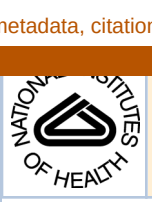

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Association of Genetic Variants and Incident Coronary Heart Disease in Multi-Ethnic Cohorts. The PAGE Study

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Abstract

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Background—Genome wide association studies identified several single nucleotide polymorphisms (SNPs) associated with prevalent coronary heart disease (CHD) but less is known of associations with incident CHD. The association of thirteen published CHD SNPs was examined in five ancestry groups of four large US prospective cohorts.

Methods and Results—The analyses included incident coronary events over 9.1 to 15.7 average follow-up times in up to 26,617 white individuals (6,626 events), 8,018 African Americans (914 events), 1,903 Hispanics (113 events), 3,669 American Indians (595 events) and 885 Asian/Pacific Islanders (66 events). We used Cox proportional hazards models (with additive mode of inheritance) adjusted for age, sex and ancestry (as needed). Nine loci were statistically associated with incident CHD events in whites: 9p21 (rs10757278, $p=4.7 \times 10^{-41}$), 16q23.1 (rs2549513, p=0.0004), 6p24.1 (rs499818, p=0.0002), 2q36.3 (rs2943634, $p=6.7 \times 10^{-6}$), *MTHFDIL* (rs6922269, $p=5.1 \times 10^{-10}$), *APOE* (rs429358, $p=2.7 \times 10^{-18}$), *ZNF627* (rs4804611, $p=5.0 \times 10^{-8}$), *CXCL12* (rs501120, $p=1.4 \times 10^{-6}$) and *LPL* (rs268, $p=2.7 \times 10^{-17}$). The 9p21 region showed significant between-study heterogeneity, with larger effects in individuals aged 55 years or younger and in women. Inclusion of coronary revascularization procedures among the incident CHD events introduced heterogeneity. The SNPs were not associated with CHD in African Americans and associations varied in other US minorities.

Conclusions—Prospective analyses of white individuals replicated several reported cross-sectional CHD-SNP associations.

Keywords

9p21 locus; incident coronary heart disease; genetic polymorphisms

Recent genome wide association studies (GWAS) identified several single nucleotide polymorphisms (SNPs) in genes or regions associated with coronary heart disease (CHD). Most were retrospective case-control comparisons and little is known about the contribution of these genetic variants to the risk of incident CHD in the general population.

One of the loci most studied for CHD is the 9p21 region near the *CDKN2A-2B* genes. This 58 kb region, in high linkage disequilibrium (LD) in individuals of European ancestry, overlaps a large noncoding RNA (antisense noncoding RNA in the *INK* locus – *ANRIL*), which may contribute to atherosclerosis through regulatory function on the *CDKN2A-2B* genes[1]. In addition to clinical CHD, variants in the 9p21 region (i.e., rs10757274 and rs2383206) have shown associations with coronary artery calcification[2, 3], premature atherosclerosis[2], ischemic stroke [4], heart failure [5], peripheral artery disease[6], abdominal aortic aneurysm and intracranial aneurysm [7] in white populations. Helgadottir et al reported early age of onset effects for CHD for this locus[8]. A recent meta-analysis of 22 studies including 35,872 cases and 95,837 controls, mostly whites, also suggested a larger effect (magnitude of association) of 9p21 variants in studies with earlier age of onset of clinical CHD[9]. Generalization of the genetic effects of this locus to populations of Asian ancestry was also observed in the latter meta-analysis.

A limited number of prospective studies of CHD have replicated the associations of the 9p21 region with incident CHD in white individuals, such as participants of the Atherosclerosis Risk in Communities (ARIC) study[2]. However, the associations did not replicate in studies of African Americans, including the ARIC study participants [2, 10] although it must be noted that the number of observations was suboptimal in several of these studies [3]. To our knowledge, a meta-analysis of the association of the 9p21 locus with incident CHD in other US minorities has not been done. We also note that additional loci for CHD have been recently described [11-13](*CELSR2/PSRC1, MTHFD1L, MIA3* among others); these associations have not been replicated in US minorities.

The Population Architecture using Genomics and Epidemiology (PAGE) Study enables a comprehensive analysis of the association of previously validated CHD variants with incident CHD in data from multiple prospective cohort studies, diverse cultural, ancestral and socio-economic settings. We examined whether CHD-related SNPs are associated with incident CHD in individuals of European ancestry in PAGE, and evaluated whether these SNPs are associated with incident CHD in US minorities, specifically in individuals self-identified as African American, American Indian, Hispanic and Asian/Pacific Islanders.

Methods

Population and definition of CHD events

The PAGE Study includes four large ongoing NIH-funded population based studies or consortia: EAGLE (Epidemiologic Architecture for Genes Linked to Environment, based on three National Health and Nutrition Examination Surveys [14], NHANES), MEC (the Multiethnic Cohort study[15]), WHI (Women's Health Initiative[16]), and CALiCo (Causal Variants Across the Life Course, a consortium of 5 cohort studies: Atherosclerosis Risk in Communities (ARIC)[17], Coronary Artery Risk in Young Adults [CARDIA][18], Cardiovascular Health Study **[**CHS][19], Hispanic Community Health Study/Study of Latinos [HCHS/SOL], and Strong Heart Study [SHS] [20, 21]). This report is based on prospective data on incident CHD from the ARIC, CHS, SHS and WHI studies. Fatal and non-fatal incident CHD events were defined as acute myocardial infarction (MI), fatal CHD, ECG diagnosis of MI (except in the WHI), and/or documented coronary revascularization procedures, based on record abstraction and confirmed by a panel of physician reviewers. Prevalent cases of CHD at the respective baseline visits were excluded.

The design of the parent studies has been previously described. Briefly, the **ARIC** study is a multi-center prospective investigation of atherosclerotic disease in a bi-racial population (white and African Americans).[17] ARIC recruited 15,792 individuals aged 45-64 years from four communities for a baseline examination in 1987-1989, with follow-up examinations in approximate 3-year intervals, during 1990-1992, 1993-1995, and 1996-1998. CHD events were ascertained from annual follow-up morbidity and mortality surveillance including hospitalizations and deaths. Events were reviewed by two physicians and differences adjudicated. CHD events were defined as non-fatal acute (definitive or probable) MI, fatal CHD and coronary revascularization procedures (coronary angioplasty or coronary artery bypass graft) through December 31st, 2005. All subjects provided written informed consent. **CHS** is a population-based cohort study of risk factors for CHD and stroke in adults ≥65 years conducted across four field centers in the United States [19]. The original predominantly Caucasian cohort of 5201 persons was recruited in 1989-1990 from a random sample of people on Medicare eligibility lists and 687 African-Americans were enrolled subsequently in 1991-1992 for a total sample of 5888. CHS participants completed standardized clinical examinations and questionnaires at study baseline and 9 annual follow-up visits. Follow-up for clinical events occurred every 6 months. CHD was classified by the CHS endpoints committee; suspected events were further investigated by a physician review panel. CHD events were defined as nonfatal MI, coronary artery angioplasty, coronary bypass surgery, or CVD death caused by "atherosclerotic CHD". CHD follow-up was available through June 30, 2007. **SHS** recruited 4,549 American Indians aged 45 to 74 years from 1989 to 1992 from 13 tribes at three study centers: Oklahoma, North and South Dakota and Arizona [20]. Events were determined from medical records, autopsy reports, and informant interviews; all materials were independently reviewed by physician members of the SHS study's morbidity and mortality committees. CHD events were defined by the occurrence of nonfatal definite MI, definite CHD, ECG-evident definite MI, fatal definite MI, definite CHD, and sudden death. Follow-up went from 1989-91 through December 31st, 2006. The Indian Health Service Institutional Review Board and institutional review boards of the participating

institutions and participating tribes approved the study; informed consent was obtained from all participants. **WHI** is a prospective cohort study investigating post-menopausal women's health in the U.S [16]. A total of 161, 838 women aged 50–79 years old were recruited from 40 US clinical centers between 1993 and 1998 to participate in the observational study (OS) and in clinical trials (CT): postmenopausal hormone therapy (estrogen alone or estrogen plus progestin), a calcium and vitamin D supplement trial, and a dietary modification trial [16]. Study protocols and consent forms were approved by the institutional review boards at all participating institutions. Annual (OS) and semi-annual (CT) follow-up identifies self-reported events which are then adjudicated following medical record review [22]. CHD was defined as acute (definitive or probable) MI requiring overnight hospitalization, fatal CHD or coronary revascularization (coronary angioplasty or coronary artery bypass graft) procedures. Acute MI was determined according to standardized criteria that included cardiac pain, cardiac enzyme (and troponin levels) and ECG readings. Events as of August 2009 are included in these analyses. A subset of 21,000 WHI women was selected for genotyping and for inclusion in the current study. Women were selected based on self-reported history of disease, incident event outcomes, DNA availability and consent, and racial/ethnic diversity. Follow-up was through August 2009.

SNP selection and Genotyping

SNPs (in genes or regions) were identified from published GWAS for their association with CHD and those available as per January 2009 were genotyped in PAGE studies [23-28]. SNPs were available in at least one study for the following loci: the 9p21 region near the *CDKN2A-2B* gene (rs1333049, rs2383207, rs10757274), *CELSR2/PSRC1/SORT1* (rs599839), *SMAD3* (rs17228212), 6p24.1 (rs499818), 16q23.1 (rs2549513), *MIA3* (rs17465637), *MTHFD1L (*rs6922269), 2q36.3 (rs2943634), *APOE* (rs429358, rs7412), *ZNF627* (rs4804611), *CXCL12* (rs501120), *LPL* (rs268) and *PCSK9* (rs11206510). We examined the correlation among SNPs in the same region using pair-wise r^2 and D' statistics[29] for linkage disequilibrium (LD). For three SNPs located in the 9p21 region (rs10757278, rs1333049, rs2383207) in African Americans (ARIC) and in the American Indians (SHS), the correlation among SNPs was 0.85 or more in African Americans and in American Indians (Supplemental Figures 1A-D). Therefore, we only reported associations for one SNP in the region (rs10757278). For the two *APOE* SNPs, correlation was 0.99 in all ethnicities and therefore we reported only associations for rs429358.

Genotyping was performed in two centers: the CALiCo Core Genotyping Laboratory at Human Genetics Center The University of Texas (Houston, TX) genotyped samples from ARIC, CHS and SHS using Taqman assays (Applied Biosystems); the Translational Genomics Research Institute (Phoenix, AZ) genotyped WHI samples using Illumina's Veracode GoldenGate genotyping assays. Each laboratory genotyped 360 HapMap samples for cross-lab and cross-platform quality control. Quality control assessments included sample call rates (> 95%), concordance of blinded replicates (>98%) and deviation from Hardy-Weinberg equilibrium among controls within self-reported ethnic group ($p<0.01$). In CALiCo populations with prior GWAS characterization (ARIC and CHS), genotyped SNPs were used (Affymetrix 6.0 for ARIC and Illumina Human 370CNV BeadChip)[30]. Quality control for GWAS has been described[30]. All alleles were aligned to positive strands.

Statistical analysis

All analyses were stratified by study and self-reported ethnicity. Cox proportional hazard models (implemented in SAS 9.2 using Proc PHREG procedure or in R)[31] were used to estimate ethnic-specific associations of SNPs with CHD (hazard ratios, HR, and 95% confidence intervals) while adjusting for baseline levels of covariates age and sex, study site (as applicable) and population stratification. We also performed an analysis adjusting for

body mass index (BMI), ever smoking, type 2 diabetes, systolic blood pressure, blood pressure-lowering medication use and education (12 years or equivalent versus less than 12 years). Results were unchanged in fully adjusted models and therefore we report minimally adjusted models except for 9p21 region in whites. Lipid measures were only available in a subset of women in the WHI and therefore were not included in analyses. To explore phenotypic heterogeneity as described by Kitsios et al.[32], we also performed sensitivity analyses using an alternative CHD definition which included only fatal CHD and non-fatal MI.

PAGE studies used the following strategies to adjust for population stratification: ARIC whites and WHI used principal components (PC) estimated from pre-existing GWAS data, adjusting only for PCs significantly associated with the outcome (alpha=0.05). For ARIC African Americans a set of 1536 ancestry informative markers genotyped among 3965 black ARIC participants was used to derive mean percentage of European ancestry using the software ANCESTRYMAP [33]. This variable was included as a covariate in the regression analyses. SHS used self-reported percentage of Indian blood.

All analyses used additive genetic models as described in the initial publications for these SNPs and/or the replication studies in whites. WHI used weighted analyses to account for the sampling schema, with sample weights ranging from 1 to 60.4. The study-specific log(HR) estimates were combined within each ethnicity using inverse-weighted variance meta-analyses[34]. Summary estimates and 95% confidence intervals (CI) are reported. A nominal replication was considered a p-value < 0.05 and a Bonferroni adjusted replication was considered p-value < 0.0008 (13 SNPs and 5 races for an α=0.05).We also tested for evidence of between-study heterogeneity [35] by estimating the between-study variance and the I^2 metric, which is a measure of the percentage of the total variation across studies due to heterogeneity rather than chance [36]. For the 9p21 SNP association, which showed significant between-study heterogeneity (p-value<0.10), we performed stratified analyses by age and sex.

We also constructed an additive genetic risk score (0-18 alleles) comprising the number of risk alleles at the nine loci showing significant replication in white individuals (p<0.0008) using data from the following SNPs (coded allele): rs10757278 (G), rs2549513(A), rs499818(A), rs6922269(A), rs429358(C), rs4804611(A), rs501120(T), rs268(G) and rs2943634(C). We then tested the association of categories or continuous risk scores with incident CHD in WHI, the study that had data on all the SNPs.

For associations in samples of US minorities, we estimated power assuming an additive genetic effect, magnitude of effect estimate of reported associations in white individuals, an alpha = 0.05, a two-sided test, and population baseline prevalence of 0.10 (Quanto v.1.2.4).

Results

Associations with incident CHD events were examined in the prospective cohorts of whites (up to 26,617 individuals and 6,626 events), African Americans (8,018 individuals and 914 events), Hispanics (1,903 individuals and 113 events), American Indians (3,669 individuals and 595 events) and Asian/Pacific Islanders (885 individuals and 66 events). The mean follow-up of studies varied from 9.1 to 15.7 person-years (Supplemental Table 1). CHS participants were older than individuals in other studies and WHI recruited only women (Table 1). CHD risk factors varied across studies and ethnicities.

We first evaluated replication of SNP associations with incident CHD in samples of white individuals. Nine of 13 investigated loci associations showed replication by our a priori criteria (p<0.0008) including the 9p21 region (Table 2), 16q23.1 (rs2549513, p=0.0004),

6p24.1 (rs499818, p=0.0002), *MTHFDIL* (rs6922269, $p=5.1 \times 10^{-10}$), 2q26.3 (rs2943634, $p=6.7 \times 10^{-6}$), *APOE* (rs429358, $p=2.7 \times 10^{-18}$), *ZNF627* (rs4804611, $p=5.0 \times 10^{-8}$), *CXCL12* (rs501120, $p=1.4 \times 10^{-6}$) and *LPL* (rs268, $p=2.7 \times 10^{-17}$) (Table 3). Meta-analyses including incident findings from published variants in the ARIC study are shown in Supplemental Table 2. Replicated loci had similar direction and magnitude of effects as reported. Using an additive genetic score from these nine SNPs, we observed an increased hazard of CHD across increasing categories of genetic risk score (Table 5). *CELSR2/SORT*, *MIA3* and *SMAD3* SNPs were not associated with incident events in white individuals.

In the 9p21 region the proxy SNP rs10757278 had associations of similar direction and magnitude as in published studies, but we noted significant between-study heterogeneity at this locus (Table 2). Each copy of the rs10757278 G allele was associated with increased hazard of CHD that varied from 10% (CHS) to 22% (WHI) (p for heterogeneity = 0.03, I^2=71.5%). Study-specific cumulative incidence findings by genotypes are shown in Supplemental Table 3. Estimates were unchanged in analyses adjusted for multiple CHD risk factors (Table 2). Sex-specificity and the varying age at recruitment were investigated as possible sources of the heterogeneity of effects. The hazard ratios for CHD by decade of age at study recruitment summarized in Figure 1A show a notably greater risk of CHD for individuals 55 years or younger. The magnitude of the risk estimates decreased with increasing decade of age, although large heterogeneity remained across age-strata (Figure 1A, Supplemental Table 4). In sex-specific analysis the CHD hazard was greater in women compared to men, with large between-sex heterogeneity of effects (Figure 1B, Supplemental Table 5). In analyses stratified by age and sex, the risk of CHD was greater in women than men at earlier ages (Figure 1C and Supplemental Table 6). To investigate CHD phenotypic heterogeneity due to inclusion of revascularization procedures, we performed sensitivity analyses using only non-fatal MI and CHD fatal events. Between-study heterogeneity for the CHD associations of rs10757278 was no longer present in these analyses (Table 2). Supplemental Table 7 shows the findings using this definition for additional loci showing heterogeneity.

We also examined whether SNP associations with CHD generalize to samples of US minority groups (Tables 4). Because of high correlation between the SNPs in the 9p21 region in African Americans and SHS American Indians (Supplemental Figures 1A-D), we only report findings for rs10757278. The association of rs10757278 with incident CHD did not replicate in African Americans despite large sample sizes and greater than 80% power to detect an effect of similar magnitude to whites (Table 4). SNPs in the 9p21 region were nominally associated with CHD in American Indians with similar magnitude and direction of effect as in whites. However, we also observed significant between-study heterogeneity of effect across the four groups of American Indians, as well as in analyses including only SHS participants (Table 4). We were unable to evaluate the associations in the 9p21 region in Hispanics and Asian/Pacific Islander due to limited sample sizes. Other loci showing nominal associations in minorities are shown in Table 4.

Discussion

Replication of the association of SNPs in the 9p21 region with incident CHD was found in white individuals, as well as generalization of these associations to American Indians but not to African Americans, Hispanics or Asian/Pacific Islanders. Our study size was limited for Hispanics and Asians but prior studies have shown associations of this region with CHD in Pakistanis (1851 MI cases and 1903 controls) [37], Han Chinese (510 CHD cases and 557 controls) [38], Japanese and Koreans [39, 40] and US Hispanics (82 cases and 108 controls)[3]. This locus has been recently shown to disrupt a transcription-factor-biding site involved in the inflammatory response (STAT1) which affects the expression of the *CDKN2A-2B*

genes [41]. Consistent with previously reports, we identified age- and sex-specific effects for the 9p21 locus on CHD risk. The rs10757278 G risk variant showed a larger effect in younger compared to older cohort participants, more evident for individuals aged 55 years or younger and in women. Heterogeneity in the estimates for 9p21 in white individuals persisted even after adjusting for multiple risk factors for CHD, but was no longer present in analyses that excluded revascularization procedures from the definition of incident CHD events. Because the use of CHD revascularization procedures (coronary angioplasty or coronary artery bypass graft) may vary in subgroups of individuals due to indication (younger symptomatic individuals and men are more likely to be offered procedures) and to socio-economic factors (access to care), our results highlight the importance of using strict and homogeneous definition of CHD events when combining data from multiple studies for evaluation of genetic effects. Thus, our results do not support sex- and age-specific effects for 9p21 locus for incident CHD.

We were able to replicate multiple other loci in white individuals including *MTHFD1L*, 16q23.1, 6p24.1, 2q36.3, *APOE, ZNF627, CXCL12* and *LPL*. Most of these loci were initially identified in the Welcome Trust Case Control Consortium (WTCCC) [24]. In general, we noticed a modestly greater CHD risk in our prospective analysis compared to the magnitude of association described using a case-control design in prior studies [24, 28, 42]. However, the risk score using these variants showed consistent graded increased CHD risk. In addition, we found significant between-study heterogeneity for the *MTHFD1L* and *CXCL12* loci (Supplemental Table 2), similarly to findings recently described in the case-control meta-analysis of the CARDIoGRAM consortium for the *MTHFD1L* associations [42].

The direction of effect of the remaining SNPs evaluated in whites was consistent with those in the discovery studies. Interestingly, three loci, *CELSR2/SORT1*, *MIA3* and *PCSK9*, which have been associated with early-onset MI in whites[28], did not replicate in our study and in a recently published study of Finland and Sweden individuals[43] . Recent experimental evidence suggests a role of *SORT1* product, sortilin 1, in the hepatic metabolism of lipoproteins containing apolipoprotein B [44]. We had over 80% power to detect an effect for this locus in whites, assuming effects similar in magnitude to those published. Because our incident cases were first CHD events occurring after age 45 and the studies that identified these SNPs were cases of early MI compared to controls, our negative findings are not inconsistent with the previously reported associations in white individuals.

None of the SNPs tested was significantly associated with CHD in African Americans after adjustments for multiple testing and our samples in Hispanics and Asians were limited preventing conclusions. SNPs in the 9p21 region available for this analysis are highly correlated (r^2>0.85) in African Americans. However, the genotyped SNPs may not be ideal proxy for functional SNPs in the region for populations of recent African ancestry. In addition, three other well powered SNP associations (rs599839, rs6922269 and rs2943634) were not significant in African Americans. Testing additional SNPs at these and other loci may help to elucidate if LD patterns account for some of the negative findings in African Americans.

The strengths of this study are the prospective evaluation of the genetic effect on CHD risk in large population based studies and the multi-ethnic evaluation of CHD loci. Our analyses were limited to SNPs previously identified in individuals of European ancestry, reported by January 2009, when SNP selection was done for genotyping in PAGE. Therefore, we did not evaluate recently discovered variants and we were unable to evaluate race-specific variants in these loci which will require fine mapping of the regions.

In summary, we identified significant heterogeneity of the 9p21 locus and other loci in individuals of European ancestry in prospective analyses of CHD risk, some of which could be explained by differences in event definition. Overall, some SNP associations replicated in longitudinal analysis of white individuals but effects were modest and none of the SNPs was associated with incident CHD in large sample sizes of African Americans. Fine-mapping of these regions may help to clarify these negative findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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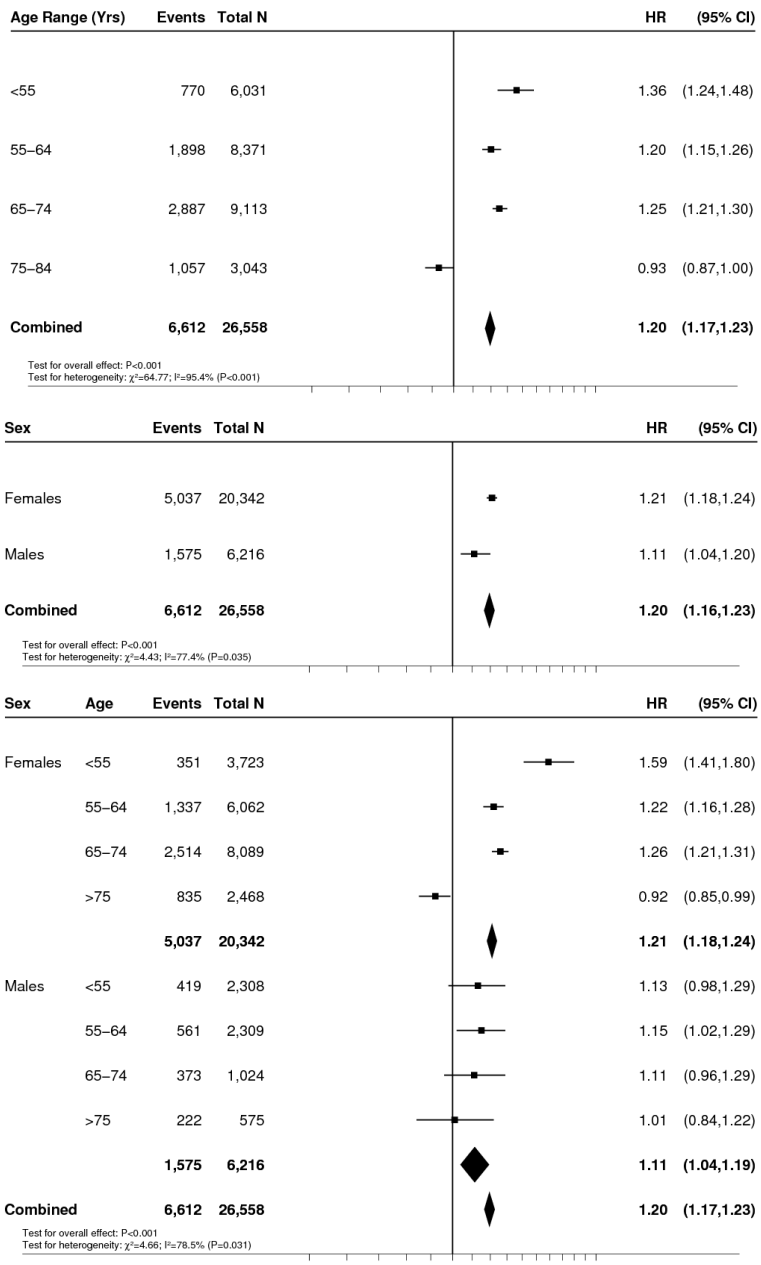

Figure 1A-C.
Meta-analysis results of the association of rs10757278 9p21 variant by age of study recruitment (A), sex (B) and age and sex strata (C).

Table 1

Study characteristics by self-reported race/ethnicity

	Strong Heart Study											
Studies	**ARIC**		**CHS**		**Dakotas**	**Arizona**	**Oklahoma**	**WHI***				
Race (number)	**White (10,247)**	**Black (3943)**	**White (4313)**	**Black (837)**	**American Indian (1186)**	**American Indian (1170)**	**American Indian (1124)**	**White (12410)**	**Black (3972)**	**Hispanic (1905)**	**American Indian (203)**	**Asian-Pacific Islander (886)**
Age (yrs)	54.2(5.7)	53.4(5.8)	72.7(5.6)	72.9(5.8)	56.4(8.1)	55.7(7.9)	56.7(8.3)	66.9(6.9)	61.3(7.1)	60.2(6.6)	61.3(7.5)	64.1(7.5)
% Male	45.4	37.5	40.0	36.7	42.1	35.9	40.0	0.0	0.0	0.0	0.0	0.0
% Education<12 yrs	16.3	41.3	26.1	45.2	52.5	61.9	30.8	4.5	12.2	27.8	18.2	5.9
BMI (kg/m^2)	26.9(4.9)	29.6(6.1)	26.3(4.5)	28.5(5.6)	29.3(5.4)	32.5(7.2)	30.8(6.0)	28.8(6.7)	33.1(7.8)	30.6(7.0)	30.5(6.4)	25.5(4.6)
% Hypertension†	26.0	55.3	55.4	72.9	29.6	43.8	43.5	46.3	55.6	33.0	43.4	50.7
% Diabetes mellitus‡	8.4	19.2	7.1	8.2	32.7	64.3	36.1	2.7	4.9	2.9	9.4	4.4
SBP (mmHg)	118.3 (16.9)	128.7 (21.2)	135.8 (21.4)	141.9 (23.0)	122.9 (18.5)	130.9 (20.7)	128.8 (18.5)	131.9 (18.4)	132.3 17.4)	126.9 (17)	128.5 (17.5)	132.9 (18.6)
DBP (mmHg)	71.6 (10.0)	79.7 (12.1)	70.2 (11.2)	75.2 (11.3)	75.3 (10.3)	77.7 (9.8)	77.2 (10.1)	75.3 (9.5)	78.5 (9.6)	75.7 (9.3)	76.2 (8.6)	78.7 (9.4)
LDL cholesterol (mg/dl) §	136.8 (37.6)	137.5 (43.1)	130.0 (35.5)	128.9 (36.6)	125.6 (33.2)	104.3 (32.4)	119.9 (32.2)	132.5 (34.3)	134.4 (39.3)	128.4 (34.9)	124.8 (37.6)	121.8 (32.3)
HDL cholesterol (mg/dl) §	51.0 (16.8)	55.3 (43.1)	54.6 (15.8)	57.9 (15.3)	46.9 (14.7)	44.8 (12.8)	46.6 (13.9)	58.6 (15.6)	57.6 (15.2)	54.0 (14.5)	52.6 (13.4)	59.2 (15.8)
Smoking, ever	59.0	53.1	52.4	51.6	78.2	57.2	68.9	50.2	50.1	37.4	50.7	28.1
% Anti-hypertensive drugs	18.6	39.7	40.8	60.8	16.4	26.4	26.6	30.1	43.6	21.3	30.1	35.3
% Lipid-lowering drugs	3.0	1.2	4.5	6.1	0.3	0.0	0.8	8.7	7.0	6.3	6.9	17.2

Numbers are mean (standard deviation) or percentages. BMI, body mass index; CHD, coronary heart disease; SBP, systolic blood pressure; DBP, diastolic blood pressure. For study abbreviations see text.

*WHI results are from un-weighted data.

†SBP ≥140 mm Hg, DBP ≥ 90 mm Hg or self-reported use of anti-hypertensive medications

‡Fasting blood glucose level ≥ 7 mmol/L after a 8-hour fasting period or the self-reported use of oral hyperglycemic medication or insulin

§LDL cholesterol was available in 5056 WHI women and HDL cholesterol was available in 7256 WHI women across race.

Table 2

Study-specific and meta-analysis findings in white individuals for the association of rs10757278 (9p21 locus) with incident coronary heart disease using different event definitions

Models	SNP	Coded/ Other allele	Study	Events	Total number	MAF	HR	95% CI	P value	I^2 (P value)
Model 1*	rs10757278	G/A	ARIC	1453	10247	0.48	1.14	1.06, 1.23	0.0003	
			CHS	1173	3978	0.49	1.10	1.01, 1.19	0.02	
			WHI	4000	12392	0.50	1.22	1.18, 1.25	2.5×10^{-40}	
			Combined†	6626	26617		1.19	1.16, 1.23	4.7×10^{-41}	71.5% (0.03)
Model 2	rs10757278		ARIC	864	10247	0.48	1.14	1.04, 1.25	0.006	
			CHS	821	3978	0.49	1.11	1.01, 1.22	0.03	
			WHI	3578	12393	0.50	1.15	1.10, 1.20	3.2×10^{-11}	
			Combined†	5263	26618		1.14	1.10, 1.18	4.5 × 10-13	0.0% (0.79)

Model 1, an event was defined as non-fatal acute myocardial infarct (MI), fatal coronary heart disease (CHD) and coronary revascularization procedures (coronary angioplasty or coronary artery bypass graft). Model 2, an event was defined as non-fatal acute MI and fatal CHD. Both models are adjusted for age, sex (except WHI), site or region and population stratification.

*HR 1.21, 95% CI 1.17, 1.24 for model adjusted for age, sex (except WHI), site or region, body mass index (BMI), ever smoking, type 2 diabetes, systolic blood pressure, anti-hypertensive medication use, education (12 years or equivalent versus less 12 years), HDL and LDL (except WHI where lipids were available in only a subset of the sample, see Table 1) and population stratification.

†Meta-analyses using fixed effect models Abbreviations: SNP, single nucleotide polymorphism; MAF, minor allele frequency; HR, hazard ratio; CI, confidence interval. For study abbreviations see text.

Abbreviations: SNP, single nucleotide polymorphism; MAF, minor allele frequency; HR, hazard ratio; CI, confidence interval. For study abbreviations see text.

Table 3

Replication of associations in white individuals for additional coronary heart disease loci

SNP	Gene/region	Coded/ Other allele	Study	Coded Alelle Frequency	Event	Total number	HR	95% CI	P value	P-hetero	I^2
rs599839	*CELSR2-SORT1* *	A/G	CHS-WHI	0.77	4965	16367	1.02	0.98, 1.05	0.37	0.27	18.7%
rs2549513	16q23.1	A/C	CHS-WHI	0.86-0.87	4746	15658	1.08	1.04 1.13	0.0004	0.87	0.0%
rs499818	6p25.1	A/G	CHS-WHI	0.25	4962	15718	1.06	1.03, 1.10	0.0002	0.74	0.0%
rs6922269	*MTHFD1L* *	A/G	WHI	0.26	3995	12394	1.11	1.07, 1.15	5.1×10^{-10}		
rs2943634	2q36.3*	C/A	WHI	0.67	3963	12316	1.07	1.04, 1.11	6.7×10^{-6}		
rs17465637	*MIA3* *	C/A	WHI	0.72	4003	12408	1.01	0.98, 1.04	0.63		
rs17228212	*SMAD3* *	T/C	WHI	0.72	4003	12404	0.97	0.94, 1.00	0.09		
rs429358	*APOE* †	C/T	WHI	0.13	3969	12313	1.20	1.15, 1.25	2.7×10^{-18}		
rs4804611	*ZNF627*	A/G	WHI-CHS	0.73	4485	15536	1.09	1.06, 1.13	5.0×10^{-8}	0.45	0.0%
rs501120	*CXCL12*	A/G	WHI-CHS	0.86	5117	15591	1.10	1.06, 1.15	1.4×10^{-6}	0.05	74.7%
rs268	*LPL*	G/A	WHI	0.02	4004	12408	1.46	1.34, 1.60	2.7×10^{-17}		
rs11206510	*PCSK9*	T/C	ARIC-WHI-CHS	0.82	6203	25051	1.00	0.96, 1.03	0.84	0.42	0.0%

Data is either one study estimates or meta-analyses of multiple listed studies.

*These SNP associations with incident CHD have been previously published in ARIC and therefore ARIC estimates are not included in the analyses (but included in Supplemental Table 2). For WHI, estimates were weighted based on sampling.

†For rs7412, C allele coded, HR=1.14, 95% CI 1.08, 1.21, p-value=2.4×10^{-6}, N=12312, events=3979.

Abbreviations: SNP, single nucleotide polymorphism; MAF, minor allele frequency; HR, hazard ratio; CI, confidence interval; P-hetero, p-values for between study heterogeneity. For study abbreviations see text.

Table 4

Associations of coronary heart disease loci in US minorities

SNP/gene	Race/ ethnicity	Coded/ Other allele	Studies	Event	Total number	Coded Allele Frequency	HR	95% CI	P-value	I^2 (P-hetero)	Power‡
rs10757278 (9p21)	African American	G/A	ARIC-CHS-WHI	914	8018	0.20-0.23	0.94	0.85, 1.04	0.22	73.9% (0.02)	0.82
	American Indian		SHS-WHI†	595	3669	0.43-0.45	1.15	1.03, 1.29	0.02	83.7% (0.002)	0.84
	Hispanic		WHI	113	1903	0.47	1.17	0.97, 1.41	0.11		0.22
	Asian/Pacific Islander		WHI	66	885	0.51	1.50	1.19, 1.89	0.0005		0.14
rs599839 (*CELSR2*)	African American	A/G	ARIC-CHS-WHI	838	7211	0.26-0.31	1.04	0.96, 1.14	0.35	0.0% (0.40)	0.89
	American Indian		SHS-WHI †	596	3659	0.17-0.33	1.14	0.99, 1.31	0.08	18.1% (0.30)	0.72
	Hispanic		WHI	112	1888	0.25	1.03	0.83, 1.29	0.76		0.17
	Asian/Pacific Islander		WHI	66	885	0.08	0.72	0.51, 1.03	0.07		0.08
rs2549513 (16q23.1)	African American	A/C	CHS-WHI	485	4559	0.75	0.98	0.87, 1.10	0.70	0.0% (0.92)	0.58
	American Indian		WHI	20	203	0.88	0.93	0.49, 1.80	0.04		0.06
	Hispanic		WHI	113	1905	0.90	1.00	0.73, 1.37	1.0		0.12
	Asian/Pacific Islander		WHI	66	886	0.93	2.77	1.33, 5.77	0.007		0.08
rs499818 (6p25.1)	African American	A/G	CHS-WHI	485	4565	0.13	1.09	0.95, 1.26	0.23	52.2% (0.15)	0.43
	American Indian		WHI	20	203	0.30	0.94	0.57, 1.55	0.81		0.07
	Hispanic		WHI	113	1903	0.30	0.77	0.61, 0.96	0.02		0.22
	Asian/Pacific Islander		WHI	66	884	0.12	1.31	0.95, 1.81	0.11		0.10
rs6922269 (*MTHFD1L*)	African American	A/G	ARIC-WHI	682	6523	0.54	1.03	0.95, 1.13	0.46	0.0% (0.48)	0.82
	American Indian		WHI	20	203	0.34	0.76	0.47, 1.23	0.27		0.07
	Hispanic		WHI	112	1904	0.36	1.16	0.96, 1.42	0.13		0.23
	Asian/Pacific Islander		WHI	66	885	0.04	0.34	0.11, 1.02	0.06		0.07

SNP/gene	Race/ ethnicity	Coded/ Other allele	Studies	Event	Total number	Coded Allele Frequency	HR	95% CI	P-value	I^2 (P-hetero)	Power‡
rs2943634 (2q36.3)	African American	C/A	ARIC-WHI	677	6502	0.41-0.43	1.02	0.93, 1.12	0.68	0.0% (0.98)	0.81
	American Indian		WHI	20	203	0.71	1.29	0.76, 2.16	0.34		0.07
	Hispanic		WHI	113	1897	0.75	0.95	0.76, 1.18	0.64		0.19
	Asian/Pacific Islander		WHI	65	883	0.90	2.00	1.20, 3.33	0.008		0.08
rs17465637 (*MIA3*)	African American	C/A	ARIC-WHI	681	6514	0.25-0.28	1.06	0.96, 1.17	0.23	0.0% (0.99)	0.47
	American Indian		WHI	20	203	0.59	1.49	0.96, 2.30	0.07		0.07
	Hispanic		WHI	113	1905	0.55	0.91	0.76, 1.09	0.31		0.24
	Asian/Pacific Islander		WHI	66	886	0.57	0.85	0.67, 1.07	0.17		0.15
rs17228212 (*SMAD3*)*	African American	T/C	ARIC-WHI	683	6526	0.87	0.87	0.77, 0.99	0.03	63.5% (0.10)	0.51
	American Indian		WHI	20	203	0.80	1.54	0.86, 2.76	0.15		0.07
	Hispanic		WHI	113	1904	0.83	0.75	0.59, 0.95	0.02		0.16
rs429358 (*APOE*)	African American	C/T	WHI	376	3930	0.20	1.06	0.93, 1.21	0.35		0.41
	American Indian		WHI	20	201	0.14	0.50	0.22, 1.15	0.10		0.06
	Hispanic		WHI	112	1892	0.12	1.04	0.77, 1.39	0.81		0.10
	Asian/Pacific Islander		WHI	65	880	0.10	1.04	0.71, 1.52	0.83		0.07
rs4804611 (*ZNF627*)	African American	A/G	CHS-WHI	561	4557	0.82	1.05	0.93, 1.19	0.41	0.0% (0.75)	0.99
	American Indian		WHI	20	203	0.75	1.50	0.87, 2.61	0.15		0.44
	Hispanic		WHI	113	1905	0.72	0.91	0.74, 1.11	0.33		0.99
	Asian/Pacific Islander		WHI	66	886	0.72	0.78	0.56, 1.09	0.14		0.98
rs501120 (*CXCL12*)	African American	A/G	CHS-WHI	566	4547	0.60	1.10	0.97, 1.21	0.06	55.1% (0.14)	0.54
	American Indian		WHI	20	203	0.77	1.87	0.92, 3.83	0.09		0.07
	Hispanic		WHI	113	1904	0.75	1.00	0.80, 1.24	1.00		0.20
	Asian/Pacific Islander		WHI	66	885	0.67	1.20	0.93, 1.54	0.15		0.15
rs11206510 (*PCSK9*)	African American	T/C	ARIC-CHS-WHI	818	7168	0.86	0.99	0.88, 1.12	0.88	0.0% (0.41)	0.99

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SNP/gene	Race/ ethnicity	Coded/ Other allele	Studies	Event	Total number	Coded Allele Frequency	HR	95% CI	P-value	I^2 (P-hetero)	Power‡
	American Indian		SHS	576	3472	0.95-0.99	1.10	0.80, 1.52	0.56	0.0% (0.85)	0.18-0.62
	Hispanic			113	1904	0.86	1.06	0.80, 1.40	0.70		0.81
	Asian/Pacific Islander			66	886	0.95	0.83	0.53, 1.29	0.41		0.22

Data is either one study estimates or combined estimates from meta-analyses of multiple listed studies.

*Estimates not reported in Asian/Pacific Islanders due to MAF= 0.006. LPL SNP estimates not reported due to low MAF (0.003 in African Americans, 0.007 in Hispanics, 0.0005 in Asian/Pacific Islanders and 0.01 in American Indians).

†Results were unchanged in meta-analysis excluding WHI American Indians.

‡Power was estimated assuming an additive genetic effect, effect estimate size of associations in white individuals, an alpha = 0.05, MAF of the target population, a two-sided test and population risk of 0.10. For rs599839 and rs499818, effect size is not available so we used estimates of 1.20.

Abbreviations: SNP, single nucleotide polymorphism; MAF, minor allele frequency; HR, hazard ratio; CI, confidence interval. For study abbreviations see text.

Table 5

Additive genetic score comprised of eight significant single nucleotide polymorphisms and the risk of incident coronary heart disease in white individuals

Risk score categories	Total number	Events	HR	95% CI	P-value
(0,5)	645	187	1.0		
(6,7)	3,722	1,136	1.15	1.03, 1.28	0.02
(8,9)	5,615	1,806	1.50	1.35, 1.67	1.7×10^{-13}
(10,16)	2,143	757	1.91	1.71, 2.14	2.2×10^{-29}
Continuous	12,125	3,886	1.13	1.12, 1.15	4.4×10^{-77}

The following SNPs (coded allele) were included in the risk score: rs10757278 (G), rs2549513(A), rs499818(A), rs6922269(A), rs429358(C), rs4804611(A), rs501120(T), rs268(G) and rs2943634(C).