SUPPLEMENTAL MATERIAL

Common genetic variation in the 3'-*BCL11B* gene desert is associated with carotid-femoral pulse wave velocity and excess cardiovascular disease risk: The AortaGen Consortium

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Supplementary Methods

GWAS Sites

The Reykjavik Study cohort originally comprised a random sample of 30,795 men and women born in 1907-1935 and living in Reykjavik in 1967. A total of 19,381 people attended, resulting in 71% recruitment rate. The study sample was divided into six groups by birth year and birth date within month. One group was designated for longitudinal follow up and was examined in all stages. One group was designated a control group and was not included in examinations until 1991. Other groups were invited to participate in specific stages of the study. Between 2002 and 2006, the Age, Gene/Environment Susceptibility-Reykjavik Study (AGES-RS) reexamined 5,764 survivors of the original cohort who had participated before in the Reykjavik Study.[1] A reexamination of survivors from AGES-RS is presently being conducted (AGES-II). Arterial tonometry was added to the original AGES-RS study protocol for all participants beginning January 12, 2005 and continuing through to the end of the examination cycle. Arterial tonometry is being assessed in all participants in AGES-II. Carotid-femoral pulse wave velocity (CFPWV) was evaluated by tonometry and calculated from the foot-to-foot delay between carotid and femoral waveforms and body surface measurements that adjusted for parallel transmission in the carotid and aorta by using the suprasternal notch as a fiducial point. Successful genotyping was available for 3,219 AGES-RS participants, of whom 967 had successful measurement of CFPWV. The AGES-RS GWAS was approved by the National Bioethics Committee and the Data Protection Authority.

The Baltimore Longitudinal Study of Aging (BLSA) is an observational study that began in 1958 to investigate normative aging in community dwelling adults who were healthy at study entry.[2] Participants are examined every one to four years depending on their age. Currently there are approximately 1,100 active participants enrolled in the study. Blood samples were collected for DNA extraction, and genome-wide genotyping was completed for 1,231 subjects using Illumina 550K. This analysis focused on a subset of the participants (N=610) of European ancestry for whom CFPW was available. CFPWV was evaluated initially using nondirectional transcutaneous Doppler probes and subsequently with the Complior® SP device (Artech Medical, Pantin, France). CFPWV was calculated from the foot-to-foot delay between carotid and femoral waveforms and body surface measurements that adjusted for parallel transmission in the carotid and aorta by using the manubrium as a fiducial point.

The Erasmus Rucphen Family (ERF) study is comprised of a family-based cohort embedded in the Genetic Research in Isolated Populations (GRIP) program in the Southwest of the Netherlands.[3] The aim of this program is to identify genetic risk factors for the development of complex disorders. In ERF, twenty-two families that had a minimum of five children baptized in the community church between 1850 and 1900 were identified with the help of detailed genealogical records. All living descendants of these couples and their spouses were invited to take part in the study. Comprehensive interviews, questionnaires, and examinations were completed at a research center in the area; approximately 3,200 individuals participated. CFPWV was assessed with an automatic device (Complior® Artech Medical, Pantin, France) and calculated from the simultaneously measured foot-to-foot time delay between carotid and femoral pressure

waves and body surface measurement of the distance between carotid and femoral pulse recording site. Data collection started in June 2002 and was completed in February 2005. In the current analyses, 1,970 participants for whom complete phenotypic, genotypic and genealogical information was available were studied.

The Framingham Heart Study (FHS) began in 1948 with the recruitment of an Original cohort of 5,209 men and women who were 28 to 62 years of age (mean age 44 years; 55 percent women) at entry. In 1971 enrollment of a second generation of study participants took place; this cohort consisted of 5,124 children and spouses of children of the original cohort. The mean age of the Offspring cohort was 37 years; 52 percent were women. A Third Generation cohort of 4,095 children (mean age 40 years; 53 percent women) of offspring cohort participants was enrolled beginning in 2002. Details of study designs for the three cohorts are summarized elsewhere.[4-6] Tonometry was performed during Examination 26 (1999-2001), Examination 7 (1998-2001) and Examination 1 (2002-2005) for the Original, Offspring and Third Generation cohorts, respectively. CFPWV was evaluated by tonometry and calculated from the foot-to-foot delay between carotid and femoral waveforms and body surface measurements that adjusted for parallel transmission in the carotid and aorta by using the suprasternal notch as a fiducial point. Successful genotyping was available for 8,508 FHS participants, of whom 6,033 had successful measurement of CFPWV.

The Health, Aging and Body Composition (Health ABC) Study is a National Institute on Aging-sponsored ongoing cohort study of the factors that contribute to incident disability and the decline in function of healthier older persons, with a particular emphasis on changes in body composition in old age.[7] Between 4/15/97 and 6/5/98 the

Health ABC study recruited 3,075 participants who were 70-79 year old community-dwelling adults (41% African-American), who were initially free of disabilities that limited mobility and activities of daily living. CFPWV was measured from simultaneous Doppler flow signals obtained from the right carotid and right femoral arteries with nondirectional transcutaneous Doppler flow probes (model 810A, 9.0- to 10-MHz probes, Parks Medical Electronics, Inc). Digitized data were recorded by custom programming for subsequent analysis. A minimum of 10 beats were averaged for each simultaneous recording site using the QRS for synchronization. Three separate runs were recorded for each participant, and all usable runs were averaged. The distance between the carotid and femoral sampling sites was measured above the surface of the body with a metal tape measure. This was done to avoid overestimation of the distance portion of the PWV equation. This distance was divided by the time differentials between the onset of flow at carotid and femoral sites (defined as foot of the flow tracing at each site) to produce CFPWV. Successful genome wide genotyping was available for 2,800 participants, of whom 2,292 had successful measurement of CFPWV. For the purposes of this study, white participants of European descent were included in the analyses, resulting in a final sample of 1,398 participants with CFPWV and genotype data.

The Old Order Amish individuals included in this study were participants of several ongoing studies of cardiovascular health carried out at the University of Maryland. Most participants were from the Heredity and Phenotype Intervention (HAPI) Heart Study and were relatively healthy volunteers from the Old Order Amish community of Lancaster County, Pennsylvania and their family members.[8] Examinations were conducted at the Amish Research Clinic in Strasburg, PA. All protocols were

approved by the Institutional Review Board at the University of Maryland and informed consent was obtained, including permission to use their DNA for genetic studies. Study participants were enrolled within the 2003-2008 time period. CFPWV was measured with a Complior® device (Artech Medical, Pantin, France). All subjects were measured in the morning after an overnight fast and abstained from smoking or exercise prior to the test. In brief, after a 10 minute rest period and while in the supine position the carotid and femoral pulse wave were recorded simultaneously. The time delay between the rapid upstroke of the carotid and femoral waveforms represented the transit time for the wave. The average of ten cycles was used to determine transit time. The distance traveled by the pulse wave from the carotid to the femoral site was measured by tape. The final PWV for each subject was then calculated by dividing the distance traveled over the average transit time and expressed in meters per second. Measures with variance of over 9% were excluded. All scans were scored by a single blinded experienced cardiologist.

The Rotterdam Study (RS) is a prospective population-based cohort study comprising 7,983 subjects (78% response rate) aged 55 years or older.[9] Baseline data (RS-I) were collected between 1990 and 1993. In 1999, inhabitants who turned 55 years of age or moved into the study district since the start of the study were invited to participate in an extension of the RS (RS-II) of whom 3,011 participated (67% response rate). The rationale and design of the RS have been described in detail elsewhere.[9] During the third examination phase from the RS-I (1997-1999) and the first examination phase from the RS-II (1999-2001), measurements of arterial stiffness were performed.[1] CFPWV was assessed with an automatic device (Complior® Artech Medical, Pantin,

France) and calculated from the simultaneously measured foot-to-foot time delay between carotid and femoral pressure waves and body surface measurement of the distance between carotid and femoral pulse recording site. All RS participants with available DNA were genotyped using Illumina Infinium II HumanHap BeadChips at the Department of Internal Medicine, Erasmus Medical Center following manufacturer's protocols. Participants with call rate < 97.5%, excess autosomal heterozygosity, sex mismatch, or outlying identity-by-state clustering estimates were excluded. After quality control 5,974 RS-I participants and 2,157 RS-II participants were included. Of these, 3,011 RS-I participants and 1,657 RS-II participants had successful measurement of CFPWV.

The SardiNIA Study is a longitudinal study of aging-related quantitative traits comprising individuals from a circumscribed region on the island of Sardinia, Italy. The sample constitutes a genetically isolated founder population by virtue of their geographic isolation and ethnic homogeneity. In the SardiNIA study, 6,148 men and women over the age of 14 were recruited from a cluster of four towns in the Lanusuei Valley in the Ogliastra province of the island, which has a total population of 11,000.[10] In this cohort, 3,329 and 1,412 individuals were genotyped with the Affymetrix 10K and Affymetrix 500K Mapping array set, respectively, with 436 individuals generating an overlapping dataset. Given the relatedness among individuals, the full genotypes on the 2,893 individuals typed with only the 10K panel were imputed based on stretches of shared haplotype, permitting analyses on 4,305 individuals. CFPWV was evaluated with nondirectional transcutaneous Doppler probes and calculated from the foot-to-foot delay between carotid and femoral waveforms and body surface measurements that

adjusted for parallel transmission in the carotid and aorta by using the manubrium as a fiducial point. CFPWV and genotype data was available in 4,216 subjects.

Replication Sites

The Anglo Cardiff Collaborative Trial (ACCT) consists of ~12,000 community-derived individuals selected at random from the local general practice lists and open-access cardiovascular risk assessment clinics across East Anglia and Wales in the United Kingdom.[11] Approval for studies was obtained from the local research ethics committees, and written informed consent obtained from each participant. The overall response rate was 85%. All participants completed a detailed questionnaire and measurements were conducted in a quiet temperature controlled room. Height and weight were assessed with standard methods. After 20 minutes of supine rest, peripheral blood pressure was recorded in the brachial artery of the non-dominant arm using a validated oscillometric technique (HEM-705 CP, Omron Corporation). CFPWV was measured using tonometry and calculated from the foot-to-foot delay between carotid and femoral waveforms and body surface measurements that adjusted for parallel transmission in the carotid and aorta by using the suprasternal notch as a fiducial point as described previously.[12] A subset of the ACCT sample (n=3,101) aged 16-87 years were selected for replication of the hits from the primary GWAS. The age distribution of the cohort was bimodal, so the dataset was analyzed globally and separately based on individuals aged <55 years and ≥55 years. Quantitative associations were investigated using the program PLINK (version 1.05) (http://pnug.mgh.harvard.edu/~purcell/plink) and SPSS (version 15.0).

The Asklepios Study (AS) is a prospective population-based cohort study. Participants are a random sample (n=2,524) drawn from the Belgian population who were 35-55 years old and free from overt cardiovascular disease. Subjects were extensively phenotyped including echocardiography, carotid and femoral vascular ultrasound and arterial tonometry. All measurements were single observer, site, protocol and device. Baseline data were collected between 2002 and 2004. The rationale and design of the AS have been described in detail elsewhere.[13] CFPWV was evaluated by Doppler and calculated from the foot-to-foot delay between carotid and femoral waveforms and body surface measurements that adjusted for parallel transmission in the carotid and aorta by using the suprasternal notch as a fiducial point. Successful genotyping and measurement of CFPWV was available for 2,380 participants. Quantitative associations were investigated using SPSS (version 17.0).

Clinical Endpoint Consortia

The Coronary ARtery DIsease Genome-wide Replication And Meta-analysis (CARDIoGRAM) consortium combines data from 14 GWAS in individuals with European ancestry including >22,000 cases with coronary artery disease (CAD) or myocardial infarction (MI) and >60,000 controls, and unifies samples from Atherosclerotic Disease VAscular functioN and genetiC Epidemiology study, CADomics, Cohorts for Heart and Aging Research in Genomic Epidemiology, deCODE, the German Myocardial Infarction Family Studies I, II, and III, Ludwigshafen Risk and Cardiovascular Heath Study/AtheroRemo, MedStar, Myocardial Infarction Genetics Consortium, Ottawa Heart Genomics Study, PennCath, and the Wellcome Trust Case Control Consortium. These

studies have a case-control design or are prospective cohort studies both having detailed phenotyping for CAD or MI as previously described.[14] Control subjects have been derived from population-based studies in most investigations. For all of the participating studies, genome-wide scans were performed in the years 2006-2009 using either Affymetrix or Illumina platforms followed by imputation of genotypes in most studies. Statistical methods have been standardized across the studies, and an analysis platform has been created to allow summarized analyses on CAD, MI, and related phenotypes.

The Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium heart failure GWAS included data from 4 prospective, population-based cohorts of adults in the USA and the Netherlands: the Atherosclerosis Risk in Communities (ARIC) Study, the Cardiovascular Health Study (CHS), the Framingham Heart Study (FHS), and the Rotterdam Study (RS).[15] In brief, ARIC recruited 15,792 participants 45 to 64 years of age from 1987 to 1989 from 4 US communities. The CHS recruited participants 65 years of age and older from 4 US communities in 2 waves: 5201 participants in 1989–1990 and an additional 687 African Americans in 1992–1993. The FHS recruited 2 generations of participants 28 to 72 years of age in 2 time periods: 5209 Original Cohort participants were recruited in 1948 from Framingham, Massachusetts, and 5124 Offspring Cohort participants were recruited in 1971–1975. The RS recruited 7983 participants 55 years of age and older from 1990 to 1993 from Rotterdam, the Netherlands. The ARIC and CHS studies included African American participants, 27% and 15%, respectively, whereas FHS and RS included almost exclusively participants of European ancestry. Each study independently conducted

genome-wide scans of study participants, and within-study analyses were conducted according to a prespecified plan.

The Pulse Pressure/Mean Arterial Pressure working group on behalf of the International Consortium for Blood Pressure GWAS combines data from 35 GWAS in individuals with European ancestry, including AGES, ARIC, ASPS, B58C-T1DGC, B58C-WTCCC, BLSA, BHS, CARL, CHS, CoLaus, CROATIA, DGI controls, EGCUT, EPIC Norfolk, ERF, Fenland, FHS, FUSION controls, FVG, INCHIANTI, KORA S3, MICROS, MIGen controls, NESDA, NFBC1966, NTR, NSPHS, ORKNEY, ProCARDIS controls, RSI, RSII, SHIP, SUVIMAX, TwinsUK and Val Borbera.[16;17] For all participating studies, genome-wide scans were performed using either Affymetrix or Illumina platforms followed by imputation of genotypes. Statistical methods have been standardized across studies. Pulse pressure was defined as systolic blood pressure minus diastolic blood pressure. The combined analyses included 74,011 subjects.

The neuroCHARGE consortium combined data from white participants in four large, prospective population based cohort studies: ARIC, CHS, FHS, and RS to study the genetics of stroke.[18] All participating studies approved guidelines for collaboration, and a neurology working-group arrived at a consensus on phenotype harmonization, covariate selection and analytic plans for within-study analyses and meta-analysis of results. Each study has an Institutional Review Board that approved the consent procedures, examination and surveillance components, data security processes, genotyping protocols and current study design. Stroke was defined as a focal neurologic deficit of presumed vascular cause with a sudden onset and lasting for at least 24 hours or until death if the participant died less than 24 hours after the onset of

symptoms. The combined analysis included 1544 incident strokes (1164 ischemic strokes) among 19,602 persons followed for an average of 11 years.

The CKDGen consortium performed a genome-wide association meta-analysis in 67,093 study participants of European ancestry from 20 predominantly population-based cohorts.[19] They analyzed glomerular filtration rate (GFR) estimated from serum creatinine by the Modification of Diet in Renal Disease (MDRD) Study equation (eGFRcrea) as well as chronic kidney disease (CKD). To discriminate true susceptibility loci for renal function from those related to creatinine production and secretion, they used GFR estimated from a second serum marker of kidney function, cystatin c (eGFRcys).

Expression Methods

Commercially available cultured human aortic smooth muscle cells, adult human cardiac fibroblasts and human umbilical vein endothelial cells (HUVEC) were purchased and cultured according to the protocol recommended by the manufacturer (Cell Application Inc); CD3+ enriched cells from a healthy donor were provided by Dr. P. Olkhanud (NIA, Baltimore, USA). Aortic tissue samples were obtained from cadaveric donors or beating heart donors through transplant coordinators from the Addenbrooke's Hospital, Cambridge. Fresh thoracic and abdominal aorta removed by the surgical team at the time of organ donation was immediately placed in tissue medium and transported to the Addenbrooke's Hospital, where it was processed immediately. Each specimen was trimmed free of blood vessels, fat and any surrounding deposits. A sample of tissue from the ascending aortic rings was chopped into small pieces and preserved

overnight at 4°C in a tube containing RNA*later* solution. Solution was removed the following day and sample stored at -80°C for RNA extraction. All samples and patient data were handled in accordance with the policies and procedures of the Human Tissue Act, and the study was approved by the Local and Regional Ethics Committees. Informed consent was also obtained from the relatives.

RNA Extraction, cDNA Preparation, PCR Amplification and Sequencing

Total RNA was extracted using RNeasy Mini Kit (Qiagen Inc) with an additional on-column DNAse digestion step, according to the protocol recommended by the manufacturer. Commercially available total RNA samples extracted from human heart, human skeletal muscles, human kidney and human brain were obtained from Cell Application Inc. For cDNA synthesis, 2 μg of total RNA were used with the cDNA Archive Kit (Applied Biosystems Inc) using oligo (dT) primers in 25 μg of final volume. A control sample lacking reverse transcriptase was processed along with each cDNA synthesis in order to detect genomic DNA contamination. For subsequent PCR reactions, 1 μl of cDNA mixture was used together with Platinum Taq-Polymerase (Invitrogen Inc) or KOD-polymerase (Novagen Inc) in a final volume of 25 μl. Primers were designed with Vector NTI 11.0 software (**Supplementary Table S2**). Amplification products of appropriate size were excised from agarose gel and purified with QIAquick Gel Extraction Kit (Qiagen Inc); recovered DNA fragments were cloned with TOPO TA Cloning® Kit (Invitrogen Inc). Three independent clones for each sample were selected for follow-up sequencing to avoid possible reading errors. We used BigDye® Terminator

v1.1 kit (Applied Biosystems Inc) for sequencing reaction and samples were analyzed on a 3130xl Genetic Analyzer (Applied Biosystems Inc).

Total RNA was extracted from human aortic tissue using the TRIzol® Plus RNA Purification System (Invitrogen). Extraction of RNA was conducted according to the manufacturer's protocol and was further purified using PureLink™ silica-gel spin columns followed by DNase I digestion to minimize genomic DNA contamination (Invitrogen, PureLink™ RNA Mini Kit). First strand cDNA synthesis was performed on 1 μg total RNA using AMV reverse transcriptase according to manufacturer instructions (Reverse Transcription System, Promega). Reverse transcription was initiated using random hexamer primers and the reaction carried out at 42°C for 60 min, followed by heat inactivation at 95°C for 5 min. PCR primers were designed to target BP432414, DB129663, *BCL11B* and *VRK1* (Supplementary Table S2). A 5 μl aliquot of cDNA was used as template DNA in a 25 μl PCR reaction. Each reaction contained 5 pmol of each primer, 0.1 mM dNTPs, 1 U AGSGold™ DNA polymerase, 2.5 mM $MgCl_2$, 75 mmol/L Tris-HCL (pH 9.0), 20 mM $(NH_4)_2SO_2$, and 0.01% TWEEN-20. The PCR protocol consisted of 10 min at 95°C, followed by a touchdown procedure of 15 cycles of 95°C for 15 s, 68°C for 15 s, and 72°C for 15 s, decreasing annealing by 1°C per cycle. Following the initial 15 cycles the method consisted of 30 cycles of 95°C for 15 s, 55°C for 15 s, and 72°C for 15 s, with a final extension of 72°C for 10 min. To verify that the amplified product was the targeted gene, the product from one sample was sequenced at Geneservice (www.geneservice.co.uk) using Sanger sequencing followed by analysis on Applied Biosystems 3730 DNA Analyzer.

Supplementary Table S1. Genotyping methods.

Study	Genotyping Platform	Genotype Calling Algorithm	Exclusion of SNPs used for Imputation	Imputation Method	Imputation Backbone (NCBI Build)	Filtering of Imputed Genotypes	Data Handling and Statistical Tests
AGES	Illumina 370CNV	Beadstudio	Call rate <97% HWE p $<10^{-6}$ MAF <1%	MACH 1.0.16	Hapmap CEU Release 22 Build 35	None	PLINK and R
BLSA	Illumina 550K	Beadstudio	Call rate <99% HWE p $\leq 10^{-4}$ MAF ≤1%	Mach 1.0.15	Hapmap CEU Release 22 Build 35	r2_hat (imputation quality) >0.3	Merlin (--fastassoc)
ERF	Illumina 318 and 370	Beadstudio	Call rate <98% HWE p $<10^{-6}$ MAF <1%	MACH 1.0.15	Hapmap CEU Release 22 Build 36	None	R, GenABEL and ProbABEL
FHS	Affymetrix 500K and MIPS 50K combined	BRLMM	Call rate <97% HWE p $<10^{-6}$ Mishap p $<10^{-9}$ Mendelian errors >100	MACH 1.0.15	Hapmap CEU Release 22 Build 36	None	LMEKIN package in R – linear mixed effects model incorporating familial covariance based on degree of relatedness
HABC	Illumina Human1M-Duo	BeadStudio	Call rate <97% HWE p $<10^{-6}$ MAF <1%	MACH (version 1.0.16)	Hapmap CEU release 22 build 36	r2_hat (imputation quality) >0.3	R
HAPI	Affymetrix 500K	BRLMM	Call rate <97% HWE p $<10^{-6}$ MAF <1% Non-HapMap	MACH version 1.0.15	Phased CEU haplotypes, HapMap Release 22 Build 36	none	Measured genotype accounting for polygenic component

Supplementary Table S1 (continued).

Study	Genotyping Platform	Genotype Calling Algorithm	Exclusion of SNPs used for Imputation	Imputation Method	Imputation Backbone (NCBI build)	Filtering of Imputed Genotypes	Data Handling and Statistical Tests
RS-I	Illumina 550K	Beadstudio	Call rate <98% HWE p $<10^{-6}$ MAF <1%	MACH 1.0.15	Hapmap CEU Release 22 Build 36	None	ProbABEL
RS-II	Illumina 550K	Beadstudio	Call rate <98% HWE p $<10^{-6}$ MAF <1%	Mach 1.0.16	Hapmap CEU Release 22 Build 36	None	ProbABEL
SARDINIA	Affymetrix 500K and 10K	BRLMM	Call rate <90% HWE p $\leq 10^{-6}$ MAF ≤5% Excess Mendelian inconsistencies	Mach 1.0.10	Hapmap CEU Release 22 Build 35	r2_hat (imputation quality) >0.3	Merlin (--fastassoc)
Replication Cohorts							
ACCT	ABI Prism 7900						
Asklepios	Sequenom						

Supplementary Table S2. Primers used for reverse transcriptase polymerase chain reaction (RT-PCR) amplification.

Primer Name	Primer Sequence	Product Size (bp)
DB129663_F1	GCTATGACAAGTTCCACTGTGG	330
DB129663_R1	CACTGGGTGGAGTTAGCAGGTT	
DB129663_F2	CAACAACCTTCTTCTCCATGC	520
DB129663_R2	AGGGTGCCTGAGGATGGTGA	
BP432414.1_F	ACCACACACGTGAGATGCAC	161
BP432414.1_R	CCATTCCCCACGTTGTCTAC	
BCL11B_F1	CAGATGCCCTTCAGCGTCTAC	161
BCL11B_R1	CTCGGTTGGCAACGGTTC	
BCL11B_F2	CACCTGCTCTCACCCACGA	161/374*
BCL11B_R2	AGGTAGATGCGGAAGCCGT	
VRK1_F1	ATGGCAAATTGGACCTCAGTG	161
VRK1_R1	TCTTGAACGGGTCTGTATGGC	
VRK1_F2	GCCGCTGCCGAGTTACGAGT	200
VRK1_R2	CCTTGGCCAATGGGTAATCC	

*Isoform 2 / Isoform 1

Supplementary Table S3. Genome wide association results for CFPWV with additional adjustment for mean arterial pressure.

	Chromosome		Allele		Meta-analysis*			Closest Gene
SNP	Number	Position	Coded	Freq	Beta	SE	P	
rs9323989	14	97,657,422	C	0.416	-0.057	0.010	5.0×10^{-9}	*C14orf64*
rs987514	14	97,698,696	T	0.431	-0.055	0.010	1.3×10^{-8}	*C14orf64*
rs2225442	14	97,692,347	C	0.323	-0.058	0.011	9.4×10^{-8}	*C14orf64*
rs17773233	14	97,652,412	T	0.225	-0.061	0.012	1.4×10^{-7}	*C14orf64*
rs698050	14	97,607,147	T	0.485	-0.051	0.010	1.7×10^{-7}	*C14orf64*
rs10764094	10	19,950,544	C	0.473	0.049	0.010	3.9×10^{-7}	*C10orf112*
rs4888416	16	74,006,677	C	0.429	-0.051	0.010	9.3×10^{-7}	*CFDP1*
rs1461587	14	97,673,604	G	0.256	-0.057	0.012	1.3×10^{-6}	*C14orf64*
rs11199964	10	123,183,988	C	0.279	-0.052	0.011	1.3×10^{-6}	*FGFR2*
rs17699249	15	80,196,915	A	0.038	-0.119	0.025	1.7×10^{-6}	*EFTUD1*
rs7898799	10	19,929,473	C	0.460	-0.048	0.010	1.8×10^{-6}	*C10orf112*
rs766522	16	73,981,611	T	0.222	-0.059	0.012	1.9×10^{-6}	*CFDP1*
rs8015529	14	97,571,972	G	0.360	-0.053	0.011	2.3×10^{-6}	*C14orf64*
rs6485690	11	46,755,207	A	0.308	-0.049	0.010	3.0×10^{-6}	*CKAP5*†

Supplementary Table S3 (continued).

SNP	Chromosome		Allele		Meta-analysis*			Closest Gene
	Number	Position	Coded	Freq	Beta	SE	P	
rs16952146	13	106,862,505	T	0.287	0.050	0.011	3.7×10^{-6}	*LOC728215*
rs10515673	5	151,876,702	A	0.117	0.068	0.015	3.7×10^{-6}	*NMUR2*
rs4778983	15	80,290,133	C	0.301	0.047	0.010	3.9×10^{-6}	*EFTUD1*
rs7084101	10	123,184,245	A	0.240	-0.053	0.012	4.2×10^{-6}	*FGFR2*
rs1381273	14	97,718,813	T	0.469	-0.045	0.010	5.8×10^{-6}	*C14orf64*
rs7321644	13	106,863,235	T	0.446	-0.049	0.011	5.8×10^{-6}	*LOC728215*
rs17676096	3	64,690,507	A	0.344	0.050	0.011	6.7×10^{-6}	*ADAMTS9*
rs3097534	15	25,584,292	T	0.018	-0.203	0.045	8.2×10^{-6}	*OCA2*
rs10827649	10	19,949,776	G	0.437	-0.043	0.010	8.9×10^{-6}	*C10orf112*
rs7160893	14	97,677,047	T	0.215	-0.053	0.012	9.0×10^{-6}	*C14orf64*
rs193031	2	64,113,962	C	0.179	-0.056	0.013	9.6×10^{-6}	*VPS54*

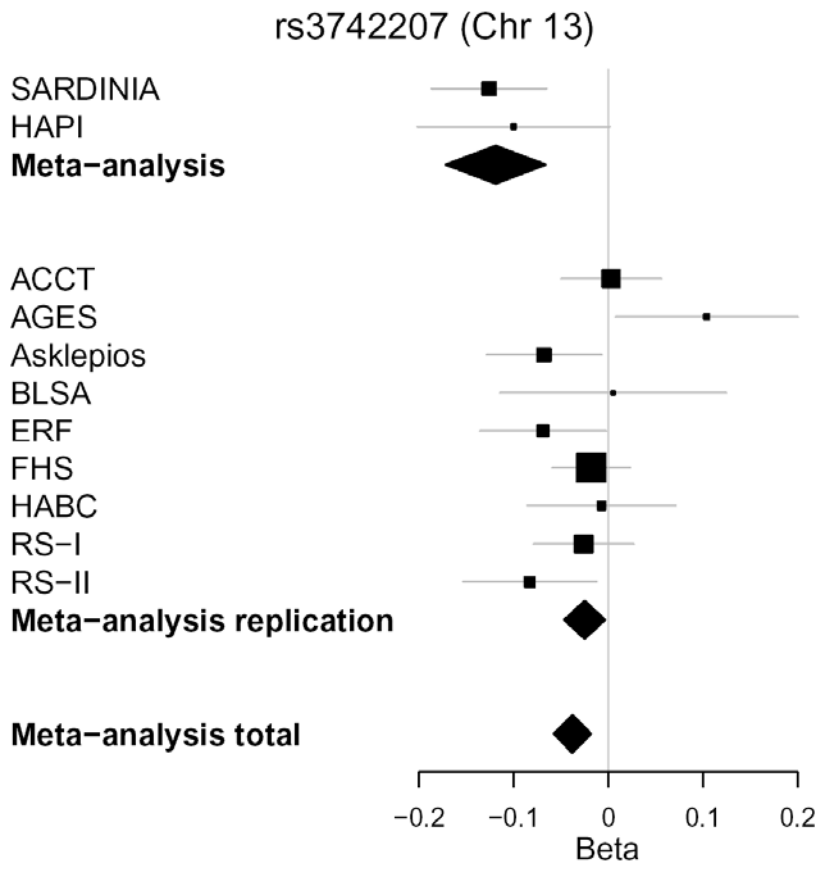
*Individual analyses were adjusted for age, age^2, sex, height, weight and mean arterial pressure. †LD block includes *ARHGAP1*, *ZNF408*, *F2*, *CKAP5* and *LRP4*.

Supplementary Table S4. Summary of expression data for genes and expressed sequence tags (EST's) associated with the chromosome 14 locus in human tissues and cell lines.

RNA source	DB129663	BP432414	*BCL11B*	*VRK1*
Aortic smooth muscle cells	+	-	+*	+
Umbilical vein endothelial cells	+	-	+	+
Cardiac fibroblasts, adult	+	-	-	+
Human aortic rings	+	+	+	+
Heart	-	-	+	+
Brain	-	-	NT	NT
Kidney	+	+	NT	NT
Skeletal muscle	-	-	NT	NT
CD3+ cells	+	+	+	+

DB129663 and BP432414 are human EST's. *****Detected only with primers BCL11B_F1/_R1. NT, not tested.

Supplementary Figure S1. Forest plot for *COL4A1.*



Results for individual cohorts are plotted against the cohort effect size (beta coefficient). Sardinia and HAPI were involved in the original report and are therefore presented separately from the 9 replication cohorts. The final point represents the meta-analysis of all 11 cohorts. The size of each box is inversely proportional to the estimated variance of the effect-size estimator. Horizontal lines are the 95% confidence intervals. Diamonds represent the results of meta-analyses.

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Replication Sites

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