Supplementary Appendix

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

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1. Support

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2. Introduction

This investigation stemmed from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium, an ongoing investigator-driven collaboration among several large, population based cohort studies that have genome-wide genotype data plus comprehensive individual phenotyping for a variety of endpoints. Details of this collaboration have been described previously.[1]

We employed a 2-stage analysis to first discover and then validate the association of genetic loci with the presence of aortic valve calcium (AVC) and mitral annular calcium (MAC). The initial discovery meta-analysis included genome-wide association study (GWAS) data from 3 large population-based cohorts including the Framingham Heart Study (FHS), the Age, Gene-Environment Susceptibility-Reykjavik Study (AGES-RS), and the Multi-Ethnic Study of Atherosclerosis (MESA). Discovery cohort participants were of White European ancestry, and had undergone genotyping and computed tomography (CT) scanning to assess the presence of AVC and/or MAC.

In stage 2, significant findings from this initial meta-analysis were tested in additional White European and multi-ethnic cohorts, including German participants of White European descent in the Heinz Nixdorf Recall Study (HNR), and African American and Hispanic American participants from the MESA and MESA Family Studies. Additional validation analyses were performed among Swedish participants in the Malmö Diet and Cancer Study (MDCS) and Danish participants in the Copenhagen City Heart Study (CCHS), in whom data for clinical aortic stenosis and aortic valve replacement were available.

Further details about the discovery and validation cohorts are described below.

3. Discovery Cohorts

3.A. Framingham Heart Study

The Framingham Heart Study (FHS) is a prospective, community based cohort study that was initiated in 1948 and now spans 3 generations, including the original cohort, their offspring and spouses of the offspring (Offspring Cohort, enrollment- beginning in 1971), and children from the largest offspring families (Generation 3 Cohort, enrollment beginning in 2000). Details regarding study recruitment and design have been reported previously.[2,3] Between June 2002 and April 2005, 1,422 of the 5,124 Offspring Study participants also took part in a substudy to examine subclinical cardiovascular disease using cardiac multidetector computed tomography (MDCT). Exclusion criteria for this substudy included pregnancy, age <40 years for women and age <35 years for men, and weight >320 pounds. All study protocols were approved by the Institutional Review Board for Boston University Medical Center and Massachusetts General Hospital. All study participants provided informed written consent prior to enrollment.

Computed Tomography and Analysis

Each participant in the FHS Offspring substudy underwent cardiac MDCT scanning, using a 8-slice Lightspeed Ultra scanner (GE Healthcare, Milwaukee, Wisconsin, USA) with a slice collimation of 8mm x 2.5mm. Two scans were performed for each individual using prospective gating, and each set of images was reviewed by one of two trained readers using offline software (Aquarius workstation, TeraRecon, San Mateo, California). If valve calcium was present on at least 1 of the 2 scans, the scans underwent a second, blinded read by an independent, trained radiologist. Disagreements regarding the presence or absence of valve calcium were resolved by consensus. Interobserver agreement for the presence or absence of AVC and MAC was kappa = 0.95 and kappa = 1.00. A total of 28 participants were excluded due to uninterpretable CT scans or the presence of prior valve surgery.

Genotyping and Imputation

Framingham Offspring Cohort participants provided consent for genotyping in the 1990s and had DNA extracted at that time. Genotyping was performed at Affymetrix (Santa Clara, CA) using the Affymetrix GeneChip Human Mapping 500K Array Set and 50K Human Gene Focused Panel, with funding provided by the NHLBI SNP-Health Association Resource (SHARe) project. Genotype results were imputed to 2.5 million SNPs using the Marchov Chain Haplotyping software (MACH, http://www.sph.umich.edu/csg/yli/software.html). Participant-specific quality controls included filters for call rate, heterozygosity, and number of Mendelian errors per individual. SNP-specific quality controls included filters for call rate and minor allele frequency. Additional high-density genotype results were available for 50K SNPs in ~2000 candidate genes on the Illumina iSelect IBC Chip (Illumina Inc., San Diego, CA, USA) from the NHLBI Candidate Gene Association Resource (CARe), as described previously.[4] These directly-genotyped IBC chip data were used to confirm the discovery meta-analysis results (which were based on imputed SNP data). The association between each SNP and the presence of valvular calcium was analyzed using logistic regression via generalized estimating equations (GEE) with each pedigree as a cluster and independent working correlation matrix, adjusting for age and sex.

3.B. Age, Gene-Environment Susceptibility-Reykjavik Study

The Age, Gene/Environment Susceptibility-Reykjavik Study (AGES-RS) is a prospective, population-based study of elderly Icelandic subjects, and represents a joint epidemiologic effort between the US National Institutes of Health, National Institute on Aging (Bethesda, MD) and the Icelandic Heart Association (Kopavogur, Iceland). Full details regarding AGES-RS design, recruitment and testing have been previously described.[5] Founded in 1967 and recruiting a total of 30,750 randomly selected Icelandic subjects born 1907-1935, the Reykjavik Study was stratified into sub-cohorts for sequential follow up. AGES-RS, initiated in 2002, is an attempt to comprehensively phenotype surviving members of the original Reykjavik Study. Between 2002-

2005, a total of 5764 original participants in the Reykjavik Study (representing a 72% response rate, all of White European descent) were recruited back for three days of comprehensive testing, including questionnaires, anthropomorphic assessment, blood testing for standard laboratory measures, chest computed tomography (CT), and both genetic and proteomic assessment. AGES-RS was approved by the National Bioethics Committee in Iceland that acts as the institutional review board for the Icelandic Heart Association (approval number VSN-00-063) and by the US National Institutes of Health, National Institute on Aging Intramural Institutional Review Board. All participants in this study gave informed, multistage consent prior to enrollment.

Computed Tomography and Analysis

All participants in AGES-RS underwent chest CT scanning (Siemens Somatom Sensation 4, Siemens Medical Solutions, Erlangen, Germany) during baseline enrollment and testing. Spatial resolution for these scans was 0.68 x 0.68 x 2.50 mm. The CT scan images were retrospectively analyzed for the presence of AVC using proprietary offline software that employed standard Agatston methods (and the same software as that used in the MESA analyses).[6] Calcium scoring was performed by a single independent reader (DSO) previously trained in valve calcium scoring. Subjects with valve replacements or scans that were uninterpretable due to artifact (e.g., from pacemaker leads) were excluded from analysis.

Genotyping and Imputation

Of the 5764 AGES-RS participants, 3664 participants were randomly selected for genome wide association testing. Genotyping of stored samples was performed at the Laboratory of Neurogenetics, Intramural Research Program, at the National Institute of Aging (Bethesda, Maryland) using the Illumina Human 370CNV-Duo Bead Chip platform (Illumina, Inc, San Diego, California, USA). Genotype results were imputed to 2.5 million SNPs using the Marchov Chain

Haplotyping software (MACH v.1.0.16, http://www.sph.umich.edu/csg/yli/software.html). Participant-specific quality controls included filters for call rate, heterozygosity, and number of Mendelian errors per individual. SNP-specific quality controls included filters for call rate and minor allele frequency. The association between each SNP and the presence of valvular calcium included adjustments for age and sex.

3.C. Multi-Ethnic Study of Atherosclerosis (MESA)

The Multi-Ethnic Study of Atherosclerosis (MESA) is a National Heart, Lung and Blood Institute-sponsored, population-based investigation of subclinical cardiovascular disease and its progression. A full description of the design and recruitment process has been reported previously.[7] In brief, a total of 6,814 individuals, aged 45 to 84 years, were recruited from six US communities (Baltimore City and County, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; New York, NY; and St. Paul, MN) between July 2000 and August 2002. Participants were excluded if they had physician-diagnosed cardiovascular disease prior to enrollment, including angina, myocardial infarction, heart failure, stroke or TIA, resuscitated cardiac arrest or a cardiovascular intervention (e.g., CABG, angioplasty, valve replacement, or pacemaker/defibrillator placement). Participants with subclinical valve disease were eligible for participation. Pre-specified recruitment plans identified four racial/ethnic groups (White European-American, African-American, Hispanic-American, and Chinese-American) for enrollment, with targeted oversampling of minority groups to enhance statistical power. The comprehensive baseline MESA examination included a clinic visit, serum analyses, and computed tomography (CT) examination of the heart. Information regarding the participants' demographic data and medical history, including medication use, was obtained by questionnaire. Ethnicity was self-reported. Only the 2622 participants who were self-identified as being of White European descent were eligible for inclusion in the discovery portion of these analyses. Other racial/ethnic groups were used for validation analyses. The institutional review

boards at each participating institution approved MESA and each individual participant provided informed written consent prior to enrollment.

Computed Tomography and Analysis

All MESA participants underwent baseline CT scans, which were analyzed for MAC and AVC. Three institutions used an electron beam computed tomography (EBCT) Imatron C150 scanner (GE Medical Systems, Milwaukee, WI), while three institutions used 4-slice multidetector CT (MDCT) scanners. Spatial resolution was 1.38 mm^3 for EBT (0.68 x 0.68 x 3.00 mm) and 1.15 mm^3 for MDCT (0.68 x 0.68 x 2.50 mm). Full details concerning the equipment, scanning methods, and CT quality control in MESA, including image calibration, phantom adjustment and inter-scanner reproducibility, have been reported previously.[8,9]. All scans were sent to a central MESA CT reading center (Harbor-UCLA Research and Education Institute, Los Angeles, CA) where they were analyzed by a single reader using proprietary offline software that utilizes the Agatston methodology. This software is the same as that used in the AGES-RS analyses.

Genotyping and Imputation

MESA participants provided consent for genotyping and had DNA extracted at the time of baseline enrollment between 2000-2002. Genotyping was performed at the Broad Institute of Harvard and MIT (Boston, Massachusetts, USA) and at the Affymetrix Laboratory (Santa Clara, CA, USA) using the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, California, USA). Genotype results were imputed to 2.5 million SNPs using IMPUTE v.2.1.0 software (http://mathgen.stats.ox.ac.uk/impute/impute.html).[10] Participant-specific quality controls included filters for call rate and number of Mendelian errors per individual. SNP-specific quality controls included filters for call rate and heterozygosity. Additional high-density genotype results were available for 50K SNPs in ~2000 candidate genes on the Illumina iSelect IBC Chip (Illumina Inc, San Diego, CA, USA) from the NHLBI Candidate Gene Association

Resource (CARe), as described previously.[4] This directly-genotyped IBC chip data were used to confirm the discovery meta-analysis results (which were based on imputed SNP data).

4. Validation Cohorts

4.A. MESA and MESA Family

MESA aimed to study subclinical atherosclerosis and its progression within a multiethnic population, and undertook targeted oversampling of African-American (n=1893), Hispanic (n=1496) and Chinese (n=803) participants to enhance statistical power. These participants of non-European descent underwent the same genotyping and phenotyping as did MESA participants of European descent described above.

The MESA Family Study was established in 2004 as an extension of MESA, recruiting siblings and sibpair families of index subjects from the original MESA cohort and additional families with a proband who matched the original MESA inclusion/exclusion criteria, for the goal of identifying genes contributing to subclinical cardiovascular disease in U.S. minority populations. A total of 2129 individuals from 595 families (yielding 3026 sibpairs) were recruited, with participants divided between African- and Hispanic-Americans. Between May 2004 and May 2007, MESA Family participants underwent similar phenotyping to the original MESA participants, including heart CT scanning and genotyping. Methods for calcium assessment, genotyping, imputation and analysis were identical to the methods described above for the MESA discovery cohort.

Validation analyses included participants in MESA and MESA Family who were not of European descent. Analyses were performed in each race/ethnicity separately and used GEE to account for familial correlations. Statistical models were adjusted for age, gender, and principal components of global ancestry.

4.B. Heinz Nixdorf Recall Study

The Heinz Nixdorf Recall Study (HNR) is a large, population-based investigation of cardiovascular disease among individuals living in the industrial Ruhr region of Northwestern Germany. The rationale and design of this study has been reported previously.[11] In brief, a total of 4814 randomly selected individuals (4487 of whom were free of self-reported cardiovascular disease), aged 45-74 years, were recruited between December 2000 and August 2003 with a recruitment efficacy of 56%.[12] All subjects underwent baseline questionnaires and testing including screening for subclinical cardiovascular disease using ECG, ankle-brachial indices, carotid ultrasound, and electron beam computed tomography (EBCT) for coronary and valvular calcium scoring. All subjects provided informed written consent prior to enrollment. The HNR was approved by the ethics committee of the University of Duisburg-Essen Medical School after the German Federal Office for Radiation Protection in Munich authorized the use of the EBCT.

Computed Tomography and Analysis

All HNR participants underwent EBCT scanning using an Imatron C-150 scanner (GE Medical Systems, Milwaukee, WI), 3mm slice thickness and prospective ECG gating at 1 of 2 radiologic centers in Germany. Scans were retrospectively scored for the presence of valve calcium using standard Agatston methods.

Genotyping and Analysis

Participants in the HNR gave informed written consent for genetic testing. A variety of genotype platforms were utilized within the cohort, including Illumina Hap300 (n=380), Illumina Hap550 (n=380), Illumina Human660W-Quad (n=133) and Illumina HumanOmni1-Quad (n=811). The SNP of interest (rs10455872) was directly measured in the HumanOmni1-Quad platform, but imputed on the other platforms. Because of potential false positive results when utilizing imputations from multiple platforms within a single cohort, only the n=811 individuals with the

directly measured SNP of interest were included in the validation cohort. Logistic regression analyses were adjusted for age and gender.

4.C. Malmö Diet and Cancer Study

The Malmö Diet and Cancer study (MDCS) is a prospective, population-based cohort study from the city of Malmö in southern Sweden. Data collection, sample characteristics and clinical definitions for MDCS have been described previously.[13] Briefly, 30,447 randomly selected men born between 1923 and 1945 and women born between 1923 and 1950 attended a baseline examination between 1991 and 1996. Participants underwent sampling of peripheral venous blood, measurement of blood pressure and anthropometric measures, and filled out a questionnaire. Informed consent was obtained from all participants and the study was approved by the ethics committee of Lund University, Sweden.

Aortic Stenosis and Valve Replacement

Prevalent and incident diagnoses of aortic stenosis or aortic valve replacement were identified by record linkage to national registers using personal identification numbers[14], The Swedish Hospital Discharge Register (HDR) and the Swedish Cause of Death Register (CDR).[15] Both registers are administered by the Swedish National Board of Health and Welfare. Data collection in the HDR was started in the 1960s and includes dates of admission and discharge as well as primary and contributory diagnoses from all public hospitals in Sweden. Reporting to the HDR has been compulsory since 1987 but the only hospital in Malmö (Malmö University Hospital) has reported since 1969. The CDR includes diagnoses from death certificates since 1952, regardless if death occurred outside of Sweden. Diagnoses in the HDR are coded as primary or contributory and in the CDR as underlying or contributory cause of death, both using the International Classification of Disease (ICD). The 8th edition (ICD-8) was used until the end of 1986, the 9th edition (ICD-9) between 1987 and 1996 and the 10th edition (ICD-10) from 1997

until present. Surgical procedures in the HDR are coded based on a national classification of surgical procedures, KKÅ from 1963 until 1989 and Op6 since then.

Aortic stenosis was defined as diagnosis codes 424.10, 424.11, 424.19 (ICD-8), 424B, 424BA, 424BB (ICD-9), I35.0 or I35.2 (ICD-10) as primary or contributory diagnosis. Aortic valve replacement for aortic stenosis was defined as a diagnosis code of AS in combination with a diagnosis code for aortic valve replacement; FMA or FMD for KKÅ and 3074, 3075, 3116, 3117 or 3078 for Op6.

To estimate the diagnostic validity of aortic stenosis in national registers, we reviewed patient records for 100 randomly selected patients diagnosed with prevalent or incident aortic stenosis and abstracted information on findings from diagnostic modalities. Patient records describing the aortic stenosis diagnosis were available for 98 patients. Of those, the diagnosis was based on observation of an increased transvalvular gradient upon echocardiography in 87 patients and upon cardiac catheterization in 2 patients (diagnosed in 1980 and 1983). Nine patients did not have diagnostic information supporting aortic stenosis but instead had echocardiographic evidence of aortic sclerosis without a transvalvular gradient (n=5) or miscoded insufficiency of the aortic (n=3) or mitral valve (n=1) without aortic stenosis or sclerosis. The median peak transvalvular gradient in patients with evidence for aortic stenosis was 58 mm Hg (IQR 40-90). Thus, the diagnostic validity for aortic stenosis diagnoses in national registers was high and most patients with evidence of aortic stenosis had moderate to severe stenosis. The majority of miscoded patients had echocardiographic evidence of aortic sclerosis, which does not have a diagnosis code, instead of aortic stenosis.

Genotyping

DNA extracted from peripheral blood cells was assigned to batches without regard to aortic stenosis status or personal identity. Batches were genotyped with the same set of reagents using real-time polymerase chain reaction (PCR) with 2.5 ng DNA as PCR template for allelic

discrimination on an ABI 7900HT (Applied Biosystems, Life Technologies, Carlsbad, CA, USA), according to the manufacturer's instructions. Genotype calls were obtained using SDS 2.3 software (Life Technologies, Carlsbad, CA, USA) and fluorescence intensity plots were manually inspected and curated.

4.D. Copenhagen City Heart Study

The Copenhagen City Heart Study (CCHS) is a prospective cardiovascular study of the Danish Copenhagen general population initiated in 1976-1978 with follow-up examinations in 1981-1983, 1991-1994, and 2001-2003. Data collection, sample characteristics, and clinical definitions for CCHS have been described previously[16]. Briefly, participants were randomly selected within 5-year age and sex strata from the Copenhagen Civil Registration System to reflect the population of Copenhagen aged 20-80+ years. Blood samples for DNA analysis were collected at the 1991-1994 and at the 2001-2003 examination of the cohort. A total of 10,400 CCHS participants were included, representing all participants of Danish descent with an available rs10455872 genotype and no history of aortic stenosis; 9,287 from the 1991-1994 examination and an additional 1,113 from the 2001-2003 examination. Examinations included, in addition to blood sampling, a self-administered questionnaire and a physical examination. Diabetes mellitus was self-reported disease, use of insulin or oral hypoglycemic drugs, and/or a non-fasting plasma glucose >11 mmol/L. We followed all individuals from DNA sampling (1991-94 or 2001-03 examination) and censored at the occurrence of aortic stenosis, death, or May 2011, whichever came first. Follow-up was 100% complete, and the mean and maximum follow-up times were 14 years and 20 years. Written informed consent was obtained from all participants and the study was approved by a local ethics committee (No. 100.2039/91. Copenhagen and Frederiksberg committee).

Aortic Stenosis and Valve Replacement

Prevalent and incident diagnoses of aortic stenosis, myocardial infarction, or aortic valve replacement were identified by record linkage to national registers using personal identification numbers. Information on aortic stenosis (ICD-8 codes 424.10, 424.11, 424.19 and ICD-10 codes I35.0 and I35.2) and myocardial infarction (ICD8 codes 410 and ICD-10 codes I21 to I22) were ascertained from the National Danish Patient Registry and the National Danish Causes of Death Registry; public registers administered by the Danish National Board of Health, and to which all hospitalizations and deaths in Denmark are reported. Aortic valve replacement for aortic stenosis was defined as a diagnosis code of aortic stenosis in combination with a diagnosis code for aortic valve replacement. Information on aortic valve replacement was ascertained from national classifications of surgical procedures (Surgery and Treatment procedures from 1971 to 1995, codes 30780, 30810, 31268, 31269, and SKS surgery procedures codes KFMD00 to KFMD20 and KFMD96) administered by the Danish National Board of Health.

Genotyping

DNA was extracted from peripheral blood cells. Genotyping for rs10455872 was performed by TaqMan SNP analysis. Enzymatic assays were used on fresh samples to measure plasma levels of total cholesterol and high-density lipoprotein (HDL) cholesterol.

5. Assessment of Valve Calcium and Clinical Aortic Stenosis

A similar methodology for assessing valvular calcium was used in each of the cohorts, according to established standards. Calcium strongly attenuates x-rays, appears bright on CT scans and is readily differentiated from surrounding tissue. Using standard Agatston methodology,[17] a threshold of ≥3 contiguous pixels of ≥ 130 Hounsfield units brightness was used to define calcium (except in the HNRS, where ≥4 pixels were needed). Lesions were classified as AVC if they resided within the aortic valve leaflets or commissures, exclusive of the

aortic annulus, aortic sinuses, aortic wall, or coronary arteries. Lesions were defined as MAC if they resided along the mitral annulus circumferentially, exclusive of the mitral leaflets (except in FHS where leaflet calcification was not excluded). If no lesions reached threshold values, calcium was deemed not present.

While individual study and recruitment sites used different scanner types, ranging from EBCT to 8-slice MDCT, the equivalency of various scanner types for the detection of valve calcium has been established previously.[8,9] Additionally, all sites used calcium phantoms to enhance quality control during image acquisition and analysis.

Clinical aortic stenosis and valve replacement surgery in the MDCS and CCHS were defined according to clinical diagnosis and procedure codes in national registries, as detailed above. The presence and severity of aortic valve disease is generally defined according to results of echocardiography and/or invasive hemodynamic assessment. International guidelines define the cutpoint between aortic sclerosis (valve calcification without stenosis) and aortic stenosis as a peak transvalvular velocity of 2.5 m/s by Doppler echocardiography or a calculated aortic valve area of <2.0 cm^2.[18] The most common indication for aortic valve replacement is the development of symptoms or reduced left ventricular systolic function in the setting of severe aortic stenosis, defined as a peak transvalvular velocity >4.0 m/s, mean gradient >40 mmHg or a calculated aortic valve area of <1.0 cm^2 (Class I indications). However, valve replacement is also recommended in other circumstances, including patients with moderate aortic stenosis undergoing other cardiac surgery (e.g., cardiopulmonary bypass grafting or aortic aneurysm repair) or those with asymptomatic very severe aortic stenosis (Class IIa indications). The adherence to guideline indications for valve replacement surgery in the MDCS and CCHS is unknown.

6. Measurement of Lipoprotein (a)

Lipoprotein (a) is comprised of an LDL-like particle that is covalently bound to an apolipoprotein (a) protein. Apo(a) proteins vary in size due to a variable number of kringle IV repeats, a reflection of the variable number of kringle IV repeats in the *LPA* gene. Lp(a) concentrations are highly heritable and remarkably constant over an individual's lifespan.[19,20] In general, there is an inverse relationship between the size of the apo(a) isoform and Lp(a) concentrations, potentially due to rate of clearance and metabolism.

Lipoprotein (a) levels were measured in FHS Offspring participants during the 3rd examination cycle (1991-1995). Lp(a) concentrations were measured using ELISA (enzyme-linked immunosorbent assay) methodology, in which a monoclonal antibody against apo(a) in a manner that is independent of apo(a) isoform size. Lp(a) concentrations were measured at a central laboratory at the Northwest Lipid Metabolism and Diabetes Research Laboratories (University of Washington, Seattle, Washington, USA). Lp(a) concentrations were measured in 18,000 members of the original Reykjavik cohort using a turbidimetric immunoassay (Denka Seiken; Tokyo, Japan); this assay is not affected by apo(a) isoform size (i.e., the number of kringle IV type 2 repeats).

7. Genotyping and Imputation

Each of the individual studies participating in this meta-analysis independently performed subject-level genotyping using different genetic platforms (described above). Genotype quality control and data cleaning were performed independently by each of the studies. Overall methods were similar across studies and reflect current standards of genotyping and analysis.

To standardize genotyping across cohorts and to allow for meta-analysis of cohort data, subject-level genotype data was imputed to the 2.5 million single-nucleotide polymorphisms (SNPs) described in the HapMap Centre d'Etude du Polymorphisme Humain samples (CEU population).[21,22] Methods for imputation differed across cohorts, but conformed to current standards. These 2.5 million SNPs were then used to analyze the association between genetic

loci and the presence of valvular calcium. Associations for imputed SNPs used expected allele dosages for the linear predictor to account for uncertainty in the imputed SNPs.

8. Statistical Analyses

8.A. Discovery Analyses

For the discovery stage (stage 1), the association between each SNP and the presence of valve calcium was analyzed in each individual cohort independently, using logistic regression with adjustments for age sex, and ancestry (if necessary). GEE was used in the FHS analyses to account for familial correlations. While the FHS, AGES-RS and MESA cohorts provided data for AVC, only the FHS and MESA provided data for MAC.

Individual study results were combined using fixed effects meta-analyses by inverse-variance weighting and METAL software.[23] Study-specific results were genomic control corrected, and SNPs with minor allele frequency <0.01 or an imputation ratio <0.3 were excluded prior to meta-analyses. Meta-analysis results were filtered to ensure that $\geq$2 cohorts contributed to the statistics for each SNP. For additional quality assessment, quantile-quantile (Q-Q) plots of the distribution of observed versus predicted p-values were created (**Supplementary Figures S1A and S1B**), as were Manhattan plots of the SNP p-values relative to the SNPs chromosomal location (**Figures 1A and 1B**). Regional plots (**Supplementary Figures S2A and S2B**) were generated using LocusZoom software.[24] To limit the number of false-positive associations given the large number of analyzed SNPs, the threshold for statistical genome-wide significance for individual SNP results in the discovery analysis was defined *a priori* as $p<5.0 \times 10^{-8}$. SNPs with $p<1.0 \times 10^{-5}$ are also reported as these may generate hypotheses for future studies.

The discovery meta-analysis was performed using imputed SNP data. To confirm these findings and exclude spurious association due to imputation, the association between valve

calcium and directly genotyped SNP data was also performed using the CARe high-density IBC chip SNP results (described above) that were available for the FHS and MESA cohorts.

8.B. Validation Analyses

In the replication stage (stage 2), significant SNPs identified in the initial meta-analysis were then tested in additional cohorts, including a White European population (HNR), a multi-ethnic population (MESA and MESA Family participants of non-European descent) and two large, population-based cohort with information on incident clinical aortic stenosis and valve replacement (MDCS and CCHS). As only single SNPs were tested for the validation phase, statistical significance was defined as $p < 0.05$, with a consistent direction of association.

Logistic regression analyses were performed for the association of these SNPs with the presence of valvular calcium in HNR, adjusted for age and sex. In order to account for familial correlations, GEE was used instead of logistic regression for the validation analyses for the non-European participants in MESA and MESA Family (**Supplementary Table S3**). These analyses were also adjusted for age, sex, and ancestry (as required). The statistical methodology for replication analyses in populations with incident aortic stenosis are described in detail below (**Supplementary Section 8.E.**).

8.C. Analyses Accounting for Prevalent Coronary Artery Disease

To exclude the possibility of spurious association between the identified LPA SNP and AVC due to coexistent coronary artery disease (clinical or subclinical), several supplemental analyses were performed. First, the intra-participant agreement for AVC and coronary artery calcium (CAC) was determined in each of the discovery cohorts using both kappa statistics for dichotomous agreement and correlation coefficients for continuous calcium scores. Second, the association between the identified SNP and AVC was determined after statistical adjustment for presence of CAC. We also examined the association among participants with low (or absent)

CAC burden. Third, the prevalence of the SNP minor allele frequency was determined among the 4 subgroups categorically defined by the presence or absence of AVC and CAC. Fourth, we excluded participants with known myocardial infarction at baseline in FHS and AGES-RS (MESA excluded known CAD at baseline). Overall, these analyses were intended to assess whether the identified SNP is independently associated with AVC by describing the relative strength of associations between the identified LPA SNP and both CAC and AVC, and by determining the residual strength of association with AVC after adjusting for or excluding coronary artery disease (clinical or subclinical). The results of these analyses are described below (**Supplementary Section 9**).

8.D. Instrumental Variable Analysis

To support the possibility of a causal association between *LPA* SNPs and AVC, an instrumental variable analysis (i.e., Mendelian randomization analysis)[25] was performed. At the time of gamete formation, genes not in linkage disequilibrium become randomly sorted ("Mendelian randomization"). Populations of individuals with different SNP genotypes will thus have similar distributions of known and unknown genetic confounders, providing a relatively unbiased method of determining if genetically determined risk factors are causally related to outcome. Along these lines, the relationships between *LPA* SNP genotype, plasma Lp(a) levels and the presence of AVC were assessed. Mendelian Randomization analysis takes advantage of the lifelong association of the *LPA* risk allele with a quantitative measures of Lp(a) and its association with AVC to estimate whether there is evidence for a causal association of Lp(a) with AVC.

For these analyses, genetically determined Lp(a) for each participant was calculated as the mean predicted Lp(a) concentration (based on each participant's genotype 0, 1 and 2 SNP copies of rs10455872) using the linear regression equation:.

$$Lp(a) = c + \beta_1(age) + \beta_2(sex) + \beta_3(\textit{LPA}\ genotype)$$

Genetically determined Lp(a) concentrations (as predicted by SNP data) were then regressed against dichotomous AVC presence in FHS and AGES-RS, respectively (Lp(a) has not yet been measured in MESA). The resultant OR can be interpreted as the (multiplicative) increase in odds for the presence of valve calcium per log-unit change in genetically determined Lp(a). A 2-stage Murphy-Topel variance estimator was used to compute 95% confidence intervals (CI). Individual study results were then combined using a random effects meta-analysis with inverse variance weighting, due to heterogeneity across cohorts.

The associations between other SNPs within the *LPA* gene (identified from the common set of imputed SNPs in the GWAS dataset) and Lp(a) levels were determined in FHS and AGES-RS, in order to test whether the SNP identified through the discovery meta-analysis demonstrated the strongest association with clinical Lp(a) levels. Cohort-specific, age and sex-adjusted linear regression analyses were performed, with β-coefficients and p-values displayed in **Supplementary Table S4**.

8.E. Aortic Stenosis Association Analyses

In MDCS, the association between the identified *LPA* SNP and incident clinical aortic stenosis was examined in participants free from aortic stenosis at baseline, using Cox proportional hazards regression with censoring at death from other causes, emigration or aortic valve replacement from other causes, adjusting for age, sex, current smoking and BMI (**Supplementary Table S5A**). A total of 60 participants (0.2%) were diagnosed with clinical aortic stenosis prior to baseline and were excluded from analyses. Additional sensitivity analyses were also performed: (a) restricted to participants who underwent aortic valve

replacement due to aortic stenosis during follow-up; and (b) who were free of myocardial infarction (at baseline and during follow-up).

In CCHS, the association between the identified *LPA* SNP and incident aortic stenosis was examined in participants free from aortic stenosis at baseline. Two sets of Cox proportional regression analyses were performed, censoring at death from other causes and adjusting for (a) age and sex; and (b) age, sex, total cholesterol, HDL cholesterol, systolic blood pressure, current smoking, and diabetes mellitus (**Supplementary Table S5B**). A total of 16 participants (0.2%) were diagnosed with clinical aortic stenosis prior to baseline and were excluded from analyses. Additional sensitivity analyses included: (a) analyses restricted to participants who underwent aortic valve replacement due to aortic stenosis during follow-up; and (b) analyses restricted to participants who were free of myocardial infarction (at baseline and at baseline as well as during follow-up).

9. Additional Results: Independence of the Association Between rs10455872 and Aortic-Valve Calcification from Coronary-Artery Calcification and Coronary Artery Disease

Several discovery-cohort analyses were performed to determine whether the association of rs10455872 with aortic-valve calcification was independent from coronary artery calcification and clinical coronary artery disease. First, there were only low levels of agreement (for calcium presence) and correlation (for calcium severity) between coronary-artery calcification and aortic valve calcification (**Supplementary Table S6**). Second, the association between rs10455872 and aortic-valve calcification remained significant after adjustment for the presence of coronary-artery calcification (**Supplementary Table S7**). In participants with low levels of coronary-artery calcification (<25th percentile for age and sex), rs10455872 remained associated with aortic-valve calcification in FHS and MESA. Third, there was no significant association between rs10455872 and coronary-artery calcification (**Supplementary Table S7**). Among participants

discordant for coronary-artery calcification and aortic-valve calcification, allele frequencies for rs10455872 were consistently higher in individuals with aortic-valve calcification rather than coronary-artery calcification **(Supplementary Table S8**). Lastly, exclusion of participants with prior myocardial infarction from FHS (n=53) and AGES-RS (n=231) did not materially change our results (FHS: $p=9.5x10^{-5}$, OR, 2.27; AGES-RS: $p=7.8x10^{-7}$, OR, 2.16).

10. Figures:

Figure S1A: QQ Plots Showing Observed vs. Expected Distribution of P-values for Associations of Single Nucleotide Polymorphisms with Aortic Valve Calcium. The genomic control inflation factor (λ), a measure of deviation from the uniform distribution, is near unity, suggesting minimal confounding from population substructure.

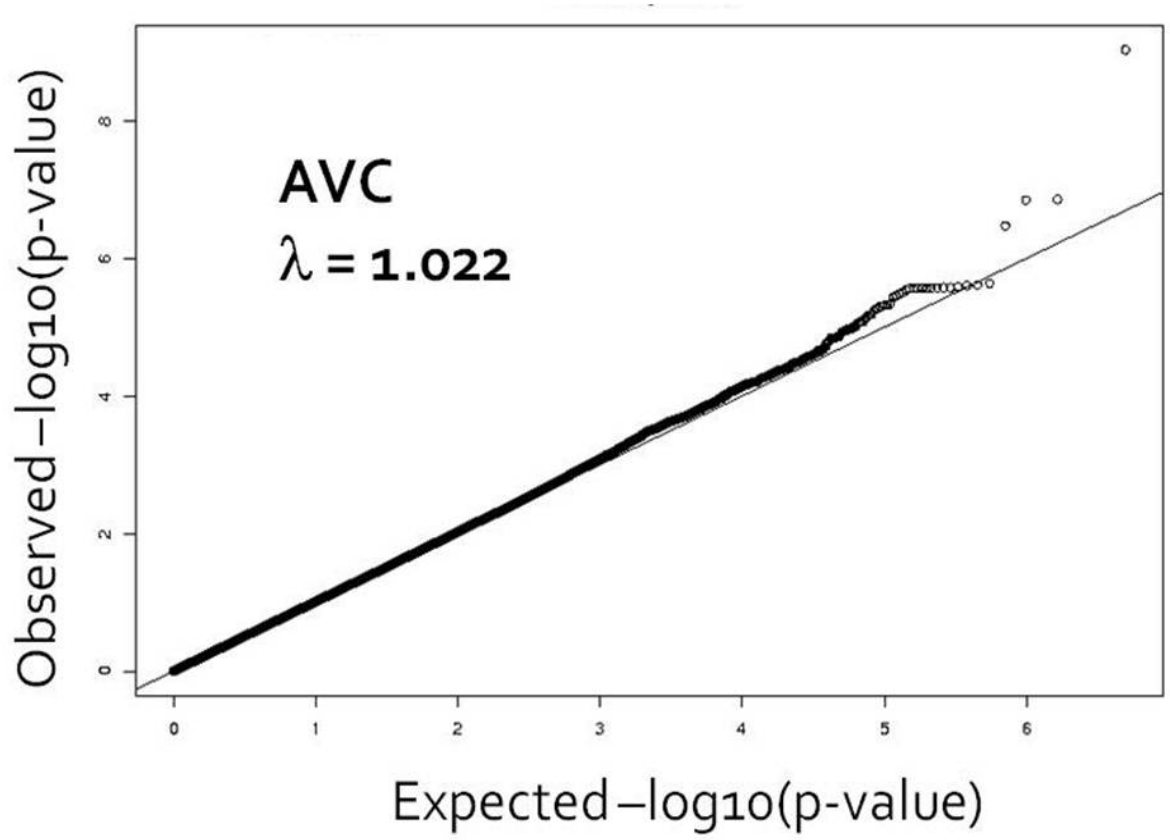


Figure S1B: QQ Plots Showing Observed vs. Expected Distribution of P-values for Associations of Single Nucleotide Polymorphisms with Mitral Annular Calcium. The genomic control inflation factor (λ), a measure of deviation from the uniform distribution, is near unity, suggesting minimal confounding from population substructure.

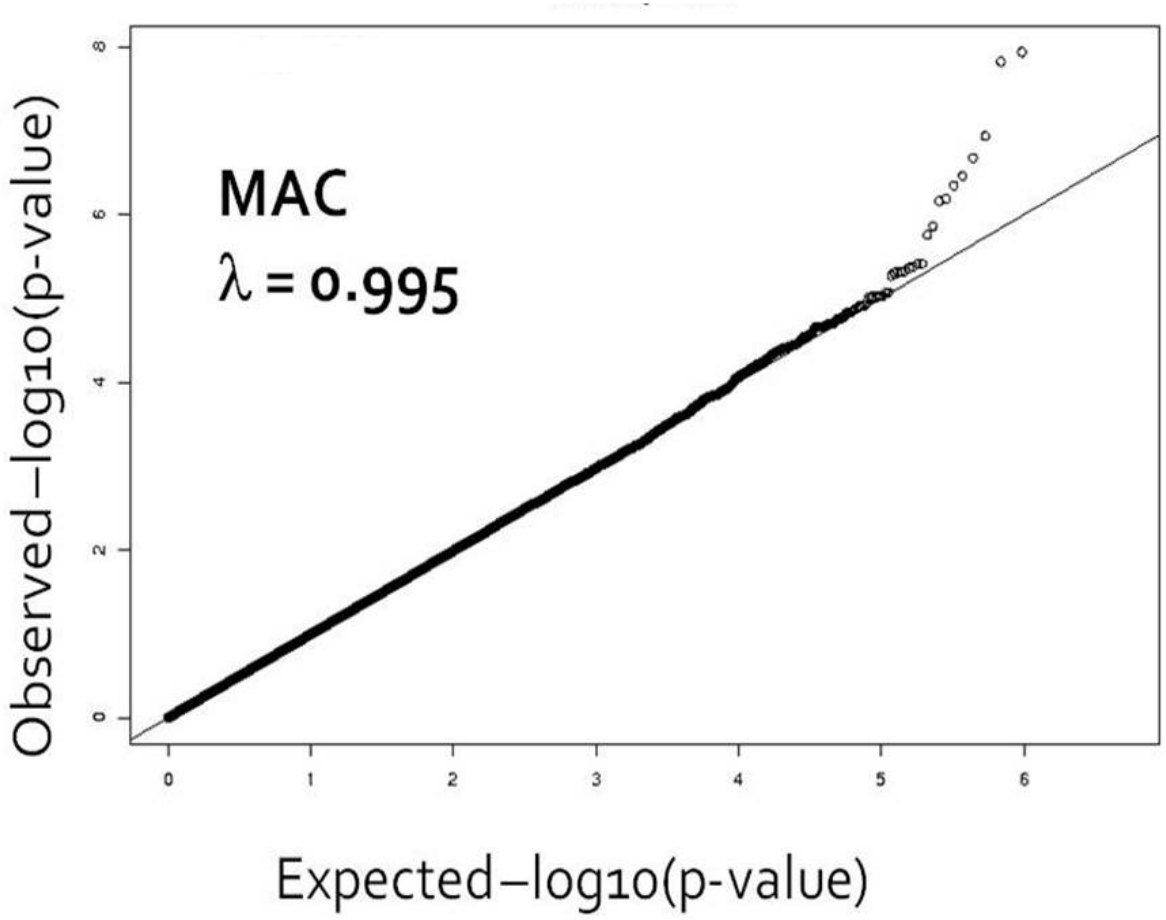


Figure S2A: Regional Plot of rs10455872, the Top Single Nucleotide Polymorphism (SNP) Associated with Aortic Valve Calcification. The chromosomal position (x-axis) of SNPs near rs10455872 along chromosome 6, is plotted against their association with aortic valve calcification (y-axis). The degree of linkage disequilibrium (r^2) for each SNP, relative to rs10455872, is color coded.

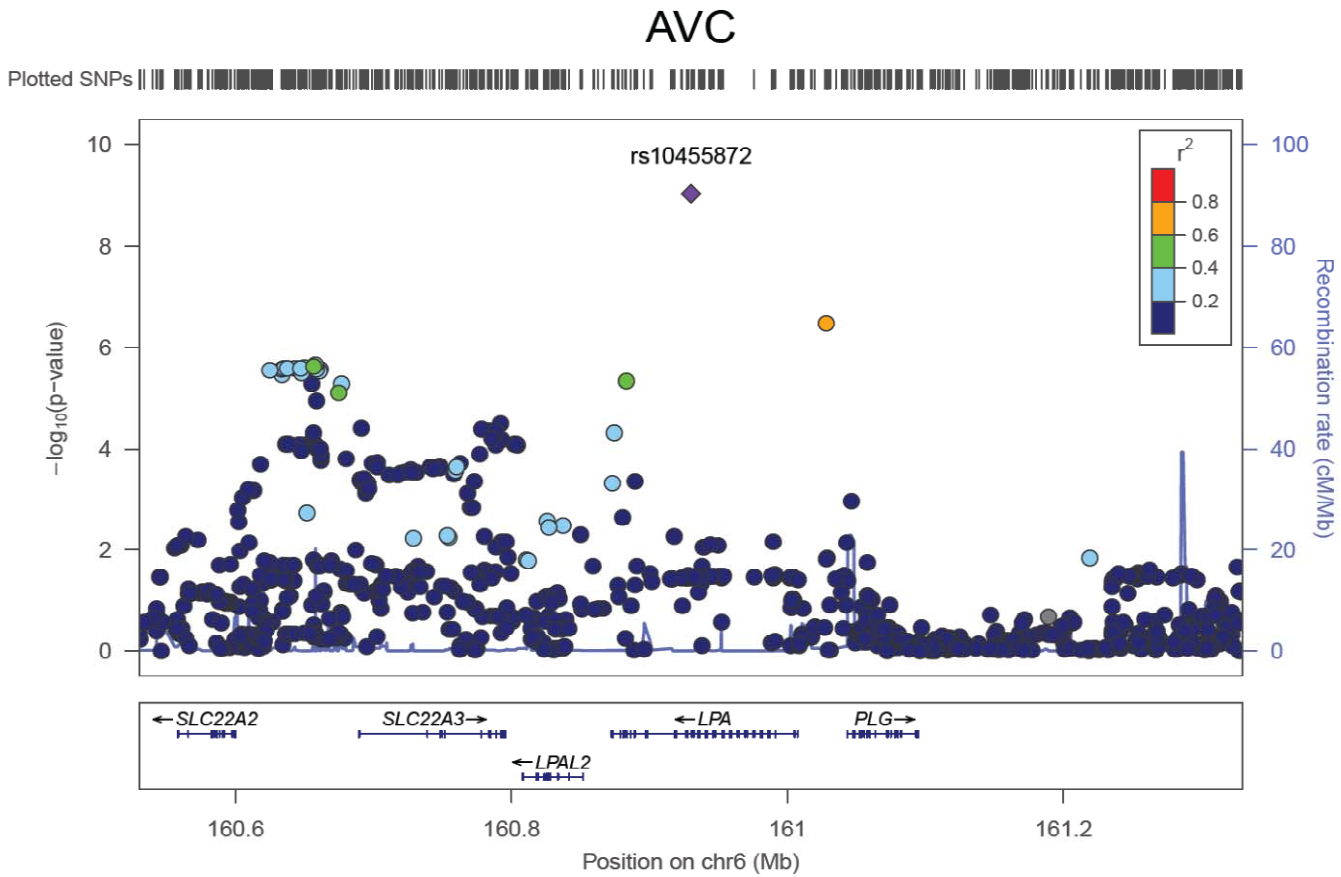
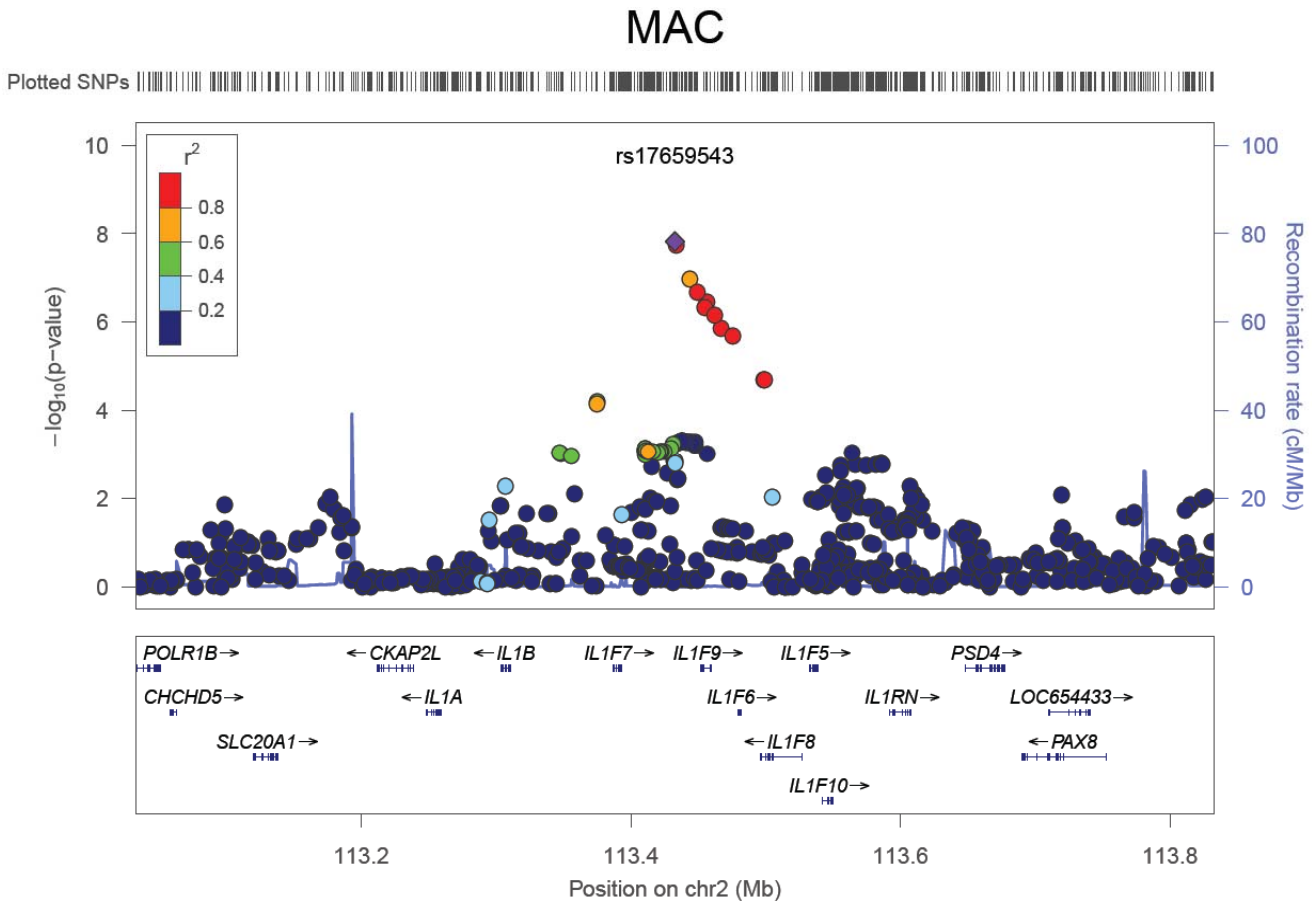


Figure S2B: Regional Plot of rs17659543, the Top Single Nucleotide Polymorphism (SNP) Associated With Mitral Annular Calcium. The chromosomal position (x-axis) of SNPs near rs17659543 along chromosome 2, is plotted against their association with mitral annular calcification (y-axis). The degree of linkage disequilibrium (r^2) for each SNP relative to rs17659543, is color coded.



11. Supplementary Tables

Table S1. SNPs with suggestive evidence for association ($5x10^{-8}<p<1x10^{-5}$) with aortic valve calcium (AVC).

SNP	Chromosome	Physical Location	Minor Allele	MAF	Beta coefficient	SE	*P-value*	Candidate genes*
rs2315065	6	161028134	A	0.08	0.62	0.12	3.26×10^{-7}	LPA;PLG
rs1146591	1	76005757	T	0.04	0.51	0.11	1.71×10^{-6}	MSH4;RABGGTB;ACADM
rs12724819	1	75907950	G	0.04	0.51	0.11	2.06×10^{-6}	SLC44A5;ACADM
rs16888478	8	39964422	T	0.16	0.27	0.06	2.15×10^{-6}	INDOL1;INDO
rs3127591	6	160657792	T	0.12	0.30	0.06	2.24×10^{-6}	SLC22A2;SLC22A3
rs3125055	6	160656777	A	0.10	0.40	0.08	2.38×10^{-6}	SLC22A2;SLC22A3
rs3106170	6	160649914	A	0.12	0.29	0.06	2.54×10^{-6}	SLC22A2;SLC22A3
rs3106167	6	160657380	G	0.12	0.29	0.06	2.61×10^{-6}	SLC22A2;SLC22A3
rs3103350	6	160642995	C	0.12	0.29	0.06	2.62×10^{-6}	SLC22A2;SLC22A3
rs2183470	6	160635083	T	0.12	0.29	0.06	2.64×10^{-6}	SLC22A2;SLC22A3
rs3127583	6	160647121	A	0.12	0.29	0.06	2.65×10^{-6}	SLC22A2;SLC22A3
rs3120151	6	160637462	T	0.12	0.29	0.06	2.65×10^{-6}	SLC22A2;SLC22A3
rs3125052	6	160658363	C	0.12	0.29	0.06	2.66×10^{-6}	SLC22A2;SLC22A3
rs3103349	6	160660711	T	0.12	0.29	0.06	2.67×10^{-6}	SLC22A3
rs12206585	6	160633514	A	0.12	0.29	0.06	2.69×10^{-6}	SLC22A2;SLC22A3
rs3120139	6	160661612	A	0.12	0.29	0.06	2.70×10^{-6}	SLC22A3
rs3127586	6	160624902	C	0.12	0.29	0.06	2.87×10^{-6}	SLC22A2
rs3125050	6	160660826	C	0.12	0.29	0.06	2.95×10^{-6}	SLC22A3
rs5745403	1	76085536	T	0.04	0.49	0.10	3.06×10^{-6}	MSH4;RABGGTB
rs3106172	6	160647965	G	0.12	0.29	0.06	3.19×10^{-6}	SLC22A2;SLC22A3
rs12203303	6	160633686	C	0.12	0.29	0.06	3.41×10^{-6}	SLC22A2;SLC22A3
rs12731410	1	76237313	A	0.04	0.50	0.11	4.12×10^{-6}	
rs11751605	6	160883220	C	0.12	0.29	0.06	4.65×10^{-6}	LPA;LPAL2
rs7823148	8	39969809	G	0.19	0.25	0.05	4.84×10^{-6}	INDOL1
rs1251566	1	76225851	T	0.04	0.49	0.11	4.97×10^{-6}	ASB17
rs12209517	6	160676726	G	0.13	0.29	0.06	5.19×10^{-6}	SLC22A3
rs3125056	6	160655271	C	0.11	0.30	0.07	5.32×10^{-6}	SLC22A2;SLC22A3
rs30149	16	14247206	C	0.06	0.43	0.09	5.74×10^{-6}	MKL2
rs1946819	1	76216173	G	0.04	0.48	0.11	6.06×10^{-6}	ASB17

rs675443	5	122672597	A	0.22	0.26	0.06	6.45×10^{-6}	CCDC100
rs11699517	20	5823786	T	0.03	0.51	0.11	6.78×10^{-6}	CHGB;MCM8;TRMT6;C20orf196
rs10835507	11	29268995	A	0.31	0.21	0.05	6.81×10^{-6}	
rs8016484	14	82937149	G	0.36	0.20	0.05	7.29×10^{-6}	
rs1251549	1	76216467	C	0.04	0.48	0.11	7.34×10^{-6}	ASB17
rs12739653	1	76214176	T	0.04	0.48	0.11	7.38×10^{-6}	ASB17
rs1832984	20	5818360	A	0.03	0.52	0.12	7.60×10^{-6}	CHGB;TRMT6;C20orf196
rs3125049	6	160674702	A	0.13	0.27	0.06	7.92×10^{-6}	SLC22A3
rs2179550	6	20644727	C	0.58	-0.19	0.04	8.19×10^{-6}	E2F3;CDKAL1
rs1612901	1	76230063	T	0.04	0.48	0.11	8.32×10^{-6}	ASB17
rs2870410	4	11304050	C	0.40	-0.19	0.04	8.41×10^{-6}	
rs2215233	4	11305152	G	0.40	-0.19	0.04	8.71×10^{-6}	
rs6847396	4	11299243	T	0.40	-0.19	0.04	9.49×10^{-6}	
rs10002275	4	11298547	A	0.40	-0.19	0.04	9.79×10^{-6}	
rs1704848	4	185471077	G	0.44	-0.20	0.05	9.86×10^{-6}	

Abbreviations: MAF, minor allele frequency; SE, standard error

*Candidate genes are within 60 kB from the SNP

Table S2. SNPs with suggestive evidence for association ($5x10^{-8}<p<1x10^{-5}$) with mitral annular calcium (MAC).

SNP	Chromosome	Physical Location	Minor Allele	MAF	Beta coefficient	SE	*P-value*	Candidate genes*
rs17607787	2	113443353	C	0.1559	0.4854	0.0912	1.01×10^{-7}	IL1F6;IL1F9;IL1F8;IL1F7
rs6542107	2	113449031	G	0.1588	0.4733	0.0912	2.10×10^{-7}	IL1F6;IL1F9;IL1F8;IL1F7
rs13392494	2	113456146	T	0.1588	0.4633	0.0909	3.47×10^{-7}	IL1F6;IL1F9;IL1F8
rs13410389	2	113454494	A	0.1587	0.458	0.0908	4.52×10^{-7}	IL1F6;IL1F9;IL1F8
rs17054136	5	156405403	A	0.0346	0.9303	0.187	6.48×10^{-7}	HAVCR1;HAVCR2
rs17042691	2	113462283	T	0.1592	0.4535	0.0914	6.91×10^{-7}	IL1F6;IL1F9;IL1F8
rs1446516	2	113466941	G	0.1595	0.4415	0.0914	1.37×10^{-6}	IL1F6;IL1F9;IL1F8
rs6714534	2	113475801	C	0.1595	0.4355	0.0916	2.00×10^{-6}	IL1F6;IL1F9;IL1F5;IL1F8
rs9376256	6	137474983	T	0.103	0.5076	0.1076	2.40×10^{-6}	IL22RA2
rs2951868	8	6703754	T	0.4851	-0.4018	0.087	3.86×10^{-6}	DEFB1;XKR5
rs2426509	20	52489243	C	0.1884	0.4243	0.0919	3.88×10^{-6}	DOK5
rs7209400	17	44805056	C	0.4572	-0.34	0.0737	3.97×10^{-6}	PHB;ZNF652
rs6916938	6	137494423	A	0.0999	0.5063	0.11	4.21×10^{-6}	IL22RA2
rs12881682	14	62461098	T	0.497	0.3466	0.0754	4.30×10^{-6}	KCNH5
rs9376259	6	137486496	G	0.1	0.4961	0.1085	4.83×10^{-6}	IL22RA2
rs10220279	14	72227033	A	0.46	-0.3442	0.0753	4.83×10^{-6}	DPF3
rs9376258	6	137480759	T	0.1015	0.4959	0.1086	4.92×10^{-6}	IL22RA2
rs12205573	6	137454920	A	0.0736	0.6291	0.1383	5.37×10^{-6}	IL22RA2;IL20RA
rs2332909	14	72233067	C	0.4453	-0.3386	0.0761	8.55×10^{-6}	DPF3
rs9389471	6	137473666	T	0.1034	0.4809	0.1081	8.56×10^{-6}	IL22RA2
rs2426511	20	52493290	A	0.1822	0.4102	0.0925	9.27×10^{-6}	DOK5
rs9773838	8	6703692	A	0.4948	0.3736	0.0843	9.40×10^{-6}	DEFB1;XKR5
rs7201037	16	20150981	C	0.0753	-0.7501	0.1694	9.49×10^{-6}	
rs1956024	14	62460765	A	0.4959	-0.3247	0.0733	9.52×10^{-6}	KCNH5
rs12588293	14	62459961	C	0.4958	-0.3246	0.0733	9.59×10^{-6}	KCNH5
rs1950572	14	62462608	C	0.4962	-0.3246	0.0734	9.67×10^{-6}	KCNH5

Abbreviations: MAF, minor allele frequency; SD, standard error

*Candidate genes are within 60 kB from the SNP

Table S3. Cross-ethnicity Replication in MESA of Genome Wide Significant SNPs for Aortic Valve and Mitral Annular Calcium.

AVC: rs10455872

Ethnicity (cohort)	N	Minor Allele	MAF	OR (95% CI)	P-value
African-American	2496	G	0.02	3.57 (1.42-8.99)	0.0068
Hispanic-American	2026	G	0.02	2.75 (1.39-5.45)	0.0037
Chinese-American	774	G	0.005	1.13	0.96

MAC: rs17659543

Ethnicity (cohort)	N	Minor Allele	MAF	OR (95% CI)	P-value
African-American	2495	T	0.23	1.15 (0.88-1.50)	0.30
Hispanic-American	2025	T	0.14	1.44 (1.02-2.02)	0.04
Chinese-American	774	T	0.01	n/a	n/a

*African-American and Hispanic-American samples include MESA and MESA Family participants.

Abbreviations: AVC, aortic valve calcium; MAC, mitral annular calcium; N, number; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval.

Table S4. Association between all available SNPs in the *LPA* locus (from the set of imputed SNPs used for the discovery meta-analysis) and plasma Lp(a) among participants in the Framingham Heart Study (FHS) and the Age, Gene/Environment Susceptibility-Reykjavik Study (AGES-RS). Analyses are age/sex adjusted and results are ranked by AGES-RS p-value. Rs10455872 demonstrates both the strongest beta coefficient and the smallest p-value.

		FHS		AGES-RS	
rs_ID	Location	beta	P-value	beta	P-value
rs10455872	1.61E+08	22.45	1.36E-31	18.2	3.17E-138
rs2315065	1.61E+08	17.87	1.51E-18	16.8	1.23E-97
rs11751605	1.61E+08	6.8	2.58E-09	6.64	1.12E-52
rs3124785	1.61E+08	3.65	1.02E-04	4.26	8.60E-39
rs3127596	1.61E+08	2.48	6.52E-03	3.72	9.67E-31
rs3127599	1.61E+08	2.21	0.01	3.55	8.23E-29
rs3123629	1.61E+08	2.22	0.01	3.55	8.39E-29
rs3127569	1.61E+08	2.25	0.01	3.56	9.39E-29
rs7770628	1.61E+08	2.59	1.01E-03	2.8	1.95E-21
rs6913833	1.61E+08	-2.1	0.01	-2.62	3.35E-17
rs9456552	1.61E+08	-1.94	0.02	-2.49	7.11E-17
rs7771129	1.61E+08	-1.94	0.02	-2.46	9.81E-17
rs6415085	1.61E+08	-1.95	0.02	-2.45	1.15E-16
rs9295130	1.61E+08	-1.96	0.02	-2.45	1.20E-16
rs10945682	1.61E+08	-1.96	0.02	-2.45	1.20E-16
rs6923917	1.61E+08	-1.96	0.02	-2.45	1.20E-16
rs6929299	1.61E+08	-1.96	0.02	-2.45	1.20E-16
rs7760585	1.61E+08	-1.96	0.02	-2.45	1.20E-16
rs9355813	1.61E+08	-1.96	0.02	-2.45	1.20E-16
rs6932014	1.61E+08	-1.96	0.02	-2.45	1.20E-16
rs6902102	1.61E+08	-1.96	0.02	-2.45	1.20E-16
rs9365179	1.61E+08	-1.95	0.02	-2.45	1.20E-16
rs1569933	1.61E+08	-1.9	0.02	-2.45	1.20E-16
rs1321196	1.61E+08	-1.79	0.03	-2.45	1.20E-16
rs9355814	1.61E+08	-1.99	0.01	-2.45	1.24E-16
rs6455696	1.61E+08	-1.9	0.02	-2.45	1.74E-16
rs7761377	1.61E+08	-1.9	0.02	-2.45	1.74E-16
rs7746273	1.61E+08	-1.93	0.02	-2.45	1.81E-16
rs10755578	1.61E+08	3.78	2.27E-06	2.35	2.65E-16
rs6919346	1.61E+08	-4.47	1.96E-06	-3.22	2.90E-16
rs7772437	1.61E+08	-0.93	0.33	-2.7	8.91E-16
rs2144723	1.61E+08	-0.93	0.33	-2.7	9.95E-16
rs2314852	1.61E+08	-4.38	1.44E-03	-3.42	2.59E-15

rs9355817	1.61E+08	-2.03	0.01	-2.37	3.15E-15
rs783147	1.61E+08	-3.24	2.99E-05	-2.27	3.84E-15
rs6415084	1.61E+08	1.1	0.17	2.44	9.55E-15
rs6935921	1.61E+08	-2.75	9.96E-03	-2.74	2.19E-14
rs6455698	1.61E+08	-2.72	0.01	-2.73	2.38E-14
rs783144	1.61E+08	-3.02	6.55E-03	-2.78	2.69E-14
rs9365171	1.61E+08	-0.47	0.57	-2.25	5.98E-14
rs12175867	1.61E+08	-3.03	1.57E-03	-2.46	9.30E-14
rs6926458	1.61E+08	-3.03	1.57E-03	-2.46	9.36E-14
rs6940254	1.61E+08	-3.02	1.57E-03	-2.45	1.02E-13
rs7770685	1.61E+08	-3.01	1.60E-03	-2.45	1.02E-13
rs13202636	1.61E+08	-3.02	1.58E-03	-2.45	1.03E-13
rs9457946	1.61E+08	-1.09	0.18	-2.23	1.82E-13
rs7453899	1.61E+08	-1.08	0.19	-2.22	1.85E-13
rs7771801	1.61E+08	-1.08	0.19	-2.22	1.86E-13
rs7765803	1.61E+08	-1.08	0.19	-2.22	1.87E-13
rs7765781	1.61E+08	-1.08	0.19	-2.22	1.88E-13
rs6455689	1.61E+08	-1.08	0.19	-2.22	1.88E-13
rs9355295	1.61E+08	-1.08	0.19	-2.22	1.98E-13
rs9457943	1.61E+08	-1.09	0.19	-2.21	2.02E-13
rs6455688	1.61E+08	-1.09	0.19	-2.21	2.02E-13
rs6923877	1.61E+08	-1.09	0.19	-2.21	2.02E-13
rs3127595	1.61E+08	-0.57	0.47	-2.09	4.99E-13
rs4708871	1.61E+08	-16.46	1.06E-05	-11.9	8.39E-13
rs1950562	1.61E+08	-0.89	0.37	-2.42	9.54E-13
rs4252065	1.61E+08	-0.88	0.37	-2.42	9.60E-13
rs9364558	1.61E+08	2.96	2.01E-04	2.05	1.27E-12
rs7761293	1.61E+08	-0.7	0.38	-2.04	1.38E-12
rs3798221	1.61E+08	-2.39	0.03	-2.48	3.07E-12
rs9346818	1.61E+08	-0.71	0.37	-1.98	8.98E-12
rs4252109	1.61E+08	-4.77	1.76E-08	-2.22	1.13E-11
rs4252096	1.61E+08	-4.77	1.76E-08	-2.22	1.13E-11
rs4252093	1.61E+08	-4.77	1.76E-08	-2.22	1.13E-11
rs4252087	1.61E+08	-4.78	1.76E-08	-2.22	1.15E-11
rs1853017	1.61E+08	-4.78	1.78E-08	-2.22	1.15E-11
rs4252078	1.61E+08	-4.83	1.80E-08	-2.23	1.15E-11
rs13231	1.61E+08	-4.97	1.38E-08	-2.22	1.24E-11
rs4252072	1.61E+08	-4.86	1.84E-08	-2.26	1.30E-11
rs4252117	1.61E+08	-4.48	1.46E-07	-2.14	5.82E-11
rs4252120	1.61E+08	-4.48	1.46E-07	-2.14	5.89E-11
rs9456578	1.61E+08	-4.48	1.50E-07	-2.13	6.90E-11
rs9365200	1.61E+08	-1.63	0.04	-1.86	2.35E-10
rs9346833	1.61E+08	-1.63	0.04	-1.86	2.35E-10
rs9365201	1.61E+08	-1.63	0.04	-1.86	2.36E-10

rs9347438	1.61E+08	-1.63	0.04	-1.86	2.36E-10
rs1897108	1.61E+08	-3.77	7.09E-06	-2.04	4.25E-10
rs4252107	1.61E+08	-5.28	1.44E-08	-2.2	6.33E-10
rs1406888	1.61E+08	-1.41	0.12	-1.99	1.15E-09
rs6921516	1.61E+08	-0.85	0.31	-1.89	1.17E-09
rs9347407	1.61E+08	0.21	0.79	-1.74	1.61E-09
rs4129086	1.61E+08	0.2	0.79	-1.74	1.64E-09
rs9365166	1.61E+08	0.18	0.82	-1.66	9.49E-09
rs9347408	1.61E+08	0.17	0.83	-1.66	9.57E-09
rs9457930	1.61E+08	0.17	0.83	-1.66	9.69E-09
rs1367211	1.61E+08	-1.02	0.23	-1.73	4.50E-08
rs1367209	1.61E+08	-1.02	0.23	-1.73	4.51E-08
rs9456577	1.61E+08	1.08	0.76	4.66	6.14E-07
rs12214416	1.61E+08	-2.85	0.16	-3.52	7.93E-07
rs4252055	1.61E+08	-0.23	0.91	-3.23	2.06E-06
rs7449650	1.61E+08	-1.76	0.03	-1.48	2.61E-06
rs9365205	1.61E+08	-1.66	0.42	-3.11	2.67E-06
rs1367210	1.61E+08	-2.3	0.04	-1.78	3.71E-06
rs1321195	1.61E+08	-2.33	0.04	-1.78	3.79E-06
rs9457933	1.61E+08	-3.09	2.00E-03	-1.63	3.88E-06
rs783148	1.61E+08	-2.33	0.04	-1.78	3.92E-06
rs4708869	1.61E+08	-3.12	1.85E-03	-1.6	5.25E-06
rs3124787	1.61E+08	-1.91	0.02	-1.45	5.27E-06
rs3123630	1.61E+08	-3.11	1.87E-03	-1.6	5.32E-06
rs2941382	1.61E+08	-3.11	1.91E-03	-1.6	5.46E-06
rs1009324	1.61E+08	-3.05	2.49E-03	-1.6	5.48E-06
rs13213129	1.61E+08	-1.35	0.61	-2.83	8.60E-06
rs1801693	1.61E+08	-1.83	0.02	-1.4	9.88E-06
rs6939089	1.61E+08	-2.1	0.12	2.35	1.59E-05
rs2457548	1.61E+08	-2.88	5.16E-03	-1.52	1.96E-05
rs2136675	1.61E+08	-2.01	0.02	-1.23	1.40E-04
rs9457935	1.61E+08	-1.08	0.18	-1.18	2.53E-04
rs9458011	1.61E+08	-1.67	0.51	-2.88	2.74E-04
rs7774579	1.61E+08	-1.03	0.2	-1.16	2.87E-04
rs12207325	1.61E+08	-2.21	0.74	-5.05	2.87E-04
rs7453836	1.61E+08	-1.03	0.2	-1.16	2.98E-04
rs9365168	1.61E+08	-1.03	0.2	-1.16	3.03E-04
rs4709458	1.61E+08	1.25	0.11	1.06	3.15E-04
rs2295368	1.61E+08	1.26	0.11	1.05	3.21E-04
rs4252092	1.61E+08	1.29	0.1	1.05	3.23E-04
rs1321201	1.61E+08	1.28	0.1	1.05	3.23E-04
rs1130656	1.61E+08	1.27	0.11	1.05	3.23E-04
rs14224	1.61E+08	1.28	0.1	1.05	3.25E-04
rs1830519	1.61E+08	4.12	2.28E-05	1.23	3.67E-04

rs7754014	1.61E+08	1.73	0.07	-1.21	4.04E-04
rs7754188	1.61E+08	1.73	0.07	-1.21	4.04E-04
rs2457569	1.61E+08	-1.55	0.06	-1.1	6.80E-04
rs9364559	1.61E+08	1.84	0.07	-1.19	7.87E-04
rs2504921	1.61E+08	0.13	0.87	-0.968	8.46E-04
rs12212724	1.61E+08	1.83	0.06	-1.14	1.12E-03
rs7749836	1.61E+08	1.84	0.06	-1.14	1.14E-03
rs10945673	1.61E+08	1.9	0.05	-1.14	1.15E-03
rs7767084	1.61E+08	2.42	0.02	-1.18	1.36E-03
rs9355804	1.61E+08	2.21	0.03	-1.11	1.55E-03
rs9355803	1.61E+08	2.23	0.02	-1.11	1.61E-03
rs2255830	1.61E+08	-0.31	0.7	-0.896	2.08E-03
rs4252186	1.61E+08	46.28	0.01	30.3	2.33E-03
rs1465620	1.61E+08	3.43	1.08E-04	0.8	0.01
rs9295131	1.61E+08	3.43	1.08E-04	0.8	0.01
rs3823055	1.61E+08	3.41	1.16E-04	0.8	0.01
rs1594307	1.61E+08	3.4	1.24E-04	0.798	0.01
rs9457931	1.61E+08	4.58	0.03	1.54	0.01
rs2457550	1.61E+08	-1.73	0.05	-0.865	0.01
rs4252116	1.61E+08	3.99	0.08	7.8	0.01
rs1321200	1.61E+08	3.99	0.08	7.6	0.01
rs4252121	1.61E+08	3.99	0.08	7.72	0.01
rs1853025	1.61E+08	3.99	0.08	7.56	0.01
rs1321199	1.61E+08	3.99	0.08	7.56	0.01
rs1321198	1.61E+08	3.99	0.08	7.48	0.01
rs12662959	1.61E+08	1.57	0.46	2.66	0.01
rs1897107	1.61E+08	3.99	0.08	7.22	0.02
rs9355816	1.61E+08	-1.59	0.66	-4.1	0.02
rs783146	1.61E+08	1.39	0.21	-0.8	0.03
rs12526465	1.61E+08	0.36	0.75	-0.876	0.03
rs7759633	1.61E+08	0.36	0.76	-0.876	0.03
rs1084651	1.61E+08	-1.67	0.15	-0.84	0.04
rs17557261	1.61E+08	0.73	0.53	-0.868	0.04
rs1652508	1.61E+08	4.27	4.90E-05	0.828	0.05
rs6914575	1.61E+08	4.76	3.84E-03	-1.03	0.06
rs4252058	1.61E+08	-3.25	0.44	-3.91	0.06
rs783149	1.61E+08	-1.66	0.16	-0.669	0.09
rs9458009	1.61E+08	1.67	0.13	0.655	0.1
rs1652507	1.61E+08	-1.58	0.15	-0.66	0.1
rs9457997	1.61E+08	1.93	0.07	0.613	0.11
rs9355296	1.61E+08	-1.52	0.17	-0.64	0.11
rs2115868	1.61E+08	1.93	0.07	0.572	0.14
rs5014650	1.61E+08	1.92	0.07	0.561	0.15
rs12525588	1.61E+08	1.92	0.07	0.548	0.16

rs12523781	1.61E+08	1.92	0.07	0.54	0.17
rs2048328	1.61E+08	2.32	0.02	-0.417	0.23
rs9456544	1.61E+08	2.87	0.43	1.02	0.25
rs7767705	1.61E+08	1.35	0.19	-0.37	0.3
rs9457999	1.61E+08	1.27	0.21	-0.373	0.3
rs4252187	1.61E+08	-0.15	0.99	-20.3	0.34
rs1358754	1.61E+08	1.13	0.34	0.354	0.38
rs1358753	1.61E+08	1.84	0.11	0.263	0.52
rs9364557	1.61E+08	2.35	0.02	0.129	0.71
rs7743535	1.61E+08	3.4	0.73	-3.12	0.76
rs7767342	1.61E+08	3.37	0.73	-3.13	0.76
rs6907156	1.61E+08	3.42	0.73	-3.12	0.76
rs7453965	1.61E+08	3.31	0.74	-3.05	0.76
rs6455697	1.61E+08	3.31	0.74	-3.05	0.76
rs7756317	1.61E+08	3.3	0.74	-3.04	0.76
rs7755463	1.61E+08	3.83	0.71	-2.35	0.83
rs7754526	1.61E+08	3.8	0.71	-2.35	0.83

Table S5. Association between rs10455872 and incident aortic stenosis (AS) in the Malmö Diet and Cancer Study (A) and the Copenhagen City Heart Study (B).

A. Malmö Diet and Cancer Study (median follow-up 14 years)

Phenotype	Exclusions	Covariates	N (events)	N (total)	HR* (95% CI)	P-value
Incident AS	None	age, sex, BMI, current smoking	308	28193	1.68 (1.32-2.15)	$3x10^{-5}$
Incident AS + AVR	None	age, sex, BMI, current smoking	133	28193	1.54 (1.05-2.27)	0.03
Incident AS	MI^	age, sex, BMI, current smoking	241	28126	1.69 (1.28-2.22)	0.0002
Incident AS + AVR	MI^	age, sex, BMI, current smoking	111	28126	1.82 (1.22-2.69)	0.003

* Hazard ratio (HR) per risk allele
^ Only participants with both MI (at baseline or during follow up) and incident AS were excluded.

B. Copenhagen City Heart Study Cohort (median follow-up 17 years)

Phenotype	Exclusions	Covariates	N (events)	N (total)	HR† (95% CI)	P-value
Incident AS	None	age, sex	192	10400	1.61 (1.13-2.30)	0.008
		age, sex, risk factors*	191	10291	1.60 (1.12-2.28)	0.010
Incident AS + AVR	None	age, sex	54	10262	1.68 (0.86-3.25)	0.126
		age, sex, risk factors*	54	10154	1.64 (0.84-3.18)	0.145
Incident AS	MI at baseline	age, sex	185	10103	1.56 (1.09-2.25)	0.016
		age, sex, risk factors*	184	9995	1.55 (1.08-2.23)	0.018

† HR for presence of at least one risk allele
Abbreviations: AS, aortic stenosis; AVR, aortic valve replacement; MI, myocardial infarction; HR, hazard ratio; BMI, body mass index
*Included adjustments for age, sex, total cholesterol, HDL cholesterol, systolic blood pressure, current smoking, and diabetes mellitus.

Table S6. Aortic valve calcium (AVC) and coronary artery calcium (CAC) prevalence, kappa statistic and correlation coefficient for the agreement between AVC and CAC within the discovery cohorts.

Test Parameter	FHS	AGES-RS	MESA
AVC prevalence (%)	37.7	42.8	15.7
CAC prevalence (%)	69.4	88.2	56.7
Kappa statistic (for dichotomous AVC and CAC agreement)	0.25	0.10	0.18
Correlation coefficient (for continuous AVC and CAC agreement)	0.27	0.19	0.18

Abbreviations: FHS, Framingham Heart Study; AGES-RS, Age, Gene/Environment Susceptibility – Reykjavik Study; MESA, Multi-Ethnic Study of Atherosclerosis; AVC, aortic valve calcium; CAC, coronary artery calcium.

Table S7. Additional analyses for the association between the rs10455872 SNP and the presence of coronary artery calcium (CAC), the presence of aortic valve calcium (AVC), or the presence of AVC among those with low CAC burden.

Calcium Phenotype	Covariates	FHS				AGES-RS				MESA			
		N (total)	N (cases)	OR (95% CI)	P-value	N (total)	N (cases)	OR (95% CI)	P-value	N (total)	N (cases)	OR (95% CI)	P-value
CAC >0	Age, sex*	1085	749	1.37 (0.87-2.16)	0.17	3144	1365	1.51 (0.92-2.48)	0.10	2480	1405	1.07 (0.79-1.46)	0.67
AVC >0	Age, sex, CAC>0*	1085	407	2.22 (1.45-3.39)	0.0002	3113	1334	1.98 (1.48-2.66)	$5.5x10^{-6}$	2480	393	1.95 (1.37-2.78)	0.0002
AVC > 0 among those with low CAC†	Age, sex*	377	72	3.06 (1.36-6.92)	0.007	786	221	1.77 (0.85-3.7)	0.13	1171	78	3.39 (1.81-6.36)	0.0001

Abbreviations: FHS, Framingham Heart Study; AGES-RS, Age, Gene/Environment Susceptibility – Reykjavik Study; MESA, Multi-Ethnic Study of Atherosclerosis; AVC, aortic valve calcium; CAC, coronary artery calcium.

*MESA analyses also included adjustment for study site.

†Low CAC was defined by CAC =0 or CAC<25% percentile for age and sex.

Table S8. Prevalence of the rs10455872 SNP in subgroups of participants with and without aortic valve calcium (AVC) and coronary artery calcium (CAC).

Cohort	Subgroup Phenotype		N	MAF	P-value*
	AVC	CAC			
FHS	>0	>0	360	0.08	0.04
	>0	0	52	0.09	0.12
	0	>0	401	0.05	0.41
	0	0	286	0.05	*Reference*
AGES-RS	>0	>0	1262	0.08	0.02
	>0	0	72	0.08	0.26
	0	>0	1485	0.06	0.46
	0	0	294	0.05	*Reference*
MESA	>0	>0	342	0.06	0.42
	>0	0	51	0.12	0.01
	0	>0	1063	0.05	0.26
	0	0	1024	0.05	*Reference*

Abbreviations: FHS, Framingham Heart Study; AGES-RS, Age, Gene/Environment Susceptibility – Reykjavik Study; MESA, Multi-Ethnic Study of Atherosclerosis; AVC, aortic valve calcium; CAC, coronary artery calcium; MAF, minor allele frequency.

* P-value compared to subgroup defined by AVC=0 and CAC=0.

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