for AS using summary-level data from ten cohorts of European ancestry. I discovered nine novel loci for AS and confirmed all seven previously identified loci, bringing the total number of AS risk loci to 16. I demonstrated that a polygenic risk score (PRS) composed of independent ($r^2 \leq 0.01$), genome-wide significant variants from these loci was associated with increased odds of AS in a model containing cardiovascular risk factors as covariates. The PRS also improved the ability to discriminate between AS cases and controls, when added to a model that contained cardiovascular risk factors. In gene-based and gene-set analyses, I identified additional loci and co-regulated gene sets that were associated with AS. These discoveries expanded the set of loci which had been associated with the odds of AS, implicated lipid metabolism, inflammation, and calcification as key processes contributing to the disease, and demonstrated that genetic data can improve the assessment of a patient's probability of disease beyond information that is available within a routine clinical visit.

Contributions of Authors

Chapter Two

Hao Yu Chen, James C. Engert, George Thanassoulis. Risk factors for valvular calcification. *Current Opinion in Endocrinology, Diabetes and Obesity.* 2019;26(2):96-102. Digital object identifier (DOI): 10.1097/MED.0000000000000471.

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Chapter Four

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Association of *LPA* Variants With Aortic Stenosis: A Large-Scale Study Using Diagnostic and Procedural Codes From Electronic Health Records. *JAMA Cardiology.* 2018;3(1):18-23. DOI: 10.1001/jamacardio.2017.4266.

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Chapter 1. General Introduction

1.1 Natural History of Aortic Stenosis

Calcific aortic valve disease encompasses a continuum of disease involving calcification of the leaflets of the aortic valve. Early in the disease process, during aortic sclerosis, the leaflets begin to thicken and develop lesions with microscopic calcium deposits[1], the infiltration of inflammatory cells[2], and the accumulation of apolipoproteins B and (a)[1]. Although blood flow across the valve is unimpeded[3], aortic sclerosis patients are at an elevated risk of incident myocardial infarction, congestive heart failure, stroke, cardiovascular mortality, and all-cause mortality, including after adjustment for cardiovascular risk factors and coronary artery disease at baseline[3].

In a subset of patients with aortic sclerosis, continued extracellular matrix remodelling[4] and calcification[5] lead to increasingly immobile leaflets, obstructing the flow of blood across the aortic valve[6]. At this stage, AS has developed. While aortic sclerosis precedes AS, not all sclerotic individuals develop AS. In a retrospective analysis of an echocardiography database, 338 (16%) of 2,131 participants with aortic sclerosis at baseline developed AS over a mean follow-up of more than 7 years[7]. In contrast, one out of the 100 (1%) age- and time-matched controls developed AS during follow-up. A similar study followed 400 sclerotic patients for a mean of 3.7 years and observed that 131 individuals (33%) developed AS[8]. Moreover, our understanding of the factors which contribute to the progression of sclerosis to stenosis is limited. In an analysis of echocardiographic variables, Cosmi and colleagues observed that mitral annular calcification was the only independent predictor of progression to AS[7]. It remains unclear whether cardiovascular risk factors, lifestyle, or genetic variants also contribute to this progression.

AS is the clinically-relevant end stage of calcific aortic valve disease. Identification of a potentially stenotic patient occurs during an auscultation of the heart, in which a loud murmur can be heard during systole[9]. A formal diagnosis is made using Doppler echocardiography to estimate the peak velocity of blood flow across the aortic valve[10], with 2.6 to 3 m/s classified as mild AS, 3 to 4 m/s classified as moderate, and greater than 4 m/s classified as severe[11]. Contemporary treatment involves a transcatheter or surgical replacement of the aortic valve, with the choice of approach dependent on the severity of disease and the presence or absence of symptoms[12]. However, not all patients undergo aortic valve replacement due to patient refusal, misclassification of disease severity, misattribution of the AS symptoms, mildness of disease, or excessive surgical risk[13]. In the absence of an aortic valve replacement, patient prognosis is poor. No medical treatment has been approved for AS, thus medical therapy is limited to managing symptoms and comorbidities[14]. The three-year survival in medically-managed patients 70 years or older is 49%, as compared to 80% in patients who have undergone surgical aortic valve replacement[15]. The majority of patients with severe AS require hospice care (28%) or home nursing care (52%), and five-year survival is only 11.6%[16].

Abnormal valve morphology is a key accelerant of AS disease progression. The normal aortic valve contains three semilunar leaflets, and each pair of leaflets meets to form a commissure[17]. However, fusion of some or all of the commissures may occur *in utero*, leading to the formation of a congenitally bicuspid or unicuspid aortic valve[18]. Valvulitis, including from rheumatic fever, can also cause commissural fusion in native tricuspid valves and hence, acquired bicuspid aortic valves[19]. Whereas tricuspid AS patients most commonly undergo aortic valve replacement between 71 and 80 years of age, aortic valve replacement is most common a decade earlier in

bicuspid patients and three decades earlier in unicuspid patients[20]. Among medically-managed patients, mortality also occurs earlier in those with unicuspid or bicuspid valves, with a mean age of 52, 63, and 70 years in unicuspid, bicuspid, and tricuspid patients, respectively[21].

1.2 Prevalence and Risk Factors for Aortic Stenosis

AS is one of the most common valvular heart diseases affecting the elderly in developed nations[22], with a prevalence of 12.4% in individuals over the age of 75[23]. It is also the most rapidly growing disease affecting the valves of the heart[24], with the number of severe AS patients over 70 years old expected to more than double by the year 2040[25]. Calcification of the aortic valve, which can be detected prior to the onset of clinical AS, is even more prevalent: 53% of individuals between 55 and 86 years of age have AVC[26].

The association of cardiovascular risk factors with AS and AVC has been well studied in the last few decades. Age is perhaps the strongest risk factor, with each standard deviation increase in age (approximately nine years) associated with 3.25 times the odds of prevalent AVC, adjusted for other cardiovascular risk factors[27]. Similarly, each decade increase in age is associated with 1.18 times greater odds of aortic sclerosis or stenosis in multivariable regression accounting for the contributions of other risk factors[28]. This is consistent with observations that AS is uncommon in young and middle-aged adults, but becomes increasingly more prevalent above the age of 60 years[22,28,29]. Male gender, smoking, higher body mass index, elevated blood pressure, and increased LDL-C also confer increased risk of prevalent or incident AS[28,30,31] and AVC[27,32]. Elevated blood pressure also accelerates AS progression, with a more rapid increase in peak aortic jet velocity among hypertensive versus normotensive AS patients (0.026 m/s/year versus 0.017

m/s/year, respectively)[33]. Similarly, AVC accumulates more rapidly in AS patients with systolic hypertension, adjusted for cardiovascular risk factors and baseline AS severity[34].

Recent studies have identified additional risk factors for AS and AVC, although replication of these findings is warranted. Patel and colleagues observed that African-Americans have 59% lower odds of severe AS compared to individuals of European descent, adjusted for cardiovascular risk factors and concomitant coronary artery disease[35]. If the aetiologies of AS indeed differ by ancestry, then the discoveries made in European-ancestry populations, which predominate AS research, may not be transferable to other ancestries. A greater emphasis on cross-ancestry replication and novel discoveries in non-European ancestries could expand our understanding of AS and highlight important differences in pathogenesis between ancestries.

Family history is another emerging risk factor for AS. In an analysis of the Utah Population Database, which features linkage of genealogical records and death certificates, Horne and colleagues observed that the average relatedness among deaths from aortic valve disease was higher than matched controls (genealogical index of familiarity, 3.44 among cases versus 2.62 among controls)[36]. Consistent with this finding, Martinsson and colleagues observed that individuals whose siblings had AS had 3.41 times the risk of developing AS, adjusted for cardiovascular risk factors and cardiovascular disease. In contrast, only a small increase in the hazard rate was observed in individuals whose spouse had AS (16% if the spouse were male and 18% if the spouse were female)[37], suggesting that the increased risk of AS is attributable to shared genetics rather than a shared environment.

Risk factors for AS and AVC are discussed in greater detail in chapter two.

1.3 Genetic Studies for Aortic Stenosis

Several lines of evidence support a heritable component for AS. Firstly, the aforementioned population-based studies demonstrated greater relatedness among AS cases than expected by chance. Secondly, Garg and colleagues reported two independent families with aortic valve disease inherited in an autosomal dominant manner. A genome-wide scan and subsequent targeted sequencing identified deleterious mutations in the *NOTCH1* gene as the likely cause, with experimental work highlighting *Notch1* expression during aortic valve development in mice[38]. Additionally, *APOB*[39], *APOE*[40], *ESR1*[41], and *VDR*[42], among others, have been identified in candidate gene studies as associated with AS, although replication of these findings has seen mixed success[43-45].

The advent of GWAS heralded a new era in AS genomics. A GWAS separately tests the association of all available genetic variants with the outcome of interest, often adjusted for age and sex. To account for the large number of tests conducted, the genome-wide significant threshold of $p \leq 5 \times 10^{-8}$ is used, which is equivalent to a Bonferroni correction for one million independent tests. In contrast to candidate gene studies, where a small set of variants are examined on the basis of a potential mechanistic link with the outcome of interest, a GWAS is hypothesis-free. All variants are tested and significant variants can be further analyzed using other approaches, as appropriate, to elucidate the biology underlying their association with the outcome. Replication of significant associations in independent cohorts is also a critical step in the GWAS approach, to reduce the number of false positives reported.

The first GWAS for calcific aortic valve disease was performed by Thanassoulis and colleagues, who identified the *LPA* locus as associated with prevalent AVC and incident AS[46]. This association has since been robustly replicated in other cohorts[47-49]. The *LPA* gene codes for apolipoprotein(a), which binds to the apolipoprotein B moiety of a cholesterol-rich lipoprotein to form Lp(a)[50]. Following the discovery of the *LPA* locus, several studies demonstrated that elevated levels of Lp(a) were associated with a greater risk of developing AS. Arsenault and colleagues observed that participants in the top tertile of Lp(a) levels were at 57% higher hazard of AS compared to participants in the lowest tertile, adjusted for age, sex, and smoking[51]. A separate study by Kamstrup and colleagues similarly observed that individuals with Lp(a) levels above the 67th percentile had 1.6 to 2.9 times the hazard of AS relative to individuals with Lp(a) levels below the 22nd percentile, adjusted for cardiovascular risk factors[47]. Individuals free of AS, but with elevated Lp(a) levels (≥ 75 nmol/L), have on average 40% higher microcalcification in their aortic valves[52], which may provide a mechanistic explanation for their increased hazard of AS. Disease progression is also accelerated among AS patients in the top tertile of Lp(a) levels, relative to patients in the bottom two tertiles (annualized change in peak aortic jet velocity, +0.26 m/s/year versus +0.17 m/s/year)[53]. Clinical trials have demonstrated that pharmacological lowering of Lp(a) levels is attainable and results in very few adverse effects[54-56], but it remains to be seen whether Lp(a) lowering will be an effective treatment option in delaying or reversing the progression of AS.

Recent genome- and transcriptome-wide association studies have identified additional genetic loci associated with AS. Thériault and colleagues[57], and Helgadottir and colleagues[58], separately

identified the *PALMD* locus. In their publication, Helgadottir and colleagues also identified the *TEX41* locus. The lead variants in these loci were likewise associated with bicuspid aortic valves and atrial septal defect[58], two common congenital defects, suggesting these associations with AS may be mediated through impaired embryonic development of cardiac structure leading to morphological valvular abnormalities. Further study is required to assess whether these variants remain associated with AS among individuals with tricuspid aortic valves, and to investigate the therapeutic potential of targeting these loci if indeed their effects on AS are mediated by congenital abnormalities.

1.4 Post Genome-Wide Association Study Approaches

Translating genetic discovery to clinical practice remains a key focus for AS research. The variants identified in a GWAS can inform the construction of a GRS or PRS which reflects genetic predisposition to the outcome tested in the GWAS. Specifically, for a list of usually genome-wide significant variants in low linkage disequilibrium, the number of risk-increasing alleles are summed in each individual to create an unweighted risk score. A weighted risk score is calculated by multiplying the number of risk alleles of each variant by its effect size before summation. Due to the flexibility of the GWAS method, the instrumentation of genetic predisposition to a risk factor for disease, or to the disease itself, is possible. Moreover, the use of genetic variation, which is determined at conception, allows risk scores to be calculated in early life, before the development of risk factors[59,60]. GRS have been associated with incident cardiovascular events[61-64], with a comparable area under the curve (AUC) to non-genetic risk scores after adjustment for age and sex[65], and may improve the net reclassification index when used in conjunction with non-genetic risk scores[66]. For a late-onset disease such as AS, a GRS or PRS could identify high-risk

individuals decades prior to disease development, potentially allowing for behavioural modification or clinical intervention to delay or avoid the onset of disease.

Furthermore, the widespread application of GWAS has spurred the development of methods which integrate GWAS summary statistics with external datasets to enhance our functional understanding of implicated loci. Variant-based annotation approaches such as RegulomeDB[67] and Combined Annotation Dependent Deletion[68] integrate observed data or predictions regarding transcription factor binding, chromatin state, evolutionary conservation, and other inputs to score the deleteriousness of variants. Gene-based approaches aggregate all genetic variants near or within a specific gene, and assess the average effect of these variants for association with the outcome. Approaches such as MAGMA[69] define genes using the genetic variants found between the transcription start and end sites of the gene, while MetaXcan[70] and other similar approaches construct genes from the genetic variants associated with expression of the gene (*i.e.* expression quantitative trait loci). Gene set approaches such as MGSEA[71] further aggregate genes based on membership in the same pathway, related biological function, or co-regulation. The public availability of data from the Genotype-Tissue Expression (GTEx) project[72], the Kyoto Encyclopedia of Genes and Genomes (KEGG)[73], the Functional Annotation of the Mammalian Genome (FANTOM)[74], and many other databases have greatly informed downstream analyses of GWAS results. The large numbers of post-GWAS analytic methods and publicly available datasets have started to bridge the gap between identifying risk loci and a functional understanding of how these loci confer disease risk.

The biomarkers and pathways identified using post-GWAS methods form the short list of candidates to identify causal mechanisms, essential knowledge for the development of pharmaceutical therapies. In case-control studies, a popular study design for genetic studies, causal inference is difficult as the temporality of the exposure-outcome relationship remains unclear[75]. Even in genetic case-control studies, establishing causality remains challenging. While the genetic variation clearly precedes the outcome, satisfying the temporality criterion, the variant may not be causal, being instead in linkage disequilibrium with the causal variant[76]. Thus, the association of genetic variation at a locus coding for a biomarker or pathway with a plausible biological link to the disease is not satisfactory evidence that the biomarker is causal.

Mendelian randomization is a method that permits causal inference of a risk factor for a disease or other trait. A form of instrumental variables analysis, this method uses genetic variants as an instrument (or proxy) for an exposure of interest. Since genetic variation is determined at conception, the variants precede the outcome and are unaffected by many confounders such as socioeconomic status and environmental factors. Similar to the selection of variants for a GRS, the genetic instrument is usually constructed from variants established to be associated with the exposure, such as genome-wide significant variants in a GWAS or expression quantitative trait loci. That is, the variants correlate with observable changes in the exposure and the genetic instrument reflects a continuum of risk for this exposure. If the variants are assumed not to directly associated with the outcome nor with potential confounders, then an association between the genetic instrument and the outcome indicates the exposure is causal.

For studying continuous outcomes where the exposure and outcome have been measured within the same cohort, MR can be performed using two-stage least squares regression[77]. When either the exposure and outcomes data are not available or the research question involves discrete outcomes, summary-level estimates from two different samples can be used instead[78,79]. With the increasing availability of GWAS summary statistics for many traits, MR can be used to assess the causality of a wide variety of exposure-outcome associations – without having measured the exposure or outcome oneself. Even when individual-level data are available, it may be preferable to use summary statistics from large meta-analyses as the large number of participants may mean more precise estimates.

As alluded to above, three assumptions underpin the MR framework: the instrument is associated with the exposure, the instrument is independent of the outcome conditional on the exposure and confounders, and the instrument is independent of all possible confounders of the exposure-outcome relationship[78]. Two additional assumptions are required if the causal effect is to be quantified: the exposure has a linear effect on the outcome and this effect is not subject to effect modification[80]. When these assumptions are violated, the presence of a causal effect can still be ascertained but the quantification of its magnitude may be erroneous[80].

Steps can be taken to mitigate the violation of these assumptions. If allele frequencies and rates of the outcome vary in different ancestral populations, *i.e.* population stratification, estimates from a single ancestral group should be used or population ancestry should be included in the models producing the estimates[81]. Variants in the instrument may be in linkage disequilibrium with other variants that have indirect or direct effects on the outcome[82]. Variants may also be pleiotropic, and

be associated with a confounder or with another exposure also associated with the outcome[82]. It is often difficult to identify variants in these two conditions but if the associations are known, they can be removed or accounted for in the analysis. For instance, multivariable MR can be used to simultaneously test for the causal effect of multiple exposures with shared genetic aetiology[78]. In robust MR methods such as the weighted median or Egger approaches, pleiotropic variants can also be identified through outlier detection and downweighted or removed[83,84]. Lastly, canalization, or the compensatory response of the body to the effects of a genotype, may mean that the predicted causal effect on the outcome may not occur[81].

1.5 Research Objectives and Hypotheses

The genetic etiology of AS remains poorly understood. Evolving genetic and bioinformatic approaches and growing functional databases are promising resources for developing mechanistic insight into known or novel genetic associations.

In this thesis, I hypothesized that:

1. Additional genetic loci are associated with AS, beyond *LPA*, *PALMD*, and *TEX41*
2. Genetic and bioinformatic approaches can provide mechanistic insight into how known or newly identified genetic loci confer increased risk of AS

Accordingly, my objectives were to:

1. Conduct a narrative review of the modifiable and genetic risk factors for AS
2. Quantify the association of modifiable and genetic factors with AS and assess for evidence of a causal relationship

3. Perform GWAS to identify additional loci for AS
4. Apply bioinformatic approaches and incorporate functional data to better understand how genetic loci contribute to AS development

1.6 Organization of the Chapters

Following the introduction to AS and genetic approaches found in this chapter, evidence for established and emerging risk factors are described in chapter two. Chapter three quantifies the observational and causal contributions to AS made by risk factors, while chapter four examines how genetic variants in the *LPA* locus associate with the disease. Chapters five and six use the GWAS approach to identify additional loci for AS. A general discussion of our findings as they relate to external AS research is undertaken in chapter seven, with conclusions and future directions explored in chapter eight.

Chapter 2. Established and Emerging Risk Factors for Aortic Stenosis

2.1 Preface to Chapter 2

Over the last several decades, the aetiology of AS has changed. Among 374 AS patients who underwent aortic valve replacement at the Mayo Clinic in the years 1965, 1970, 1975, and 1980, the most frequent causes of AS were congenitally bicuspid valves (46%) and rheumatic disease and other inflammatory conditions (35%), with so-called degenerative calcification accounting for only 10% of cases[85]. Between 1981 and 1985, however, the proportion of AS cases at the institution caused by congenital bicuspid valves and inflammatory conditions fell to 38% and 24%, respectively. Degenerative calcification became the second most common cause of AS, accounting for 33% of the 646 AS cases seen over the five year period[86].

This increasingly prevalent form of AS was termed degenerative because it was thought to be a consequence of aging[87]. Immunohistochemical studies thereafter demonstrated this to be a misnomer: diseased aortic valves were found to feature subendothelial thickening, lipid accumulation, mineral deposition, and macrophage infiltration[1,2], indicating an active disease process rather the wear-and-tear hypothesis previously advanced[88]. With this understanding came a change in the nomenclature, with degenerative AS renamed more appropriately as calcific AS.

The discovery of an active process underlying calcific AS development also implied the existence of initiation and progression factors. In the years following the pivotal discovery, many studies have investigated genetic and clinical risk factors for calcification and stenosis of the aortic valve. In the subsequent manuscript, which was an invited narrative review on the risk factors for valvular calcification, I summarised the evidence supporting established risk factors of AVC and calcific

AS. I also highlighted potential risk factors for which additional research is required. Lastly, I summarised the literature concerning risk factors for mitral annular calcification.

Risk Factors for Valvular Calcification

Hao Yu Chen[1,2], James C. Engert[1,2,3], George Thanassoulis[1,2]

[1]Division of Experimental Medicine, McGill University, Montreal, Canada

[2]Preventive and Genomic Cardiology, McGill University Health Centre and Research Institute, Montreal, Canada

[3]Department of Human Genetics, McGill University, Montreal, Canada

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2.2 Abstract

Purpose of Review: Recent literature is examined to identify established and emerging risk factors for valvular calcification, specifically aortic valve disease and mitral annular calcification.

Recent Findings: Strong evidence implicates older age, male sex, cigarette smoking, elevated blood pressure, dyslipidemia, adiposity, and mineral metabolism as risk factors for calcific aortic valve disease. Emerging evidence suggests family history and lipoprotein(a) are additional risk factors. Recently, large-scale genome-wide analyses have identified robust associations for *LPA*, *PALMD*, and *TEX41* with aortic stenosis. Factors predisposing to mitral annular calcification are less well characterized. Older age, cigarette smoking, increased body mass index, kidney dysfunction, and elevated triglycerides are associated with greater risk of mitral annular calcification, but conflicting evidence exists for sex and C-reactive protein.

Summary: Established and emerging risk factors for calcific aortic valve disease, including some that overlap with atherosclerosis, may represent targets for pharmacological intervention. Mitral annular calcification is comparatively less well understood though some atherosclerosis risk factors do appear to increase risk.

Keywords

Calcific Aortic Valve Disease, Aortic Stenosis, Mitral Annular Calcification, Risk Factor, Genomics

Abbreviations

AVC: Aortic Valve Calcium

CAVD: Calcific Aortic Valve Disease

CHS: Cardiovascular Health Study

CI: Confidence Interval

FOS: Framingham Offspring Study

HR: Hazard Ratio

LDL-C: Low-Density Lipoprotein Cholesterol

MAC: Mitral Annular Calcification

MESA: Multi-Ethnic Study of Atherosclerosis

OR: Odds Ratio

RR: Risk Ratio

SD: Standard Deviation

2.3 Main Text

Introduction

In calcific aortic valve disease (CAVD), valvular calcification is observed on the cusps of the aortic valve and ranges from aortic sclerosis, when calcific nodule formation and leaflet thickening are relatively mild and individuals are asymptomatic, to aortic stenosis, where substantial nodular development and stiffened leaflets lead to severe obstruction of blood flow through the valve and individuals become symptomatic. Prognosis is poor: aortic sclerosis is associated with 68% higher risk of coronary events, 69% higher risk of cardiovascular mortality, and 36% higher risk of all-cause mortality[89], and without aortic valve replacement, less than one-third of patients with severe aortic stenosis survive beyond 5 years[16,90].

In developed nations, CAVD is the most prevalent valvular heart disease among the elderly[91]. Aortic sclerosis afflicts between 2.3% and 51.7% of older individuals, with the prevalence of sclerosis increasing dramatically with age[89]. Aortic stenosis is observed in 1.7% of individuals 65 years or older[92] but 12.4% of individuals greater than 75 years of age[23]. The prevalence of aortic stenosis demonstrates a similar dependency on age[29] and is expected to more than double by the year 2040 and triple by the year 2060[25] due to the ageing population. Healthcare costs are expected to increase in tandem and in the United States, the cost of medically-managed severe aortic stenosis is already estimated to be $1.3 billion per year[16].

Mitral annular calcification (MAC) is a less common form of valvular calcification that occurs at the fibrous base of the mitral valve. It affects 9-20% of the population[27,93] and likewise increases in prevalence with age. MAC has been associated with 50% greater risk of incident cardiovascular

disease, 60% greater risk of cardiovascular death, and 30% greater risk of all-cause death, adjusted for cardiovascular risk factors[94]. Individuals with MAC are also more likely to have severe coronary artery disease[95] and to sustain a stroke[96].

Currently, no medical therapy exists for either CAVD or MAC. Epidemiological studies spanning the last three decades have implicated a number of risk factors which associate with valve calcification. Recent studies, including large-scale genome-wide studies, have identified novel risk factors. In this review, we discuss established and emerging risk factors for both CAVD and MAC.

Established Risk Factors for CAVD

Older Age

Due to the advanced ages of CAVD patients, the disease was thought to be a degenerative condition acquired due to ageing. Although this view has been overturned, age remains a strong risk factor for CAVD. Each 10-year increase in age among participants of the Cardiovascular Health Study (CHS) was associated with double the odds of CAVD (odds ratio [OR], 2.18; 95% confidence interval [CI], 2.15-2.20; $p<0.001$), adjusted for clinical factors[28]. In the Multi-Ethnic Study of Atherosclerosis (MESA), the risk for aortic valve calcium (AVC) more than doubled per decade (risk ratio [RR], 2.19; 95% CI, 1.84-2.61; $p<0.001$)[32] (Table 2.1).

Male Sex

With the exception of Boon *et al*[97], male sex has been consistently associated with increased risk for AVC and aortic stenosis. Relative to women, men in the Framingham Offspring Study (FOS) had 56% higher odds of AVC (95% CI, 1.15-2.13; $p=0.005$) in models adjusted for atherosclerotic

risk factors[27]. Similarly, men were more likely than women to have aortic sclerosis or stenosis in the CHS (OR, 2.03; 95% CI, 1.7, 2.5; $p<0.001$), independent of other clinical factors[28].

Cigarette Smoking

Smoking has been strongly associated with increased risk of CAVD. In the FOS, greater cigarette use was associated with elevated odds for AVC (OR per standard deviation [SD] of cigarettes per day, 1.22; 95% CI, 1.06-1.39; $p=0.005$), adjusted for other cardiovascular factors[27]. In the MESA, current smokers more than doubled their risk of incident AVC relative to never smokers independent of other clinical factors (RR, 2.49; 95% CI, 1.49-4.15; $p=0.001$), though smoking was not associated with the progression of calcification[32]. Current smokers also had higher odds for aortic stenosis in the CHS (OR, 1.35; 95% CI 1.1-1.7; $p=0.006$)[28].

Blood Pressure and Vascular Stiffness

A number of studies have demonstrated an association between hypertension and CAVD. In the Helsinki Ageing Study, hypertension was associated with AVC independent of the effects of age and body mass index (OR, 1.74; 95% CI, 1.19-2.55; $p=0.005$)[87]. A more modest effect was observed in the CHS, which accounted for additional clinical factors (OR, 1.23; 95% CI, 1.1-1.4; $p=0.002$). Within the MESA, a graded correlation was observed between the stages of hypertension, as defined by the Joint National Committee 7, and AVC: calcification was observed in 6% of normotensive individuals, 11% of borderline hypertensive individuals, 17% of individuals with stage I hypertension, and 16% of individuals with stage II hypertension The association of hypertension with prevalent AVC was also modified by age (interaction $p=0.041$),

such that hypertensive individuals doubled their odds of calcification, but only before 65 years of age (adjusted OR, 2.31; 95% CI, 1.35-3.94).

Hypertension has also been associated with more rapid progression of CAVD. Peak aortic jet velocity, a measure of aortic stenosis progression, increased more rapidly in hypertensive versus non-hypertensive individuals (annualized change in velocity, 0.26±0.23 m/s versus 0.17±0.20 m/s; $p<0.01$) in a retrospective, echocardiography study[33]. Faster progression was also observed among individuals with systolic hypertension in the PROGRESSA study (2-year change in median [25th-75th percentile] AVC, 370 [126-824] versus 157 [58-303] among normotensives; $p=0.007$), and this association persisted following adjustment for clinical factors and baseline AVC[34].

In the MESA, a comparison of blood pressure measures and their association with prevalent AVC demonstrated that the largest effect was observed for pulse pressure (OR per 10 mmHg, 1.41; 95% CI, 1.21-1.64 among individuals <65 years old and 1.14; 95% CI, 1.05-1.23 among individuals ≥65 years old)[98]. Pulse pressure is a measure of arterial stiffness and wave reflection, suggesting that abnormal hemodynamics may promote calcification. Consistent with this hypothesis, greater wave reflection, as represented by the augmentation index, was associated with elevated odds for moderate to severe diffuse AVC independent of demographic variables and glomerular filtration rate in the Cardiac Abnormalities and Brain Lesions cohort (OR per 1% increase in augmentation index, 1.08; 95% CI, 1.02-1.14; $p=0.005$)[99]. Increased wave reflection leads to greater tensile stress on the cusps of the aortic valve, and exposure of porcine aortic valve interstitial cells to such stress initiates extracellular matrix remodelling[100], which precedes thickening and calcification of the aortic valve.

Dyslipidemia

Whether elevated low-density lipoprotein cholesterol (LDL-C) is a risk factor for CAVD remains controversial in light of apparently discrepant findings between observational studies and randomized controlled trials. An immunohistological study found that apolipoprotein B and apolipoprotein(a) co-localized with calcium in the lesions of stenotic valves, but neither apolipoprotein was detected in normal valves[1]. The distribution of apolipoprotein B was also more diffuse than that of apolipoprotein(a), suggesting LDL-C is found in the diseased aortic valve in addition to lipoprotein(a) (see section on lipoprotein[a]). This finding was supported by observations in the CHS that higher levels of LDL-C were associated with greater odds of developing aortic sclerosis (OR, 1.00; 95% CI, 1.00-1.01; p=0.002)[101] and increased odds for prevalent aortic sclerosis or stenosis (OR per mg/dl, 1.12; 95% CI, 1.03-1.23; p=0.008)[28], adjusted for other clinical factors. In the FOS, levels of total cholesterol, which correlate highly with levels of LDL-C, in early adulthood strongly predicted the presence of AVC in later life (adjusted OR per SD, 1.81; 95% CI, 1.55-2.11; p<0.0001)[27]. Retrospective studies have also shown that aortic stenosis patients treated with statins progressed less rapidly[102-104].

Building upon this body of evidence, three randomized controlled trials investigated the use of lipid-lowering therapies in delaying or reversing aortic stenosis progression. In the Scottish Aortic Stenosis and Lipid Lowering Trial, Impact on Regression (SALTIRE), 155 patients randomized to either atorvastatin (80 mg) or placebo were followed for a median of 25 months[105]. The Simvastatin and Ezetimibe in Aortic Stenosis (SEAS) trial followed 1,873 patients with mild to moderate, asymptomatic aortic stenosis for a median of 52.2 months following randomization to

simvastatin (40 mg) plus ezetimibe (10 mg) or placebo daily[106]. In the Aortic Stenosis Progression Observation: Measuring Effects of Rosuvastatin (ASTRONOMER) trial, 269 patients were randomized to rosuvastatin (40 mg) or placebo daily and followed for a median of 3.5 years. No reductions in aortic stenosis progression were observed in any of these trials.

Although the results of these trials were disappointing, it would be premature to dismiss the therapeutic potential of statins for CAVD. In all three trials, patients had relatively advanced disease with elevated transaortic pressure gradients and mean aortic valve areas ≤1.5 cm^2. Lipids may exert a larger influence on the initiation of the disease process and earlier intervention, perhaps among patients with aortic sclerosis, may be more effective. Mendelian randomization analysis performed in the Malmö Diet and Cancer Study demonstrated that genetically-elevated levels of LDL-C, reflecting a lifetime exposure to higher LDL-C, were causally associated with the incidence of aortic stenosis (hazard ratio [HR] per genetic risk score unit, 2.78; 95% CI, 1.22-6.37; p=0.02)[107]. Moreover, individuals were not eligible to participate in the trials if they had clinical indications for cholesterol lowering, but these individuals would benefit most from intensive lipid-lowering therapies and their exclusion could underestimate the effectiveness of such regimens. While lipid-lowering therapies may be ineffective at delaying progression in advanced aortic stenosis cases, additional studies are warranted to assess the effectiveness of statins in early stage CAVD.

Adiposity

Several adiposity measures have been associated with greater risk for CAVD. In the MESA, metabolic syndrome and diabetes separately increased odds of developing AVC (OR, 1.67; 95%

CI, 1.21-2.31 and 2.06; 95% CI, 1.39-3.06, respectively), adjusted for clinical factors, but neither were associated with progression among participants with calcification at baseline[108]. Diabetes was also associated with greater risk of incident aortic stenosis in the Cardiovascular Health in Ambulatory Care Research Team cohort, which featured a 13-year median follow-up period (HR, 1.49; 95% CI, 1.44-1.54; $p<0.001$)[31]. Long-term mean of body mass index in adulthood was associated with the presence of AVC (OR per SD, 1.21; 95% CI, 1.05-1.40; $p=0.008$) in the FOS, adjusted for clinical factors. Being overweight or obese also conferred increased risk of incident aortic stenosis relative to normal weight (HR, 1.24; 95% CI, 1.05-1.48 and 1.81; 95% CI, 1.47-2.23, respectively). These findings indicate that increased adiposity confers risk for CAVD, but additional work is required to identify possible mechanisms, such as dysglycemia or dyslipidemia.

Kidney Dysfunction and Mineral Metabolism

CAVD is commonly observed among patients with renal failure, with AVC reported in 28% of individuals ≤70 years old with end stage renal disease[109]. Kidney dysfunction has also been linked to more rapid progression of, and more severe, disease. Aortic stenosis patients undergoing dialysis progress more rapidly (change in valve area in cm^2/year, -0.19; range, -1.45 to 0.20 versus -0.07; range, -1.1 to 0.37; $p<0.001$), and dialysis remains an independent predictor of annualized changes in aortic valve area following adjustment for clinical variables (cm^2/year, -0.92; $p=0.02$)[110]. Calcium, phosphate, and the calcium × phosphate product were elevated among end-stage renal disease patients with AVC (all $p<0.05$)[109]. Among aortic stenosis patients treated with hemodialysis, greater serum calcium levels predicted faster progression (OR, 6.08; 95% CI, 1.28-28.8; $p=0.02$). In community cohorts, higher serum phosphate has also been associated with greater

risk for AVC (RR per mg/dl, 1.3; 95% CI, 1.1-1.5; $p<0.001$)[111] as well as aortic sclerosis (OR per 0.5 mg/dl, 1.17; 95% CI 1.04-1.31; $p=0.01$)[112].

Emerging Risk Factors for CAVD

Family History

Recently, a few studies have suggested that aortic stenosis has a familial component. Using national registry data on 6.1 million Swedish siblings, including 13,442 cases of aortic stenosis, Martinsson and colleagues observed that 4.8% of aortic stenosis patients had a sibling with aortic stenosis, compared to 0.5% among the general population[37]. Following adjustment for age, sex, family size, and comorbidities, individuals with a sibling history of aortic stenosis had more than three times the risk (HR, 3.41; 95% CI, 2.23-5.21). In contrast, having a spouse with aortic stenosis was associated with a smaller increase in risk (HR, 1.16; 95% CI, 1.05-1.28 for husbands and 1.18; 95% CI, 1.07-1.30 for wives), suggesting that shared environment in adulthood explains less risk than genetics. Using the Utah Population Database, which features linkage of genealogical records with death certificates, Horne and colleagues demonstrated that individuals who died of non-rheumatic aortic stenosis were on average more related than the average relatedness observed among matched controls (genealogical index of familiality, 3.44 in cases versus 2.62 in controls; $p<0.001$)[36]. When restricted to deaths before 65 years of age, the higher relatedness among cases was more pronounced, consistent with other partially heritable disorders (genealogical index of familiality, 8.47 in cases versus 2.43 in controls; $p<0.001$). In regions of France with little population movement, the distribution of aortic stenosis cases who undergo aortic valve replacement is heterogeneous and some families have a higher than expected disease prevalence[113,114], but these lines of evidence are weaker.

Lipoprotein(a)

A rapidly-growing body of evidence points to lipoprotein(a) as an important, potentially causal contributor to CAVD. A genome-wide association study (GWAS) performed in the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium associated a variant at the *LPA* locus, rs10455872, with both prevalent AVC (OR per G allele, 2.05; 95% CI, 1.66-2.53; $p=2.8\times10^{-11}$) and incident aortic stenosis (HR per G allele, 1.68; 95% CI, 1.32-2.15; $p=3\times10^{-5}$)[46]. The association of this variant with aortic stenosis has since been robustly demonstrated in a number of cohorts[47-49,51]. The *LPA* gene codes for apolipoprotein(a), and >90% of the variation in lipoprotein(a) levels has been attributed to genetic variation in *LPA*[115]. The association of *LPA* with AVC and aortic stenosis therefore implicates elevated lipoprotein(a) as a risk factor for CAVD. Mendelian randomization studies have confirmed that this association is likely causal[46,47], pointing to lipoprotein(a) as a therapeutic target. Lipoprotein(a)-lowering agents are currently in development and randomized controlled trials have demonstrated remarkable reductions in lipoprotein(a) levels[55,116], paving the way for future trials investigating lipoprotein(a)-targeting therapeutics for arresting aortic stenosis progression.

Findings from Large-Scale Genetic Association Studies

Two large-scale GWAS for aortic stenosis have been reported in the years following the discovery of *LPA*. A study in the deCODE cohort and a transcriptome-wide association study in the QUEBEC-Calcific Aortic Valve Stenosis cohort independently identified a variant in the *PALMD* locus as associated with aortic stenosis, with each copy of the risk allele conferring 20-28% greater odds for the disease[57,58]. The genome-wide association study in deCODE additionally identified

the *TEX41* variant rs1830321, which was associated with 15% greater odds for aortic stenosis per T allele (95% CI, 1.11-1.20; $p=1.8\times10^{-13}$). Both the *PALMD* and *TEX41* variants were also associated with bicuspid aortic valves and atrial septal defects (all $p\leq5.9\times10^{-3}$), implicating congenital cardiac malformations as a mechanism for CAVD.

Risk Factors for MAC

Our understanding of the risk factors for MAC remains poor due to the relatively low prevalence of MAC in the general population leading to very few adequately-powered cohorts. As observed for CAVD, the most critical risk factor is older age. In the MESA, each 10-year increase was associated with 3.58 times the odds of MAC adjusted for clinical factors (95% CI, 3.18-4.03; $p<0.01$)[93]. Body mass index and cigarette smoking also conferred elevated odds for MAC in both the MESA and the FOS, while diabetes was only associated in the MESA (adjusted OR, 1.58; 95% CI, 1.25-1.99)[27,93]. In the FOS, chronic kidney disease was associated with 60% greater odds for MAC independent of clinical factors (95% CI, 1.03-2.5; $p<0.05$; Table 2.2)[117].

Unlike CAVD, women may be more likely than men to have MAC. In the MESA, the odds were 41% higher for women (95% CI, 1.15-1.75)[93]. However, female sex was not a predictor of MAC in the FOS[27]. Conversely, C-reactive protein was associated with MAC in the FOS (adjusted OR per SD, 1.29; 95% CI, 1.10-1.52; $p=0.002$) but not in the MESA, though it has not been associated with AVC in either cohort[27,32]. Also notable was the lack of association for MAC with LDL-C. Rather, Mendelian randomization analyses supported a causal association with triglycerides in the Cohorts for Heart and Aging Research in Genomic Epidemiology cohort (OR per genetic risk score unit, 1.73; 95% CI, 1.24-2.41; $p=0.0013$) that persisted in sensitivity analyses accounting for

genetic pleiotropy or the inclusion of Hispanic-Americans[118]. This finding suggests that triglycerides may represent a new therapeutic target for the treatment or prevention of MAC.

Conclusion

Valvular calcification, manifesting as either CAVD or MAC, is particularly prevalent among the elderly. Strong evidence implicates atherosclerotic risk factors as increasing the risk of CAVD, and emerging evidence indicates family history, lipoprotein(a), and some genetic variants are also likely contributors. Risk factors for MAC overlap with those for CAVD, suggesting a shared aetiology, but female sex, C-reactive protein, and triglycerides may be more important for MAC. Among these are risk factors with potential for therapeutic targeting, though a better characterization of their contributions is warranted given the failure of statins in randomized controlled trials for aortic stenosis. Large-scale genetic analyses and Mendelian randomization may provide valuable insight in this regard.

Table 2.1. Summary of Evidence for Risk Factors for Calcific Aortic Valve Disease.

Risk Factor	Strength of Evidence	
	Observational Studies	**Genetic Studies**
Established Risk Factors		
Older Age	+++	n/a
Male Sex	+++	n/a
Cigarette Smoking	++	n/a
Blood Pressure and Vascular Stiffness	++	n/a
Dyslipidemia	+[a]	+++
Adiposity	++	n/a
Kidney Dysfunction and Mineral Metabolism	++	n/a
Emerging Risk Factors		
Family History	+	n/a
Lipoprotein(a)	++	+++
Findings from Large-Scale Genetic Association Studies	n/a	++

+++, strong evidence of increased risk; ++, moderate evidence of increased risk; +, weak evidence of increased risk; n/a, not available.

[a]Discrepant findings between observational studies and randomized controlled trials.

Table 2.2. Summary of Evidence for Risk Factors for Mitral Annular Calcification.

Risk Factor	Strength of Evidence	
	Observational Studies	**Genetic Studies**
Established Risk Factors		
Older Age	+++	n/a
Adiposity	+	n/a
Cigarette Smoking	++	n/a
Triglycerides	n/a	++
Emerging Risk Factors		
Kidney Dysfunction	+	n/a
Female Sex	+	n/a
C-Reactive Protein	+	n/a

+++, strong evidence of increased risk; ++, moderate evidence of increased risk; +, weak evidence of increased risk; n/a, not available.

Key Points

- Atherosclerotic risk factors have been well established as increasing the risk of calcific aortic valve disease
- Emerging risk factors for calcific aortic valve disease include a family history of the disease, lipoprotein(a), and genetic variants
- Large-scale genetic analyses and Mendelian randomization studies have identified risk loci with strong evidence of association with aortic stenosis
- Current understanding of the risk factors for mitral annular calcification is limited. While a subset of atherosclerotic risk factors has been shown to increase mitral annular calcification risk, the evidence was inconsistent for some others and non-atherosclerotic risk factors have not been well studied

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Conflicts of interest

G.T. has received consulting fees from Ionis Pharmaceuticals and has participated in advisory boards for Amgen and Sanofi. The other authors report no relevant disclosures.

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Chapter 3. Associations of Risk Factors with Aortic Stenosis

3.1 Preface to Chapter 3

My review of the calcific aortic valve disease literature in the preceding chapter revealed that risk factors associated with other cardiovascular diseases, namely older age, male sex, smoking, higher LDL-C, and elevated adiposity, were also associated with increased odds of AVC and AS. The evidence for these cardiovascular risk factors often came from studies with low numbers of AS cases, which may lead to imprecise estimates of disease odds. The low prevalence of AS in the general population (less than 1%) can impede the recruitment of larger numbers of AS cases. Diagnosis codes derived from electronic health records (EHR) have been shown to be a cost-effective and efficient alternative for identifying cases, which has facilitated the study of AS in larger study samples and with greater numbers of cases.

The literature review also highlighted the limited genetic evidence supporting observational associations. Particularly in cross-sectional studies where causality cannot be determined using traditional epidemiological analysis, genetic analysis using MR can provide insight into whether risk factors are causal contributors to AS. Large GWAS have been conducted for risk factors such as diabetes, smoking, and blood pressure, permitting the creation of PRS for each risk factor which can be applied to a cohort of interest, even if the risk factor is not available in the cohort.

Using an EHR-derived definition, I identified in the GERA cohort one of the largest collections of AS cases in the world. In the following manuscript, I estimated the association of cardiovascular risk factors with AS in the GERA cohort, adjusted for the concomitant effects of other risk factors. The larger case numbers could lead to improved precision in the odds of AS estimated for each

risk factor. I also performed MR to assess the causal contribution of risk factors, allowing for a direct comparison of genetic and observational results.

Observational and Genetic Associations of Cardiovascular Risk Factors with Aortic Stenosis

Hao Yu Chen[1,2], Line Dufresne[2], Hannah A. Burr[2], Athithan Ambikkumar[2], Katia Desbiens[2], Dilrini K. Ranatunga[3], Rachel A. Whitmer[4], Mark Lathrop[5,6], James C. Engert[1,2,5*], George Thanassoulis[1,2*]

[1]Division of Experimental Medicine, McGill University, Montreal, Canada

[2]Preventive and Genomic Cardiology, McGill University Health Centre Research Institute, Montreal, Canada

[3]Division of Research, Kaiser Permanente of Northern California, Oakland, United States

[4]Department of Public Health Sciences, University of California Davis, Davis, United States

[5]Department of Human Genetics, McGill University, Montreal, Canada

[6]McGill University and Genome Quebec Innovation Centre, Montreal, Canada

*Corresponding authors. The last two authors contributed equally to the manuscript.

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3.2 Abstract

Background: Aortic stenosis (AS) patients have a higher prevalence of cardiovascular risk factors, and using genetic instruments, Mendelian randomization (MR) has shown several of these risk factors have a causal role. The replication of observational and genetic associations within the same cohort could provide insight into the relative contribution of risk factors.

Methods: We included 44,703 European-ancestry participants (3,469 AS cases) aged 55 years or older from the Genetic Epidemiology Research on Adult Health and Aging (GERA) cohort. The observational associations of all seven available cardiovascular risk factors with AS were estimated using logistic regression adjusted for age, age^2, and sex, and additionally adjusted for dyslipidemia, hypertension, smoking, and diabetes mellitus in the full models. To assess the causal contributions of risk factors, we constructed genetic instruments from genome-wide association studies and performed Mendelian randomization using the inverse-variance weighted method. The Egger and penalized weighted median methods were used in secondary MR analyses.

Results: Male sex, age, diabetes mellitus, body mass index, hypertension, dyslipidemia, and smoking were independently associated with AS when considering age, age^2, sex, and other risk factors (all $p < 0.05$). Low-density lipoprotein cholesterol (odds ratio [OR] per SD, 1.27; 95% CI, 1.07 to 1.51; $p = 6.6 \times 10^{-3}$), triglycerides (OR per SD, 1.27; 95% CI, 1.01 to 1.59; $p = 0.041$), apolipoprotein B (OR per SD, 1.19; 95% CI, 1.01 to 1.40; $p = 0.035$), and systolic blood pressure (OR per SD, 1.44; 95% CI 1.05 to 1.98; $p = 0.022$) were causally associated with AS. In sensitivity analyses allowing for up to 50% of variants to be pleiotropic, only triglycerides remained causally associated with AS (OR per SD, 1.46; 95% CI, 1.13 to 1.88; $p = 3.8 \times 10^{-3}$).

Conclusions: Several cardiovascular risk factors were observationally associated with AS. MR confirmed causal contributions to AS of low-density lipoprotein cholesterol, triglycerides, and systolic blood pressure, and a novel causative role of apolipoprotein B.

3.3 Main Text

Introduction

Aortic stenosis (AS) remains the most prevalent valvular heart disease in the developed world, affecting approximately 12% of adults over the age of 75[23]. Replacement of the aortic valve is the only approved treatment for AS but is not indicated for mild stenosis[119], and may not be as effective in prolonging survival in older patients or those with multiple comorbidities[120-122]. The absence of an approved medical treatment reflects a marked gap in effective therapeutic options for AS.

Several cardiovascular risk factors are associated with the presence or progression of AS and aortic valve calcium (AVC), a subclinical phase which precedes clinical calcific AS. These include low-density lipoprotein cholesterol (LDL-C), cigarette smoking, hypertension, and diabetes[27,28,31,123]. The quantification of risk factors for AS, particularly in multivariable models account for the simultaneous effects of other risk factors, could highlight key pathways. Furthermore, Mendelian randomization (MR) can be used to ascertain modifiable factors which contribute causally to disease, and thus may be suitable targets of new or repurposed pharmaceutical agents. MR assesses the association between an exposure and an outcome using genetic variants known to associate with the exposure, mitigating issues of confounding and reverse causality[124] even in a cross-sectional study design. Recent MR studies have reported the causative roles of LDL-C[107], triglycerides[125], systolic blood pressure (SBP)[126], and body mass index (BMI)[127,128] for AS. In the present study, we estimate the observational and causal associations of several cardiovascular risk factors with AS, including adiposity, blood pressure, lipid, and smoking traits, to replicate previously reported associations and potentially identify novel causal relationships.

Methods

Study Participants

The Genetic Epidemiology Research on Adult Health and Aging (GERA) cohort consists of more than 100,000 individuals living in northern California, who are plan members of the Kaiser Permanente integrated health care delivery system. The composition of this cohort has been described elsewhere (NCBI Database of Genotypes and Phenotypes, phs000788.v2.p3). Self-administered surveys of demographic and behavioural characteristics were linked with genome-wide genotyping, de-identified electronic health records (EHR), and a prescriptions database. All participants provided written, informed consent.

The current AS case-control study is a subset of the GERA cohort and has been previously described[129]. In brief, the study consists of 44,703 participants of self-reported European ancestry, aged 55 years or older. Individuals with congenital valvular heart disease (*International Classification of Disease, 9th Revision [ICD-9]* 746-747) were excluded. The study has been approved by the appropriate institutional review boards at Kaiser Permanente Northern California and the Research Institute of the McGill University Health Centre.

Disease and Risk Factor Definitions in the GERA Cohort

Aortic stenosis cases were defined as the presence of either a diagnosis code for aortic stenosis (*ICD-9* 424.1) or a procedure code for aortic valve replacement in the EHR between January 1996 and December 2015, inclusive; all other participants were defined as AS controls. This is a validated approach that has a positive predictive value greater than 90%[46]. Cases of coronary artery disease were identified as the presence of a diagnosis code in the EHR for myocardial infarction

or coronary artery disease (*ICD-9* 410-414), a procedure code in the EHR for percutaneous coronary intervention or coronary artery bypass graft, or self-reported myocardial infarction, angina, or revascularization.

Dyslipidemia was defined as at least two diagnostic codes for disorders of lipid metabolism (*ICD-9* 272) in the EHR and at least one prescription for statins in the prescriptions database. BMI was calculated as an individual's self-reported weight divided by the square of their self-reported height, in kg/m^2. Hypertension, smoking status (past, present, or never smoker), and diabetes mellitus were self-reported.

Genetic Data and Construction of the Genetic Risk Scores

Following standard quality control of the genome-wide genotyped data, SHAPEIT2[130] (University of Oxford) and IMPUTE2[131,132] (University of Oxford) were used to impute the genotypes using the 1000 Genomes[133] reference panel. From summary statistics of genome-wide association studies published as of December 2017, we constructed genetic instruments for an expanded set of risk factors: type 2 diabetes (n = 35 variants), BMI (n = 94 variants), waist-to-hip ratio adjusted for body mass index (WHRadjBMI; n = 39 variants), LDL-C (n = 57 variants), high-density lipoprotein cholesterol (HDL-C; n = 70 variants), triglycerides (n = 39 variants), apolipoprotein B (apoB; n = 19 variants), SBP (n = 87 variants), diastolic blood pressure (n = 99 variants), pulse pressure (n = 64 variants), hypertension (n = 11 variants), and cigarettes smoked per day (n = 6 variants) (Supplementary Table 3.1-Supplementary Table 3.12). For each instrument, a maximum of one variant was included for any gene, and all variants were in linkage equilibrium ($r^2 < 0.3$), common (minor allele frequency > 0.01), and associated with the trait in an original publication at

$p \leq 5 \times 10^{-8}$. If a variant was not available or poorly imputed (information score < 0.3), then a variant in high linkage disequilibrium ($r^2 \geq 0.8$) was used instead.

Observational Associations of Risk Factors

To test for differences in clinical characteristics between the AS cases and controls, we applied the Fisher exact test for categorical traits and the Welch t-test for continuous traits. We estimated the observational associations of sex, age, BMI, dyslipidemia, hypertension, smoking, and diabetes with AS in separate logistic regression models with age, age^2, and sex as covariates. Effect sizes for age and sex were reported as the corresponding coefficients in models containing age, age^2, and sex. Specifically, age and age^2 terms were constructed from centered age and were therefore uncorrelated, so the effect size for age can be interpreted as the change in odds of AS per 10-year increase in age. In the fully-adjusted models, further adjustments for dyslipidemia, hypertension, smoking, and diabetes mellitus were performed.

Causal Associations of Risk Factors

To assess whether cardiovascular risk factors were causally associated with AS, we performed MR for the expanded set of risk factors described above. We used the R package MendelianRandomization[134], with the inverse-variance weighted method as our primary analysis and the penalized weighted median and Egger methods as secondary analyses to assess for pleiotropy in the instruments. We performed multivariable MR[135] using data from the Global Lipids Genetics Consortium[136] for two lipid traits known to have overlapping genetic components. The proportion of variance explained by each instrument was calculated using the minor allele

frequencies and effect sizes of the variants comprising the score[137] and post-hoc power calculations for the MR analyses were performed using mRnd[138].

All statistical analyses were performed using R, versions 3.4.1 and 3.4.3 (R Foundation)[139]. Due to the hypothesis-generating nature of these analyses, multiple-testing corrections were not applied. Two-sided p-values ≤ 0.05 were considered significant.

Results

The clinical characteristics of the GERA cohort are described in Table 3.1. Compared to the controls (n = 41,234), the AS cases (n = 3,469) were more likely to be male (56% [n = 1,943] in cases versus 49% [n = 20,076] in controls; $p < 0.001$) and older (mean [SD] of 74.6 [8.5] in cases versus 69.3 [8.3] in controls; $p < 0.001$). A greater proportion of the AS cases had coronary artery disease, dyslipidemia, hypertension, smoking, and diabetes mellitus ($p < 0.001$ for all comparisons; Table 3.1).

Observational Associations of Risk Factors

All cardiovascular risk factors were associated with AS in models with age, age^2, and sex as covariates (Table 3.2). Effect sizes for age and sex are the corresponding coefficients in a model containing age, age^2, and sex. Among the dichotomous risk factors, dyslipidemia was associated with the greatest odds of AS (odds ratio [OR], 2.18; 95% CI, 2.01 to 2.37; $p = 2.7 \times 10^{-76}$), followed by hypertension (OR, 1.63; 95% CI, 1.51 to 1.75; $p = 1.3 \times 10^{-40}$), diabetes (OR, 1.55; 95% CI, 1.41 to 1.70; $p = 3.2 \times 10^{-19}$), male sex (OR, 1.53; 95% CI, 1.43 to 1.64; $p = 1.3 \times 10^{-31}$), and smoking (OR, 1.16; 95% CI, 1.08 to 1.25; $p = 6.2 \times 10^{-5}$). In models additionally adjusted for

dyslipidemia, hypertension, smoking, and diabetes mellitus, all risk factors remained associated with AS (Table 3.2). The magnitude of these associations did not materially change except diabetes, where the association for AS became attenuated (OR 1.19; 95% CI, 1.08 to 1.32; $p = 7.0 \times 10^{-4}$).

Causal Associations of Risk Factors

MR indicated that LDL-C, triglycerides, apoB, and SBP contributed causally to AS. Each SD of genetically-elevated LDL-C was associated with 27% higher causal odds of AS (OR per SD, 1.27; 95% CI, 1.07 to 1.51; $p = 6.6 \times 10^{-3}$). We estimated a similar causal effect for triglycerides (OR per SD, 1.27; 95% CI, 1.01 to 1.59; $p = 0.041$). For apoB, each genetically-elevated SD was associated with a 19% increase in causal odds of AS (OR per SD, 1.19; 95% CI, 1.01 to 1.40; $p = 0.035$). Per SD of genetically-elevated SBP, we estimated a 44% increase in causal odds (OR per SD, 1.44; 95% CI, 1.05 to 1.98; $p = 0.022$). No other risk factors demonstrated evidence of a causal contribution to AS (all $p > 0.05$; Table 3.3).

In secondary analyses, we used the penalized weighted median method to allow up to 50% of the variants in each genetic instrument to be pleiotropic, that is, associated with AS through pathways other than the one under investigation. Triglycerides was the only risk factor which remained causally associated with AS (OR per SD, 1.46; 95% CI, 1.13 to 1.88; $p = 3.8 \times 10^{-3}$); we observed no association by SBP, LDL-C, and apolipoprotein B ($p > 0.05$ (Table 3.4). When we used multivariable MR to account for the simultaneous association with LDL-C of the variants in the genetic instrument for triglycerides, a phenomenon known as horizontal pleiotropy, the OR for genetically-elevated triglycerides became attenuated (OR per SD, 1.17; 95% CI, 0.93 to 1.46; $p =$

0.18) but remained indicative of a causative role. We did not observe directional pleiotropy for any risk factors which were significant in the primary analysis (Egger intercept $p > 0.05$; Table 3.4). For each genetic instrument, the proportion of variance explained is in Supplementary Table 3.13 and the minimal detectable odds ratio at 80% power is in Supplementary Table 3.14.

Discussion

In this case-control study, we provide large-scale confirmation that several cardiovascular risk factors are observationally associated with clinical AS, including age, sex, and risk factors related to adiposity, blood pressure, lipids, and smoking. For several cardiovascular risk factors, we performed MR analysis to assess whether they contribute causally to the development of AS. We demonstrate that genetic predisposition to higher LDL-C, triglycerides, apoB, and SBP is causally associated with AS. While we observed no evidence of directional pleiotropy in the MR-Egger analyses, only the association of genetically-elevated triglycerides with AS persisted after allowing for up to 50% of the variants used in the genetic instruments to be pleiotropic, in secondary analyses using the penalized weighted median method. Our results therefore indicate that triglycerides, LDL-C, apoB, and SBP contribute to the development of AS, and provide new evidence that improved management of these specific risk factors could reduce the incidence of AS in the population.

Previous epidemiological studies have demonstrated that several cardiovascular risk factors associate with AS. Stewart and colleagues showed that older age, male sex, hypertension, and present smoking are independently associated with a composite outcome of prevalent aortic sclerosis and stenosis, in addition to lipoprotein(a), height, and LDL-C[28]. These findings are

supported by Yan and colleagues, who identified hypertension, diabetes, and dyslipidemia as independently associating with incident severe AS. Older age, male sex, BMI, and smoking, as well as total cholesterol and lower levels of HDL-C, were likewise independently associated with AVC[27]. In the present large-scale study, we confirm and quantify these associations with prevalent AS. We show that dyslipidemia is associated with the greatest increase in the odds for AS. Furthermore, hypertension, diabetes, and male sex are each associated with a substantial increase of approximately 50% in the odds for AS. Our results suggest that men with any combination of dyslipidemia, hypertension, or diabetes are at high risk of AS and should therefore be more closely monitored for the development and progression to AS, particularly at older ages.

Our data also confirm the role of LDL-C as a causal risk factor for the development of AS. Smith and colleagues have shown that per 2.4 mmol/L of genetically-elevated LDL-C, there was a 38% increase in the odds of prevalent AVC. This association persisted when genetic variants that are also associated with other lipid traits were excluded, or when the outcome was incident AS[107], providing further support for a causal role for LDL-C in AS pathology. Calcification of the aortic valve is frequently observed among patients with familial hypercholesterolemia (FH), an autosomal disorder characterized by mutations in genes relevant to LDL-C metabolism, including low-density lipoprotein receptor (*LDLR*), apolipoprotein B (*APOB*), and proprotein convertase subtilisin/kexin type 9 (*PCSK9*). Compared to healthy individuals, the prevalence of AVC is elevated among individuals with heterozygous FH[140], and reaches 100% amongst individuals with homozygous FH[141]. Reduced function of *PCSK9* is also protective for AS, with a 36% reduction in the odds of AS among individuals who are heterozygous or homozygous for a loss-of-function

mutation[142]. Our findings add to mounting evidence from several independent lines of investigation that LDL-C, but not HDL-C, plays a causal role in the development of AS.

Our observation of a causal contribution by triglycerides to AS confirms the recent discovery of this causal relationship in the Copenhagen General Population Study[125]. We have previously shown that triglycerides, but not other lipoproteins, were associated with mitral annular calcification[118], suggesting that increased triglycerides may represent a common mechanism for both aortic and mitral calcification. Triglycerides are carried primarily in very-low-density lipoprotein, an apoB-containing particle. Given that LDL-C[107], Lp(a)[143], and triglycerides[125] (which are all primarily carried by apoB-containing particles) are causally implicated in AS, our results point to a potential common pathway based on the accumulation of apoB-related particles in valve tissues leading to calcification. Our results may also provide a mechanistic link to explain the observational associations between AS and several dysmetabolic states, such as elevated BMI and metabolic syndrome[144,145], which are characterized by high apoB. These findings are consistent with recent MR studies demonstrating a causal association of BMI with AS in separate cohorts[127,128], although we did not observe supporting evidence in our present analyses.

Our MR findings, which support the causal contribution to AS of LDL-C and triglycerides, potentially through apoB-containing lipoproteins, are inconsistent with the results of randomized controlled trials which have not shown any reduction in the progression of AS or the occurrence of cardiovascular outcomes among AS patients when administered statins[105,106,146]. However, the associations identified through MR reflect the lifelong exposure to genetically-elevated levels of LDL-C, triglycerides, and increased circulating apoB particles, as opposed to the shorter exposure

times examined within these trials. These trials also examined patients who had already progressed to clinical AS, and it remains unclear whether the earlier targeting of individuals in the subclinical phase may be more effective. It is also important to consider that statins have been shown to increase calcification in the cardiovascular system[147,148], and may have specific pro-calcific effects which may suggest that statins may not be an optimal strategy for AS prevention or treatment. Novel agents such as PCSK9 inhibitors, which target LDL-C, or ANGPTL3 inhibitors, which target triglycerides, also significantly lower apoB particles. Whether these agents have secondary benefits in preventing or treating AS remains unknown but should be considered in future randomized trials.

In addition to lipoproteins, our study confirms SBP to be a causative factor for AS, providing independent replication of previous study[126]. Higher SBP, systolic hypertension, and isolated systolic hypertension are associated with faster AVC progression among individuals with AS[34], whereas AS patients treated with angiotensin-receptor blockers demonstrated slower AS progression than either normotensive or untreated hypertensive AS patients[33]. In conjunction with epidemiological and genetic studies examining the contribution of SBP to AS, our results suggest that more aggressive blood pressure control among individuals with early valve disease, using inhibitors of the angiotensin-renin-aldosterone system or other agents, may represent an additional therapeutic option for the prevention and treatment of AS and warrants additional investigation.

Strengths and Limitations

Our study features the simultaneous assessment of observational and genetic associations of risk factors with AS within the same cohort, facilitating comparison of the relative contributions of risk

factors. However, there are several limitations to our study. Typical of large-scale genetic studies, our study is a case-control design so our observational associations reflect correlations between risk factor and disease prevalence rather than a temporal relationship suggestive of causality. Our study is also restricted to European-ancestry participants due to the smaller numbers of participants of other ancestries and the construction of genetic instruments from external studies which were likewise composed of predominantly European-ancestry study subjects. Consequently, the transferability of our findings to other ancestries may be limited. Lastly, we identified AS cases using a combination of diagnosis and procedure codes extracted from EHR rather than echocardiography, which is the gold standard for diagnosing AS. Although this approach has been validated to have a positive predictive value greater than 90% in another cohort[46], the positive predictive value may be lower in GERA. We also imposed congenital valvular heart disease as an exclusion criterion but did not exclude other aetiologies of valvular heart disease such as radiation or rheumatic fever, which may bias our findings. As these aetiologies are comparatively rare, we do not anticipate the bias to be large.

Conclusions

We confirm that age, sex, and risk factors related to adiposity, blood pressure, lipids, and smoking are observationally associated with clinical AS. Genetic data provide supporting evidence that LDL-C, triglycerides, SBP, and apoB contribute causally to AS development. Future work is required to assess the therapeutic potential of targeting these potentially causal risk factors in the treatment or prevention of AS.

Table 3.1. Clinical Characteristics of the GERA Cohort.

Characteristic	Aortic Stenosis, No. (%)		*p*
	Controls	Cases	
n	41,234	3,469	N/A
Sex, male	20,076 (49)	1,943 (56)	< 0.001
Age, mean [SD], y	69.3 [8.3]	74.6 [8.5]	< 0.001
Body mass index, mean [SD], kg/m^{2}[a]	26.8 [4.9]	27.4 [5.4]	< 0.001
Dyslipidemia	23,926 (58)	2,666 (77)	< 0.001
Coronary artery disease	10,007 (24)	2,131 (61)	< 0.001
Hypertension	16,961 (41)	1,945 (56)	< 0.001
Ever smoked[b]	20,247 (52)	1,834 (56)	< 0.001
Diabetes mellitus	4,491 (11)	606 (17)	< 0.001
Aortic valve replacement	NA	400 (12)	N/A

Abbreviations: GERA, Genetic Epidemiology Research on Aging (n = 44,703); N/A, not available.

[a]Data on body mass index were available for 42,962 individuals.

[b]Data on smoking were available for 42,535 individuals.

Table 3.2. Observational Associations of Cardiovascular Risk Factors with Aortic Stenosis.

Risk Factor	Modelled With Age, Age^2, and Sex as Covariates (n = 44,703; 3,469 cases)		Fully Adjusted[a] (n = 42,535; 3,260 cases)	
	OR (95% CI)	***p***	**OR (95% CI)**	***p***
Demographic				
Sex, male	1.53 (1.43, 1.64)	1.3×10^{-31}	1.47 (1.37, 1.59)	5.3×10^{-24}
Age, 10 y	2.17 (2.06, 2.30)	9.5×10^{-166}	2.00 (1.89, 2.12)	2.8×10^{-127}
Adiposity				
Diabetes mellitus	1.55 (1.41, 1.70)	3.2×10^{-19}	1.19 (1.08, 1.32)	7.0×10^{-4}
Body mass index, SD[b]	1.27 (1.23, 1.32)	2.2×10^{-41}	1.17 (1.13, 1.22)	1.6×10^{-15}
Blood pressure				
Hypertension	1.63 (1.51, 1.75)	1.3×10^{-40}	1.44 (1.34, 1.55)	2.8×10^{-21}
Lipids				
Dyslipidemia	2.18 (2.01, 2.37)	2.7×10^{-76}	1.92 (1.76, 2.10)	1.5×10^{-48}
Smoking				
Ever smoked[c]	1.16 (1.08, 1.25)	6.2×10^{-5}	1.12 (1.04, 1.21)	2.6×10^{-3}

Abbreviations: OR, odds ratio.

[a]Fully adjusted models were adjusted for dyslipidemia, hypertension, smoking, and diabetes mellitus, in addition to age, age^2, and sex.

[b]In the age, age^2, and sex model for body mass index, 42,962 individuals were included (3,309 AS cases).

[c]In the age, age^2, and sex model for smoking, 42,535 individuals were included (3,260 AS cases).

Table 3.3. Mendelian Randomization for the Causal Contribution of Risk Factors to Aortic Stenosis.

Risk Factor	No. Variants	Inverse-Variance Weighted	
		Causal Odds Ratio (95% CI)	*p*
Adiposity			
Type 2 diabetes	35	1.02 (0.91, 1.13)	0.77
Body mass index, SD	94	1.58 (0.78, 3.21)	0.21
WHRadjBMI, SD	39	1.01 (0.70, 1.47)	0.95
Blood pressure			
Systolic blood pressure, SD	87	1.44 (1.05, 1.98)	0.022
Diastolic blood pressure, SD	99	1.14 (0.88, 1.48)	0.34
Pulse pressure, SD	64	1.16 (0.83, 1.62)	0.38
Hypertension	11	0.97 (0.80, 1.18)	0.75
Lipids			
LDL-C, SD	57	1.27 (1.07, 1.51)	6.6×10^{-3}
HDL-C, SD	70	0.94 (0.78, 1.13)	0.49
Triglycerides, SD	39	1.27 (1.01, 1.59)	0.041
Apolipoprotein B, SD	19	1.19 (1.01, 1.40)	0.035
Smoking			
Cigarettes per day	6	1.02 (0.97, 1.07)	0.41

Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; WHRadjBMI: waist-to-hip ratio adjusted for body mass index.

Table 3.4. Sensitivity Analyses for the Causal Contribution of Cardiovascular Risk Factors to Aortic Stenosis.

Risk Factor	Penalized Weighted Median		Egger Intercept *p*
	Causal Odds Ratio (95% CI)	*p*	
Blood pressure			
Systolic blood pressure, SD	0.98 (0.64, 1.50)	0.92	0.34
Lipids			
LDL-C, SD	1.12 (0.90, 1.39)	0.32	0.37
Triglycerides, SD	1.46 (1.13, 1.88)	3.8×10^{-3}	0.25
Apolipoprotein B, SD	1.10 (0.89, 1.36)	0.39	0.29

Abbreviations: LDL-C, low-density lipoprotein cholesterol.

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3.4 Supplement

Supplementary Table 3.1. Input for the Mendelian Randomization of Type 2 Diabetes.

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Type 2 Diabetes			PMID
					Beta[a]	SE	*p*	Beta[a]	SE	*p*	
rs10923931	1:120517959	*NOTCH2*	T/G	0.100	-0.079	0.044	0.068	0.122	0.020	4.1E-08	18372903
rs7578597	2:43732823	*THADA*	T/C	0.892	-0.018	0.041	0.658	0.140	0.022	1.1E-09	18372903
rs243021	2:60584819	*BCL11A*	A/G	0.467	0.050	0.026	0.051	0.077	0.009	2.9E-15	20581827
rs13389219	2:165528876	*GRB14*	C/T	0.604	0.036	0.026	0.174	0.068	0.012	1.0E-08	22885922
rs7578326	2:227020653	*IRS1*	A/G	0.649	0.049	0.027	0.067	0.104	0.012	5.4E-20	20581827
rs4607103	3:64711904	*ADAMTS9*	C/T	0.737	-0.028	0.029	0.333	0.086	0.014	1.2E-08	18372903
rs4402960	3:185511687	*IGF2BP2*	T/G	0.314	-0.014	0.028	0.605	0.131	0.016	9.0E-16	17463249
rs459193	5:55806751	*ANKRD55*	G/A	0.746	0.014	0.029	0.629	0.077	0.014	6.0E-09	22885922
rs4457053	5:76424949	*ZBED3*	G/A	0.310	-0.011	0.028	0.687	0.077	0.012	2.8E-12	20581827
rs6931514	6:20703952	*CDKAL1*	G/A	0.270	0.038	0.029	0.186	0.223	0.033	1.3E-11	17463249
rs864745	7:28180556	*JAZF1*	T/C	0.495	-0.037	0.025	0.143	0.095	0.014	5.0E-14	18372903
rs972283	7:130466854	*KLF14*	G/A	0.525	0.037	0.026	0.144	0.068	0.012	2.2E-10	20581827
rs516946	8:41519248	*ANK1*	C/T	0.755	-0.097	0.029	0.001	0.086	0.014	2.5E-10	22885922
rs896854	8:95960511	*TP53INP1*	T/C	0.499	-0.024	0.025	0.346	0.058	0.012	9.9E-10	20581827
rs13266634	8:118184783	*SLC30A8*	C/T	0.697	0.027	0.028	0.331	0.113	0.021	5.0E-08	17463249
rs13292136	9:81952128	*CHCHD9*	C/T	0.936	0.001	0.052	0.985	0.104	0.018	2.8E-08	20581827
rs2796441	9:84308948	*TLE1*	G/A	0.592	-0.003	0.026	0.904	0.068	0.012	5.4E-09	22885922

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Type 2 Diabetes			PMID
					Beta[a]	SE	*p*	Beta[a]	SE	*p*	
rs12779790	10:12328010	*CDC123/CAMK1D*	G/A	0.152	0.007	0.049	0.880	0.104	0.016	1.2E-10	18372903
rs12571751	10:80942631	*ZMIZ1*	A/G	0.542	0.018	0.031	0.559	0.077	0.012	1.0E-10	22885922
rs7903146	10:114758349	*TCF7L2*	T/C	0.298	-0.021	0.028	0.452	0.315	0.035	3.0E-23	17463249
rs231362	11:2691471	*KCNQ1*	G/A	0.528	-0.011	0.030	0.705	0.077	0.009	2.8E-13	20581827
rs5215	11:17408630	*KCNJ11*	C/T	0.370	-0.006	0.027	0.831	0.131	0.020	5.0E-11	17463249
rs1552224	11:72433098	*CENTD2*	A/C	0.856	-0.016	0.036	0.661	0.131	0.013	1.4E-22	20581827
rs1387153	11:92673828	*MTNR1B*	T/C	0.289	-0.046	0.028	0.101	0.086	0.012	7.8E-15	20581827
rs10842994	12:27965150	*KLHDC5*	C/T	0.805	0.042	0.033	0.200	0.095	0.016	6.1E-10	22885922
rs1531343	12:66174894	*HMGA2*	C/G	0.105	-0.040	0.043	0.344	0.095	0.016	3.6E-09	20581827
rs7961581	12:71663102	*TSPAN8/LGR5*	C/T	0.281	0.023	0.028	0.415	0.086	0.014	1.1E-09	18372903
rs7957197	12:121460686	*HNF1A*	T/A	0.813	-0.054	0.033	0.102	0.068	0.012	2.4E-08	20581827
rs7177055	15:77832762	*HMG20A*	A/G	0.709	0.045	0.029	0.117	0.077	0.012	4.6E-09	22885922
rs11634397	15:80432222	*ZFAND6*	G/A	0.657	-0.021	0.027	0.430	0.058	0.010	2.4E-09	20581827
rs8042680	15:91521337	*PRC1*	A/C	0.327	-0.030	0.027	0.273	0.068	0.010	2.4E-10	20581827
rs8050136	16:53816275	*FTO*	A/C	0.403	0.048	0.026	0.066	0.207	0.029	7.0E-14	17463249
rs7202877	16:75247245	*BCAR1*	T/G	0.898	0.111	0.044	0.010	0.113	0.021	3.5E-08	22885922

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Type 2 Diabetes			PMID
					Beta[a]	SE	*p*	Beta[a]	SE	*p*	
rs12970134	18:57884750	*MC4R*	A/G	0.264	-0.016	0.029	0.581	0.077	0.014	1.2E-08	22885922
rs10401969	19:19407718	*CILP2*	C/T	0.074	-0.029	0.050	0.552	0.122	0.020	7.0E-09	22885922

Abbreviations: EAF, effect allele frequency; PMID, PubMed ID.

[a] Log odds per effect allele.

Supplementary Table 3.2. Input for the Mendelian Randomization of Body Mass Index.

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Body Mass Index			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs977747	1:47684677	*TAL1*	T/G	0.40	0.029	0.026	0.26	0.017	0.0031	2.2E-08	25673413
rs657452	1:49589847	*AGBL4*	A/G	0.39	0.004	0.026	0.89	0.023	0.0031	5.5E-13	25673413
rs3101336	1:72751185	*NEGR1*	C/T	0.63	0.011	0.026	0.67	0.033	0.0031	2.7E-26	25673413
rs12566985	1:75002193	*FPGT_TNNI3K*	G/A	0.44	-0.013	0.026	0.61	0.024	0.0031	3.3E-15	25673413
rs12401738	1:78446761	*FUBP1*	A/G	0.34	-0.027	0.027	0.31	0.021	0.0033	1.2E-10	25673413
rs11165643	1:96924097	*PTBP2*	T/C	0.58	-0.041	0.026	0.11	0.022	0.0031	2.1E-12	25673413
rs17024393	1:110154688	*GNAT2*	C/T	0.03	-0.032	0.078	0.68	0.066	0.0087	7.0E-14	25673413
rs543874	1:177889480	*SEC16B*	G/A	0.19	-0.009	0.032	0.77	0.048	0.0040	2.6E-35	25673413
rs2820292	1:201784287	*NAV1*	C/A	0.54	-0.011	0.026	0.66	0.02	0.0031	1.8E-10	25673413
rs13021737	2:632348	*TMEM18*	G/A	0.83	-0.002	0.033	0.95	0.06	0.0038	1.1E-50	25673413
rs10182181	2:25150296	*ADCY3*	G/A	0.47	0.000	0.026	1.00	0.031	0.0031	8.8E-24	25673413
rs11126666	2:26928811	*KCNK3*	A/G	0.26	0.005	0.030	0.86	0.021	0.0031	1.3E-09	25673413
rs887912	2:59302877	*LINC01122*	T/C	0.29	0.026	0.028	0.36	0.1	0.0204	1.8E-12	20935630
rs11688816	2:63053048	*EHBP1*	G/A	0.51	-0.020	0.026	0.43	0.017	0.0031	1.9E-08	25673413
rs2890652	2:142959931	*RPS16P3_KYNU*	C/T	0.16	-0.011	0.035	0.74	0.09	0.0306	1.4E-10	20935630
rs1460676	2:164567689	*FIGN*	C/T	0.16	-0.013	0.035	0.70	0.021	0.0038	5.0E-08	25673413

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Body Mass Index			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs1528435	2:181550962	*UBE2E3*	T/C	0.62	-0.012	0.026	0.64	0.018	0.0031	1.2E-08	25673413
rs17203016	2:208255518	*CREB1*	G/A	0.18	-0.032	0.034	0.33	0.021	0.0038	3.4E-08	25673413
rs7599312	2:213413231	*ERBB4*	G/A	0.73	0.021	0.029	0.48	0.022	0.0031	1.2E-10	25673413
rs492400	2:219349752	*USP37*	C/T	0.42	0.008	0.026	0.75	0.024	0.0041	6.8E-09	25673413
rs6804842	3:25106437	*RARB*	G/A	0.58	0.021	0.026	0.42	0.019	0.0031	2.5E-09	25673413
rs2365389	3:61236462	*FHIT*	C/T	0.59	-0.001	0.026	0.96	0.02	0.0031	1.6E-10	25673413
rs3849570	3:81792112	*GBE1*	A/C	0.33	-0.019	0.027	0.49	0.019	0.0031	2.6E-08	25673413
rs13078960	3:85807590	*CADM2*	G/T	0.21	-0.023	0.032	0.47	0.03	0.0040	1.7E-14	25673413
rs16851483	3:141275436	*RASA2*	T/G	0.06	-0.018	0.053	0.73	0.048	0.0082	3.6E-10	25673413
rs1516725	3:185824004	*ETV5*	C/T	0.87	0.046	0.038	0.22	0.045	0.0043	1.9E-22	25673413
rs10938397	4:45182527	*GNPDA2*	G/A	0.43	-0.003	0.026	0.90	0.04	0.0031	3.2E-38	25673413
rs17001654	4:77129568	*NUP54/ SCARB2*	G/C	0.16	0.016	0.036	0.65	0.031	0.0051	7.8E-09	25673413
rs13107325	4:103188709	*SLC39A8*	T/C	0.07	-0.096	0.050	0.05	0.19	0.0408	1.5E-13	20935630
rs11727676	4:145659064	*HHIP*	T/C	0.90	0.000	0.043	1.00	0.036	0.0061	2.6E-08	25673413
rs2112347	5:75015242	*POC5*	T/G	0.63	-0.002	0.026	0.94	0.026	0.0030	6.2E-17	25673413
rs4836133	5:124332103	*RPL28P3/H MGB1P22*	A/C	0.50	0.001	0.026	0.96	0.07	0.0204	2.0E-09	20935630

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Body Mass Index			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs7715256	5:153537893	*GALNT10*	G/T	0.43	-0.031	0.026	0.23	0.017	0.0028	8.9E-09	25673413
rs206936	6:34302869	*NUDT3/RPS10*	G/A	0.20	0.030	0.031	0.33	0.06	0.0204	3.0E-08	20935630
rs205262	6:34563164	*C6orf106*	G/A	0.28	0.050	0.028	0.08	0.022	0.0036	1.8E-10	25673413
rs2033529	6:40348653	*TDRG1/LRFN2*	G/A	0.29	0.023	0.029	0.42	0.019	0.0031	1.4E-08	25673413
rs2207139	6:50845490	*TFAP2B*	G/A	0.17	0.038	0.034	0.26	0.045	0.0038	4.1E-29	25673413
rs9400239	6:108977663	*FOXO3/HSS00296402*	C/T	0.70	-0.020	0.028	0.47	0.019	0.0031	1.6E-08	25673413
rs9374842	6:120185665	*LOC285762*	T/C	0.76	-0.023	0.029	0.44	0.023	0.0040	2.7E-08	25673413
rs13201877	6:137675541	*IFNGR1*	G/A	0.14	-0.027	0.038	0.47	0.024	0.0043	4.3E-08	25673413
rs13191362	6:163033350	*PARK2*	A/G	0.88	0.008	0.039	0.84	0.028	0.0051	7.3E-09	25673413
rs1167827	7:75163169	*HIP1/PMS2L3/PMS2P5*	G/A	0.53	-0.018	0.027	0.51	0.02	0.0031	6.3E-10	25673413
rs2245368	7:76608143	*PMS2L11*	C/T	0.19	0.059	0.036	0.10	0.032	0.0061	3.2E-08	25673413
rs6465468	7:95169514	*ASB4*	T/G	0.30	-0.041	0.032	0.20	0.025	0.0043	5.0E-08	25673413
rs17405819	8:76806584	*HNF4G*	T/C	0.70	0.050	0.028	0.07	0.022	0.0033	2.1E-11	25673413
rs16907751	8:81375457	*ZBTB10*	C/T	0.89	-0.018	0.041	0.66	0.047	0.0084	3.9E-08	25673413

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Body Mass Index			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs2033732	8:85079709	*RALYL*	C/T	0.75	-0.030	0.029	0.31	0.019	0.0041	4.9E-08	25673413
rs4740619	9:15634326	*C9orf93*	T/C	0.54	0.054	0.026	0.03	0.018	0.0031	4.6E-09	25673413
rs10968576	9:28414339	*LINGO2*	G/A	0.31	-0.004	0.028	0.89	0.025	0.0033	6.6E-14	25673413
rs6477694	9:111932342	*EPB41L4B/ C9orf4*	C/T	0.35	0.006	0.027	0.83	0.017	0.0031	2.7E-08	25673413
rs1928295	9:120378483	*TLR4*	T/C	0.55	-0.033	0.026	0.20	0.019	0.0031	7.9E-10	25673413
rs10733682	9:129460914	*LMX1B*	A/G	0.48	0.057	0.029	0.05	0.017	0.0031	1.8E-08	25673413
rs7899106	10:87410904	*GRID1*	G/A	0.05	0.042	0.060	0.49	0.04	0.0071	3.0E-08	25673413
rs17094222	10:102395440	*HIF1AN*	C/T	0.22	0.013	0.033	0.70	0.025	0.0041	5.9E-11	25673413
rs11191560	10:104869038	*NT5C2/ CYP17A1/ SFXN2*	C/T	0.09	-0.052	0.046	0.25	0.031	0.0051	8.5E-09	25673413
rs7903146	10:114758349	*TCF7L2*	C/T	0.70	0.021	0.028	0.45	0.023	0.0031	1.1E-11	25673413
rs4256980	11:8673939	*TRIM66*	G/C	0.64	-0.022	0.027	0.40	0.021	0.0028	2.9E-11	25673413
rs11030104	11:27684517	*BDNF*	A/G	0.79	0.047	0.032	0.13	0.051	0.0040	1.3E-31	25673413
rs2176598	11:43864278	*HSD17B12*	T/C	0.25	0.015	0.029	0.61	0.02	0.0041	3.0E-08	25673413
rs3817334	11:47650993	*MTCH2*	T/C	0.41	-0.029	0.026	0.27	0.026	0.0028	5.2E-17	25673413
rs7138803	12:50247468	*BCDIN3D*	A/G	0.38	0.040	0.026	0.13	0.032	0.0031	8.2E-24	25673413

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Body Mass Index			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs11057405	12:122781897	*CLIP1*	G/A	0.90	-0.039	0.048	0.42	0.031	0.0061	2.0E-08	25673413
rs9581854	13:28017782	*MTIF3*	T/C	0.18	0.000	0.033	1.00	0.03	0.0046	2.3E-10	25673413
rs12429545	13:54102206	*OLFM4*	A/G	0.13	0.020	0.038	0.59	0.033	0.0050	1.1E-12	25673413
rs9540493	13:66205704	*MIR548X2*	A/G	0.44	0.008	0.026	0.75	0.021	0.0040	5.0E-08	25673413
rs1441264	13:79580919	*MIR548A2*	A/G	0.61	0.015	0.027	0.58	0.017	0.0031	3.0E-08	25673413
rs10132280	14:25928179	*STXBP6*	C/A	0.69	-0.034	0.028	0.22	0.023	0.0031	1.1E-11	25673413
rs11847697	14:30515112	*PRKD1/ RPS6P24*	T/C	0.04	0.010	0.063	0.87	0.17	0.0510	5.8E-11	20935630
rs7141420	14:79899454	*NRXN3*	T/C	0.52	0.009	0.026	0.72	0.024	0.0030	1.2E-14	25673413
rs7151545	14:103177644	*RCOR1*	G/A	0.06	0.037	0.054	0.49	0.063	0.0117	4.1E-08	22982992
rs3736485	15:51748610	*SCG3/ DMXL2*	A/G	0.47	-0.008	0.026	0.77	0.018	0.0031	7.4E-09	25673413
rs2241423	15:68086838	*MAP2K5*	G/A	0.76	0.003	0.030	0.92	0.13	0.0204	1.2E-18	20935630
rs7164727	15:73093991	*LOC10028 7559*	T/C	0.68	-0.001	0.027	0.97	0.019	0.0031	3.9E-09	25673413
rs758747	16:3627358	*NLRC3*	T/C	0.30	-0.024	0.031	0.45	0.023	0.0041	7.5E-10	25673413
rs12444979	16:19933600	*GPRC5B/ GPR139*	C/T	0.85	0.057	0.037	0.12	0.17	0.0306	2.9E-21	20935630

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Body Mass Index			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs3888190	16:28889486	*ATP2A1*	A/C	0.39	-0.040	0.026	0.13	0.031	0.0031	3.1E-23	25673413
rs9925964	16:31129895	*KAT8/ ZNF646/ VKORC1/ ZNF668/ STX1B/ FBXL19*	A/G	0.63	0.018	0.027	0.50	0.019	0.0031	8.1E-10	25673413
rs2080454	16:49062590	*CBLN1*	C/A	0.37	-0.051	0.027	0.05	0.017	0.0031	8.6E-09	25673413
rs1558902	16:53803574	*FTO*	A/T	0.41	0.038	0.026	0.14	0.082	0.0030	7.5E-153	25673413
rs9914578	17:2005136	*SMG6*	G/C	0.20	0.036	0.032	0.26	0.02	0.0036	2.1E-08	25673413
rs1000940	17:5283252	*RABEP1*	G/A	0.30	0.040	0.027	0.15	0.019	0.0031	1.3E-08	25673413
rs12940622	17:78615571	*RPTOR*	G/A	0.57	0.021	0.026	0.41	0.018	0.0028	2.5E-09	25673413
rs1808579	18:21104888	*C18orf8*	C/T	0.53	0.004	0.026	0.86	0.017	0.0030	4.2E-08	25673413
rs7239883	18:40147671	*LOC284260*	G/A	0.39	0.012	0.027	0.64	0.023	0.0041	1.5E-08	25673413
rs7243357	18:56883319	*GRP*	T/G	0.82	0.047	0.034	0.17	0.022	0.0041	3.9E-08	25673413
rs6567160	18:57829135	*MC4R*	C/T	0.23	-0.006	0.030	0.84	0.056	0.0036	3.9E-53	25673413
rs17724992	19:18454825	*GDF15/ PGPEP1*	A/G	0.70	0.010	0.040	0.80	0.019	0.0041	3.4E-08	25673413

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Body Mass Index			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs29941	19:34309532	*KCTD15/ RPS4XP20*	G/A	0.68	0.032	0.028	0.24	0.06	0.0204	3.0E-09	20935630
rs2075650	19:45395619	*TOMM40*	A/G	0.84	0.018	0.039	0.65	0.026	0.0043	1.3E-08	25673413
rs2287019	19:46202172	*QPCTL*	C/T	0.80	0.025	0.038	0.51	0.036	0.0040	4.6E-18	25673413
rs3810291	19:47569003	*ZC3H4*	A/G	0.65	-0.020	0.029	0.50	0.028	0.0040	4.8E-15	25673413
rs6091540	20:51087862	*ZFP64*	C/T	0.71	-0.029	0.028	0.30	0.03	0.0043	2.2E-11	25673413
rs2836754	21:40291740	*ETS2*	C/T	0.63	-0.017	0.026	0.53	0.017	0.0031	1.6E-08	25673413

Abbreviations: EAF, effect allele frequency; PMID, PubMed ID.

[a] Log odds per effect allele.

[b] Kg/m^2 per effect allele. For ease of comparison, the results of the Mendelian randomization are shown per SD increase using the conversion 4.8 kg/m^2 = 1 SD[149].

Supplementary Table 3.3. Input for the Mendelian Randomization of WHRadjBMI.

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			WHRadjBMI			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs2645294	1:119574587	*TBX15/ WARS2*	T/C	0.55	-0.029	0.025	0.25	0.031	0.004	1.7E-19	28196256
rs905938	1:154991389	*DCST2*	T/C	0.74	-0.013	0.029	0.66	0.025	0.004	7.3E-10	28196256
rs10919388	1:170372503	*GORAB*	C/A	0.73	0.013	0.029	0.66	0.024	0.004	3.2E-09	28196256
rs714515	1:172352990	*DNM3/ PIGC*	G/A	0.43	-0.024	0.026	0.36	0.027	0.003	4.4E-15	28196256
rs2820443	1:219753509	*LYPLAL1*	T/C	0.72	-0.042	0.028	0.14	0.035	0.004	5.3E-21	28196256
rs1385167	2:66200648	*MEIS1*	G/A	0.13	0.011	0.038	0.77	0.029	0.005	1.9E-09	28196256
rs10195252	2:165513091	*GRB14/ COBLL1*	T/C	0.59	0.036	0.026	0.16	0.027	0.004	5.9E-15	28196256
rs1569135	2:188115398	*CALCRL*	A/G	0.52	-0.024	0.025	0.33	0.021	0.003	5.6E-10	28196256
rs17819328	3:12489342	*PPARG*	G/T	0.42	-0.029	0.026	0.28	0.021	0.004	2.4E-09	28196256
rs2276824	3:52637486	*PBRM1*	C/G	0.44	-0.010	0.027	0.70	0.024	0.004	3.2E-11	28196256
rs2371767	3:64718258	*ADAMTS9*	G/C	0.70	-0.046	0.027	0.09	0.036	0.004	1.6E-20	28196256
rs10804591	3:129334233	*PLXND1*	A/C	0.78	-0.024	0.032	0.46	0.025	0.004	6.6E-09	28196256
rs17451107	3:156797609	*LEKR1*	T/C	0.58	-0.004	0.026	0.87	0.026	0.004	1.1E-12	28196256
rs9991328	4:89713121	*FAM13A*	T/C	0.48	0.057	0.026	0.03	0.019	0.003	4.5E-08	28196256
rs303084	4:124066948	*SPATA5/ FGF2*	A/G	0.78	-0.004	0.031	0.90	0.023	0.004	3.9E-08	28196256
rs7705502	5:173320815	*CPEB4*	A/G	0.30	0.013	0.028	0.63	0.027	0.004	4.7E-14	28196256

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			WHRadjBMI			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs6556301	5:176527577	*FGFR4*	T/G	0.37	0.035	0.032	0.28	0.022	0.004	2.6E-08	28196256
rs1294410	6:6738752	*LY86*	C/T	0.63	-0.028	0.026	0.29	0.031	0.004	2.0E-18	28196256
rs7759742	6:32381736	*BTNL2*	A/T	0.52	-0.008	0.026	0.75	0.023	0.003	4.4E-11	28196256
rs1358980	6:43764551	*VEGFA*	T/C	0.50	0.064	0.034	0.06	0.039	0.004	3.1E-27	28196256
rs1936805	6:127452116	*RSPO3*	T/C	0.52	-0.022	0.026	0.39	0.043	0.003	3.6E-35	28196256
rs10245353	7:25858614	*NFE2L3*	A/C	0.18	0.006	0.033	0.85	0.035	0.004	8.4E-16	28196256
rs7801581	7:27223771	*HOXA11*	T/C	0.25	0.005	0.033	0.88	0.027	0.004	3.7E-10	28196256
rs12679556	8:72514228	*MSC*	G/T	0.25	0.003	0.030	0.92	0.027	0.004	2.1E-11	28196256
rs10991437	9:107735920	*ABCA1*	A/C	0.12	0.035	0.039	0.37	0.031	0.005	1.0E-08	28196256
rs11231693	11:63862612	*MACROD1 /VEGFB*	A/G	0.06	-0.013	0.064	0.84	0.041	0.008	4.5E-08	28196256
rs10842707	12:26471364	*ITPR2/ SSPN*	T/C	0.22	-0.010	0.031	0.74	0.032	0.004	4.4E-16	28196256
rs1443512	12:54342684	*HOXC13*	A/C	0.23	-0.049	0.030	0.10	0.028	0.004	6.9E-13	28196256
rs4765219	12:124440110	*CCDC92*	C/A	0.66	-0.006	0.027	0.81	0.028	0.004	1.6E-15	28196256
rs8042543	15:31708263	*KLF13*	C/T	0.79	0.021	0.032	0.50	0.026	0.004	1.2E-09	28196256
rs8030605	15:56504598	*RFX7*	A/G	0.13	-0.038	0.038	0.33	0.030	0.005	8.8E-09	28196256

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			WHRadjBMI			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs1440372	15:67033151	*SMAD6*	C/T	0.71	0.072	0.029	0.01	0.024	0.004	1.1E-10	28196256
rs4646404	17:17420199	*PEMT*	G/A	0.65	-0.017	0.031	0.58	0.027	0.004	1.4E-11	28196256
rs12608504	19:18389135	*JUND*	A/G	0.31	-0.046	0.039	0.23	0.022	0.004	8.8E-10	28196256
rs4081724	19:33824946	*CEBPA*	G/A	0.86	0.034	0.037	0.35	0.035	0.005	7.4E-12	28196256
rs979012	20:6623374	*BMP2*	T/C	0.36	0.060	0.026	0.02	0.027	0.004	3.3E-14	28196256
rs224333	20:34023962	*GDF5*	G/A	0.62	0.027	0.026	0.30	0.020	0.004	2.6E-08	28196256
rs6090583	20:45558831	*EYA2*	A/G	0.49	0.030	0.030	0.32	0.022	0.003	6.2E-11	28196256
rs2294239	22:29449477	*ZNRF3/KREMEN1*	A/G	0.57	0.043	0.026	0.10	0.025	0.004	7.2E-13	28196256

Abbreviations: EAF, effect allele frequency; PMID, PubMed ID; WHRadjBMI, waist-to-hip ratio adjusted for body mass index.

[a] Log odds per effect allele.

[b] SD per effect allele.

Supplementary Table 3.4. Input for the Mendelian Randomization of Systolic Blood Pressure.

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Systolic Blood Pressure			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs9662255	1:9441949	*SPSB1*	C/A	0.61	0.030	0.033	0.35	0.38	0.07	8.3E-09	27841878
rs880315	1:10796866	*CASZ1*	T/C	0.65	0.022	0.031	0.47	-0.48	0.06	2.1E-14	27618452
rs17037390	1:11860843	*MTHFR/ NPPB*	A/G	0.16	-0.048	0.036	0.18	-0.91	0.08	6.0E-29	27618452
rs3820068	1:15798197	*CELA2A/ CELA2B*	A/G	0.80	0.046	0.033	0.16	0.47	0.08	1.1E-08	27841878
rs7515635	1:42408070	*HIVEP3*	T/C	0.46	0.061	0.025	0.02	0.31	0.04	4.8E-12	27618452
rs786919	1:89281529	*PKN2/ RNU6/ 125P*	A/G	0.40	0.024	0.026	0.35	0.36	0.07	5.0E-08	27841878
rs1620668	1:113023980	*ST7L/ CAPZA1/ MOV10*	A/G	0.84	0.016	0.038	0.67	-0.54	0.08	1.5E-12	27618452
rs2493134	1:230849359	*AGT*	T/C	0.58	0.003	0.026	0.91	-0.41	0.06	9.7E-13	27618452
rs2586886	2:26932031	*KCNK3*	T/C	0.62	-0.005	0.028	0.84	-0.40	0.06	5.9E-12	27618452
rs7562	2:28635740	*FOSL2*	T/C	0.53	0.016	0.026	0.54	0.26	0.05	1.9E-08	28135244
rs3731818	2:86368804	*PTCD3/ IMMT*	G/A	0.69	0.012	0.028	0.67	0.39	0.07	2.6E-08	27841878
rs1371182	2:165099215	*FIGN/ GRB14*	T/C	0.45	-0.027	0.026	0.30	-0.44	0.06	1.9E-14	27618452
rs1250247	2:216299629	*FN1*	C/G	0.27	0.043	0.029	0.13	0.43	0.07	3.1E-09	27841878
rs2594992	3:11360997	*HRH1/ ATG7*	A/C	0.61	0.016	0.027	0.54	-0.33	0.06	2.3E-08	27618452

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			WHRadjBMI			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs11128722	3:14958126	*FGD5*	A/G	0.55	-0.052	0.027	0.05	-0.31	0.05	3.6E-11	27618452
rs711737	3:27543655	*SLC4A7*	A/C	0.61	0.016	0.026	0.54	0.33	0.06	9.9E-09	27618452
rs6442101	3:48130893	*MAP4*	T/C	0.68	0.012	0.028	0.66	0.40	0.06	1.6E-10	27618452
rs113161639	3:154615819	*LOC105374171*	G/T	0.90	0.035	0.043	0.41	0.63	0.11	4.7E-09	27841878
rs6779380	3:169111915	*MECOM*	T/C	0.53	0.021	0.026	0.42	-0.44	0.06	1.9E-13	27618452
rs2291435	4:38387395	*TBC1D1/ FLJ13197*	T/C	0.54	-0.030	0.026	0.25	-0.34	0.04	1.9E-14	27618452
rs1458038	4:81164723	*FGF5*	T/C	0.29	-0.018	0.028	0.52	0.66	0.07	5.4E-24	27618452
rs17010957	4:86719165	*ARHGAP24*	T/C	0.86	0.017	0.038	0.65	-0.50	0.08	1.5E-09	27618452
rs13107325	4:103188709	*SLC39A8*	T/C	0.07	-0.096	0.050	0.05	-0.98	0.13	3.3E-14	21909115
rs4691707	4:156441314	*GUCY1A3/ GUCY1B3*	A/G	0.67	0.042	0.027	0.12	-0.35	0.06	7.1E-09	27618452
rs1173771	5:32815028	*NPR3/ C5orf23*	G/A	0.60	-0.002	0.026	0.94	0.50	0.06	1.8E-16	21909115
rs10077885	5:114390121	*TRIM36*	A/C	0.51	-0.046	0.026	0.07	-0.28	0.04	1.6E-10	27618452
rs1008058	5:122435627	*PRDM6*	A/G	0.12	0.084	0.041	0.04	0.55	0.09	3.0E-10	27618447
rs6595838	5:127868199	*FBN2*	G/A	0.71	-0.008	0.028	0.76	-0.40	0.07	1.1E-08	27841878
rs11953630	5:157845402	*EBF1*	T/C	0.37	0.008	0.026	0.76	-0.41	0.06	3.0E-11	21909115

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			WHRadjBMI			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs9349379	6:12903957	*PHACTR1*	A/G	0.59	0.032	0.027	0.24	0.29	0.05	8.8E-10	27618447
rs1799945	6:26091179	*HFE*	C/G	0.85	-0.058	0.035	0.10	-0.60	0.09	3.3E-12	27618452
rs6919440	6:43352898	*ZNF318/ ABCC10*	A/G	0.56	0.034	0.026	0.19	-0.34	0.06	4.9E-09	27618452
rs78648104	6:50683009	*TFAP2D*	T/C	0.91	-0.048	0.045	0.29	-0.48	0.08	1.3E-08	28135244
rs1361831	6:127181089	*RSPO3*	T/C	0.56	-0.021	0.026	0.41	-0.48	0.06	7.4E-17	27618452
rs2969070	7:2512545	*CHST12/ LFNG*	A/G	0.68	0.005	0.038	0.90	-0.30	0.05	1.4E-10	27618452
rs3735533	7:27245893	*HOTTIP/ EVX*	T/C	0.07	-0.051	0.054	0.34	-0.80	0.11	6.5E-14	27618452
rs12705390	7:106410777	*PIK3CG*	A/G	0.20	-0.041	0.032	0.20	0.62	0.07	2.7E-19	27618452
rs11556924	7:129663496	*ZC3HC1*	T/C	0.35	0.016	0.029	0.58	-0.27	0.05	7.6E-09	27618452
rs13238550	7:131059056	*MKLN1*	A/G	0.39	0.010	0.026	0.71	0.33	0.05	1.9E-12	28135244
rs1011018	7:139463264	*HIPK2*	A/G	0.20	0.010	0.032	0.76	-0.33	0.06	1.5E-08	28135244
rs2898290	8:11433909	*BLK/ GATA4*	T/C	0.47	0.042	0.026	0.11	0.38	0.06	8.9E-11	27618452
rs894344	8:135612745	*ZFAT*	A/G	0.59	0.003	0.026	0.90	-0.26	0.05	3.2E-08	28135244
rs34591516	8:142367087	*GPR20*	T/C	0.04	0.239	0.079	0.00	0.64	0.10	6.1E-10	27618447
rs10760117	9:123586737	*PSMD5*	T/G	0.41	-0.041	0.026	0.11	0.28	0.05	6.1E-10	27618452

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			WHRadjBMI			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs6271	9:136522274	*DBH*	T/C	0.07	0.023	0.084	0.79	-0.59	0.09	4.9E-11	27618452
rs1813353	10:18707448	*CACNB2*	T/C	0.65	-0.028	0.027	0.29	0.57	0.08	2.6E-12	21909115
rs7076398	10:63533663	*C10orf107*	A/T	0.19	-0.046	0.033	0.16	-0.56	0.08	1.7E-13	27618452
rs1848797	10:64552934	*AL133417.1*	A/G	0.61	0.052	0.026	0.05	0.40	0.07	1.1E-09	27841878
rs12247028	10:75410052	*SYNPO2L*	A/G	0.64	-0.003	0.028	0.91	-0.36	0.06	8.2E-09	27618452
rs932764	10:95895940	*PLCE1*	A/G	0.57	-0.016	0.026	0.54	-0.50	0.06	6.9E-17	27618452
rs4551692	10:102556453	*PAX2*	G/A	0.11	-0.028	0.041	0.49	-0.68	0.10	3.3E-11	27841878
rs943037	10:104835919	*CYP17A1/NT5C2*	T/C	0.09	-0.052	0.046	0.25	-1.13	0.10	2.4E-27	27618452
rs740746	10:115792787	*ADRB1*	A/G	0.72	0.041	0.029	0.15	0.49	0.07	4.6E-13	27618452
rs592373	11:1890990	*LSP1/TNNT3*	A/G	0.65	0.039	0.038	0.31	0.48	0.06	2.0E-14	27618452
rs7129220	11:10350538	*ADM*	G/A	0.89	0.000	0.041	1.00	-0.62	0.09	3.0E-12	21909115
rs381815	11:16902268	*PLEKHA7*	T/C	0.27	-0.022	0.029	0.45	0.58	0.09	5.3E-11	21909115
rs757081	11:17351683	*NUCB2*	G/C	0.33	-0.024	0.027	0.38	0.40	0.06	1.7E-10	24560520
rs7103648	11:47461783	*RAPSN/PSMC3/SLC39A13*	A/G	0.61	-0.030	0.026	0.25	-0.33	0.05	4.4E-13	27618452
rs1938598	11:58413910	*GLYAT*	T/C	0.75	-0.042	0.029	0.15	0.44	0.08	7.5E-09	27841878

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			WHRadjBMI			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs751984	11:61278246	*LRRC10B*	T/C	0.87	-0.017	0.048	0.73	0.41	0.07	3.8E-09	27618452
rs3741378	11:65408937	*RELA/ SIPA1*	T/C	0.14	-0.069	0.040	0.08	-0.55	0.09	3.4E-10	24560520
rs633185	11:100593538	*FLJ32810/ TMEM133*	G/C	0.29	0.011	0.028	0.70	-0.57	0.07	1.2E-17	21909115
rs7951348	11:107081841	*LOC105369477*	C/T	0.52	-0.001	0.026	0.97	-0.40	0.06	7.7E-10	27841878
rs6487543	12:26438189	*SSPN*	A/G	0.76	0.046	0.030	0.12	0.30	0.05	6.3E-10	28135244
rs7302981	12:50537815	*CERS5*	A/G	0.39	0.037	0.026	0.16	0.34	0.05	6.1E-13	27618447
rs10784502	12:66343810	*HMGA2*	C/T	0.48	-0.036	0.025	0.16	-0.37	0.06	1.3E-08	27841878
rs11105354	12:90026523	*ATP2B1*	A/G	0.83	-0.042	0.033	0.21	0.91	0.08	3.9E-29	27618452
rs3184504	12:111884608	*SH2B3*	T/C	0.51	-0.013	0.025	0.60	0.60	0.07	3.8E-18	21909115
rs8016306	14:63928546	*PPP2R5E*	A/G	0.79	0.013	0.031	0.68	0.34	0.06	3.7E-09	28135244
rs1378942	15:75077367	*CYP1A1/ ULK3*	C/A	0.35	-0.005	0.028	0.84	0.61	0.06	5.7E-23	21909115
rs2521501	15:91437388	*FURIN/ FES*	A/T	0.67	0.002	0.033	0.95	-0.64	0.07	3.4E-20	27618452
rs140004517	16:65265702	*AC009055.2*	C/T	0.49	-0.029	0.026	0.26	-0.39	0.07	2.5E-09	27841878
rs35261357	16:75444572	*SNORA76/ CFDP1*	C/T	0.41	-0.080	0.026	0.00	-0.43	0.07	6.4E-11	27841878

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			WHRadjBMI			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs7226020	17:6473828	*KIAA0753*	T/C	0.63	0.001	0.028	0.98	-0.42	0.07	3.9E-10	27841878
rs7213273	17:43155914	*PLCD3*	A/G	0.66	-0.016	0.027	0.56	-0.41	0.07	4.7E-10	27618452
rs17608766	17:45013271	*GOSR2*	T/C	0.87	0.038	0.038	0.32	-0.66	0.08	2.3E-15	27618452
rs7406910	17:46688256	*HOXB7*	T/C	0.09	0.020	0.045	0.66	-0.46	0.08	3.8E-08	27618447
rs12940887	17:47402807	*ZNF652*	T/C	0.37	-0.043	0.026	0.10	0.36	0.06	1.8E-10	21909115
rs8068318	17:59483766	*TBX2*	T/C	0.69	-0.012	0.032	0.71	0.42	0.05	1.3E-15	27618447
rs12958173	18:42141977	*SETBP1*	A/C	0.30	-0.016	0.028	0.56	0.36	0.05	1.4E-13	27618452
rs4247374	19:7252756	*INSR*	T/C	0.14	0.037	0.036	0.31	-0.59	0.07	1.2E-18	27618452
rs167479	19:11526765	*RGL3*	T/G	0.48	-0.029	0.036	0.42	-0.50	0.05	1.5E-26	27618447
rs1327235	20:10969030	*JAG1*	A/G	0.54	0.014	0.025	0.58	-0.40	0.06	2.2E-11	27618452
rs6015450	20:57751117	*GNAS/ EDN3*	G/A	0.12	-0.024	0.040	0.54	0.90	0.09	3.9E-23	21909115
rs6090040	20:62692060	*TCEA2*	A/C	0.45	0.012	0.038	0.74	0.40	0.07	7.6E-10	27841878
rs13050325	21:16343812	*NRIP1*	A/G	0.75	0.025	0.030	0.39	-0.46	0.07	1.0E-09	27841878

Abbreviations: EAF, effect allele frequency; PMID, PubMed ID.

[a] Log odds per effect allele.

[b] mmHg per effect allele. For ease of comparison, the results of the Mendelian randomization are shown per SD increase using the conversion 20.7 mmHg = 1 SD[149].

Supplementary Table 3.5. Input for the Mendelian Randomization of Diastolic Blood Pressure.

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Diastolic Blood Pressure			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs880315	1:10796866	*CASZ1*	T/C	0.65	0.022	0.031	0.475	-0.257	0.038	1.3E-11	27618452
rs17037390	1:11860843	*MTHFR/ NPPB*	A/G	0.16	-0.048	0.036	0.177	-0.499	0.050	1.2E-23	27618452
rs6686889	1:25030470	*chr1mb25*	T/C	0.27	0.084	0.029	0.004	0.185	0.031	3.6E-09	28135244
rs1620668	1:113023980	*ST7L/ CAPZA1/ MOV10*	A/G	0.84	0.016	0.038	0.670	-0.285	0.047	9.0E-10	27618452
rs11102916	1:115836746	*NGF*	C/A	0.98	0.128	0.105	0.216	-0.705	0.126	2.1E-08	27841878
rs12405515	1:172357441	*PIGC\ DNM3*	G/T	0.42	-0.025	0.026	0.326	0.207	0.037	1.7E-08	27841878
rs4245739	1:204518842	*MDM4*	A/C	0.72	0.022	0.029	0.440	0.243	0.041	4.6E-09	27618452
rs10916082	1:227252626	*CDC42BPA*	A/G	0.73	-0.034	0.029	0.240	-0.177	0.031	8.4E-09	28135244
rs2493134	1:230849359	*AGT*	T/C	0.58	0.003	0.026	0.907	-0.275	0.036	9.5E-15	27618452
rs6429422	1:243472801	*SDCCAG8*	T/G	0.68	-0.022	0.027	0.422	-0.255	0.039	4.1E-11	27841878
rs55701159	2:25139596	*ADCY3*	T/G	0.89	-0.003	0.041	0.951	0.285	0.044	7.2E-11	28135244
rs2586886	2:26932031	*KCNK3*	T/C	0.62	-0.005	0.028	0.843	-0.254	0.036	1.9E-12	27618452
rs4952611	2:40567743	*SLC8A1*	T/C	0.59	-0.009	0.027	0.746	-0.157	0.029	4.0E-08	28135244
rs76326501	2:43167878	*AC016735.1*	A/C	0.91	0.002	0.047	0.959	0.419	0.048	3.6E-18	28135244
rs1975487	2:55809054	*PNPT1*	A/G	0.49	-0.010	0.026	0.688	-0.160	0.027	1.8E-09	27618452

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Diastolic Blood Pressure			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs2579519	2:96675166	*GPAT2/ FAHD2CP*	T/C	0.64	0.031	0.026	0.235	-0.197	0.029	4.8E-12	28135244
rs1446468	2:164963486	*FIGN*	T/C	0.47	-0.007	0.026	0.782	-0.265	0.046	6.9E-09	27618448
rs16823124	2:183224127	*PDE1A*	A/G	0.30	-0.028	0.028	0.305	0.262	0.041	2.0E-10	24560520
rs7592578	2:191439591	*TMEM194 B*	T/G	0.19	-0.042	0.033	0.200	-0.240	0.035	9.5E-12	28135244
rs2972146	2:227100698	*2q36.3*	T/G	0.64	0.046	0.027	0.085	0.172	0.030	8.4E-09	27618447
rs11128722	3:14958126	*FGD5*	A/G	0.55	-0.052	0.027	0.053	-0.173	0.028	5.2E-10	27618452
rs13082711	3:27537909	*SLC4A7*	T/C	0.78	0.020	0.030	0.500	-0.238	0.040	3.8E-09	21909115
rs3774372	3:41877414	*ULK4*	T/C	0.83	-0.058	0.033	0.083	-0.367	0.049	9.0E-14	21909115
rs6442101	3:48130893	*MAP4*	T/C	0.68	0.012	0.028	0.656	0.303	0.038	1.6E-15	27618452
rs918466	3:64710253	*ADAMTS9*	A/G	0.44	0.066	0.025	0.009	-0.182	0.027	1.7E-11	27618452
rs16851397	3:141134818	*ZBTB38*	A/G	0.96	0.017	0.063	0.785	-0.375	0.068	3.0E-08	27618447
rs113161639	3:154615819	*LOC10537 4171*	G/T	0.90	0.035	0.043	0.412	0.418	0.060	2.6E-12	27841878
rs419076	3:169100886	*MECOM*	T/C	0.48	-0.023	0.026	0.371	0.241	0.034	2.1E-12	21909115
rs4686683	3:185307363	*SENP2*	T/G	0.59	0.034	0.026	0.194	-0.215	0.037	4.8E-09	27841878
rs2291435	4:38387395	*TBC1D1/ FLJ13197*	T/C	0.54	-0.030	0.026	0.248	-0.156	0.027	4.3E-09	27618452

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Diastolic Blood Pressure			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs1458038	4:81164723	*FGF5*	T/C	0.29	-0.018	0.028	0.515	0.457	0.044	8.5E-25	21909115
rs13107325	4:103188709	*SLC39A8*	T/C	0.07	-0.096	0.050	0.053	-0.684	0.081	2.3E-17	21909115
rs66887589	4:120509279	*PDE5A*	T/C	0.51	-0.038	0.026	0.132	-0.289	0.036	1.2E-15	27841878
rs13139571	4:156645513	*GUCY1A3/ GUCY1B3*	C/A	0.76	-0.008	0.029	0.783	0.260	0.041	2.2E-10	21909115
rs1173771	5:32815028	*NPR3/ C5orf23*	G/A	0.60	-0.002	0.026	0.939	0.261	0.038	9.1E-12	21909115
rs258494	5:75038718	*LOC441087*	C/G	0.37	-0.003	0.027	0.910	0.247	0.037	4.1E-11	27841878
rs10077885	5:114390121	*TRIM36*	A/C	0.51	-0.046	0.026	0.071	-0.174	0.026	4.0E-11	27618452
rs6891344	5:123136656	*CSNK1G3*	A/G	0.81	-0.001	0.032	0.984	0.231	0.034	1.6E-11	27618452
rs12521868	5:131784393	*C5orf56*	T/G	0.42	-0.011	0.026	0.658	-0.191	0.029	6.1E-11	27618447
rs11953630	5:157845402	*EBF1*	T/C	0.37	0.008	0.026	0.759	-0.281	0.039	3.8E-13	21909115
rs72812846	5:173377636	*CPEB4*	T/A	0.74	-0.041	0.030	0.171	0.224	0.041	3.8E-08	27841878
rs1799945	6:26091179	*HFE*	C/G	0.85	-0.058	0.035	0.097	-0.430	0.053	3.1E-16	27618452
rs169287	6:27854760	*HIST1H3J*	C/A	0.84	0.036	0.035	0.306	0.265	0.046	7.0E-09	27841878
rs2854275	6:32628428	*HLA/DQB1*	A/C	0.12	0.007	0.039	0.858	-0.562	0.082	5.5E-12	24560520
rs13205180	6:51832494	*PKHD1*	C/T	0.51	0.065	0.026	0.011	-0.214	0.036	4.4E-09	27841878

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Diastolic Blood Pressure			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs2050663	6:79753394	*PHIP*	T/C	0.50	-0.023	0.026	0.366	-0.250	0.036	4.7E-12	27841878
rs1361831	6:127181089	*RSPO3*	T/C	0.56	-0.021	0.026	0.409	-0.271	0.036	2.3E-14	27618452
rs17080093	6:150997440	*PLEKHG1*	T/C	0.07	-0.090	0.052	0.083	-0.411	0.068	1.7E-09	27618452
rs2969070	7:2512545	*CHST12/ LFNG*	A/G	0.68	0.005	0.038	0.900	-0.182	0.027	2.9E-11	27618452
rs3735533	7:27245893	*HOTTIP/ EVX*	T/C	0.07	-0.051	0.054	0.343	-0.445	0.065	1.1E-11	27618452
rs11556924	7:129663496	*ZC3HC1*	T/C	0.35	0.016	0.029	0.584	-0.214	0.028	8.2E-15	27618452
rs111630016	7:158048396	*PTPRN2*	C/T	0.95	-0.021	0.068	0.756	0.443	0.081	4.4E-08	27841878
rs2978098	8:101676675	*SNX31*	A/C	0.54	0.003	0.026	0.901	0.165	0.027	1.5E-09	28135244
rs4364717	9:21801530	*MTAP*	A/G	0.55	0.011	0.026	0.681	-0.175	0.027	1.3E-10	28135244
rs687621	9:136137065	*ABO*	A/G	0.66	-0.010	0.027	0.700	0.188	0.030	5.5E-10	27618447
rs6271	9:136522274	*DBH*	T/C	0.07	0.023	0.084	0.790	-0.465	0.053	2.4E-18	27618452
rs12243859	10:18740632	*CACNB2*	T/C	0.34	0.022	0.027	0.414	-0.335	0.038	8.1E-19	27618452
rs7076398	10:63533663	*C10orf107*	A/T	0.19	-0.046	0.033	0.161	-0.409	0.047	2.6E-18	27618452
rs10995311	10:64564934	*ADO*	C/G	0.56	0.042	0.026	0.106	0.210	0.030	1.1E-12	27618447
rs932764	10:95895940	*PLCE1*	A/G	0.57	-0.016	0.026	0.536	-0.224	0.036	6.3E-10	27618452

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Diastolic Blood Pressure			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs943037	10:104835919	*CYP17A1/ NT5C2*	T/C	0.09	-0.052	0.046	0.251	-0.482	0.064	4.5E-14	27618452
rs740746	10:115792787	*ADRB1*	A/G	0.72	0.041	0.029	0.153	0.320	0.041	8.6E-15	27618452
rs592373	11:1890990	*LSP1/ TNNT3*	A/G	0.65	0.039	0.038	0.308	0.282	0.039	3.6E-13	27618452
rs110419	11:8252853	*LMO1*	A/G	0.49	-0.017	0.032	0.596	0.159	0.029	3.0E-08	27618447
rs1450271	11:10356115	*ADM*	T/C	0.47	-0.058	0.026	0.024	0.199	0.036	4.1E-08	27618452
rs1156725	11:16307700	*PLEKHA7*	T/C	0.80	-0.021	0.032	0.498	-0.292	0.044	3.7E-11	27618452
rs7103648	11:47461783	*RAPSN/ PSMC3/ SLC39A13*	A/G	0.61	-0.030	0.026	0.254	-0.241	0.027	9.0E-19	27618452
rs751984	11:61278246	*LRRC10B*	T/C	0.87	-0.017	0.048	0.727	0.376	0.041	4.2E-20	27618452
rs633185	11:100593538	*FLJ32810/ TMEM133*	G/C	0.29	0.011	0.028	0.701	-0.328	0.041	2.0E-15	21909115
rs3752728	12:20192972	*PDE3A*	A/G	0.72	0.002	0.028	0.944	0.319	0.040	2.4E-15	27618452
rs7302981	12:50537815	*CERS5*	A/G	0.39	0.037	0.026	0.159	0.249	0.029	1.4E-17	27618447
rs11105354	12:90026523	*ATP2B1*	A/G	0.83	-0.042	0.033	0.210	0.459	0.050	2.6E-20	27618452
rs3184504	12:111884608	*SH2B3*	T/C	0.51	-0.013	0.025	0.604	0.448	0.043	3.6E-25	21909115
rs2891546	12:115552499	*TBX5/ TBX3*	A/G	0.10	-0.035	0.042	0.398	-0.380	0.061	4.7E-10	27618452
rs1060105	12:123806219	*SBNO1*	T/C	0.21	0.000	0.032	0.988	-0.182	0.035	2.8E-07	27618447

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Diastolic Blood Pressure			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs7178615	15:66869072	*RP11/ 321F6.1*	A/G	0.38	-0.005	0.026	0.855	-0.179	0.028	2.6E-10	28135244
rs117638970	15:69675605	*PAQR5*	C/T	0.97	0.031	0.089	0.723	0.655	0.120	4.5E-08	27841878
rs1378942	15:75077367	*CYP1A1/ ULK3*	C/A	0.35	-0.005	0.028	0.843	0.416	0.039	2.7E-26	21909115
rs62012628	15:79070000	*ADAMTS7*	T/C	0.29	0.055	0.029	0.062	-0.238	0.034	5.1E-12	28135244
rs2521501	15:91437388	*FURIN/ FES*	A/T	0.67	0.002	0.033	0.950	-0.358	0.042	1.9E-17	27618452
rs12906962	15:95312071	*LOC105370988*	T/C	0.67	-0.006	0.027	0.838	-0.248	0.039	1.9E-10	27841878
rs4984497	15:96635899	*AC012409.2*	T/C	0.34	0.011	0.027	0.678	0.216	0.039	2.2E-08	27841878
rs8059962	16:81574197	*CMIP*	T/C	0.44	0.045	0.026	0.085	-0.170	0.028	1.3E-09	28135244
rs1126464	16:89704365	*DPEP1*	C/G	0.24	0.005	0.033	0.876	0.275	0.034	1.0E-15	27618447
rs12940887	17:47402807	*ZNF652*	T/C	0.37	-0.043	0.026	0.100	0.270	0.035	2.3E-14	21909115
rs8068318	17:59483766	*TBX2*	T/C	0.69	-0.012	0.032	0.715	0.262	0.032	1.9E-16	27618447
rs12958173	18:42141977	*SETBP1*	A/C	0.30	-0.016	0.028	0.558	0.179	0.029	5.9E-10	27618452
rs745821	18:48142854	*MAPK4*	T/G	0.76	0.000	0.030	0.999	0.189	0.031	1.4E-09	28135244
rs4247374	19:7252756	*INSR*	T/C	0.14	0.037	0.036	0.306	-0.385	0.040	2.1E-22	27618452
rs167479	19:11526765	*RGL3*	T/G	0.48	-0.029	0.036	0.422	-0.330	0.028	2.0E-31	27618447

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Diastolic Blood Pressure			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs17638167	19:11584818	*ELAVL3*	T/C	0.05	-0.006	0.061	0.919	-0.348	0.063	3.7E-08	27618452
rs2304130	19:19789528	*ZNF101*	A/G	0.91	0.032	0.050	0.517	-0.292	0.052	1.5E-08	27618447
rs6108168	20:8626271	*PLCB1*	A/C	0.25	0.000	0.029	0.989	-0.211	0.031	1.1E-11	28135244
rs1327235	20:10969030	*JAG1*	A/G	0.54	0.014	0.025	0.578	-0.308	0.036	1.8E-17	27618452
rs867186	20:33764554	*PROCR*	A/G	0.90	0.085	0.044	0.051	0.265	0.046	1.2E-08	27618447
rs6095241	20:47308798	*PREX1*	A/G	0.44	0.025	0.026	0.336	-0.168	0.029	4.8E-09	27618447
rs6026748	20:57745815	*GNAS/ EDN3*	A/G	0.12	-0.028	0.040	0.476	0.552	0.055	4.9E-24	27618452
rs12627651	21:44760603	*CRYAA/ SIK1*	A/G	0.29	-0.088	0.051	0.083	0.204	0.030	1.4E-11	27618452
rs9306160	21:45107562	*RRP1B*	T/C	0.38	-0.028	0.027	0.304	-0.179	0.031	6.8E-09	27618447

Abbreviations: EAF, effect allele frequency; PMID, PubMed ID.

[a] Log odds per effect allele.

[b] mmHg per effect allele. For ease of comparison, the results of the Mendelian randomization are shown per SD increase using the conversion 11.3 mmHg = 1 SD[149].

Supplementary Table 3.6. Input for the Mendelian Randomization of Pulse Pressure.

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Pulse Pressure			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs709209	1:6278414	*RNF207*	A/G	0.68	0.023	0.035	0.504	0.199	0.035	9.6E-09	27618447
rs9662255	1:9441949	*SPSB1*	C/A	0.61	0.030	0.033	0.354	0.307	0.046	3.5E-11	27841878
rs112557609	1:56576924	*RP4/7 10M16.1/ PPAP2B*	A/G	0.34	0.009	0.027	0.740	0.227	0.030	6.8E-12	28135244
rs60199046	1:59663341	*HSD52/ LINC01358*	A/G	0.73	0.030	0.029	0.303	0.448	0.050	3.6E-19	27841878
rs786919	1:89281529	*PKN2/ RNU6/ 125P*	A/G	0.40	0.024	0.026	0.353	0.261	0.046	1.5E-08	27841878
rs12731740	1:208024820	*CD34*	T/C	0.11	-0.051	0.042	0.228	-0.249	0.040	1.1E-08	28135244
rs11690961	2:46363336	*PRKCE*	A/C	0.89	-0.037	0.039	0.350	0.446	0.071	2.7E-10	27841878
rs2540950	2:65279223	*CEP68*	C/T	0.63	-0.037	0.026	0.161	0.265	0.048	2.9E-08	27841878
rs13002573	2:164915208	*FIGN*	G/A	0.23	0.013	0.030	0.678	-0.310	0.055	1.8E-08	21909110
rs1250247	2:216299629	*FN1*	C/G	0.27	0.043	0.029	0.135	0.398	0.051	8.6E-15	27841878
rs62270945	3:128201889	*GATA2*	C/T	0.97	0.035	0.090	0.698	-0.818	0.138	2.9E-09	27841878
rs11708647	3:142617353	*PCOLCE2*	G/A	0.65	-0.013	0.027	0.636	0.260	0.048	4.9E-08	27841878
rs871606	4:54799245	*CHIC2*	T/C	0.89	0.008	0.042	0.852	0.429	0.075	1.3E-08	21909110
rs1878406	4:148393664	*EDNRA*	C/T	0.86	0.033	0.037	0.369	-0.452	0.066	5.3E-12	27841878
rs869396	4:169688000	*PALLD*	C/A	0.52	0.045	0.026	0.078	0.292	0.046	2.4E-10	27841878

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Pulse Pressure			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs17059668	4:174584663	*chr4mb174*	C/G	0.92	0.036	0.048	0.456	-0.332	0.060	2.8E-08	28135244
rs1173756	5:32789852	*NPR3/ C5orf23*	T/C	0.47	-0.010	0.026	0.701	-0.267	0.046	6.9E-09	27841878
rs9349379	6:12903957	*PHACTR1*	A/G	0.59	0.032	0.027	0.242	0.342	0.046	1.8E-13	27841878
rs169287	6:27854760	*HIST1H3J*	C/A	0.84	0.036	0.035	0.306	-0.360	0.058	4.9E-10	27841878
rs4140574	6:56099424	*COL21A1*	T/A	0.45	0.033	0.026	0.205	0.330	0.046	9.1E-13	27841878
rs11154027	6:121781390	*GJA1*	T/C	0.46	-0.018	0.026	0.488	0.207	0.030	1.1E-10	28135244
rs1570350	6:143592386	*AIG1*	A/G	0.56	0.007	0.026	0.777	0.286	0.046	6.2E-10	27841878
rs13192976	6:152312415	*ESR1*	A/T	0.89	0.054	0.042	0.190	-0.644	0.075	8.5E-18	27841878
rs516143	6:159696185	*FNDC1*	G/C	0.13	0.043	0.037	0.247	0.466	0.065	1.0E-12	27841878
rs1322639	6:169587103	*AL136129. 1*	G/A	0.22	0.019	0.031	0.528	-0.401	0.055	4.2E-13	27841878
rs2282978	7:92264410	*CDK6*	C/T	0.35	-0.022	0.027	0.412	-0.268	0.049	4.5E-08	24560520
rs34489224	7:100525559	*RPS29P15*	C/G	0.73	0.002	0.030	0.937	0.296	0.054	4.4E-08	27841878
rs17477177	7:106411858	*PIK3CG*	T/C	0.80	0.040	0.032	0.207	-0.418	0.057	2.3E-13	21909110
rs2071518	8:120435812	*NOV*	T/C	0.27	0.017	0.028	0.560	0.312	0.053	3.7E-09	21909110
rs4454254	8:141060027	*TRAPPC9*	A/G	0.64	0.029	0.027	0.271	-0.261	0.030	5.1E-16	28135244

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Pulse Pressure			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs1333047	9:22124504	*CDKN2B-AS1*	A/T	0.52	0.056	0.027	0.035	-0.253	0.046	3.1E-08	27841878
rs9337951	10:30317073	*KIAA1462*	G/A	0.73	-0.014	0.032	0.660	-0.295	0.050	2.7E-09	27841878
rs813412	10:32284825	*RN7SL825P*	C/T	0.25	-0.032	0.030	0.278	0.297	0.053	2.0E-08	27841878
rs9663362	10:95895177	*PLCE1*	G/C	0.54	-0.026	0.026	0.309	-0.271	0.046	5.2E-09	27618448
rs3824755	10:104595849	*CYP17A1/NT5C3*	G/C	0.90	0.038	0.043	0.379	0.477	0.077	7.3E-10	27618448
rs7126805	11:828916	*CRACR2B*	A/G	0.72	-0.040	0.040	0.326	0.222	0.040	3.3E-09	28135244
rs757081	11:17351683	*NUCB2*	G/C	0.33	-0.024	0.027	0.377	0.321	0.050	8.9E-11	24560520
rs685149	11:57657413	*CTNND1*	A/G	0.35	-0.030	0.027	0.271	-0.262	0.048	3.9E-08	27841878
rs2289125	11:89224453	*NOX4*	A/C	0.22	-0.055	0.032	0.080	-0.483	0.056	6.6E-18	27841878
rs1261744	11:117218460	*CEP164*	T/C	0.81	0.003	0.032	0.937	-0.316	0.057	2.9E-08	27841878
rs11222084	11:130273230	*ADAMTS8*	T/A	0.34	-0.002	0.027	0.954	0.337	0.050	1.9E-11	21909110
rs10784502	12:66343810	*HMGA2*	C/T	0.48	-0.036	0.025	0.161	-0.289	0.045	2.1E-10	27841878
rs17249754	12:90060586	*ATP2B1*	G/A	0.83	-0.038	0.033	0.256	0.392	0.063	5.5E-10	27618448
rs452036	14:23865885	*MYH6*	A/G	0.35	-0.038	0.032	0.246	-0.282	0.035	3.0E-16	27618447
rs1381289	14:98592364	*C14orf64*	T/C	0.43	0.014	0.026	0.578	0.073	0.011	5.6E-11	22068335

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Pulse Pressure			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs62011052	15:79156983	*MORF4L1*	T/C	0.85	0.011	0.036	0.762	-0.358	0.064	2.2E-08	27841878
rs140004517	16:65265702	*AC009055.2*	C/T	0.49	-0.029	0.026	0.263	-0.336	0.046	2.7E-13	27841878
rs117006983	16:70755610	*VAC14*	A/G	0.02	0.005	0.101	0.959	0.986	0.140	4.1E-12	28135244
rs35261357	16:75444572	*SNORA76/CFDP1*	C/T	0.41	-0.080	0.026	0.002	-0.302	0.046	7.4E-11	27841878
rs7500448	16:83045790	*CDH13*	A/G	0.74	0.003	0.029	0.918	0.416	0.053	4.5E-15	27841878
rs34457140	17:1353920	*CRK*	T/G	0.51	0.030	0.026	0.250	-0.258	0.046	1.7E-08	27841878
rs7226020	17:6473828	*KIAA0753*	T/C	0.63	0.001	0.028	0.984	-0.349	0.047	8.2E-14	27841878
rs78378222	17:7571752	*TP53*	T/G	0.99	-0.018	0.120	0.879	1.575	0.214	1.9E-13	27841878
rs79089478	17:40317241	*KCNH4/HSD17B1*	T/C	0.97	0.009	0.080	0.913	0.584	0.100	3.1E-09	28135244
rs62080325	17:42060631	*PYY*	A/G	0.69	-0.035	0.028	0.219	-0.186	0.030	4.0E-08	28135244
rs17608766	17:45013271	*GOSR2*	T/C	0.87	0.038	0.038	0.318	-0.534	0.068	6.4E-15	27618448
rs740698	17:60767151	*MRC2*	T/C	0.57	-0.054	0.029	0.066	-0.228	0.030	3.1E-12	28135244
rs2193635	18:43096236	*SLC14A2*	C/T	0.82	0.003	0.033	0.922	-0.432	0.060	6.1E-13	27841878
rs10418305	19:15278808	*NOTCH3*	C/G	0.10	-0.019	0.043	0.648	-0.455	0.078	4.5E-09	27841878
rs3790227	20:19469002	*SLC24A3*	C/A	0.71	0.034	0.029	0.234	-0.311	0.051	1.2E-09	27841878

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Pulse Pressure			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs6090040	20:62692060	*TCEA2*	A/C	0.45	0.012	0.038	0.744	0.283	0.046	7.2E-10	27841878
rs13050325	21:16343812	*NRIP1*	A/G	0.75	0.025	0.030	0.393	-0.290	0.053	4.1E-08	27841878
rs470113	22:40729614	*TNRC6B*	A/G	0.82	0.036	0.034	0.289	-0.253	0.042	1.7E-09	27618447
rs73161324	22:42038786	*XRCC6*	T/C	0.05	-0.036	0.060	0.554	0.496	0.070	2.8E-11	28135244

Abbreviations: EAF, effect allele frequency; PMID, PubMed ID.

[a] Log odds per effect allele.

[b] mmHg per effect allele. For ease of comparison, the results of the Mendelian randomization are shown per SD increase using the conversion 14.2 mmHg = 1 SD[149].

Supplementary Table 3.7. Input for the Mendelian Randomization of Hypertension.

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Hypertension			PMID
					Beta[a]	SE	*p*	Beta[a]	SE	*p*	
rs17367504	1:11862778	*MTHFR/ NPPB*	A/G	0.84	0.040	0.036	0.258	0.103	0.016	2.3E-10	21909115
rs1173771	5:32815028	*NPR3/ C5orf23*	G/A	0.60	0.002	0.026	0.939	0.062	0.010	3.2E-10	21909115
rs1799945	6:26091179	*HFE*	G/C	0.15	0.058	0.035	0.097	0.095	0.015	1.8E-10	21909115
rs1813353	10:18707448	*CACNB2*	T/C	0.65	-0.028	0.027	0.286	0.078	0.013	6.2E-10	21909115
rs4590817	10:63467553	*C10orf107*	G/C	0.83	0.032	0.035	0.359	0.096	0.017	9.8E-09	21909115
rs932764	10:95895940	*PLCE1*	G/A	0.43	0.016	0.026	0.536	0.055	0.010	9.4E-09	21909115
rs633185	11:100593538	*FLJ32810/ TMEM133*	C/G	0.71	-0.011	0.028	0.701	0.070	0.011	5.4E-11	21909115
rs17249754	12:90060586	*ATP2B1*	G/A	0.83	-0.038	0.033	0.256	0.126	0.016	1.1E-14	21909115
rs1378942	15:75077367	*CYP1A1/ ULK3*	C/A	0.35	-0.005	0.028	0.843	0.073	0.013	1.0E-08	21909115
rs13333226	16:20365654	*UMOD*	A/G	0.82	-0.030	0.033	0.368	0.163	0.022	1.5E-13	21082022
rs6015450	20:57751117	*GNAS/ EDN3*	G/A	0.12	-0.024	0.040	0.537	0.110	0.015	4.2E-14	21909115

Abbreviations: EAF, effect allele frequency; PMID, PubMed ID.

[a] Log odds per effect allele.

Supplementary Table 3.8. Input for the Mendelian Randomization of LDL-C.

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			LDL-C			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs12027135	1:25775733	*LDLRAP1*	A/T	0.46	-0.012	0.026	0.646	-0.030	0.004	2.0E-14	24097068
rs12748152	1:27138393	*PIGV/ NR0B2*	T/C	0.07	-0.005	0.049	0.921	0.050	0.007	3.0E-12	24097068
rs2479409	1:55504650	*PCSK9*	G/A	0.36	0.042	0.031	0.172	0.064	0.004	3.0E-50	24097068
rs2131925	1:63025942	*ANGPTL3*	G/T	0.33	0.042	0.027	0.122	-0.049	0.004	3.0E-32	24097068
rs629301	1:109818306	*SORT1*	G/T	0.22	-0.107	0.035	0.002	-0.167	0.005	5.0E-241	24097068
rs267733	1:150958836	*ANXA9/ CERS2*	G/A	0.15	0.008	0.037	0.837	-0.033	0.006	5.0E-09	24097068
rs2642442	1:220973563	*MOSC1*	C/T	0.31	0.028	0.028	0.309	-0.036	0.005	5.0E-11	24097068
rs514230	1:234858597	*IRF2BP2*	A/T	0.48	-0.025	0.026	0.322	-0.036	0.005	9.0E-12	24097068
rs1367117	2:21263900	*APOB*	A/G	0.31	0.006	0.027	0.821	0.119	0.004	1.0E-182	24097068
rs4299376	2:44072576	*ABCG5*	G/T	0.32	0.067	0.027	0.014	0.081	0.005	4.0E-72	24097068
rs2710642	2:63149557	*EHBP1*	G/A	0.35	0.022	0.027	0.415	-0.024	0.004	6.0E-09	24097068
rs10490626	2:118835841	*INSIG2*	A/G	0.08	-0.092	0.049	0.057	-0.051	0.007	2.0E-12	24097068
rs2030746	2:121309488	*LOC84931*	T/C	0.42	0.056	0.026	0.034	0.021	0.004	9.0E-09	24097068
rs1250229	2:216304384	*FN1*	T/C	0.26	0.054	0.029	0.064	-0.024	0.004	3.0E-08	24097068
rs11563251	2:234679384	*UGT1A1*	T/C	0.11	0.055	0.040	0.179	0.034	0.006	5.0E-08	24097068

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			LDL-C			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs7640978	3:32533010	*CMTM6*	T/C	0.09	0.017	0.045	0.709	-0.039	0.007	1.0E-08	24097068
rs17404153	3:132163200	*ACAD11*	T/G	0.12	0.004	0.039	0.924	-0.034	0.006	2.0E-09	24097068
rs6831256	4:3473139	*LRPAP1*	G/A	0.43	0.045	0.033	0.179	-0.025	0.004	2.0E-08	24097068
rs12916	5:74656539	*HMGCR*	C/T	0.41	-0.029	0.026	0.266	0.073	0.004	8.0E-78	24097068
rs4530754	5:122855416	*CSNK1G3*	G/A	0.44	0.024	0.026	0.354	-0.028	0.004	4.0E-12	24097068
rs6882076	5:156390297	*TIMD4*	T/C	0.37	-0.027	0.027	0.311	-0.046	0.004	3.0E-31	24097068
rs3757354	6:16127407	*MYLIP*	T/C	0.21	0.016	0.031	0.610	-0.038	0.004	2.0E-17	24097068
rs1800562	6:26093141	*HFE*	A/G	0.06	0.041	0.053	0.442	-0.062	0.008	8.0E-14	24097068
rs3177928	6:32412435	*HLA*	A/G	0.14	-0.011	0.036	0.769	0.045	0.005	3.0E-17	24097068
rs9488822	6:116312893	*FRK*	T/A	0.35	-0.072	0.027	0.008	0.031	0.006	2.0E-07	24097068
rs1564348	6:160578860	*LPA*	C/T	0.16	-0.037	0.035	0.283	0.048	0.005	3.0E-21	24097068
rs12670798	7:21607352	*DNAH11*	C/T	0.23	-0.021	0.030	0.491	0.034	0.005	5.0E-14	24097068
rs4722551	7:25991826	*MIR148A*	C/T	0.16	0.022	0.035	0.530	0.039	0.005	4.0E-14	24097068
rs2072183	7:44579180	*NPC1L1*	C/G	0.23	0.046	0.033	0.159	0.039	0.005	7.0E-16	24097068
rs9987289	8:9183358	*PPP1R3B*	A/G	0.09	0.020	0.045	0.658	-0.071	0.007	9.0E-24	24097068
rs10102164	8:55421614	*SOX17*	A/G	0.20	-0.009	0.032	0.778	0.032	0.005	4.0E-11	24097068

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			LDL-C			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs2081687	8:59388565	*CYP7A1*	T/C	0.34	0.025	0.027	0.358	0.031	0.006	1.0E-07	24097068
rs2954029	8:126490972	*TRIB1*	T/A	0.46	-0.039	0.026	0.125	-0.056	0.004	2.0E-50	24097068
rs11136341	8:145043543	*PLEC1*	G/A	0.36	0.023	0.029	0.429	0.045	0.007	7.0E-12	24097068
rs3780181	9:2640759	*VLDLR*	G/A	0.07	0.028	0.052	0.586	-0.044	0.007	2.0E-09	24097068
rs635634	9:136155000	*ABO*	T/C	0.19	0.019	0.032	0.553	0.077	0.006	2.0E-41	24097068
rs2255141	10:113933886	*GPAM*	A/G	0.30	-0.032	0.028	0.250	0.030	0.004	1.0E-13	24097068
rs174546	11:61569830	*FADS1/2/3*	T/C	0.33	-0.131	0.027	0.000	-0.051	0.004	2.0E-39	24097068
rs964184	11:116648917	*APOA1*	C/G	0.86	-0.091	0.036	0.011	-0.086	0.008	2.0E-26	24097068
rs11220462	11:126243952	*ST3GAL4*	A/G	0.12	0.043	0.041	0.293	0.059	0.006	7.0E-21	24097068
rs11065987	12:112072424	*BRAP*	G/A	0.44	0.009	0.026	0.720	-0.027	0.004	1.0E-11	24097068
rs1169288	12:121416650	*HNF1A*	C/A	0.33	0.050	0.028	0.068	0.038	0.004	6.0E-21	24097068
rs4942486	13:32953388	*BRCA2*	T/C	0.49	-0.004	0.025	0.860	0.024	0.004	2.0E-11	24097068
rs8017377	14:24883887	*NYNRIN*	A/G	0.47	-0.026	0.027	0.343	0.030	0.004	3.0E-15	24097068
rs3764261	16:56993324	*CETP*	A/C	0.32	-0.021	0.027	0.433	-0.053	0.004	2.0E-34	24097068
rs2000999	16:72108093	*HPR*	A/G	0.20	0.028	0.032	0.372	0.065	0.005	4.0E-41	24097068
rs314253	17:7091650	*DLG4*	C/T	0.34	-0.003	0.027	0.921	-0.024	0.004	3.0E-10	24097068

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			LDL-C			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs7206971	17:45425115	*OSBPL7*	A/G	0.48	-0.020	0.026	0.430	0.029	0.006	3.0E-07	24097068
rs6511720	19:11202306	*LDLR*	T/G	0.12	-0.023	0.040	0.566	-0.221	0.006	4.0E-262	24097068
rs10401969	19:19407718	*CILP2*	C/T	0.07	-0.029	0.050	0.552	-0.118	0.008	3.0E-54	24097068
rs4420638	19:45422946	*APOE*	G/A	0.16	0.026	0.045	0.567	0.225	0.008	2.0E-178	24097068
rs364585	20:12962718	*SPTLC3*	A/G	0.38	-0.035	0.026	0.186	-0.025	0.004	4.0E-10	24097068
rs2328223	20:17845921	*SNX5*	C/A	0.19	0.015	0.032	0.633	0.030	0.005	6.0E-09	24097068
rs2902940	20:39091487	*MAFB*	G/A	0.29	-0.055	0.029	0.052	-0.027	0.004	2.0E-11	24097068
rs6029526	20:39672618	*TOP1*	A/T	0.47	-0.015	0.026	0.562	0.044	0.005	5.0E-18	24097068
rs5763662	22:30378703	*MTMR3*	T/C	0.02	-0.031	0.085	0.718	0.077	0.013	1.0E-08	24097068
rs4253772	22:46627603	*PPARA*	T/C	0.11	0.042	0.041	0.304	-0.031	0.006	3.0E-08	24097068

Abbreviations: EAF, effect allele frequency; LDL-C, low-density lipoprotein cholesterol; PMID, PubMed ID.

[a] Log odds per effect allele.

[b] SD per effect allele.

Supplementary Table 3.9. Input for the Mendelian Randomization of HDL-C.

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			HDL-C			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs12748152	1:27138393	*PIGV/ NR0B2*	T/C	0.07	-0.005	0.049	0.921	-0.051	0.006	1.0E-15	24097068
rs4660293	1:40028180	*PABPC4*	G/A	0.24	0.053	0.030	0.078	-0.035	0.004	3.0E-18	24097068
rs12145743	1:156700651	*HDGF/ PMVK*	G/T	0.33	0.024	0.030	0.414	0.020	0.004	2.0E-08	24097068
rs4650994	1:178515312	*ANGPTL1*	G/A	0.48	-0.007	0.026	0.798	0.021	0.004	7.0E-09	24097068
rs1689800	1:182168885	*ZNF648*	G/A	0.36	0.042	0.027	0.116	-0.034	0.004	5.0E-20	24097068
rs4846914	1:230295691	*GALNT2*	G/A	0.39	-0.009	0.026	0.742	-0.048	0.004	4.0E-41	24097068
rs12328675	2:165540800	*COBLL1*	C/T	0.13	-0.014	0.038	0.708	0.045	0.006	2.0E-15	24097068
rs2972146	2:227100698	*IRS1*	G/T	0.36	-0.046	0.027	0.085	0.032	0.004	2.0E-17	24097068
rs2606736	3:11400249	*ATG7*	C/T	0.38	-0.013	0.026	0.620	0.025	0.005	5.0E-08	24097068
rs2290547	3:47061183	*SETD2*	A/G	0.17	-0.007	0.034	0.829	-0.030	0.005	4.0E-09	24097068
rs2013208	3:50129399	*RBM5*	T/C	0.50	0.016	0.026	0.522	0.025	0.004	9.0E-12	24097068
rs13326165	3:52532118	*STAB1*	A/G	0.20	-0.077	0.035	0.028	0.029	0.004	9.0E-11	24097068
rs6805251	3:119560606	*GSK3B*	T/C	0.40	0.003	0.026	0.907	0.020	0.003	1.0E-08	24097068
rs17404153	3:132163200	*ACAD11*	T/G	0.12	0.004	0.039	0.924	0.028	0.005	5.0E-09	24097068
rs10019888	4:26062990	*C4orf52*	G/A	0.17	-0.083	0.035	0.017	-0.027	0.005	5.0E-08	24097068
rs3822072	4:89741269	*FAM13A*	A/G	0.47	0.059	0.026	0.023	-0.025	0.004	4.0E-12	24097068

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			HDL-C			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs2602836	4:100014805	*ADH5*	A/G	0.43	0.001	0.026	0.960	0.019	0.003	5.0E-08	24097068
rs13107325	4:103188709	*SLC39A8*	T/C	0.07	-0.096	0.050	0.053	-0.071	0.009	1.0E-15	24097068
rs6450176	5:53298025	*ARL15*	A/G	0.25	-0.039	0.029	0.187	-0.025	0.004	7.0E-10	24097068
rs998584	6:43757896	*VEGFA*	A/C	0.51	0.067	0.035	0.054	-0.026	0.004	2.0E-11	24097068
rs1936800	6:127436064	*RSPO3*	C/T	0.49	0.035	0.026	0.171	0.020	0.003	3.0E-10	24097068
rs605066	6:139829666	*CITED2*	C/T	0.41	0.054	0.026	0.035	-0.028	0.005	3.0E-08	24097068
rs702485	7:6449272	*DAGLB*	G/A	0.51	-0.019	0.028	0.501	0.024	0.003	7.0E-12	24097068
rs4142995	7:17919258	*SNX13*	T/G	0.41	0.040	0.026	0.122	-0.026	0.004	9.0E-12	24097068
rs4917014	7:50305863	*IKZF1*	G/T	0.33	0.037	0.027	0.166	0.022	0.004	1.0E-08	24097068
rs17145738	7:72982874	*MLXIPL*	T/C	0.12	-0.029	0.039	0.467	0.041	0.006	5.0E-13	24097068
rs4731702	7:130433384	*KLF14*	T/C	0.48	-0.043	0.025	0.093	0.029	0.003	5.0E-17	24097068
rs17173637	7:150529449	*TMEM176A*	C/T	0.09	0.030	0.044	0.499	-0.036	0.006	2.0E-08	24097068
rs9987289	8:9183358	*PPP1R3B*	A/G	0.09	0.020	0.045	0.658	-0.082	0.006	2.0E-41	24097068
rs12678919	8:19844222	*LPL*	G/A	0.10	0.006	0.042	0.885	0.155	0.006	1.0E-149	24097068
rs2293889	8:116599199	*TRPS1*	T/G	0.42	-0.015	0.026	0.567	-0.031	0.004	4.0E-17	24097068
rs2954029	8:126490972	*TRIB1*	T/A	0.46	-0.039	0.026	0.125	0.040	0.004	3.0E-29	24097068

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			HDL-C			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs581080	9:15305378	*TTC39B*	G/C	0.19	0.033	0.034	0.324	-0.042	0.005	1.0E-19	24097068
rs1883025	9:107664301	*ABCA1*	T/C	0.26	-0.027	0.029	0.354	-0.070	0.004	2.0E-65	24097068
rs970548	10:46013277	*MARCH8/ ALOX5*	C/A	0.24	-0.017	0.030	0.577	0.026	0.004	2.0E-10	24097068
rs2923084	11:10388782	*AMPD3*	G/A	0.18	0.063	0.033	0.061	-0.026	0.005	5.0E-08	24097068
rs3136441	11:46743247	*LRP4*	C/T	0.13	-0.060	0.039	0.124	0.054	0.005	7.0E-29	24097068
rs11246602	11:51512090	*OR4C46*	C/T	0.12	0.002	0.039	0.954	0.034	0.005	2.0E-10	24097068
rs174546	11:61569830	*FADS1/2/3*	T/C	0.33	-0.131	0.027	0.000	-0.039	0.004	8.0E-28	24097068
rs12801636	11:65391317	*KAT5*	A/G	0.23	-0.057	0.032	0.080	0.024	0.004	3.0E-08	24097068
rs499974	11:75455021	*MOGAT2/ DGAT2*	A/C	0.17	-0.095	0.035	0.007	-0.026	0.005	1.0E-08	24097068
rs964184	11:116648917	*APOA1*	C/G	0.86	-0.091	0.036	0.011	0.106	0.007	6.0E-48	24097068
rs7941030	11:122522375	*UBASH3B*	C/T	0.38	-0.056	0.027	0.036	0.027	0.003	1.0E-14	24097068
rs7134375	12:20473758	*PDE3A*	A/C	0.43	-0.003	0.026	0.916	0.021	0.004	1.0E-08	24097068
rs11613352	12:57792580	*LRP1*	T/C	0.23	-0.011	0.030	0.709	0.028	0.004	2.0E-13	24097068
rs7134594	12:110000193	*MVK*	C/T	0.47	-0.009	0.026	0.739	-0.035	0.005	2.0E-13	24097068
rs4759375	12:123796238	*SBNO1*	T/C	0.09	0.069	0.043	0.111	0.056	0.010	3.0E-08	24097068

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			HDL-C			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs4765127	12:124460167	*ZNF664*	T/G	0.35	0.005	0.027	0.851	0.032	0.005	8.0E-10	24097068
rs838880	12:125261593	*SCARB1*	C/T	0.30	0.030	0.036	0.402	0.048	0.004	6.0E-32	24097068
rs4983559	14:105277209	*ZBTB42/ AKT1*	G/A	0.41	-0.037	0.038	0.325	0.020	0.003	1.0E-08	24097068
rs1532085	15:58683366	*LIPC*	A/G	0.38	0.042	0.026	0.113	0.107	0.004	1.0E-188	24097068
rs2652834	15:63396867	*LACTB*	A/G	0.20	-0.001	0.032	0.972	-0.028	0.004	4.0E-11	24097068
rs1121980	16:53809247	*FTO*	A/G	0.43	0.020	0.026	0.442	-0.020	0.003	7.0E-09	24097068
rs3764261	16:56993324	*CETP*	A/C	0.32	-0.021	0.027	0.433	0.241	0.007	0.0E+00	24097068
rs16942887	16:67928042	*LCAT*	A/G	0.12	0.019	0.039	0.635	0.083	0.005	8.0E-54	24097068
rs2925979	16:81534790	*CMIP*	T/C	0.29	-0.073	0.032	0.020	-0.035	0.004	1.0E-19	24097068
rs11869286	17:37813856	*STARD3*	G/C	0.34	-0.057	0.028	0.042	-0.032	0.004	3.0E-17	24097068
rs4148008	17:66875294	*ABCA8*	G/C	0.32	-0.002	0.027	0.937	-0.028	0.004	1.0E-12	24097068
rs4129767	17:76403984	*PGS1*	G/A	0.50	-0.001	0.026	0.981	-0.024	0.004	2.0E-11	24097068
rs7241918	18:47160953	*LIPG*	G/T	0.16	0.011	0.035	0.749	-0.090	0.006	1.0E-44	24097068
rs12967135	18:57849023	*MC4R*	A/G	0.23	-0.008	0.030	0.788	-0.026	0.005	4.0E-08	24097068
rs7255436	19:8433196	*ANGPTL4*	C/A	0.49	0.018	0.028	0.510	-0.032	0.006	2.0E-08	24097068
rs737337	19:11347493	*ANGPTL8*	C/T	0.09	0.044	0.047	0.347	-0.056	0.007	5.0E-17	24097068

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			HDL-C			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs731839	19:33899065	*PEPD*	G/A	0.34	0.045	0.029	0.118	-0.022	0.004	3.0E-09	24097068
rs4420638	19:45422946	*APOE*	G/A	0.16	0.026	0.045	0.567	-0.067	0.007	2.0E-21	24097068
rs17695224	19:52324216	*HAS1*	A/G	0.27	-0.001	0.029	0.962	-0.029	0.004	2.0E-13	24097068
rs386000	19:54792761	*LILRA3*	C/G	0.20	-0.014	0.032	0.666	0.048	0.005	3.0E-23	24097068
rs1800961	20:43042364	*HNF4A*	T/C	0.03	-0.035	0.075	0.639	-0.127	0.010	2.0E-34	24097068
rs6065906	20:44554015	*PLTP*	C/T	0.19	-0.010	0.033	0.773	-0.059	0.004	5.0E-40	24097068
rs181362	22:21932068	*UBE2L3*	T/C	0.20	0.008	0.032	0.793	-0.038	0.004	4.0E-18	24097068

Abbreviations: EAF, effect allele frequency; HDL-C, high-density lipoprotein cholesterol; PMID, PubMed ID.

[a] Log odds per effect allele.

[b] SD per effect allele.

Supplementary Table 3.10. Input for the Mendelian Randomization of Triglycerides.

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Triglycerides			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs12748152	1:27138393	*PIGV/ NR0B2*	T/C	0.07	-0.005	0.049	0.921	0.037	0.006	1.0E-09	24097068
rs2131925	1:63025942	*ANGPTL3*	G/T	0.33	0.042	0.027	0.122	-0.066	0.004	3.0E-74	24097068
rs4846914	1:230295691	*GALNT2*	G/A	0.39	-0.009	0.026	0.742	0.040	0.003	7.0E-31	24097068
rs1260326	2:27730940	*GCKR*	T/C	0.42	0.076	0.026	0.003	0.115	0.003	2.0E-239	24097068
rs2972146	2:227100698	*IRS1*	G/T	0.36	-0.046	0.027	0.085	-0.028	0.004	3.0E-15	24097068
rs645040	3:135926622	*MSL2L1*	G/T	0.23	-0.039	0.031	0.206	-0.029	0.004	2.0E-12	24097068
rs6831256	4:3473139	*LRPAP1*	G/A	0.43	0.045	0.033	0.179	0.026	0.004	2.0E-12	24097068
rs442177	4:88030261	*KLHL8*	G/T	0.40	-0.012	0.026	0.630	-0.031	0.004	1.0E-18	24097068
rs9686661	5:55861786	*MAP3K1*	T/C	0.20	-0.015	0.032	0.647	0.038	0.005	3.0E-16	24097068
rs6882076	5:156390297	*TIMD4*	T/C	0.37	-0.027	0.027	0.311	-0.029	0.004	2.0E-15	24097068
rs998584	6:43757896	*VEGFA*	A/C	0.51	0.067	0.035	0.054	0.029	0.004	3.0E-15	24097068
rs1936800	6:127436064	*RSPO3*	C/T	0.49	0.035	0.026	0.171	-0.020	0.004	3.0E-08	24097068
rs4722551	7:25991826	*MIR148A*	C/T	0.16	0.022	0.035	0.530	0.029	0.004	9.0E-11	24097068
rs13238203	7:72129667	*TYW1B*	T/C	0.03	-0.079	0.082	0.331	-0.059	0.013	3.0E-06	24097068
rs17145738	7:72982874	*MLXIPL*	T/C	0.12	-0.029	0.039	0.467	-0.115	0.005	9.0E-99	24097068
rs38855	7:116358044	*MET*	G/A	0.47	-0.023	0.026	0.377	-0.019	0.003	2.0E-08	24097068

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Triglycerides			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs11776767	8:10683929	*PINX1*	C/G	0.38	-0.046	0.026	0.078	0.022	0.003	3.0E-11	24097068
rs1495741	8:18272881	*NAT2*	G/A	0.22	-0.007	0.030	0.819	0.040	0.006	3.0E-12	24097068
rs12678919	8:19844222	*LPL*	G/A	0.10	0.006	0.042	0.885	-0.170	0.006	2.0E-199	24097068
rs2954029	8:126490972	*TRIB1*	T/A	0.46	-0.039	0.026	0.125	-0.076	0.003	1.0E-107	24097068
rs1832007	10:5254847	*AKR1C4*	G/A	0.15	-0.043	0.036	0.240	-0.033	0.005	2.0E-12	24097068
rs10761731	10:65027610	*JMJD1C*	T/A	0.43	0.017	0.026	0.496	-0.031	0.005	8.0E-12	24097068
rs2068888	10:94839642	*CYP26A1*	A/G	0.46	0.020	0.027	0.454	-0.024	0.004	2.0E-11	24097068
rs174546	11:61569830	*FADS1/2/3*	T/C	0.33	-0.131	0.027	0.000	0.045	0.003	7.0E-38	24097068
rs964184	11:116648917	*APOA1*	C/G	0.86	-0.091	0.036	0.011	-0.234	0.007	7.0E-224	24097068
rs11613352	12:57792580	*LRP1*	T/C	0.23	-0.011	0.030	0.709	-0.028	0.004	9.0E-14	24097068
rs2412710	15:42683787	*CAPN3*	A/G	0.02	0.179	0.086	0.040	0.099	0.015	2.0E-11	24097068
rs2929282	15:44245931	*FRMD5*	T/A	0.05	-0.011	0.061	0.855	0.072	0.012	2.0E-09	24097068
rs1532085	15:58683366	*LIPC*	A/G	0.38	0.042	0.026	0.113	0.031	0.004	2.0E-18	24097068
rs3198697	16:15129940	*PDXDC1*	T/C	0.41	0.023	0.026	0.374	-0.020	0.004	2.0E-08	24097068
rs11649653	16:30918487	*CTF1*	G/C	0.40	0.032	0.026	0.217	-0.027	0.005	2.0E-07	24097068
rs1121980	16:53809247	*FTO*	A/G	0.43	0.020	0.026	0.442	-0.021	0.004	3.0E-08	24097068

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Triglycerides			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs3764261	16:56993324	*CETP*	A/C	0.32	-0.021	0.027	0.433	-0.040	0.004	2.0E-25	24097068
rs8077889	17:41878166	*MPP3*	C/A	0.22	0.048	0.030	0.117	0.025	0.004	1.0E-08	24097068
rs7248104	19:7224431	*INSR*	A/G	0.41	0.014	0.026	0.584	-0.022	0.004	5.0E-10	24097068
rs10401969	19:19407718	*CILP2*	C/T	0.07	-0.029	0.050	0.552	-0.121	0.007	1.0E-69	24097068
rs731839	19:33899065	*PEPD*	G/A	0.34	0.045	0.029	0.118	0.022	0.004	3.0E-09	24097068
rs6065906	20:44554015	*PLTP*	C/T	0.19	-0.010	0.033	0.773	0.053	0.004	2.0E-34	24097068
rs5756931	22:38546033	*PLA2G6*	C/T	0.37	0.036	0.027	0.175	-0.020	0.004	3.0E-08	24097068

Abbreviations: EAF, effect allele frequency; PMID, PubMed ID.

[a] Log odds per effect allele.

[b] SD per effect allele.

Supplementary Table 3.11. Input for the Mendelian Randomization of Apolipoprotein B.

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Apolipoprotein B			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs11591147	1:55505647	*PCSK9*	T/G	0.02	0.001	0.119	0.994	-0.438	0.035	2.5E-34	27005778
rs72660594	1:55636240	*USP24*	C/T	0.01	0.100	0.115	0.386	-0.356	0.029	4.1E-33	27005778
rs190934192	1:55334001	*DHCR24*	A/G	0.02	0.070	0.110	0.530	-0.320	0.041	1.3E-14	27005778
rs4609471	1:55493584	*PARS2*	A/C	0.02	-0.196	0.123	0.102	-0.322	0.030	9.2E-26	27005778
rs4926673	1:55713671	*USP24*	A/G	0.08	0.087	0.046	0.059	-0.086	0.014	7.7E-10	27005778
rs141970801	2:21152755	*C2orf43*	A/G	0.04	-0.047	0.068	0.490	-0.175	0.023	2.1E-13	27005778
rs1367117	2:21263900	*APOB*	A/G	0.31	0.006	0.027	0.821	0.109	0.011	1.0E-21	27005778
rs1260326	2:27730940	*GCKR*	C/T	0.58	-0.076	0.026	0.003	-0.067	0.010	2.5E-10	27005778
rs6756629	2:44065090	*ABCG5*	A/G	0.07	0.018	0.052	0.736	-0.113	0.019	1.9E-09	27005778
rs10056811	5:74605220	*HMGRC*	A/G	0.35	0.008	0.027	0.771	0.086	0.011	1.4E-15	27005778
rs4722043	7:21697529	*DNAH11*	C/G	0.44	-0.007	0.027	0.782	-0.066	0.011	1.2E-09	27005778
rs2980875	8:126481747	*RP11/ 136O12.2*	G/A	0.46	-0.037	0.026	0.148	-0.070	0.010	6.7E-12	27005778
rs115849089	8:19912370	*LPL*	A/G	0.12	-0.005	0.039	0.898	-0.100	0.017	1.0E-08	27005778
rs635634	9:136155000	*ABO*	T/C	0.19	0.019	0.032	0.553	0.074	0.013	7.7E-09	27005778
rs964184	11:116648917	*ZNF259*	C/G	0.86	-0.091	0.036	0.011	-0.166	0.014	2.6E-30	27005778
rs142130958	19:111190652	*SMARCA4*	A/G	0.12	-0.021	0.040	0.596	-0.200	0.017	7.8E-32	27005778

Table Continued

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Apolipoprotein B			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs157594	19:45425175	*PVRL2*	G/T	0.49	0.008	0.034	0.825	0.125	0.012	1.7E-24	27005778
rs189741280	19:19624481	*TSSK6*	G/A	0.01	-0.100	0.138	0.464	-0.176	0.031	1.5E-08	27005778
rs1883711	20:39179822	*LOC105372618*	C/G	0.04	0.067	0.067	0.315	0.144	0.025	2.0E-08	27005778

Abbreviations: EAF, effect allele frequency; PMID, PubMed ID.

[a] Log odds per effect allele.

[b] SD per effect allele.

Supplementary Table 3.12. Input for the Mendelian Randomization of Cigarettes per Day.

Variant	Position (GRCh37)	Gene	Effect/Other Allele	EAF	Aortic Stenosis			Apolipoprotein B			PMID
					Beta[a]	SE	*p*	Beta[b]	SE	*p*	
rs215605	7:32336965	*PDE1C*	G/T	0.38	0.020	0.026	0.450	0.260	0.040	5.4E-09	20418888
rs13280604	8:42559586	*CHRNB3*	A/G	0.77	0.013	0.030	0.673	0.310	0.050	1.3E-08	20418888
rs3025343	9:136478355	*DBH*	G/A	0.89	0.045	0.041	0.270	0.121	0.022	3.6E-08	20418890
rs1329650	10:93348120	*SEC13*	T/G	0.28	0.001	0.029	0.965	-0.367	0.059	5.7E-10	20418890
rs16969968	15:78882925	*CHRNA3*	G/A	0.66	-0.009	0.027	0.736	-1.003	0.056	5.6E-72	20418890
rs7937	19:41302706	*MIA/ RAB4B/ RAB4B/ RAB4B/ EGLN2*	T/C	0.56	0.024	0.026	0.346	0.240	0.040	2.4E-09	20418888

Abbreviations: EAF, effect allele frequency; PMID, PubMed ID.

[a] Log odds per effect allele.

[b] Cigarettes per day per effect allele.

Supplementary Table 3.13. Proportion of Variance Explained by Genetic Instruments.

Risk Factor	R^2 by Genetic Instrument (%)
Adiposity	
Type 2 diabetes	2.1
Body mass index	1.7
WHRadjBMI	0.7
Blood pressure	
Systolic blood pressure	2.3
Diastolic blood pressure	2.7
Pulse pressure	2.0
Hypertension	0.5
Lipids	
LDL-C	5.7
HDL-C	4.6
Triglycerides	4.3
Apolipoprotein B	7.4
Smoking	
Cigarettes per day	1.4

Supplementary Table 3.14. Minimum Detectable Odds Ratio in the Mendelian Randomization.

Risk Factor	Detectable Odds Ratio per SD at 80% Power[a]
Adiposity	
Type 2 diabetes	1.35
Body mass index	1.39
WHRadjBMI	1.64
Blood pressure	
Systolic blood pressure	1.34
Diastolic blood pressure	1.31
Pulse pressure	1.37
Hypertension[b]	1.71
Lipids	
LDL-C	1.21
HDL-C	1.24
Triglycerides	1.25
Apolipoprotein B	1.19
Smoking	
Cigarettes per day	1.44

[a] All odds ratios are shown as risk-increasing to facilitate comparison between risk factors.

[b] The odds ratio is presented per SD change of a continuous genetic instrument for the risk factor.

3.5 References

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Chapter 4. The *LPA* Locus and Aortic Stenosis

4.1 Preface to Chapter 4

In chapter three, genetic analyses indicated the causal contribution of several cardiovascular risk factors to AS, consistent with observational associations noted in previous studies. However, I did not observe a causative effect for several cardiovascular risk factors, despite post hoc power calculations suggesting adequate statistical power, resulting in discrepant findings between genetic and observational analyses.

To date, lipoprotein(a) remains the only risk factor for CAVD with robust observational, experimental, and genetic evidence from a multitude of studies[52,53,143,150,151]. Several randomized controlled trials have also achieved remarkable lowering of lipoprotein(a) levels in healthy volunteers and cardiovascular patients[54-56,116], suggesting lipoprotein(a) may become a modifiable risk factor for AS should lipoprotein(a)-targeting therapies become approved. Levels of lipoprotein(a) are highly heritable, with an h^2 estimate of 67% by Lamon-Fava and colleagues[152] and 94% by Austin and colleagues[153], and much of the variation in lipoprotein(a) levels is attributable to the *LPA* gene[154].

The most well-known variants in the *LPA* gene are rs10455872 and rs3798220, two variants in linkage equilibrium which explain 36% of the variation in lipoprotein(a) levels and are strongly associated with coronary artery disease[155]. While studies have examined the association of these variants with AS, the small numbers of cases has precluded a detailed examination, including estimating the per-allele odds ratio for the less common rs3798200 (minor allele frequency of 1% in the European participants of the 1000 Genomes Project[133]) or the effect associated with possessing various combinations of the risk alleles of both variants. In addition, no study has

investigated whether the association of these variants with AS is modified by age, sex, or other cardiovascular risk factors. In the following study, I estimated the association with AS of the two *LPA* variants and an *LPA* risk score, examined the odds of AS in individuals with specific combinations of risk alleles, and investigated interactions of the *LPA* variants with risk factors.

Association of *LPA* Variants with Aortic Stenosis

A Large-Scale Study Using Diagnostic and Procedural Codes from Electronic Health Records

Hao Yu Chen[1,2], Line Dufresne[2], Hannah Burr[2], Athithan Ambikkumar[2], Niko Yasui[2], Kevin Luk[2], Dilrini K. Ranatunga[3], Rachel A. Whitmer[3], Mark Lathrop[4,5], James C. Engert[1,2,4*], George Thanassoulis[1,2*]

[1]Department of Medicine, McGill University, Quebec, Canada

[2]Preventive and Genomic Cardiology, McGill University Health Centre and Research Institute, Quebec, Canada

[3]Division of Research, Kaiser Permanente of Northern California, Oakland, USA

[4]Department of Human Genetics, McGill University, Quebec, Canada

[5]McGill University and Genome Quebec Innovation Centre, Quebec, Canada

*Corresponding authors. The last two authors contributed equally to the manuscript.

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4.2 Abstract

Importance: Elevated lipoprotein(a) levels are a risk factor for aortic stenosis (AS). However, large-scale replication of associations between *LPA* variants and AS, their interactions with risk factors, and the impact of multiple risk alleles is not well established.

Objective: To replicate the association between *LPA* variants with AS and identify subgroups who are at higher risk of AS.

Design, Setting, and Participants: This case-control study of AS included 44,703 individuals (3469 cases) 55 years or older who were enrolled in the Genetic Epidemiology Research on Aging cohort and who were members of the Kaiser Permanente Northern California health care delivery system. The study leveraged the linkage of administrative health data, electronic medical records, genotypes, and self-reported questionnaire data. The 3469 AS cases were diagnosed between January 1996 and December 2015. Individuals with valvular congenital valvular heart disease were excluded.

Exposures: Two single nucleotide polymorphisms in the *LPA* locus, r10455872 and rs3798220, that are known to associate with circulating plasma lipoprotein(a) levels and an *LPA* risk score.

Main Outcomes and Measures: Aortic stenosis or aortic valve replacement.

Results: The 44,703 participants were of European ancestry, of whom 22,019 (49.3%) were men. The mean (SD) age for the control group was 69.3 (8.3) years and the mean (SD) age for AS cases

was 74.6 (8.5) years. Both *LPA* variants were associated with AS, with a per risk allele odds ratio of 1.34 (95% CI, 1.23-1.47; $p = 1.7 \times 10^{-10}$) for rs10455872 and 1.31 (95% CI, 1.09-1.58; $p = 3.6 \times 10^{-3}$) for rs3798220, adjusted for age, age^2, and sex. The results remained significant after adjustment for risk factors. The estimates were similar for an *LPA* risk score. Individuals with 2 risk alleles had a 2-fold or greater odds of AS compared to individuals with no risk alleles (for rs10455872, homozygous odds ratio, 2.05; 95% CI, 1.37-3.07; $p = 5.3 \times 10^{-4}$; for rs3798220, homozygous odds ratio, 3.74; 95% CI, 1.03-13.62; p = 0.05; and for compound heterozygotes, odds ratio, 2.00; 95% CI, 1.17-3.44; p = 0.01). For rs10455872, the odds ratio for AS was greatest in individuals aged 55-64 and declined with age (interaction p = 0.03). Each rs10455872 risk allele was also associated with AS diagnosed 0.71 years earlier (95% CI, -1.42 to 0; p = 0.049).

Conclusions: We provide a large-scale confirmation of the association between 2 *LPA* variants and AS, reaching genome-wide significance. In addition, individuals with 2 risk alleles have 2-fold or greater odds of developing AS. Age may modify these associations and identify subgroups who are at greater risk of developing AS.

Key Points

Question: Can the association between *LPA* variants and aortic stenosis be detected using electronic health record-derived data?

Findings: In this case-control study, individuals with 2 *LPA* risk alleles in any combination possessed had 2-fold or greater odds of developing aortic stenosis compared to individuals with no risk alleles. In addition, age significantly modified the association of the *LPA* variant rs10455872 with aortic stenosis.

Meaning: Variation at the *LPA* locus increases the odds of aortic stenosis, with 2-fold or greater odds in individuals with the highest genetic risk, but this association may be modified by risk factors.

4.3 Main Text

Introduction

Lipoprotein(a) [Lp(a)], a complex of apolipoprotein(a) [apo(a)] and a low-density lipoprotein-like particle, is a risk factor for cardiovascular disease[143,156-159]. Plasma levels of Lp(a) have a strong genetic basis, with more than half of the variation in Lp(a) levels attributable to the *LPA* gene, which encodes apo(a)[115]. To date, *LPA* is also the only locus which has been robustly associated with valve calcification and aortic stenosis (AS)[46], the most common valve disease in the United States[22]. These associations, when combined with instrumental variables analyses, provide evidence that elevated Lp(a) levels promote AS[51,143]. No medical therapy exists which effectively slows or reverses the progression of AS, leading to interest in the *LPA* locus as a therapeutic target. Indeed, elevated levels of Lp(a) have recently been associated with faster disease progression[53], highlighting the potential for Lp(a) as a target for medical therapy.

Prior genetic studies of AS have been limited to smaller cohorts with limited power to provide a detailed examination of these genetic associations with clinical AS, including the association of multiple alleles at the *LPA* locus and possible interactions. A better understanding of these associations could identify vulnerable groups at higher risk for Lp(a)-mediated AS, which could better inform the specific targeting of therapeutics. Accordingly, we used electronic health records (EHRs) from a large health care organization in northern California to establish a case-control study of AS. We provide large-scale replication of the association between *LPA* single nucleotide polymorphisms (SNPs) and AS, as well as investigate the interaction of the *LPA* SNPs rs10455872 and rs3798220 with age, sex, and traditional cardiovascular risk factors.

Methods

The Genetic Epidemiology Research on Aging (GERA) Cohort is a population-based cohort of > 100,000 adults living in Northern California. All participants are members of the Kaiser Permanente Northern California Integrated Health Care Delivery who provided written, informed consent (database of Genotypes and Phenotypes study accession phs000674.v2.p2). The study was approved by the relevant internal review boards at Kaiser Permanente Northern California and the McGill University Health Centre.

Participants completed a detailed, self-administered survey of behavioural and demographic variables. Using DNA extracted from saliva, genome-wide genotyping was performed on customized, ethnicity-specific Affymetrix Axiom arrays that have been described elsewhere[160,161]. Responses from the Kaiser Permanente Research Program on Genes, Environment and Health (2007-2010) or California Men's Health Study (2002-2003) questionnaires, genotypes, and EHRs were subsequently linked together using an anonymized, unique patient identifier, to protect the participants' privacy (database of Genotypes and Phenotypes study accession phs000788.v1.p2). AS cases, determined through extraction of EHR data from 1996 to 2015, inclusive, were defined based on the presence of either: (1) an *International Classification of Diseases, Ninth Revision (ICD-9)* code for AS (*ICD-9* 424.1), or (2) a procedure code for a prior aortic valve replacement (AVR), an approach that has been previously validated to have a positive predictive value of more than 90%[46]. Coronary artery disease (CAD) cases were defined using a diagnosis of myocardial infarction (MI) or CAD (ICD-9 410-414); procedure codes for percutaneous coronary intervention or coronary artery bypass surgery; or self-reported angina, MI, or revascularization. Individuals with congenital heart disease (*ICD-9* 746-747) were excluded. Dyslipidemia was defined as 2 or

more diagnoses of disorders of lipid metabolism (*ICD-9* 272) and 1 or more prescription for a statin, as noted in the Kaiser Permanente prescriptions database. Smoking (ever/never), diabetes mellitus, and hypertension were self-reported in the questionnaire. Ages greater than 89 years old were rounded down to 90, to further protect the privacy of those participants (n = 389). Controls were GERA participants without an *ICD-9* code for AS or a procedure code for AVR. For the present case-control study of AS, we included individuals aged ≥ 55 years old, with known AS disease status from the years 1996 to 2015, inclusive (n = 44,703). We restricted our analyses to participants who self-reported only European descent, as there were insufficient numbers of individuals available of other races/ethnicities.

We selected for study the *LPA* SNPs rs10455872 and rs3798220, which are strongly associated with Lp(a) levels[155] and have been recently examined for their association with AS in studies with smaller collections of AS cases (n < 1,800)[47,48]. The SNP rs10455872 had been genotyped with the Affymetrix Axiom array, while the SNP rs3798220 required imputation. Following standard quality control of the genotyped data, we imputed rs10455872 (to fill in missing genotypes) and rs3798220 using SHAPEIT2[130] (University of Oxford) and IMPUTE2[131,132] (University of Oxford) with the 1000 Genomes Project[133] serving as the reference panel. A continuous dosage value was created for each individual by summing 2 times the probability of the homozygous minor allele genotype with the probability of the heterozygous genotype. Thus, dosage values ranged from 0 to 2. Imputed dosages were 100% complete for rs10455872 and rs3798220. Where necessary, the dosage was converted to hard calls by rounding dosages of 0.5 or less to 0 and dosages of 1.5 or more to 2, with the remaining dosages being set to 1. Carriers of a variant were defined as individuals with at least one risk allele. An *LPA* risk score was constructed by summing the number

of rs10455872 and rs3798220 risk alleles (minor allele G for rs10455872 and minor allele C for rs3798220) that a participant possessed.

Statistical analyses were conducted using R version 3.3.0 (R Foundation)[139]. Differences between the AS cases and controls were calculated using Welch's t-test for continuous traits and Pearson's chi-square test for categorical traits. All logistic and linear regression models were adjusted for age, age^2, and sex, except where age was the outcome. Multivariable models were further adjusted for dyslipidemia, smoking, diabetes, and hypertension. Additionally, we adjusted the multivariable models for CAD status, to examine whether the observed associations were an epiphenomenon due to the high prevalence of concomitant CAD in AS cases. To assess whether the association of the *LPA* locus with AS is modified by clinical risk factors, interactions of the 2 *LPA* variants and the *LPA* risk score with age, sex, dyslipidemia, smoking, diabetes, and hypertension were separately modelled by introducing multiplicative, interaction terms in the models. Models with interaction terms were only adjusted for age, age^2, and sex, due to the similar estimates arising from models of main effects with, and without, adjustment for the additional clinical risk factors. No multiple testing corrections were applied due to the hypothesis-generating nature of the analyses. Two-tailed tests with $p < 0.05$ were considered significant.

Results

Descriptive characteristics of the study participants are shown in Table 1. Compared with the controls, AS cases (n = 3,469) were older (mean [SD] of 74.6 [8.5] in cases vs 69.3 [8.3] in controls; $p < 0.001$) and were more likely to be male (56% [n = 1,943] of cases vs 49% [n = 20,076]

of controls; $p < 0.001$). As expected, a greater proportion of cases had dyslipidemia or comorbid CAD, and were more likely to be hypertensive, diabetic, and a past or present smoker (Table 4.1).

The minor allele frequencies for rs10455872 and rs3798220 were 0.07 and 0.02, respectively. Both SNPs were in Hardy-Weinberg Equilibrium ($p \geq 0.31$). The theoretical range for the *LPA* risk score that combined both SNPs was 0 to 4, but we observed a range of 0 to 2. That is, individuals possessed at most 2 risk alleles, either from the same SNP (homozygous), or 1 from each SNP (compound heterozygous).

Association of LPA Variants and the LPA Risk Score with AS

Both *LPA* SNPs and the *LPA* risk score were significantly associated with AS in logistic regression models adjusted for age, age^2, and sex (Table 4.2). Per risk allele, the odds ratio (OR) was 1.34 (95% CI, 1.23-1.47; $p = 1.7 \times 10^{-10}$) for rs10455872, 1.31 (95% CI, 1.09-1.58; $p = 3.6 \times 10^{-3}$) for rs3798220, and 1.35 (95% CI, 1.24-1.46; 1.3×10^{-12}) for the risk score. Both the *LPA* SNPs and the risk score remained significant after adjustment for additional clinical risk factors. The magnitude of these associations did not materially differ when further adjusted for the presence of CAD (Supplementary Table 4.1).

Possessing 2 risk alleles in any combination led to a 2-fold or greater increase in the odds of AS, relative to individuals without any risk alleles (

Table 4.3). The OR for individuals who were homozygous for rs10455872 was 2.05 (95% CI, 1.37-3.07; $p = 5.3 \times 10^{-4}$), and for rs3798220 was 3.74 (95% CI, 1.03-13.62; $p = 0.05$). For compound heterozygotes, the OR was 2.00 (95% CI, 1.17-3.44; $p = 0.01$).

In sensitivity analyses, we evaluated the association of the *LPA* SNPs and the risk score with AVR. Both rs10455872 and the *LPA* risk score were significantly associated with AVR: per risk allele, the OR for rs10455872 was 1.57 (95% CI, 1.24-1.99; $p = 1.5 \times 10^{-4}$) and for the risk score was 1.53 (95% CI, 1.23-1.90; $p = 1.2 \times 10^{-4}$). The SNP rs3798220 did not reach statistical significance for association with AVR (per risk allele OR, 1.27; 95% CI, 0.75-2.13; p = 0.37).

Interaction of LPA Variants with Risk Factors for AS

The association between rs10455872 and AS was modified by age (interaction p = 0.025) (Figure 4.1). In participants aged 55 to 64 years old, the per risk allele OR for rs10455872 was 1.59 (95% CI, 1.29-1.97; $p = 1.7 \times 10^{-5}$). In participants 65 to 74 years old, the OR declined to 1.37 (95% CI, 1.17-1.60; $p = 9.1 \times 10^{-5}$), and in participants 75 years of age or older, the OR was 1.24 (95% CI, 1.09-1.41; $p = 1.1 \times 10^{-3}$). No age interaction was observed for either rs3798220 or the *LPA* risk score (data not shown).

Consistent with these analyses, rs10455872 was associated with the age that a patient received an AS diagnosis, with each risk allele corresponding to a reduction of 0.71 years in the age at disease diagnosis (95% CI, -1.42 to 0; p = 0.05). Neither rs3798220 nor the *LPA* risk score were significantly associated with age at disease (

Supplementary Table 4.2).

We also observed an interaction between sex and rs3798220 for AS (interaction p = 0.05) (Figure 4.1). In men, each risk allele of rs3798220 was associated with an OR of 1.55 (95% CI, 1.22-1.98;

$p = 3.4 \times 10^{-4}$). In women, the association was not significant (OR per risk allele, 1.07; 95% CI, 0.80-1.42; p = 0.66). No sex interaction was observed for either rs10455872 or the *LPA* risk score (data not shown). No significant interactions were identified between the *LPA* variants or the risk score and dyslipidemia, smoking, diabetes mellitus, or hypertension for AS (Supplementary Table 4.3).

Discussion

In this case-control study of 44,703 individuals, we have confirmed that variants in the *LPA* locus are strongly associated with AS. Our data provide large-scale replication of the rs10455872 association, reaching genome-wide significance, using a single large cohort based on EHR-derived diagnostic and procedure codes. Associations were similar even when we restricted our analysis to AVR, a robust valve outcome, providing strong validation of this association. In addition, we provide new evidence that rs3798220, a rare variant in the *LPA* locus for which limited data exists, is also associated with AS. Given the large sample size, we were able to extend these associations to evaluate individuals who possessed 2 risk alleles of either rs10455872 or rs3798220 (including compound heterozygotes who possessed one risk allele from each SNP) and demonstrated a 2-fold or greater increase in their odds of developing AS, relative to individuals who possessed zero risk alleles. These results suggest that homozygous (or compound heterozygous) individuals at the *LPA* locus have a markedly elevated risk for AS, likely due to higher circulating Lp(a)[47,143,155,162], and may therefore be candidates for future Lp(a)-lowering therapies. We further demonstrated that the association of rs10455872 with AS was modified by age, but not by other clinical risk factors. Indeed, the association between rs10455872 and AS declined with increasing age, suggesting that increased Lp(a) may be more relevant in younger cases of AS, and that other aetiologies may

predominate at older ages. We also observed that rs3798220 associated with AS only in men, but this requires validation in other cohorts. Finally, our confirmation of the association between *LPA* and AS using EHRs provides important validation of our methodology for determining AS disease status, based on diagnostic (*ICD-9*) codes for AS and procedure codes for AVR in participants' EHRs. This validation supports the construction of phenotypes from health records, and points to healthcare organizations as being valuable sources of data for answering questions about the genetic aetiology of complex diseases that may have a relatively low prevalence in the general population, such as AS.

Although prior studies evaluating *LPA* and calcific aortic valve disease have used computed tomographic measures of valve calcium and/or echocardiographic data for the diagnosis of AS, our estimates for the association between *LPA* variants and AS, using diagnostic and procedural codes, are consistent with these prior studies and add to the evidence that this association is highly robust and consistent[46,47,51,159]. While *LPA* variants have been linked at a genome-wide significance level with aortic valve calcium[46], associations with clinical AS and valve replacement have been limited by small samples, precluding the evaluation of rare variants such as rs3798220. Herein, we demonstrate that rs3798220, which strongly associates with Lp(a) levels[47,143,155,162], also associates with AS[47], with homozygous individuals possessing a 3.7-fold increase in the odds of AS. These results are concordant with a recent meta-analysis, which demonstrated a significant association between rs3798220 and AS in a limited number of participants[48]. To date, we are not aware of other studies which have evaluated age or sex interactions with *LPA* variants for AS and these results will require validation by independent cohorts.

Given the consistent association between *LPA* variants, Lp(a) levels and AS, Mendelian randomization studies suggest that elevated Lp(a) levels contribute to the development of AS[46,47,51,143]. Recent data also suggest that Lp(a) is involved in disease progression, with individuals having mild to moderate AS and high Lp(a) levels possessing a more than 2-fold increase in their odds of AVR or cardiac death[53]. Although limited treatment options exist at the current time, Lp(a)-lowering therapies are in development[116,163]. Identifying which patients to target with such therapies in future randomized control trials will be an important consideration. Our results, if validated by others, would suggest that younger patients (less than 65 years of age) and those with markedly high Lp(a) levels (e.g. homozygous or compound heterozygotes for *LPA* variants) are at highest risk for AS, and would therefore likely derive the greatest benefit from Lp(a)-lowering therapy.

Our study has several strengths, including having one of the largest samples of patients with AS and genome-wide genotyping worldwide, as well as being clinically representative of a large US integrated healthcare delivery organization. Nonetheless, our study has several limitations that deserve mention. First, AS was defined based on *ICD-9* codes and prior AVR rather than detailed echocardiographic assessment, which is the gold standard. However, we have previously validated the ICD code for AS with echocardiography, showing a positive predictive value greater than 90%[46]. We acknowledge that the accuracy of diagnostic codes may vary between different healthcare delivery systems and the misclassification of AS status in our study may be greater than previously reported. In addition, the inclusion of bicuspid aortic valve and/or other aetiologies (e.g. radiation-associated AS) among cases remains possible, but would represent only a small proportion of the included cases. Nonetheless, any such misclassification of disease status would

be non-differential by *LPA* status and would tend to bias our reported associations towards the null. Additionally, sensitivity analyses using AVR, a more robust valve outcome than *ICD-9* coding, demonstrated consistent results. Second, quantitative data on other risk factors for AS, such as low-density lipoprotein cholesterol and blood pressure were unavailable. However, adjustments for available clinical risk factors, including presence of hypertension, dyslipidemia, diabetes, or smoking did not materially change the estimates of effect, which was expected since *LPA* variants are not known to confer AS risk through any other risk factor except genetically-elevated Lp(a) levels[47,51,143]. Third, AS cases were, on average, older than the controls, allowing for the possibility that some of the controls may develop AS in the future. However, this would have led to an underestimation of the association we observed between *LPA* variants and AS. Fourth, as is frequently performed for large-scale genetic studies, our analysis was conducted as a case-control study and therefore longitudinal estimates of absolute risks cannot be directly calculated; nonetheless, given that the prevalence of AS is less than 10% in the general population, odds ratios approximate risk ratios. Lastly, our study was limited to individuals of European ancestry. However, the rs10455872 risk allele is rare in East Asian and African populations (allele frequency < 0.01), and owing to the greatly reduced numbers of non-European participants in the GERA cohort, those analyses would have low statistical power.

Conclusion

In this large-scale, case-control study of AS we confirmed the association between *LPA* SNPs rs10455872 and rs3798220 with AS, and demonstrated that individuals with 2 risk alleles have 2-fold or greater odds of developing AS. Age may modify these associations, and could identify

subgroups at greatest risk of developing AS, and the most likely to benefit from Lp(a)-lowering therapies.

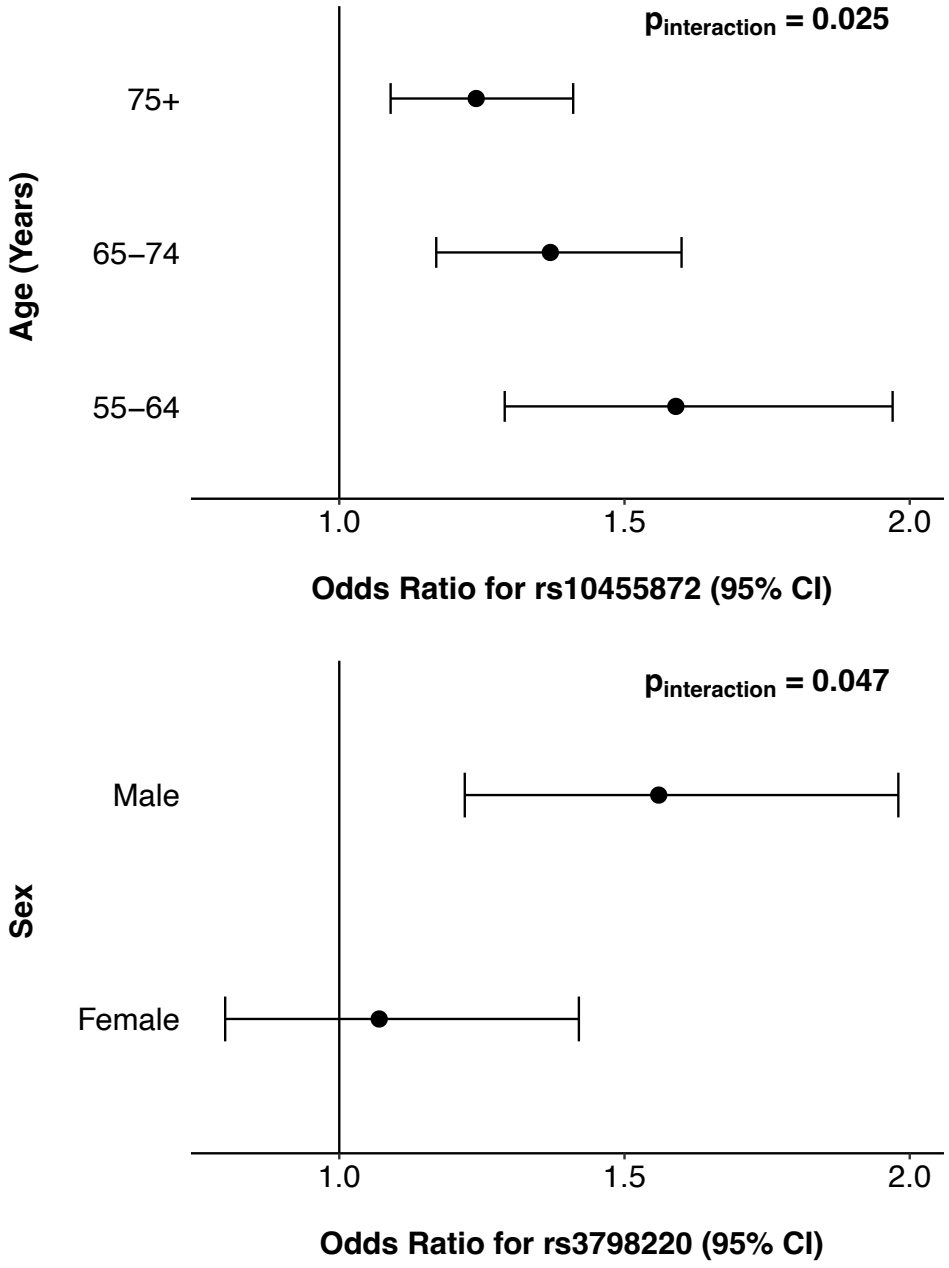

Figure 4.1. Interactions of *LPA* Variants with Clinical Risk Factors.

Interaction for aortic stenosis of *LPA* variants with age (p = 0.03) (top) and sex (p = 0.05) (bottom). Odds ratios are presented per risk allele.

Table 4.1. Characteristics of the GERA Cohort (n = 44,703).

Characteristic	Aortic Stenosis		
	No. (%)		
	Controls	Cases	p-Value
n	41,234	3,469	--
Males	20,076 (49)	1,943 (56)	< 0.001
Age, mean (SD), years	69.3 ± 8.3	74.6 ± 8.5	< 0.001
Body mass index[a], mean (SD)	26.8 ± 4.9	27.4 ± 5.4	< 0.001
Dyslipidemia	23,926 (58)	2,666 (77)	< 0.001
Coronary artery disease	10,007 (24)	2,131 (61)	< 0.001
Hypertension	16,961 (41)	1,945 (56)	< 0.001
Ever smoked[b]	20,247 (52)	1,834 (56)	< 0.001
Diabetes mellitus	4,491 (11)	606 (17)	< 0.001
LPA rs10455872, carrier	5,240 (13)	563 (16)	< 0.001
LPA rs3798220, carrier	1,330 (3)	137 (4)	0.03
Aortic valve replacement	NA	400 (12)	NA

Abbreviations: GERA, Genetic Epidemiology Research on Aging; NA, not applicable.

[a]Data on body mass index were available for 42,962 participants.

[b]Data on smoking were available for 42,535 participants.

Table 4.2. Association of *LPA* Variants with AS[a].

LPA Variant	Adjusted for Age and Sex (n = 44,703)		Adjusted for Age, Sex, and Risk Factors (n = 42,535)	
	OR (95% CI)	**p Value**	**OR (95% CI)**	**p Value**
rs10455872	1.34 (1.23-1.47)	1.7×10^{-10}	1.30 (1.18-1.43)	4.9×10^{-8}
rs3798220	1.31 (1.09-1.58)	3.6×10^{-3}	1.26 (1.04-1.52)	0.02
Risk score	1.35 (1.24-1.46)	1.3×10^{-12}	1.30 (1.19-1.42)	1.7×10^{-9}

Abbreviations: AS, aortic stenosis; CI, confidence interval; OR, odds ratio.

[a]Multivariable models were adjusted for dyslipidemia, smoking, diabetes mellitus, and hypertension, in addition to age and sex. Odds ratios are per risk allele.

Table 4.3. *LPA* Risk Allele Combinations and Their Corresponding Associations with AS[a].

Number of Risk Alleles		n			
rs10455872	**rs3798220**	**Total**	**Cases**	**OR (95% CI)**	**p Value**
0	0	37,554	2,785	Reference	Reference
Reference versus any carrier					
1+	0	5,682	547	1.35 (1.22-1.49)	2.3×10^{-9}
0	1+	1,346	121	1.26 (1.04-1.53)	0.02
Reference versus 1 risk allele					
1	0	5,477	518	1.32 (1.20-1.49)	4.4×10^{-8}
0	1	1,330	118	1.24 (1.02-1.51)	0.03
Reference versus 2 risk alleles					
2	0	205	29	2.05 (1.37-3.07)	5.3×10^{-4}
1	1	121	16	2.00 (1.17-3.44)	0.01
0	2	16	3	3.74 (1.03-13.62)	0.05

Abbreviations: AS, aortic stenosis; CI, confidence interval; OR, odds ratio.

[a]Models were adjusted for age, age^2, and sex.

Author Contributions

Ms Chen and Dr Thanassoulis had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs Engert and Thanassoulis also contributed equally to the manuscript.

Conflict of Interest Disclosures

All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Thanassoulis has received consulting fees from IONIS Pharmaceuticals and has participated in advisory boards for Amgen. Dr Thanassoulis reports receiving grant support from National Institutes of Health, the Heart and Stroke Foundation of Canada, the Canadian Institutes of Health Research; grants and personal fees from Ionis Pharmaceuticals; and personal fees from Amgen, Regeneron, and Servier. No other disclosures were reported.

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Role of the Funder/Sponsor

The funding organizations had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

4.4 Supplement

Supplementary Table 4.1. Association of *LPA* Variants with AS in Multivariable Models Adjusted for Cardiovascular Risk Factors and CAD.

	Association with AS (n = 42,353)	
***LPA* Variant**	**OR (95% CI)**	**p Value**
rs10455872	1.26 (1.14-1.38)	2.5×10^{-6}
rs3798220	1.26 (1.04-1.53)	0.017
Risk score	1.27 (1.16-1.38)	9.1×10^{-8}

Models were adjusted for age, age^2, sex, dyslipidemia, smoking, diabetes mellitus, hypertension, and CAD. Odds ratios are per risk allele. AS: aortic stenosis; CAD: coronary artery disease; CI: confidence interval; OR: odds ratio.

Supplementary Table 4.2. Association of *LPA* Variants with Age of Diagnosis of AS.

***LPA* Variant**	**Effect Size in Years (95% CI)**	**p Value**
rs10455872	-0.71 (-1.42 to 0.00)	0.049
rs3798220	-0.25 (-1.68 to 1.19)	0.74
Risk score	-0.64 (-1.28 to 0.01)	0.052

Models were adjusted for sex. AS: aortic stenosis; CI: confidence interval; OR: odds ratio.

Supplementary Table 4.3. Interaction of *LPA* Variants with Clinical Risk Factors, for Associations with AS.

Risk Factor	n	Interaction Term with *LPA* Variant					
		rs10455872		rs3798220		Risk Score	
		OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value
Dyslipidemia	44,703	0.91 (0.73-1.13)	0.4	0.94 (0.59-1.50)	0.8	0.92 (0.75-1.12)	0.39
Smoking	42,353	1.12 (0.93-1.36)	0.23	1.10 (0.75-1.60)	0.64	1.12 (0.95-1.33)	0.18
Diabetes mellitus	44,703	0.92 (0.71-1.18)	0.5	0.86 (0.51-1.46)	0.58	0.90 (0.72-1.14)	0.38
Hypertension	44,703	0.96 (0.80-1.15)	0.64	0.72 (0.50-1.04)	0.077	0.90 (0.76-1.06)	0.22

The reference groups for dyslipidemia, smoking, diabetes mellitus, and hypertension were individuals without dyslipidemia, non-smokers, individuals without diabetes mellitus, and individuals without hypertension, respectively. Models were adjusted for age, age^2, and sex. AS: aortic stenosis; CI: confidence interval; OR: odds ratio.

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Chapter 5. Polyunsaturated Fatty Acids and Aortic Stenosis

5.1 Preface to Chapter 5

In chapter four, I replicated the association of common genetic variation at the *LPA* locus with AS and further demonstrated the association of this locus with replacement of the aortic valve, which was inferred to be a proxy for severe AS. The *LPA* locus has been the focus of many AS studies[47,51,164,165], reflecting the relatively well understood mechanism linking genetic variation to AS risk through lipoprotein(a) levels[154] and the therapeutic potential of targeting this lipoprotein as demonstrated by recent randomized clinical trials[54-56].

Despite the encouraging reductions in lipoprotein(a) levels seen in these clinical trials, identifying additional targets for pharmacological intervention could expand prophylactic and remedial treatment of AS. While genetic studies have identified additional loci for the disease, promising targets are lacking. Notably, the discoveries of *PALMD* and *TEX41* suggest congenital heart defects may contribute to disease[57,58], though this explanation remains speculative and prophylactic intervention in early life might be untenable.

The GWAS remains a promising approach for agnostically identifying regions of the genome associated with AS. In the following study, I performed a GWAS for AS in the GERA cohort, which has one of the largest collections of AS cases in the world. Due to the higher number of cases, there is greater statistical power. As significant variants in a GWAS may tag loci involved in disease development, and the magnitude of effect observed in a GWAS does not reflect the magnitude of risk reduction achievable through pharmacological targeting of the same locus, the increased statistical power in GERA offered an opportunity to identify novel loci for AS, each of which have the potential for pharmacological targeting.

Association of *FADS1/2* Locus Variants and Polyunsaturated Fatty Acids With Aortic Stenosis

Hao Yu Chen, MSc[1,2], Benjamin J. Cairns, PhD[3], Aeron M. Small, MD[4], Hannah A. Burr, BSc[1,2], Athithan Ambikkumar, BSc[2], Andreas Martinsson, MD[5], Sébastien Thériault, MD[6], Hans Markus Munter, PhD[7], Brian Steffen, PhD[8], Richard Zhang, BSc[2], Rebecca T. Levinson, PhD[9], Christian M. Shaffer, Bsc[9], Jian Rong, PhD[10], Emily Sonestedt, PhD[11], Line Dufresne, MSc[2], Johan Ljungberg, MD[12], Ulf Näslund, MD[12], Bengt Johansson, MD[12], Dilrini K. Ranatunga, BA[13], Rachel A. Whitmer, PhD[14], Matthew J. Budoff, MD[15], Albert Nguyen, PhD[2], Ramachandran S. Vasan, MD[10], Martin G. Larson, SD[10], William S. Harris, PhD[16], Scott M. Damrauer, MD[17], Ken D. Stark, PhD[18], S. Matthijs Boekholdt, MD[19], Nicholas J. Wareham, MD[20], Philippe Pibarot, PhD[6], Benoit J. Arsenault, PhD[6], Patrick Mathieu, MD, MSc[6], Vilmundur Gudnason, MD[21], Christopher J. O'Donnell, MD[10], Jerome I. Rotter, MD[15], Michael Y. Tsai, PhD[8], Wendy S. Post, MD[22], Robert Clarke, MD[3], Stefan Söderberg, MD[12], Yohan Bossé, PhD[6], Quinn S. Wells, MD[9], J. Gustav Smith, MD[5,23,24], Daniel J. Rader, MD[25], Mark Lathrop, PhD[7,26], James C. Engert, PhD[1,2,26]*, George Thanassoulis, MD[1,2]*

*co-senior authors

[1] Division of Experimental Medicine, McGill University, Montreal, Canada

[2] Preventive and Genomic Cardiology, McGill University Health Centre and Research Institute, Montreal, Canada

[3] MRC Population Health Research Unit, Clinical Trial Service Unit and Epidemiological Studies Unit, Nuffield Department of Population Health, University of Oxford, Oxford, United Kingdom

[4] Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, United States

[5] Department of Cardiology, Clinical Sciences, Lund University, Lund, Sweden and Skåne University Hospital, Lund, Sweden

[6] Quebec Heart and Lung Institute, Laval University, Quebec City, Canada

[7] McGill University and Genome Quebec Innovation Centre, Montreal, Canada

[8] Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, United States

[9] Vanderbilt Translational and Clinical Cardiovascular Research Center, Vanderbilt University Medical Center, Nashville, United States

[10] National Heart, Lung, and Blood Institute's and Boston University's Framingham Heart Study, Boston, United States

[11] Nutritional Epidemiology, Department of Clinical Sciences Malmö, Lund University, Malmö, Sweden

[12] Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden

[13] Division of Research, Kaiser Permanente of Northern California, Oakland, United States

[14] Department of Public Health Sciences, University of California Davis, Davis, United States

[15] Los Angeles Biomedical Research Institute and Departments of Pediatrics and Medicine at Harbor-UCLA Medical Center, Torrance, United States

[16] Sanford School of Medicine, University of South Dakota and OmegaQuant Analytics, LLC, Sioux Falls, United States

[17] Department of Surgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, United States

[18] Department of Kinesiology, University of Waterloo, Waterloo, Canada

[19] Department of Cardiology, Amsterdam University Medical Center, University of Amsterdam, Amsterdam, The Netherlands

[20] MRC Epidemiology Unit, University of Cambridge, Cambridge, United Kingdom

[21] Faculty of Medicine, University of Iceland, Reykjavík, Iceland

[22] Division of Cardiology, Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, United States

[23] Wallenberg Center for Molecular Medicine, Lund University, Lund, Sweden

[24] Lund University Diabetes Center, Lund University, Lund, Sweden

[25] Departments of Genetics and Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, United States

[26] Department of Human Genetics, McGill University, Montreal, Canada

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5.2 Abstract

Importance: Aortic stenosis (AS) has no approved medical treatment. Identifying etiological pathways for AS could identify pharmacological targets.

Objective: To identify novel genetic loci and pathways associated with AS.

Design, Setting, and Participants: This genome-wide association study used a case-control design to evaluate 44 703 participants (3469 cases of AS) of self-reported European ancestry from the Genetic Epidemiology Research on Adult Health and Aging (GERA) cohort (from January 1, 1996, to December 31, 2015). Replication was performed in 7 other cohorts totaling 256 926 participants (5926 cases of AS), with additional analyses performed in 6942 participants from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium. Follow-up biomarker analyses with aortic valve calcium (AVC) were also performed. Data were analyzed from May 1, 2017, to December 5, 2019.

Exposures: Genetic variants (615,643 variants) and polyunsaturated fatty acids (n-6 and n-3) measured in blood.

Main Outcomes and Measures: Aortic stenosis and aortic valve replacement defined by electronic health records, surgical records, or echocardiography and the presence of AVC measured by computed tomography.

Results: The mean (SD) age of the 44,703 GERA participants was 69.7 (8.4) years, and 22,019 (49.3%) were men. The rs174547 variant at the *FADS1/2* locus was associated with AS (odds ratio [OR] per C allele, 0.88; 95% CI, 0.83-0.93; p=3.0×10^{-6}), with genome-wide significant after meta-analysis with seven replication cohorts totalling 312,118 individuals (9,395 AS cases) (OR, 0.91; 95% CI, 0.88-0.94; p=2.5×10^{-8}). A consistent association with AVC was also observed (OR, 0.91; 95% CI, 0.83-0.99; p=0.026). A higher ratio of arachidonic acid to linoleic acid was associated with AVC (OR per SD of the natural logarithm, 1.19; 95% CI, 1.09-1.30; p=6.6×10^{-5}). In Mendelian randomization, increased *FADS1* liver expression and arachidonic acid were associated with AS (OR per unit of normalized expression, 1.31 [95% CI, 1.17-1.48; p=7.4×10^{-6}]; OR per 5-percentage point increase in arachidonic acid for AVC, 1.23 [95% CI, 1.01-1.49; p=0.035], OR per 5-percentage point increase in arachidonic acid for AS, 1.08 [95% CI, 1.04-1.13; p=4.1×10^{-4} for AS]).

Conclusions and Relevance: Variation at the *FADS1/2* locus is associated with AS and AVC. Findings from biomarker measurements and Mendelian randomization appear to link n-6 fatty acid biosynthesis to AS, which may represent a therapeutic target.

Key Points

Question: Can genetics identify additional etiologies for aortic stenosis?

Findings: In a genome-wide case-control association study of 44,703 participants, each copy of a *FADS1/2* (fatty acid desaturase) genetic variant was associated with a 13% decrease in the odds of aortic stenosis. Meta-analysis including seven replication cohorts was genome-wide significant, with biomarker and Mendelian randomization analyses implicating elevated n-6 fatty acid levels as having a potentially causal association with aortic valve calcium and aortic stenosis.

Meaning: The *FADS1/2* locus and fatty acid biosynthesis are associated with aortic stenosis and should be examined further for their potential as therapeutic targets.

5.3 Main Text

Introduction

Aortic stenosis (AS) remains the leading cause of clinical valve disease in the developed world[24]. Contemporary treatment is limited to replacement of the aortic valve as no approved medical therapy currently exists. The development of such therapy would expand options for treating AS, but is hindered by a limited understanding of the causal contributors.

A genome-wide association study (GWAS) conducted in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium (2,245 aortic valve calcium [AVC] cases) demonstrated that the *LPA* locus, which codes for the apolipoprotein(a) moiety of lipoprotein(a), is causally associated with both incident AS and prevalent AVC, a subclinical phenotype that precedes AS[46]. This association with AS has been robustly confirmed in multiple cohorts[48,49,143]. Recent clinical trials have demonstrated significant reductions in lipoprotein(a) levels are achievable[54,56,116], which may represent a novel AS prevention strategy. Two recent GWAS (≤ 2,457 AS cases) have identified *TEX41* and *PALMD* variants as associated with AS, implicating abnormal cardiac development in disease etiology[57,58].

A GWAS with a greater number of cases could have improved statistical power to discover additional genetic loci for AS, and identify novel pathways as pharmacological targets. Accordingly, we performed a GWAS for AS among 44,703 participants (3,469 AS cases) of the Genetic Epidemiology Research on Adult Health and Aging (GERA) cohort, one of the largest collections of AS cases in the world. We validated our finding in seven additional cohorts totalling

256,926 participants (5,926 AS cases) and performed genetic and plasma biomarker analyses to describe a novel mechanism underlying AS, with potential therapeutic implications.

Methods

Genetic Discovery and Replication

In the GERA cohort (NCBI Database of Genotypes and Phenotypes, phs000788.v2.p3), we performed a GWAS for prevalent AS (615,643 variants), adjusted for age, age squared, and sex, among 44,703 unrelated individuals (3,469 AS cases) of self-reported European ancestry, aged 55 years or older (Supplementary Table 5.1). We restricted our analysis to European participants due to the small numbers of non-European GERA participants, as differences in genetic structure between ancestries may confound our findings. AS status was ascertained through electronic health records (January 1996 to December 2015, inclusive), using the diagnosis code for AS (*International Classification of Diseases, 9th Revision [ICD-9]* 424.1) or a procedure code for aortic valve replacement to designate cases; all other individuals were designated as controls. Individuals with congenital valvular disease (*ICD-9* 746-747) were excluded. Details of this case-control study are in the supplement.

We later received updated GERA data for 55,192 unrelated, European-ancestry participants aged 55 years and older (3,469 AS cases). Due to a small number of participants who withdrew consent after our initial analysis, the composition of the cases changed slightly but the significant change was the addition of 10,489 controls. We imputed the one region which contained a variant that demonstrated a novel, suggestive ($p \leq 1 \times 10^{-6}$) association with AS (see supplement) and reassessed the association of the variant with AS, first adjusted for age, age^2, and sex, and then further adjusted

for 1) dyslipidemia, hypertension, smoking (ever/never), and diabetes; 2) the *LPA* variant rs10455872; or 3) 10 principal components in separate models. In seven replication cohorts totalling 256,926 participants (5,926 AS cases; Supplementary Table 5.2), we estimated the association of this variant with AS. To assess the overall association of the variant with AS, we performed a fixed effects meta-analysis using estimates from discovery and replication cohorts, weighted by the inverse of their variance. As a sensitivity analysis, we performed a fixed effects meta-analysis using estimates from only the replication cohorts.

In the CHARGE Consortium, we estimated the association of the variant with prevalent AVC among 6,942 predominantly-European participants (2,245 AVC cases). AVC was quantified using computed tomography and dichotomized into presence (Agatston score>0) or absence (Agatston score=0) of AVC (see supplement). We also identified previously-reported, genome-wide significant associations with PhenoScanner[166] (retrieved September 23, 2018) and accessed results for this variant in a GWAS for coronary artery disease (CARDIoGRAMplusC4D consortium)[167].

Fatty Acid Associations with AS and AVC

To investigate mediation of the lead variant through polyunsaturated fatty acid biosynthesis, we estimated the associations of four polyunsaturated fatty acids (arachidonic acid, linoleic acid, eicosapentaenoic acid, and α-linolenic acid) and two polyunsaturated fatty acid ratios reflecting fatty acid desaturation activity (arachidonic acid to linoleic acid, and eicosapentaenoic acid to α-linolenic acid) with AVC in the Framingham Offspring Study (FOS; n=1,310 [492 AVC cases]) and European-ancestry participants in the Multi-Ethnic Study of Atherosclerosis (MESA; n=2,415 [387 AVC cases]) (see supplement), as fatty acid measurements were unavailable in the GERA

cohort. We also assessed whether the association of dietary linoleic acid or α-linolenic acid with incident AS in the MDCS, and prevalent AVC in the FOS and MESA cohorts, was modified by our lead variant (see supplement).

Mendelian Randomization of Arachidonic Acid, FADS1 Expression, and FADS2 Expression with AS and AVC

Using Mendelian randomization, we estimated the association of a plasma arachidonic acid genetic risk score with both AS (32 variants; Supplementary Table 5.3) and AVC (24 variants; Supplementary Table 5.4). Additional sensitivity analyses were 1) removal of the lead variant, and 2) inverse variance-weighted, penalized weighted median, and Egger extension methods, to assess for robustness of the findings. We also assessed whether elevated *FADS1/2* expression in the liver was causally associated with prevalent AS (5 variants for *FADS1* [Supplementary Table 5.4]) and AVC (6 variants for *FADS1*; Supplementary Table 5.6) using Mendelian randomization. Details are provided in the supplement.

Statistical Analyses

Genome- and locus-wide genetic associations in GERA were computed using PLINK 2.0[168]. The associations of fatty acids with AVC in the MESA and FOS were estimated using R version 3.5.1 and SAS version 9.4, respectively. Meta-analyses of the lead variant and Mendelian randomization analyses were performed in R version 3.5.1. Two-sided $p \leq 5 \times 10^{-8}$ was deemed significant in the GWAS and $p \leq 0.05$ was considered significant in other analyses.

Ethics

All participants have provided written, informed consent and all relevant internal review boards have approved of the study.

Results

Association of the FADS1/2 Locus with AS and AVC

The discovery cohort consisted of 49% men (n=22,019). There was no evidence of inflated test statistics in the GWAS for AS (genomic inflation factor [λ]=1.03; Supplementary Figure 5.2). We confirmed the associations previously reported for *LPA* and *PALMD* variants (Supplementary Table 5.7). The intronic variant, rs174547, at the *FADS1/2* (fatty acid desaturase 1 and 2) locus on chromosome 11 was the only variant which demonstrated a novel association with AS at $p \leq 1 \times 10^{-6}$ (Table 5.1), with each copy of the minor (C) allele (frequency=33%) conferring 13% lower odds of AS (odds ratio [OR] per minor allele, 0.87; 95% CI, 0.83-0.92; $p=8.5 \times 10^{-7}$). Following imputation of the locus in the larger set of GERA participants, the association of rs174547 with AS was essentially unchanged (OR per minor allele, 0.88; 95% CI, 0.83-0.93; $p=3.0 \times 10^{-6}$), with variants in high linkage disequilibrium also associated with AS (Supplementary Figure 5.3). Further adjustment for cardiovascular risk factors, the *LPA* variant rs10455872, or population sub-stratification did not materially change these estimates (Supplementary Table 5.8). Participants who were homozygous for the minor allele (11% of the cohort) had 26% lower odds of AS (95% CI, 0.65-0.84; $p=2.8 \times 10^{-6}$) relative to participants homozygous for the major allele, adjusted for age, age^2, and sex.

When we combined this result with seven replication cohorts, in a meta-analysis totalling 312,118 individuals (9,395 AS cases), the overall association reached genome-wide significance (OR per

minor allele, 0.91; 95% CI, 0.88-0.94; $p=2.5\times10^{-8}$; Figure 5.2) and we observed no heterogeneity in the estimates ($I^2=0\%$). When only the replication cohorts were meta-analyzed, the magnitude of association was similar and significant (OR per minor allele, 0.93; 95% CI, 0.89-0.97; $p=7.4\times10^{-4}$), and there remained no heterogeneity ($I^2=0\%$).

The rs174547 variant demonstrated a consistent association with AVC in the CHARGE Consortium (OR per minor allele, 0.91; 95% CI, 0.83-0.99; $p=0.026$). Results accessed from PhenoScanner (Supplementary Table 5.9) indicated this variant was also associated with other biochemical phenotypes including fatty acid and lipid measures, but only one disease entity, asthma, which is characterized by eicosanoid-mediated inflammation. In the CARDIoGRAMplusC4D consortium, there was a nominally-significant association with coronary artery disease (OR per minor allele, 0.98; 95% CI, 0.96-1.00; $p=0.027$).

Associations of Fatty Acids with AS and AVC

Due to the role of the *FADS1/2* locus in n-6 and n-3 fatty acid biosynthesis (Figure 5.3), we examined the association of n-6 and n-3 fatty acids as well as fatty acid ratios reflecting n-6 and n-3 desaturation activity with AVC in the FOS and MESA cohorts (Table 5.1 and Supplementary Table 5.10). Higher levels of arachidonic acid and a higher ratio of arachidonic acid to linoleic acid, reflecting increased conversion of linoleic acid to arachidonic acid, were associated with increased odds for AVC, adjusted for age and sex (combined OR per SD of the natural logarithm, 1.12; 95% CI, 1.03-1.22; $p=0.010$ for arachidonic acid and 1.19; 95% CI, 1.09-1.30; $p=6.6\times10^{-5}$ for the ratio). These associations were materially unchanged after adjustment for low-density lipoprotein cholesterol, systolic blood pressure, smoking, and diabetes (Table 5.1). Neither

eicosapentaenoic acid nor the ratio of eicosapentaenoic acid to α-linolenic acid, reflecting increased conversion of α-linolenic acid to eicosapentaenoic acid, were associated with AVC.

We did not observe interactions between dietary linoleic acid and rs174547 for their associations with incident AS or prevalent AVC (Supplementary Table 5.11-Supplementary Table 5.12).

Mendelian Randomization of Arachidonic Acid and FADS1 Expression with AS and AVC

To evaluate causality of n-6 fatty acids in AS and AVC, we used Mendelian randomization to separately estimate the associations between a plasma arachidonic acid genetic risk score and AS and AVC. Genetically-elevated arachidonic acid was associated with a higher prevalence of AS and AVC, with a 5 percentage point increase of arachidonic acid among total fatty acids corresponding to an 8% increase in the odds for AS (95% CI, 1.04-1.13; p=4.1×10^{-4}) and a 23% increase in the odds for AVC (95% CI; 1.01-1.49; p=0.035). In sensitivity analyses excluding the rs174547 variant or accounting for the effects of the genetic risk score through mechanisms other than arachidonic acid (i.e. genetic pleiotropy), we observed attenuation under some conditions (Table 5.2) though the intercept in the Egger regressions was not significant.

Mendelian randomization analysis also indicated that genetically-elevated *FADS1* expression in the liver conferred increased odds of AS and AVC (OR per unit increase of normalized expression, 1.31; 95% CI, 1.17-1.48; p=7.4×10^{-6} and 1.25; 95% CI, 1.02-1.52; p = 0.031, respectively). Sensitivity analyses supported a potentially causal association of elevated *FADS1* expression with AS and AVC, with no evidence of directional pleiotropy (Supplementary Table 5.13). No significant *FADS2* liver expression quantitative trait loci were available.

Discussion

We conducted a GWAS for AS in the GERA cohort, with one of the largest collections of cases to date. The *FADS1/2* variant rs174547 demonstrated association with prevalent AS, with each copy of the minor allele conferring >10% lower odds of the disease. In individuals homozygous for the minor allele, we observed a 26% reduction in the odds of AS. The association persisted following adjustments for *LPA* rs10455872, cardiovascular risk factors or population stratification. When the discovery and seven replication cohorts comprising 312,118 individuals (9,395 AS cases) were combined, rs174547 reached genome-wide significance. The association also persisted in a sensitivity analysis excluding the discovery cohort, supporting a robust association with AS. The rs174547 variant was also associated with prevalent AVC in the CHARGE Consortium, providing additional evidence for a role in early valvular calcification. Since the *FADS1/2* locus is a key regulator of polyunsaturated fatty acid biosynthesis[169], we assessed the association of several n-6 and n-3 fatty acids with AVC. Increased production of the n-6 arachidonic acid, but not the n-3 eicosapentaenoic acid, was associated with AVC, with highly consistent results in two cohorts. We further observed that genetically-elevated *FADS1* expression in the liver was associated with increased odds of AS and AVC. Additional Mendelian randomization analyses provided evidence of a potentially causal association between plasma arachidonic acid, the product of the n-6 pathway, and both AS and AVC, although we were unable to entirely exclude the possibility of pleiotropy. Therefore, our results indicate that *FADS1/2* variation is a key determinant of valve calcification, demonstrate that plasma n-6 fatty acids are associated with valve calcium, and suggest that increased n-6 fatty acid biosynthesis may be a causal pathway for AS.

The rs174547 variant is located in an intron of *FADS1*, a member of the *FADS1/2/3* gene cluster. The function of *FADS3* is unknown, while *FADS1* and *FADS2* encode fatty acid desaturases with key functions in the conversion of dietary linoleic and α-linolenic acids into arachidonic and eicosapentaenoic acids, respectively (Figure 5.3)[169]. Notably, the rs174547 variant resides on a haplotype which extends across *FADS1* and part of *FADS2*, and the minor allele is associated with decreased transcription of *FADS1* and increased transcription of *FADS2* across most tissue types[170]. The net result is lower arachidonic acid levels and higher linoleic acid levels[171,172], indicating that the conversion of dietary n-6 fatty acids to longer chain polyunsaturated fatty acids is less active in minor allele carriers. The minor allele is also associated with lower levels of eicosapentaenoic acid and higher levels of α-linolenic acid[173], mirroring the effects observed for n-6 long chain fatty acid synthesis. However, the conversion of α-linolenic acid to downstream products is inefficient and levels of long-chain n-3 fatty acids are highly dependent on diet[174]. Arachidonic acid is a precursor for pro-inflammatory prostaglandins and leukotrienes, whereas leukotrienes and resolvins derived from eicosapentaenoic acid are anti-inflammatory[175], and thus the enzymatic activities of FADS1 and 2 may have pro- and anti-inflammatory effects.

Although higher linoleic acid levels have been associated with reduced risk of all-cause mortality and myocardial infarction[176], many prior studies have not evaluated the contribution of the arachidonic to linoleic acid ratio (or *FADS1/2* genotypes), thereby overlooking inter-individual variation in the production of arachidonic acid. A higher arachidonic acid to linoleic acid ratio has been associated with cardiovascular and all-cause mortality after adjustment for risk factors[177], which suggests that FADS1 and 2 activity may be an independent contributor to cardiovascular outcomes.

Several lines of evidence point to n-6 fatty acids as possible causal mediators for AS. We found that a higher ratio of arachidonic acid to linoleic acid (reflecting n-6 desaturation), but not the ratio of eicosapentaenoic acid to α-linolenic acid (reflecting n-3 desaturation), was associated with AVC. This was consistent with our Mendelian randomization findings, which demonstrated that genetically-elevated *FADS1* expression, as well as arachidonic acid, were associated with AS and AVC, providing evidence of a causal link. A greater conversion of linoleic acid to arachidonic acid may be associated with a local pro-inflammatory state via increased leukotrienes[178,179]. Increased inflammation has been demonstrated among AS patients, as denoted by overexpression of interleukin-6[180] and interleukin-1β[4] at the valve. Indeed, local levels of leukotrienes B_4 and C_4, downstream metabolites of arachidonic acid, are associated with the extent of valve calcification[181] and aortic valve area[25], providing a mechanistic link between production of arachidonic acid and valve calcification and AS. Higher levels of arachidonic acid in phospholipids, observed in explanted stenotic aortic valves[182], may also increase their susceptibility to oxidation, and promote local inflammation and subsequent calcification.

Linking variation at the *FADS1/2* locus to AS is complicated by the multitude of identified biomarker associations, which are all likely secondary to polyunsaturated fatty acid biosynthesis. The association of rs174547 with AS in the GERA cohort persisted when adjusted for dyslipidemia, hypertension, smoking, and diabetes. In the FOS and MESA cohorts, the association between AVC and greater conversion of linoleic acid to arachidonic acid also remained after adjustment for low-density lipoprotein cholesterol, systolic blood pressure, smoking, and diabetes. Thus, the associations of rs174547 and increased arachidonic acid with aortic valve outcomes are

likely to be independent of the effects of rs174547 on these risk factors. Our Mendelian randomization of *FADS1* expression further demonstrates the key role of FADS1 and implicates fatty acid desaturation in valve calcification. Finally, Mendelian randomization analyses for arachidonic acid were robust to pleiotropy under certain assumptions such as penalized weighted median, which allows for up to 50% of the variants in the genetic risk score to be pleiotropic. As the intercept did not differ significantly from zero in our Egger regression for AS or AVC, there was no strong evidence for alternate pleiotropic pathways. Taken together, the present findings link FADS1/2 activity with AS and identify arachidonic acid as having a likely causal association with disease. Further investigation will be needed to delineate the downstream processes that link the *FADS1/2* locus and arachidonic acid to aortic valve pathology.

Our results point to the *FADS1/2* locus and n-6 fatty acid biosynthesis as potential therapeutic targets. Direct therapeutic alteration of *FADS1/2* expression, to mimic the observed genetic effects and reduce fatty acid desaturation, may represent a therapeutic strategy for AS, which is supported by our Mendelian randomization of *FADS1* expression. Alternatively, we speculate the role of FADS1/2 in the conversion of linoleic acid to arachidonic acid raises the possibility of dietary modification as a preventive strategy for AS. Both approaches warrant further study as possible treatments for AS.

Strengths and Limitations

Our discovery GWAS was performed in the GERA cohort with one of the largest collections of AS cases assembled to date. Data from several large-scale cohorts provided robust replication for this novel association, and extended it to relevant fatty acid measures. Nonetheless, there are

several limitations to note. First, not all participants underwent echocardiography, the gold standard for diagnosing AS. We relied on a heterogeneous definition of AS across cohorts that may not have captured all cases, but misclassification of undiagnosed AS cases as controls is likely to bias our results towards the null, as is heterogeneity in our definition of controls. Our use of diagnosis and procedure codes to define AS also precludes an assessment of disease severity. However, our case-finding approach permits the study of AS in large cohorts without echocardiographic data, has previously led to the discovery and robust replication of the *LPA* locus (including in many of the cohorts in the current study[46,48,49,57,58]), and has a positive predictive value exceeding 90%[46]. We also observed no heterogeneity in our meta-analysis of rs174547, suggesting the various definitions for AS are concordant. Second, some Mendelian randomization sensitivity analyses lacked statistical power, including Egger regression, which is a known limitation of this approach. Third, we restricted our GWAS to participants with self-reported European ancestry as the number of non-Europeans was low and the frequency of *FADS1/2* variants varies markedly across ethnicities[183]. Fourth, while all measured fatty acids were derived from blood, the measurements were taken in red blood cells in the FOS and in plasma in the MESA, and associations with AVC in both cohorts were cross-sectional. However, results were highly consistent across the two cohorts despite the different approaches. Fifth, we focused on the *FADS1/2* genes as likely candidates in the locus and provide evidence in favour of this pathway. We acknowledge that other genes nearby could also play a role, but these are unlikely candidates based on their known biology. Lastly, although we observed modest reductions in the odds of AS among participants with one or two copies of the minor allele, this reflects the natural genetic variation at a locus with important biological function; targeting this locus by pharmacological means could achieve larger reductions in AS odds.

Conclusions

We demonstrate that a common variant in the *FADS1/2* locus is associated with AS and AVC. Concordant findings from biomarker measurements and Mendelian randomization link increased n-6 fatty acid biosynthesis to the development of AS, which may represent a novel therapeutic target.

Figures

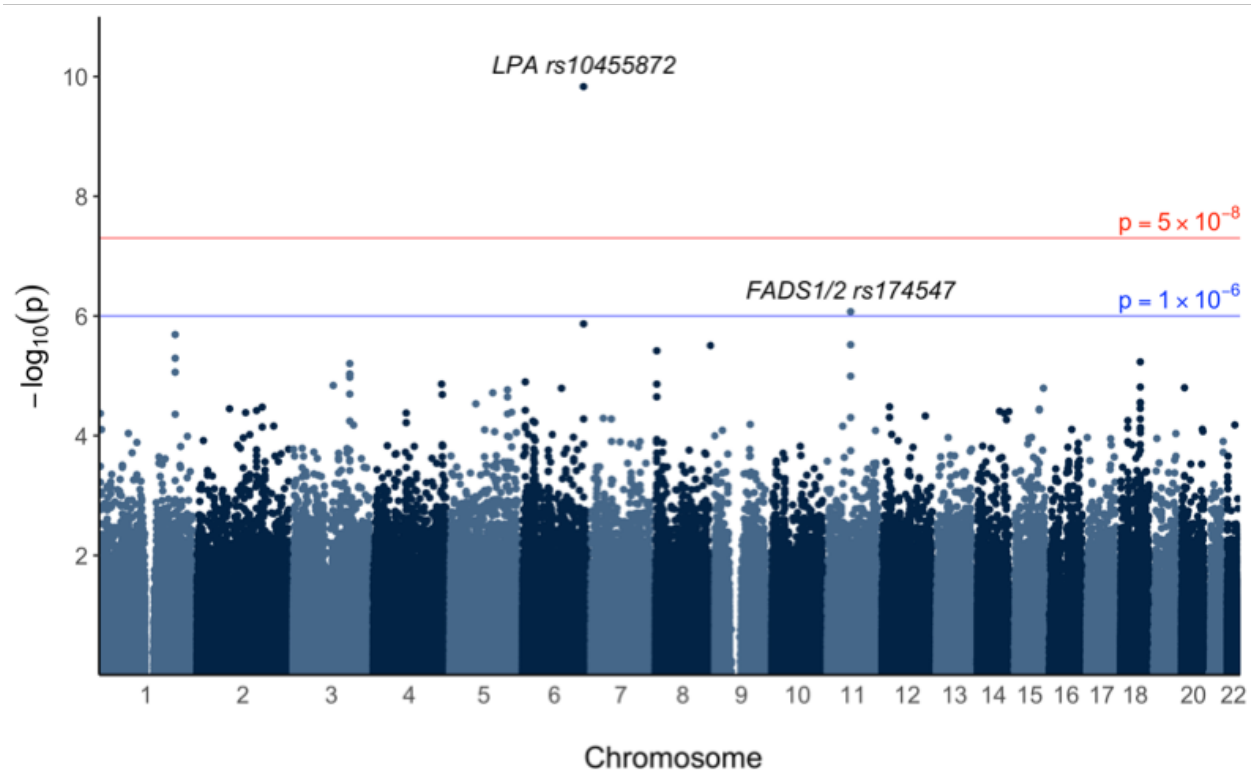

Figure 5.1. *P* Values for the Association of 615,643 Genetic Variants with Aortic Stenosis in the GERA Cohort, by Chromosome.

The red line indicates genome-wide significance ($p \leq 5\times10^{-8}$) while the blue lines indicates suggestive evidence of association ($5\times10^{-8} < p \leq 1\times10^{-6}$).

Abbreviations: GERA, Genetic Epidemiology Research on Adult Health and Aging.

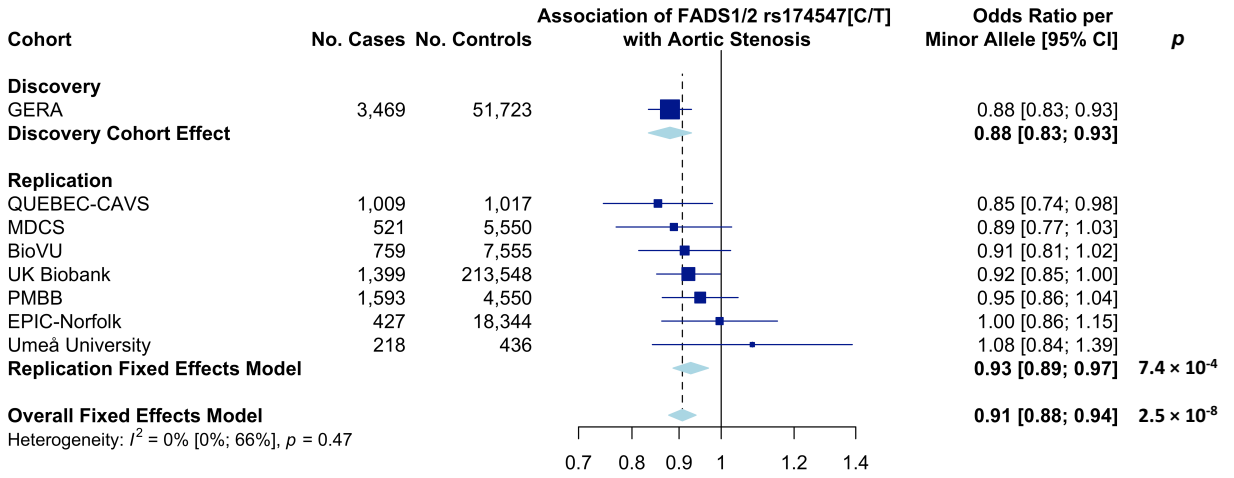

Figure 5.2. Association of FADS1/2 rs174547 with Aortic Stenosis in the Discovery and Replication Cohorts.

For rs174547, C and T are the minor and major alleles, respectively. The sizes of the dark blue squares reflect the weight of the cohorts in the fixed effects meta-analysis.

Abbreviations: GERA, Genetic Epidemiology on Adult Health and Aging; QUEBEC-CAVS, Quebec City Case-Control Calcific Aortic Valve Stenosis; MDCS, Malmö Diet and Cancer Study; BioVU, Vanderbilt DNA Biobank; PMBB, Penn Medicine BioBank; EPIC-Norfolk, European Prospective Investigation of Cancer and Nutrition – Norfolk.

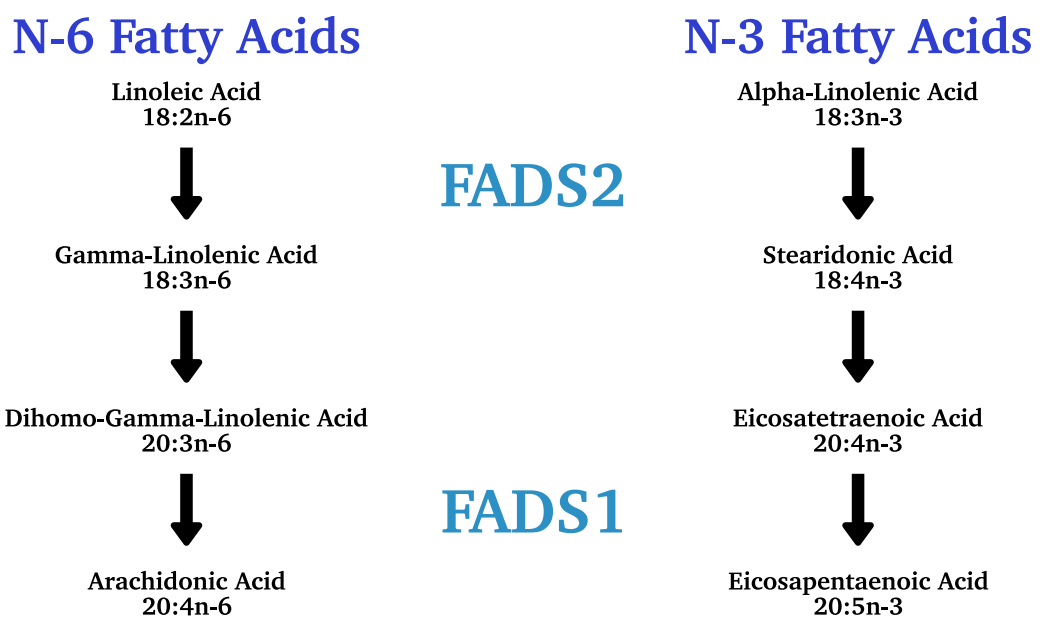

Figure 5.3. Roles of FADS1 and FADS2 in the Conversion of 18-Carbon n-6 and n-3 Fatty Acids to Arachidonic and Eicosapentaenoic Acids.

FADS1 and FADS2 perform the desaturation steps in the conversion of 18-carbon n-6 and n-3 fatty acids to arachidonic and eicosapentaenoic acids.

Tables

Table 5.1. Associations of N-6 and N-3 Fatty Acids with Aortic Valve Calcium.

Fatty Acid	Cohort	Age and Sex Adjusted		Fully Adjusted	
		OR (95% CI)	*p*	OR (95% CI)	*p*
N-6					
AA	FOS	1.10 (0.97-1.25)	0.13	1.13 (0.98-1.29)	0.086
	MESA	1.13 (1.01-1.27)	0.039	1.14 (1.01-1.29)	0.031
	Combined	1.12 (1.03-1.22)	0.010	1.14 (1.04-1.24)	5.8×10^{-3}
AA/LA Ratio	FOS	1.20 (1.06-1.37)	5.2×10^{-3}	1.22 (1.06-1.39)	4.6×10^{-3}
	MESA	1.19 (1.06-1.34)	4.4×10^{-3}	1.22 (1.08-1.38)	1.6×10^{-3}
	Combined	1.19 (1.09-1.30)	6.6×10^{-5}	1.22 (1.11-1.34)	2.2×10^{-5}
N-3					
EPA	FOS	0.91 (0.80-1.04)	0.16	0.91 (0.80-1.04)	0.18
	MESA	1.04 (0.92-1.16)	0.54	1.07 (0.95-1.21)	0.25
	Combined	0.98 (0.90-1.07)	0.63	1.00 (0.91-1.09)	0.97
EPA/ALA Ratio	FOS	0.99 (0.87-1.12)	0.85	1.00 (0.88-1.15)	0.95
	MESA	1.08 (0.96-1.22)	0.19	1.12 (0.99-1.26)	0.080
	Combined	1.04 (0.95-1.13)	0.40	1.06 (0.97-1.16)	0.18

OR presented per SD of the natural logarithm for AA and EPA, and per SD of the natural logarithm of the ratio of the fatty acids, for the AA/LA and EPA/ALA ratios. Fully adjusted models were adjusted for low-density lipoprotein cholesterol, systolic blood pressure, current smoking, and diabetes, in addition to age and sex. Estimates were combined via fixed effects meta-analysis weighted by the inverse of their variance.

Abbreviations: OR, odds ratio; LA, linoleic acid; FOS, Framingham Offspring Study; MESA, Multi-Ethnic Study of Atherosclerosis; AA, arachidonic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid.

Table 5.2. Genetic Associations of Arachidonic Acid with Aortic Stenosis and Aortic Valve Calcium.

Method	OR per 5 Percentage Point Increase of AA Among Total Fatty Acids (95% CI)	*p*
Aortic Stenosis		
Mendelian Randomization[a]	1.08 (1.04-1.13)	4.1×10^{-4}
Mendelian Randomization, Excluding rs174547[a]	1.03 (0.99-1.08)	0.15
Inverse Variance-Weighted	1.08 (1.02-1.15)	8.9×10^{-3}
Penalized Weighted Median	1.11 (1.03-1.20)	4.9×10^{-3}
Egger Extension to Mendelian Randomization	1.02 (0.92-1.13)	0.72
Intercept for the Egger Extension	-	0.15
Aortic Valve Calcium		
Mendelian Randomization[a]	1.23 (1.01-1.49)	0.035
Mendelian Randomization, Excluding rs174547[a]	1.10 (0.82-1.49)	0.52
Inverse Variance-Weighted	1.23 (1.01-1.49)	0.035
Penalized Weighted Median	1.32 (1.04-1.68)	0.022
Egger Extension to Mendelian Randomization	1.08 (0.78-1.49)	0.63
Intercept for the Egger Extension	-	0.32

[a] Genetic risk score is not associated with eicosapentaenoic acid.

Abbreviations: AA, arachidonic acid; OR, odds ratio.

Author Contributions

Drs Engert and Thanassoulis served as co-senior authors. Ms Chen and Dr Thanassoulis had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Concept and design: Chen, Näslund, Whitmer, Vasan, Arsenault, Mathieu, O'Donnell, Smith, Lathrop, Engert, Thanassoulis.

Acquisition, analysis, or interpretation of data: Chen, Cairns, Small, Burr, Ambikkumar, Martinsson, Thériault, Munter, Steffen, Zhang, Levinson, Shaffer, Rong, Sonestedt, Dufresne, Ljungberg, Näslund, Johansson, Ranatunga, Whitmer, Budoff, Nguyen, Vasan, Larson, Harris, Damrauer, Stark, Boekholdt, Wareham, Pibarot, Gudnason, Rotter, Tsai, Post, Clarke, Söderberg, Bossé, Wells, Smith, Rader, Lathrop, Engert, Thanassoulis.

Drafting of the manuscript: Chen.

Critical revision of the manuscript for important intellectual content: Chen, Cairns, Small, Burr, Ambikkumar, Martinsson, Thériault, Munter, Steffen, Zhang, Levinson, Shaffer, Rong, Sonestedt, Dufresne, Ljungberg, Näslund, Johansson, Ranatunga, Whitmer, Budoff, Nguyen, Vasan, Larson, Harris, Damrauer, Stark, Boekholdt, Wareham, Pibarot, Arsenault, Mathieu, Gudnason, O'Donnell, Rotter, Tsai, Post, Clarke, Söderberg, Bossé, Wells, Smith, Rader, Lathrop, Engert, Thanassoulis.

Statistical analysis: Chen, Small, Burr, Ambikkumar, Martinsson, Thériault, Zhang, Levinson, Shaffer, Rong, Sonestedt, Dufresne, Whitmer, Larson, Smith.

Obtained funding: Näslund, Vasan, Wareham, Gudnason, Rotter, Post, Söderberg, Smith, Lathrop, Engert, Thanassoulis.

Administrative, technical, or material support: Ljungberg, Näslund, Ranatunga, Whitmer, Vasan, Harris, Stark, Wareham, Rotter, Tsai, Söderberg, Bossé, Smith, Lathrop, Thanassoulis. *Supervision:* Johansson, Budoff, Vasan, Larson, Damrauer, Clarke, Smith, Rader, Engert, Thanassoulis.

Conflict of Interest Disclosures

Ms Chen reported receiving grants from McGill University Faculty of Medicine and McGill University Health Centre Foundation during the conduct of the study. Dr Cairns reported receiving grants from the Medical Research Council (MRC) UK during the conduct of the study. Dr Thériault reported receiving grants from Fonds de Recherche du Québec-Santé (FRQS) during the conduct of the study. Dr Budoff reported receiving grants from General Electric outside the submitted work. Dr Vasan reported receiving grants from the National Institutes of Health (NIH) during the conduct of the study and support from the Evans Medical Foundation and the Jay and Louis Coffman Endowment from the Department of Medicine, Boston University School of Medicine. Dr Damrauer reported receiving grants from RenalytixAI plc and the US Department of Veterans Affairs outside the submitted work. Dr Stark reported receiving salary support from the Canada Research Chairs program for a Chair in Nutritional Lipidomics. Dr Pibarot reported receiving grants from Edwards Lifesciences and Medtronic plc outside the submitted work. Dr Arsenault reported receiving grants from Ionis Pharmaceuticals and Pfizer, Inc, and personal fees from Novartis International AG outside the submitted work. Dr Mathieu reported holding a Fonds de Recherche du Québec-Santé Research Chair on the Pathobiology of Calcific Aortic Valve Disease. Dr Rotter reported receiving grants from the NIH during the conduct of the study. Dr Post reported receiving grants from the NIH during the conduct of the study. Dr Söderberg reported

receiving personal fees from Actelion Ltd outside the submitted work and support from the Swedish Heart–Lung Foundation (grant numbers 20140799, 20120631 and 20100635), the County Council of Västerbotten (ALF, VLL-548791), Umeå University, and the Heart Foundation of Northern Sweden. Dr Smith reported receiving grants from the Swedish Heart-Lung Foundation (2016-0134 and 2016-0315), the Swedish Research Council (2017-02554), the European Research Council (ERC-STG-2015-679242), the Crafoord Foundation, Skåne University Hospital, Scania County, governmental funding of clinical research within the Swedish National Health Service, the Knut and Alice Wallenberg Foundation to the Wallenberg Center for Molecular Medicine in Lund, and funding from the Swedish Research Council (Linnaeus grant 349-2006-237, Strategic Research Area Exodiab 2009-1039), and Swedish Foundation for Strategic Research (IRC15-0067) to the Lund University Diabetes Center. Dr Rader reported receiving personal fees from Alnylam Pharmaceuticals, Inc, Novartis International AG, Pfizer, Inc, Verve Therapeutics, and AstraZeneca plc outside the submitted work. Dr Thanassoulis reported receiving grants from the Canadian Institutes of Health Research, the National Heart, Lung, and Blood Institute (NHLBI) of the NIH, Heart and Stroke Foundation of Canada, Fonds de Recherche Québec-Santé, Doggone Foundation, Courtois Foundation, and the Ingram Family Foundation during the conduct of the study; participating on the advisory boards of Amgen, Inc, and Regeneron/ Sanofi, personal fees from HLS Therapeutics, Inc, grants and personal fees from Servier Laboratories, and consulting fees from Ionis Pharmaceuticals outside the submitted work. No other disclosures were reported.

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The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contributions

Genotyping was performed at Affymetrix, Santa Clara, California, and the Broad Institute of Harvard and Massachusetts Institute of Technology, Boston, using the Affymetrix Genome-Wide Human SNP Array 6.0. We thank the investigators, staff, and participants from all the cohorts for their contributions.

Additional Information

A full list of participating Multi-Ethnic Study of Atherosclerosis investigators and institutions can be found at http://www.mesa- nhlbi.org. Data supporting this publication were made available to the investigators according to the data sharing policies of the contributing studies. Please contact the individual studies for more information.

5.4 Supplement

Chen HY, Cairns BJ, Small AM, et al. Association of *FADS1/2* locus variants with polyunsaturated fatty acids and aortic stenosis. Published online March 18, 2020. *JAMA Cardiology*. doi:10.1001/jamacardio.2020.0246

Supplementary Figure 5.1. Overview of the Discovery, Replication, and Follow-up Analyses Undertaken.

Supplementary Figure 5.2. Distribution of Observed Vs Expected *P* Values in the Genome-Wide Association Study for Aortic Stenosis.

Supplementary Figure 5.3. Association of Variants in the *FADS1/2* Locus With Aortic Stenosis.

Supplementary Table 5.1. Clinical Characteristics of the GERA Cohort.

Supplementary Table 5.2. Overview of Discovery and Replication Cohorts.

Supplementary Table 5.3. Variants in the Mendelian Randomization Analyses of the Association of Plasma Arachidonic Acid With Aortic Stenosis.

Supplementary Table 5.4. Variants in the Mendelian Randomization Analyses of the Association of Plasma Arachidonic Acid With Aortic Valve Calcium.

Supplementary Table 5.5. Variants in the Mendelian Randomization Analysis of the Association of Liver FADS1 Expression With Aortic Stenosis.

Supplementary Table 5.6. Variants in the Mendelian Randomization Analysis of the Association of Liver FADS1 Expression With Aortic Valve Calcium.

Supplementary Table 5.7. Variants Identified in Previous Genome-Wide Association Studies for Aortic Stenosis and Their Associations With Aortic Stenosis in the GERA Cohort.

Supplementary Table 5.8. Additional Covariate Adjustments for the Association of FADS1/2 rs174547 With Aortic Stenosis in the GERA Cohort.

Supplementary Table 5.9. Associations of FADS1/2 rs174547 With Traits at a Genome-Wide Level of Significance.

Supplementary Table 5.10. Associations of ω-6 and ω-3 Fatty Acids With Aortic Valve Calcium.

Supplementary Table 5.11. Associations of Dietary Fatty Acids With Aortic Stenosis by FADS1/2 rs174546 Genotype in the Malmö Diet and Cancer Study.

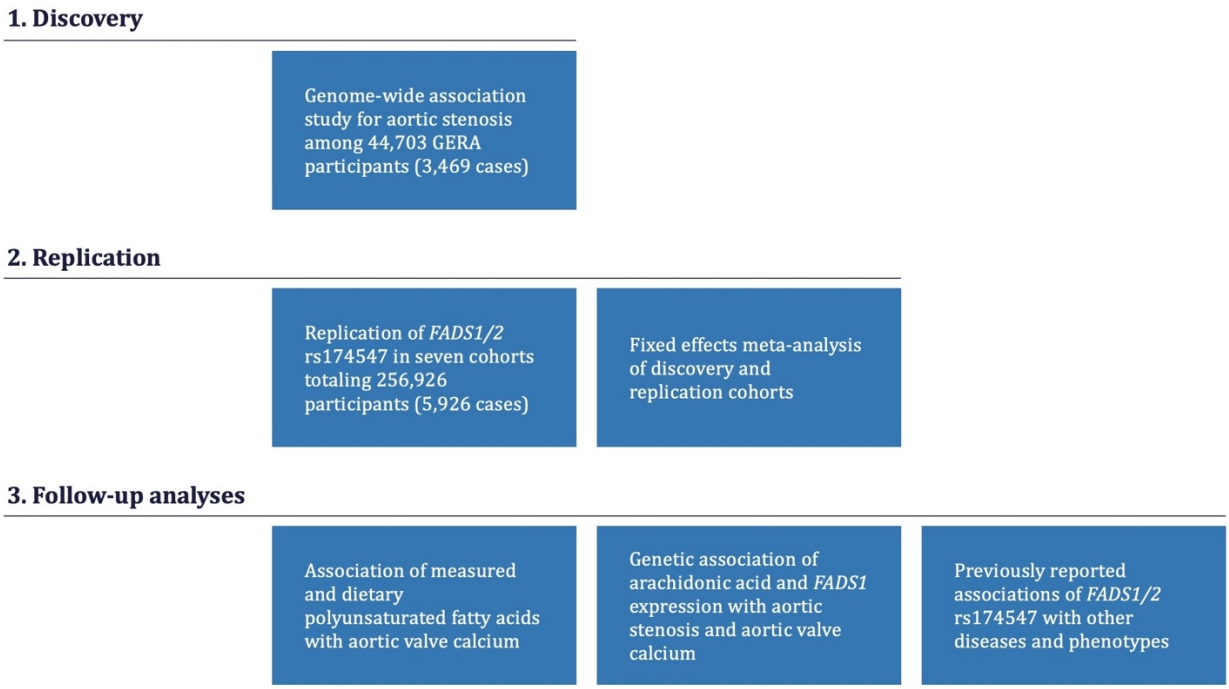
Supplementary Table 5.12. Associations of Dietary Fatty Acids With Aortic Valve Calcium by FADS1/2 rs174547 Genotype.

Supplementary Table 5.13. Sensitivity Analyses for the Genetic Associations of Liver FADS1 Expression With Aortic Stenosis and Aortic Valve Calcium.

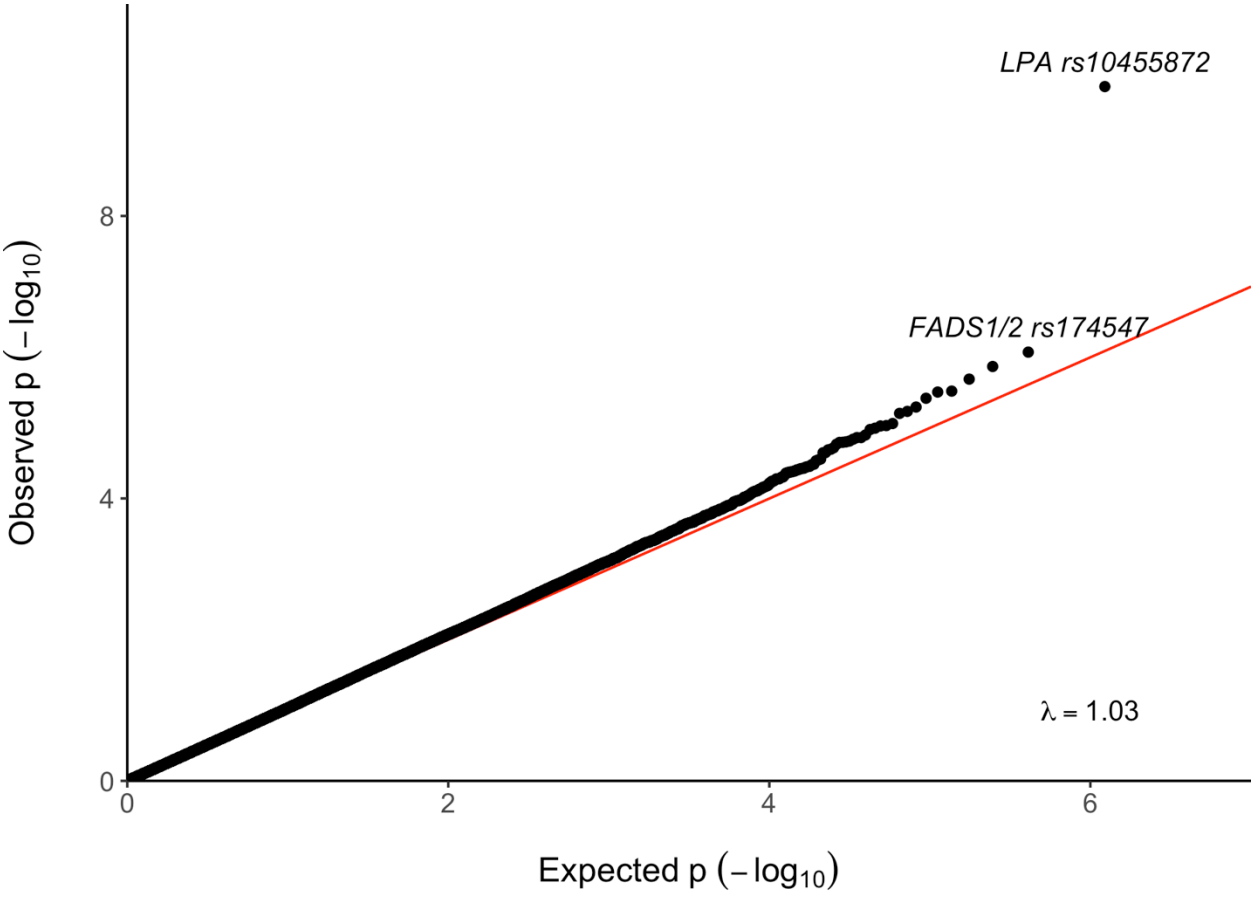
Supplementary Methods. Discovery and Replication Cohorts, Aortic Valve Calcium Cohorts, Gene-Diet Interactions for Aortic Stenosis and Aortic Valve Calcium, and Genetic Associations With Aortic Stenosis and Aortic Valve Calcium

This supplementary material has been provided by the authors to give readers additional information about their work.

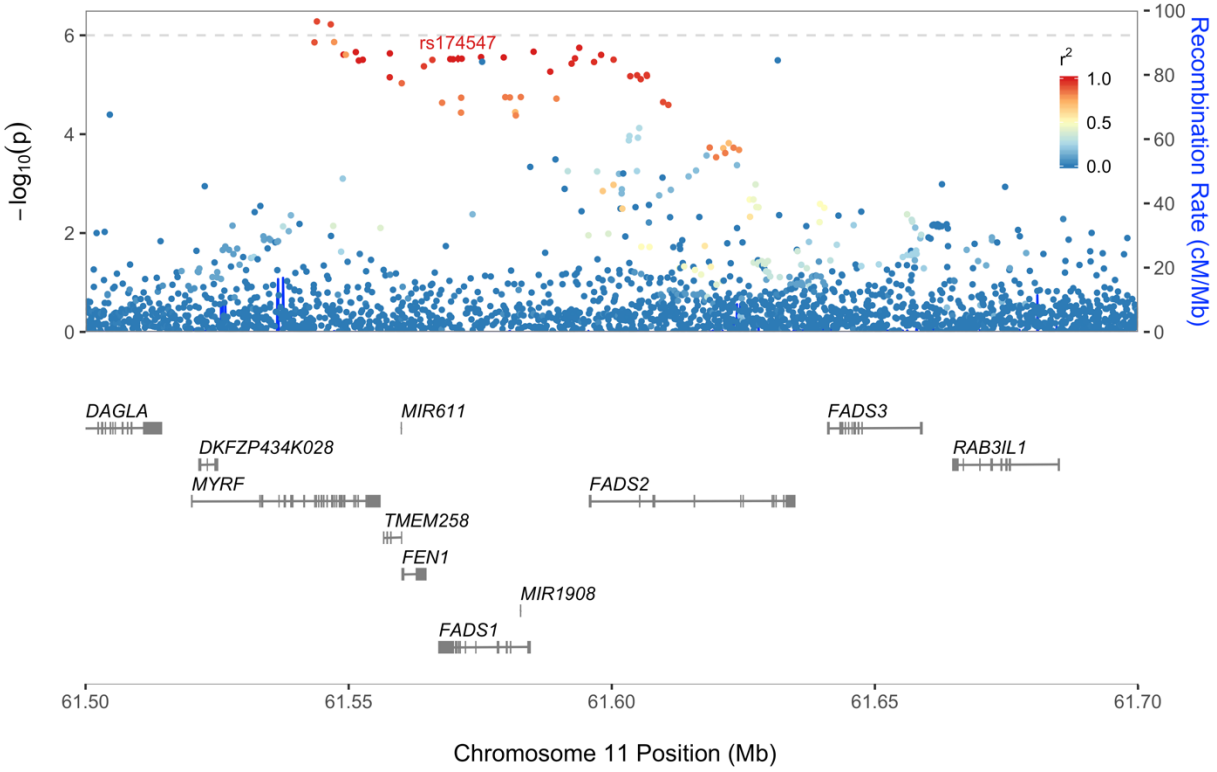
Supplementary Figure 5.1. Overview of the Discovery, Replication, and Follow-up Analyses Undertaken.



Supplementary Figure 5.2. Distribution of Observed Vs Expected *P* Values in the Genome-Wide Association Study for Aortic Stenosis.



Supplementary Figure 5.3. Association of Variants in the *FADS1/2* Locus With Aortic Stenosis.



Associations with aortic stenosis were estimated following imputation of the locus among 55,192 GERA participants (3,469 cases).

Abbreviations: GERA, Genetic Epidemiology Research on Adult Health and Aging.

Supplementary Table 5.1. Clinical Characteristics of the GERA Cohort.

Characteristic	Aortic Stenosis, n (%)		*p*
	Controls	Cases	
n	41,234	3,469	--
Sex, Male	20,076 (49)	1,943 (56)	<0.001
Age, Year (mean [SD])	69.3 [8.3]	74.6 [8.5]	<0.001
Body Mass Index, kg/m^2 (mean [SD])[a]	26.8 [4.9]	27.4 [5.4]	<0.001
Dyslipidemia	23,926 (58)	2,666 (77)	<0.001
Coronary Artery Disease	10,007 (24)	2,131 (61)	<0.001
Hypertension	16,961 (41)	1,945 (56)	<0.001
Ever Smoked[b]	20,247 (52)	1,834 (56)	<0.001
Diabetes	4,491 (11)	606 (17)	<0.001
Aortic Valve Replacement	0 (0)	400 (12)	--

[a] n=42,962

[b] n=42,535

Abbreviations: GERA, Genetic Epidemiology Research on Adult Health and Aging.

Supplementary Table 5.2. Overview of Discovery and Replication Cohorts.

Cohort	Countries	Aortic Stenosis			Genotyping Array	Imputation Reference Panel
		No. Cases	No. Controls	Case Definition		
Discovery						
GERA[a]	United States	3,469	51,723	Electronic health records	Affymetrix Axiom	Haplotype Reference Consortium version r1.1
Replication						
QUEBEC-CAVS	Canada	1,009	1,017	Aortic valve replacement	Illumina HumanOmni Express BeadChip	Haplotype Reference Consortium version r1.1
MDCS	Sweden	521	5,550	Electronic health records	Illumina Human Omni Express Exome BeadChip	Haplotype Reference Consortium version r1.1
BioVU	United States	759	7,555	Electronic health records	Illumina Multi-Ethnic Genotyping Array	Haplotype Reference Consortium version r1.1
UK Biobank	England, Scotland, and Wales	1,399	213,548	Electronic health records	Affymetrix Axiom	Haplotype Reference Consortium version r1.1, UK10K, and the 1000 Genomes Project phase 3
PMBB	United States	1,593	4,550	Electronic health records	Illumina Quad Omni Genotyping Chip	Haplotype Reference Consortium version r1.1
EPIC-Norfolk	England	427	18,344	Electronic health records and death records	Affymetrix UK Biobank Axiom Array	1000 Genomes Project phase 3
Umeå University	Sweden	218	436	Aortic valve replacement	Affymetrix UK Biobank Axiom Array r3	Haplotype Reference Consortium version r1.1

[a] Number of cases and controls after receiving updated data for the GERA cohort. The discovery genome-wide association study was conducted in an earlier dataset composed of 3,469 cases and 41,234 controls (see Supplementary Table 2).

Abbreviations: GERA, Genetic Epidemiology on Adult Health and Aging; QUEBEC-CAVS, Quebec City Case-Control Calcific Aortic Valve Stenosis; MDCS, Malmö Diet and Cancer Study; BioVU, Vanderbilt DNA Biobank; PMBB, Penn Medicine BioBank; EPIC-Norfolk, European Prospective Investigation of Cancer and Nutrition – Norfolk.

Supplementary Table 5.3. Variants in the Mendelian Randomization Analyses of the Association of Plasma Arachidonic Acid With Aortic Stenosis.

Chr.	Base Pair (GRCh37)	Genes (±50 kb)	Variant	Allele		Arachidonic Acid				Aortic Stenosis			
				Effect	Other	EAF	Beta	SE	*p*	EAF	Beta	SE	*p*
1	91,944,137	*CDC7*	rs12747494	T	C	0.99	-2.64	0.41	1.10E-10	0.95	0.04	0.04	0.28
1	102,489,339	*OLFM3*	rs11578575	T	C	0.02	2.55	0.36	1.00E-12	0.02	0.09	0.07	0.2
1	118,028,688	*MAN1A2*	rs7546429	A	G	0.98	2.75	0.15	4.00E-78	0.98	-0.07	0.07	0.29
2	99,485,920	*KIAA1211L*	rs12623171	T	C	0.01	3.2	0.45	1.90E-12	0.02	-0.07	0.06	0.21
3	41,139,829	--	rs13314643	T	C	0.01	1.44	0.15	6.30E-21	0.02	-0.02	0.07	0.75
3	119,147,958	*ARHGAP31, TMEM39A, POGLUT1*	rs16829840	T	C	0.01	0.46	0.08	2.50E-08	0.02	-0.1	0.06	0.08
3	133,893,303	*RYK*	rs11927316	A	T	0.02	0.71	0.13	4.50E-08	0.02	0.05	0.07	0.45
4	25,874,805	*SEL1L3, SMIM20*	rs11726352	A	G	0.01	2.69	0.43	3.10E-10	0.03	0.06	0.06	0.35
4	104,380,632	--	rs340480	A	G	0.98	-2.91	0.39	1.20E-13	0.96	-0.05	0.05	0.33
5	147,240,678	*SPINK1, SCGB3A2, C5orf46*	rs1432985	A	G	0.98	-2.13	0.35	1.10E-09	0.95	0	0.05	0.98
6	31,345,815	*HLA-B, MICA*	rs13193697	T	C	0.01	-0.72	0.13	4.10E-08	0.01	0	0.07	0.99
6	32,667,820	*HLA-DQB1, HLA-DQA2*	rs9275354	T	G	0.99	2.68	0.23	8.00E-31	0.77	0.07	0.02	2.70E-03
6	165,413,927	--	rs9356335	A	G	0.98	-1.16	0.1	6.10E-32	0.97	0.07	0.05	0.21
7	126,052,078	*GRM8*	rs7778698	A	C	0.02	-0.69	0.11	4.70E-10	0.03	-0.07	0.05	0.22
7	149,620,802	*ATP6V0E2-AS1, ATP6V0E2, DQ590227*	rs7456249	T	C	0.98	-2.68	0.41	9.40E-11	0.97	0.07	0.05	0.15
10	9,705,976	--	rs7916429	C	G	0.96	-0.51	0.09	1.30E-08	0.95	0.02	0.04	0.56

Table Continued

Chr.	Base Pair (GRCh37)	Genes (±50 kb)	Variant	Allele		Arachidonic Acid				Aortic Stenosis			
				Effect	Other	EAF	Beta	SE	*p*	EAF	Beta	SE	*p*
10	68,212,659	*CTNNA3, SnoU40*	rs12416578	T	C	0.99	-0.96	0.12	1.30E-14	0.99	-0.07	0.07	0.32
10	81,236,036	*ZCCHC24, AK302451, EIF5AL1*	rs7080747	T	C	0.01	-1.16	0.16	3.80E-13	0.02	-0.03	0.06	0.6
11	61,570,783	*MYRF, DKFZP434K028, BC020196, TMEM258, MIR611, FEN1, FADS1, MIR1908, FADS2*	rs174547	T	C	0.68	1.69	0.03	≈ 0	0.67	0.1	0.02	5.80E-09
11	61,823,386	--	rs11230889	A	C	0.83	0.29	0.05	9.30E-10	0.83	-0.02	0.02	0.49
11	125,259,904	*PKNOX2*	rs890455	T	G	0.02	1.85	0.32	5.10E-09	0.02	0	0.07	0.97
12	38,998,940	*BC040886, CPNE8*	rs2653765	A	C	0.01	-1.27	0.14	5.50E-21	0.01	-0.1	0.09	0.29
12	129,881,993	*TMEM132D*	rs7970058	A	G	0.01	-0.87	0.11	7.70E-15	0.02	-0.05	0.07	0.48
12	133,462,329	*CHFR, AK055957, ZNF605*	rs11147144	A	C	0.01	0.89	0.13	1.90E-12	0.02	0	0.07	0.97
13	96,870,398	*HS6ST3, Metazoa_SRP*	rs16951711	T	C	0.01	-1.8	0.13	1.60E-45	0.02	-0.08	0.06	0.18
14	20,377,949	*OR4K2, OR4K5, OR4K1*	rs4359352	A	C	0.03	2.45	0.36	7.00E-12	0.02	-0.02	0.07	0.82
14	20,994,759	*PNP, RNASE10, RNASE9*	rs2150349	T	C	0.02	2.01	0.35	1.10E-08	0.02	-0.04	0.07	0.58
14	41,497,960	--	rs12894905	A	C	0.02	-0.92	0.11	5.90E-17	0.02	0.01	0.05	0.8
16	15,130,351	*PDXDC1, NTAN1, RRN3*	rs1741	C	G	0.31	-0.2	0.03	1.60E-10	0.3	-0.05	0.02	2.80E-03

Table Continued

Chr.	Base Pair (GRCh37)	Genes (±50 kb)	Variant	Allele		Arachidonic Acid				Aortic Stenosis			
				Effect	Other	EAF	Beta	SE	*p*	EAF	Beta	SE	*p*
18	30,030,309	*GAREM*	rs17811780	T	G	0.98	-0.68	0.12	1.20E-08	0.98	0.05	0.06	0.36
18	45,488,690	*SMAD2*	rs12456907	A	C	0.03	2.37	0.29	1.50E-16	0.02	-0.02	0.07	0.82
18	68,738,240	--	rs7231821	A	T	0.99	-0.82	0.13	2.00E-10	0.98	-0.05	0.06	0.45

The effect size for arachidonic acid is expressed as the percentage point change among total plasma fatty acids while the effect size for aortic stenosis is expressed as the natural logarithm of the odds ratio. For aortic stenosis, the EAF is from the GERA cohort.

Abbreviations: EAF, effect allele frequency; GERA, Genetic Epidemiology Research on Adult Health and Aging.

Supplementary Table 5.4. Variants in the Mendelian Randomization Analyses of the Association of Plasma Arachidonic Acid With Aortic Valve Calcium.

Chr.	Base Pair (GRCh37)	Genes (±50 kb)	Variant	Allele		Arachidonic Acid				Aortic Valve Calcium			
				Effect	Other	EAF	Beta	SE	*p*	EAF	Beta	SE	*p*
1	118,028,688	*MAN1A2*	rs7546429	A	G	0.98	2.75	0.15	4.00E-78	0.96	0.01	0.15	0.93
3	41,139,829	--	rs13314643	T	C	0.01	1.44	0.15	6.30E-21	0.02	0.07	0.24	0.76
3	119,147,958	*ARHGAP31, TMEM39A, POGLUT1*	rs16829840	T	C	0.01	0.46	0.08	2.50E-08	0.02	-0.01	0.14	0.96
3	133,893,303	*RYK*	rs11927316	A	T	0.02	0.71	0.13	4.50E-08	0.02	-0.01	0.16	0.94
4	118,437,442	--	rs17862012	A	C	0.01	2.65	0.46	1.20E-08	0.01	0.51	0.33	0.13
6	31,345,815	*HLA-B, MICA*	rs13193697	T	C	0.01	-0.72	0.13	4.10E-08	0.02	-0.12	0.15	0.43
6	32,667,820	*HLA-DQB1, HLA-DQA2*	rs9275354	T	G	0.99	2.68	0.23	8.00E-31	0.99	-0.16	0.27	0.56
6	165,413,927	--	rs9356335	A	G	0.98	-1.16	0.1	6.10E-32	0.97	-0.15	0.12	0.22
7	126,052,078	*GRM8*	rs7778698	A	C	0.02	-0.69	0.11	4.70E-10	0.03	0.11	0.18	0.55
10	9,705,976	--	rs7916429	C	G	0.96	-0.51	0.09	1.30E-08	0.95	0.12	0.1	0.21
10	68,212,659	*CTNNA3, SnoU40*	rs12416578	T	C	0.99	-0.96	0.12	1.30E-14	0.98	0.13	0.17	0.44
10	81,236,036	*ZCCHC24, AK302451, EIF5AL1*	rs7080747	T	C	0.01	-1.16	0.16	3.80E-13	0.01	0.12	0.39	0.76
10	109,928,793	--	rs10082453	A	T	0.98	0.94	0.11	1.10E-18	0.98	-0.37	0.25	0.14
11	61,570,783	*MYRF, DKFZP434K028, BC020196, TMEM258, MIR611, FEN1, FADS1, MIR1908, FADS2*	rs174547	T	C	0.68	1.69	0.03	≈ 0	0.63	0.1	0.04	0.026

Table Continued

Chr.	Base Pair (GRCh37)	Genes (±50 kb)	Variant	Allele		Arachidonic Acid				Aortic Valve Calcium			
				Effect	Other	EAF	Beta	SE	*p*	EAF	Beta	SE	*p*
1	118,028,688	*MAN1A2*	rs7546429	A	G	0.98	2.75	0.15	4.00E-78	0.96	0.01	0.15	0.93
3	41,139,829	--	rs13314643	T	C	0.01	1.44	0.15	6.30E-21	0.02	0.07	0.24	0.76
3	119,147,958	*ARHGAP31, TMEM39A, POGLUT1*	rs16829840	T	C	0.01	0.46	0.08	2.50E-08	0.02	-0.01	0.14	0.96
3	133,893,303	*RYK*	rs11927316	A	T	0.02	0.71	0.13	4.50E-08	0.02	-0.01	0.16	0.94
4	118,437,442	--	rs17862012	A	C	0.01	2.65	0.46	1.20E-08	0.01	0.51	0.33	0.13
6	31,345,815	*HLA-B, MICA*	rs13193697	T	C	0.01	-0.72	0.13	4.10E-08	0.02	-0.12	0.15	0.43
6	32,667,820	*HLA-DQB1, HLA-DQA2*	rs9275354	T	G	0.99	2.68	0.23	8.00E-31	0.99	-0.16	0.27	0.56
6	165,413,927	--	rs9356335	A	G	0.98	-1.16	0.1	6.10E-32	0.97	-0.15	0.12	0.22
7	126,052,078	*GRM8*	rs7778698	A	C	0.02	-0.69	0.11	4.70E-10	0.03	0.11	0.18	0.55
10	9,705,976	--	rs7916429	C	G	0.96	-0.51	0.09	1.30E-08	0.95	0.12	0.1	0.21
10	68,212,659	*CTNNA3, SnoU40*	rs12416578	T	C	0.99	-0.96	0.12	1.30E-14	0.98	0.13	0.17	0.44
10	81,236,036	*ZCCHC24, AK302451, EIF5AL1*	rs7080747	T	C	0.01	-1.16	0.16	3.80E-13	0.01	0.12	0.39	0.76
10	109,928,793	--	rs10082453	A	T	0.98	0.94	0.11	1.10E-18	0.98	-0.37	0.25	0.14
11	61,570,783	*MYRF, DKFZP434K028, BC020196, TMEM258, MIR611, FEN1, FADS1, MIR1908, FADS2*	rs174547	T	C	0.68	1.69	0.03	≈ 0	0.63	0.1	0.04	0.026

Table Continued

Chr.	Base Pair (GRCh37)	Genes (±50 kb)	Variant	Allele		Arachidonic Acid				Aortic Valve Calcium			
				Effect	Other	EAF	Beta	SE	*p*	EAF	Beta	SE	*p*
11	61,823,386	*NA*	rs11230889	A	C	0.83	0.29	0.05	9.30E-10	0.83	0.04	0.06	0.53
12	33,798,136	*NA*	rs16921193	A	T	0.01	1.06	0.11	5.40E-21	0.06	0	0.23	0.98
12	38,998,940	*BC040886, CPNE8*	rs2653765	A	C	0.01	-1.27	0.14	5.50E-21	0.01	0.15	0.2	0.45
12	129,881,993	*TMEM132D*	rs7970058	A	G	0.01	-0.87	0.11	7.70E-15	0.02	-0.03	0.18	0.86
12	133,462,329	*CHFR, AK055957, ZNF605*	rs11147144	A	C	0.01	0.89	0.13	1.90E-12	0.01	0.07	0.18	0.7
13	96,870,398	*HS6ST3, Metazoa_SRP*	rs16951711	T	C	0.01	-1.8	0.13	1.60E-45	0.01	-0.11	0.26	0.66
14	41,497,960	--	rs12894905	A	C	0.02	-0.92	0.11	5.90E-17	0.03	-0.07	0.13	0.58
16	15,130,351	*PDXDC1, NTAN1, RRN3*	rs1741	C	G	0.31	-0.2	0.03	1.60E-10	0.28	-0.1	0.05	0.036
18	30,030,309	*GAREM*	rs17811780	T	G	0.98	-0.68	0.12	1.20E-08	0.97	0.03	0.13	0.81
18	68,738,240	--	rs7231821	A	T	0.99	-0.82	0.13	2.00E-10	0.98	-0.06	0.25	0.8

The effect size for arachidonic acid is expressed as the percentage point change among total plasma fatty acids while the effect size for aortic valve calcium is expressed as the natural logarithm of the odds ratio.

Abbreviations: EAF, effect allele frequency.

Supplementary Table 5.5. Variants in the Mendelian Randomization Analysis of the Association of Liver FADS1 Expression With Aortic Stenosis.

Chrom.	Base Pair (GRCh37)	Variant	Allele		*FADS1* Expression			Aortic Stenosis		
			Effect	Other	Beta	SE	p	Beta	SE	P
11	61,560,081	rs174538	G	A	0.36	0.09	4.3E-05	0.13	0.03	9.3E-06
11	61,581,656	rs174559	G	A	0.39	0.09	1.9E-05	0.12	0.03	3.6E-05
11	61,581,764	rs174560	T	C	0.42	0.08	6.2E-07	0.12	0.03	4.2E-05
11	61,588,305	rs174564	A	G	0.41	0.08	4.3E-07	0.12	0.03	5.4E-06
11	61,619,829	rs174594	A	C	0.35	0.08	8.7E-06	0.10	0.03	2.9E-04

The effect size for *FADS1* gene expression is terms of the normalized effect size while the effect size for aortic stenosis is expressed as the natural logarithm of the odds ratio.

Supplementary Table 5.6. Variants in the Mendelian Randomization Analysis of the Association of Liver FADS1 Expression With Aortic Valve Calcium.

Chrom.	Base Pair (GRCh37)	Variant	Allele		*FADS1* Expression			Aortic Valve Calcium		
			Effect	Other	Beta	SE	p	Beta	SE	p
11	61,543,499	rs174528	T	C	0.37	0.08	2.5e-06	0.11	0.04	9.4e-03
11	61,560,081	rs174538	G	A	0.36	0.09	4.3e-05	0.08	0.04	0.075
11	61,564,299	rs4246215	G	T	0.35	0.08	2.7e-05	0.10	0.04	0.021
11	61,579,760	rs174555	T	C	0.41	0.09	1.8e-06	0.11	0.05	0.019
11	61,609,750	rs174583	C	T	0.39	0.08	8.7e-07	0.10	0.04	0.019
11	61,623,140	rs174601	C	T	0.34	0.08	1.7e-05	0.11	0.05	0.021

The effect size for *FADS1* gene expression is terms of the normalized effect size while the effect size for aortic valve calcium is expressed as the natural logarithm of the odds ratio.

Supplementary Table 5.7. Variants Identified in Previous Genome-Wide Association Studies for Aortic Stenosis and Their Associations With Aortic Stenosis in the GERA Cohort.

Chromosome	Base Pair (GRCh37)	Locus	Variant	Effect Allele	Other Allele	OR per Effect Allele (95% CI)	*p*
1	100,049,785	*PALMD*	rs7543130[a]	A	C	1.06 (1.01-1.11)	0.021
2	145,825,555	*TEX41*	rs1830321[b]	T	C	1.05 (1.00-1.11)	0.054
6	161,010,118	*LPA*	rs10455872	G	A	1.34 (1.23-1.47)	1.5×10^{-10}

[a] In perfect linkage disequilibrium (r^2=1) with rs6702619 among 1000 Genomes Project European populations using LDLink[184].

[b] This estimate was for rs6725803, a variant in perfect linkage disequilibrium with rs1830321 among 1000 Genomes Project European populations using LDLink[184].

Abbreviations: GERA, Genetic Epidemiology Research on Adult Health and Aging; OR, odds ratio.

Supplementary Table 5.8. Additional Covariate Adjustments for the Association of FADS1/2 rs174547 With Aortic Stenosis in the GERA Cohort.

Adjusted for[a]	No. Participants	OR per Minor Allele (95% CI)	*p*
Dyslipidemia, Hypertension, Smoking, and Diabetes	52,708	0.90 (0.85-0.95)	1.1×10^{-4}
LPA rs10455872	55,192	0.88 (0.83-0.93)	3.0×10^{-6}
10 Principal Components	55,180	0.88 (0.84-0.93)	5.6×10^{-6}

[a] All models were also adjusted for age, age^2, and sex.

Abbreviations: GERA, Genetic Epidemiology Research on Adult Health and Aging.

Supplementary Table 5.9. Associations of FADS1/2 rs174547 With Traits at a Genome-Wide Level of Significance.

Trait	No. Participants	Effect Size	Standard Error	*p*	PubMed ID
Protective Allele for Aortic Stenosis (C) Decreases Levels or Risk of Trait					
Lipids					
Low-Density Lipoprotein	170,015	-0.0505	0.0038	7.99×10^{-38}	24097068
Total Cholesterol	184,184	-0.0472	0.0037	1.35×10^{-35}	24097068
High-Density Lipoprotein	184,044	-0.0389	0.0035	4.05×10^{-27}	24097068
Fatty Acids and Phospholipids					
Plasma Arachidonic Acid Level	-	-1.69	NA	≈ 0	24823311
Glycerophospholipid Levels	-	-0.1652	0.00585	2.00×10^{-175}	26068415
Docosapentaenoic Acid	8,866	-0.0746	0.0028	3.79×10^{-154}	21829377
Phospholipid Levels Plasma	-	-0.07	0.002647	4.00×10^{-154}	21829377
Adrenic Acid	8,631	-0.0483	0.0019	6.26×10^{-140}	24823311
Plasma Gamma-Linolenic Acid Levels	-	-0.02	0.001111	2.00×10^{-72}	24823311
Eicosapentaenoic Acid	8,866	-0.082	0.0051	1.83×10^{-57}	21829377
Stearic Acid	8,961	-0.1773	0.0193	4.42×10^{-20}	23362303
Plasma Linoleic Acid Levels	-	-0.048	0.00578	1.00×10^{-16}	26584805
Trans Fatty Acid Levels	-	-0.0032	0.0004262	6.00×10^{-14}	25646338
Sphingolipid Levels	-	-0.026	0.003765	5.00×10^{-12}	26068415
Blood Cells					
Red Cell Distribution Width	173,480	-0.04908	0.003713	6.77×10^{-40}	27863252
Mean Platelet Volume	173,480	-0.03889	0.003792	1.14×10^{-24}	27863252
Granulocyte Percentage of Myeloid White Cells	173,480	-0.03025	0.003749	7.06×10^{-16}	27863252
Granulocyte Count	173,480	-0.02593	0.003767	5.82×10^{-12}	27863252
Sum Neutrophil Eosinophil Counts	173,480	-0.02578	0.003761	7.15×10^{-12}	27863252
Eosinophil Count	173,480	-0.02407	0.003741	1.24×10^{-10}	27863252
Mean Corpuscular Volume	173,480	-0.02382	0.003704	1.26×10^{-10}	27863252

Sum Basophil Neutrophil Counts	173,480	-0.02375	0.003765	2.81×10^{-10}	27863252
Neutrophil Count	173,480	-0.02351	0.003758	3.94×10^{-10}	27863252
Myeloid White Cell Count	173,480	-0.02348	0.003778	5.15×10^{-10}	27863252
White Blood Cell Count	173,480	-0.0231	0.003761	8.16×10^{-10}	27863252
Sum Eosinophil Basophil Counts	173,480	-0.02255	0.003745	1.74×10^{-9}	27863252
Other					
Height	-	-0.037	0.004286	6.00×10^{-18}	25429064
Fasting Glucose	133,010	-0.019	0.0022	1.33×10^{-17}	22885924
Self-Reported Asthma	337,159	-0.005349	0.0008199	6.86×10^{-11}	UKB
Protective Allele for Aortic Stenosis (C) Increases Levels or Risk of Trait					
Lipids					
Triglycerides	174,696	0.0469	0.0035	1.04×10^{-40}	24097068
Fatty Acids and Phospholipids					
Linoleic Acid	8,631	1.474	0.0417	4.98×10^{-274}	24823311
Dihomo-Gamma-Linolenic Acid	8,631	0.355	0.0136	2.63×10^{-151}	24823311
Alpha-Linolenic Acid	8,866	0.0159	9.00E-04	3.47×10^{-64}	21829377
Oleic Acid	8,961	0.2282	0.0194	5.57×10^{-32}	23362303
Cis-Trans-18:2	8,013	0.0032	4.00E-04	6.17×10^{-14}	25646338
Palmitoleic Acid	8,961	0.023	0.0033	2.97×10^{-12}	23362303
Blood Cells					
Platelet Count	173,480	0.03721	0.003824	2.21×10^{-22}	27863252
Red Blood Cell Count	173,480	0.03451	0.003732	2.29×10^{-20}	27863252
Monocyte Percentage of White Cells	173,480	0.0283	0.003739	3.77×10^{-14}	27863252
Hemoglobin Concentration	173,480	0.0275	0.003721	1.47×10^{-13}	27863252
Hematocrit	173,480	0.02423	0.003705	6.16×10^{-11}	27863252
Plateletcrit	173,480	0.02208	0.003837	8.64×10^{-9}	27863252
Reticulocyte Count	173,480	0.02155	0.00377	1.09×10^{-8}	27863252
Other					
Pulse Rate	317,756	0.03383	0.002632	8.59×10^{-38}	UKB
Resting Heart Rate	-	6.2	1.034	2.00×10^{-9}	20639392

Abbreviations: UKB, UK Biobank.

Supplementary Table 5.10. Associations of ω-6 and ω-3 Fatty Acids With Aortic Valve Calcium.

Fatty Acid	Cohort	Age and Sex Adjusted		Fully Adjusted	
		OR (95% CI)	*p*	OR (95% CI)	*p*
N-6					
LA	FOS	0.82 (0.72-0.93)	2.5×10^{-3}	0.82 (0.71-0.94)	3.5×10^{-3}
	MESA	0.85 (0.76-0.96)	8.3×10^{-3}	0.82 (0.73-0.93)	2.0×10^{-5}
	Combined	0.84 (0.77-0.91)	6.7×10^{-5}	0.82 (0.75-0.90)	2.2×10^{-5}
N-3					
ALA	FOS	0.91 (0.80-1.04)	0.17	0.89 (0.78-1.02)	0.093
	MESA	0.92 (0.82-1.04)	0.19	0.93 (0.82-1.05)	0.25
	Combined	0.92 (0.84-1.00)	0.058	0.91 (0.83-1.00)	0.048

OR presented per SD of the natural logarithm. Fully adjusted models were adjusted for low-density lipoprotein cholesterol, systolic blood pressure, current smoking, and diabetes, in addition to age and sex. Estimates were combined via fixed effects meta-analysis weighted by the inverse of their variance.

Abbreviations: OR, odds ratio; LA, linoleic acid; FOS, Framingham Offspring Study; MESA, Multi-Ethnic Study of Atherosclerosis; ALA, α-linolenic acid.

Supplementary Table 5.11. Associations of Dietary Fatty Acids With Aortic Stenosis by FADS1/2 rs174546 Genotype in the Malmö Diet and Cancer Study.

Fatty Acid	Hazard Ratio per SD of Natural Logarithm (95% CI)				$p_{interaction}$
	Overall	Homozygous Major	Heterozygous	Homozygous Minor	
N-6					
LA	1.03 (0.95-1.13)	1.14 (1.01-1.29)	0.88 (0.77-1.01)	1.08 (0.80-1.45)	0.06
N-3					
ALA	1.00 (0.92-1.09)	1.02 (0.90-1.16)	0.98 (0.86-1.12)	0.98 (0.73-1.33)	0.71

The rs174546 variant is in perfect linkage disequilibrium (r^2=1) with rs174547 among 1000 Genomes Project European populations using LDLink[184]. Models were adjusted for age, sex, energy intake, season, and diet method.

Abbreviations: LA, linoleic acid; ALA, α-linolenic acid.

Supplementary Table 5.12. Associations of Dietary Fatty Acids With Aortic Valve Calcium by FADS1/2 rs174547 Genotype.

Fatty Acid	Cohort	Odds Ratio per SD of Natural Logarithm (95% CI)				$p_{interaction}$
		Overall	Homozygous Major	Heterozygous	Homozygous Minor	
N-6						
LA	MESA	1.04 (0.92-1.18)	1.03 (0.86-1.23)	1.02 (0.83-1.25)	1.14 (0.79-1.63)	0.95
	FOS	0.86 (0.78-1.03)	1.04 (0.84-1.29)	0.80 (0.65-0.98)	0.78 (0.47-1.12)	0.065
N-3						
ALA	MESA	1.05 (0.93-1.19)	1.02 (0.85-1.22)	1.01 (0.83-1.23)	1.28 (0.87-1.87)	0.69
	FOS	0.86 (0.75-0.98)	0.93 (0.75-1.14)	0.79 (0.65-0.98)	0.76 (0.49-1.18)	0.29

Models were adjusted for age and sex.

Abbreviations: LA, linoleic acid; MESA, Multi-Ethnic Study of Atherosclerosis; FOS, Framingham Offspring Study; ALA, α-linolenic acid.

Supplementary Table 5.13. Sensitivity Analyses for the Genetic Associations of Liver FADS1 Expression With Aortic Stenosis and Aortic Valve Calcium.

Method	Odds Ratio per Unit Increase of Normalized Expression (95% CI)	*p*
Aortic Stenosis		
Inverse Variance-Weighted	1.31 (1.17-1.48)	7.4×10^{-6}
Penalized Weighted Median	1.35 (1.21-1.50)	4.1×10^{-8}
Egger Extension to Mendelian Randomization	1.25 (1.00-1.56)	0.054
Intercept for the Egger Extension	-	0.60
Aortic Valve Calcium		
Inverse Variance-Weighted	1.25 (1.02-1.52)	0.031
Penalized Weighted Median	1.32 (1.16-1.50)	2.2×10^{-5}
Egger Extension to Mendelian Randomization	1.02 (0.61-1.72)	0.94
Intercept for the Egger Extension	-	0.40

Supplementary Methods. Discovery and Replication Cohorts, Aortic Valve Calcium Cohorts, Gene-Diet Interactions for Aortic Stenosis and Aortic Valve Calcium, and Genetic Associations With Aortic Stenosis and Aortic Valve Calcium

Discovery Cohort

The Genetic Epidemiology Research on Adult Health and Aging (GERA) cohort contains more than 100,000 adults who are plan members of the Kaiser Permanente Medical Care Plan, Northern California Region and who are participants in the Kaiser Permanente Research Program on Genes, Environment, and Health. The GERA cohort features de-identified linkage of electronic health records, survey data of demographic variables, and genome-wide genotyping. The cohort is ethnically diverse and its racial composition has been previously described[185]. Genotyping of DNA extracted from saliva was performed on customized, ethnicity-specific Affymetrix Axiom arrays[186].

In the present study, we restricted our analyses to unrelated individuals of self-reported European ancestry, aged 55 years or older, free of congenital valvular heart disease (*International Classification of Diseases, Ninth Revision [ICD-9]* 746-747. We defined cases of aortic stenosis (AS) as individuals with either a diagnosis code for aortic stenosis (*ICD-9* 424.1) or a procedure code for an aortic valve replacement in their electronic health records between January 1996 and December 2015, inclusive; all other participants were designated controls. This approach was validated in another health care delivery system to have a positive predictive value greater than 90%[46]. Dyslipidemic participants were identified as participants with two or more diagnoses of disorders of lipid metabolism (*ICD-9* 272) in the electronic health records and one or more statin

prescriptions in the Kaiser Permanente prescriptions database. Coronary artery disease was defined as a diagnosis of myocardial infarction or coronary artery disease (*ICD-9* 410-414) or a procedure code for coronary artery bypass surgery or percutaneous coronary intervention in the electronic health records, or self-reported revascularization, angina, or myocardial infarction. Hypertension, smoking, and diabetes were self-reported in questionnaire data and the body mass index was calculated from self-reported height and weight. Ages greater than 90 years had been rounded down to 90, to enhance the privacy of these participants (n=389).

Our genome-wide association study was performed among 44,703 unrelated participants (3,469 AS cases) using logistic regression models adjusted for age, age^2, and sex. We later received updated data, including genetic data for additional GERA participants and excluding participants who had withdrawn consent. We imputed the locus which contained a variant that demonstrated evidence of association with AS using the Michigan Imputation Server[187] with the Haplotype Reference Consortium version r1.1[188] as reference, among 55,192 European-ancestry GERA participants aged 55 years and older (3,469 AS cases) and our subsequent analyses were performed on this dataset.

All participants have provided written, informed consent and this study was approved by the internal review boards of Kaiser Permanente Northern California and the McGill University Health Centre Research Institute.

Replication Cohorts

All participants provided written, informed consent and each study was approved by the relevant institutional review boards.

1. Quebec City Case-Control Calcific Aortic Valve Stenosis

The Quebec City Case-Control Calcific Aortic Valve Stenosis cohort is an AS case-control cohort composed of 2,026 participants recruited from patients undergoing cardiac surgery at the Quebec Heart and Lung Institute. Aortic stenosis cases (n=1,009) were individuals with severe, non-rheumatic, tricuspid AS undergoing replacement of their aortic valve. Controls were patients undergoing surgery for other reasons, mostly for isolated coronary artery bypass, and were matched on age, sex, ethnicity, type 2 diabetes, and hypertension in a 1:1 scheme with the AS cases. Blood samples were genotyped using the Illumina HumanOmniExpress BeadChip and following standard quality control, 613,862 variants were used for imputation using the Michigan Imputation Server[187] with the Haplotype Reference Consortium version r.1.1[188] as the reference panel. Associations between 7,732,680 variants and AS were estimated using logistic regression models in SNPTEST version 2.5.2[189], adjusted for age, sex, and 10 principal components to account for population sub-stratification. Details of this cohort are available elsewhere[57].

2. Malmö Diet and Cancer Study

The Malmö Diet and Cancer Study is a prospective, population-based cohort of 30,447 individuals living in Malmö, Sweden. This cohort has been described previously[107], but briefly, blood samples from a nested random sub-cohort and sets of cases for a range of incident diseases collectively comprising more than half the cohort were genotyped on the Illumina Human Omni Express Exome BeadChip platform. The samples which passed genotyping quality control were imputed

using the Michigan Imputation Server[187] with the Haplotype Reference Consortium version r1.1[188] as the reference panel, yielding up to 7,272,618 variants (after excluding variants with ≥5% missingness, minor allele frequency ≤0.01, and *p* for Hardy-Weinberg disequilibrium <0.0001) in 16,168 individuals. AS cases were defined from nation-wide registers based on hospital diagnosis codes for AS (*International Classification of Diseases [ICD], 8th revision* 424.10, 424.11, or 424.19; *ICD-9* 424B, 424BA, or 424BB; *ICD-10* I35.0 or I35.2), with the remaining participants designated as controls. We restricted our analysis of the lead variant to 6,071 participants representing a nested randomly-selected sub-cohort (n=5,550 controls) and all incident cases of AS (n=521 AS cases), and modelled the association of the variant with AS in R version 3.5.0 using a logistic regression model adjusted for age, age^2, and sex. The association with AS of the 32 variants in the arachidonic acid (AA) genetic risk score (GRS) were estimated using PLINK 2.0 alpha[168] among 5,342 participants (n=464 AS cases) using genetic data that had been genotyped using Illumina Omni Express Exome and subsequently imputed using the Michigan Imputation Server[187] with the Haplotype Reference Consortium version r1.1[188] as the reference panel.

3. Vanderbilt DNA Biobank (BioVU)

The Vanderbilt DNA Biobank, or BioVU, is a biorepository featuring de-identified linkage of electronic health records and genetic data for patients undergoing treatment at the Vanderbilt University Medical Center. Described elsewhere[190], the biobank contains more than 225,000 DNA samples, of which 13,569 were genotyped on the Illumina Multi-Ethnic Genotyping Array (MEGA) platform. Following standard quality control, genotypes were imputed using the Haplotype Reference Consortium version r1.1 panel as reference. AS cases were defined as subjects who had at least one Transthoracic Echocardiogram (TTE) report in Vanderbilt's EHR

system with an aortic stenosis severity (determined by natural language processing) of moderate, moderate-to-severe, or severe after the age of 55. Subjects with mild or mild-moderate AS were not included as cases and were also excluded from the control group. Controls were defined as subjects older than 55 years with at least one TTE, but who had an aortic valve peak velocity < 1.5 m/s and no degree of AS. Among 8,314 unrelated participants of European ancestry (n=759 AS cases), we modelled the association of variants with AS in logistic regression models adjusted for age, age^2, and sex using SNPTEST version 2.5.4-beta3[189].

4. UK Biobank

The UK Biobank is a longitudinal cohort of more than 500,000 predominantly white inhabitants of the United Kingdom, aged 40-79 years at recruitment in 2006-2010. Previously described elsewhere[191], participants had provided blood samples for genotyping, completed questionnaires regarding demographic and behavioural attributes, and attended medical examinations. Genotyping of 487,409 samples had been performed on the Affymetrix Axiom platform, and following standard quality control, the genotypes were imputed using the Haplotype Reference Consortium version r1.1[188], the UK10K[192], and the 1000 Genomes Project phase 3[133] as references. Cases of AS were identified by combining EHRs for diagnosis codes for AS (*ICD-10* I35.0 or I35.2); the remaining participants were designated AS controls. We limited our analysis to 214,947 unrelated individuals of white British ancestry (n=1,399 AS cases), and using PLINK 2.0 alpha[168], assessed the association of variants with AS in logistic regression models adjusted for age, age^2, sex, recruiting centre, and 40 principal components to account for population stratification.

5. Penn Medicine BioBank

The Penn Medicine BioBank (PMBB) consists of multi-ethnic participants (~60,000) recruited throughout the University of Pennsylvania Health System who have consented to genotyping/sequencing and access to electronic health record phenotype data. This cohort has been described previously[193]. Cases of CAVD and disease-free controls were ascertained through electronic health record text mining, genotyping was performed using the Illumina Quad Omni Genotyping Chip, and imputation was performed using the Michigan Imputation Server[187] using the Haplotype Reference Consortium version r1.1[188] as the reference panel. AS cases were defined as the presence of a diagnosis code for AS (*ICD-9* 424.1 or *ICD-10* I35.0) or a procedure code for AVR in the EHR, or a validated procedure of text mining echocardiography reports[194]. The remaining individuals were designated AS controls. Participants with congenital heart disease (*ICD-9* 746-747 or *ICD-10* Q20-Q22) were excluded. We restricted our analysis to 6,143 European-ancestry participants (n=1,593 AS cases) and modeled the association of variants with AS in PLINK 2.0 alpha[168] using logistic regression models adjusted for age, age^2, and sex.

6. European Prospective Investigation of Cancer and Nutrition – Norfolk

The European Prospective Investigation of Cancer and Nutrition – Norfolk is a prospective, population-based cohort of 25,639 individuals living in Norfolk, United Kingdom and the construction of this cohort has been described in detail elsewhere[195]. AS cases were defined as individuals coded with AS (*ICD-10* I35*)* or who died with AS as an underlying cause according to their death certificate. All other participants were defined as controls. Genotyping of blood samples was performed using the Affymetrix UK Biobank Axiom Array platform and following standard quality control, imputation was performed using IMPUTE software[132] with the 1000 Genomes

Project Phase 3[133] as the reference panel. We estimated the association of rs174547 with AS in a logistic regression model adjusted for age and sex using SPSS version 25.

7. Umeå University

The Umeå University cohort is a case-control study composed of 3,597 participants from northern Sweden who were previous participants of population-based surveys of health. Of these participants, 725 were cases who had undergone surgery for valvular heart disease and/or disease of the ascending aorta and 2,872 were controls randomly matched on a 1:4 scheme to individuals from the pool of health survey participants on the basis of age, sex, survey, questionnaire completion date, and geography[196]. Genotyping for 1,853 samples was performed on the Affymetrix UK Biobank Axiom Array r3, with 760,637 variants and 1,699 individuals passing standard genotyping quality control. We imputed using the Michigan Imputation Server[187] using the Haplotype Reference Consortium version r.1.1[188] as the reference panel. For our replication analysis, we considered only the 218 AS cases and their 436 matched controls. Since an unmatched analysis with adjustment for the matched factors can be as valid as a matched analysis[197], we modelled the association of variants with AS in unmatched, logistic regression models adjusted for age and sex in PLINK 2.0 alpha[168].

Replication of *FADS1/2* rs174547

Each cohort estimated the association of the variant with AS using logistic regression adjusted for age and sex, as well as age^2 if deemed appropriate.

Previously Reported Associations for *FADS1/2* rs174547

Results were obtained from PhenoScanner[166] on September 23, 2018 with a p-value cut-off of $p \leq 5 \times 10^{-8}$ (Supplementary Table 5.9). Associations without a direction of effect were removed, as were associations with unclear descriptions or irrelevant to disease pathology. For traits estimated in multiple studies, only the most significant association has been shown.

Aortic Valve Calcium Cohorts

Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium

The Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium was established to identify contributors to cardiovascular and aging phenotypes through the meta-analysis of findings from large, longitudinal cohorts[198]. Among the cohorts in the CHARGE Consortium are the Multi-Ethnic Study of Atherosclerosis (MESA) and the Framingham Heart Study, which includes the Framingham Offspring Study (FOS).

1. Multi-Ethnic Study of Atherosclerosis

The MESA is a prospective study composed of 6,814 participants of white, African American, Hispanic American, and Chinese individuals, and has been previously described[199]. The cohort was established to identify the prevalence of, and contributors to, subclinical cardiovascular disease. Recruitment occurred from 2000-2002 and individuals were eligible for inclusion if they were 45-84 years, free of clinical cardiovascular disease, and living in one of six American communities (Baltimore, Maryland; Chicago, Illinois; Forsyth County, North Carolina; Los Angeles County, California; Northern Manhattan and Southern Bronx, New York; and Saint Paul, Minnesota).

Fatty Acid Measurements

Lipids were extracted using a chloroform-methanol mixture from plasma, obtained at baseline, that had been stored at -70 ºC. Thin layer chromatography was used to separate cholesterol, triglycerides, and phospholipids, and fatty acid methyl esters were obtained from the phospholipid band. Separation and identification of fatty acid methyl esters were achieved using gas chromatography with a flame ionization detector. This method has been described in detail elsewhere[200].

Computed Tomography Scans

Computed tomography scans were performed using either an electron beam computed tomography (EBCT) scanner with a spatial resolution of 1.38 mm^3 (Imatron C150; General Electric Medical Systems, Milwaukee, Wisconsin, US), or a four-slice multidetector computed tomography (MDCT) scanner with a spatial resolution of 1.15 mm^3. Agatston scoring for aortic valve calcium (AVC) was performed by a single reader using proprietary offline software at a central MESA computed tomography reading centre (Los Angeles Biomedical Research Institute at Harbor-UCLA, Torrance, California, US). Details regarding the equipment used and quality control performed have been described previously[201].

Genetic Data

Genotyping was performed on the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, California, US) and following standard quality control, the genotypes were imputed using the Michigan Imputation Server[187] using the Haplotype Reference Consortium version r.1.1[188] as the reference panel.

2. Framingham Offspring Study

The FOS is a prospective study established in 1971, and consists of 5,124 individuals who were either the offspring, or the spouses of the offspring, of the original Framingham Heart Study. Details of the cohort have been described previously[202]. A majority of the FOS participants are of European descent.

Fatty Acid Measurements

Red blood cells from fasted blood, obtained during Examination 8 and previously frozen at -80 ºC, were methylated in a mixture of boron trifluoride and hexane. Fatty acid methyl esters were separated via gas chromatography and identified by comparing with a fatty acid mixture characteristic of red blood cells (GLC 727, NuCheck Prep). Details of this process have been previously reported[203].

Computed Tomography Scans

At Examination 7, two cardiac scans were performed for each participant using an eight-slice MDCT scanner (Lightspeed Ultra; General Electric Medical Systems, Milwaukee, Wisconsin, US). One of two trained readers reviewed each set of images (Aquarius workstation; TeraRecon, San Mateo, California, US), and if valve calcium was present on one or both scans, the set was read by an independent, blinded, trained radiologist. AVC was then quantified in Agatston units. Interobserver agreement was high (κ=0.95)[46]. Disagreement was resolved through consensus.

Genetic Data

Genotyping was performed on the Affymetrix GeneChip Human Mapping 500K Array and 50K Human Gene Focused Panel (Affymetrix, Santa Clara, California, US). Following standard quality control, the genotypes were imputed using MaCH[204] with HapMap as the reference panel[205].

3. Associations with Aortic Valve Calcium

Prevalent Aortic Valve Calcium

A prevalent AVC variable was constructed by dichotomizing the quantitative AVC measures into no presence of AVC (Agatston score=0) or presence of AVC (Agatston score>0).

Meta-Analysis for FADS1/2 rs174547 in CHARGE Cohorts

To estimate the association of *FADS1/2* rs174547 with prevalent AVC, we performed a fixed effects meta-analysis of the FOS, MESA, and the Age, Gene/Environment Susceptibility-Reykjavik Study totalling 6,942 participants (2,245 AVC cases).

Associations Between Fatty Acids and AVC in CHARGE Cohorts

The association of four polyunsaturated fatty acids (linoleic acid, arachidonic acid, α-linolenic acid, and eicosapentaenoic acid) and two polyunsaturated fatty acid ratios (arachidonic acid to linoleic acid, and eicosapentaenoic acid to α-linolenic acid) with AVC were modelled separately in MESA (2,415 European-ancestry participants [387 AVC cases]) and FOS (1,310 predominantly European participants [492 AVC cases]) using logistic regression adjusted for age and sex, with further adjustment for low-density lipoprotein cholesterol, systolic blood pressure, current smoking, and diabetes in the full models. The fatty acids were modelled per SD of the natural logarithm of the percentage of total fatty acids and the fatty acid ratios were modelled per SD of

the natural logarithm of the ratio of the fatty acids. To estimate overall effects of the fatty acids on AVC, we performed fixed effects, inverse variance-weighted meta-analyses as no heterogeneity was observed across cohorts (heterogeneity $p>0.05$ for all comparisons).

Gene-Diet Interactions for Aortic Stenosis and Aortic Valve Calcium

Statistical Analysis

Among 28,041 participants with complete diet data from the MDCS, we examined whether dietary linoleic acid or α-linolenic acid intake was associated with incident AS in a Cox proportional-hazards model adjusted for age, sex, energy intake, season, and diet method. Linoleic acid intake was measured by a modified diet history methodology combining a 168-item dietary questionnaire, a 7-day menu book, and a 1-h diet history interview specifically designed for the MDCS, as previously described[206]. In 25,477 MDCS participants with available genetic data, we assessed whether this association was modified by rs174546, a variant in perfect linkage disequilibrium (r^2=1) with our lead variant, and then estimated the association of dietary LA and ALA (in percentage of energy intake) with AS in each genotypic class of the variant.

Among 2,416 MESA participants (380 AVC cases) and 1,220 FOS participants (452 AVC cases) with available food frequency data, we separately estimated the overall association of dietary LA and ALA intake (in grams) with AVC. Again, both LA and ALA were natural log transformed. We also assessed whether the association between the transformed dietary LA and ALA with AVC was modified by the lead variant among participants with available genetic data (MESA: 2,416 participants, of which 380 were AVC cases; FOS: 1,149 participants, of which 427 were AVC cases), and quantified the effect of dietary LA or ALA on AVC within each genotypic class

Genetic Associations With Aortic Stenosis and Aortic Valve Calcium

Risk Score Construction

The arachidonic acid GRS was constructed from independent variants ($r^2 \leq 0.001$) associated with plasma arachidonic acid (percentage of total fatty acids) at a genome-wide level of significance ($p \leq 5 \times 10^{-8}$) in a previous publication[171]. If a variant was not available, a proxy in high linkage disequilibrium ($r^2 \geq 0.95$) was used if available.

The estimates used for aortic stenosis were obtained through meta-analysis of estimates from seven cohorts: GERA (imputed using the Michigan Imputation Server[187] with the Haplotype Reference Consortium version r1.1[188] as the reference panel), QUEBEC-CAVS, MDCS, BioVU, UKB, PMBB, and Umeå. To account for differences in minor allele frequencies, the GRS was limited to 32 variants with a minor allele frequency≥0.01 in both the AA publication and the GERA cohort. Meta-analysis was performed using PLINK version 1.9[168].

The aortic valve calcium estimates for each variant in the GRS were obtained from a prior genome-wide meta-analysis[46]. The Mendelian randomization analysis for aortic valve calcium was performed using 24 variants with available association results and a minor allele frequency≥0.01 in both the arachidonic and AVC studies.

Genetic Association of *FADS1* Expression with Aortic Stenosis and Aortic Valve Calcium

Risk Score Construction and Analysis

We performed Mendelian randomization to assess whether elevated *FADS1* or *FADS2* expression was causally associated with AS and AVC. Estimates for significant *FADS1* expression quantitative trait loci (eQTL) in the liver were extracted from the Gene-Tissue Expression (GTEx) Project, release V8 (dbGaP accession phs000424.v8.p2)[72]. No significant *FADS2* liver eQTL were available. Variants were included in the genetic risk score if they were non-ambiguous, bi-allelic, not in very high linkage disequilibrium with another variant in the risk score (r^2<0.9) in the GERA cohort, and an effect estimate was also available for the outcome (5 variants for *FADS1* with AS [Supplementary Table 5.11], 6 variants for *FADS1* with AVC [Supplementary Table 5.12]). The association of these variants with AS were estimated in the GERA cohort using logistic regression models adjusted for age, age^2, and sex, while their associations with AVC were extracted from a prior meta-analysis[46]. All variants were well imputed in the GERA cohort (info score≥0.3). Imputation quality metrics were not available for the AVC meta-analysis.

To estimate the causal association of *FADS1* expression with the odds of AS and AVC, we applied the inverse variance-weighted meta-analysis method of Mendelian randomization and accounted for correlation between variants in the risk score using *r* values computed in the GERA cohort using PLINK 2.0 alpha[168]. This approach to account for a correlated genetic risk score has been described previously[80]. As sensitivity analyses, we performed inverse-variance weighted, penalized weighted median, and Egger extension methods if the primary analysis was significant (Supplementary Table 5.13).

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Chapter 6. Additional Loci for Aortic Stenosis

6.1 Preface to Chapter 6

In the preceding chapter, a GWAS in the GERA cohort identified the *FADS1/2* locus as associated with AS. This association was subsequently confirmed through replication in seven additional cohorts. Given the roles of the fatty acid desaturases 1 and 2 in polyunsaturated fatty acid metabolism, I postulated that the association of genetic variation at the *FADS1/2* locus with AS was mediated by n-6 polyunsaturated fatty acids. Our Mendelian randomization analyses for AS and AVC, as well as the observational associations of n-6 fatty acid levels with AVC, provided strong evidence that elevated n-6 fatty acids contribute to calcification of the aortic valve and stenosis.

Despite the GERA cohort having one of the largest collections of AS cases, the association of *FADS1/2* rs174547 with AS only became genome-wide significant for AS ($p \leq 5 \times 10^{-8}$) after meta-analysis with seven replication cohorts, together totalling 311,118 participants (9,395 cases). While genome- or transcriptome-wide efforts in one or a few cohorts have identified new loci for AS[57,58,207], the number of additional loci that could be discovered by any single cohort may be limited by the number of AS cases. Collaborations between a larger number of cohorts could improve the statistical power to detect novel loci. Furthermore, if the participating cohorts are from different countries, the loci identified may apply more broadly to participants of European ancestry.

The established role of fatty acid desaturases 1 and 2 in polyunsaturated fatty acid metabolism was pivotal in developing a mechanistic understanding of the link between the *FADS1/2* locus and aortic stenosis. Importantly, I was able to demonstrate that the conversion of dietary n-6 fatty acids

to their longer chain counterparts could be a potential target for AS treatment or prevention. Supplementing the discovery of a new locus with strong functional and mechanistic data therefore augmented the utility of the discovery from solely improving risk prediction (for example, via a polygenic risk score) to a new pathway to target via pharmacological intervention.

In the subsequent manuscript, I performed a genome-wide meta-analysis for AS in 10 European-ancestry cohorts from five countries. This study was the largest genome-wide meta-analysis for AS to date, and benefiting from the improved statistical power, identified several new loci for AS. I also integrated functional, expression, and cross-phenotype data to enhance our understanding of these loci, including key causal pathways which should be examined further as potential targets for pharmacological agents.

Genome-Wide Analyses Highlight the Roles of Dyslipidemia, Inflammation, Calcification, and Adiposity in Aortic Stenosis

Hao Yu Chen, MSc, Christian Dina, PhD, Aeron M. Small, MD, Christian M. Shaffer, BSc, Rebecca T. Levinson, PhD, Anna Helgadóttir, MD, PhD, Romain Capoulade, PhD, Hans Markus Munter, PhD, Andreas Martinsson, MD, Benjamin J. Cairns, PhD, Mary Hoekstra, MSc, Hannah A. Burr, MSc, Thomas W. Marsh, BA, Scott M. Damrauer, MD, Line Dufresne, MSc, Solena Le Scouarnec, PhD, Dilrini K. Ranatunga, BA, Rachel A. Whitmer, PhD, Amélie Bonnefond, PhD, Garðar Sveinbjornsson, MSc, Ragnar Daníelsen, MD, PhD, David O. Arnar, MD, PhD, Gudmundur Thorgeirsson, MD, PhD, Unnur Thorsteinsdottir, PhD, Daníel F. Gudbjartsson, PhD, Kári Stefánsson, MD, PhD, Christopher J. O'Donnell, MD, Matthew J. Budoff, MD, Vilmundur Gudnason, MD, Wendy S. Post, MD, Jerome I. Rotter, MD, Mark Lathrop, PhD, Bengt Johansson, MD, Johan Ljungberg, MD, Ulf Näslund, MD, Thierry Le Tourneau, MD, J. Gustav Smith, MD, Quinn S. Wells, MD, Hilma Hólm, MD, Stefan Söderberg, MD, Jean-Jacques Schott, PhD, Daniel J. Rader, MD, Robert Clarke, MD, James C. Engert, PhD*, and George Thanassoulis, MD* *on behalf of the Therapeutic targets for AoRtic stenosis using GEneTics (TARGET) Consortium*

* Co-senior authors

Affiliations

Division of Experimental Medicine, McGill University, Montreal, Canada

Hao Yu Chen, Mary Hoekstra, Hannah A. Burr, James C. Engert & George Thanassoulis

Preventive and Genomic Cardiology, McGill University Health Centre and Research Institute, Montreal, Canada

Hao Yu Chen, Mary Hoekstra, Hannah A. Burr, Thomas W. Marsh, Line Dufresne, James C. Engert & George Thanassoulis

Université de Nantes, CHU Nantes, CNRS, INSERM, l'institut du thorax, F-44000 Nantes, France

Christian Dina, Romain Capoulade, Solena Le Scouarnec, Thierry Le Tourneau & Jean-Jacques Schott

Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, United States

Aeron M. Small

Vanderbilt Translational and Clinical Cardiovascular Research Center, Vanderbilt University Medical Center, Nashville, United States

Christian M. Shaffer, Rebecca T. Levinson & Quinn S. Wells

deCODE genetics/Amgen Inc., Reykjavik, Iceland

Anna Helgadóttir, Garðar Sveinbjornsson, David O. Arnar, Gudmundur Thorgeirsson, Unnur Thorsteinsdottir, Daníel F. Gudbjartsson, Kári Stefánsson & Hilma Hólm

McGill University and Genome Quebec Innovation Centre, Montreal, Canada

Hans Markus Munter & Mark Lathrop

Department of Cardiology, Clinical Sciences, Lund University, Lund, Sweden and Skåne University Hospital, Lund, Sweden

Andreas Martinsson & J. Gustav Smith

MRC Population Health Research Unit, Clinical Trial Service Unit and Epidemiological Studies Unit, Nuffield Department of Population Health, University of Oxford, Oxford, United Kingdom

Benjamin J. Cairns & Robert Clarke

Department of Surgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, United States

Scott M. Damrauer

Division of Research, Kaiser Permanente of Northern California, Oakland, United States

Dilrini K. Ranatunga

Department of Public Health Sciences, University of California Davis, Davis, United States

Rachel A. Whitmer

CNRS UMR 8199, European Genomic Institute for Diabetes, Institut Pasteur de Lille, University of Lille, Lille, France

Amélie Bonnefond

Department of Metabolism, Section of Genomics of Common Disease, Imperial College London, London, United Kingdom

Amélie Bonnefond

Internal Medicine and Emergency Services, Landspitali – The National University Hospital of Iceland, Reykjavik, Iceland

Ragnar Daníelsen & David O. Arnar

School of Health Sciences, Faculty of Medicine, University of Iceland, Reykjavik, Iceland

David O. Arnar, Gudmundur Thorgeirsson, Unnur Thorsteinsdottir & Kári Stefánsson

School of Engineering and Natural Sciences, University of Iceland, Reykjavik, Iceland

Daníel F. Gudbjartsson

National Heart, Lung, and Blood Institute's and Boston University's Framingham Heart Study, Boston, United States

Christopher J. O'Donnell

The Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, Torrance, USA

Matthew J. Budoff

Faculty of Medicine, University of Iceland, Reykjavík, Iceland

Vilmundur Gudnason

Division of Cardiology, Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, United States

Wendy S. Post

The Institute for Translational Genomics and Population Sciences, Department of Pediatrics, The Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, Torrance, USA

Jerome I. Rotter

Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden

Bengt Johansson, Johan Ljungberg, Ulf Näslund & Stefan Söderberg

Wallenberg Center for Molecular Medicine and Lund University Diabetes Center, Lund, Sweden

J. Gustav Smith

The Wallenberg Laboratory/Department of Molecular and Clinical Medicine, Institute of Medicine, Gothenburg University and the Department of Cardiology, Sahlgrenska University Hospital, Gothenburg, Sweden

Andreas Martinsson & J. Gustav Smith

Department of Human Genetics, McGill University, Montreal, Canada

Thomas W. Marsh, Mark Lathrop & James C. Engert

Departments of Genetics and Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, United States

Daniel J. Rader

Status:

In preparation

6.2 Abstract

Recent evidence indicates calcific aortic stenosis is highly heritable, yet the genetic etiology of the disease remains incompletely understood. We performed a genome-wide meta-analysis of 11.6 million variants in 10 cohorts involving 653,867 European participants (13,765 cases). We observed 16 loci (18 variants) associated with aortic stenosis, of which nine loci (10 variants) had not previously been reported at a genome-wide level of significance ($p \leq 5 \times 10^{-8}$), including *CELSR2-SORT1*, *BET1L*, and *HMGB1*. A genetic risk score comprised of the 18 variants was strongly associated with both aortic valve calcium and aortic stenosis, and a phenome-wide association study indicated multiple associations with coronary artery disease, apolipoprotein B, triglycerides, and C-reactive protein. Mendelian randomization analyses identified a causal role for apolipoprotein B in aortic stenosis and replicated previous findings of causality for lipoprotein(a), low-density lipoprotein cholesterol, and body mass index. Additional analyses predicted differential expression of genes in AS cases, including *NOTCH4* and *IL6R*, with gene set analyses indicating differential hepatic expression of genes targeted by microRNAs including microRNA-21. Our results demonstrated that aortic stenosis is a disease in which dyslipidemia, inflammation, calcification, and adiposity play important roles. These results may have implications for novel treatments and prevention strategies.

6.3 Main Text

Introduction

Calcific aortic stenosis (AS) is the leading form of incident valvular heart disease in industrialized nations[24]. In individuals over 75 years, the prevalence of AS is 12.4% and is expected to more than double by 2040[25]. Although replacement of the aortic valve is indicated and effective for severe AS cases[12,208], there are no specific 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 times the risk of AS[37]. Several families have also been identified with many affected members, including one extended family with 48 cases of severe AS[114]. Previous genome- and transcriptome-wide association efforts have identified seven genetic loci associated with AS[46,57,58,207,209], including *LPA*[46] (which codes for the apolipoprotein[a] moiety of lipoprotein[a]), *IL6*[207] (which codes for interleukin 6), and the *FADS1/2* gene cluster[209] (which codes for desaturases involved in fatty acid biosynthesis). Additionally, Mendelian randomization studies have supported a causal contribution of low-density lipoprotein cholesterol (LDL-C)[107], non-high-density lipoprotein cholesterol[58], lipoprotein(a)[51], body mass index (BMI)[128], and possibly arachidonic acid[209] to AS, suggesting some of the susceptibility to AS is 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. Finally, we investigated whether individual genes or gene sets were predicted to be differentially expressed in AS cases.

Methods

Genome-wide meta-analysis for aortic stenosis

We performed centralized, cohort-specific quality control of genome-wide summary statistics for prevalent AS from 10 European cohorts totalling 653,867 participants (13,765 cases) (Supplementary Table 6.1). 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 $p \geq 1 \times 10^{-4}$, and deCODE, which excluded variants not found on the Haplotype Reference Consortium version r1.1 panel, quality control was performed using a single set of criteria for both imputed and genotyped variants. 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 were estimated successfully with standard errors less than or equal to the median standard error plus five times the interquartile range of the standard error, calculated from all summary statistics from each cohort. Using PLINK version 1.9, we performed inverse variance-weighted, fixed effects meta-analysis for each of 11,591,806 variants with summary statistics that passed quality control and the allele frequency threshold in at least two cohorts. For variants which were genome-wide significant ($p \leq 5 \times 10^{-8}$) and independent ($r^2 \leq 0.01$), *i.e.* index variants, as well as variants in high 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[133], we queried the University of California Santa Cruz (UCSC) database[210] to obtain information on genomic location.

Functional analysis of variants

We used ANNOVAR[211] to extract predicted function and pathogenicity of the variants, including scores generated by CADD[68], DANN[212], LINSIGHT[213], EIGEN-PC[214], and FATHMM-MKL non-coding[215]. While the DANN annotations were developed by training on the same dataset as CADD, the CADD method used a support vector machine whereas the DANN methodology used a deep neural network[212], and the two methods have been shown to identify different candidate genes for breast and lung cancers[216]. From GTEx version eight[72], we extracted significant expression quantitative trait loci in the aorta, left ventricle, liver, and whole blood.

Genetic risk score

Using all index variants, we constructed a weighted genetic risk score by summing the products of each risk allele and the log odds of the association with AS. We assessed the association of this risk score with AS in 257,231 unrelated White British participants in the UK Biobank aged 55 years or older (2,213 AS cases) using a logistic regression model adjusted for age, age^2, and sex, and then further adjusted for diabetes, LDL-C, systolic blood pressure, smoking (ever/never), and coronary artery disease. The UK Biobank participant numbers in these and subsequent analyses differed slightly from the discovery analysis as updated versions of the data had become available. We also assessed the association of the risk score with the presence of aortic valve calcium using logistic regression on 2,488 unrelated European participants of the Multi-Ethnic Study of Atherosclerosis (389 aortic valve calcium cases), adjusted for age and sex and then fully adjusted

for fasting glucose, LDL-C, systolic blood pressure, smoking (ever/never), and coronary artery calcium.

Cross-ancestry and cross-phenotype associations

In the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium, we extracted summary statistics previously reported[46] for the association of the index variants with prevalent aortic valve calcium in an inverse variance-weighted fixed effects meta-analysis of three independent cohorts totalling 6,942 European participants (2,245 cases), with each cohort adjusted for age and sex. If an index variant was not available, we used a proxy in high LD ($r^2 \geq 0.8$) if one were available.

To examine the transferability of the index variants to other ancestries, we estimated their effects on AS among 1,917 African-American participants (86 cases) and 3,482 Latin-American participants (159 cases) of the Genetic Epidemiology Research on Adult Health and Aging Cohort, adjusted for age, age^2, and sex.

In a phenome-wide association study, we examined the association of the index variants with 58 serum biomarkers, physiological measurements, and diseases among 257,231 unrelated White British UK Biobank participants aged 55 years or older. Diseases were defined using hospital inpatient diagnosis codes, procedure codes, and causes of death. Levels of alkaline phosphatase, C-reactive protein, gamma-glutamyl transferase, lipoprotein(a), and triglycerides were transformed using the natural logarithm. Logistic and linear regression models were adjusted for age, age^2, and sex, except for breast cancer, which was analyzed only in women and adjusted for

age and age^2. 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 determine whether these biomarkers were causal contributors to AS. 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 in a set of unrelated UK Biobank White British participants (up to 383,533 participants). We then used the R package MendelianRandomization version 0.4.2[134] to estimate the inverse-variance weighted association with AS. Although the summary statistics for the biomarkers and AS were generated on partially overlapping samples (up to 215,036 UK Biobank participants), previous simulations suggested that any introduced bias was unlikely to be large[217]. In secondary analyses allowing for pleiotropy in the instrument, we applied the penalized weighted median and Egger approaches.

Gene and gene-set associations

To identify gene regions associated with AS, we employed the single nucleotide polymorphism (SNP)-wise mean approach of MAGMA[69] that examines if variants of gene regions showed enriched association for AS. Each gene region was defined as the transcription boundary of the gene ± 50 kb, and linkage disequilibrium between variants was accounted for using imputed data from the European participants of the 1000 Genomes Project, Phase Three[133]. We tested 18,539 protein-coding gene regions and applied a Bonferroni correction to identify significant regions ($p \leq 0.05/18{,}539 \approx 2.7 \times 10^{-6}$).

We employed MetaXcan[70] to identify genetic variants whose effects on AS may be mediated by gene expression. We inferred gene expression in the aorta, left ventricle, liver, and whole blood using prediction models built from expression quantitative trait loci in GTEx version seven[72], and tested the association of the predicted gene expression with AS. A false discovery rate correction of 5% was applied to account for multiple testing. To identify index variants in high LD ($r^2 \geq 0.95$) with the top eQTL of the gene or genes named in the locus associated to the index variants, we extracted the top aorta, left ventricle, liver, and whole blood eQTL for these genes from GTEx version eight[72].

We used the Genotype Imputed Gene Set Enrichment Analysis (GIGSEA)[218] approach, which leverages predicted gene expression, to assess whether sets of genes with shared function or regulation demonstrate differential expression. We determined the presence of inferred differential expression of gene sets defined by the Kyoto Encyclopedia of Genes and Genomes (KEGG)[73], composed of genes in the same pathway; Gene Ontology (GO)[219], containing genes with related functions; Functional ANnoTation Of the Mammalian genome, version Five (FANTOM5)[220], reflecting genes with shared transcription factor binding sites; and miRBase[221], containing genes with the same microRNA seed sequence in their 3' untranslated region. Analyses were conducted using the matrix option of GIGSEA, which simultaneously accounts for genes found in more than one gene set and generates empirical *p* values through permutation. We applied a false discovery rate correction of 5% across gene sets for each combination of tissue and gene set source.

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[222] as implemented on LD Hub[223]. We selected GWAS or meta-analyses which had been performed in European populations and applied a false discovery rate correction of 5%.

To identify additional variants associated with AS, we used the conditional and joint analysis method[224] from the Genome-wide Complex Trait Analysis (GCTA) software[225] to re-estimate the summary statistics from our genome-wide meta-analysis conditioned upon the index variants. A variant which was not genome-wide significant in the original meta-analysis but which 1) became genome-wide significant in the conditional analysis and 2) was independent ($r^2 \leq 0.01$) was deemed to be a novel association.

Results

Genome-wide meta-analysis identifies nine novel loci for AS

We performed a genome-wide meta-analysis for AS using GWAS summary statistics from 10 European-ancestry cohorts totalling 653,867 participants (13,765 cases) (Supplementary Table 6.1). Following study-specific, centralized quality control of GWAS summary statistics, estimates for each of 11,591,806 variants were combined in an inverse variance-weighted, fixed effects model (see Figure 6.1 for an overview of the study design). We observed no evidence of inflated test statistics in the meta-analysis (genomic inflation factor [λ]= 1.04; Supplementary Figure 6.1).

We identified 16 genetic loci containing one or more independent variants ($r^2 \leq 0.01$) which satisfied the conventional genome-wide significance threshold ($p \leq 5 \times 10^{-8}$) for association with AS

(Figure 6.2), confirming all seven previously identified loci and identifying nine new loci not previously reported to be genome-wide significant. After pruning for variants in linkage disequilibrium (LD), we identified 18 independent variants ($r^2 \leq 0.01$) (see meta-analysis results in Table 6.1 and forest plots in Supplementary Figure 6.2-Supplementary Figure 6.19). The association with AS of variants surrounding the index variants are provided in the supplement (Supplementary Figure 6.20-Supplementary Figure 6.37). The *HMGB1* variant rs181753401, which was the only rare variant (minor allele frequency=2.4×10^{-3}), had the largest effect associated with AS (odds ratio [OR] per minor allele, 2.29; 95% CI, 1.74 to 3.02; $p=4.2\times10^{-9}$).

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 (OR per T allele, 1.57; 95% CI, 1.34 to 1.83; $p=9.6\times10^{-9}$). Given the proximity of this variant to *LPA*, we tested the association of this variant with AS conditioned on its association with levels of lipoprotein(a) and observed substantial attenuation (OR per T allele, 1.20; 95% CI, 1.03 to 1.40; $p=0.019$).

We examined publicly available databases for functional and regulatory effects of the index variants and their proxies in high LD ($r^2 \geq 0.95$) (Supplementary Table 6.2). While most variants were genic, there was only one coding variant: *ARGHEF26* rs6794263 was in high LD ($r^2=0.99$)[184] with the missense variant rs13096373 (p.Phe203Ser). This substitution was predicted by the Combined Annotation Dependent Depletion (CADD) software[226] to be in the top 4% most deleterious substitutions (scaled C-score=14.5). CADD also predicted variants in high LD with *ARGHEF26* rs6794263 to be among the top 5% substitutions, in addition to the index variants or

variants in high LD from the *CELSR2-SORT1, PRRX1, BET1L loci* and the intergenic region at 18q11.2. Furthermore, the index variants or variants in high LD at the *PRRX1, ACTR2,* and *BET1L* loci were among the top 5% most pathogenic variants as predicted by the Deleterious Annotation of Genetic Variants Using Neural Networks (DANN)[212] (ranked score≥0.95), and index variants or variants in high LD at the *CELSR2-SORT1, PRRX1,* and 18q11.2 loci were classified as deleterious using the FATHMM-MKL non-coding approach (p≥0.5)[215] and were under negative selection according to LINSIGHT[213] analysis (p≥0.5). The index variant at the *TMEM170A* locus had the highest Eigen-PC score[214] (phred score=38.8), followed by rs99780, a proxy of the *FADS1/2* index variant (phred score=35.3).

Variants are also associated with aortic valve calcium

From a previous meta-analysis for prevalent aortic valve calcium involving 6,942 European participants from three cohorts in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium (2,245 participants with aortic valve calcium>0)[46], we extracted fixed effects associations with prevalent aortic valve calcium for our index variants. Where the exact variant was not available, a proxy in high LD (r^2≥0.8) was used, though no proxies were found for *LPA* rs140570886 and *HMGB1* rs181753401. Five variants associated nominally with aortic valve calcium (p≤0.05): *PRRX1* rs61817383 (OR per minor allele, 1.10; 95% CI, 1.01 to 1.21; p=0.035), *ACTR2* rs62139062 (OR per minor allele, 1.11; 95% CI, 1.01 to 1.21; p=0.029), *LPA* rs10455872 (OR per minor allele, 2.05; 95% CI, 1.63 to 2.57; $p=9.0\times10^{-10}$), *BET1L* rs17156153 (OR per minor allele, 1.17; 95% CI, 1.02 to 1.35; p=0.028) and *FADS1/2* rs174533 (OR per minor allele, 0.91; 95% CI, 0.83 to 0.99; p=0.024) (Supplementary Table 6.3).

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

We constructed a weighted genetic risk score for AS from the index variants, using weights re-estimated from a meta-analysis excluding the UK Biobank, and applied it to an updated UK Biobank dataset with 257,231 participants (2,213 AS cases; see Supplementary Table 6.1). The prevalence of AS increased across risk score tertiles, beginning at 0.6% in the lowest tertile and reaching 1.2% in the highest (Figure 6.3A). Each 1 SD higher risk score was associated with 38% higher odds of AS (OR per SD, 1.38; 95% CI, 1.33 to 1.44; $p=4.6\times10^{-57}$) when adjusted for age, age^2, and sex, with a similar estimate after adjustment for diabetes, LDL-C, systolic blood pressure, smoking, and coronary artery disease (OR per SD, 1.31; 95% CI, 1.25 to 1.37; $p=2.7\times10^{-34}$). The area under the receiver operating characteristic curve, also known as the C-statistic, for this full model was 0.71 (95% CI, 0.70 to 0.72), with the addition of the risk score only modestly improving the area under the curve compared to a model with just the cardiovascular risk factors (0.69; 95% CI, 0.68 to 0.70; $p_{difference}=2.0\times10^{-11}$).

When we applied the risk score to 2,488 unrelated European-ancestry participants (389 cases of aortic valve calcium>0) from the Multi-Ethnic Study of Atherosclerosis, the prevalence of aortic valve calcium was 14.9%, 14.0%, and 18.0% in tertiles one, two and three, respectively (Figure 6.3B). After adjustment for age and sex, each 1 SD higher risk score was associated with 23% higher odds of aortic valve calcium (OR per SD, 1.23; 95% CI, 1.09 to 1.39; $p=6.3\times10^{-4}$); this association persisted after additional adjustment for glucose, LDL-C, systolic blood pressure, smoking, and coronary artery calcium (OR per SD, 1.23; 95% CI, 1.08 to 1.39; $p=1.2\times10^{-3}$).

A subset of variants replicates in African- and Latin-Americans

When we examined the index variants among 1,917 African-American participants (86 cases) and 3,482 Latin-American participants (159 cases) from the Genetic Epidemiology Research on Adult Health and Aging Cohort, we observed replication ($p \leq 0.05$ and with a concordant direction of effect) of *CELSR2-SORT1* rs12740374 in both ancestries, of *ALPL* rs6696066 and *BET1L* rs17156153 in Latin Americans, and of *LPA* rs10455872 in African Americans (Supplementary Table 6.4).

Region-based analysis identifies additional associations with AS

We considered the joint contribution of all variants in a gene region, to identify additional loci. For each of 18,539 protein-coding genes, we used the Multi-marker Analysis of GenoMic Annotation (MAGMA)[69] to test the mean association with AS of variants within 50 kb, correcting for LD between variants. We identified 95 regions associated with AS after Bonferroni correction (Supplementary Table 6.5), including regions spanning 9 of the 16 loci identified with single variant analysis (*IL6*, *LPA*, *ALPL*, *NAV1*, *TMEM170A*, *ARHGEF26*, *BET1L*, *FADS1/2*, and *PRRX1*). Among these known loci, only *TMEM170A* was less significant than an overlapping region defined by another gene ($p=2.9\times10^{-10}$ for *CFDP1* versus $p=4.5\times10^{-10}$ for *TMEM170A*). The three most significant regions not identified in the single variant-based approaches were *LDLR* ($p=2.3\times10^{-10}$), *AGO2* ($p=5.9\times10^{-10}$), and *XKR6* ($p=9.8\times10^{-10}$). Although this approach did not account for LD between regions, these three regions were independent (>100 Mb away from another association).

Expression-based analyses

We used MetaXcan[70] to analyze gene expression from expression quantitative trait loci (eQTL) extracted from the Genotype-Tissue Expression Project (GTEx)[72]. We examined four tissues which may be involved in the AS disease process: the 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 (Supplementary Figure 6.38). Increased *LPA* expression in the liver was predicted for AS cases (Z score=5.25; $p=1.5\times10^{-7}$). Expression of *IL6R* was inferred to be increased in the blood of cases (Z score=4.66; $p=3.1\times10^{-6}$). *NOTCH4* signalling in the aorta was predicted to be decreased in cases (Z score=-4.07; $p=4.7\times10^{-5}$). In contrast to these single tissue effects, inferred *RPS23* expression was lower in the blood, left ventricle, and aorta in AS cases ($p\leq3.3\times10^{-5}$). The most significant, predicted differential expression was decreased *ZEB2* expression in the aorta of individuals with AS (Z score= -7.14; $p=9.2\times10^{-13}$).

The index variants rs62139062 at the *ACTR2* locus and rs73386631 at the *BET1L* locus were in high LD ($r^2\geq0.97$) with the top eQTL in the aorta for *ACTR2* and *BET1L*, respectively. The index variant at the *ARHGEF26* locus was in high LD ($r^2=0.97$) with the top eQTL for *ARHGEF26* in the left ventricle. While the index variant at the *SMC2* locus was associated with *SMC2* expression in the left ventricle, it was not the top eQTL for the gene. Index variants and proxies at the *CELSR2-SORT1*, *TMEM170A, IL6,* and *FADS1/2* loci were eQTL for one or more genes in two or more of the four tissues examined (Supplementary Table 6.2), though the index variant at the *CELSR2-SORT1* locus was in perfect LD ($r^2=1.0$) with the top hepatic eQTL for *CELSR2* and *SORT1*, and the index variant at the *FADS1/2* locus was in high LD ($r^2\geq0.96$) with the top eQTL for *FADS2* in the aorta and liver. Neither the index variant at the *IL6* locus or any variants in high LD ($r^2\geq0.95$) were associated with *IL6* expression in the tissues examined. However, the index variant or a

variant in high LD (r^2=0.95) was the top eQTL for IL6 antisense RNA 1 (*IL6-AS1*) in the aorta and left ventricle. No eQTLs were observed for the other index variants or their proxies.

Co-regulated groups of genes are differentially expressed in AS

Using gene set enrichment analyses (GIGSEA[218]), we inferred the differential expression of genes in the same pathway (Kyoto Encyclopedia of Genes and Genomes[73]); with related function (Gene Ontology[219]); bound by the same transcription factor (Functional Annotation of the Mammalian Genome, Version 5[220]), or targeted by the same microRNA (miRBase[221]). With a 5% false discovery rate, the hepatic expression of genes in the neutrophin signaling pathway and genes bound by transcription factor 7 was predicted to be lower in AS cases (T statistic=-6.44; p=1.1×10^{-4} and T statistic=-5.17; p=2.8×10^{-5}, respectively). Genes bound by four microRNAs were also inferred to be differentially expressed in the liver, with greater expression in AS cases of miR-219, miR-491, and miR-19a/b gene targets (T statistic≥4.32; p≤0.030) but lower expression of genes targeted by miR-21 (T statistic=-6.90; p=6.2×10^{-5}). In whole blood, expression of the Polycomb Group protein complex, proteasome complex, and receptor complex genes were predicted to be higher in controls (T statistic≤-4.85; p≤ 4.4×10^{-6}).

Aortic stenosis is genetically correlated with adiposity

Using LD Hub[223], we computed the genetic correlation of AS with 157 traits related to cardiovascular risk factors, metabolites, and immunological diseases using GWAS summary statistics provided by the web interface. With a false discovery rate of 5%, we observed 34 (21.7%) traits that were genetically correlated with AS, most of which involved adiposity, glycemia, or lipids (Supplementary Table 6.6). 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=6.0×10^{-4}).

Pleiotropic effects of variants associated with AS

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≤0.05 (Supplementary Table 6.7). The traits with the greatest number of associations were height (8 variants); coronary artery disease, albumin, and triglycerides (6 variants each); C-reactive protein and apolipoprotein B (5 variants each); and heel bone mineral density, diastolic blood pressure, and LDL-C cholesterol (4 variants each) (Figure 6.4; Supplementary Table 6.7). The five most significant associations involved apolipoprotein B, lipoprotein(a), LDL-C, or triglycerides (Supplementary Table 6.7).

Blood-based biomarkers are causal contributors to AS

We performed Mendelian randomization for six traits associated with several variants in the phenome-wide association study. We constructed genetic instruments using summary statistics of GWAS we performed in unrelated White British participants of the UK Biobank and assessed the association of these instruments with AS using the summary statistics from our meta-analysis. Genetically-elevated levels of apolipoprotein B, lipoprotein(a), LDL-C, and BMI were causally associated with increased odds of AS (OR per g/L of apolipoprotein B, 4.16; 95% CI, 3.16 to 5.46;

p=1.7×10^{-24}; OR per natural logarithm of lipoprotein[a], 1.22; 95% CI, 1.19 to 1.24; p=4.8×10^{-73}; OR per mmol/L of LDL-C, 1.61; 95% CI, 1.48 to 1.75; p=1.3×10^{-30}; OR per kg/m^2 of BMI, 1.08; 95% CI, 1.06, 1.10; p=5.8×10^{-20}, respectively), with no evidence of pleiotropy (all Egger intercepts p>0.05) (Supplementary Table 6.8). The association between higher genetically-predicted levels of C-reactive protein and AS (OR per natural logarithm, 1.08; 95% CI, 1.02 to 1.15; p=0.013) was pleiotropic (Egger intercept p=4.0×10^{-3}). We did not observe a causal association of alkaline phosphatase with AS (OR per natural logarithm, 1.10; 95% CI, 0.91 to 1.32; p=0.34).

Discussion

In this genome-wide meta-analysis, we combined summary statistics from 10 cohorts of European ancestry and discovered nine loci not previously reported to be genome-wide significant for AS. We observed modest changes in AS odds (9% to 55% increased odds per risk allele), except for the *HMGB1* variant for which the odds more than doubled. Several variants were associated with aortic valve calcium or with AS in other ancestries, and a genetic risk score with all 18 index variants was a predictor of AS in a model where other risk factors were covariates. Further analysis using gene- and gene-set 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 confirmed a causal contribution of lipoprotein(a), apolipoprotein B, LDL-C, and BMI to AS.

Our findings build upon previous work to support four central mechanisms for AS etiology: calcification, lipid metabolism, adiposity, and inflammation. In the present study, variants at the *PRRX1*, *ACTR2*, *LPA*, *BET1L*, and *FADS1/2* loci were associated with aortic valve calcification

identified by computed tomography, with the predisposing allele conferring higher odds of AS. We also observed associations of the index variants at the *CELSR2-SORT1*, *PRRX1*, *TEX41*, and *FADS1/2* loci with heel bone mineral density, suggesting systemic effects of these loci on calcification.

Located in the 3' untranslated region of *CELSR2*, rs12740374 affects expression of *SORT1*[227], reported to decrease hepatic excretion of apolipoprotein B and increase catabolism of LDL-C[228]. Multiple studies have reported lower LDL-C[229,230] and odds of coronary artery disease[167,231] 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=3.4×10^{-4})[58]. Our meta-analysis showed that a *CELSR2-SORT1* variant was associated with AS at genome-wide significance. Consistent with its effects on LDL-C and apolipoprotein B, the minor allele conferred a 10% reduction in the odds of AS. Further evidence that lipid metabolism is a causal mechanism for AS was provided by Mendelian randomization analyses, which confirmed the role of LDL-C[107] and identified a novel, causal contribution of apolipoprotein B, which is present in all atherogenic lipoprotein particles. This is consistent with prior work demonstrating an association of a non-high density lipoprotein cholesterol genetic risk score with AS[58].

Overall and abdominal obesity has been previously associated with a higher incidence of AS[30], and a recent Mendelian randomization study identified a causal association between BMI and AS[128]. Our Mendelian randomization analyses replicate the causal contribution of BMI to AS and were supported by the genetic correlations we observed 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 overall and central adiposity in our phenome-wide analysis. Another missense variant rs12493885 (p.Val29Leu) in *ARHGEF26* was previously associated with coronary artery disease[232], 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[232]. However, this variant is independent of our *ARHGEF26* rs6794263 (r^2=0.018)[184] and was not associated with AS in our meta-analysis (OR per T allele, 1.02; 95% CI, 0.98 to 1.06; p=0.44 for rs1713812, which is in perfect LD with rs12493885[184]). Conversely, the AS-lowering allele of rs6794263 has been associated with lower odds of coronary artery disease, but not at genome-wide significance (OR per C allele, 0.96; 95% CI, 0.92 to 1.00; p=0.037)[233]. The presence of two independent, missense mutations in *ARHGEF26* with discordant effects on AS and coronary artery disease is intriguing and suggests possible pleiotropic effects of the protein on cardiovascular diseases.

The accumulation of inflammatory cells in the aortic valve is strongly associated with remodelling and fibrosis[234], suggesting a role for inflammation in disease progression. We confirmed that the *FADS1/2* locus was associated with AS[209]. *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[175]. Moreover, we identified a rare (minor allele frequency=2.4×10^{-3}), intronic variant in *HMGB1*. High mobility group box protein 1 (HMGB1) participates in chromatin remodelling[235] but is also a signaling molecule secreted by macrophages[236] and other immune cells in response to cytokines and cellular stress[237], that is elevated in inflammatory diseases[238,239]. In AS, HMGB1 is found in the endothelial and interstitial cells of the aortic valve,

and its expression is increased in response to osteopontin[240]. HMGB1 activates Toll-like receptor 4 to increase interleukin-6 production[241], and consistent with this, we replicated the association previously reported between an *IL6* variant and AS[207] and the *IL6* variant rs2069832 (r^2=0.95 with our index variant) colocalizes with *IL6-AS1* expression[207]. Notably, the risk-increasing alleles are associated with increased expression of both *IL6* and *IL6-AS1* in fibroblasts in GTEx[72]. Our analyses indicated an association with AS in the *IL6* region but also higher predicted *IL6R* expression in the cells of the blood in AS cases. Furthermore, HMGB1 upregulates interleukin-6 and miR-21 expression levels concordantly[242]. MiR-21 is overexpressed in the aortic valves[243] and plasma[244] of AS patients, and our prediction of decreased hepatic expression of miR-21 gene targets in cases supports the involvement of miR-21 in AS pathophysiology. Thus, our findings provide several orthogonal signals indicating that an increase in the pro-inflammatory interleukin-6 signaling pathway is associated with AS, consistent with what has been observed for other cardiovascular diseases[245,246], and more specifically, may directly implicate HMGB1 and miR-21.

The index variant and proxies at the *TMEM170A* locus were eQTLs for *BCAR1*, *CFDP1,* and/or RP11-252K23.2 in the four tissues we examined. The *BCAR1-CFDP1-TMEM170A* locus has been previously associated with carotid intima-media thickness and coronary artery disease, with the most significant variant being *CFDP1* rs4888378[247] (r^2=0.69 with our *TMEM170A* index variant[184]). This association has been proposed to be due to altered transcription factor binding affecting *BCAR1* expression[248]. However, *CFDP1,* an established gene for aortic root diameter[249], has been shown to be nominally significant for AS in prior work[58]. Interestingly, *CFDP1* participates in chromatin remodelling and affects the localization of Structural Maintenance of

Proteins 2[250], which is coded for by one of our novel AS loci, *SMC2*. Thus, our analyses support the possible contribution of chromatin regulation to AS.

Several of our findings highlight the role of vascular remodelling in AS. Leukocyte infiltration and co-localized tumour necrosis factor-α (TNF-α) expression have been observed in the aortic valves of AS patients[251]. The paired-related homeobox protein 1, the protein product of the novel locus *PRRX1*, is a transcription factor required for the inhibition of osteoblast differentiation by TNF-α[252] and is an inducer of the epithelial-mesenchymal transition[253]. Zinc finger e-box binding homeobox 2, coded by *ZEB2*, is a transcriptional repressor of the process[254]. *ZEB2* expression in the aorta was the most significant differentially expressed gene our analysis predicted; consistent with this finding was a prior report that candidate causal variants at *TEX41* may be associated with AS through long-range chromatin interactions with the promoter region of *ZEB2*, including in the aorta[58]. The Notch signalling pathway is essential for remodelling of the vasculature[255], and *NOTCH1* haploinsufficiency causes familial aortic valve disease and severe calcification[38]. We observed decreased *NOTCH4* expression in the aorta of AS cases. The *Notch4* knockout mouse has been associated with delayed and less extensive vessel growth[256], and in humans, *NOTCH4* expression is also decreased in the aorta of individuals with bicuspid aortic valves[257,258].

In this genome-wide meta-analysis involving 653,867 participants (13,765 cases), we applied variant-, gene-, and gene set-based analyses to identify additional risk loci and mechanisms. Despite the strengths of the study, there are several limitations. Although we attempted cross-ancestry replication of genome-wide significant variants, we observed limited reproducibility likely due to the low numbers of African- and Latin-American participants. In addition, because

our discovery cohorts were of European ancestry, the transferability of our results to other ancestries groups may be limited[259-261]. Future studies should focus on genetic loci in non-European populations. Our analyses also made exclusive use of bioinformatic methods to identify genetic loci. For the expression-based analyses in particular, we relied on genetically predicted levels of gene expression. These results require confirmation using complementary approaches.

In conclusion, our results identify novel genetic contributors to the etiology of AS and confirm the disease etiology is characterized by the effects of calcification, altered lipid metabolism, adiposity, and inflammation. An AS genetic risk score was a predictor of both clinical and subclinical disease, providing additional discriminatory ability when added to clinical risk factors. Additional analyses of established and novel genetic loci warrant investigation as potential therapeutic targets to prevent the initiation of aortic calcification and progression to stenosis.

Figures

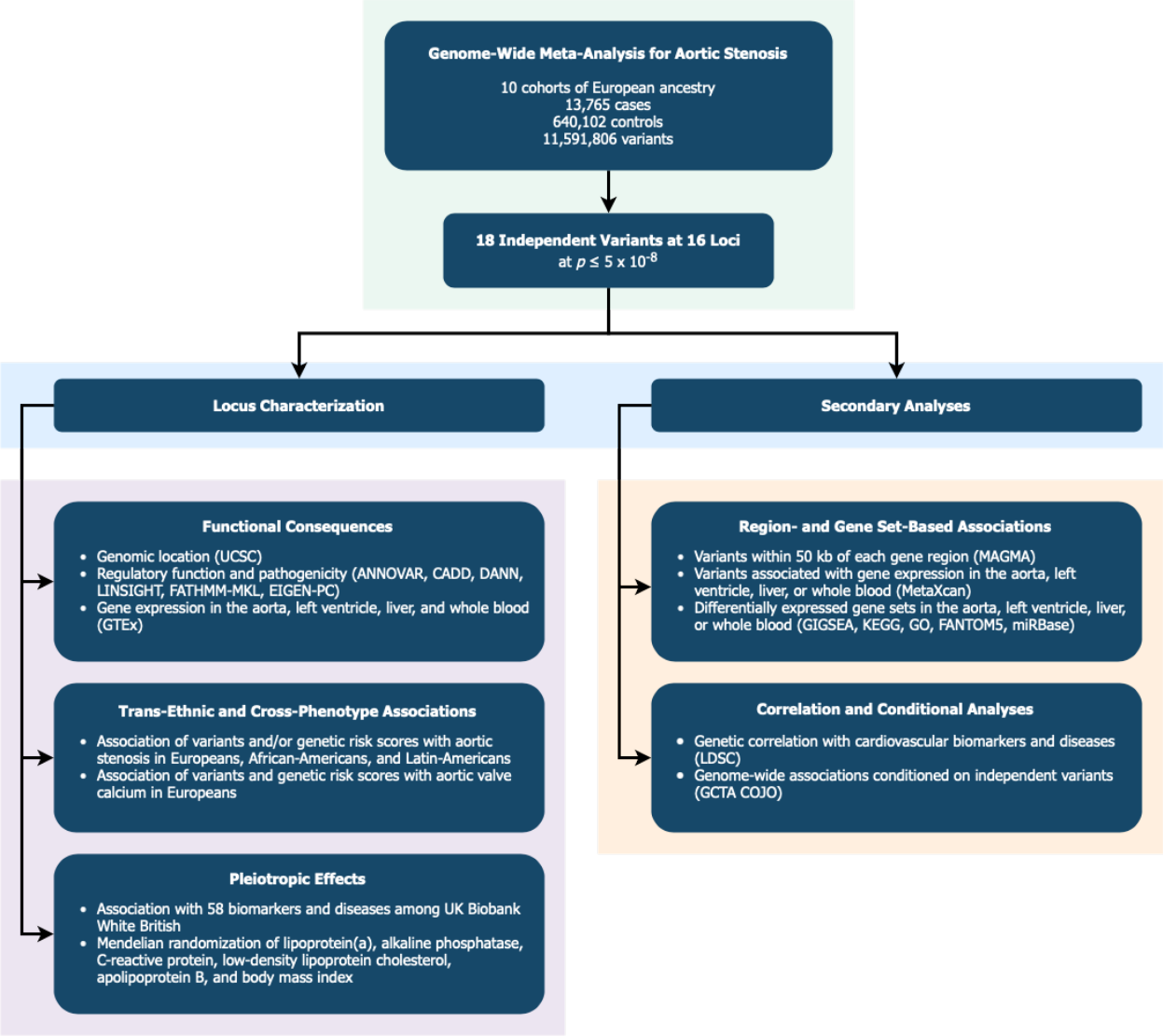


Figure 6.1. Design of the Genome-Wide Meta-Analysis and Follow-Up Analyses.

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 Five; 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.

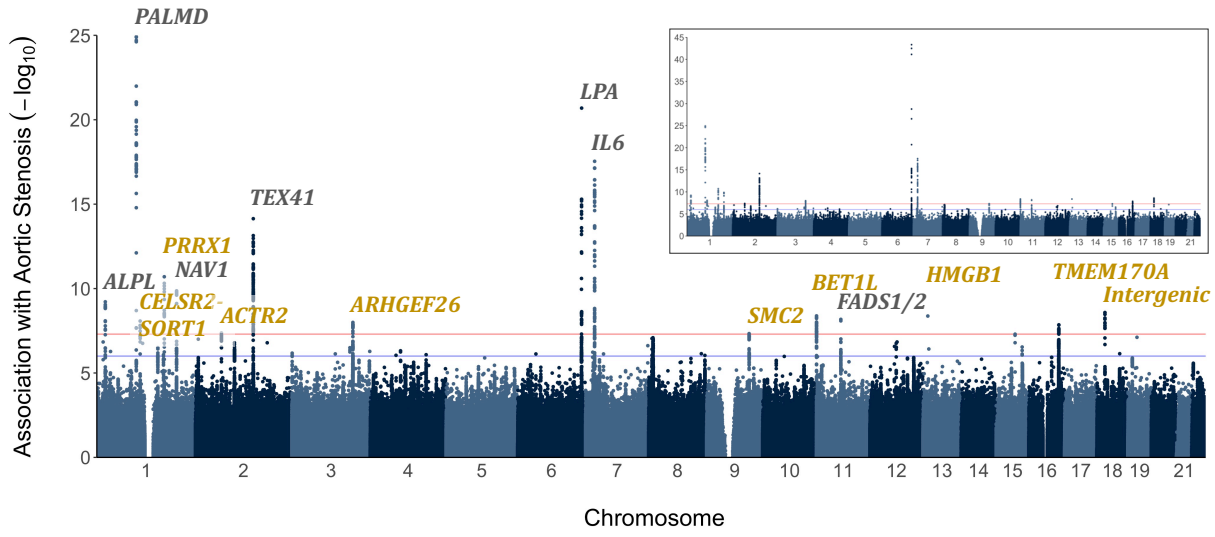
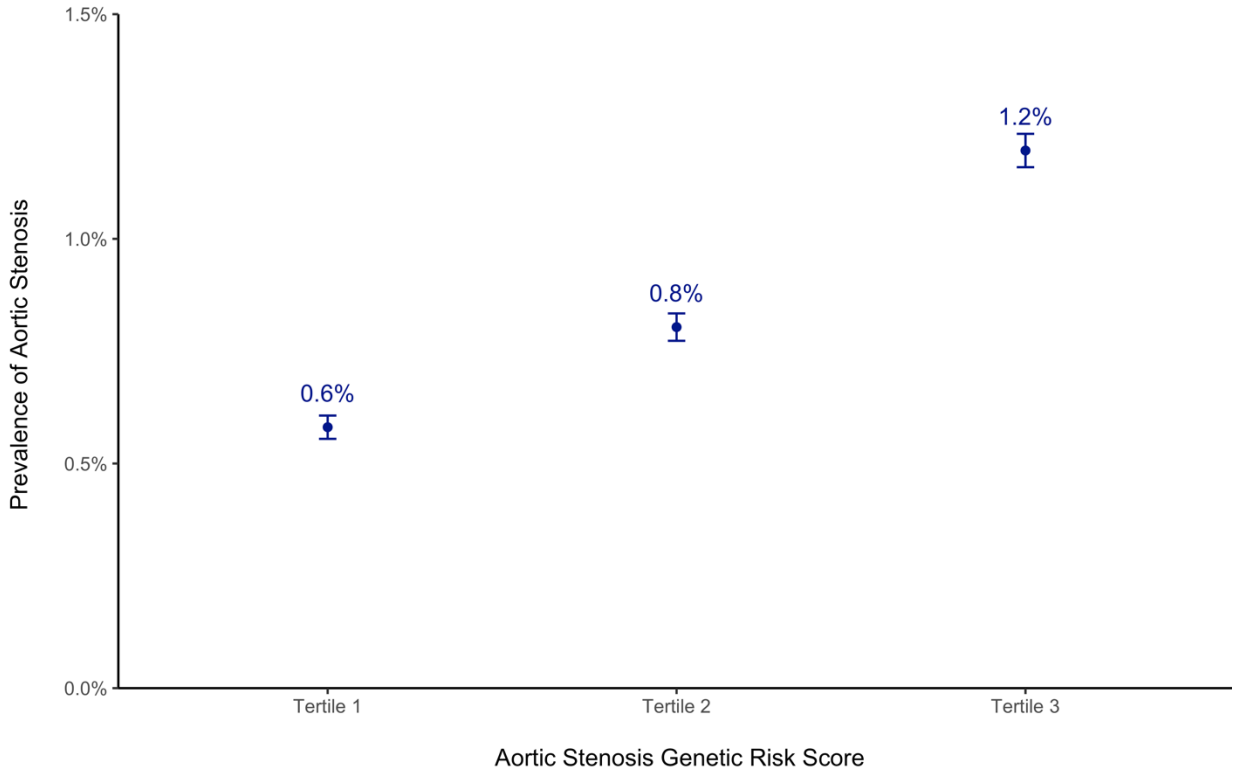


Figure 6.2. *P* for the Association of 11,591,806 Variants with Aortic Stenosis in the Meta-Analysis.

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.

A


1.5%
1.0%
0.5%
0.0%
Prevalence of Aortic Stenosis
0.6%
0.8%
1.2%
Tertile 1
Tertile 2
Tertile 3
Aortic Stenosis Genetic Risk Score

B

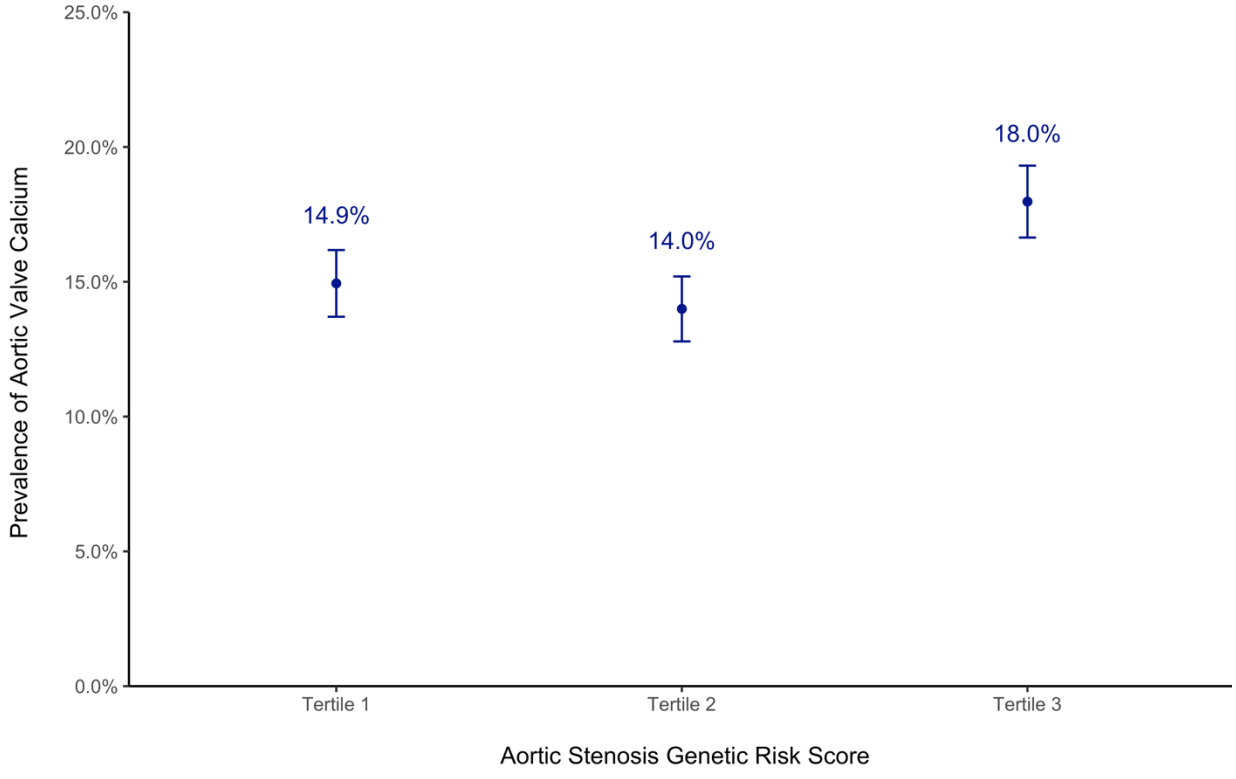

25.0%
20.0%
15.0%
10.0%
5.0%
0.0%
Prevalence of Aortic Valve Calcium
14.9%
14.0%
18.0%
Tertile 1
Tertile 2
Tertile 3
Aortic Stenosis Genetic Risk Score

Figure 6.3. Disease Prevalence Versus Genetic Risk Score for Aortic Stenosis.

Aortic stenosis prevalence by tertiles of the aortic stenosis genetic risk score among White British participants of the UK Biobank (**A**). Aortic valve calcium prevalence by tertiles of the aortic stenosis genetic risk score among European participants of the Multi-Ethnic Study of Atherosclerosis (**B**).

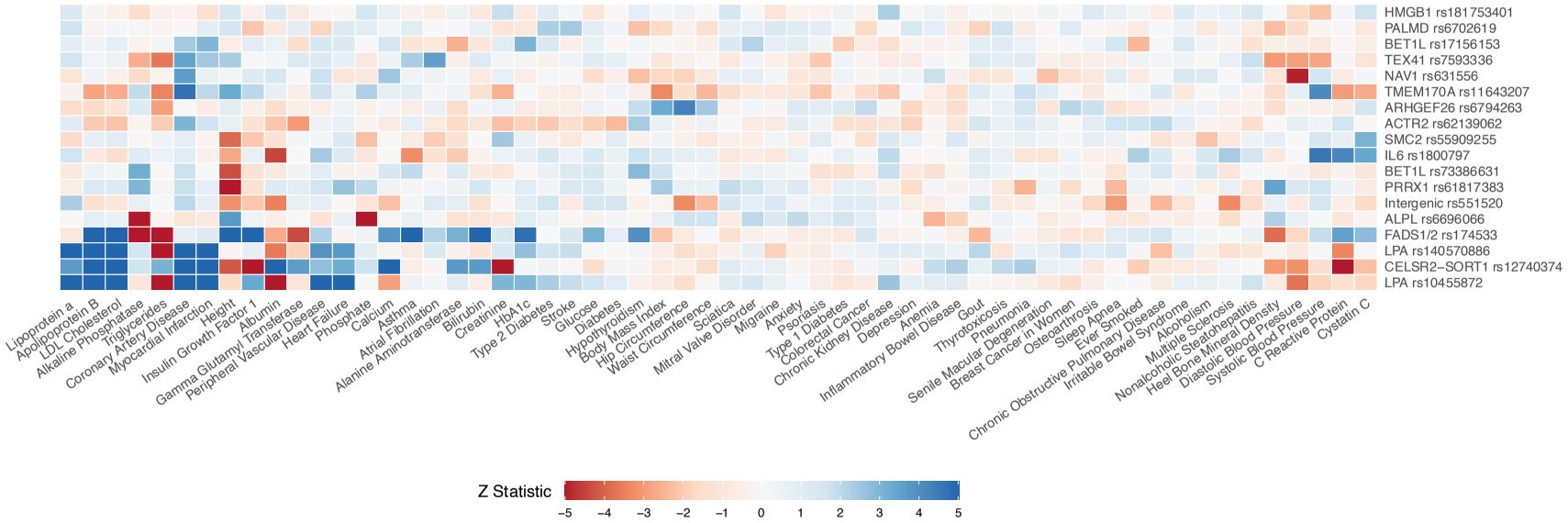

HMGB1 rs181753401
PALMD rs6702619
BET1L rs17156153
TEX41 rs7593336
NAV1 rs631556
TMEM170A rs11643207
ARHGEF26 rs6794263
ACTR2 rs62139062
SMC2 rs55909255
IL6 rs1800797
BET1L rs73386631
PRRX1 rs61817383
Intergenic rs551520
ALPL rs6696066
FADS1/2 rs174533
LPA rs140570886
CELSR2–SORT1 rs12740374
LPA rs10455872
Lipoprotein a
Apolipoprotein B
LDL Cholesterol
Alkaline Phosphatase
Triglycerides
Coronary Artery Disease
Myocardial Infarction
Height
Insulin Growth Factor 1
Albumin
Gamma Glutamyl Transferase
Peripheral Vascular Disease
Heart Failure
Phosphate
Calcium
Asthma
Atrial Fibrillation
Alanine Aminotransferase
Bilirubin
Creatinine
HbA1c
Type 2 Diabetes
Stroke
Glucose
Diabetes
Hypothyrodism
Body Mass Index
Hip Circumference
Waist Circumference
Sciatica
Mitral Valve Disorder
Migraine
Anxiety
Psoriasis
Type 1 Diabetes
Colorectal Cancer
Chronic Kidney Disease
Depression
Anemia
Inflammatory Bowel Disease
Gout
Thyrdoxicosis
Pneumonia
Senile Macular Degeneration
Breast Cancer in Women
Osteoarthrosis
Sleep Apnea
Ever Smoked
Pulmonary Disease
Chronic Obstructive Pulmonary Disease
Irritable Bowel Syndrome
Alcoholism
Multiple Sclerosis
Nonalcoholic Steatohepatitis
Heel Bone Mineral Density
Diastolic Blood Pressure
Systolic Blood Pressure
C Reactive Protein
Cystatin C
Z Statistic
−5
−4
−3
−2
−1
0
1
2
3
4
5

Figure 6.4. Association of Aortic Stenosis Variants with a Variety of 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 colours, with blue cells indicating consistent, positive effects on the odds of aortic stenosis and red cells indicating inverse associations. For ease of visualization, Z statistics greater than 5 or less than -5 were rounded to 5 and -5, respectively.

Tables

Table 6.1. New and Previously Identified Genetic Loci for Aortic Stenosis.

Chr.	Locus	Variant	Minor Allele (Frequency)	Odds Ratio per Minor Allele (95% CI)	*p*	I^2 % (95% CI)	Imputation Quality Score Range
New Loci							
1	*CELSR2-SORT1*	rs12740374	T (0.21)	0.90 (0.87, 0.94)	8.4×10^{-9}	0 (0, 0)	0.77 to 1.00
1	*PRRX1*	rs61817383	T (0.26)	1.11 (1.08, 1.14)	2.0×10^{-11}	10 (0, 66)	0.94 to 1.00
2	*ACTR2*	rs62139062	T (0.28)	1.09 (1.06, 1.12)	4.2×10^{-8}	32 (0, 68)	0.92 to 1.00
3	*ARHGEF26*	rs6794263	C (0.11)	0.88 (0.84, 0.92)	1.0×10^{-8}	39 (0, 71)	0.98 to 1.00
9	*SMC2*	rs55909255	C (0.39)	1.08 (1.05, 1.11)	4.7×10^{-8}	53 (3, 77)	0.99 to 1.00
11	*BET1L*	rs73386631	T (0.043)	1.22 (1.14, 1.31)	2.8×10^{-8}	20 (0, 62)	0.37 to 1.00
		rs17156153	T (0.085)	1.16 (1.10, 1.22)	4.2×10^{-9}	0 (0, 56)	0.73 to 1.00
13	*HMGB1*	rs181753401	A (2.4×10^{-3})	2.29 (1.74, 3.02)	4.2×10^{-9}	36 (0, 73)	0.40 to 1.00
16	*TMEM170A*	rs11643207	C (0.38)	0.92 (0.89, 0.95)	1.4×10^{-8}	50 (0, 76)	0.98 to 1.00
18	*Intergenic* (18q11.2)	rs551520	T (0.23)	0.91 (0.88, 0.94)	2.6×10^{-9}	27 (0, 65)	0.99 to 1.01
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
1	*PALMD*	rs6702619	G (0.49)	1.16 (1.13, 1.19)	1.2×10^{-25}	59 (18, 80)	0.97 to 1.00
1	*NAV1*	rs631556	A (0.41)	1.10 (1.07, 1.13)	1.4×10^{-10}	0 (0, 44)	0.98 to 1.00
2	*TEX41*	rs7593336	G (0.38)	1.12 (1.09, 1.15)	7.3×10^{-15}	59 (17, 79)	0.95 to 1.00
6	*LPA*	rs10455872	G (0.069)	1.42 (1.35, 1.49)	4.6×10^{-44}	28 (0, 65)	0.76 to 1.00
		rs140570886	C (0.014)	1.55 (1.40, 1.73)	5.1×10^{-16}	24 (0, 64)	0.83 to 1.00
7	*IL6*	rs1800797	A (0.45)	1.13 (1.10, 1.16)	2.9×10^{-18}	45 (0, 73)	0.88 to 1.00
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

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6.4 Supplement

Supplementary Table 6.1. Cohorts in the Genome-Wide Meta-Analysis.

Cohort	Country	AS Definition	No. Cases	No. Controls	Median Age (Quartile 1, Quartile 3)	Genotyping Array	Imputation Reference Panel	No. Variants
BioVU	US	EHR	759	7,555	68 (62, 75)	Illumina Multi-Ethnic Genotyping Array	HRC version r1.1	10,689,407
CAVS-France1	France	ECG	1,261	1,305	75 (70,79)[a]	Affymetrix Axiom Genome-Wide CEU-1 Array	HRC version r1.1	10,395,306
CAVS-France2	France	ECG	1,495	2,707	77 (70, 83)[a]	Affymetrix Axiom Genome-Wide Precision Medicine	HRC version r1.1	10,031,533
CAVS-France3	France	ECG	367	2,519	74 (67,79)[a]	Affymetrix Axiom Genome-Wide Precision Medicine	HRC version r1.1	9,884,426
deCODE	Iceland	EHR	2,464	351,068	77 (68, 83)[a]	Illumina Chips	Icelandic reference panel by deCODE	9,812,907
GERA	US	EHR	3,469	51,723	66 (61, 74)	Affymetrix Axiom Genome-Wide EUR	HRC version r1.1	10,578,354
Malmö Diet and Cancer Study	Sweden	EHR	464	4,878	58 (53, 63)	Illumina Human Omni Express Exome BeadChip	HRC version r1.1	6,130,056
Penn Medicine Biobank	US	EHR	1,593	4,550	71 (62, 79)	Illumina Quad Omni Genotyping Chip	HRC version r.1.1	11,016,108
UK Biobank[b]	UK	EHR	1,675	213,361	63 (59, 66)	Affymetrix UK Biobank Axiom Array	HRC version r1.1 and UK10K + 1000	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

Abbreviations: AS, aortic stenosis; CAVS, calcific aortic valve stenosis; ECG, echocardiography; EHR, electronic health records; EUR, European; GERA, Genetic Epidemiology Research on Adult Health and Aging; HRC, Haplotype Reference Consortium; UK, United Kingdom; US, United States.

[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 2,213 cases and 255,018 controls was used. See the UK Biobank cohort description in this document for more details.

Supplementary Table 6.2. Functional and Regulatory Effects of Index Variants and Their Proxies ($r^2 \geq 0.95$).

Proxy	Location	CADD Scaled C-Score	DANN Rank Score	FATHMM-MKL Noncoding Score	Eigen-PC Phred Score	LIN-SIGHT Score	Aorta eQTL[a]	Left Ventricle eQTL[a]	Liver eQTL[a]	Whole Blood eQTL[a]
New Loci	*CELSR2-SORT1* rs12740374									
rs7528419	PSRC1(5):downstream(5)|CELSR2(1):UTR3(1)	1.18	0.23	0.1	20.78	0.21		*PSRC1*	*CELSR2, PSRC1, SORT1*	*PSRC1*
rs12740374	CELSR2(1):UTR3(1)	4.72	0.36	0.58	24.11	0.08		*PSRC1*	*CELSR2, PSRC1, SORT1*	*PSRC1*
rs660240	PSRC1(5):downstream(5)|CELSR2(1):UTR3(1)	18.47	0.88	0.96	22.44	0.48		*PSRC1*	*CELSR2, PSRC1, SORT1*	*PSRC1*
rs629301	PSRC1(5):downstream(5)|CELSR2(1):UTR3(1)	13.49	0.77	0.14	15.88	0.51		*PSRC1*	*CELSR2, PSRC1, SORT1*	*PSRC1*
rs646776	PSRC1(5):downstream(5)|CELSR2(1):downstream(1)	2.44	0.26	0.14	15.72	0.08		*PSRC1*	*CELSR2, PSRC1, SORT1*	*PSRC1*
rs583104	PSRC1(5):downstream(5)|CELSR2(1):downstream(1)	4.97	0.77	0.23	5.25	0.05			*CELSR2, PSRC1, SORT1*	*PSRC1*
rs4970836	PSRC1(5):downstream(5)|CELSR2(1):downstream(1)	2.6	0.57	0.09	0.74	0.05				

Table Continued

Proxy	Location	CADD Scaled C-Score	DANN Rank Score	FATHMM-MKL Noncoding Score	Eigen-PC Phred Score	LINSIGHT Score	Aorta eQTL[a]	Left Ventricle eQTL[a]	Liver eQTL[a]	Whole Blood eQTL[a]
PRRX1 rs61817383										
rs4656221	PRRX1(2):intronic(2)	13.02	0.95	0.35	7.34	0.54				
rs4656222	PRRX1(2):intronic(2)	8.45	0.46	0.35	10.21	0.56				
rs61817382	PRRX1(2):intronic(2)	17.55	0.87	0.92	11.2	0.4				
rs61817383	PRRX1(2):intronic(2)	6.49	0.66	0.22	6.53	0.21				
ACTR2 rs62139062										
rs62142478	ACTR2(4):intronic(4)	1.37	0.47	0.11	1.26	0.05	*ACTR2*			
rs115375526	ACTR2(4):intronic(4)	11.81	0.98	0.47	4.88	0.05	*ACTR2*			
rs62139061	ACTR2(4):downstream(3)	6.56	0.53	0.2	5.74	0.09	*ACTR2*			

Table Continued

Proxy	Location	CADD Scaled C-Score	DANN Rank Score	FATHMM-MKL Noncoding Score	Eigen-PC Phred Score	LINSIGHT Score	Aorta eQTL[a]	Left Ventricle eQTL[a]	Liver eQTL[a]	Whole Blood eQTL[a]
rs62139062	ACTR2(4):downstream(3)	0.7	0.48	0.15	3.12	0.05	*ACTR2*			
rs72822411	ACTR2(4):downstream(3)	0.05	0.54	0.04	0.08	0.05	*ACTR2*			
rs62139085	.(0):intergenic(1)	1.49	0.47	0.07	0.69	0.05	*ACTR2*			
rs7556894	.(0):intergenic(1)	0.45	0.73	0.16	7.45	0.05	*ACTR2*			
rs72822419	.(0):intergenic(1)	0.56	0.82	0.05	1.06	0.04	*ACTR2*			
ARHGEF26 rs6794263										
rs56198593	ARHGEF26-AS1(1):ncRNA_intronic(1)	5.16	0.56	0.15	4.15	0.04				
rs35526449	ARHGEF26-AS1(1):ncRNA_intronic(1)	1.8	0.67	0.08	1.38	0.05				
rs12630687	ARHGEF26-AS1(1):ncRNA_intronic(1)	0.36	0.08	0.04	0.12	0.04				

Table Continued

Proxy	Location	CADD Scaled C-Score	DANN Rank Score	FATHMM-MKL Noncoding Score	Eigen-PC Phred Score	LINSIGHT Score	Aorta eQTL[a]	Left Ventricle eQTL[a]	Liver eQTL[a]	Whole Blood eQTL[a]
rs11710405	ARHGEF26-AS1(1):ncRNA_intronic(1)	1.72	0.15	0.07	0.58	0.04				
rs7616315	ARHGEF26-AS1(1):ncRNA_intronic(1)	1.31	0.81	0.07	0.26	0.04				
rs1470666	ARHGEF26-AS1(1):ncRNA_intronic(1)	1.35	0.15	0.11	2.66	0.05				
rs1527794	ARHGEF26-AS1(1):ncRNA_intronic(1)	0.72	0.77	0.11	1.21	0.05				
rs1318182	ARHGEF26-AS1(1):ncRNA_intronic(1)	5.96	0.83	0.15	3.87	0.05				
rs7648816	ARHGEF26-AS1(1):ncRNA_intronic(1)|ARHGEF26(4)	5.61	0.17	0.15	3.15	0.05				
rs6796569	ARHGEF26-AS1(1):upstream(1)|ARHGEF26(4):UTR5	10.96	0.28	0.35	19.44	0.1				
rs12496515	ARHGEF26-AS1(1):upstream(1)|ARHGEF26(4):intron	7.92	0.22	0.2	19.98	0.11				
rs13096373	ARHGEF26(4):nonsynonymous(3)	14.47	0.72	0.13	0.25					

Table Continued

Proxy	Location	CADD Scaled C-Score	DANN Rank Score	FATHMM-MKL Noncoding Score	Eigen-PC Phred Score	LINSIGHT Score	Aorta eQTL[a]	Left Ventricle eQTL[a]	Liver eQTL[a]	Whole Blood eQTL[a]
rs7642221	ARHGEF26-AS1(1):upstream(1)|ARHGEF26(4):intron	3.47	0.64	0.18	12.88	0.14				
rs881435	ARHGEF26-AS1(1):upstream(1)|ARHGEF26(4):intron	7.6	0.85	0.12	7.57	0.06				
rs59868320	ARHGEF26-AS1(1):upstream(1)|ARHGEF26(4):intron	3.82	0.71	0.16	3	0.05				
rs2122361	ARHGEF26(4):intronic(3)	1.27	0.07	0.15	3.53	0.05				
rs10935975	ARHGEF26(4):intronic(3)	4.79	0.45	0.15	2.72	0.05				
rs6763911	ARHGEF26(4):intronic(3)	2.56	0.05	0.17	5.17	0.05				
rs16846895	ARHGEF26(4):intronic(3)	10.4	0.66	0.31	10.59	0.25				
rs6786617	ARHGEF26(4):intronic(3)	0.64	0.2	0.19	8.25	0.05				
rs6762384	ARHGEF26(4):intronic(3)	0.08	0.27	0.16	7.98	0.12				

Table Continued

Proxy	Location	CADD Scaled C-Score	DANN Rank Score	FATHMM-MKL Noncoding Score	Eigen-PC Phred Score	LINSIGHT Score	Aorta eQTL[a]	Left Ventricle eQTL[a]	Liver eQTL[a]	Whole Blood eQTL[a]
rs12498021	ARHGEF26(4):intronic(3)	3.37	0.8	0.12	2.45	0.05				
rs6794263	ARHGEF26(4):intronic(3)	9.04	0.89	0.32	6.66	0.12				
rs10935976	ARHGEF26(4):intronic(3)	2.75	0.12	0.07	1	0.04				
rs13066925	ARHGEF26(4):intronic(3)	2.29	0.28	0.08	0.99	0.04				
rs12496189	ARHGEF26(4):intronic(3)	0.24	0.16	0.08	1.75	0.04				
rs12490468	ARHGEF26(4):intronic(3)	6.51	0.52	0.14	5.03	0.04				
rs991467	ARHGEF26(4):intronic(3)	4.6	0.35	0.16	6.08	0.04				
rs1405894	ARHGEF26(4):intronic(3)	2.64	0.44	0.19	4.32	0.04				
rs896012	ARHGEF26(4):intronic(3)	2.88	0.83	0.07	1.67	0.04				

Table Continued

Proxy	Location	CADD Scaled C-Score	DANN Rank Score	FATHMM-MKL Noncoding Score	Eigen-PC Phred Score	LINSIGHT Score	Aorta eQTL[a]	Left Ventricle eQTL[a]	Liver eQTL[a]	Whole Blood eQTL[a]
SMC2 rs55909255										
rs55909255	SMC2(7):intronic(7)	6.51	0.51	0.1	2.2	0.04		*SMC2*		
BET1L rs73386631										
rs73386631	BET1L(2):downstream(2)|ODF3(3):downstream(3)	2.65	0.13	0.1	1.54	0.05	*BET1L*			
rs11245990	ODF3(3):downstream(3)|BET1L(2):downstream(2)	1.77	0.2	0.1	1.22	0.05	*BET1L*			
rs79902640	ODF3(3):downstream(3)|BET1L(2):downstream(2)	0.79	0.35	0.14	6.88	0.06	*BET1L*			
rs73386635	ODF3(3):downstream(3)|BET1L(2):downstream(2)	1.32	0.62	0.08	6.79	0.09	*BET1L*			
rs73386640	ODF3(3):downstream(3)|BET1L(2):UTR3(2)	3.87	0.72	0.13	3.48	0.06	*BET1L*			
rs2280543	RIC8A(3):upstream(2)|ODF3(3):downstream(3)|BET1L(2):UTR3(2)	2.8	0.32	0.15	3.62	0.07	*BET1L*			

Table Continued

Proxy	Location	CADD Scaled C-Score	DANN Rank Score	FATHMM-MKL Noncoding Score	Eigen-PC Phred Score	LINSIGHT Score	Aorta eQTL[a]	Left Ventricle eQTL[a]	Liver eQTL[a]	Whole Blood eQTL[a]
rs11245997	RIC8A(3):upstream(3)|ODF3(3):downstream(3)|BET1L(2):UTR	0.21	0.4	0.1	2.47	0.11	*BET1L*			
rs3782122	RIC8A(3):upstream(3)|BET1L(2):intronic(2)	2.12	0.66	0.11	7.62	0.11	*BET1L*			
rs3782121	RIC8A(3):upstream(3)|BET1L(2):intronic(2)	2.05	0.7	0.14	8.86	0.11	*BET1L*			
rs11246001	RIC8A(3):intronic(3)|Z49985(1):downstream(1)|BET1L(2):upstream(2)|SIRT3(7):downstream(7)	0.65	0.3	0.05	7.11	0.05	*BET1L*			
rs11246002	RIC8A(3):intronic(3)|Z49985(1):downstream(1)|BET1L(2):upstream(2)|SIRT3(7):downstream(7)	4.33	0.8	0.12	6.64	0.12	*BET1L*			
rs11246003	RIC8A(3):intronic(3)|Z49985(1):downstream(1)|SIRT3(7):downstream(7)	2.72	0.41	0.07	12.63	0.05	*BET1L*			
rs12226698	RIC8A(3):intronic(3)|Z49985(1):downstream(1)|SIRT3(7):downstream(7)	4.38	0.92	0.1	12.71	0.05	*BET1L*			

Table Continued

Proxy	Location	CADD Scaled C-Score	DANN Rank Score	FATHMM-MKL Noncoding Score	Eigen-PC Phred Score	LINSIGHT Score	Aorta eQTL[a]	Left Ventricle eQTL[a]	Liver eQTL[a]	Whole Blood eQTL[a]
rs12226402	RIC8A(3):downstream(3)|Z49985(1):upstream(1)|SIRT3(7):UTR3(7)	0.02	0.52	0.12	13.32	0.05	*BET1L*			
rs12226697	RIC8A(3):downstream(3)|Z49985(1):upstream(1)|SIRT3(7):UTR3(7)	4.37	0.59	0.19	13.53	0.05	*BET1L*			
rs12222188	SIRT3(7):intronic(7)	5.19	0.2	0.18	5.73	0.06				
rs12225799	PSMD13(3):intronic(3)|SIRT3(7):upstream(7)	1.85	0.42	0.08	0.84	0.05	*BET1L*			
BET1L rs17156153										
rs559422	PSMD13(3):intronic(3)	4.46	0.68	0.12	2.47	0.12				
rs17156153	.(0):intergenic(1)	1.22	0.46	0.08	5.12	0.05				
rs11246043	NLRP6(2):upstream(2)	9.4	0.88	0.24	2.57	0.05				

Table Continued

Proxy	Location	CADD Scaled C-Score	DANN Rank Score	FATHMM-MKL Noncoding Score	Eigen-PC Phred Score	LINSIGHT Score	Aorta eQTL[a]	Left Ventricle eQTL[a]	Liver eQTL[a]	Whole Blood eQTL[a]
rs11246044	NLRP6(2):upstream(2)	13.84	0.98	0.66	6.61	0.09				
rs11246045	NLRP6(2):upstream(2)	8.28	0.89	0.19	2.73	0.05				
rs12419714	NLRP6(2):upstream(2)	3.34	0.4	0.1	1.79	0.05				
HMGB1 rs181753401										
rs181753401	HMGB1(5):intronic(1)	1.97	0.12	0.12	3.26	0.05				
TMEM170A rs11643207										
rs4888430	TMEM170A(3):intronic(3)	2.75	0.59	0.08	4.42	0.06	*BCAR1, ENSG00000261783.1*	*ENSG00000261783.1*	*ENSG00000261783.1*	
rs1834013	TMEM170A(3):intronic(3)	0.12	0.53	0.05	0.49	0.05	*BCAR1, ENSG00000261783.1*	*ENSG00000261783.1*	*ENSG00000261783.1*	
rs8055490	TMEM170A(3):intronic(3)	6.5	0.41	0.16	22.44	0.07	*BCAR1, ENSG00000261783.1*	*ENSG00000261783.1*	*ENSG00000261783.1*	

Table Continued

Proxy	Location	CADD Scaled C-Score	DANN Rank Score	FATHMM-MKL Noncoding Score	Eigen-PC Phred Score	LINSIGHT Score	Aorta eQTL[a]	Left Ventricle eQTL[a]	Liver eQTL[a]	Whole Blood eQTL[a]
rs12446877	TMEM170A(3):intronic(3)	10.27	0.46	0.21	22.73	0.06	*BCAR1, ENSG00000261783.1*	*ENSG00000261783.1*	*ENSG00000261783.1*	
rs11643207	TMEM170A(3):upstream(3)	8.79	0.93	0.36	38.81	0.09	*BCAR1, ENSG00000261783.1*	*ENSG00000261783.1*	*ENSG00000261783.1*	
rs4888431	TMEM170A(3):upstream(3)	5.31	0.82	0.14	2.25	0.05	*BCAR1, ENSG00000261783.1*	*ENSG00000261783.1*	*ENSG00000261783.1*	*BCAR1*
rs1834014	TMEM170A(3):upstream(3)	2.25	0.03	0.08	0.91	0.1	*BCAR1, ENSG00000261783.1*	*ENSG00000261783.1*	*ENSG00000261783.1*	
rs62060550	CHST6(3):downstream(1)|TMEM170A(3):upstream(3)	6.95	0.84	0.18	1.14	0.12	*BCAR1, ENSG00000261783.1*	*ENSG00000261783.1*	*ENSG00000261783.1*	*BCAR1*
rs62060551	CHST6(3):downstream(1)|TMEM170A(3):upstream(3)	0.56	0.4	0.05	0.18	0.05	*BCAR1, ENSG00000261783.1*	*ENSG00000261783.1*	*ENSG00000261783.1*	*CFDP1*
rs34918083	CHST6(3):downstream(1)	1.4	0.37	0.06	0.5	0.05	*BCAR1, ENSG00000261783.1*	*ENSG00000261783.1*	*ENSG00000261783.1*	
rs12924333	CHST6(3):downstream(1)	1.83	0.52	0.06	0.37	0.05	*BCAR1, ENSG00000261783.1*	*ENSG00000261783.1*	*ENSG00000261783.1*	
rs977987	CHST6(3):downstream(2)	6.05	0.82	0.1	11.36	0.05	*BCAR1, ENSG00000261783.1*	*ENSG00000261783.1*	*ENSG00000261783.1*	

Table Continued

Proxy	Location	CADD Scaled C-Score	DANN Rank Score	FATHMM-MKL Noncoding Score	Eigen-PC Phred Score	LINSIGHT Score	Aorta eQTL[a]	Left Ventricle eQTL[a]	Liver eQTL[a]	Whole Blood eQTL[a]
rs977985	CHST6(3):downstream(2)	1.05	0.14	0.08	10.94	0.07	*BCAR1, ENSG00000261783.1*	*ENSG00000261783.1*	*ENSG00000261783.1*	
Intergenic rs551520										
rs177991	.(0):intergenic(1)	7.86	0.31	0.28	8.78	0.86				
rs177996	.(0):intergenic(1)	2.12	0.2	0.19	8.64	0.05				
rs16967814	.(0):intergenic(1)	18.63	0.82	0.96	15.76	0.31				
rs636261	.(0):intergenic(1)	3.95	0.83	0.07	0.83	0.04				
rs35630671	.(0):intergenic(1)	0.21	0.41	0.02	0.01	0.04				
rs178004	.(0):intergenic(1)	2.96	0.84	0.12	7.96	0.05				
rs178007	.(0):intergenic(1)	1.93	0.06	0.06	0.75	0.04				

Table Continued

Proxy	Location	CADD Scaled C-Score	DANN Rank Score	FATHMM-MKL Noncoding Score	Eigen-PC Phred Score	LINSIGHT Score	Aorta eQTL[a]	Left Ventricle eQTL[a]	Liver eQTL[a]	Whole Blood eQTL[a]
rs178008	.(0):intergenic(1)	3.97	0.45	0.08	0.91	0.04				
rs551520	.(0):intergenic(1)	1.5	0.36	0.07	0.15	0.04				
rs476998	.(0):intergenic(1)	4.35	0.4	0.1	1.65	0.04				
rs12958298	.(0):intergenic(1)	2.55	0.8	0.12	8.43	0.05				
rs764359	.(0):intergenic(1)	3.59	0.38	0.15	3.87	0.04				
Previously Identified Loci										
ALPL rs6696066										
rs12127343	ALPL(5):intronic(4)	1.19	0.54	0.08	1.31	0.04				
rs6666477	ALPL(5):intronic(4)	4.68	0.31	0.14	4.29	0.05				

Table Continued

Proxy	Location	CADD Scaled C-Score	DANN Rank Score	FATHMM-MKL Noncoding Score	Eigen-PC Phred Score	LINSIGHT Score	Aorta eQTL[a]	Left Ventricle eQTL[a]	Liver eQTL[a]	Whole Blood eQTL[a]
rs6696066	ALPL(5):intronic(4)	0.05	0.15	0.03	0.03	0.04				
PALMD rs6702619										
rs11166276	.(0):intergenic(1)	1.81	0.08	0.14	8.56	0.04				
rs6702619	.(0):intergenic(1)	21.8	0.79	0.93	18.39	0.96				
rs7543039	.(0):intergenic(1)	3.33	0.38	0.16	6.48	0.04				
rs7543130	.(0):intergenic(1)	4.43	0.62	0.17	4.92	0.04				
NAV1 rs631556										
rs480937	NAV1(6):intronic(5)	4.63	0.71	0.16	7.82	0.05				
rs631556	NAV1(6):intronic(5)	0.36	0.4	0.11	10.75	0.09				

Table Continued

Proxy	Location	CADD Scaled C-Score	DANN Rank Score	FATHMM-MKL Noncoding Score	Eigen-PC Phred Score	LINSIGHT Score	Aorta eQTL[a]	Left Ventricle eQTL[a]	Liver eQTL[a]	Whole Blood eQTL[a]
rs516705	NAV1(6):intronic(5)	0.02	0.11	0.12	10.97	0.08				
rs665834	NAV1(6):upstream(1) intronic(5)	1.38	0.12	0.08	5.91	0.06				
rs665770	NAV1(6):upstream(1) intronic(5)	4.94	0.51	0.18	7.31	0.06				
rs511763	NAV1(6):upstream(1) intronic(5)	3.14	0.46	0.03	5.29	0.07				
rs536070	NAV1(6):upstream(1) intronic(5)	2.22	0.09	0.1	6.7	0.1				
rs650720	NAV1(6):upstream(1) intronic(5)	0.12	0.24	0.1	1.46	0.05				
rs529581	NAV1(6):upstream(1) intronic(5)	0.2	0.37	0.09	2.2	0.12				
rs528789	NAV1(6):intronic(6)	3.16	0.83	0.13	10.42	0.07				
rs527725	NAV1(6):intronic(6)	4.26	0.83	0.13	10.74	0.06				

Table Continued

Proxy	Location	CADD Scaled C-Score	DANN Rank Score	FATHMM-MKL Noncoding Score	Eigen-PC Phred Score	LINSIGHT Score	Aorta eQTL[a]	Left Ventricle eQTL[a]	Liver eQTL[a]	Whole Blood eQTL[a]
TEX41 rs7593336										
rs7593336	TEX41(2):downstream(2)|BC040861(1):ncRNA_intronic(1)|U80	0.12	0.53	0.09	0.91	0.05				
LPA rs10455872										
rs118039278	LPA(1):intronic(1)	0.42	0.4	0.11	1.85	0.04				
rs74617384	LPA(1):intronic(1)	0.58	0.61	0.05	0.3	0.04				
rs55730499	LPA(1):intronic(1)	0.09	0.36	0.04	0.06	0.05				
rs10455872	LPA(1):intronic(1)	0.15	0.12	0.06	0.14	0.05				
LPA rs140570886										
rs140570886	LPA(1):intronic(1)	2.57	0.27	0.07	0.66	0.04				

Table Continued

Proxy	Location	CADD Scaled C-Score	DANN Rank Score	FATHMM-MKL Noncoding Score	Eigen-PC Phred Score	LINSIGHT Score	Aorta eQTL[a]	Left Ventricle eQTL[a]	Liver eQTL[a]	Whole Blood eQTL[a]
IL6 rs1800797										
rs1800797	LOC541472(1):ncRNA_exonic(1)	9.92	0.68	0.14	16.9	0.13	*IL6-AS1*	*IL6-AS1*		
rs1800795	IL6(4):upstream(4)|LOC541472(1):ncRNA_intronic(1)	16.27	0.62	0.72	23	0.27	*IL6-AS1*	*IL6-AS1*		
FADS1/2 rs174533										
rs174533	MYRF(3):intronic(3)	5.51	0.39	0.17	18.51	0.12	*TMEM258, FADS2*	*FADS2, FADS1*	*FADS3*	*TMEM258, FADS2*
rs174535	MYRF(3):synonymous(3)	12.79	0.75	0.79	13.79		*TMEM258, FADS2*	*FADS2, FADS1*	*FADS3*	*TMEM258, FADS2*
rs174536	MYRF(3):intronic(3)|TMEM258(1):downstream(1)|BC020196(1):upstream(1)	0.99	0.19	0.1	12.57	0.07	*TMEM258, FADS2*	*FADS2, FADS1*	*FADS3*	*TMEM258, FADS2*
rs174537	MYRF(3):intronic(3)|TMEM258(1):downstream(1)|BC020196(1):upstream(1)	0.32	0.05	0.09	12.16	0.06	*TMEM258, FADS2*	*FADS2, FADS1*	*FADS3*	*TMEM258, FADS2*

Table Continued

Proxy	Location	CADD Scaled C-Score	DANN Rank Score	FATHMM-MKL Noncoding Score	Eigen-PC Phred Score	LINSIGHT Score	Aorta eQTL[a]	Left Ventricle eQTL[a]	Liver eQTL[a]	Whole Blood eQTL[a]
rs102275	FEN1(2):upstream(1)|TMEM258(1):intronic(1)|MYRF(3):downstream(3)|BC020196(1):downstream(1)|MIR611(1):downstream(1)	8.91	0.86	0.1	5.58	0.19	*TMEM258, FADS2*	*FADS2, FADS1*	*FADS3*	*TMEM258, FADS2*
rs102274	FEN1(2):upstream(1)|TMEM258(1):intronic(1)|MYRF(3):downstream(3)|BC020196(1):downstream(1)|MIR611(1):downstream(1)	1.82	0.32	0.11	1.21	0.1	*TMEM258, FADS2*	*FADS2, FADS1*	*FADS3*	*TMEM258, FADS2*
rs174545	FEN1(2):downstream(1)|FADS1(3):UTR3(2)downstream(1)	12.78	0.87	0.56	9.88	0.05	*TMEM258, FADS2*	*FADS2, FADS1*	*FADS3*	*TMEM258, FADS2*
rs174546	FADS1(3):UTR3(2)downstream(1)	12.71	0.79	0.83	9.88	0.27	*TMEM258, FADS2*	*FADS2, FADS1*	*FADS3*	*TMEM258, FADS2*
rs174547	FADS1(3):downstream(1)intronic(2)	5.18	0.47	0.14	8.71	0.05	*TMEM258, FADS2*	*FADS2, FADS1*	*FADS3*	*TMEM258, FADS2*
rs174550	FADS1(3):intronic(3)	7.21	0.91	0.12	8.39	0.05	*TMEM258, FADS2*	*FADS2, FADS1*	*FADS3*	*TMEM258, FADS2*
rs174553	FADS1(3):intronic(3)	4.74	0.53	0.1	4.68	0.05	*TMEM258, FADS2*	*FADS2, FADS1*	*FADS3*	*TMEM258, FADS2*

Table Continued

Proxy	Location	CADD Scaled C-Score	DANN Rank Score	FATHMM-MKL Noncoding Score	Eigen-PC Phred Score	LINSIGHT Score	Aorta eQTL[a]	Left Ventricle eQTL[a]	Liver eQTL[a]	Whole Blood eQTL[a]
rs174554	FADS2(4):upstream(2)|MIR1908(1):downstream(1)|FADS1(3):intronic(3)	2.79	0.05	0.06	8.25	0.06	*TMEM258, FADS2*	*FADS2, FADS1*	*FADS3*	*TMEM258, FADS2*
rs174562	FADS2(4):intronic(2)|MIR1908(1):upstream(1)|FADS1(3):upstream(3)	4.77	0.15	0.12	17.34	0.06	*TMEM258, FADS2*	*FADS2, FADS1*	*FADS3*	*TMEM258, FADS2*
rs174564	FADS2(4):intronic(2)|FADS1(3):upstream(1)	2.72	0.56	0.04	1.74	0.05	*TMEM258, FADS2*	*FADS2, FADS1*	*FADS3*	*TMEM258, FADS2*
rs174566	FADS2(4):upstream(2)intronic(2)	4.5	0.55	0.15	2.3	0.05	*TMEM258, FADS2*	*FADS2, FADS1*	*FADS3*	*TMEM258, FADS2*
rs174567	FADS2(4):upstream(2)intronic(2)	1.44	0.01	0.02	0.05	0.05	*TMEM258, FADS2*	*FADS2, FADS1*	*FADS3*	*TMEM258, FADS2*
rs174568	FADS2(4):upstream(2)intronic(2)	0.34	0.22	0.15	17.69		*TMEM258, FADS2*	*FADS2, FADS1*	*FADS3*	*TMEM258, FADS2*
rs99780	FADS2(4):intronic(4)	7.72	0.95	0.08	35.34	0.13	*TMEM258, FADS2*	*FADS2, FADS1*	*FADS3*	*TMEM258, FADS2*
rs1535	FADS2(4):intronic(4)	4.82	0.26	0.12	10.89	0.1	*TMEM258, FADS2*	*FADS2, FADS1*	*FADS3*	*TMEM258, FADS2*

Abbreviations: CADD, Combined Annotated Depletion Dependent; DANN, Deleterious Annotation of Genetic Variants Using Neural Networks; eQTL, expression quantitative trait loci; UTR, untranslated region.

[a] Defined as significant by the Genotype-Tissue Expression project[72] using gene-specific, permutation-based thresholds for significance.

Supplementary Table 6.3. Association of Significant Variants with Prevalent Aortic Valve Calcium Among European-Ancestry Participants in the CHARGE Consortium.

Chr.	Position (GRCh37)	Gene	Variant	Minor Allele	Odds Ratio per Minor Allele for Aortic Stenosis	*p* for Aortic Stenosis	Odds Ratio per Minor Allele for Aortic Valve Calcium	*p* for Aortic Valve Calcium
1	21,872,004	*ALPL*	rs6696066	A	0.92 (0.89, 0.94)	6.1E-10	1.02 (0.94, 1.11)	0.65
1	100,046,246	*PALMD*	rs6702619	G	1.16 (1.13, 1.19)	1.2E-25	1.02 (0.95, 1.11)	0.56
1	109,817,590	*CELSR2-SORT1*	rs12740374	T	0.90 (0.87, 0.94)	8.4E-09	0.95 (0.86, 1.05)	0.29
1	170,665,348	*PRRX1*	rs61817383	T	1.11 (1.08, 1.14)	2.0E-11	1.10 (1.01, 1.21)	0.035
1	201,743,185	*NAV1*	rs631556	A	1.10 (1.07, 1.13)	1.4E-10	1.05 (0.96, 1.15)	0.25
2	65,498,898	*ACTR2*	rs62139062	T	1.09 (1.06, 1.12)	4.2E-08	1.11 (1.01, 1.21)	0.029
2	145,836,429	*TEX41*	rs7593336	G	1.12 (1.09, 1.15)	7.3E-15	1.03 (0.94, 1.12)	0.55
3	153,856,332	*ARHGEF26*	rs6794263	C	0.88 (0.84, 0.92)	1.0E-08	0.96 (0.84, 1.09)	0.52
6	161,010,118	*LPA*	rs10455872	G	1.42 (1.35, 1.49)	4.6E-44	2.05 (1.63, 2.57)	9.0E-10
7	22,766,221	*IL6*	rs1800797	A	1.13 (1.10, 1.16)	2.9E-18	1.05 (0.97, 1.14)	0.22
9	106,871,788	*SMC2*	rs55909255	C	1.08 (1.05, 1.11)	4.7E-08	1.03 (0.95, 1.12)	0.49
11	202,017	*BET1L*	rs73386631	T	1.22 (1.14, 1.31)	2.8E-08	1.15 (0.93, 1.43)	0.21
11	270,514	*BET1L*	rs17156153	T	1.16 (1.10, 1.22)	4.2E-09	1.17 (1.02, 1.35)	0.028
11	61,549,025	*FADS1/2*	rs174533	A	0.91 (0.88, 0.94)	6.7E-09	0.91 (0.83, 0.99)	0.024
16	75,498,793	*TMEM170A*	rs11643207	C	0.92 (0.89, 0.95)	1.4E-08	0.97 (0.89, 1.05)	0.45
18	20,094,035	Intergenic	rs551520	T	0.91 (0.88, 0.94)	2.6E-09	1.03 (0.94, 1.14)	0.47

Associations with aortic stenosis have been provided to facilitate comparison. If the exact variant was not available, a proxy in high linkage disequilibrium ($r^2 \geq 0.8$) was used if available.

Supplementary Table 6.4. Association of Significant Variants with Aortic Stenosis in Other Ancestries.

Chr.	Position (GRCh37)	Gene	Variant	Minor Allele	Odds Ratio per Minor Allele in Europeans	*p* in Europeans	Odds Ratio per Minor Allele in African-	*p* in African-Americans	Odds Ratio per Minor Allele in Latin-Americans	*p* in Latin-Americans
1	21,872,004	*ALPL*	rs6696066	A	0.92 (0.89, 0.94)	6.10E-10	0.85 (0.61, 1.19)	0.35	0.80 (0.64, 1.00)	0.049
1	100,046,246	*PALMD*	rs6702619	G	1.16 (1.13, 1.19)	1.20E-25	1.22 (0.81, 1.83)	0.33	1.20 (0.94, 1.53)	0.14
1	109,817,590	*CELSR2-SORT1*	rs12740374	T	0.90 (0.87, 0.94)	8.40E-09	0.60 (0.39, 0.93)	0.021	0.73 (0.53, 0.99)	0.044
1	170,665,348	*PRRX1*	rs61817383	T	1.11 (1.08, 1.14)	2.00E-11	1.19 (0.73, 1.95)	0.47	0.98 (0.76, 1.25)	0.85
1	201,743,185	*NAV1*	rs631556	A	1.10 (1.07, 1.13)	1.40E-10	1.01 (0.68, 1.50)	0.98	0.93 (0.73, 1.18)	0.55
2	65,498,898	*ACTR2*	rs62139062	T	1.09 (1.06, 1.12)	4.20E-08	0.65 (0.43, 0.99)	0.047	1.22 (0.92, 1.62)	0.16
2	145,836,429	*TEX41*	rs7593336	G	1.12 (1.09, 1.15)	7.30E-15	0.88 (0.63, 1.23)	0.47	0.92 (0.73, 1.16)	0.49
3	153,856,332	*ARHGEF26*	rs6794263	C	0.88 (0.84, 0.92)	1.00E-08	1.26 (0.89, 1.77)	0.19	0.98 (0.76, 1.27)	0.91
6	161,010,118	*LPA*	rs10455872	G	1.42 (1.35, 1.49)	4.60E-44	4.52 (1.50, 13.62)	7.40E-03	1.04 (0.58, 1.87)	0.89
6	161,013,013	*LPA*	rs140570886	C	1.55 (1.40, 1.73)	5.10E-16	1.50 (0.29, 7.90)	0.63	0.82 (0.57, 1.18)	0.29
7	22,766,221	*IL6*	rs1800797	A	1.13 (1.10, 1.16)	2.90E-18	1.49 (0.97, 2.29)	0.068	0.84 (0.63, 1.12)	0.23
9	106,871,788	*SMC2*	rs55909255	C	1.08 (1.05, 1.11)	4.70E-08	1.17 (0.84, 1.62)	0.35	1.06 (0.84, 1.35)	0.62
11	202,017	*BET1L*	rs73386631	T	1.22 (1.14, 1.31)	2.80E-08	0.97 (0.31, 3.02)	0.95	1.63 (0.96, 2.77)	0.071
11	270,514	*BET1L*	rs17156153	T	1.16 (1.10, 1.22)	4.20E-09	0.90 (0.46, 1.78)	0.77	1.63 (1.04, 2.56)	0.032
11	61,549,025	*FADS1/2*	rs174533	A	0.91 (0.88, 0.94)	6.70E-09	0.82 (0.45, 1.49)	0.51	1.02 (0.80, 1.30)	0.86
13	31,121,064	*HMGB1*	rs181753401	A	2.29 (1.74, 3.02)	4.20E-09	NA	NA	0.00 (0.00, ~Inf)	0.71

Chr.	Position (GRCh37)	Gene	Variant	Minor Allele	Odds Ratio per Minor Allele in Europeans	*p* in Europeans	Odds Ratio per Minor Allele in African-	*p* in African-Americans	Odds Ratio per Minor Allele in Latin-Americans	*p* in Latin-Americans
16	75,498,793	*TMEM170A*	rs11643207	C	0.92 (0.89, 0.95)	1.40E-08	0.93 (0.67, 1.27)	0.64	1.25 (0.99, 1.60)	0.066
18	20,094,035	Intergenic	rs551520	T	0.91 (0.88, 0.94)	2.60E-09	1.14 (0.81, 1.61)	0.45	0.84 (0.62, 1.14)	0.26

Associations in African- and Latin-American participants are from the Genetic Epidemiology Research on Adult Health and Aging (GERA) cohort. Associations in European-ancestry participants from the meta-analysis have been provided to facilitate comparison.

Supplementary Table 6.5. Significant Regions in the Mean-p Based Association of Genes with Aortic Stenosis.

Gene Region	Chr.	Transcription ± 50 kb (GRCh37)	No. Variants	*p*	Bonferroni *p*
NBPF3	1	21,716,583-21,861,393	610	1.7E-07	3.1E-03
ALPL	1	21,785,475-21,954,905	790	1.8E-12	3.3E-08
RAP1GAP	1	21,872,708-22,046,010	769	8.1E-07	0.015
SARS	1	109,706,515-109,830,804	460	2.9E-07	5.4E-03
CELSR2	1	109,742,641-109,868,378	409	1.1E-07	2.1E-03
PSRC1	1	109,772,176-109,875,790	345	1.2E-07	2.2E-03
MYBPHL	1	109,784,987-109,899,663	341	9.4E-07	0.017
IL6R	1	154,327,669-154,491,926	581	1.3E-06	0.025
PRRX1	1	170,583,313-170,758,541	526	1.7E-07	3.1E-03
NAV1	1	201,458,243-201,846,102	1,489	9.6E-12	1.8E-07
RNF144A	2	7,007,523-7,259,011	1,000	1.2E-07	2.2E-03
FAHD2B	2	97,699,320-97,810,603	160	2.4E-06	0.045
ZAP70	2	98,280,031-98,406,990	319	1.7E-08	3.1E-04
TMEM131	2	98,322,799-98,662,389	882	3.1E-07	5.7E-03
ARHGEF26	3	153,788,792-154,025,616	730	9.4E-09	1.7E-04
MECOM	3	168,751,287-169,431,563	2,682	1.5E-08	2.7E-04
MUC20	3	195,366,293-195,517,004	271	1.1E-07	2.1E-03
IBSP	4	88,670,702-88,783,601	487	4.5E-07	8.3E-03
MGST2	4	140,536,922-140,711,899	752	5.4E-07	0.010
MAML3	4	140,587,545-141,125,233	1,813	1.2E-06	0.022
CDKN2AIP	4	184,315,788-184,419,348	617	1.3E-06	0.024
ISL1	5	50,628,958-50,740,564	307	1.5E-06	0.027
RPS23	5	81,519,139-81,624,235	304	2.0E-06	0.037
ATP6AP1L	5	81,551,166-81,664,147	353	4.6E-07	8.5E-03
SNX24	5	122,131,149-122,397,317	1,051	9.6E-07	0.018
PPIC	5	122,309,078-122,422,425	499	1.2E-06	0.022
LOC100652758	5	151,099,354-151,201,069	272	2.6E-07	4.8E-03
G3BP1	5	151,101,476-151,234,915	363	3.4E-07	6.3E-03
GLRA1	5	151,152,074-151,354,397	574	1.3E-07	2.4E-03
NKX2-5	5	172,609,107-172,712,315	373	1.2E-06	0.022
SLC17A1	6	25,733,125-25,882,287	712	1.1E-06	0.020
ATF6B	6	32,033,045-32,146,017	378	3.2E-07	5.9E-03
FKBPL	6	32,046,484-32,148,067	339	2.4E-07	4.5E-03

PRRT1	6	32,066,140-32,169,720	350	6.3E-08	1.2E-03
PPT2	6	32,071,229-32,181,458	391	2.3E-09	4.2E-05
EGFL8	6	32,082,382-32,186,062	385	3.3E-08	6.1E-04
AGPAT1	6	32,085,983-32,195,888	471	7.9E-08	1.5E-03
RNF5	6	32,096,162-32,198,570	452	1.1E-07	2.1E-03
AGER	6	32,098,745-32,202,099	485	1.2E-06	0.022
PBX2	6	32,102,510-32,207,963	522	2.6E-07	4.9E-03
GPSM3	6	32,108,543-32,213,300	552	2.3E-06	0.042
SLC22A3	6	160,719,405-160,923,613	894	3.3E-10	6.1E-06
LPA	6	160,902,506-161,147,478	799	6.8E-13	1.3E-08
PLG	6	161,073,225-161,225,086	648	5.1E-07	9.4E-03
IL6	7	22,716,766-22,821,621	493	7.8E-18	1.5E-13
TOMM7	7	22,802,251-22,912,471	517	1.4E-06	0.025
CLDN23	8	8,509,666-8,611,617	614	3.2E-07	5.8E-03
MFHAS1	8	8,590,864-8,801,131	1,221	2.1E-07	3.8E-03
MSRA	8	9,861,830-10,336,401	2,520	5.3E-07	9.8E-03
RP1L1	8	10,413,859-10,562,617	796	2.0E-08	3.7E-04
C8orf74	8	10,480,147-10,608,103	630	2.6E-08	4.9E-04
SOX7	8	10,531,278-10,638,084	542	1.7E-07	3.1E-03
PINX1	8	10,572,473-10,747,394	900	7.7E-09	1.4E-04
XKR6	8	10,703,654-11,108,875	1,818	9.8E-10	1.8E-05
FAM167A	8	11,228,972-11,374,276	939	2.2E-08	4.1E-04
BLK	8	11,301,521-11,472,108	1,074	1.5E-09	2.8E-05
CHRAC1	8	141,471,397-141,577,252	379	2.7E-07	5.0E-03
AGO2	8	141,491,264-141,695,646	783	5.9E-10	1.1E-05
PTK2	8	141,618,481-142,061,412	1,289	1.8E-08	3.4E-04
NRP1	10	33,416,419-33,673,833	1,161	1.9E-06	0.035
TMEM180	10	104,171,149-104,289,546	376	9.9E-07	0.018
ACTR1A	10	104,188,986-104,312,580	422	3.8E-08	7.0E-04
SUFU	10	104,213,719-104,443,214	716	2.6E-07	4.8E-03
SCGB1C1	11	138,101-244,575	292	1.2E-07	2.3E-03
ODF3	11	146,761-250,273	337	2.7E-07	4.9E-03
BET1L	11	152,924-257,422	376	1.3E-08	2.5E-04
RIC8A	11	157,499-265,113	412	2.6E-07	4.8E-03
SIRT3	11	165,030-286,362	516	1.9E-08	3.5E-04
PSMD13	11	186,808-302,984	602	1.2E-07	2.3E-03
NLRP6	11	228,570-335,388	557	5.3E-07	9.9E-03
ATHL1	11	238,480-347,511	573	1.3E-06	0.024
MYRF	11	61,470,121-61,605,990	356	3.5E-07	6.5E-03

TMEM258	11	61,506,602-61,610,085	261	2.4E-07	4.4E-03
FEN1	11	61,510,109-61,614,716	277	2.0E-07	3.8E-03
FADS1	11	61,517,097-61,634,529	343	1.6E-08	3.0E-04
FADS2	11	61,533,675-61,684,826	466	6.6E-08	1.2E-03
ATP2A2	12	110,669,032-110,838,898	487	2.2E-06	0.042
ANAPC7	12	110,760,705-110,891,535	407	4.6E-07	8.5E-03
ARPC3	12	110,822,695-110,938,222	379	7.0E-07	0.013
GPN3	12	110,840,291-110,956,667	378	3.8E-08	7.0E-04
FAM216A	12	110,856,232-110,978,192	397	1.3E-07	2.3E-03
VPS29	12	110,879,330-110,989,916	369	7.6E-08	1.4E-03
RAD9B	12	110,890,005-111,023,952	432	1.1E-06	0.021
TCTN1	12	111,001,832-111,136,935	369	1.4E-06	0.026
HVCN1	12	111,036,491-111,180,276	418	2.6E-07	4.8E-03
PPP1CC	12	111,107,613-111,230,783	368	7.6E-07	0.014
RPL6	12	112,792,994-112,906,647	218	8.3E-07	0.015
CFDP1	16	75,277,608-75,517,387	1,111	2.9E-10	5.4E-06
TMEM170A	16	75,427,136-75,548,584	560	4.5E-10	8.4E-06
CHST6	16	75,457,022-75,578,926	570	4.3E-10	7.9E-06
ZFPM1	16	88,470,014-88,651,574	929	1.7E-06	0.032
SMARCA4	19	11,021,598-11,222,958	743	2.3E-08	4.3E-04
LDLR	19	11,150,037-11,294,506	682	2.3E-10	4.2E-06
SPC24	19	11,207,444-11,316,563	537	1.6E-06	0.030
DGCR8	22	20,017,755-20,149,400	574	2.6E-06	0.048

Regions are arranged by chromosomal position. Bonferroni p values were calculated by multiplying the observed p value by 18,539 (the number of gene regions tested), with a Bonferroni $p \leq 0.05$ considered significant.

Supplementary Table 6.6. Genetic Correlations with Aortic Stenosis.

Trait	Genetic Correlation (95% CI)	*p*	FDR *p*
Body Mass Index	0.25 (0.17 to 0.33)	4.8E-10	7.5E-08
Overweight	0.28 (0.19 to 0.37)	1.6E-09	1.2E-07
Waist Circumference	0.21 (0.14 to 0.29)	6.2E-08	3.0E-06
Obesity Class 1	0.24 (0.15 to 0.33)	7.7E-08	3.0E-06
Hip Circumference	0.21 (0.13 to 0.29)	4.1E-07	1.3E-05
Obesity Class 2	0.25 (0.15 to 0.36)	1.7E-06	4.6E-05
Extreme Body Mass Index	0.28 (0.16 to 0.41)	1.6E-05	3.5E-04
Total Lipids in Very Large VLDL	0.29 (0.12 to 0.45)	6.0E-04	0.010
Total Lipids in Chylomicrons and Largest VLDL Particles	0.31 (0.13 to 0.48)	6.0E-04	0.010
Triglycerides in Very Large VLDL	0.26 (0.10 to 0.42)	1.2E-03	0.019
Phospholipids in Very Large VLDL	0.28 (0.11 to 0.45)	1.4E-03	0.019
Childhood Obesity	0.20 (0.07 to 0.32)	1.6E-03	0.019
Cholesterol Esters in Large VLDL	0.26 (0.10 to 0.42)	1.6E-03	0.019
Height; Females at Age 10 and Males at Age 12	0.19 (0.07 to 0.31)	1.7E-03	0.019
Total Cholesterol in Large VLDL	0.26 (0.09 to 0.43)	2.1E-03	0.022
Free Cholesterol in Large VLDL	0.25 (0.09 to 0.40)	2.2E-03	0.022
Total Lipids in Large VLDL	0.26 (0.09 to 0.42)	2.4E-03	0.022
Concentration of Medium VLDL Particles	0.25 (0.09 to 0.42)	3.2E-03	0.025
Triglycerides in Large VLDL	0.26 (0.09 to 0.43)	3.3E-03	0.025
HbA1C	0.24 (0.08 to 0.40)	3.4E-03	0.025
Waist-to-Hip Ratio	0.11 (0.04 to 0.19)	3.4E-03	0.025
Concentration of Large VLDL Particles	0.26 (0.08 to 0.44)	4.3E-03	0.031
Phospholipids in Large VLDL	0.24 (0.07 to 0.41)	5.3E-03	0.036
Cholesterol Esters in Large HDL	-0.28 (-0.47 to -0.08)	5.8E-03	0.038
Total Lipids in Medium VLDL	0.23 (0.06 to 0.39)	6.5E-03	0.041
Triglycerides in Chylomicrons and Largest VLDL Particles	0.25 (0.07 to 0.44)	7.9E-03	0.046
Phospholipids in Chylomicrons and Largest VLDL Particles	0.23 (0.06 to 0.41)	8.0E-03	0.046
Mean Diameter for HDL Particles	-0.27 (-0.48 to -0.07)	8.3E-03	0.046
Total Cholesterol in Large HDL	-0.28 (-0.48 to -0.07)	8.6E-03	0.046
Total Lipids in Large HDL	-0.26 (-0.45 to -0.07)	8.7E-03	0.046
Total Lipids in Small VLDL	0.22 (0.05 to 0.39)	0.010	0.049

Triglycerides in Medium VLDL	0.25 (0.06 to 0.45)	0.010	0.049
Total Cholesterol in Small VLDL	0.27 (0.06 to 0.47)	0.011	0.049
Type 2 Diabetes	0.17 (0.04 to 0.30)	0.011	0.049

Abbreviations: VLDL, very low-density lipoprotein; HDL, high-density lipoprotein.

Supplementary Table 6.7. Significant Associations in the Phenome-Wide Association Study.

Trait	Gene	Variant	Effect Allele	Odds Ratio (95% CI)	Effect Size (95% CI)	*p*	FDR *p*
Alkaline Phosphatase, Natural Logarithm of U/L	*ALPL*	rs6696066	G	NA	-0.011 (-0.012, -0.093)	1.20E-45	6.90E-44
Phosphate, mmol/L	*ALPL*	rs6696066	G	NA	-0.0027 (-0.0036, -0.0018)	2.60E-09	7.60E-08
Height, cm	*ALPL*	rs6696066	G	NA	0.065 (0.031, 0.100)	2.20E-04	4.20E-03
Hip Circumference, cm	*ARHGEF26*	rs6794263	A	NA	0.17 (0.09, 0.24)	1.90E-05	1.10E-03
Body Mass Index	*ARHGEF26*	rs6794263	A	NA	0.066 (0.026, 0.100)	1.10E-03	0.031
Height, cm	*BET1L*	rs73386631	T	NA	-0.18 (-0.27, -0.10)	1.60E-05	9.10E-04
Alkaline Phosphatase, Natural Logarithm of U/L	*BET1L*	rs73386631	T	NA	0.0060 (0.0023, 0.0096)	1.30E-03	0.037
Apolipoprotein B, g/L	*CELSR2-SORT1*	rs12740374	G	NA	0.036 (0.034, 0.038)	4.1E-419	< 1E-400
LDL Cholesterol, mmol/L	*CELSR2-SORT1*	rs12740374	G	NA	0.094 (0.088, 0.100)	4.20E-213	1.20E-211
Coronary Artery Disease	*CELSR2-SORT1*	rs12740374	G	1.12 (1.09, 1.15)	NA	6.80E-23	1.30E-21
Insulin Growth Factor 1, nmol/L	*CELSR2-SORT1*	rs12740374	G	NA	-0.17 (-0.21, -0.14)	3.10E-20	4.50E-19
Myocardial Infarction	*CELSR2-SORT1*	rs12740374	G	1.13 (1.10, 1.17)	NA	7.50E-14	8.70E-13
Albumin, g/L	*CELSR2-SORT1*	rs12740374	G	NA	0.062 (0.044, 0.080)	1.10E-11	1.10E-10
C-Reactive Protein, Natural Logarithm of mg/L	*CELSR2-SORT1*	rs12740374	G	NA	-0.019 (-0.026, -0.012)	1.50E-07	1.20E-06

Table Continued

Trait	Gene	Variant	Effect Allele	Odds Ratio (95% CI)	Effect Size (95% CI)	*p*	FDR *p*
Creatinine, umol/L	*CELSR2-SORT1*	rs12740374	G	NA	-0.29 (-0.40, -0.18)	2.10E-07	1.50E-06
Calcium, mmol/L	*CELSR2-SORT1*	rs12740374	G	NA	0.0017 (0.0010, 0.0024)	4.90E-07	3.20E-06
Height, cm	*CELSR2-SORT1*	rs12740374	G	NA	-0.088 (-0.130, -0.047)	3.10E-05	1.80E-04
Alanine Aminotransferase, U/L	*CELSR2-SORT1*	rs12740374	G	NA	0.17 (0.08, 0.26)	1.90E-04	9.90E-04
Bilirubin, umol/L	*CELSR2-SORT1*	rs12740374	G	NA	0.053 (0.025, 0.080)	2.20E-04	1.10E-03
Lipoprotein(a), Natural Logarithm of nmol/L	*CELSR2-SORT1*	rs12740374	G	NA	0.016 (0.007, 0.024)	2.50E-04	1.10E-03
Gamma-Glutamyl Transferase, Natural Logarithm of U/L	*CELSR2-SORT1*	rs12740374	G	NA	0.0073 (0.0034, 0.0113)	3.00E-04	1.20E-03
Heart Failure	*CELSR2-SORT1*	rs12740374	G	1.08 (1.03, 1.14)	NA	1.20E-03	4.60E-03
Diastolic Blood Pressure, mmHg	*CELSR2-SORT1*	rs12740374	G	NA	-0.11 (-0.18, -0.04)	1.50E-03	5.40E-03
Triglycerides, Natural Logarithm of mmol/L	*CELSR2-SORT1*	rs12740374	G	NA	0.0052 (0.0019, 0.0084)	1.80E-03	6.30E-03
Heel Bone Mineral Density (Right), g/cm2	*CELSR2-SORT1*	rs12740374	G	NA	-0.0021 (-0.0035, -0.007)	2.80E-03	9.20E-03
Peripheral Vascular Disease	*CELSR2-SORT1*	rs12740374	G	1.08 (1.02, 1.14)	NA	6.30E-03	0.019
Triglycerides, Natural Logarithm of mmol/L	*FADS1/2*	rs174533	G	NA	-0.028 (-0.031, -0.026)	7.00E-85	4.10E-83

Table Continued

Trait	Gene	Variant	Effect Allele	Odds Ratio (95% CI)	Effect Size (95% CI)	*p*	FDR *p*
Alkaline Phosphatase, Natural Logarithm of U/L	*FADS1/2*	rs174533	G	NA	-0.013 (-0.014, -0.011)	7.50E-58	2.20E-56
Apolipoprotein B, g/L	*FADS1/2*	rs174533	G	NA	0.011 (0.009, 0.012)	1.10E-49	2.20E-48
LDL Cholesterol, mmol/L	*FADS1/2*	rs174533	G	NA	0.027 (0.021, 0.032)	5.70E-24	8.30E-23
Insulin Growth Factor 1, nmol/L	*FADS1/2*	rs174533	G	NA	0.10 (0.07, 0.14)	1.80E-10	2.10E-09
Height, cm	*FADS1/2*	rs174533	G	NA	0.11 (0.08, 0.15)	1.60E-09	1.50E-08
Bilirubin, umol/L	*FADS1/2*	rs174533	G	NA	0.073 (0.049, 0.097)	4.10E-09	3.40E-08
Asthma	*FADS1/2*	rs174533	G	1.06 (1.04, 1.09)	NA	2.10E-08	1.50E-07
HbA1c, mmol/mol	*FADS1/2*	rs174533	G	NA	0.093 (0.055, 0.132)	2.20E-06	1.40E-05
Gamma-Glutamyl Transferase, Natural Logarithm of U/L	*FADS1/2*	rs174533	G	NA	-0.0077 (-0.0112, -0.0043)	1.20E-05	6.90E-05
Hypothyroidism	*FADS1/2*	rs174533	G	1.05 (1.03, 1.08)	NA	7.60E-05	4.00E-04
Heel Bone Mineral Density (Right), g/cm2	*FADS1/2*	rs174533	G	NA	-0.0023 (-0.0035, -0.0011)	1.30E-04	6.00E-04
Calcium, mmol/L	*FADS1/2*	rs174533	G	NA	0.0011 (0.0006, 0.0017)	1.40E-04	6.00E-04
C-Reactive Protein, Natural Logarithm of mg/L	*FADS1/2*	rs174533	G	NA	0.011 (0.005, 0.017)	4.30E-04	1.80E-03

Table Continued

Trait	Gene	Variant	Effect Allele	Odds Ratio (95% CI)	Effect Size (95% CI)	*p*	FDR *p*
Glucose, mmol/L	*FADS1/2*	rs174533	G	NA	0.012 (0.005, 0.020)	1.80E-03	6.90E-03
Alanine Aminotransferase, U/L	*FADS1/2*	rs174533	G	NA	0.12 (0.04, 0.20)	2.00E-03	7.20E-03
Cystatin C, mg/L	*FADS1/2*	rs174533	G	NA	0.0015 (0.0004, 0.0025)	5.40E-03	0.018
Albumin, g/L	*FADS1/2*	rs174533	G	NA	-0.021 (-0.037, -0.006)	8.00E-03	0.026
Gout	*FADS1/2*	rs174533	G	0.93 (0.87, 0.99)	NA	0.016	0.049
Systolic Blood Pressure	*IL6*	rs1800797	A	NA	0.24 (0.14, 0.35)	5.00E-06	2.60E-04
Albumin, g/L	*IL6*	rs1800797	A	NA	-0.034 (-0.050, -0.019)	9.00E-06	2.60E-04
C-Reactive Protein, Natural Logarithm of mg/L	*IL6*	rs1800797	A	NA	0.013 (0.007, 0.018)	2.90E-05	5.60E-04
Cystatin C, mg/L	*IL6*	rs1800797	A	NA	0.0017 (0.0007, 0.0027)	7.50E-04	0.011
Asthma	*IL6*	rs1800797	A	0.97 (0.95, 0.99)	NA	1.10E-03	0.012
Albumin, g/L	Intergenic	rs551520	C	NA	-0.030 (-0.047, -0.013)	5.30E-04	0.018
Multiple Sclerosis	Intergenic	rs551520	C	0.83 (0.75, 0.93)	NA	8.50E-04	0.018
Hip Circumference, cm	Intergenic	rs551520	C	NA	-0.096 (-0.152, -0.039)	9.40E-04	0.018

Table Continued

Trait	Gene	Variant	Effect Allele	Odds Ratio (95% CI)	Effect Size (95% CI)	*p*	FDR *p*
Height, cm	Intergenic	rs551520	C	NA	-0.064 (-0.103, -0.024)	1.50E-03	0.022
Sleep Apnea	Intergenic	rs551520	C	0.92 (0.88, 0.97)	NA	3.00E-03	0.035
Lipoprotein(a), Natural Logarithm of nmol/L	*LPA*	rs10455872	G	NA	1.72 (1.71, 1.73)	1.6E-17084	< 1E-400
Coronary Artery Disease	*LPA*	rs10455872	G	1.32 (1.28, 1.36)	NA	1.40E-69	3.90E-68
Apolipoprotein B, g/L	*LPA*	rs10455872	G	NA	0.018 (0.015, 0.020)	5.80E-45	1.10E-43
Myocardial Infarction	*LPA*	rs10455872	G	1.34 (1.29, 1.40)	NA	1.20E-40	1.70E-39
LDL Cholesterol, mmol/L	*LPA*	rs10455872	G	NA	0.061 (0.052, 0.071)	1.80E-40	2.10E-39
Triglycerides, Natural Logarithm of mmol/L	*LPA*	rs10455872	G	NA	-0.028 (-0.033, -0.023)	4.00E-28	3.80E-27
Peripheral Vascular Disease	*LPA*	rs10455872	G	1.28 (1.19, 1.38)	NA	3.00E-11	2.50E-10
Albumin, g/L	*LPA*	rs10455872	G	NA	-0.074 (-0.102, -0.047)	9.70E-08	7.00E-07
Heart Failure	*LPA*	rs10455872	G	1.19 (1.11, 1.27)	NA	5.60E-07	3.60E-06
Diastolic Blood Pressure, mmHg	*LPA*	rs10455872	G	NA	-0.19 (-0.30, -0.09)	2.00E-04	1.20E-03
Insulin Growth Factor 1, nmol/L	*LPA*	rs10455872	G	NA	0.094 (0.039, 0.150)	9.00E-04	4.80E-03

Table Continued

Trait	Gene	Variant	Effect Allele	Odds Ratio (95% CI)	Effect Size (95% CI)	*p*	FDR *p*
HbA1c, mmol/mol	*LPA*	rs10455872	G	NA	0.10 (0.03, 0.17)	3.10E-03	0.014
Calcium, mmol/L	*LPA*	rs10455872	G	NA	-0.0015 (-0.0025, -0.0005)	3.60E-03	0.015
Chronic Kidney Disease	*LPA*	rs10455872	G	1.09 (1.02, 1.16)	NA	0.01	0.039
Lipoprotein(a), Natural Logarithm of nmol/L	*LPA*	rs140570886	C	NA	1.29 (1.23, 1.34)	3.7E-505	< 1E-400
Coronary Artery Disease	*LPA*	rs140570886	C	1.57 (1.47, 1.67)	NA	2.10E-41	6.00E-40
Triglycerides, Natural Logarithm of mmol/L	*LPA*	rs140570886	C	NA	-0.063 (-0.075, -0.052)	1.10E-28	2.10E-27
Myocardial Infarction	*LPA*	rs140570886	C	1.55 (1.41, 1.70)	NA	2.70E-21	4.00E-20
Apolipoprotein B, g/L	*LPA*	rs140570886	C	NA	0.022 (0.017, 0.028)	2.00E-15	2.40E-14
LDL Cholesterol, mmol/L	*LPA*	rs140570886	C	NA	0.076 (0.056, 0.097)	1.80E-13	1.70E-12
Peripheral Vascular Disease	*LPA*	rs140570886	C	1.37 (1.17, 1.60)	NA	1.00E-04	8.50E-04
Heart Failure	*LPA*	rs140570886	C	1.32 (1.14, 1.52)	NA	2.00E-04	1.50E-03
Albumin, g/L	*LPA*	rs140570886	C	NA	-0.11 (-0.18, -0.05)	2.90E-04	1.90E-03
C-Reactive Protein, Natural Logarithm of mg/L	*LPA*	rs140570886	C	NA	-0.041 (-0.065, -0.018)	6.60E-04	3.80E-03

Table Continued

Trait	Gene	Variant	Effect Allele	Odds Ratio (95% CI)	Effect Size (95% CI)	*p*	FDR *p*
HbA1c, mmol/mol	*LPA*	rs140570886	C	NA	0.21 (0.06, 0.36)	7.00E-03	0.037
Diastolic Blood Pressure, mmHg	*NAV1*	rs631556	A	NA	-0.14 (-0.20, -0.09)	8.20E-07	4.70E-05
Coronary Artery Disease	*NAV1*	rs631556	A	1.03 (1.02, 1.05)	NA	3.00E-04	8.70E-03
Height, cm	*PRRX1*	rs61817383	T	NA	-0.11 (-0.15, -0.07)	3.30E-08	1.90E-06
Heel Bone Mineral Density (Right), g/cm2	*PRRX1*	rs61817383	T	NA	0.0023 (0.0009, 0.0036)	5.50E-04	0.016
Height, cm	*SMC2*	rs55909255	C	NA	-0.070 (-0.105, -0.034)	1.10E-04	6.30E-03
Triglycerides, Natural Logarithm of mmol/L	*TEX41*	rs7593336	G	NA	-0.0052 (-0.0080, -0.0024)	2.60E-04	1.00E-02
Atrial Fibrillation	*TEX41*	rs7593336	G	1.17 (1.07, 1.27)	NA	3.50E-04	1.00E-02
Coronary Artery Disease	*TEX41*	rs7593336	G	1.03 (1.01, 1.05)	NA	5.20E-04	1.00E-02
Heel Bone Mineral Density (Right), g/cm2	*TEX41*	rs7593336	G	NA	-0.0018 (-0.0030, -0.0006)	2.80E-03	0.039
Diastolic Blood Pressure, mmHg	*TEX41*	rs7593336	G	NA	-0.086 (-0.143, -0.028)	3.40E-03	0.039
Coronary Artery Disease	*TMEM170A*	rs11643207	T	1.05 (1.03, 1.07)	NA	2.70E-06	1.50E-04
Systolic Blood Pressure, mmHg	*TMEM170A*	rs11643207	T	NA	0.22 (0.12, 0.33)	3.80E-05	1.10E-03

Table Continued

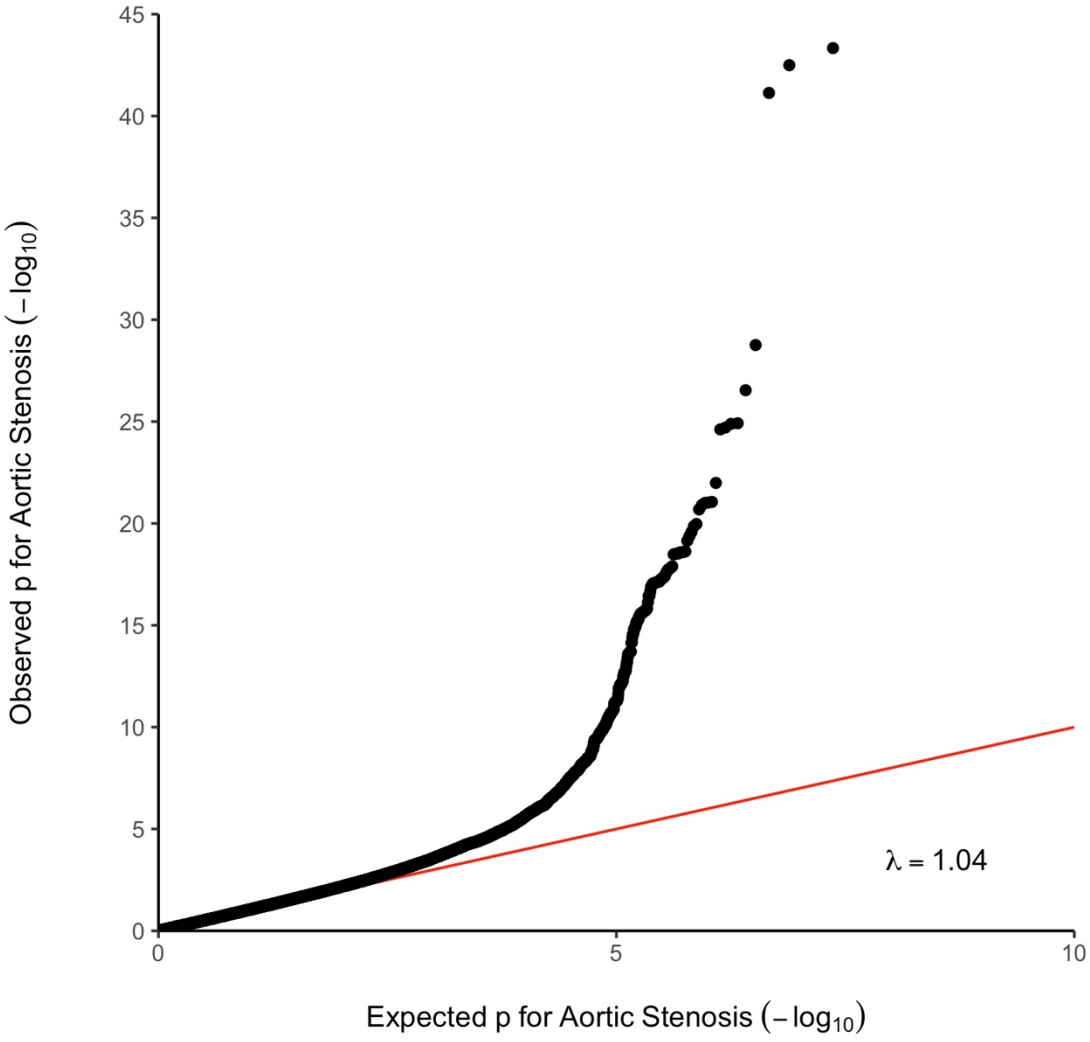
Trait	Gene	Variant	Effect Allele	Odds Ratio (95% CI)	Effect Size (95% CI)	*p*	FDR *p*
Triglycerides, Natural Logarithm of mmol/L	*TMEM170A*	rs11643207	T	NA	-0.0049 (-0.0077, -0.0021)	6.20E-04	0.011
Body Mass Index	*TMEM170A*	rs11643207	T	NA	-0.045 (-0.071, -0.019)	7.90E-04	0.011
Height, cm	*TMEM170A*	rs11643207	T	NA	0.060 (0.024, 0.096)	1.00E-03	0.012
C-Reactive Protein, Natural Logarithm of mg/L	*TMEM170A*	rs11643207	T	NA	-0.0091 (-0.0151, -0.0031)	3.00E-03	0.029
Apolipoprotein B, g/L	*TMEM170A*	rs11643207	T	NA	-0.0020 (-0.0034, -0.0006)	5.20E-03	0.043

Associations are shown if they remained significant after a 5% false discovery rate correction.

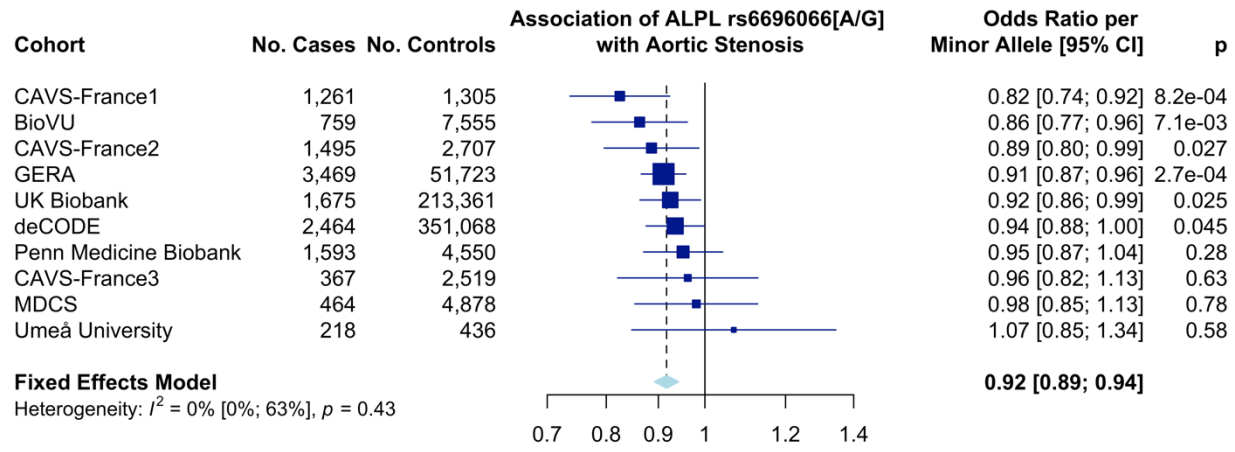
Abbreviation: FDR, false discovery rate.

Supplementary Table 6.8. Mendelian Randomization for Aortic Stenosis.

Trait	Method	Odds Ratio (95% CI)	*p*
Lipoprotein(a) (Natural Logarithm)	Inverse-Variance Weighted	1.22 (1.19, 1.24)	4.8E-73
	Penalized Weighted Median	1.23 (1.19, 1.26)	1.0E-45
	Egger	1.22 (1.19, 1.25)	3.1E-55
	Egger Intercept	NA	0.92
Alkaline Phosphatase (Natural Logarithm)	Inverse-Variance Weighted	1.10 (0.91, 1.32)	0.34
	Penalized Weighted Median	0.67 (0.48, 0.93)	0.017
	Egger	0.84 (0.65, 1.08)	0.18
	Egger Intercept	NA	3.1E-03
C-Reactive Protein (Natural Logarithm)	Inverse-Variance Weighted	1.08 (1.02, 1.15)	0.013
	Penalized Weighted Median	1.13 (1.01, 1.28)	0.038
	Egger	0.99 (0.91, 1.08)	0.80
	Egger Intercept	NA	4.0E-03
Low-Density Lipoprotein Cholesterol (mmol/L)	Inverse-Variance Weighted	1.61 (1.48, 1.75)	1.3E-30
	Penalized Weighted Median	1.57 (1.39, 1.78)	9.3E-13
	Egger	1.67 (1.49, 1.87)	1.2E-18
	Egger Intercept	NA	0.36
Apolipoprotein B (g/L)	Inverse-Variance Weighted	4.16 (3.16, 5.46)	1.7E-24
	Penalized Weighted Median	4.40 (2.91, 6.65)	2.2E-12
	Egger	4.09 (2.83, 5.91)	7.3E-14
	Egger Intercept	NA	0.90
Body Mass Index (kg/m^2)	Inverse-Variance Weighted	1.08 (1.06, 1.10)	5.8E-20
	Penalized Weighted Median	1.09 (1.07, 1.12)	2.3E-11
	Egger	1.07 (1.02, 1.12)	7.6E-03
	Egger Intercept	NA	0.61



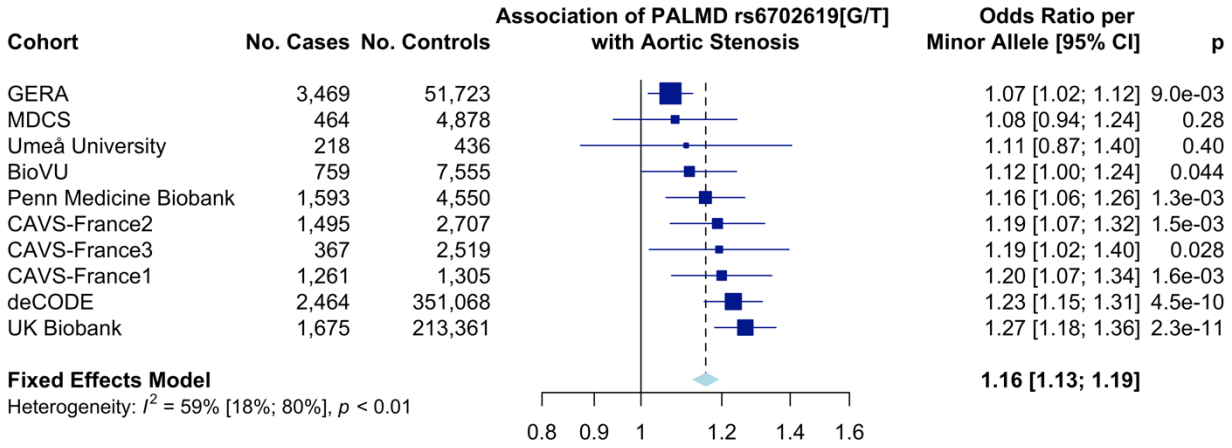
Supplementary Figure 6.1. Quantile-Quantile Plot for the Observed Versus Expected p in the Meta-Analysis.



Supplementary Figure 6.2. Meta-Analysis of the Association of *ALPL* rs6696066 with Aortic Stenosis.

Abbreviations: GERA, Genetic Epidemiology Research on Adult Health and Aging; MDCS, Malmö Diet and Cancer Study.

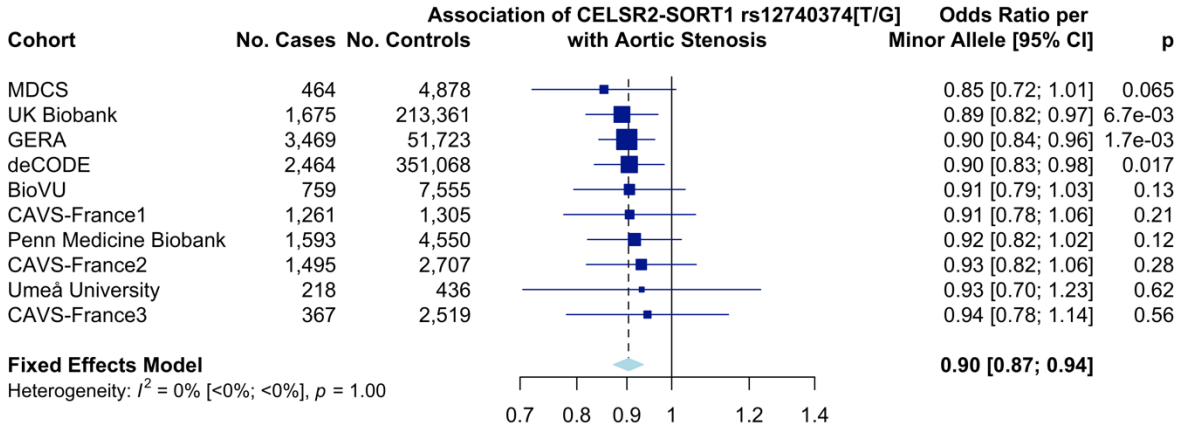
Estimates were combined using an inverse-variance weighted, fixed effects meta-analysis. Box widths are proportional to the weights of the estimates in the meta-analysis. Alleles are coded as [minor/major].



Supplementary Figure 6.3. Meta-Analysis of the Association of *PALMD* rs6702619 with Aortic Stenosis.

Abbreviations: GERA, Genetic Epidemiology Research on Adult Health and Aging; MDCS, Malmö Diet and Cancer Study.

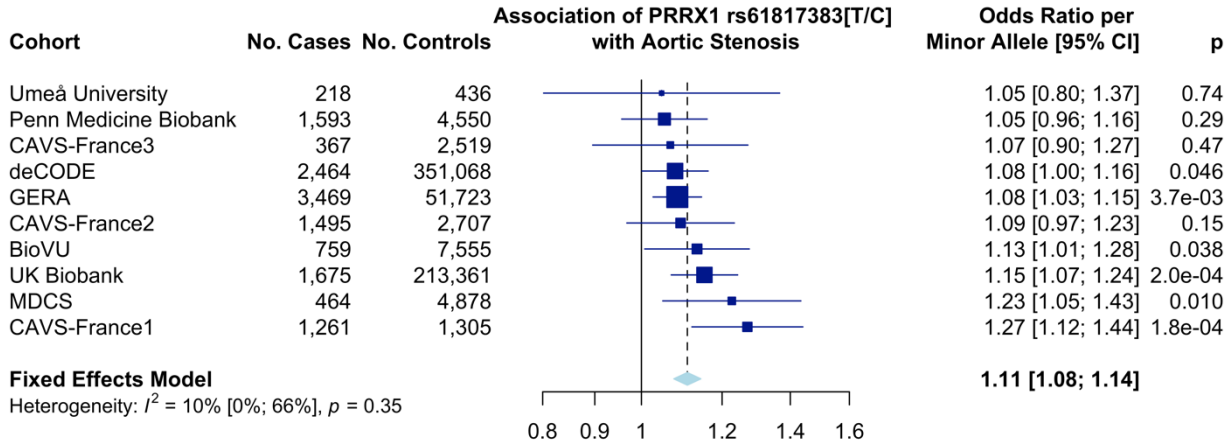
Estimates were combined using an inverse-variance weighted, fixed effects meta-analysis. Box widths are proportional to the weights of the estimates in the meta-analysis. Alleles are coded as [minor/major].



Supplementary Figure 6.4. Meta-Analysis of the Association of *CELSR2-SORT1* rs12740374 with Aortic Stenosis.

Abbreviations: GERA, Genetic Epidemiology Research on Adult Health and Aging; MDCS, Malmö Diet and Cancer Study.

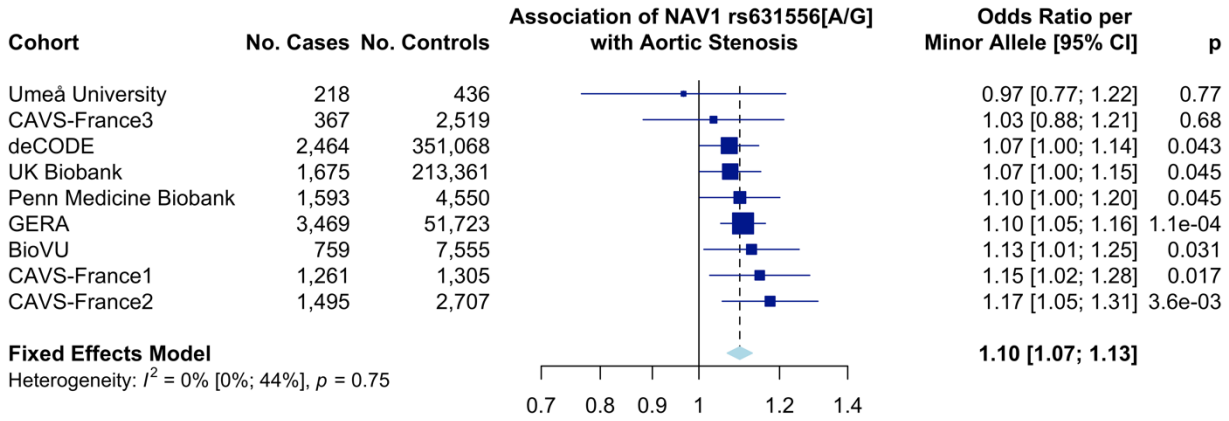
Estimates were combined using an inverse-variance weighted, fixed effects meta-analysis. Box widths are proportional to the weights of the estimates in the meta-analysis. Alleles are coded as [minor/major].



Supplementary Figure 6.5. Meta-Analysis of the Association of *PRRX1* rs61817383 with Aortic Stenosis.

Abbreviations: GERA, Genetic Epidemiology Research on Adult Health and Aging; MDCS, Malmö Diet and Cancer Study.

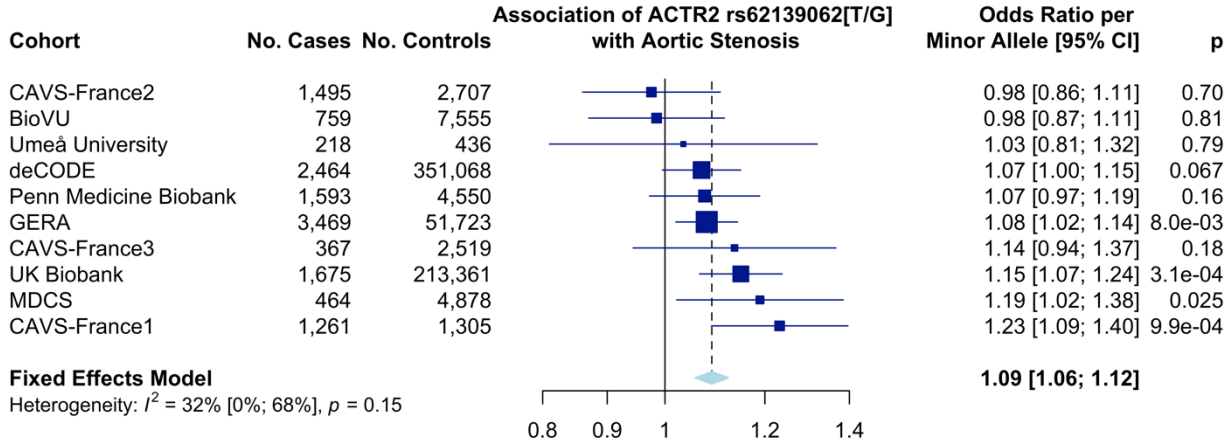
Estimates were combined using an inverse-variance weighted, fixed effects meta-analysis. Box widths are proportional to the weights of the estimates in the meta-analysis. Alleles are coded as [minor/major].



Supplementary Figure 6.6. Meta-Analysis of the Association of *NAV1* rs631556 with Aortic Stenosis.

Abbreviations: GERA, Genetic Epidemiology Research on Adult Health and Aging.

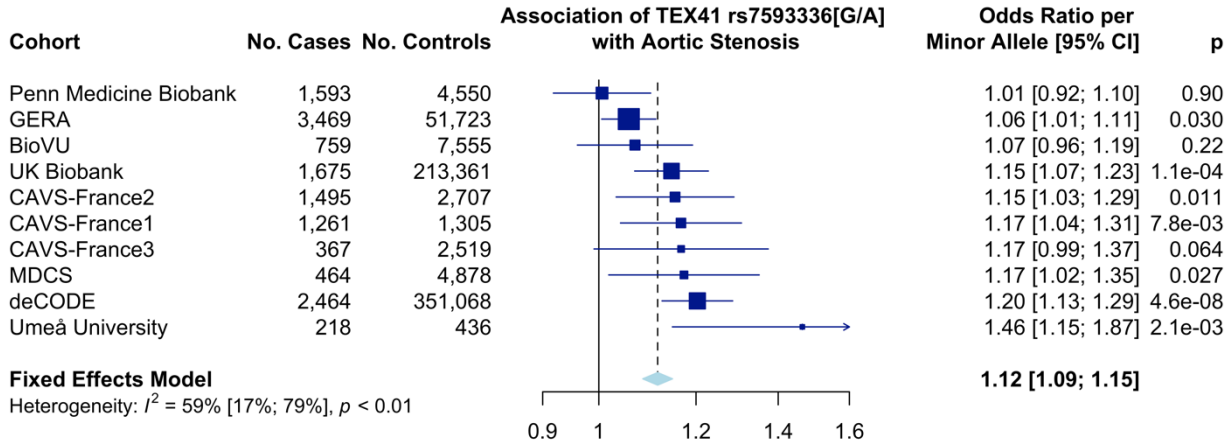
Estimates were combined using an inverse-variance weighted, fixed effects meta-analysis. Box widths are proportional to the weights of the estimates in the meta-analysis. Alleles are coded as [minor/major].



Supplementary Figure 6.7 Meta-Analysis of the Association of *ACTR2* rs62139062 with Aortic Stenosis.

Abbreviations: GERA, Genetic Epidemiology Research on Adult Health and Aging; MDCS, Malmö Diet and Cancer Study.

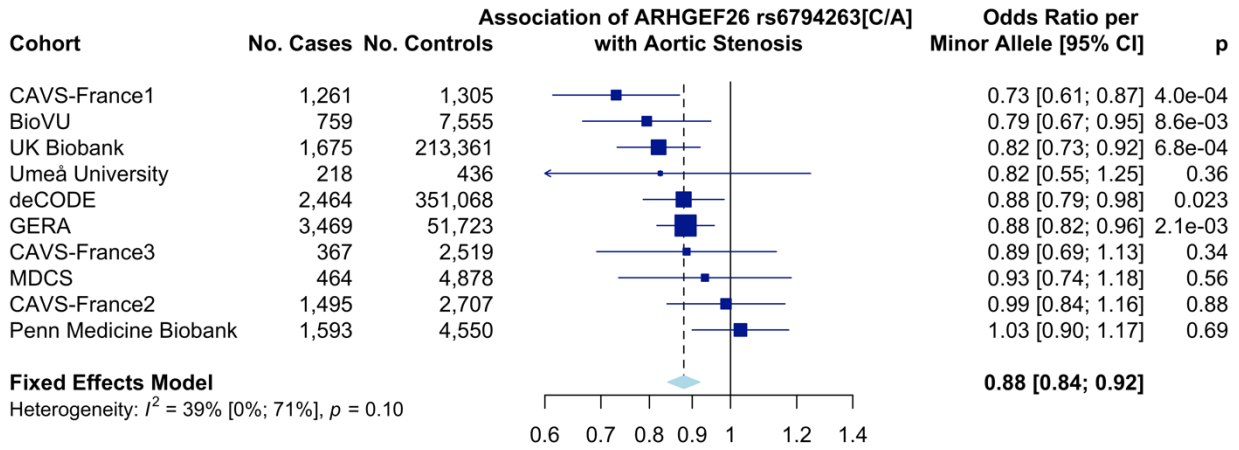
Estimates were combined using an inverse-variance weighted, fixed effects meta-analysis. Box widths are proportional to the weights of the estimates in the meta-analysis. Alleles are coded as [minor/major].



Supplementary Figure 6.8. Meta-Analysis of the Association of *TEX41* rs7593336 with Aortic Stenosis.

Abbreviations: GERA, Genetic Epidemiology Research on Adult Health and Aging; MDCS, Malmö Diet and Cancer Study.

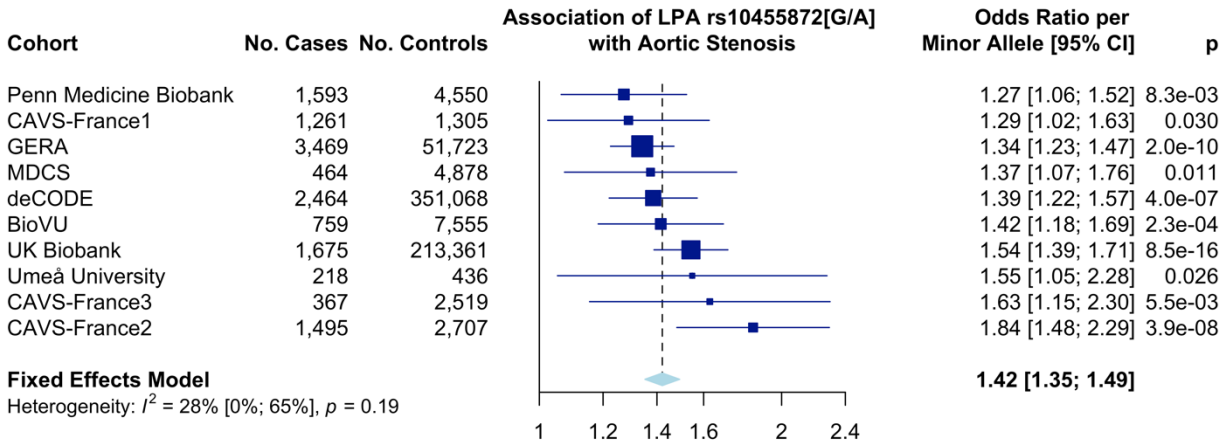
Estimates were combined using an inverse-variance weighted, fixed effects meta-analysis. Box widths are proportional to the weights of the estimates in the meta-analysis. Alleles are coded as [minor/major].



Supplementary Figure 6.9. Meta-Analysis of the Association of *ARHGEF26* rs6794263 with Aortic Stenosis.

Abbreviations: GERA, Genetic Epidemiology Research on Adult Health and Aging; MDCS, Malmö Diet and Cancer Study.

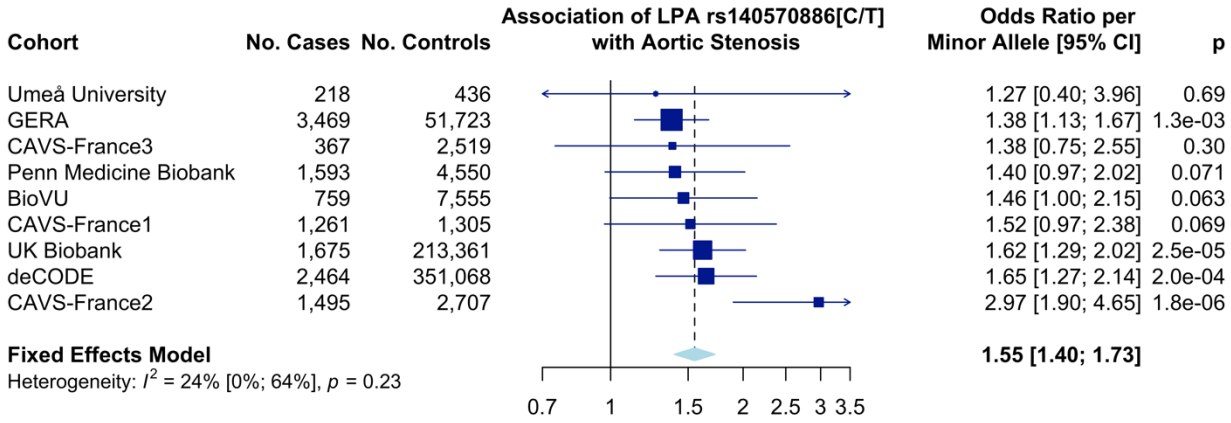
Estimates were combined using an inverse-variance weighted, fixed effects meta-analysis. Box widths are proportional to the weights of the estimates in the meta-analysis. Alleles are coded as [minor/major].



Supplementary Figure 6.10. Meta-Analysis of the Association of *LPA* rs10455872 with Aortic Stenosis.

Abbreviations: GERA, Genetic Epidemiology Research on Adult Health and Aging; MDCS, Malmö Diet and Cancer Study.

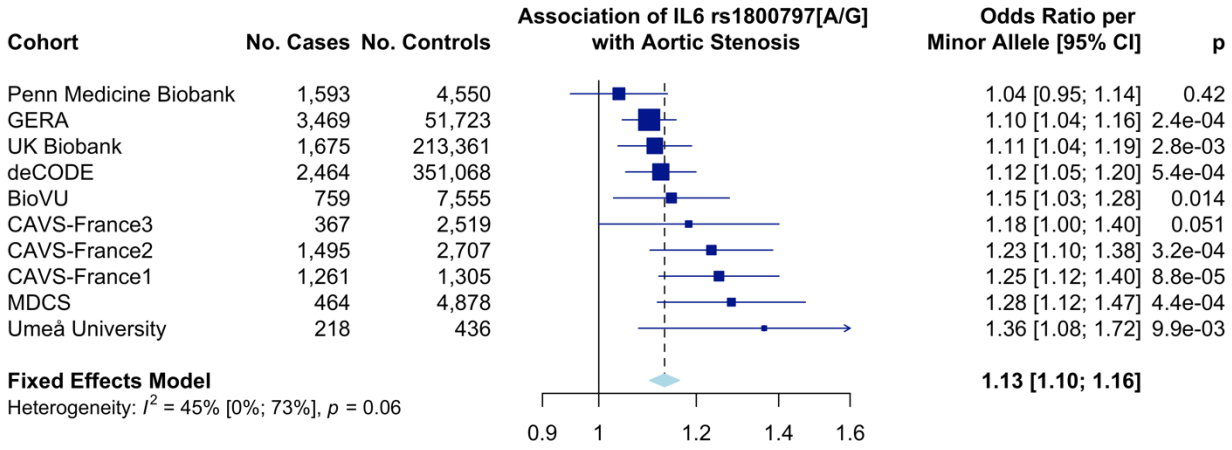
Estimates were combined using an inverse-variance weighted, fixed effects meta-analysis. Box widths are proportional to the weights of the estimates in the meta-analysis. Alleles are coded as [minor/major].



Supplementary Figure 6.11. Meta-Analysis of the Association of *LPA* rs140570886 with Aortic Stenosis.

Abbreviations: GERA, Genetic Epidemiology Research on Adult Health and Aging; MDCS, Malmö Diet and Cancer Study.

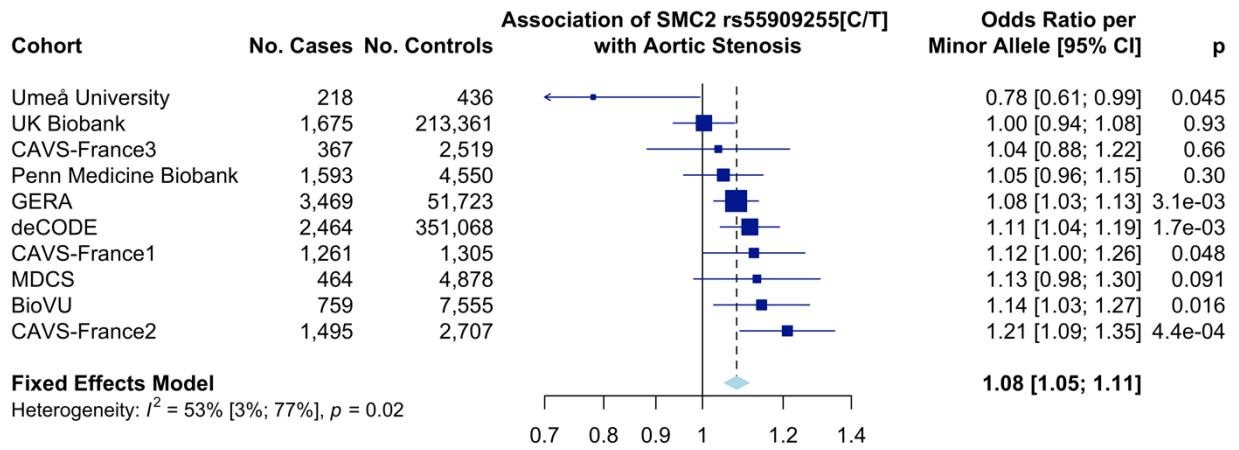
Estimates were combined using an inverse-variance weighted, fixed effects meta-analysis. Box widths are proportional to the weights of the estimates in the meta-analysis. Alleles are coded as [minor/major].



Supplementary Figure 6.12. Meta-Analysis of the Association of *IL6* rs1800797 with Aortic Stenosis.

Abbreviations: GERA, Genetic Epidemiology Research on Adult Health and Aging; MDCS, Malmö Diet and Cancer Study.

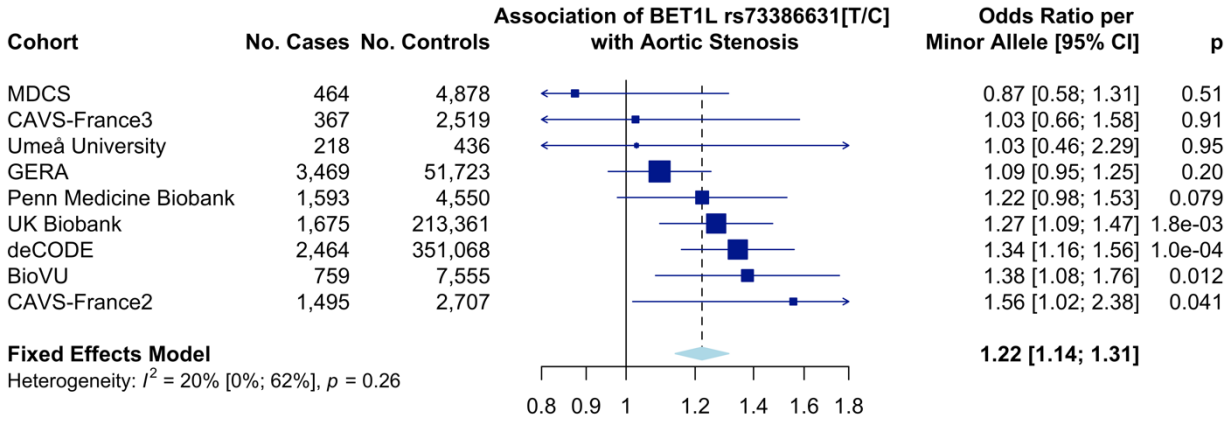
Estimates were combined using an inverse-variance weighted, fixed effects meta-analysis. Box widths are proportional to the weights of the estimates in the meta-analysis. Alleles are coded as [minor/major].



Supplementary Figure 6.13. Meta-Analysis of the Association of *SMC2* rs55909255 with Aortic Stenosis.

Abbreviations: GERA, Genetic Epidemiology Research on Adult Health and Aging; MDCS, Malmö Diet and Cancer Study.

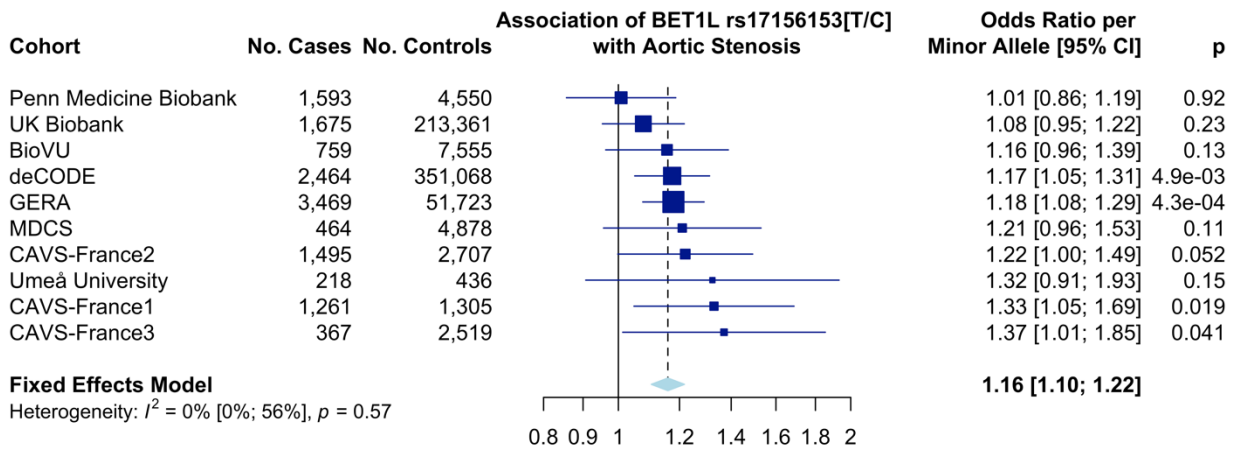
Estimates were combined using an inverse-variance weighted, fixed effects meta-analysis. Box widths are proportional to the weights of the estimates in the meta-analysis. Alleles are coded as [minor/major].



Supplementary Figure 6.14. Meta-Analysis of the Association of *BET1L* rs73386631 with Aortic Stenosis.

Abbreviations: GERA, Genetic Epidemiology Research on Adult Health and Aging; MDCS, Malmö Diet and Cancer Study.

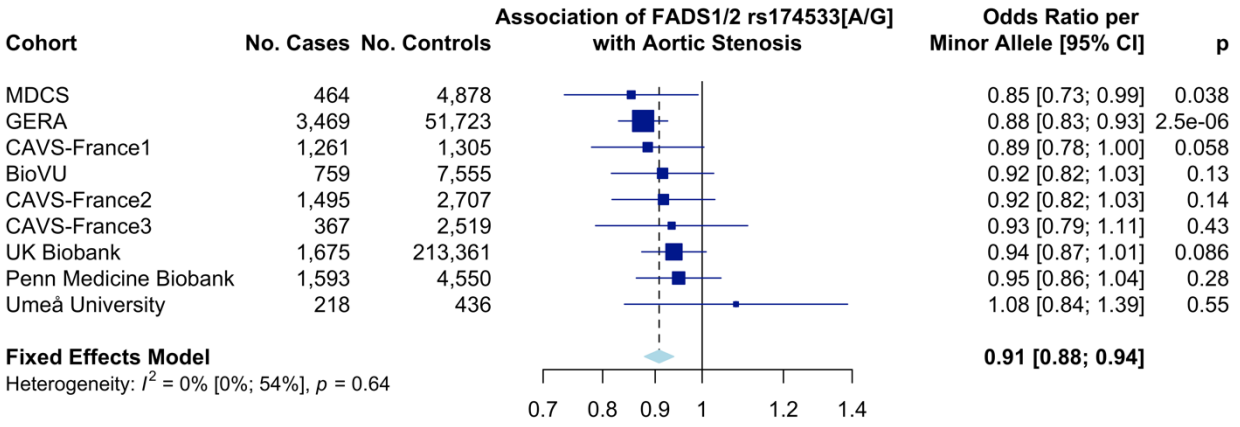
Estimates were combined using an inverse-variance weighted, fixed effects meta-analysis. Box widths are proportional to the weights of the estimates in the meta-analysis. Alleles are coded as [minor/major].



Supplementary Figure 6.15. Meta-Analysis of the Association of *BET1L* rs1715613 with Aortic Stenosis.

Abbreviations: GERA, Genetic Epidemiology Research on Adult Health and Aging; MDCS, Malmö Diet and Cancer Study.

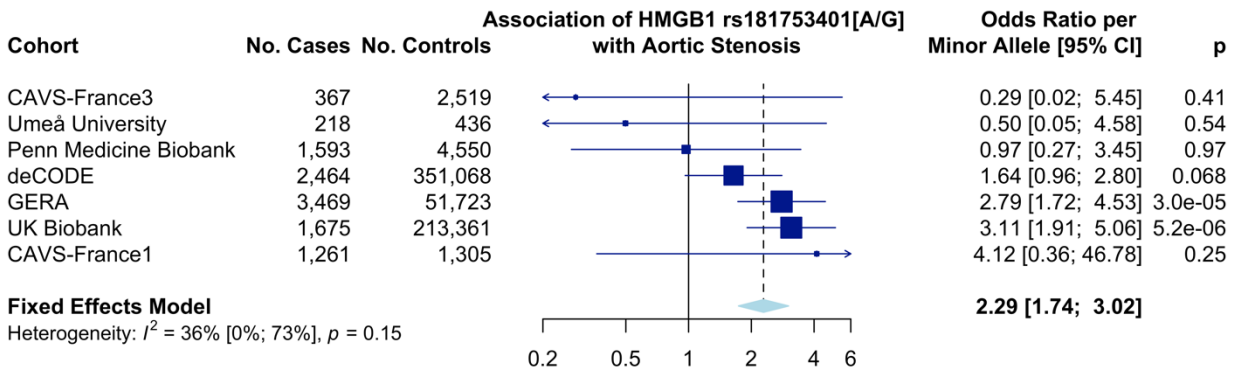
Estimates were combined using an inverse-variance weighted, fixed effects meta-analysis. Box widths are proportional to the weights of the estimates in the meta-analysis. Alleles are coded as [minor/major].



Supplementary Figure 6.16. Meta-Analysis of the Association of *FADS1/2* rs174533 with Aortic Stenosis.

Abbreviations: GERA, Genetic Epidemiology Research on Adult Health and Aging; MDCS, Malmö Diet and Cancer Study.

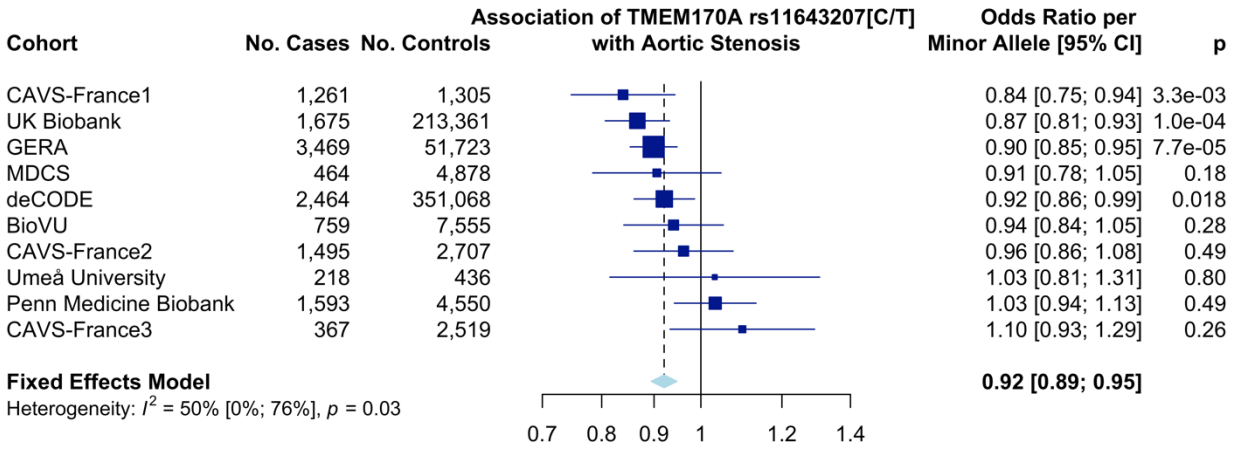
Estimates were combined using an inverse-variance weighted, fixed effects meta-analysis. Box widths are proportional to the weights of the estimates in the meta-analysis. Alleles are coded as [minor/major].



Supplementary Figure 6.17. Meta-Analysis of the Association of *HMGB1* rs181753401 with Aortic Stenosis.

Abbreviations: GERA, Genetic Epidemiology Research on Adult Health and Aging.

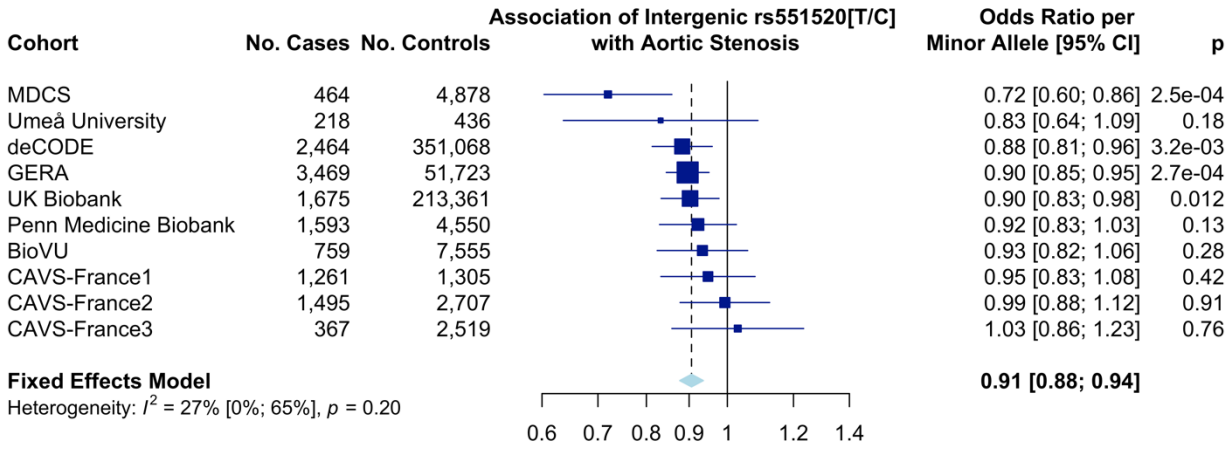
Estimates were combined using an inverse-variance weighted, fixed effects meta-analysis. Box widths are proportional to the weights of the estimates in the meta-analysis. Alleles are coded as [minor/major].



Supplementary Figure 6.18. Meta-Analysis of the Association of *TMEM170A* rs11643207 with Aortic Stenosis.

Abbreviations: GERA, Genetic Epidemiology Research on Adult Health and Aging; MDCS, Malmö Diet and Cancer Study.

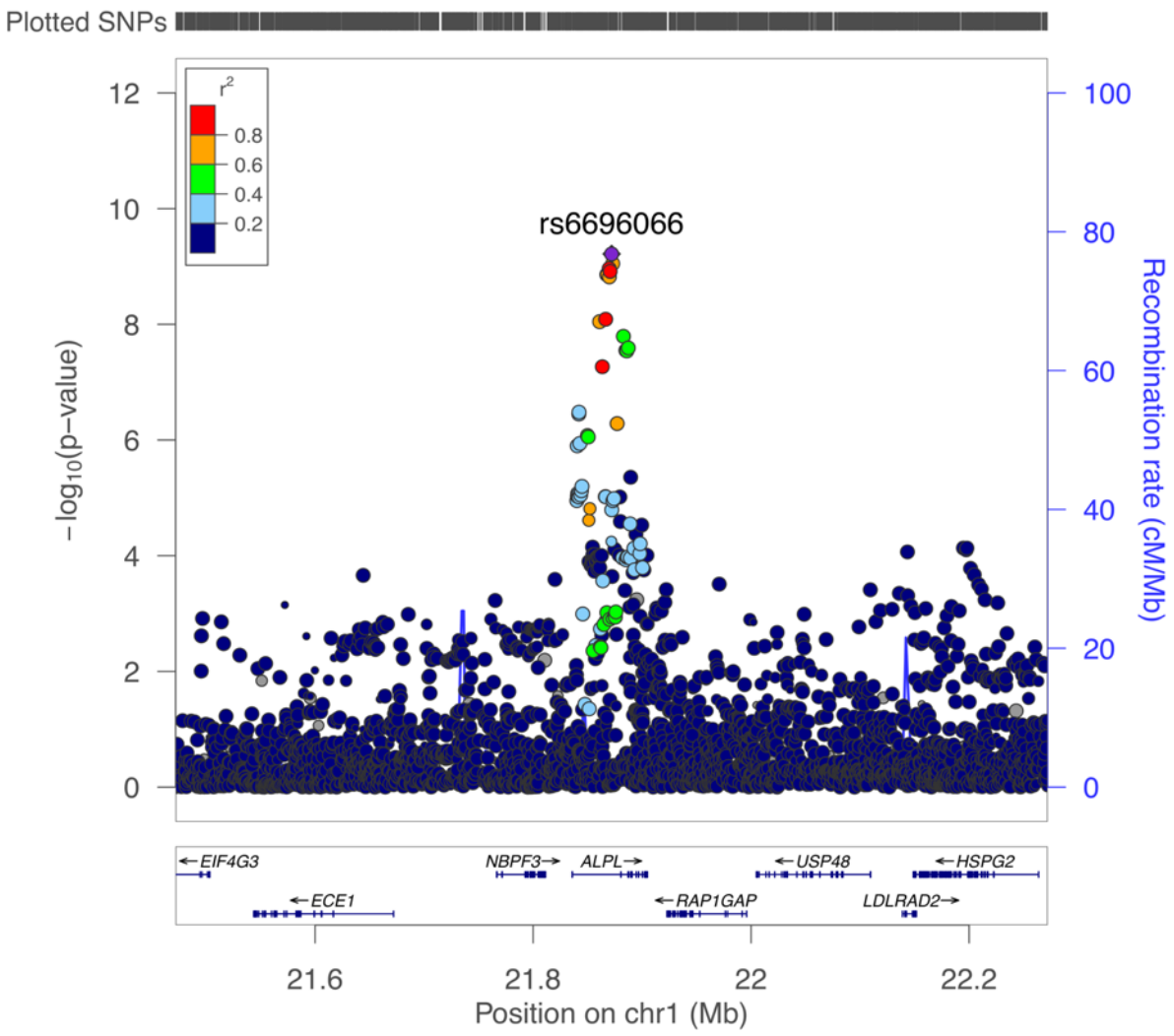
Estimates were combined using an inverse-variance weighted, fixed effects meta-analysis. Box widths are proportional to the weights of the estimates in the meta-analysis. Alleles are coded as [minor/major].



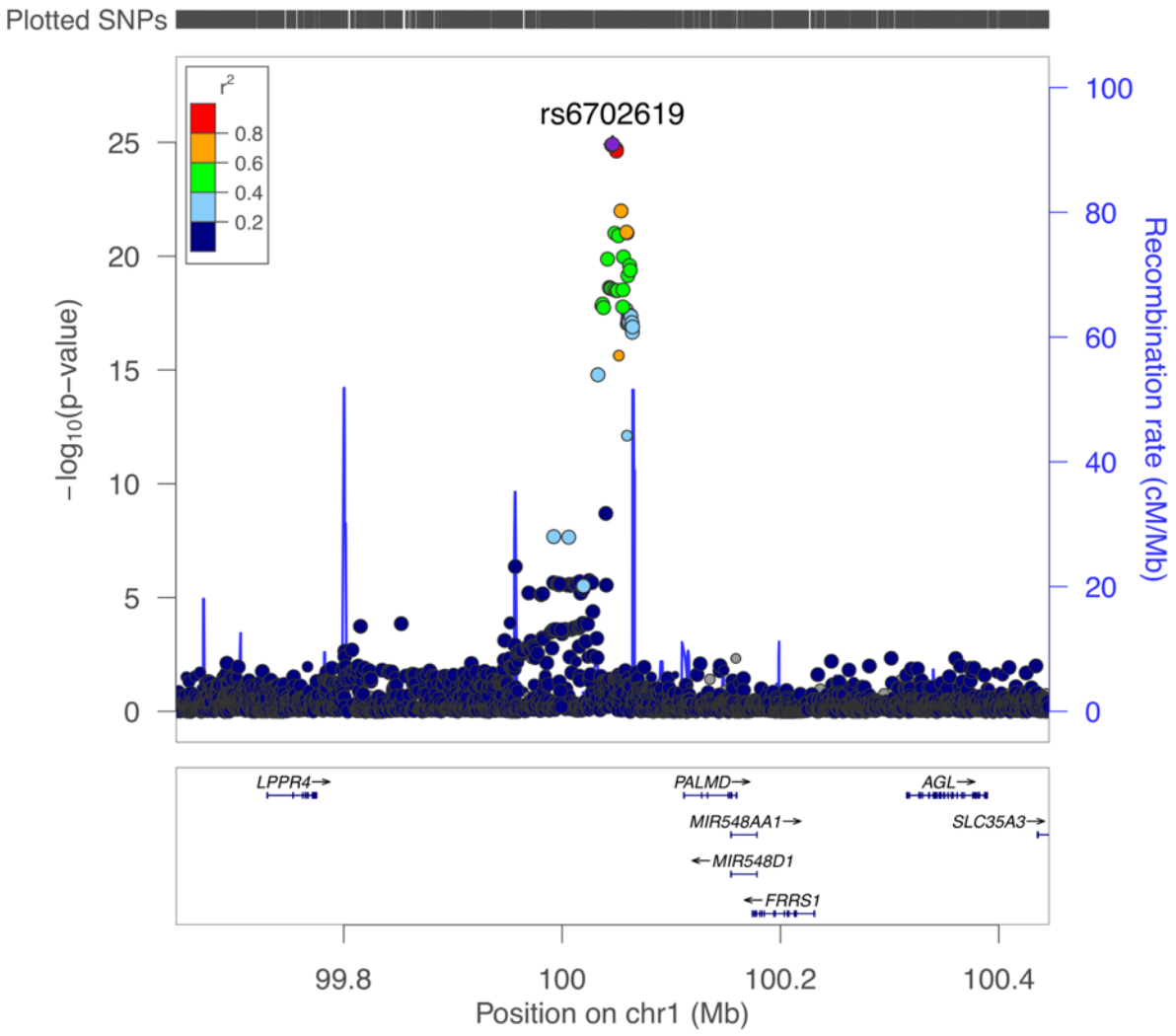
Supplementary Figure 6.19. Meta-Analysis of the Association of Intergenic rs551520 with Aortic Stenosis.

Abbreviations: GERA, Genetic Epidemiology Research on Adult Health and Aging; MDCS, Malmö Diet and Cancer Study.

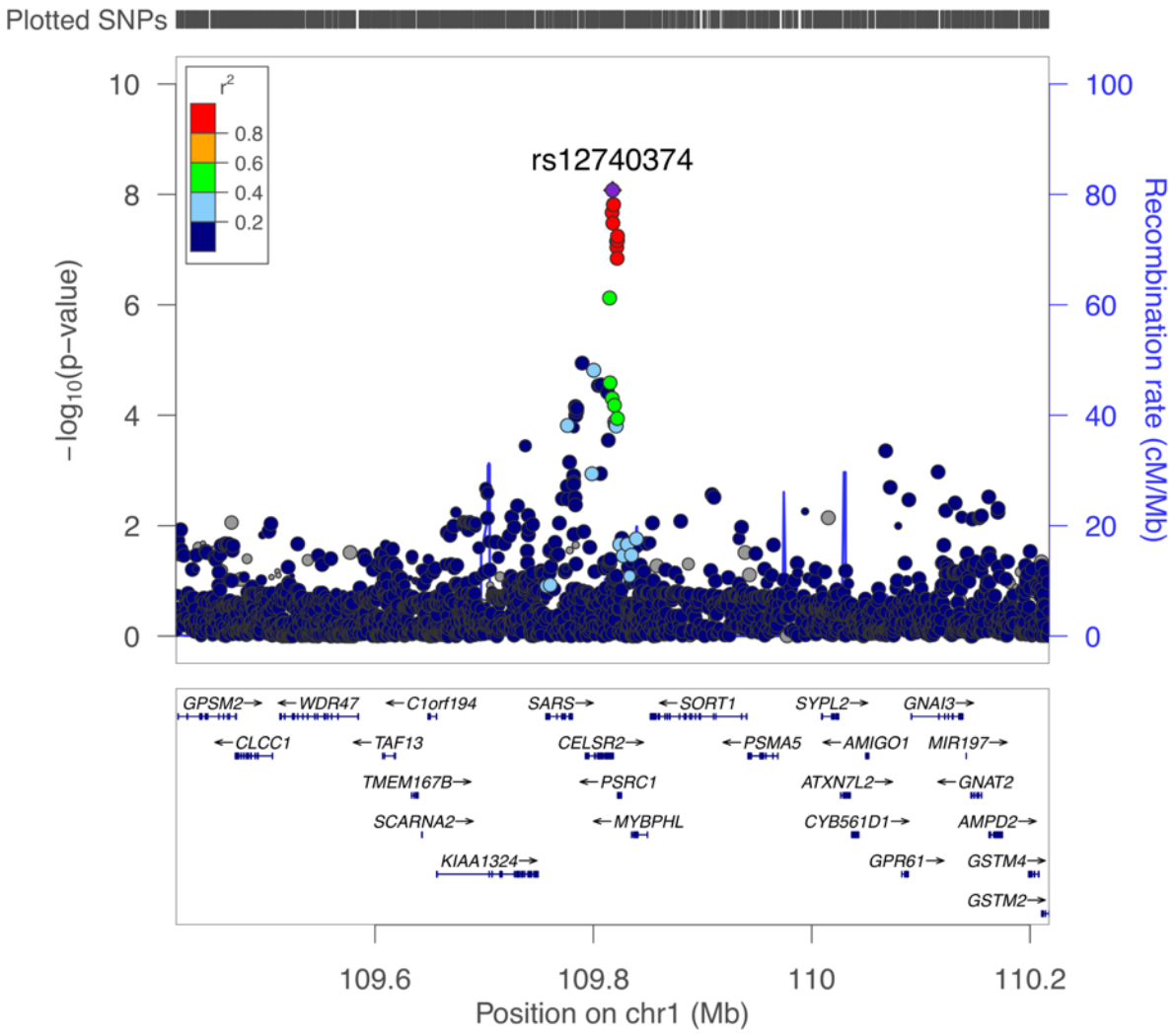
Estimates were combined using an inverse-variance weighted, fixed effects meta-analysis. Box widths are proportional to the weights of the estimates in the meta-analysis. Alleles are coded as [minor/major].



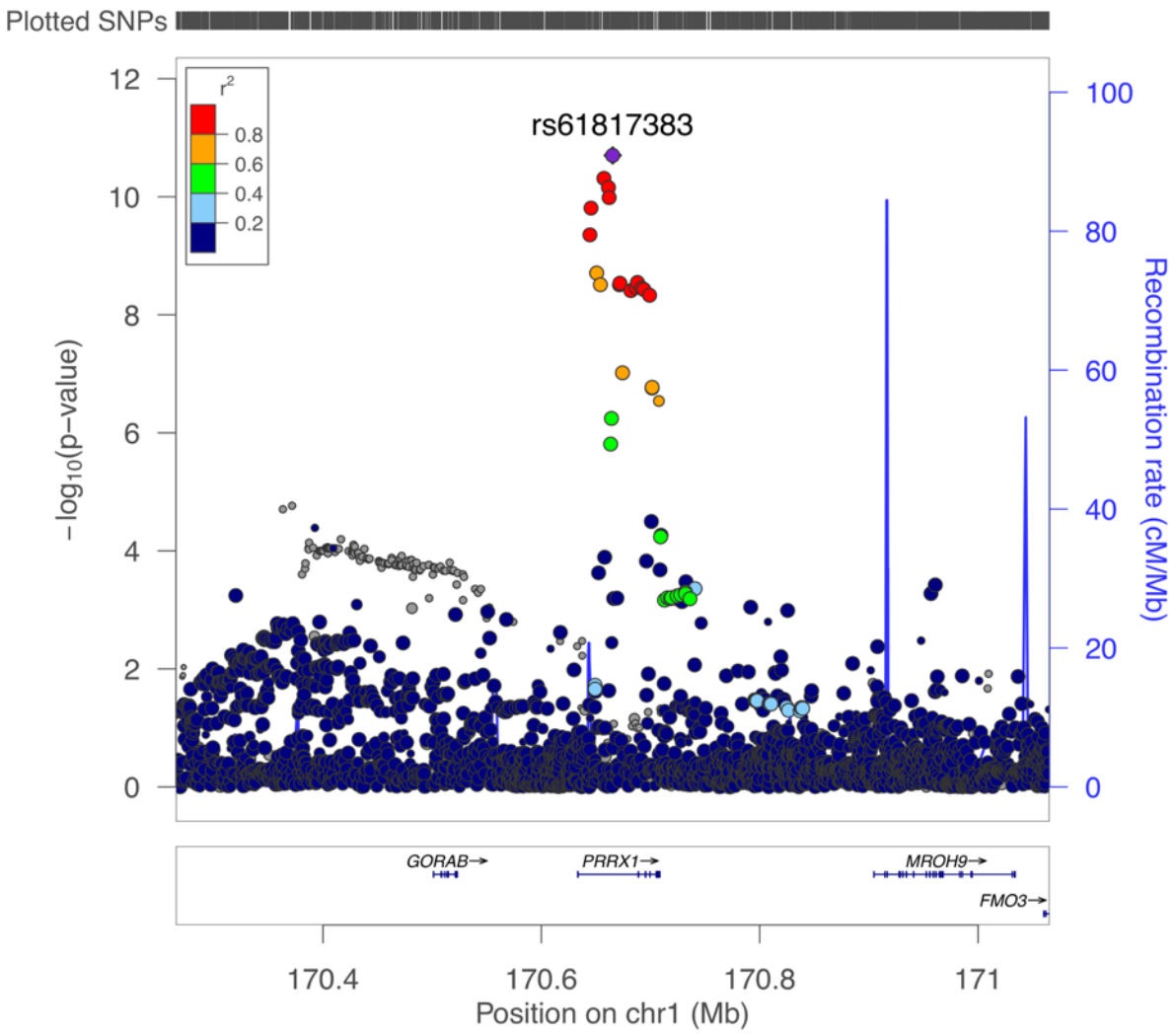
Supplementary Figure 6.20. Association of *ALPL* rs6696066 and Nearby Genetic Variants With Aortic Stenosis in the Meta-Analysis.



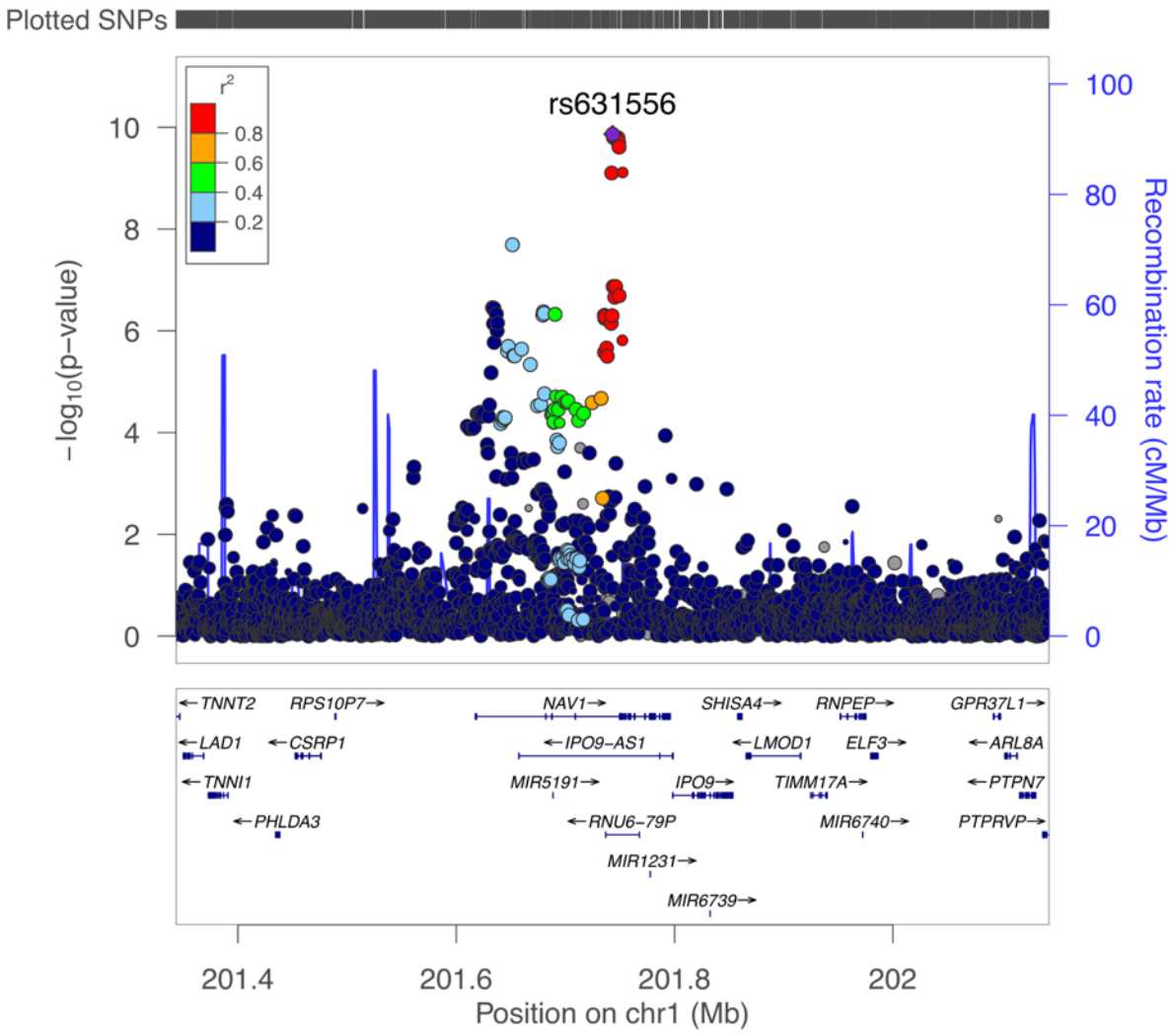
Supplementary Figure 6.21. Association of *PALMD* rs6702619 and Nearby Genetic Variants With Aortic Stenosis in the Meta-Analysis.



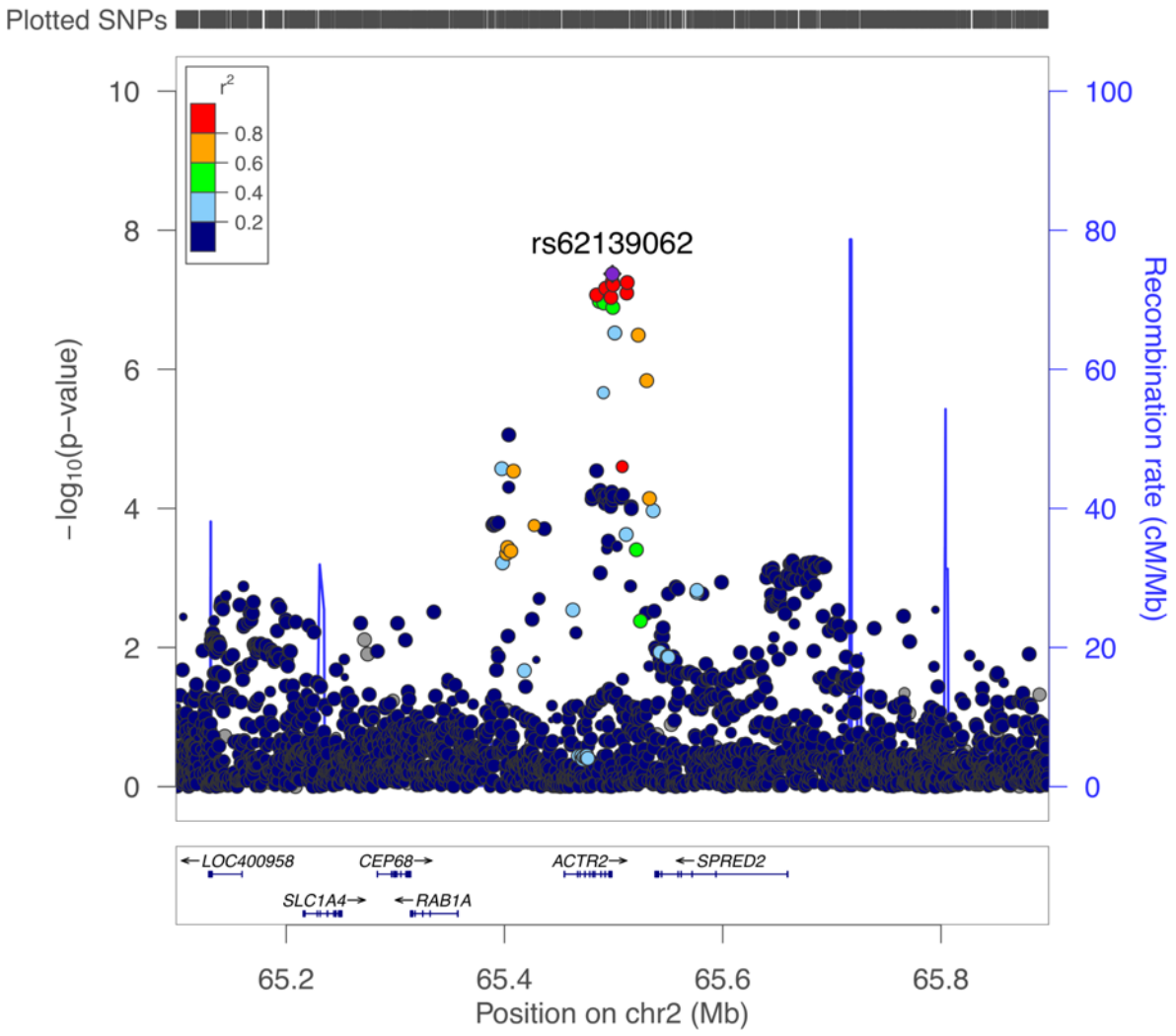
Supplementary Figure 6.22. Association of *CELSR2-SORT1* rs12740374 and Nearby Genetic Variants With Aortic Stenosis in the Meta-Analysis.



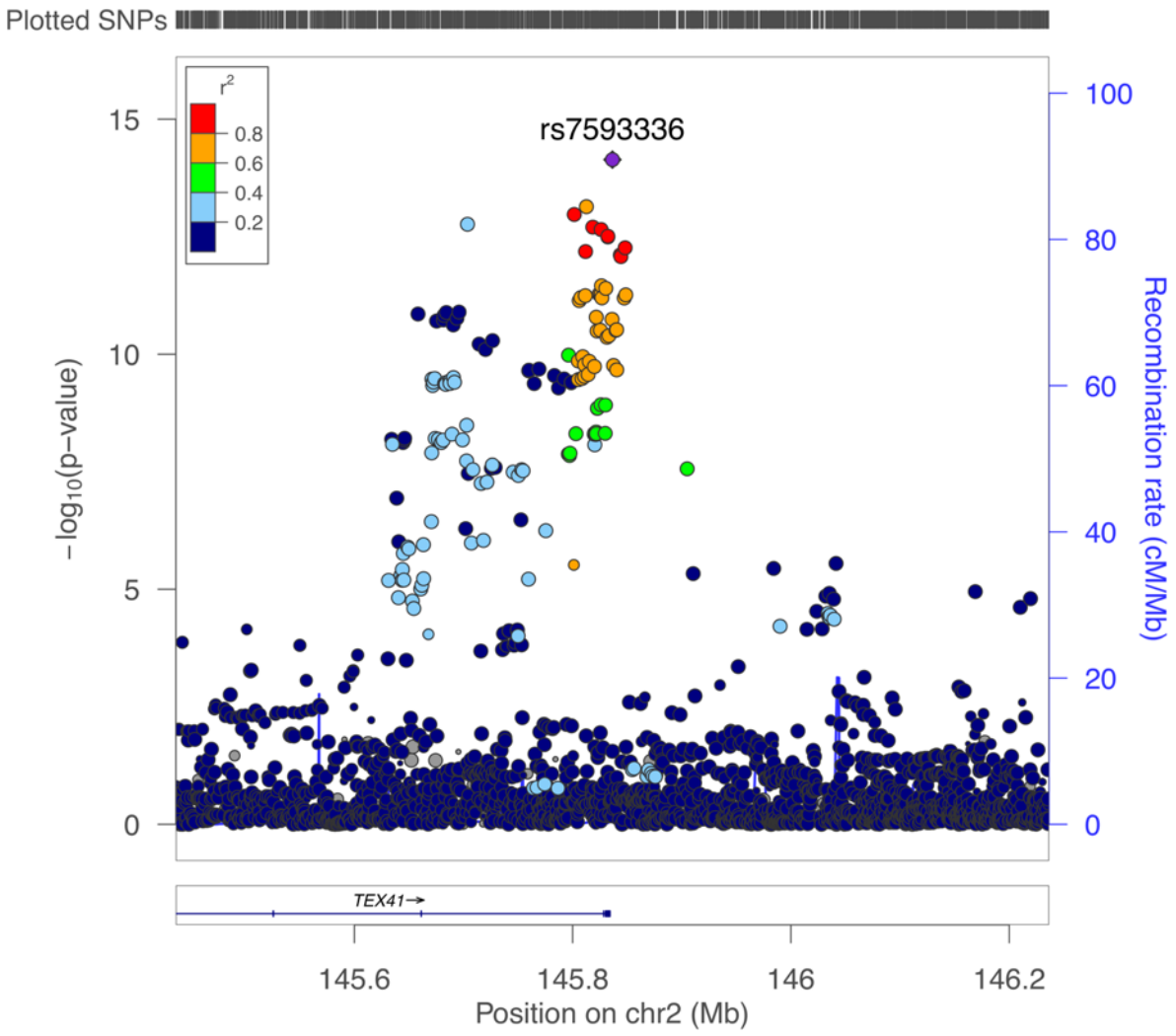
Supplementary Figure 6.23. Association of *PRRX1* rs61817383 and Nearby Genetic Variants With Aortic Stenosis in the Meta-Analysis.



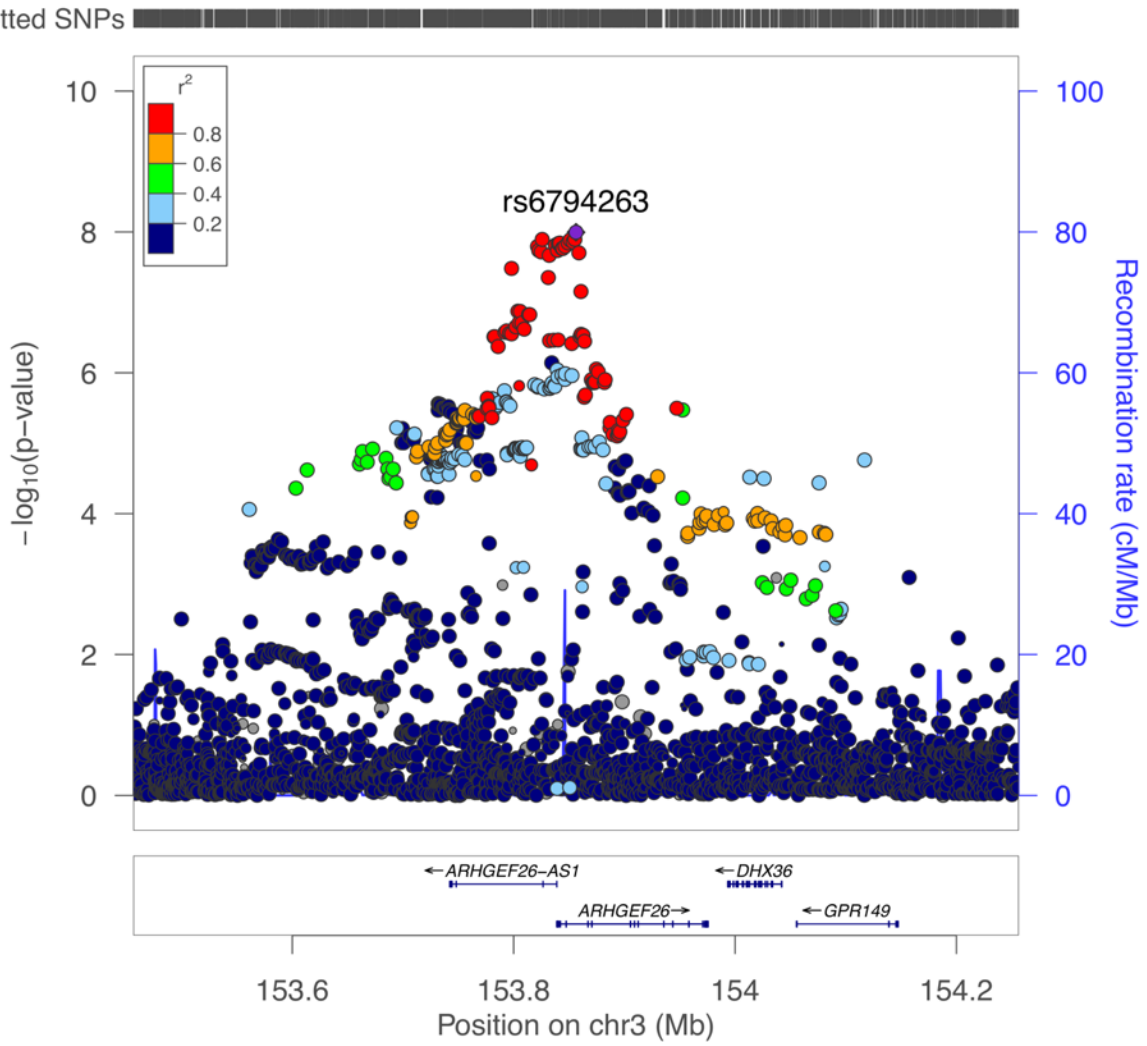
Supplementary Figure 6.24. Association of *NAV1* rs631556 and Nearby Genetic Variants With Aortic Stenosis in the Meta-Analysis.



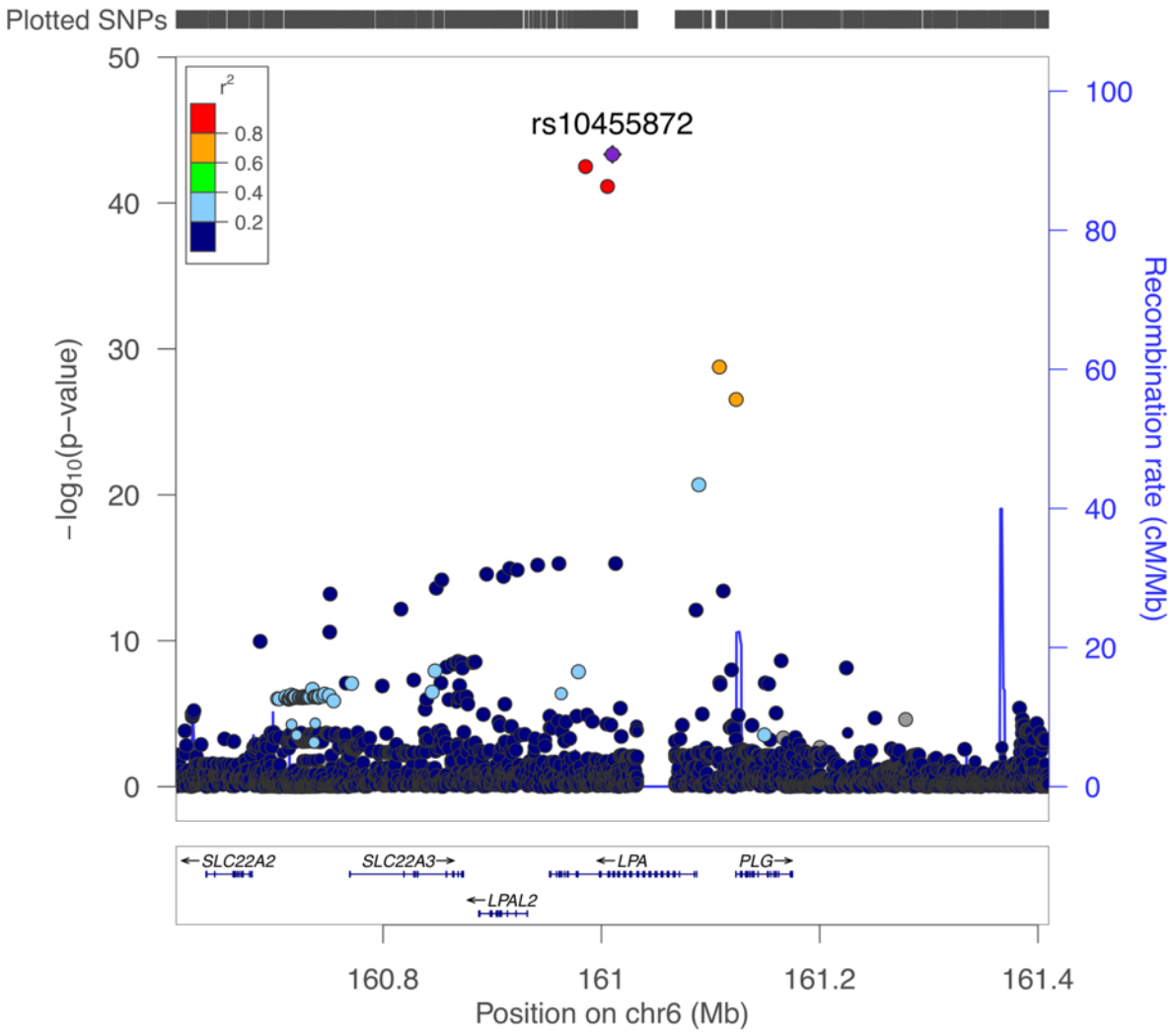
Supplementary Figure 6.25. Association of *ACTR2* rs62139062 and Nearby Genetic Variants With Aortic Stenosis in the Meta-Analysis.



Supplementary Figure 6.26. Association of *TEX41* rs7593336 and Nearby Genetic Variants With Aortic Stenosis in the Meta-Analysis.

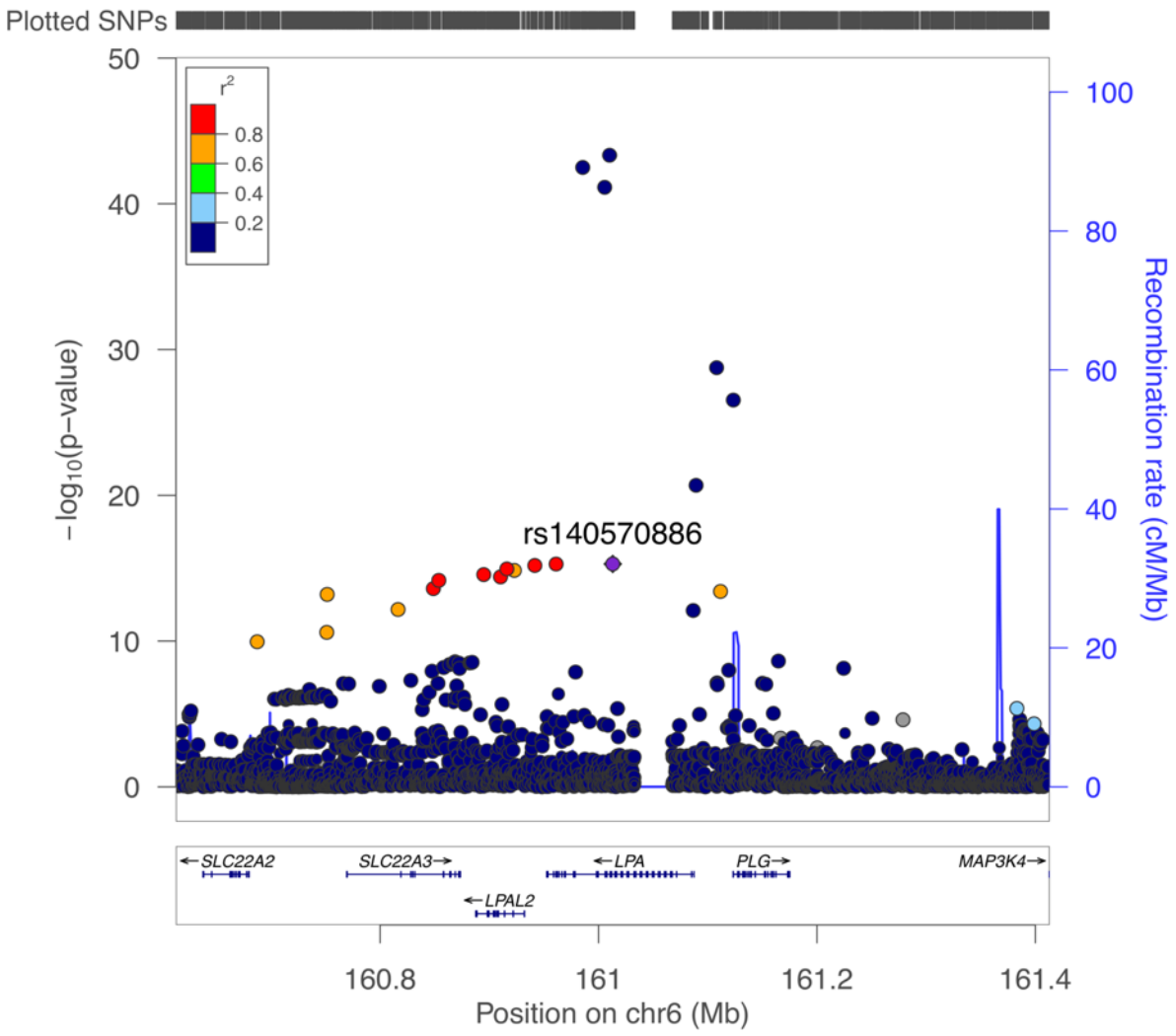


Supplementary Figure 6.27. Association of *ARHGEF26* rs6794263 and Nearby Genetic Variants With Aortic Stenosis in the Meta-Analysis.



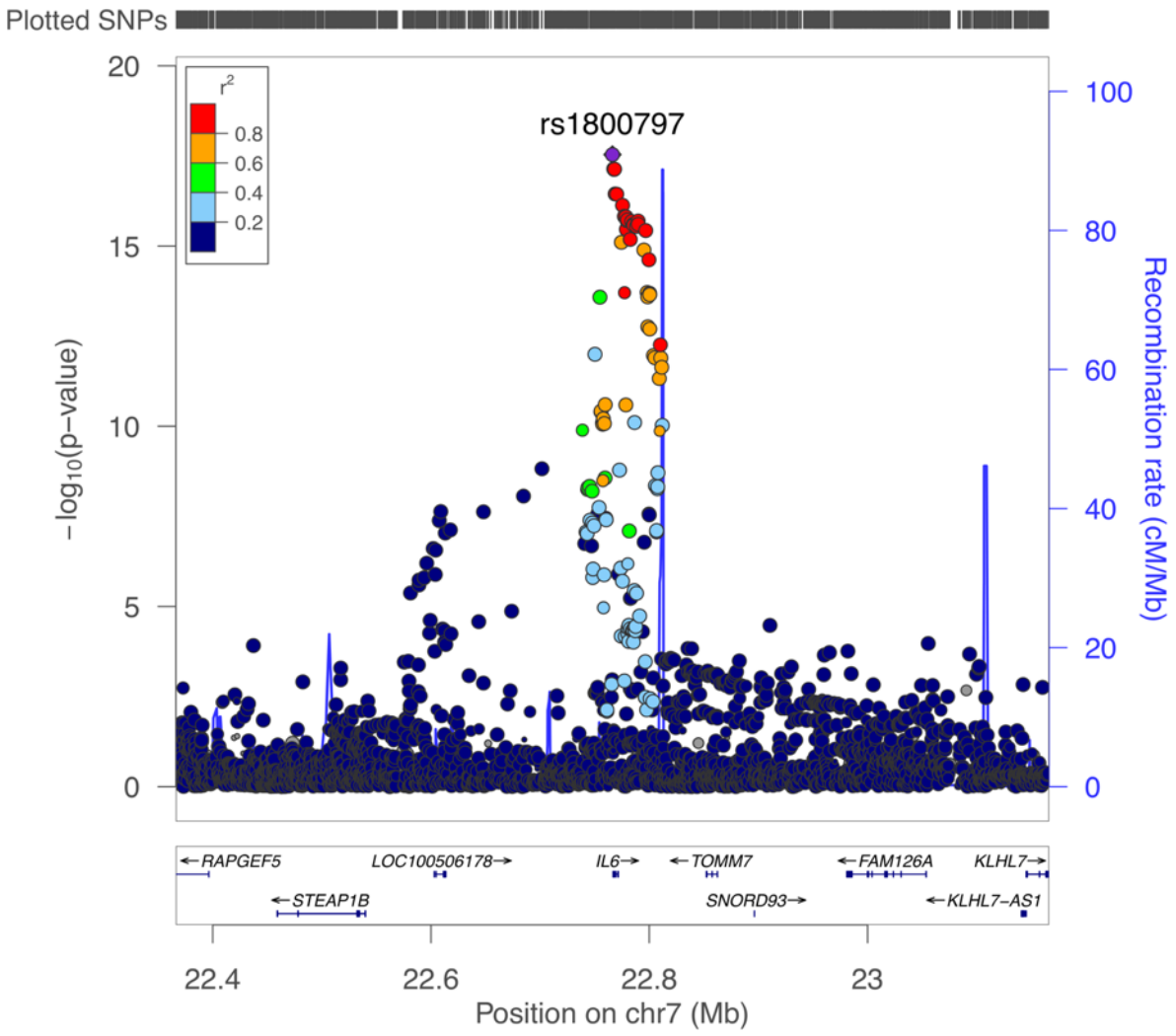
Supplementary Figure 6.28. Association of *LPA* rs10455872 and Nearby Genetic Variants With Aortic Stenosis in the Meta-Analysis.

The independent ($r^2 \leq 0.01$) stack of genome-wide significant variants is for *LPA* rs140570886 (see Supplementary Figure 6.29).

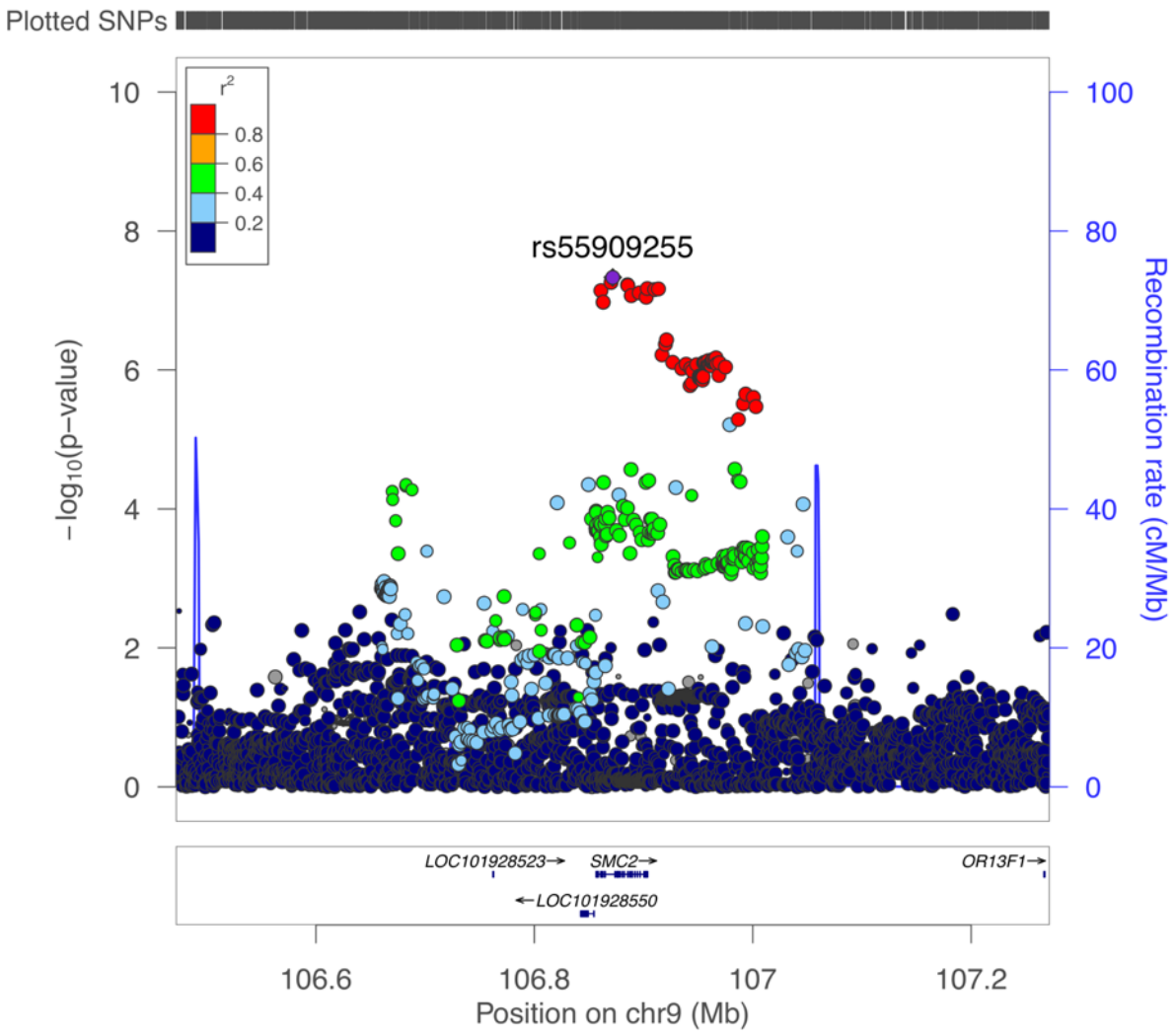


Supplementary Figure 6.29. Association of *LPA* rs140570886 and Nearby Genetic Variants With Aortic Stenosis in the Meta-Analysis.

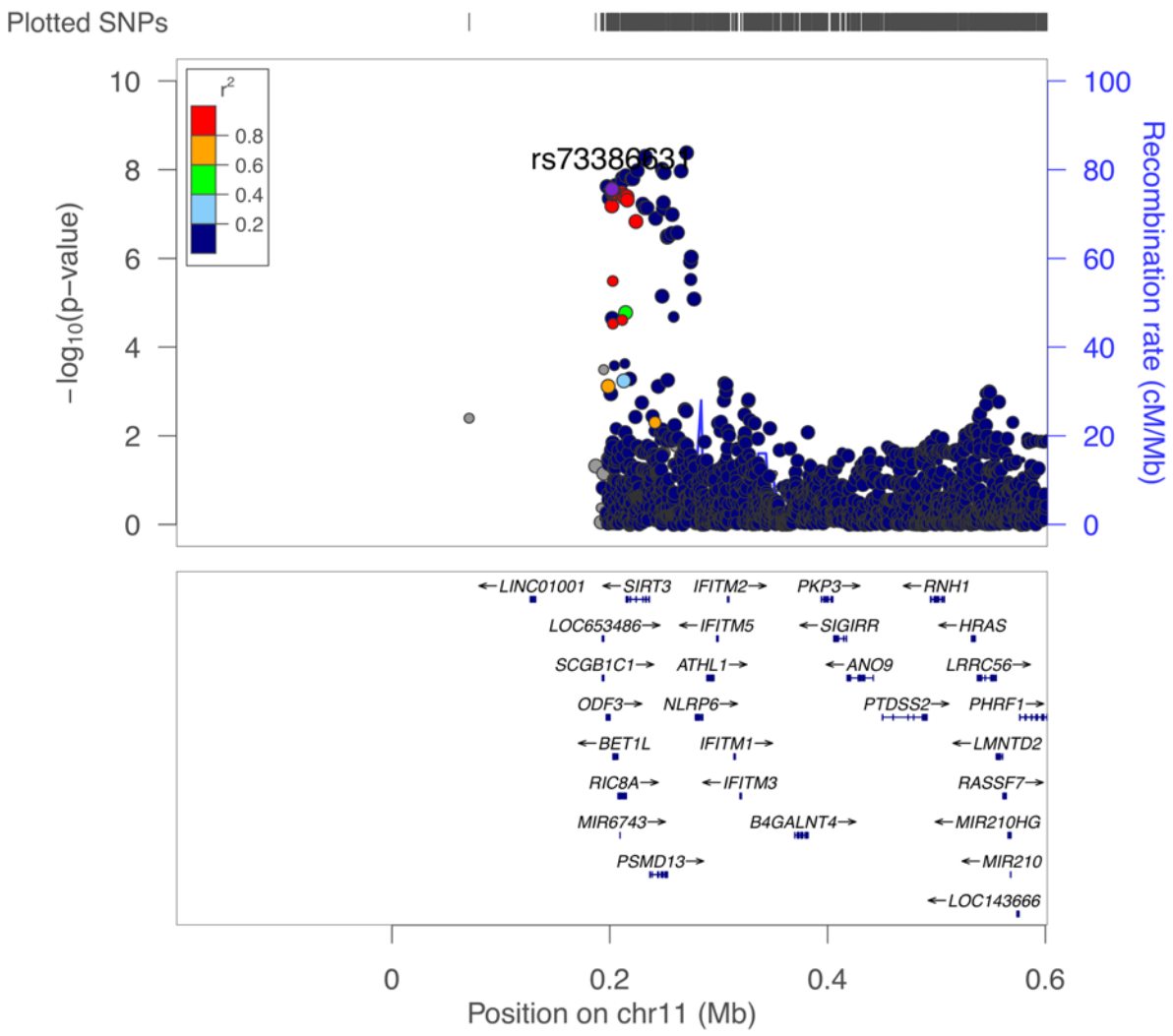
The independent ($r^2 \leq 0.01$) stack of genome-wide significant variants is for *LPA* rs10455872 (see Supplementary Figure 6.28).



Supplementary Figure 6.30. Association of *IL6* rs1800797 and Nearby Genetic Variants With Aortic Stenosis in the Meta-Analysis.

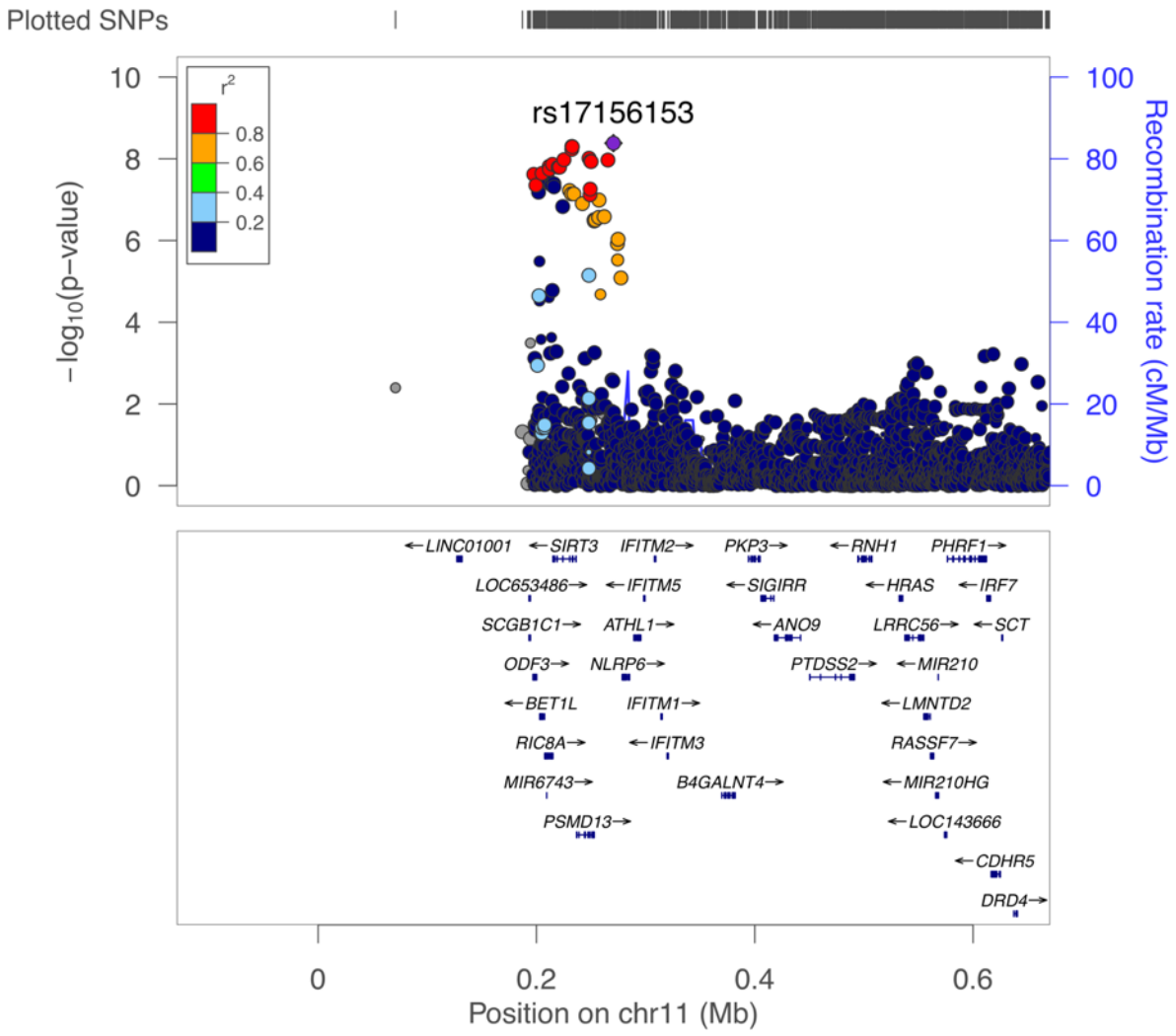


Supplementary Figure 6.31. Association of *SMC2* rs55909255 and Nearby Genetic Variants With Aortic Stenosis in the Meta-Analysis.



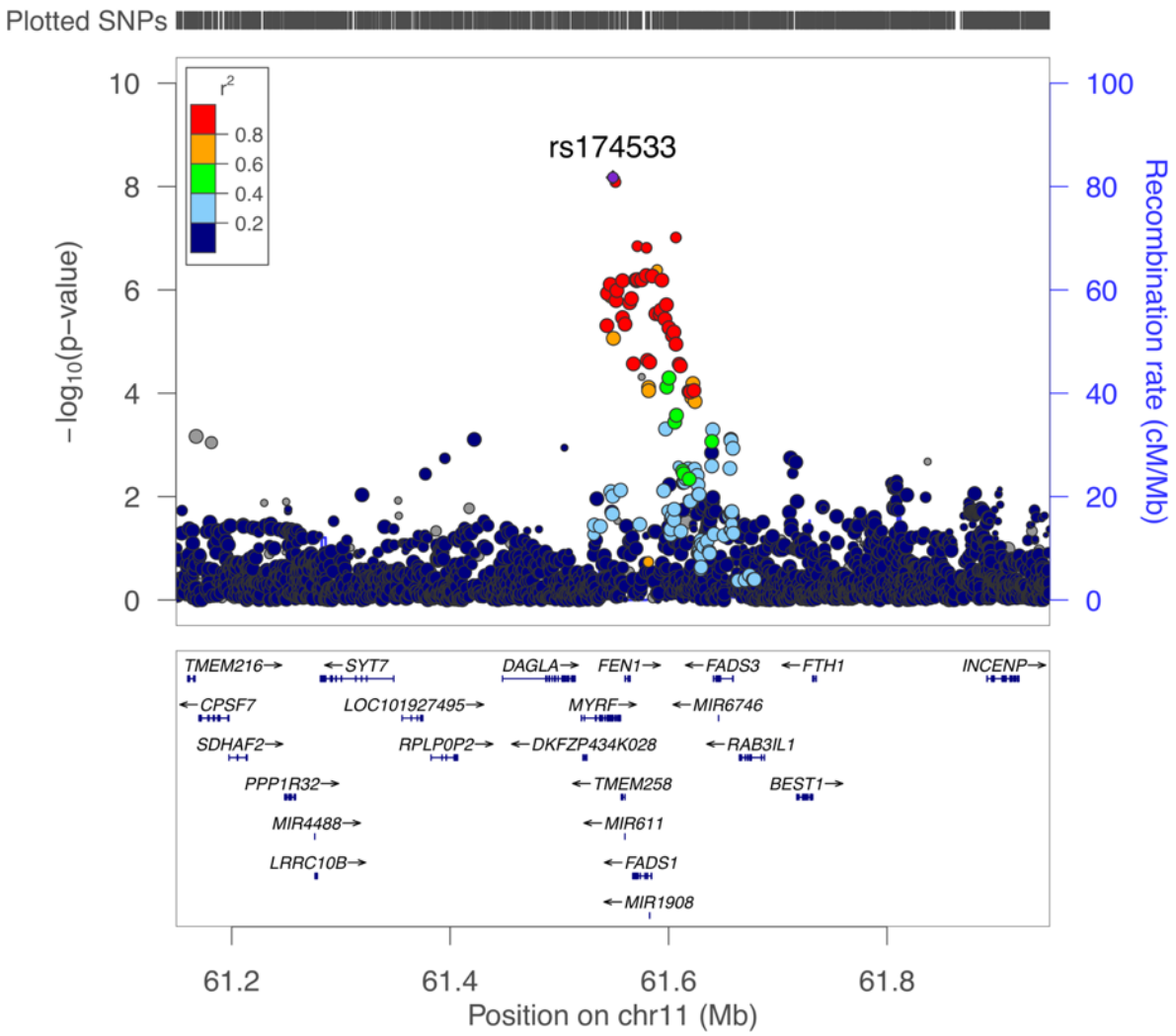
Supplementary Figure 6.32. Association of *BET1L* rs73386631 and Nearby Genetic Variants With Aortic Stenosis in the Meta-Analysis.

The independent ($r^2 \leq 0.01$) stack of genome-wide significant variants is for *BET1L* rs17156153 (see

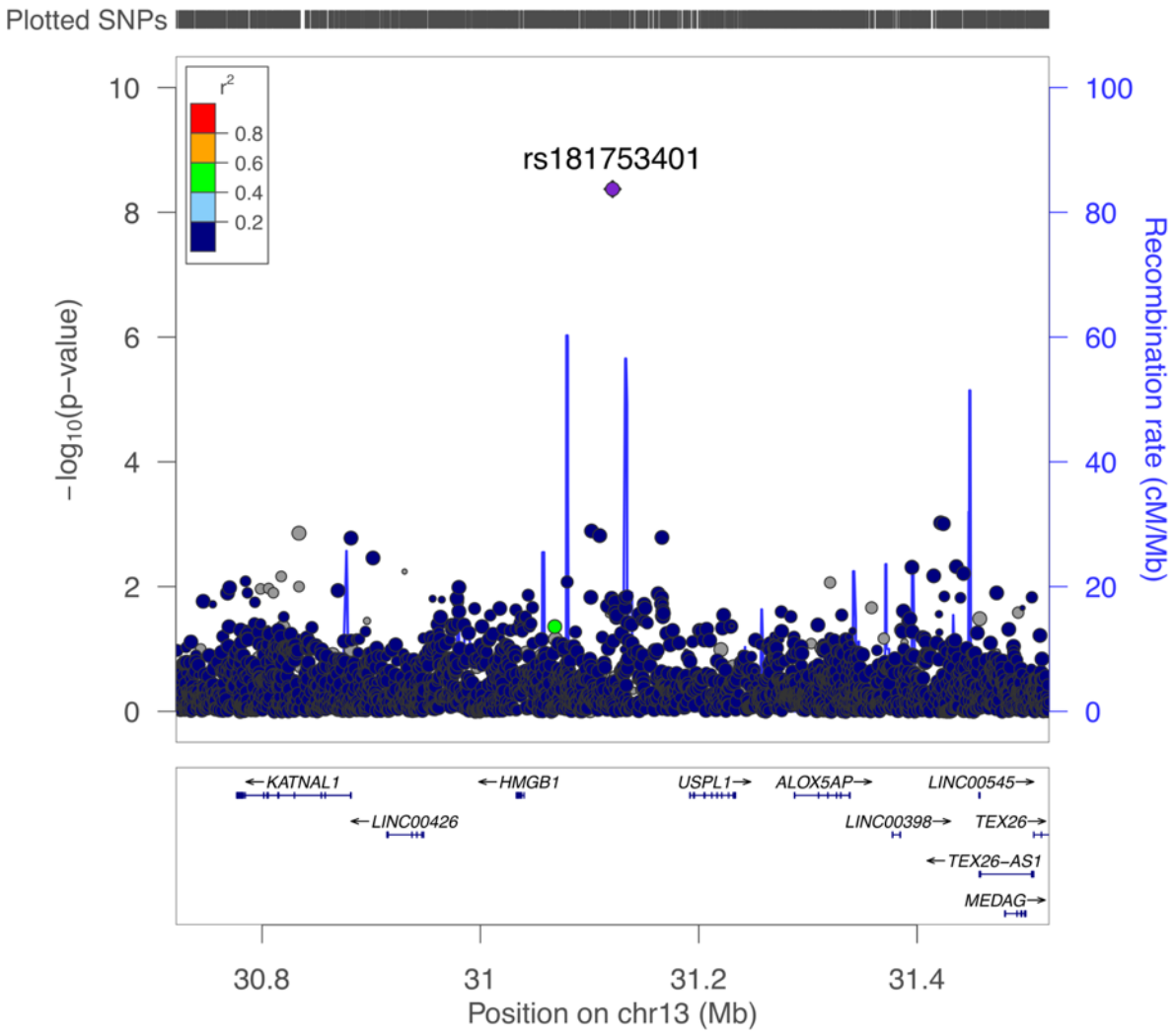


Supplementary Figure 6.33. Association of *BET1L* rs17156153 and Nearby Genetic Variants With Aortic Stenosis in the Meta-Analysis.

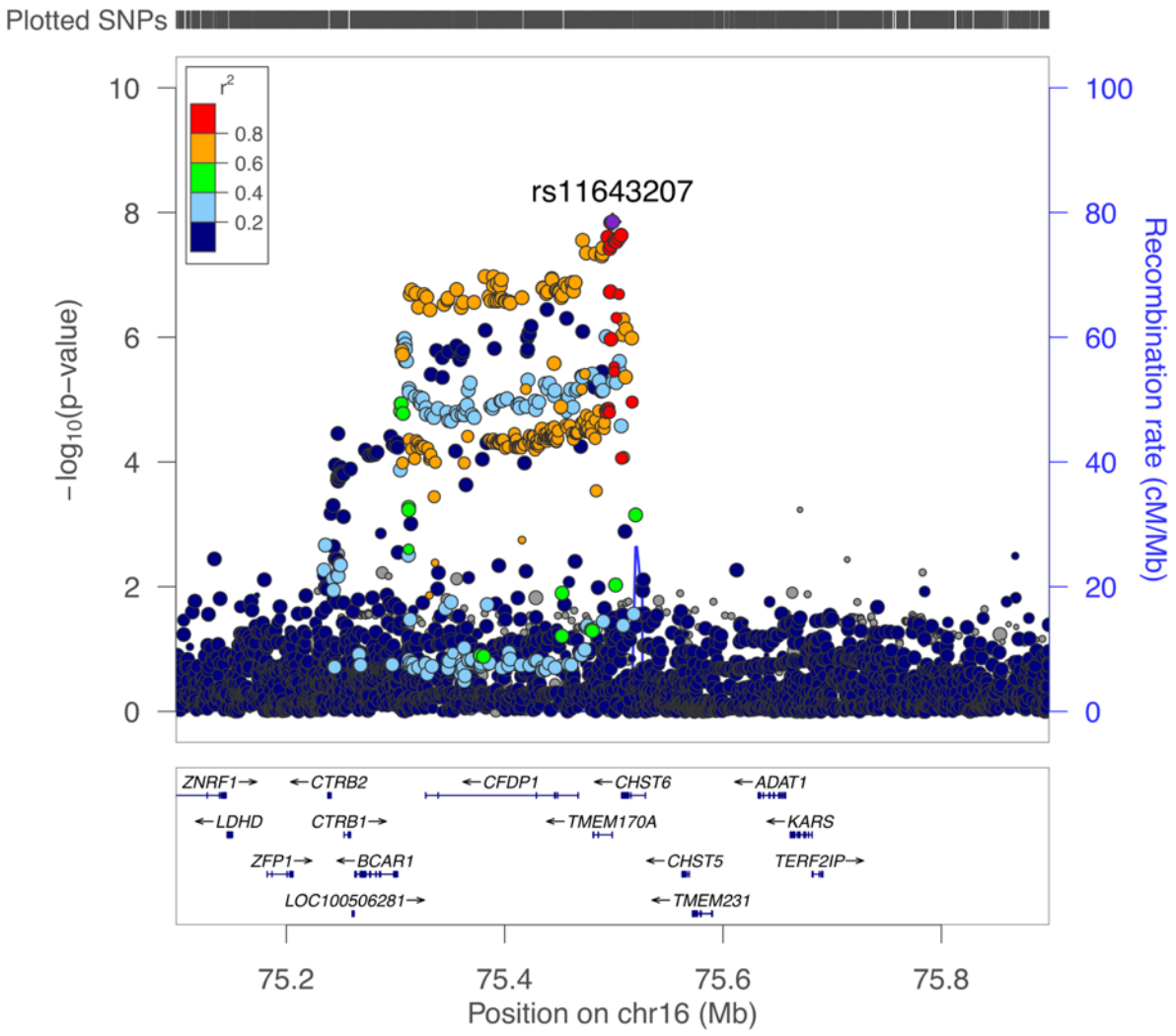
The independent ($r^2 \leq 0.01$) stack of genome-wide significant variants is for *BET1L* rs73386631 (see Supplementary Figure 6.32).



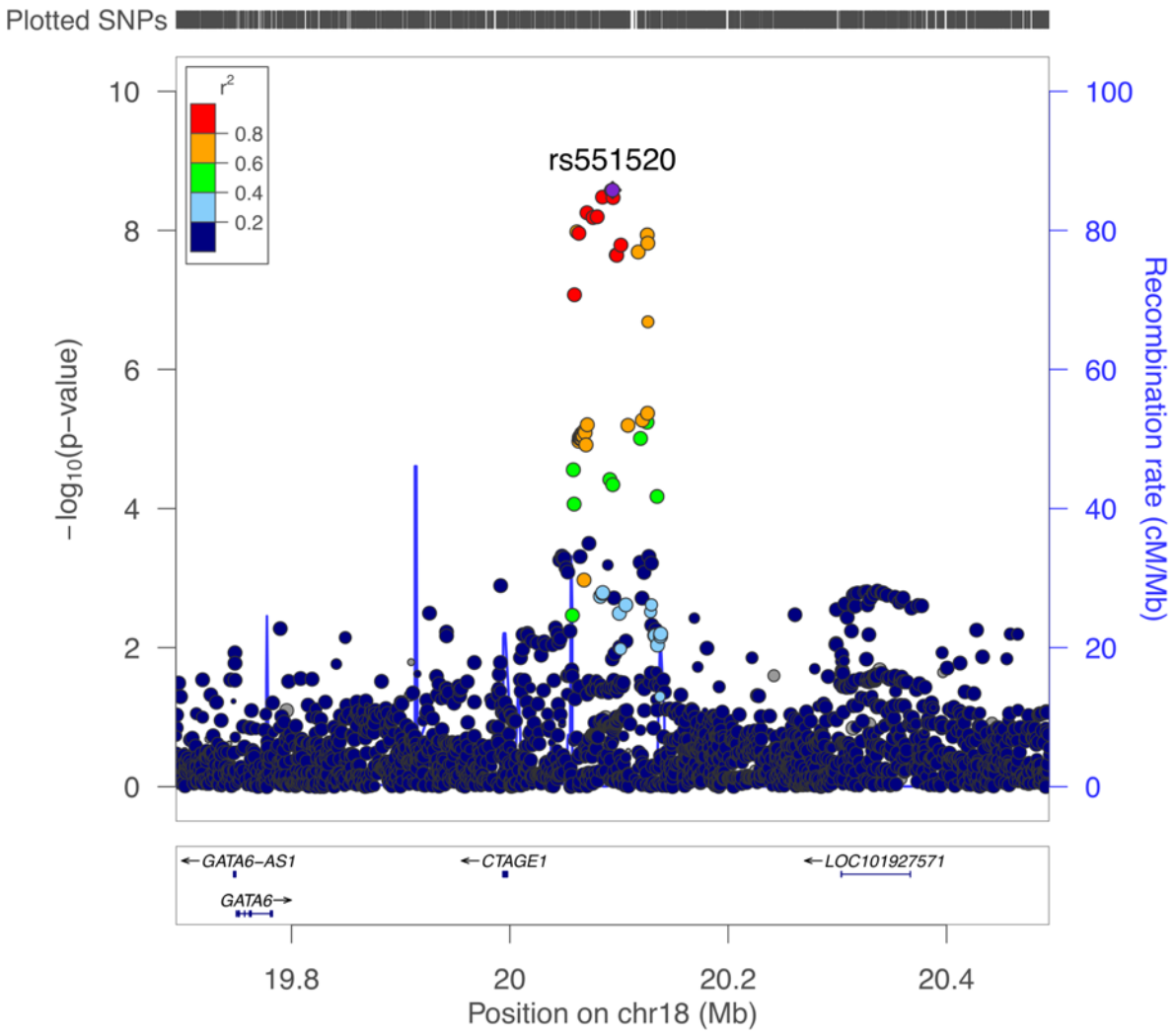
Supplementary Figure 6.34. Association of *FADS1/2* rs174533 and Nearby Genetic Variants With Aortic Stenosis in the Meta-Analysis.



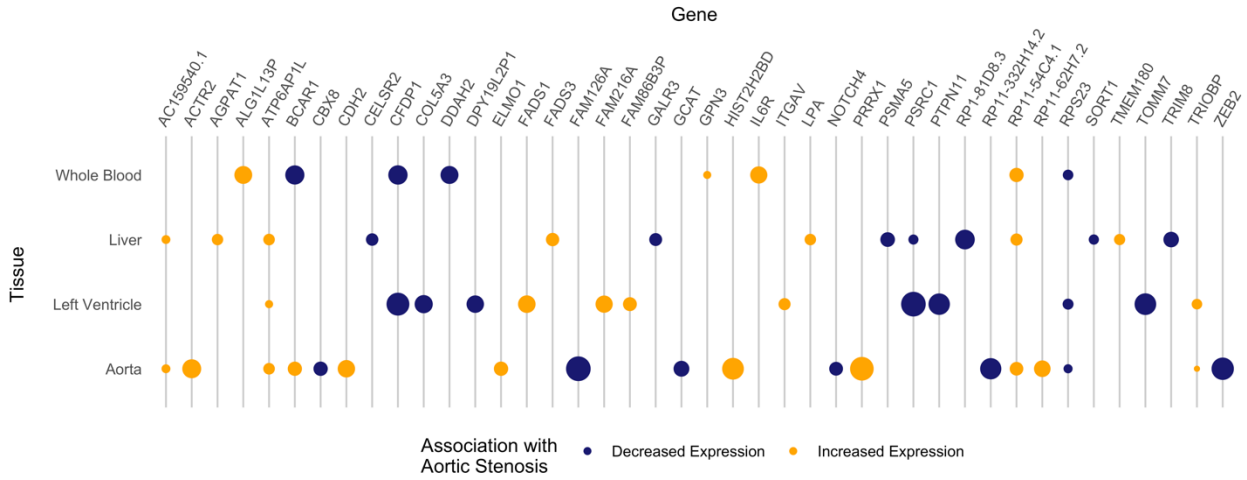
Supplementary Figure 6.35. Association of *HMGB1* rs181753401 and Nearby Genetic Variants With Aortic Stenosis in the Meta-Analysis.



Supplementary Figure 6.36. Association of *TMEM170A* rs11643207 and Nearby Genetic Variants With Aortic Stenosis in the Meta-Analysis.



Supplementary Figure 6.37. Association of the Intergenic rs551520 and Nearby Genetic Variants With Aortic Stenosis in the Meta-Analysis.



Supplementary Figure 6.38. Predicted Differential Gene Expression for Aortic Stenosis.

The area of each circle is proportional to the absolute value of the Z score of the association of gene expression with aortic stenosis.

Cohort Descriptions: Aortic Stenosis

Vanderbilt DNA Biobank

The Vanderbilt DNA Biobank is a biorepository containing de-identified genetic and electronic health record data on patients of the Vanderbilt University Medical Center[190]. Genotyping using the Illumina Multi-Ethnic Genotyping Array had been performed on 13,569 samples, and following standard quality control, the genotyped data were imputed using the Michigan Imputation Server[187] with the Haplotype Reference Consortium version r.1.1[188] as the reference panel. Cases of aortic stenosis were participants with at least one transthoracic echocardiogram report indicating moderate, moderate-to-severe, or severe aortic stenosis after 55 years of age, as determined using a natural language processing algorithm. Controls were participants older than 55 years with at least one transthoracic echocardiogram but aortic valve peak velocity < 1.5 m/s and no aortic stenosis. Individuals with mild or mild-to-moderate aortic stenosis were excluded from the study. A genome-wide association study for aortic stenosis was performed among 8,314 unrelated European-ancestry participants (759 cases) using SNPTEST version 2.5.4-beta3[189] via logistic regression models adjusted for age, age^2, and sex. There were 10,689,407 variants which entered the genome-wide meta-analysis after quality control of the imputed data and summary statistics.

Calcific Aortic Valve Stenosis-France1, Calcific Aortic Valve Stenosis-France2, and Calcific Aortic Valve Stenosis-France3

Between the years 2001 and 2017, inclusively, 1,663 severe aortic stenosis cases confirmed via echocardiography were recruited from the Angers, Rennes, and Nantes University Hospitals as part of a biobank operated by l'institut du thorax in Nantes, France. Separate recruitment by the

Bichat University Hospital enrolled 1,500 echocardiography-confirmed cases of aortic stenosis as part of the COFRASA-GENERAC study[262]. Controls were drawn from the DESIR[263] and PREGO[207] cohorts, which recruited individuals from western France. The cases and controls from these studies were genotyped in three waves, and this is reflected in the naming of the cohorts in the current study as Calcific Aortic Valve Stenosis (CAVS)-France1, CAVS-France2, and CAVS-France3.

In CAVS-France1, 1,329 cases from the institut du thorax biobank, 901 controls from DESIR, and 466 controls from PREGO were genotyped using the Affymetrix Axiom Genome-Wide CEU-1 array. Following standard quality control, the genotyped data for 1,261 cases and 1,305 controls (all of European ancestry and unrelated) were imputed using the Michigan Imputation Server[187] with the Haplotype Reference Consortium version r1.1[188] as the reference panel. A genome-wide association study for aortic stenosis was performed using SNPTEST version 2.5.2[189] through logistic regression models adjusted for the first five principal components for ancestry, in addition to the fifth to tenth principal components which were associated with aortic stenosis. After quality control of the imputed data and the summary statistics, 10,395,306 variants entered the genome-wide meta-analysis.

In CAVS-France2, 1,478 cases from the Bichat University Hospital, 319 cases from the institut du thorax biobank, and 2,828 controls from the PREGO cohort were genotyped using the Affymetrix Axiom Genome-Wide Precision Medicine Research Array. There were 1,495 cases and 2,707 controls who passed standard genotyping quality control, all of whom were unrelated and of European ancestry. Their genotyped data were imputed using the Haplotype Reference

Consortium version r1.1[188] as the reference panel at the Michigan Imputation Server[187]. The genome-wide association study for aortic stenosis was performed as described for CAVS-France1, and ensuing quality control of the imputed data and association results yielded 9,884,426 variants which were eligible for the genome-wide meta-analysis.

As part of the CAVS-France3, 379 cases from the institut du thorax biobank and 2,743 controls from the PREGO cohort were genotyped using the Affymetrix Axiom Genome-Wide Precision Medicine Research Array. There were 367 cases and 2,519 who passed standard genotyping quality control, all of whom were unrelated and of European ancestry. Their genotyped data were then imputed using the Michigan Imputation Server[187] with the Haplotype Reference Consortium version r1.1[188] as the reference panel, and a genome-wide association study for aortic stenosis was conducted using the imputed data as described for CAVS-France1. In total, 9,812,907 variants entered the genome-wide meta-analysis after quality control of the imputed data and summary statistics.

deCODE

The deCODE study sample contains patients diagnosed with aortic stenosis at the Landspitali – The National University Hospital in Reykjavik, Iceland between the years 1983 and 2016[58]. Cases were individuals with the *International Classification of Diseases* (*ICD)*, 10th revision codes *ICD-10* I35.0 or I35.2 among their diagnoses at discharge or the *Nordic Medico-Statistical Committee (NOMESCO)* classification of surgical procedure codes *FMA*, *FMSA*, *FMD*, *FMSD,* or their subcodes. Of the 2,609 aortic stenosis cases identified in their manner, the 2,464 cases for whom genotyped data were available were included in the study. The controls were 351,068 individuals

of the Icelandic genealogical database or participants of other genetic studies at deCODE genetics. Genetic data on deCODE participants were obtained through whole-genome sequencing (27,633 participants), genotyping on Illumina chips with subsequent imputation using the sequenced participants as the reference panel (155,250 participants), or familial imputation in untyped relatives informed by genealogy (198,282 participants). All participants were of Icelandic ancestry. A genome-wide association study for aortic stenosis was performed using logistic regression including year of birth, sex, and country of origin as covariates, using software written at deCODE. The test statistics were corrected for relatedness and sub-stratification among participants by regressing the χ^2 statistics from the genome-wide association study on their LD score[222] in a subset of 1.1 million variants and using the intercept of this regression as the correction factor. After restricting to variants found on the Haplotype Reference Consortium version r1.1 reference panel[188], quality control of the imputed data and the summary statistics produced 9,812,907 variants which entered the genome-wide meta-analysis.

Genetic Epidemiology Research on Adult Health and Aging

The Genetic Epidemiology Research on Adult Health and Aging cohort is composed of over 100,000 members of the Kaiser Permanente Medical Care Plan, Northern California Region and who are also participants of the Kaiser Permanente Research Program on Genes, Environment, and Health. Genome-wide genotyping was performed on ancestry-specific Affymetrix Axiom arrays, with the European-ancestry participants genotyped on the Affymetrix Axiom Genome-Wide EUR array[186]. We performed standard genotyping quality control and then imputed the genotyped data using the Michigan Imputation Server[187] with the Haplotype Reference Consortium version r1.1[188] as the reference panel. Following removal of individuals with

diagnosed congenital valvular heart disease (*ICD-9* 746-747 in their electronic health records), we identified aortic stenosis cases as participants with *ICD-9* 424.1 or a procedure code for aortic valve replacement in their electronic health records. All remaining participants were designated controls. Using PLINK version 2.0[168], we performed a genome-wide association study for aortic stenosis in 3,469 cases and 51,723 controls, adjusted for age, age^2, and sex. We restricted our analysis to unrelated individuals of European ancestry aged 55 years or older; the aortic stenosis sub-cohort of the Genetic Epidemiology Research on Adult Health and Aging has been described previously[209]. Following quality control of the imputed data and the summary statistics, 10,578,354 variants entered the genome-wide meta-analysis.

Malmö Diet and Cancer Study

The Malmö Diet and Cancer Study is a population-based, prospective cohort composed of 30,447 participants living in Malmö, Sweden[107]. Genotyping was performed using the Illumina Human Omni Express Exome BeadChip platform for a nested randomly selected sub-cohort and incident cases for a variety of diseases, together comprising more than 50% of the cohort. The genotypes which passed standard genotyping quality control were imputed using the Haplotype Reference Consortium version r1.1[188] as the reference panel at the Michigan Imputation Server[187]. Cases of aortic stenosis were participants with hospital diagnosis codes for aortic stenosis (*ICD-8* 424.10, 424.11, or 424.19; *ICD-9* 424B, 424BA, or 424BB; *ICD-10* I35.0 or I35.2) as ascertained from national registers, while all other participants were deemed controls. A genome-wide association study for aortic stenosis was performed on the imputed data in a nested, randomly selected sub-cohort of 4,878 controls and all 464 cases using logistic regression in PLINK version 2.0[168] adjusted for age and sex. All participants were unrelated and of European ancestry. After excluding

variants with genotype missingness ≥ 5%, minor allele frequency ≤ 0.01, and Hardy-Weinberg equilibrium $p \leq 1 \times 10^{-4}$, quality control was performed on the imputed data and summary statistics, resulting in 6,130,056 variants which entered the genome-wide meta-analysis.

Penn Medicine Biobank

The Penn Medicine Biobank contained 18,762 genotyped participants recruited throughout the University of Pennsylvania Health System at the time of analysis in April 2018. Genotyping was performed using the Illumina Quad Omni Genotyping Chip, and following standard genotyping quality control, the genotyped data were imputed using the Haplotype Reference Consortium version r1.1[188] at the Michigan Imputation Server[187]. Individuals with congenital valvular heart disease (*ICD-9* 746-747 or *ICD-10* Q20-Q22) were excluded, after which aortic stenosis cases were defined as participants with a diagnosis code of *ICD-9* 424.1 or *ICD-10* I35.0 or a procedure code for aortic valve replacement in their electronic health records, or who had an echocardiography report indicating aortic stenosis as determined through a validated text mining procedure[194]. All other participants were designated controls. A genome-wide association study for aortic stenosis was performed using imputed data from 1,593 cases and 4,550 controls, all of whom were unrelated and of European ancestry. The associations with aortic stenosis were modelled using logistic regression in PLINK version 2.0[168], adjusted for age, age^2, and sex. Following quality control of the imputed data and summary statistics, 11,016,108 variants were included in the genome-wide meta-analysis.

UK Biobank

The UK Biobank is a prospective cohort of more than 500,000 individuals living in the United Kingdom who were 40-79 years of age at recruitment (2006-2010)[191]. Genotyping was performed on 487,409 samples using the Affymetrix UK Biobank Axiom Array, and following standard genotyping quality control, imputation of the genotyped data was performed using the Haplotype Reference Consortium version r1.1, the 1000 Genomes Project phase 3[133], and the UK10K[192] as the reference panels. Aortic stenosis cases were participants with the diagnosis codes *ICD-10* I35.0 or I35.2 in their hospital inpatient records; all other participants were controls. Using PLINK version 2.0[168], we performed a genome-wide association study for aortic stenosis on 1,675 cases and 213,361 controls, all of whom were unrelated, of White British ancestry, and aged 55 years or older. The association of each variant with aortic stenosis was modelled using logistic regression adjusted for age, age^2, sex, genotyping batch, recruitment centre, and the first 20 principal components. After quality control of the imputed data and the summary statistics, 9,927,329 variants were used in the genome-wide meta-analysis.

For analyses of the aortic stenosis polygenic risk scores, the phenome-wide association study, and the Mendelian randomization performed in the UK Biobank, an updated version of the dataset was used with a more comprehensive definition of aortic stenosis cases. After excluding individuals with diagnosis codes for congenital valvular heart disease in their hospital inpatient records (*ICD-9* 746-747 and *ICD-10* Q20-Q23), we defined aortic stenosis cases as participants with the diagnosis codes *ICD-9* 424.1 or *ICD-10* I35.0 or *OPCS Classification of Interventions and Procedures (OPCS-4)* procedure codes K26.1, K26.2, K26.3, K26.4, K31.2, K32.2, or K35.2 in their hospital inpatient records. All other participants were designated controls. Again, we restricted our analyses to unrelated participants aged 55 years or older of White British ancestry.

Umeå University

The Umeå University cohort is composed of 3,597 European-ancestry participants living in northern Sweden who had previously participated in population-based health surveys. The participants were recruited in a case-control design, with 725 cases who had undergone surgery for disease of the ascending aorta or valvular heart disease matched on age, sex, survey, questionnaire completion date, and geography via a 1:4 scheme to 2,872 controls selected from the healthy participants who had also participated in the surveys[196]. Genotyping was performed for 1,853 participants using the Affymetrix UK Biobank Axiom Array r3, yielding 1,699 participants and 760,637 variants which passed standard genotyping quality control. We imputed the genotypes with the Haplotype Reference Consortium version r1.1[188] as reference using the Michigan Imputation Server[187]. A genome-wide association study for aortic stenosis was performed on the 218 aortic stenosis cases and their 436 matched controls, all unrelated and aged 55 years or older at the time of the survey. Associations with aortic stenosis were estimated via logistic regression models adjusted for age and sex in PLINK version 2.0[168]. We conducted an unmatched analysis as the unmatched analysis adjusted for the matching factors should be as valid as a matched analysis[197]. After quality control of the imputed data and summary statistics, the association results for 8,810,694 variants entered the genome-wide meta-analysis.

Cohort Descriptions: Aortic Valve Calcium

Multi-Ethnic Study of Atherosclerosis

The Mult-Ethnic Study of Atherosclerosis (MESA) is a prospective cohort of 6,814 participants of white, African American, Hispanic American, or Chinese ancestries[199]. Recruitment occurred

between the years 2000-2002 and individuals could participate if they were aged 45-84 years, free of clinical cardiovascular disease, and lived in one of the following communities of the United States: Baltimore, Maryland; Chicago, Illinois; Forsyth County, North Carolina; Los Angeles County, California; Northern Manhattan and Southern Bronx, New York; or Saint Paul, Minnesota.

To quantify calcification of the aortic valve, computed tomography scans were performed using either a four-slice multidetector computed tomography scanner with a spatial resolution of 1.15 mm^3 or an electron beam computed tomography scanner with a spatial resolution of 1.38 mm^3 (Imatron C150; General Electric Medical Systems, Milwaukee, Wisconsin, US). Aortic valve calcium was scored at a central MESA computed tomography reading centre (Los Angeles Biomedical Research Institute at Harbor-UCLA, Torrance, California, US) by a single reader and recorded in terms of Agatston units[201]. Genome-wide genotyping was performed using the Affymetrix Genome-Wide Human SNP Array 6.0 and following standard quality control, the genotypes were imputed using the Michigan Imputation Server[187] with the Haplotype Reference Consortium version r.1.1[188] as the reference panel.

6.5 References

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Chapter 7. General Discussion

The literature review and original research in chapters two to six contributed to the field of AS genetics, with a focus on the genetic factors underlying AS in European ancestry participants. I began in chapter two by summarizing the risk factors for aortic valve calcification and stenosis, which were predominantly cardiovascular risk factors including older age, male sex, and smoking. Genetic analyses such as GWAS and MR supported the contribution of lipids, namely LDL-C and Lp(a), to disease and suggested early valvular formation may have a causative role even in tricuspid individuals. Registry-based analyses demonstrated familial segregation of AS, confirming a heritable component and indicating the possible contribution of additional genetic loci beyond *LPA*, *PALMD*, and *TEX41*. In chapter three, I confirmed that multiple cardiovascular risk factors were associated with increased odds of AS following the inclusion of other risk factors as covariates in the model. However, MR analyses only provided evidence of causal roles for triglycerides, and possibly LDL-C, apoB, and SBP, emphasizing the contribution of abnormal lipid metabolism to disease development.

The examination of two well-known variants at the *LPA* locus in chapter four revealed that possessing two risk alleles in any combination (homozygous for either variant or as a compound heterozygous) resulted in greater than or equal to two times the odds of AS. I also demonstrated that age modified the association of *LPA* rs10455872, with the greatest effect size observed in the youngest individuals.

In chapters five and six, I identified new loci associated with AS through a GWAS and a genome-wide meta-analysis, respectively. The discovery of the *FADS1/2* locus implicated polyunsaturated fatty acid metabolism in AS pathophysiology, and informed by the roles of fatty acid desaturases

1 and 2 in n-6 and n-3 fatty acid synthesis, I demonstrated using MR that hepatic *FADS1* expression and elevated levels of AA contributed causally to AS. I also observed consistent and causal associations of variants at the *FADS1/2* locus and AA synthesis with AVC, suggesting fatty acid metabolism contributes to the spectrum of aortic valve disease, beginning with subclinical calcification and culminating in clinically-relevant stenosis. The genome-wide meta-analysis confirmed the discoveries of all previous genome-wide significant loci for AS and further identified eight new loci. A weighted PRS constructed from all independent ($r^2 \leq 0.01$) genome-wide significant variants was strongly associated with AS and improved the classification of AS beyond the performance of cardiovascular risk factors alone. In a phenome-wide association study, the individual variants were associated with multiple cardiovascular diseases and risk factors, and MR analyses supported the causal contribution of elevated lipid-related biomarkers and body mass index to AS.

At the outset of this work, the genetic architecture of AS was poorly understood. Evidence for the heritability of AS was limited: although previous studies had reported familial transmission of aortic valve disease[38,264], these findings identified rare variations in small numbers of families enriched in congenital valve disease. The discovery of *LPA* as the first locus for AS[46] was one of the earliest indications that common genetic variation may contribute to AS in tricuspid individuals. Recent genome-wide and transcriptome-wide studies have identified additional loci associated with AS[57,58,207], all of which were tagged by common genetic variants. My work further discovered new loci for AS, and likewise, many of the index variants identified had a frequency > 1% in European populations. The identification of multiple loci for AS provides strong evidence that AS is a heritable disease and that multiple common variants indeed contribute to AS risk.

Another reason for the discovery of predominantly common variants may be limitations in the statistical power to detect rarer associations in existing AS cohorts. In a genetic case-control study, power is affected by factors such as the frequency of the variant, its effect size, the number of cases, and the case-control ratio[265]. Thus for a given power, detecting a rarer variant or a variant with a smaller effect size would require increases in the number of participants. Calculations have demonstrated diminishing returns when using a case-control ratio of 1:4 as compared to 1:3, whereas large improvements in power can be achieved by increasing the absolute number of cases[266]. In my GWAS for AS in the GERA cohort, the *FADS1/2* rs174547 became genome-wide significant after a meta-analysis with 7 replication cohorts containing 5,926 cases and 251,000 controls. My genome-wide meta-analysis, which was the largest genome-wide meta-analysis for AS to date, identified the rarest variant detected in a pan-genome- or -transcriptome analysis (*HMGB1* rs181753401; MAF = 2.4×10^{-3}). The identification of rarer variants at genome-wide significance will require new large-scale cohorts or collaboration between existing AS cohorts to improve the study size and the number of cases.

A complementary approach to identifying novel loci for AS could be to prioritise variants which do not achieve genome-wide significance, but which have a known functional role or strong evidence of a biological link to disease. In chapter five, the *FADS1/2* rs174547 variant demonstrated suggestive evidence of association ($p \leq 1 \times 10^{-6}$). Its position in a locus with established roles in n-6 and n-3 polyunsaturated fatty acid metabolism[173,267] was consistent with previous reports of inflammatory markers in the aortic valves of AS cases[4,234]. Subsequent analyses confirmed AVC associations for both the index variant and the arachidonic acid to linoleic acid

ratio, reflecting the conversion of n-6 dietary fatty acids to their longer chain counterparts. MR further confirmed that levels of the n-6 AA contributed causally to AS and AVC, supporting the contribution of inflammation to AS development. These findings provided multiple lines of evidence that the *FADS1/2* locus was associated with AS, despite the initial discovery not achieving genome-wide significance. Even with compelling mechanistic evidence, the prevailing analytic approach for GWAS still emphasizes achieving genome-wide significance to identify novel loci, though there are some exceptions[268,269]. For *FADS1/2*, this was achieved in a meta-analysis with seven replication cohorts, again highlighting the need for large-scale collaboration in future variant-based analyses for AS.

Even as cohorts and collaborative studies increase in size, the number of novel variants identified in future studies may be limited by the genetic data available for analysis. Due to the prohibitive cost of performing exome or whole-genome sequencing in a large number of participants, the current iteration of genetic data for most AS cohorts has been generated through the imputation of genotyped data, usually with the Haplotype Reference Consortium reference panel[188] as was seen in chapters five and six. Compared to the 1000 Genomes Project phase 3 reference panel[133] which was widely used prior to the release of the larger Haplotype Reference Consortium reference panel (2,504 samples and 32,488 samples, respectively)[133,188], imputation using the latter panel yields a greater number of variants, and is more accurate, particularly at rare allele frequencies[188,270]. The recently released Trans-Omics for Precision Medicine (TOPmed) reference panel[271] surpasses the Haplotype Reference Consortium reference panel: it contains 97,256 samples, making it the largest panel currently available publicly. It also achieves increased imputation accuracy and yields a greater number of variants compared to its predecessors[271,272]. While the growing size of reference

panels has substantially increased the number of genetic variants which can be imputed – more than 308 million variants using TOPmed, for instance – the size of many AS cohorts precludes the observation of very rare variants. For example, for a variant with a minor allele frequency of 1×10^{-5}, a cohort of 50,000 participants is expected to observe just one carrier of the minor allele. Furthermore, the imputation quality of low frequency variants remains poor, with a mean $\underline{R^2} < 0.2$ for variants with minor allele frequencies $\leq 5 \times 10^{-4}$ when imputed using the 1000 Genomes or the Haplotype Reference Consortium[273], meaning these variants would likely be excluded from analysis. The establishment of larger imputation reference panels could substantially improve the quality of imputation for very rare variants. TOPmed reports that it is able to achieve reasonable imputation ($R^2 > 0.3$) of variants as rare as 2×10^{-5} [271], although the average imputation quality of variants at these low frequencies has not been reported for the reference panel. Alternatively, the rapidly falling price of whole-genome sequencing suggests sequencing could become an affordable option for more AS cohorts. The average cost to perform whole-genome sequencing on a sample was US $3,970 in January 2015; five years later, in February 2020, the average cost was US $645[274]. More high-quality genetic data could result in a larger number of variants eligible for GWAS, particularly among the rare variants.

To better leverage existing genetic data, alternatives to the commonly used additive, main effects model should be pursued for locus discovery. Simulations suggest the additive model is well powered to detect causal loci with additive and dominant modes of inheritance, but is poorly powered for recessive genetic loci[275]. Empirical testing confirms this: in a comparison of different models of inheritance, the additive model identified the most genome-wide significant loci. For three recessive variants examined by the authors, power calculations indicated at least three times

the number of participants would have been required to detect associations at genome-wide significance with an additive model[276]. GWAS using recessive or dominant models could detect new loci that would not be detected using an additive model. Recessive and dominant loci have been reported for type 2 diabetes[276,277] and coronary artery disease[233], which share their genetic etiology with AS as I demonstrated in chapter six, so loci with non-additive inheritance patterns may be associated with AS as well.

Gene-environment interaction analyses should be pursued to identify loci whose associations with AS differ across strata of risk factors. Identifying these interactions could provide insight into the disease process and provide an opportunity to refine risk prediction in specific sub-populations. In chapter four, I showed that *LPA* rs3798220 was associated with AS in men but not in women, suggesting the sex-specific modification of genetic effects could in part explain why male sex is a risk factor for the disease. This finding supports a previous report that Lp(a) increases in ovariectomized Lp(a) transgenic mice, and decreases following supplementation with exogenous estrogen[278]. In the same chapter, I also demonstrated that the association of *LPA* rs10455872 with AS differed by age, with the greatest effects in younger individuals. Additional gene-environment interactions are likely to contribute to AS, including at loci not yet identified for AS, and genome-wide gene-environment interactions should be undertaken to expand the scope of variants investigated for interactions. These findings could lead to the development of more precise risk assessment tools for AS, such as a sex-specific PRS.

Rare *NOTCH1* mutations have been associated with aortic valve disease[38], and in chapter six, I showed that a rare variant in *HMGB1* was genome-wide significant for AS, indicating rare variants

indeed contribute to AS. Gene burden and nonburden tests may identify additional loci in which multiple rare variants are causally associated, as has been shown for the bicuspid aortic valve[279,280]. These tests consider the aggregate effects of multiple variants within a gene, with several methodologies for how these rare variants are combined and differing assumptions regarding the causality and direction of effect of the modelled variants. By aggregating rare variants, these tests offer improved power compared to testing a single rare variant[281].

As I demonstrated in chapter six, gene- and expression-based analyses may identify additional loci associated with AS, reflecting the combined contribution of variants which may not individually be genome-wide significant. Unlike the burden and nonburden tests, these analyses do not assume that all or a subset of the composite variants are causal. In considering the *p* values of variants within a gene region using the MAGMA approach[69], I identified genes such as *LDLR*, supporting the causal contribution of LDL-C to AS reported previously[107] and which I replicated in chapter six. The approach also suggested *CFDP1* rather than *TMEM170A* as the gene mediating the effect associated with rs11643207 identified in the meta-analysis, providing insight into the biology underlying statistical associations. Furthermore, the prediction of differentially expressed genes using MetaXcan[70] and transcriptome models built from the Genotype-Tissue Expression project[72] identified or confirmed tissues where particular pathways may be dysregulated in AS cases. For example, previous studies have shown that variants at the *LPA* locus are associated with AS[46] (which I replicated in chapter four), Lp(a) levels are associated with a higher risk of incident AS[51], and variants at the *LPA* locus associated with AS are also associated with higher levels of lipoprotein(a). Our MetaXcan analysis predicted higher hepatic expression of *LPA* in AS cases, confirming that higher circulating concentrations of Lp(a) observed in cases is due to increased

expression of *LPA* and formation of Lp(a) particles rather than decreased clearance of Lp(a) from the blood. The analysis also predicted differential hepatic expression of several gene sets targeted by microRNAs, suggesting post-transcriptional regulation of certain pathways may contribute to AS development, an aspect of the disease that is currently poorly understood. Gene- and expression-based analyses were instrumental in identifying additional loci participating in disease pathophysiology, although replication of these findings is required in independent cohorts or through model organisms. These analytic methods also allowed me to identify contributing pathways in relevant tissues, furthering the mechanistic understanding of AS beyond what is achievable when identifying a significant variant in a GWAS or genome-wide meta-analysis.

A key benefit of variant discovery, as opposed to the less granular identification of a locus, is in improved risk stratification. In chapter four, the two-variant, unweighted *LPA* GRS was strongly associated with AS, with each additional risk allele conferring a modest increase in the odds of AS. This association persisted after adjustment for cardiovascular risk factors. Consistent with our findings, recent work by Trinder *et al* demonstrated that a 43-variant *LPA* GRS was associated with incident atherosclerotic cardiovascular disease in the UK Biobank, with each 120 nmol/L increase in Lp(a) associated with a 26% increase in risk[282]. Among participants with borderline to intermediate risk of atherosclerotic cardiovascular disease, the addition of their *LPA* GRS to a model containing a clinically-derived 10-year risk score was associated with a minor increase in the area under the receiver operating curve[282], suggesting enhanced risk stratification could be achieved for cardiovascular diseases by incorporating genetically-determined or measured Lp(a).

In chapter six, the weighted 18-variant PRS (which also included two variants from *LPA*) was strongly associated with AS. Not only did the association of the PRS with AS remain significant when adjusted for cardiovascular risk factors, its inclusion also increased the area under the curve of the model to 0.71 (95% CI, 0.70 to 0.72). Previous studies have not examined the AUC of classification models for AS involving PRS. There are only a limited number of studies which have examined the ability of non-genetic models to stratify AS risk. For von Willebrand factor multimers, the ratio of intermediate/high molecular weight to low molecular weight, reflecting hemostasis activity, has been reported to have high discrimination for severe AS (AUC, 0.86; 95% CI, 0.76 to 0.95)[283]. However, this was a small study (66 cases and 21 controls) and replication of the findings should be performed. Also, the generalizability of this finding to the general population may be limited as all controls had undergone AVR. The N-terminal pro-B-type natriuretic peptide, a marker of left ventricular function, has been shown to improve the classification of another severe AS endpoint, AVR, with an AUC of 0.73 (95% CI, 0.64 to 0.81)[284]. Adding the PRS, which captures genetic predisposition, to markers of heart function, dyslipidemia, blood pressure, smoking, and other risk factors considered in the clinical setting could lead to improvements in risk stratification, including earlier in the disease process.

Similar and significant associations of the 18-variant PRS with AVC highlighted the transferability of AS-derived genetic instruments to subclinical disease. While only five of 16 available variants were associated with AVC when modelled individually, the weighted PRS was associated with AVC and these associations persisted after adjustment for measured clinical risk factors. Thus, while not all effects of the AS variants may be mediated through calcification, the aggregation of the variants in a risk score does associate with calcification of the aortic valve. The successful

application of the AS PRS to AVC provides a promising entrée into the development of an AS PRS refined to predict AVC, benefiting from the larger cohorts available for AS versus AVC. As clinically-relevant AS is preceded by an asymptomatic phase of calcium accumulation in the aortic valve, the identification of individuals genetically predisposed to AVC, rather than AS, could allow for effective intervention earlier in the disease process. For instance, individuals at high risk of AVC could be counselled to adopt a healthier diet and more active lifestyle, as well as undergo more rigorous management of their clinical risk factors. Indeed, the prophylactic treatment of AS via lipid-lowering medications has been extensively examined. Whereas multiple statin trials have not demonstrated discernable effects on AS progression[105,106,285], MR indicates a causal contribution of LDL-C to AS[107], a finding I confirmed in chapter six. One explanation proposed for this apparent discrepancy is that LDL-C is involved in early-stage mineralization and calcification at the valve leaflets so lipid-lowering therapies may be more effective if administered prior to significant stenosis of the aortic valve[107].

At the other end of the disease spectrum, the contribution of genetic variants to late-stage AS also merits additional attention. In chapter four, we demonstrated that each risk allele of *LPA* rs10455872 was associated with an earlier age of diagnosis and with higher odds of AVR, suggesting individuals possessing the risk allele experience an accelerated disease process, both at the onset of clinically-significant stenosis and in severe disease requiring valve replacement. This is consistent with multiple reports indicating that lipoprotein(a) or lipoprotein(a)-associated particles are associated with faster AS progression, AVR, and cardiac death, even when cardiovascular risk factors are included as covariates[53,150,286]. Thus, genetic variants or associated

biomarkers can accelerate the onset of symptomatic severe AS, when AVR is recommended under current American College of Cardiology/American Heart Association guidelines[119].

Unfortunately, attempts to identify genetic predictors of AS progression will likely be hampered by currently available data. Not all AS cohorts with genetic data have progression data, or when available, the progression data are inconsistently defined, making comparison and replication of findings across cohorts difficult to standardize. For example, in chapter four, AVR status for the GERA cohort was ascertained from procedure codes in the EHR. In contrast, text mining has been used in the Penn Medicine Biobank to quantify disease severity as described in the echocardiogram report[194]. In the Multi-Ethnic Study of Atherosclerosis and the Framingham Offspring Study, baseline[46] and follow-up computed tomography scans have been performed, quantifying changes in AVC in the same individuals over time.

The UK Biobank offers one of the richest datasets currently available for correlating genetic variants with AS progression. In chapter six, we defined AS in the UK Biobank through a composite of diagnosis and procedure codes from the hospital inpatient records and causes of death in the death records. These components could be parsed to identify individuals who have been 1) diagnosed with AS, 2) undergone AVR, and 3) died with AS as a contributing cause, reflecting distinct stages in the disease process. Furthermore, the availability of diagnosis and death dates would allow for time to event analyses using genetic instruments, a poorly researched area in AS due to a dearth of suitable data. Despite heterogeneity in progression indicators across cohorts, future work should examine whether the loci identified for AS also associate with progression to severe disease, since the burden of clinical care is on later stages. The development of an AS

progression PRS could identify patients who are likely to progress rapidly. These patients could be prioritized for AVR if their disease is already moderate to severe, or potentially, a preventive medical therapy if their disease is identified at earlier stages. Additionally, the less rapidly progressing individuals could be targeted for more aggressive comorbidity management to reduce the number of patients who need an AVR.

A focus of the work in chapters three to six was the possible application of findings to clinical practice. I achieved large-scale replication of known loci and identified additional loci in a hypothesis-free manner with participation from many European-ancestry cohorts. The PRS demonstrated the potential use of genetic variants, either in isolation or as a composite with traditional risk factors, for clinical risk stratification. As was emphasized in chapters five and six, the identification of new pathways indicates lipid metabolism, calcification, inflammation, and adiposity all contribute to the development of AS, although the relative importance and interaction of these processes require additional investigation. Moreover, while I have identified loci and pathways which may participate in disease development, experimental work is necessary to identify downstream consequences of dysregulated pathways and to evaluate the therapeutic potential of targeting a particular locus or pathway.

The availability of suitable animal models may impede the ability of researchers to investigate these findings. For sortilin, the protein coded by *SORT1*, work in knockout mice has successfully demonstrated its involvement in the degradation of intracellular apolipoprotein B and circulating low-density lipoprotein cholesterol[228], as well as vascular calcification[287]. In contrast, knockout *FADS1* mice fail to survive past 12 weeks due to arachidonic acid deficiency, requiring dietary

supplementation for longer-term studies[288,289] as would be required for AS. For example, the apolipoprotein B-100/LDLR-deficient mouse develops AS after one year of a high cholesterol diet[290] and even the more invasive wire injury mouse model develops valvular calcification beginning at 20 weeks[291].

An important caveat to this body of work is that all discovery analyses were performed in individuals of European ancestry. I observed limited success when replicating index variants from the genome-wide meta-analysis in African- and Latin-American participants, probably due to a lack of power as the number of AS cases in each of these ancestries was less than 200. Another contributing factor could be differences in the genetic architecture of AS between ancestries. Compared to Chinese or South Asian participants, a previous study showed a stronger correlation between kringle IV type 2 copy number and lipoprotein(a) concentration, and a larger proportion of variation in lipoprotein(a) plasma levels explained by *LPA* variants, sex, LDL-C, and apolipoprotein B, in European-ancestry participants[292], suggesting the genetic basis for lipoprotein(a) is lower in East and South Asian individuals. The association of *LPA* rs10455872 with AVC is also markedly different in Chinese-, African-, and Latin-Americans[46]. These findings have serious ramifications for how we treat AS: if the genetic contributions to AS and a key risk factor, lipoprotein(a), are indeed ancestry-specific, then the development of risk stratification tools using GWAS findings from European-ancestry populations may not be appropriate in other ancestries. Additionally, identifying gene targets for medical therapies from studies performed in European-ancestry populations, without confirming their association with AS in other ancestries, could lead to therapies which are less effective in non-European individuals. These concerns also extend to the evaluation of medical therapies in randomized clinical trials which could eventually

be used for AS. A majority of participants in clinical trials for lipoprotein(a)-lowering therapies were of European ancestry[55,56,116], with the largest and most recent trial composed of 96% European-ancestry individuals[56]. Genetic studies for AS are largely conducted in European-ancestry cohorts, which hinders the application of their findings to diverse populations. A concerted focus on cross-ancestry replication and discovery in future studies is essential to the risk management and treatment of AS across ancestries.

In summary, this thesis achieved the objectives established at its outset. A review of the literature identified traditional cardiovascular risk factors to be well-established risk factors for AS, while less was known about the genetic etiology of the disease. Only three loci had been reported to be genome-wide significant at the time of the review. In collaboration with multiple international cohorts, my GWAS and genome-wide meta-analysis studies identified 10 novel loci for AS. Among these loci were *FADS1/2*, which encodes for the fatty acid desaturases 1 and 2, and *CELSR-SORT1*, involved in the catabolism of LDL-C. MR showed for the first time a causative role for n-6 fatty acid metabolism in AS, and confirmed the causality of multiple lipid and adipose biomarkers, consistent with previous reports. Lastly, an *LPA* GRS and an AS PRS were both strongly associated with AS, and remained associated following adjustment for cardiovascular risk factors, highlighting the value of incorporating genetic predisposition in patient risk stratification.

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Chapter 8. Conclusion

The genetic architecture of AS in European-ancestry individuals was incompletely understood at the onset of this work. By using the GWAS and meta-analysis methods, I replicated all known loci and identified 10 new loci not previously reported at genome-wide significance, including *FADS1/2*, *SMC2*, and *ARHGEF26*. Secondary analyses using gene- and expression-based analyses identified *IL6R*, *ZEB2*, and additional loci which may contribute to AS. A phenome-wide association study and genetic correlation analyses indicated a genetic overlap between AS and adiposity, lipid, and cardiovascular traits, and MR identified causal contributions of multiple lipid biomarkers to AS, including the novel causative effect of apolipoprotein B. Genetic loci identified from discovery and secondary analyses together support prior evidence that calcification, dyslipidemia, adiposity, and inflammation are key physiological processes underlying the development of AS.

Genetic risk scores composed of *LPA* variants or all genome-wide significant variants associated with AS, including following the inclusion of traditional cardiovascular risk factors as covariates in the model. These findings suggest improved risk stratification for AS could be achieved using disease-predisposing variants in conjunction with clinically-based assessments of risk. The interaction of two *LPA* variants with risk factors suggest prediction using PRS could be refined by considering the sex- or age-specific effects of variants. Moreover, several AS variants and the AS PRS were associated with AVC, raising the possibility of using an AS PRS to screen for individuals with calcification of the aortic valve, and thus the opportunity to prophylactically manage individuals with subclinical disease prior to the onset of clinical disease.

My identification of variants and the genes likely mediating their associations with AS may inform the development of new therapies, or the repurposing of existing therapies, to treat AS. Future work using complementary methods, such as experimental work involving model organisms, is necessary to confirm the causal genes and to provide insight into the effect of targeting these genes, thereby prioritizing the genes likely to yield the greatest therapeutic benefit if targeted using pharmaceutical agents. As all discovery analyses were performed in European-ancestry populations, and cross-ancestry replication in small numbers of African- and Latin-American individuals showed limited success, well-powered replication in other genetic ancestries should be attempted in future analyses to investigate the generalizability of these findings. Discovery analyses in populations of non-European ancestry should also be conducted to identify contributing loci. These analyses will be essential to understanding whether medical therapies developed with guidance from a predominantly European-ancestry literature will be effective in non-European or mixed individuals. Lastly, other strategies for identifying loci should be pursued, such as gene-environment GWAS, gene-burden tests, and non-additive models of inheritance.

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