Supplementary Table 1. Baseline Characteristics of Participants in the Discovery and Replication Cohorts.

	Discovery Cohorts						Validation Cohorts			
Characteristic	**FHS**	**AGES-RS**	**MESA**	**FamHS**	**HNR**	**HNR**	**MESA: African American**	**MESA: Hispanic**	**FamHS: AA**	**AA-DHS**
Ethnicity	European	European	European	European	European	European	African American	Hispanic American	African American	African American
Genotyping Platform	Affymetrix 5.0	Illumina Hu370CNV	Affymetrix 6.0	Illumina HumMap 550K, and 1M-Duov3	Illumina Human Omni-express	Illumina Human Omni1 Quad	Affymetrix 6.0	Affymetrix 6.0	Illumina Human 1M-Duov3	Illumina 5M
Imputation Software	MACH	MACH 1.0.16.b	IMPUTE 2.2.2	MACH 1.0.16	IMPUTE 2.1.2	IMPUTE 2.1.2	IMPUTE 2.2.2	IMPUTE 2.2.2	MACH 1.0.16	IMPUTE 2.2.2
Country of Origin	USA	Iceland	USA	USA	Germany	Germany	USA	USA	USA	USA
No. of AAC participants	3285	2680	760	2692	N/A	N/A	343	496	621	631
AAC present, No. (%)	1780 (54%)	2680 (100%)	601 (79%)	1873 (70%)	N/A	N/A	208 (61%)	331 (67%)	414 (67%)	570 (90%)
Extent of AAC Median (IQR)	7 (0,604)	0.76* (0.66,0.86)	467 (0,2366)	216 (0,2567)	N/A	N/A	92 (0,831)	151 (0,1200)	132 (0,1428)	1156‖ (43,6627)
Age, mean ±SD,	53 ±12	77 ± 5	63 ± 10	57 ± 13	N/A	N/A	62 ± 10	61 ± 10	53 ± 11	56 ± 10
Women, No. (%)	1572 (48%)	1534 (57%)	360 (47%)	1480 (55%)	N/A	N/A	180 (53%)	243 (49%)	408 (66%)	402 (64%)
No. of TAC participants	3241	638	2525	N/A	1273	745				
TAC present, No. (%)	783 (24%)	611 (96%)	798 (31%)	N/A	709 (56%)	415 (56%)				
Extent of TAC Median (IQR)	0 (0,0)	1967 (696,4766)	0 (0,58)	N/A	5.7 (0,55)	6.7 (0,57)				
Age, mean ±SD	53 ± 12	76 ± 5	63 ± 10	N/A	59 ± 8	60 ± 8				
Women, No. (%)	1558 (48%)	382 (60%)	1308 (52%)	N/A	633 (50%)	365 (49%)				

Abbreviations: FHS, Framingham Heart Study; AGES-RS, Age, Gene-Environment Susceptibility-Reykjavik Study; MESA, Multi-Ethnic Study of Atherosclerosis; FamHS, Family Heart Study; HNR, Heinz Nixdorf Recall Study; AA-DHS, African American-Diabetes Heart Study; SD, standard deviation; No., number; N/A, not applicable.

* The abdominal aortic calcium measurements from AGES-RS were quantified using a volumetric approach. ‖The abdominal aortic calcium measurements from AA-DHS were quantified using a calcium mass score.[1] All other sites utilized the Agatston scoring method.[2]

Supplementary Table 2. Results from Discovery Cohorts for SNP rs2107595 in the *HDAC9* Locus and rs4654975 in the *RAP1GAP* Locus and their Association with Abdominal Aortic Calcification

rs2107595	MAF	N	Z Score	P-value
Discovery Cohort Results				
FHS	0.18	3285	3.1	0.0019
AGES-RS	0.17	2680	3.2	0.0014
MESA	0.19	760	2.6	0.0084
FamHS	0.14	2692	3.3	0.00082
Discovery Meta-analysis		9417	6.02	1.7×10^{-9}

rs4654975	MAF	N	Z Score	P-value
Discovery Cohort Results				
FHS	0.32	3285	2.3	0.021
AGES-RS	0.36	2680	3.5	0.00045
MESA	0.33	760	2.3	0.020
FamHS	0.32	2692	3.2	0.0013
Discovery Meta-analysis		9417	5.55	2.8×10^{-8}

Single SNP association test was performed by each cohort using two-sided multivariate linear regression, adjusting for age, sex, study sites when appropriate, and top principal components. Abbreviations: SNP, single nucleotide polymorphism; EAF, effect allele frequency; FHS, Framingham Heart Study; AGES-RS, Age/Gene-Environment Susceptibility-Reykjavik Study; MESA, Multi-Ethnic Study of Atherosclerosis; FamHS, Family Heart Study

Supplementary Table 3. SNPs with Suggestive Evidence for Association ($p<1 \times 10^{-6}$) with Abdominal Aortic Calcification.

SNP	Chromosome	Physical Location	Minor Allele	MAF	Z Score	*P-value*	Candidate gene(s)*
rs10159452	1	21973914	C	0.19	5.43	5.78E-08	*RAP1GAP*
rs10157126	1	21973835	C	0.19	5.42	5.92E-08	*RAP1GAP*
rs150501030	3	169732746	G	0.019	5.28	1.30E-07	unknown
rs2128699	1	21974208	C	0.297	5.27	1.36E-07	*RAP1GAP*
rs117679069	9	33502473	T	0.016	5.14	2.82E-07	*SUGT1P1*
rs75940184	3	130422853	C	0.047	5.04	4.56E-07	*PIK3R4*
rs16825755	1	21975214	G	0.144	5.02	5.06E-07	*RAP1GAP*
rs7788833	7	19034191	C	0.220	4.97	6.69E-07	*HDAC9*
rs7788972	7	19034280	A	0.220	4.97	6.76E-07	*HDAC9*
rs12045185	1	21961338	A	0.109	4.94	7.84E-07	*RAP1GAP*
rs11011650	10	20153910	T	0.121	-4.94	7.94E-07	*PLXDC2*
rs6671920	1	21960032	A	0.109	4.93	8.10E-07	*RAP1GAP*
rs6660927	1	21960044	C	0.109	4.93	8.11E-07	*RAP1GAP*
rs189086340	9	19126983	A	0.022	4.92	8.47E-07	*PLIN2*
rs12039278	1	21953026	C	0.110	4.92	8.63E-07	*RAP1GAP*
rs77064115	3	130484314	A	0.047	4.92	8.81E-07	*PIK3R4*

Abbreviations: MAF, minor allele frequency
*Candidate genes are within 60 kB from the SNP

Single SNP association test was performed by each cohort using two-sided multivariate linear regression while adjusting for age, sex, study sites when appropriate, and top principal components. GWAS results from all cohorts were meta-analyzed using sample size weighted fixed-effect models as implemented in the program METAL. A genome-wide significance threshold of 5×10^{-8} was used in consideration of multiple comparisons.

Supplementary Table 4. SNPs with Suggestive Evidence for Association ($p<1 \times 10^{-6}$) with Thoracic Aortic Calcification.

SNP	Chromosome	Physical Location	Minor Allele	MAF	Z Score	*P-value*	Candidate gene(s)*
rs148988294	6	21005874	T	0.013	-5.31	1.07E-07	*CDKAL1*
rs187916214	2	184302158	A	0.013	-5.02	5.09E-07	*unknown*
rs62573203	9	79890129	G	0.219	5.02	5.10E-07	*VPS13A*
rs144740773	6	20767890	C	0.014	-5.01	5.41E-07	*CDKAL1*
rs12352759	9	80040947	T	0.224	5.00	5.72E-07	*GNA14*
rs1934725	9	79891904	T	0.219	5.00	5.84E-07	*VPS13A*
rs12352871	9	80041201	G	0.224	5.00	5.85E-07	*GNA14*
rs955306	9	79897372	T	0.218	4.99	5.90E-07	*VPS13A*
rs115249592	3	130330342	C	0.015	4.98	6.53E-07	*COL6A6*
rs12343376	9	80040921	A	0.224	4.97	6.72E-07	*GNA14*
rs10491839	9	80040897	G	0.224	4.97	6.72E-07	*GNA14*
rs12352830	9	80041132	G	0.224	4.97	6.79E-07	*GNA14*
rs12343441	9	80041116	A	0.224	4.96	7.09E-07	*GNA14*
rs12339287	9	80041115	C	0.224	4.96	7.10E-07	*GNA14*
rs7030802	9	79891237	G	0.218	4.96	7.25E-07	*VPS13A*
rs74524992	9	79879286	G	0.220	4.94	7.77E-07	*VPS13A*
rs1934726	9	79891808	C	0.218	4.93	8.06E-07	*VPS13A*
rs60620535	4	11089103	T	0.063	4.92	8.59E-07	*unknown*
rs66651760	9	79897815	C	0.218	4.90	9.37E-07	*VPS13A*
rs12351061	9	79860272	G	0.213	4.90	9.43E-07	*VPS13A*
rs6902719	6	125252364	A	0.019	-4.90	9.58E-07	*RNF217-AS1*
rs10115162	9	79862502	C	0.213	4.90	9.60E-07	*VPS13A*

Abbreviations: MAF, minor allele frequency
*Candidate genes are within 60 kB from the SNP

Single SNP association test was performed by each cohort using two-sided multivariate linear regression, adjusting for age, sex, study sites when appropriate, and top principal components. GWAS results from all cohorts were meta-analyzed using sample size weighted fixed-effect models as implemented in the program METAL. A genome-wide significance threshold of 5×10^{-8} was used in consideration of multiple comparisons.

Supplementary Table 5. Trans-ethnic Replication of Genome-Wide Significant SNPs for Abdominal Aortic Calcification in a Meta-Analysis of African Americans in MESA (n=343), the FamHS (n=621), and the AA-DHS (n=631).

African Americans (n=1595)					
SNP	**Chromosome**	**Minor Allele**	**MAF**	**Z score**	**P-value**
rs57301765	7	A	0.30	0.81	0.42
rs2107595	7	A	0.22	1.41	0.12
rs28688791	7	C	0.28	0.92	0.36
rs2023936	7	G	0.35	0.52	0.61
rs2526620	7	G	0.47	0.63	0.53
rs7798197	7	G	0.33	0.56	0.57
rs4654975	1	C	0.42	0.65	0.51
rs3767120	1	C	0.44	0.06	0.95

Results from three African American cohorts were meta-analyzed using sample size weighted fixed-effect models as implemented in the program METAL. Bonferroni correction was applied in consideration of multiple testing, i.e. $p < 0.05/6$ in the HDAC9 gene, and $P < 0.05/2$ in the RAP1GAP gene.

Supplementary Table 6. Associations of Abdominal Aortic Calcification-associated SNPs with Other Forms of Vascular Calcification, Carotid Artery Plaque, and Cardiovascular Disease.

			Thoracic Aortic Calcium (n=8422)		Coronary Artery Calcium (n=9992)[3]		Carotid Plaque (n=40,574)		Clinical Coronary Heart Disease (n=193,389)[4]	
SNP	**Chromosome**	**Minor Allele**	**Z score**	**P-value**	**Z score**	**P-value**	**Z score**	**P-value**	**Z score**	**P-value**
rs57301765	7	A	1.98	0.047			3.34	8.8×10^{-4}	6.19	6.1×10^{-10}
rs2107595	7	A	1.81	0.07	2.06	0.039	3.37	7.8×10^{-4}	6.50	8.1×10^{-11}
rs28688791	7	C	1.64	0.10			2.81	4.9×10^{-3}	6.18	6.5×10^{-10}
rs2023936	7	G	1.61	0.13	1.86	0.063	2.83	4.7×10^{-3}	6.26	3.8×10^{-10}
rs2526620	7	G	1.51	0.14			2.36	0.019	5.70	1.2×10^{-8}
rs7798197	7	G	1.27	0.11	1.91	0.057	3.11	1.8×10^{-3}	6.16	7.3×10^{-10}
rs4654975	1	C	1.50	0.21	1.63	0.10	1.03	0.30	1.30	0.19
rs3767120	1	C	1.32	0.19			1.10	0.27	0.84	0.40

Single SNP association test was performed by each cohort using two-sided multivariate linear regression, adjusting for age, sex, study sites when appropriate, and top principal components. GWAS results from all cohorts were meta-analyzed using sample size weighted fixed-effect models as implemented in the program METAL.

Supplementary Note

Splicing Quantitative Trait Loci Analyses:

The rs2107595 minor allele is associated with increased *HDAC9* mRNA levels in peripheral blood mononuclear cells of 77 healthy individuals.[5] *HDAC9* undergoes alternative splicing to generate 29 different mRNA transcripts and multiple protein isoforms that harbor distinct deacetylase activities and cellular localizations.[6] We therefore sought to determine whether genetic variants in the *HDAC9* locus affect the expression of mRNA splice variants of *HDAC9* using splicing quantitative trait loci (sQTL) analyses. RNA isolated from the whole blood of 5,257 FHS participants was used to quantify exon-level expression with the Affymetrix Human Exon Array, as previously described.[7] The minor alleles of rs7798197 and rs2023936 were associated with increased expression of *HDAC9* transcripts targeted by the Affymetrix exon array's probe set ID #2991499 (β-value=0.033, p=0.01 for both) and the minor allele of rs2107595 demonstrated a trend towards association with increased expression (β-value=0.027, p=0.06).[7] Furthermore, applying the Altrans method[8] for assessment of alternative splice variants in a whole blood RNA-sequencing analysis from 183 FHS participants, we found that the minor allele of rs2107595 was associated with increased expression of *HDAC9* transcript: ENST00000474742 (hg19. position:chr7:18330073-18536369; fraction=0.86 for the minor allele, p=0.043). Both analyses indicate that some minor alleles in the *HDAC9* locus, that are associated with increased AAC (**Table 1**), are also associated with increased expression of certain splicing transcripts of *HDAC9*. These findings indirectly suggest a possible role for the *HDAC9* SNPs associated with AAC in regulating alternative splicing of *HDAC9* and the level of *HDAC9* transcripts.

The Role of HDAC9 in an in vivo Model of Vascular Calcification

A murine model of vascular calcification caused by matrix Gla protein (MGP) deficiency[9] was used to investigate the role of HDAC9 in vascular calcification *in vivo*. In the MGP-deficient mouse model, vascular calcification occurs in the absence of vascular inflammation.[10,11] The use of this model permitted us to study the effects of HDAC9 on vascular calcification without the confounding effects of inflammation. Histologic analyses revealed greater aortic wall elastic fiber fragmentation (VVG stain, **Figure 4a**) in *Mgp*$^{-/-}$ mice compared to wild-type mice. As measured using immunofluorescence and immunoblot, HDAC9 protein levels were increased in the aortas of MGP-deficient mice compared to the aortas of age- and sex-matched wild-type mice (**Figures 4b and 4c**), whereas levels of other class IIa HDACs did not differ between *Mgp*$^{-/-}$ and wild-type mice (**Supplementary Figure 2**). HDAC9 protein co-localized with DAPI, confirming nuclear localization of the protein. HDAC9 accumulation in the aortas of *Mgp*$^{-/-}$ mice was associated with reduced SM22α expression (a marker of SMC contractility) and increased disruption of elastin fibers (VVG stain). Mice deficient in both MGP and HDAC9 (*Mgp*$^{-/-}$ *Hdac9*$^{-/-}$), however, were protected from these abnormal phenotypes and exhibited higher SM22α expression and reduced elastin fiber disruption relative to *Mgp*$^{-/-}$ *Hdac9*$^{+/+}$ mice (**Figures 4a-b**). *Mgp*$^{-/-}$ *Hdac9*$^{+/-}$ mice exhibited an intermediate phenotype between *Mgp*$^{-/-}$ *Hdac9*$^{+/+}$ and *Mgp*$^{-/-}$ *Hdac9*$^{-/-}$ mice. Compared to *Mgp*$^{-/-}$ *Hdac9*$^{+/+}$ mice, *Mgp*$^{-/-}$ *Hdac9*$^{-/-}$ mice exhibited a ~50% reduction in aortic *Runx2* mRNA levels (p=0.02) associated with a ~40% reduction in aortic calcification as assessed by near-infrared fluorescent imaging (p=0.03) (**Figure 4d-e**). Levels of matrix metalloproteinase (MMP)-9, a known activator of medial arterial calcification,[12,13] were significantly increased in the aortas of *Mgp*$^{-/-}$ *Hdac9*$^{+/+}$ and *Mgp*$^{-/-}$ *Hdac9*$^{+/-}$ mice compared to wild-type mice (**Figure 4f**). However, compared to *Mgp*$^{-/-}$ *Hdac9*$^{+/+}$ and *Mgp*$^{-/-}$ *Hdac9*$^{+/-}$ mice, there was a greater than 50% reduction in the level of MMP-9 in *Mgp*$^{-/-}$ *Hdac9*$^{-/-}$. *Mgp*$^{-/-}$ *Hdac9*$^{-/-}$

mice exhibited improved survival compared to *Mgp*$^{-/-}$ *Hdac9*$^{+/+}$ mice (log-rank p=0.0018, **Figure 4g**). Furthermore, primary aortic SMCs isolated from *Hdac9*$^{-/-}$ mice had reduced expression of osteogenic markers including *Runx2*, tissue non-specific alkaline phosphatase (*Tnap*), and *Col3a1*, were protected from calcification, and had increased contractility when grown in calcifying media compared to primary aortic SMCs isolated from wild-type mice (**Supplementary Figure 3**). These results identify HDAC9 as an important contributor to the development of vascular calcification.

Study Limitations and Discussion of TAC Findings

Variants in several genetic loci have been associated with increased calcification in other vascular beds. For instance, SNPs within the 9p21, *PHACTR1*, *MRAS*, *COL4A1/COL4A2*, and *SORT1* loci are associated with coronary artery calcification.[3,14] Similarly, polymorphisms in the *LPA* locus are associated with aortic valve calcification.[15] In the current study, SNPs in the *HDAC9* locus met a genome-wide significant threshold for association with AAC, but the aforementioned loci did not. These results support the hypothesis that the strongest genetic determinants of calcification vary across different vascular territories. There are potential limitations to our study. First, the association between *HDAC9* polymorphisms and AAC was not reproduced in all trans-ethnic replication cohorts. Although not reproduced in African Americans, the association was reproduced with strong statistical significance in Hispanic Americans, perhaps reflecting underlying genetic differences across ethnicities. In this study, trans-ethnic differences may be explained, in part, by the greater European admixture in Hispanic Americans than in African Americans. Differences in regions of linkage disequilibrium (LD) in the *HDAC9* locus also highlight genetic differences across ethnicities. Regions of LD tend to be smaller in individuals of African ancestry relative to other ethnicities.[16] The results of

our trans-ethnic validation studies are consistent with these more general LD observations. The six *HDAC9* SNPs in the white European population form two blocks with relatively high LD (D' ≥0.81) and all six SNPs are in LD in Hispanic Americans (D' ≥0.92). However, for the same region, we found four LD blocks in African Americans. Thus, trans-ethnic genetic differences are highlighted by varying degrees of LD for the SNPs in the *HDAC9* locus. Second, heterogeneity in study participants and phenotype acquisition across the different cohorts must be considered. However, a method of meta-analysis that involved weighting by sample size was applied, allowing for results to be combined when different units of measurement are used. We further examined heterogeneity in the meta-analysis using Cochran's Q-test and found no evidence of heterogeneity (p-value>0.98 for the 8 top SNPs).

We did not identify polymorphisms associated with TAC at a genome-wide level of significance. Several possible explanations may underlie the absence of association. First, it is possible that there are multiple genetic contributions to TAC, each with a smaller magnitude than those for AAC such that the present study was unable to detect an association. Second, although the meta-analysis of individuals with TAC included 8422 participants, the prevalence of TAC was markedly lower than the prevalence of AAC in the three cohorts in which both were measured (**Supplementary Table 1**). The present study was likely underpowered for this phenotype relative to our power to detect polymorphisms associated with AAC. Finally, although we employed a standard and validated methodology for the assessment of TAC, this method excluded the aortic arch. CT scans of the heart do not routinely include images of the arch of the aorta, which is a region with a high atherosclerotic plaque burden. It is possible that inclusion of the aortic arch in the TAC phenotype might have yielded a more accurate assessment of thoracic aortic calcification with stronger genetic associations.

Supplementary Methods

1. Discovery Cohorts

1.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 generation, their offspring and spouses of the offspring (Offspring Cohort, enrollment beginning in 1971, n=5124), and children from the largest offspring families (Generation 3 Cohort, enrollment beginning in 2002, n=4095). Details regarding study recruitment and design have been reported previously.[17,18] Participants underwent on-site examinations including physician examinations, laboratory sample collection, and in-person interviews that recurred every 2 years for the original cohort and every 4 years for the offspring and Gen3 cohorts. At each examination cycle, information on cardiovascular disease risk factors including details on medical treatments such as prescribed and non-prescribed medication use, hospitalization, and surgery was collected through in-person interview. Medical records, operation records, and pathological reports were requested for verification through a three-physician review panel. For genome-wide association studies, all participants were invited that provided genotyping-specific consents and blood samples were collected. Numbers of participants those had qualified genotype data were 954, 3468, and 3863 for original, offspring, and the Gen3 cohorts, respectively.

Computed Tomography and Analysis

Information on abdominal aortic calcification (AAC) and thoracic aortic calcification (TAC) for the FHS participants was derived from findings of the first FHS multidetector computed tomography (MDCT) study (from June 2002 to April 2005).[19] Participants that attended

offspring 7th examination cycle (1998-2001) and the first Gen3 examination (2002-2005) were invited to participate. Complete information on CT-scan measured calcification was available for 3285 participants including 1422 offspring and 1863 Gen3 participants. Exclusion criteria for the MDCT study included pregnancy, age <40 years for women and age <35 years for men, and weight >320 pounds. Participants were imaged on an 8-slice MDCT scanner (LightSpeed Ultra; General Electric) with prospective ECG triggering during a breath hold.[20] All MDCT scans were read independently by experienced readers using an Aquarius (Terarecon) workstation. Calcification was measured using well established, highly reproducible methods based on the Agatston score, where calcification was defined as at least three contiguous pixels with >130 Hounsfield units attenuation.[2,21] TAC was defined as calcification of the descending thoracic aorta above the diaphragm. For AAC, the top of the S1 vertebral body was selected as the most caudal border of the abdominal volume imaged. 30 continuous 5-mm slices were obtained cranial to S1 for a total coverage of 15 cm in the Z direction and analyzed for AAC using the Agatston score.[2,20] 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.

Genotyping and Imputation

Framingham Offspring Cohort participants provided consent for genotyping in the 1990s and had DNA extracted at that time. Gen3 participants were invited to participate in the genetic studies at the beginning of their FHS clinical studies (year 2002-2005). 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. Genotyping results were imputed used the 1000 Genome panel as the reference and applied the Marchov Chain Haplotyping software

(MACH, http://www.sph.umich.edu/csg/yli/software.html). For the current study, we tested for significant associations between AAC/TAC and imputed genotypes. Prior to GWAS, we excluded non-ideal samples on the basis of low call rate (<95%), high heterozygosity (>6 standard deviations), or extremely high Mendelian errors (≥4 standard deviations of study samples). In addition, we filtered genetic variants for SNP-specific quality controls and excluded variants with low call rate (<95%), low imputation quality (<30%), and extreme minor allele frequency (<0.005). For the family-based study population, we applied a linear mixed effect model to characterize significant associations for AAC that familial correlation was adjusted.

1.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.[22] 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

Participants in AGES-RS underwent chest and abdominal CT scanning (Siemens Somatom Sensation 4, Siemens Medical Solutions, Erlangen, Germany) during baseline enrollment and testing. Spatial resolution for the chest scans was 0.68 x 0.68 x 2.50 mm. The thoracic aorta was scanned in two separate sequential scans in the cranio-caudal direction during suspended inspiration (standard scan setting: slice thickness; 2.5 mm, tube voltage; 140 kilo-voltage, tube-current-time-product; 50 milli-ampere-seconds and scan time 0.361 sec). Each of the two scans included a minimum of 40 contiguous images, the first starting above the aortic arch to the bottom of the aortic bulb and the second starting above the left main coronary artery to the bottom of both ventricles. The images were reconstructed into a display field of view of 350 mm to include a calibration phantom (Image Analysis, Columbia, KY, USA) which was positioned under the thorax of each subject. The phantom contained calibration cells of 0, 50, 100, 200 mg/cm^3 equivalent concentration of calcium hydroxyapatite. Calcium in the thoracic aorta was quantified using the Agatston method[2] using a custom software for the MESA study (Multi-Ethnic Study of Atherosclerosis).[23] TAC was scored in two separate segments. The first segment included the proximal descending thoracic aorta (from the inferior border of the transverse arch to the level of the aortic bulb), and the second segment included the distal descending thoracic aorta (from the level of the aortic bulb to the bottom of the left ventricular apex). Scores from these two segments were combined to determine the extent of TAC. As

described by Agatston et al,[2] the calcium score of each lesion was calculated by multiplying the lesion area by a density factor derived from the maximum Hounsfield units (HU) within this area. TAC was scored by 4 image analysts who had received appropriate training. Inter- and intra-observer variability assessment showed high reliability of the calcium scoring. Inter-observer variability based on the re-analysis of randomly selected 365 scans from the core study population by an expert observer showed an average correlation coefficient of 0.99. Intra-observer variability based on re-analysis of 45 scans by each of the four observers resulted in an average correlation coefficient of 0.99.

Images for calcium scoring in the abdominal aorta were obtained at a level extending from the superior L1 vertebrae to the inferior L2 vertebrae. This segment of the abdominal aorta was scanned using a volumetric acquisition (120 kVp, 140 mAs, 1-mm slice thickness, pitch = 1). Spatial resolution for the abdominal aorta scans was 0.98 x 0.98 x 1.00 mm. To calibrate CT Hounsfield units to equivalent calcium concentration, all subjects lay on a calibration phantom (Image Analysis, Columbia, KY, USA). The phantom contained calibration cells of 0, 75 and 150 mg/cm^3 equivalent concentration of calcium hydroxyapatite. CT images were transferred from the CT scanner to a network of computer workstations equipped with the Linux operating system (Red Hat Version 7.2) and the AVS5 visualization program (AVS, Waltham MA, USA). Images were processed to extract measures of volumetric calcium in the segment of the aorta described above. The total abdominal aortic calcium density was expressed in g/cc.

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 the 1000 Genome data freezes from 23 Nov 2010 (low-coverage whole-genome) and 21 May 2011 (high-coverage exome), phased haplotypes released March 2012 (v3), and phased haplotypes for 1,092 individuals and 39+ million variants using the Marchov Chain Haplotyping software (MACH v.1.0.16, http://www.sph.umich.edu/csg/yli/software.html). All imputed and genotyped SNPs were aligned to the '+' strand of the human genome reference sequence (NCBI Build 37). 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 extent of calcification included adjustments for age and sex.

1.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.[24] 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). 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 chest and heart. Information regarding the participants' demographic data and medical history, including medication use, was obtained by questionnaire. Ethnicity was self-reported. Only the 2685 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 AAC and/or TAC. 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.[25,26] 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. The calcium score of each lesion was determined by multiplying the lesion area by a density factor derived from the maximal Hounsfield units (HU) within this area, as previously performed by Agatston et al.[2] Since TAC was obtained from cardiac CTs, the aortic arch was not visualized. TAC was measured in the descending thoracic aorta from the lower edge of the pulmonary artery to the cardiac apex.[26,27] For AAC, a 15-cm segment of the

distal abdominal aorta was imaged using the L5-S1 disc space from the scout film as the presumed location of the aortic bifurcation. Due to individual variability in the anatomic location of the aortic bifurcation, AAC was measured in the 8-cm segment proximal to the aortic bifurcation, as previously described, since all subjects had this 8-cm segment visualized.[28]

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). Participant-specific quality controls included filters for call rate and sample cQC, and sample heterozygosity by race at the sample level. Cryptic sample duplicates or unresolved cryptic duplicates were removed. Unresolved gender mismatches were also removed. At the SNP level, monomorphic SNPs across all samples were excluded. SNPs with a missing rate of >5% or observed heterozygosity >53% were also excluded. Genotype results were imputed to the 1000 Genomes Phase I integrated variant set (NCBI build 37 / hg19) separately in each ethnic group using IMPUTE v.2.2.2 software (http://mathgen.stats.ox.ac.uk/impute/impute.html).[29] The reference panel included phased haplotypes for 1,092 individuals and more than 39 million variants and was based on data freezes from 23 Nov 2010 (low-coverage whole-genome) and 21 May 2011 (high-coverage exome), with phased haplotypes released March 2012 (v3).

All imputed and genotyped SNPs were aligned to the '+' strand of the human genome reference sequence (NCBI Build 37). 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.[30] This directly-genotyped IBC chip data were used to confirm the discovery meta-analysis results (which were based on imputed SNP data).

1.D. Family Heart Study (FamHS)

The Family Heart Study (FamHS: https://dsgweb.wustl.edu/PROJECTS/MP1.html) was started in 1992 with the ascertainment of 1,200 families, half randomly sampled, and half specifically selected because of an excess of coronary heart disease (CHD) or risk factor abnormalities as compared with age- and sex-specific population rates.[31] The families, totaling approximately 6,000 subjects, were sampled on the basis of information on probands from four population-based parent studies: the Framingham Heart Study (field center at Boston University), the Utah Family Tree Study (field center at University of Utah), and two ARIC centers (Minneapolis, University of Minnesota, and Forsyth County, NC, University of North Carolina). Across the years 1994-1996, these subjects attended a clinic visit having provided appropriate informed consent. A broad range of phenotypes was assessed in the general domains of CHD, atherosclerosis, cardiac and vascular function, inflammation and hemostasis, lipids and lipoproteins, blood pressure, diabetes and insulin resistance, pulmonary function, diet, habitual physical activity, anthropometry, medical history and medication use. Approximately 8 years later, study participants belonging to the largest pedigrees were invited for a second clinical exam (2002-04). A total of 2,756 European Americans in 510 extended families were recruited. In addition, 633 African Americans were recruited at an additional ARIC field center at the University of Alabama in Birmingham. Informed consent was obtained from all participants, and this project was approved by the Institutional Review Boards of all participating institutions. For

the current study, 2,692 European Americans from the second clinical exam and 621 African Americans were analyzed.

Computed Tomography and Analysis

Participants underwent a cardiac multidetector CT exam with four detectors using a standardized protocol, as described previously.[23] In brief, the two sequential cardiac scans were obtained by using a single breath hold at end inspiration. This approach helped to reduce motion artifacts from breathing and improved image quality by depressing the diaphragm and liver, thereby leading to a reduction in beam attenuation. For participants weighing 100 kg (220 lbs) or greater, the milliamperes were increased by 25%. CT images from all study centers were sent electronically to the central CT reading center located at Wake Forest University Health Sciences, Winston Salem, NC, USA. CT images were analyzed using Medical Image Processing, Analysis, and Visualization (MIPAV) software[32] with custom programmed subroutines (a.k.a. “plug-ins”) developed at Wake Forest University Health Sciences. The CT abdominal aorta scans were measured by an Agatston score modified to account for slice thickness, which were calculated using a 130 CT number threshold and a minimum lesion size of 0.9 mm. The sum of the individual AAC (*i.e.*, abdominal aorta, right common iliac, left common iliac) was calculated as the total calcium score, averaged over the first and second CT scan series.

Genotyping and Imputation

The European Americans were genotyped on three Illumina platforms. In total, genotypes were available for 971 subjects with 547,353 SNP markers on the Illumina HumMap 550K chip, 1,674 subjects with 576,888 SNP markers on the Human 610-Quadv1 Illumina chip, and 1,490

subjects with 1,111,639 SNP markers on the Human 1M-Duov3 Illumina chip. Quality control was performed before imputation and included assessment of Mendelian errors (LOKI)[33] and verification of reported pedigree relationships using GRR.[34] For the 1000 Human Genome (HG) imputation, SNPs were used if: call rate >0.98; minor allele frequency (MAF) >0.01; no deviation from Hardy-Weinberg equilibrium ($p>1x10^{-6}$), allelic match with 1000 HG; and present in 1000 HG genotyping. A total of 501,404 (550K), 530,979 (610K-Quadv1), and 910,456 (1M-Duov3) SNPs were included for the haplotype phasing and imputation using MACH (version1.0.16).[35,36] The reference phased haplotypes was the Cosmopolitan panel (version 2010-11 data freeze, 2012-03-04 haplotypes; http://www.sph.umich.edu/csg/abecasis/MaCH/download/1000G.2012-03-14.html,ALL phase1_release_v3.20101123.snps_indels_svs.genotypes.refpanel.ALL.vcf.gz.tgz (4.4Gb)). We created hybrid genotype data, in which 519,261 typed SNPs replaced the imputed ones, when available, in addition to the 36,026,053 imputed SNPs. Principal components (PCs) were estimated using EIGENSTRAT.[37] Ten PCs entered into the stepwise regression analysis for phenotype adjustment to control for genetic background.

1.E. Heinz Nixdorf Recall (HNR)

The Heinz Nixdorf Recall Study (HNR) is a large, population-based study conducted in the industrial Ruhr region of Northwestern Germany. The rationale and design of this study has been reported previously.[38] 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%.[39] 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). 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), with 3-mm slice thickness and prospective ECG gating, at one of two radiology institutions in Germany. Assessment of TAC burden was possible in segments of the descending thoracic aorta that were included in the coronary calcium scan. The aortic arch and the infrarenal abdominal aorta were not included in the scan. The descending thoracic aorta ranged from the lower edge of the pulmonary artery bifurcation to the cardiac apex, as previously described.[40] A CT threshold of 4 pixels at >130 Hounsfield units was used for the identification of a calcified lesion. Scans were scored for the presence of TAC using standard Agatston methods.[40]

Genotyping and Analysis

The genotyping of HNR was done using the Illumina HumanOmni1-Quad and Illumina HumanOmniexpress. LiftOver was used to convert HumanOmni1-Quad coordinates to the reference build hg19. Quality control (QC) was performed separately for both the chips before imputing each chip using IMPUTE v2.3.1 (based on the reference sample 1000 Genomes Phase 3, October 2014). Subjects were excluded based on heterozygosity (HET>5 standard deviations of the mean), missing genotype data (>5%) and outliers identified by principle component analysis. The SNPs which were not observed in founders or with MAF<1% and missing genotype frequency >5% were also excluded. After QC the analysis cohort consisted of 745 participants from HumanOmni1-Quad and 1273 participants from HumanOmniexpress having TAC phenotype.

2. Validation Cohorts

2.A. MESA

MESA aimed to study subclinical atherosclerosis and its progression within a multiethnic population and undertook targeted oversampling of African-American (n=1594), Hispanic (n=1440) and Chinese (n=777) participants to enhance statistical power. These participants of non-European descent underwent the same genotyping and phenotyping as did MESA white Europeans. Validation analyses included participants in the MESA African American and Hispanic American cohorts but excluded Chinese participants due to the small sample size of individuals with AAC phenotyping. Analyses were performed in each race/ethnicity separately. Statistical models were adjusted for age, gender, and top two principal components of global ancestry within each ethnic group.

2.B. Family Heart Study: African Americans

Computed Tomography and Analysis

The standardized protocol for the cardiac multidetector CT exam used in European Americans from the Family Heart Study (FamHS) was also applied to the African Americans (described in **2.D).**

Genotyping and Imputation

The African Americans were genotyped with 1,111,656 SNPs on the Human 1M-Duov3 Illumina chip. The quality control procedures and genotype imputation for African Americans were performed similarly to the FamHS in European Americans (described in **2.D.**). In brief, SNPs

were removed if: minor allele frequency (MAF) <1%, deviations from Hardy-Weinberg equilibrium ($p<1x10^{-6}$), and not in 1000 Genomes. A total of 992,564 genotyped SNPs that passed quality control were used for imputation, which was performed by MACH (version 1.0.16).[35,36] The reference phased haplotypes was the Cosmopolitan panel (version 2010-11 data freeze, 2012-03-04 haplotypes). The hybrid genotype dataset was created similarly to the FamHS in European Americans (described in **2.D.**), producing 37,353,967 SNPs, of which 1,035,374 SNPs were genotyped and 36,318,593 SNPs were imputed for 622 AA subjects. Ten PCs were forced in the phenotype adjustment to account for the genetic population stratification.

2.C. African American Diabetes Heart Study

The African American Diabetes Heart Study is a genetic and epidemiologic study that recruited African Americans with Type 2 diabetes from internal medicine clinics and community advertising.[1,41] The initial examination consisted of 691 participants between 2007 and 2010. Examination components included interviews for medical history, anthropometric measures, resting blood pressure, ECG, blood draw in the fasting state, and measures of subclinical cardiovascular disease including measurement of vascular calcified plaque by computed tomography.

Computed Tomography and Analysis

AAC was measured using either a single-slice helical CT or a four-channel multidetector CT (HiSpeed LX and LightSpeed QXi with the SmartScore Cardiac scan package, General Electric), both performed with cardiac gating. The juxtarenal and infrarenal aorta, beginning 25 mm proximal to the origin of the superior mesenteric artery and extending 25 mm into the common iliac arteries below the aortic bifurcation, was measured.[42] Scan parameters included 2.5-mm slice thickness, 35 cm field of view, 120 kV, and 250 mA. A standardized scanning

protocol based on those implemented in the National Heart Lung and Blood Institute's MESA study was utilized.[24,26] This report used the calcium mass score (milligrams of calcium), which is derived from the volume score but also accounts for the density of calcified plaque on a pixel by pixel basis, as previously described.[1] Two adjacent pixels with a >90 Hounsfield unit (HU) threshold was used to define the maximum calcified lesion size.

Genotyping and Analysis

DNA in AA-DHS participants was extracted from peripheral blood using the PureGene system (Gentra Systems, Minneapolis, MN). The AA-DHS GWAS utilized the Illumina 5M chip and was imputed to 1000 Genomes (phase 1 version 3, cosmopolitan panel).

3. Carotid Arterial Plaque

Within the CHARGE consortium, 14 cohort studies performed carotid artery plaque measures: AGES-RS, MESA, FHS, the Atherosclerosis Risk in Communities (ARIC) study, the Austrian stroke prevention family study (ASPS-FAM), the Cardiovascular Health Study (CHS), the Erasmus Rucphen Family (ERF) study, the Three-City (3C) study, the Netherlands Study of Depression and Anxiety (NESDA), the Rotterdam Study I and II (RSI and RSII), the Study of Health in Pomerania (SHIP and SHIP-TREND), and the Young Finns Study (YFS). Carotid artery plaque was evaluated using high-resolution B-mode ultrasonography, as previously reported,[43] and plaque was defined as atherosclerotic thickening of the carotid artery wall or using the proxy measure of luminal stenosis greater than 25%. Each cohort conducted genome-wide imputation using a Phase 1 integrated (March 2012 release) reference panel from the 1000G Consortium[44] with Impute2[45] or MaCH/minimac,[46] and used Human Reference Genome Build 37. Statistical analyses used logistic regression and additive genetic models

adjusted for age, sex, and up to 10 principal components. Meta-analysis of summary data was performed using METAL.

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