Association of Gene Variants With Incident Myocardial Infarction in the Cardiovascular Health Study

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Objective—We asked whether single nucleotide polymorphisms (SNPs) that had been nominally associated with cardiovascular disease in antecedent studies were also associated with cardiovascular disease in a population-based prospective study of 4522 individuals aged 65 or older.

Methods and Results—Based on antecedent studies, we prespecified a risk allele and an inheritance model for each of 74 SNPs. We then tested the association of these SNPs with myocardial infarction (MI) in the Cardiovascular Health Study (CHS). The prespecified risk alleles of 8 SNPs were nominally associated (1-sided $P<0.05$) with increased risk of MI in White CHS participants. The false discovery rate for these 8 was 0.43, suggesting that about 4 of these 8 are likely to be true positives. The 4 of these 8 SNPs that had the strongest evidence for association with cardiovascular disease before testing in CHS (association in 3 antecedent studies) were in *KIF6* (CHS HR=1.29; 90%CI 1.1 to 1.52), *VAMP8* (HR=1.2; 90%CI 1.02 to 1.41), *TAS2R50* (HR=1.13; 90%CI 1 to 1.27), and *LPA* (HR=1.62; 90%CI 1.09 to 2.42).

Conclusions—Although most of the SNPs investigated were not associated with MI in CHS, evidence from this investigation combined with previous studies suggests that 4 of these SNPs are likely associated with MI. (***Arterioscler Thromb Vasc Biol*. 2008;28:173-179.)**

Key Words: coronary disease ■ myocardial infarction ■ genetics ■ polymorphisms

Cardiovascular disease is a complex disease with a genetic component,[1] and many genetic polymorphisms have been reported to be associated with cardiovascular disease.[2] However, to confirm these associations, they should be examined in other populations, ideally in population-based prospective studies that have sufficient power to detect the hypothesized associations. One such population-based prospective study is the Cardiovascular Health Study (CHS), a study of American men and women 65 years and older sponsored by the National Heart, Lung, and Blood Institute.[3,4] CHS offers several strengths, including a large population-based cohort, collection of baseline data for traditional risk factors, long follow-up, and central adjudication of cardiovascular events.

We have been investigating the association between cardiovascular disease and single nucleotide polymorphisms (SNPs) using a panel of ≈12 000 mostly nonsynonymous SNPs.[5–7] The discovery studies for these investigations were conducted in case–control studies that included patients enrolled by investigators at the Cleveland Clinic Foundation (CCF) and the University of California, San Francisco (UCSF)[5–7]; the association between 9 of these SNPs and cardiovascular disease in multiple discovery studies has been previously described.[5–9] We have used these 9 SNPs to build multiplex assays that are suitable for genotyping thousands of samples even when only a limited quantity of DNA is available for each sample. These multiplex assays also contain assays for 65 additional SNPs that were found to be associated with cardiovascular disease in one or more of the discovery studies (for these 65 SNPs, the results of the antecedent discovery studies are presented in the online supplement of this article, available online at http://atvb.ahajournals.org). We investigated whether the risk allele that was identified for each of these 74 SNPs in the antecedent studies would be associated with increased risk of MI in CHS.

Methods

Cardiovascular Health Study

CHS is a prospective observational study of risk factors for cardiovascular disease in older adults. Men and women aged 65 years and

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older were recruited from random samples of Medicare eligibility lists in 4 US communities (Sacramento County, Calif; Washington County, Md; Forsyth County, NC; and Pittsburgh, Pa) and from age-eligible participants in the same household. Potential participants were excluded if they were institutionalized, not ambulatory at home, under hospice care, receiving radiation or chemotherapy for cancer, not expected to remain in the area for at least 3 years, or unable to be interviewed. CHS enrolled 5201 participants in 1989 to 1990; an additional 687 African American participants entered the cohort in 1992 to 1993. The combined cohort of 5888 was 57.6% female and 15.7% African American. The mean age at enrollment was 72.8 years (standard deviation 5.6). Participants who did not donate DNA or who did not consent to the use of their DNA for studies by private companies (n=514) were excluded from the present study. Participants for whom DNA samples were inadequate (n=130) were also excluded. The institutional review board at each site approved the study methods, and all participants gave written informed consent. Details of CHS recruitment[3] and design[4] have been reported.

Participants completed a baseline clinic examination[4] that included a medical history interview, physical examination, and blood draw.[10] Baseline self-reports of MI or stroke were confirmed by information from the clinic examination or by review of medical records or physician questionnaires.[11] Genotypes of the CHS participants were determined using a multiplex method that combines polymerase chain reaction (PCR), allele-specific oligonucleotide ligation assays, and hybridization to oligonucleotides coupled to Luminex 100TM xMAP microspheres (Luminex, Austin, TX). See online supplemental materials for details.

Diabetes mellitus was defined by fasting serum glucose of at least 126 mg/dL or the use of insulin or oral hypoglycemic medications.[12] Impaired fasting glucose was defined as a fasting glucose of 110 to 125 mg/dL. Hypertension was defined by systolic blood pressure of at least 140 mm Hg, diastolic blood pressure of at least 90 mm Hg, or a physician's diagnosis of hypertension plus the use of antihypertensive medications. Body mass index (BMI) was defined as body weight in kilograms divided by the square of height in meters.

Cardiovascular events during follow-up were identified at semiannual contacts, which alternated between clinic visits and telephone calls. Suspected events were adjudicated according to standard criteria by a physician review panel using information from medical records and, in some cases, interviews with the physician, participant, or a proxy informant.[13] Medicare utilization files were searched to ascertain events that may have been missed. In this analysis, MI was defined as definite or probable nonfatal MI or definite fatal MI.

Prespecification of Risk Alleles and Inheritance Models for SNPs Investigated in CHS

For each of the 74 SNPs that were genotyped in CHS we prespecified a risk allele and an inheritance model based on antecedent data (supplemental Table I). For 9 of the 74 SNPs, genotypic association results have been previously published.[5–9] The remaining 65 SNPs were associated with MI in at least 1 of 2 case–control studies described in the online supplementary text (supplemental text and supplemental Table II). An inheritance model for each SNP was prespecified using the following 3 rules: (1) for SNPs that had been previously reported to be associated with cardiovascular disease the inheritance model was based on the published data; (2) for SNPs that were nominally associated ($P<0.1$) in the 2 antecedent MI case–control studies reported in the online supplement (supplemental Table I) and had the same inheritance model in both studies, we used that model; (3) for all other SNPs we used an additive inheritance model. For example, if a SNP had the same risk allele in both studies and was nominally associated with MI ($P<0.1$) using an additive model in one study and using a recessive model in the second study, we used an additive model. We also used an additive model for SNPs that were associated with MI in only one of the two antecedent studies of MI.

Table 1. Baseline Characteristics of CHS Participants in This Study

Characteristic	Whites	African Americans
No. of individuals	3849	673
Male	1575 (41)	243 (36)
Age, mean (SD), y	72.7 (5.6)	72.9 (5.7)
BMI, mean (SD), kg/m^2	26.3 (4.5)	28.5 (5.6)
Smoking, current	423 (11)	113 (17)
Diabetes	511 (13)	151 (23)
Impaired fasting glucose	522 (14)	92 (14)
Hypertension	2110 (55)	490 (73)
LDL cholesterol, mean (SD), mg/dL	130 (36)	129 (36)
HDL cholesterol, mean (SD), mg/dL	54 (16)	58 (15)
Total cholesterol, mean (SD), mg/dL	212 (39)	210 (39)

Data presented as No. of participants (%) unless otherwise indicated.

Statistical Analysis

Analyses excluded participants with a baseline history of MI (n=517 of the 5244 participants with genotype data) or stroke (n=222). Participants who were neither White nor African American were also excluded (n=30). Participant characteristics at baseline were described by counts and percents or means and standard deviations (Table 1).

Hardy–Weinberg equilibrium (HWE) tests were run for each SNP using the "genhw" procedure[14] in Stata[15] with corresponding Pearson chi-square tests; if either homozygote count was 5 or less, an exact test was used.

Because genetic risk factors can have different magnitude in Whites and in African Americans, we investigated the association of SNPs with incident MI in CHS in each race separately.

We conducted analyses of time to incident MI. Follow-up began at CHS enrollment and ended on the date of incident MI, death, loss to follow-up, or June 30, 2003, whichever occurred first. The median time at risk was 11.3 years for incident MI (12.7 years for the 1989 to 90 cohort and 10.1 years for the African American cohort).

Cox regression was used to estimate hazard ratios associated with each SNP in each race. Multivariate analyses were adjusted for baseline age and sex. Additional analyses were also adjusted for traditional risk factors: BMI, current smoking, diabetes or impaired fasting glucose, hypertension, LDL cholesterol, and HDL cholesterol. Because the expected direction of the effect (risk allele) was prespecified, we used a 1-sided probability value to test the significance of the coefficient associated with each SNP. Correspondingly, we estimated 90% confidence intervals for the hazard ratios (for hazard ratios greater than one, there is 95% confidence that a true risk estimate is greater than the lower bound of a 90% confidence interval). Data were analyzed using Stata statistical software.[15]

The expected influence of multiple testing was evaluated using 2 approaches. First, we used false discovery rates (FDR) as described by Benjamini and Hochberg[16] to estimate the expected fraction of false-positives in a group of SNPs with probability values below a given threshold. For the 8 pairs of SNPs that are located in the same gene (rs529038 and rs619203 in *ROS1*; rs11016076 and rs10082504 in *MKI67*; rs3129196 and rs3130210 in *LOC651870*; rs7439293 and rs12510359 in *PALLD;* rs3813135 and rs892145 in *PGLYRP2*; rs428785 and rs402007 in *ADAMTS1*; rs2296436 and rs1804689 in *HPS1*; rs3749817 and rs13183672 in *FSTL4*), we included only the SNP with the higher (less significant) probability value in FDR calculations, which were performed with R statistical software.[17] Second, false-positive report probabilities were calculated as described by Wacholder et al.[18] Because assigning a prior probability is subjective, we used a range of prior probabilities to calculate a range of false-positive report probabilities for each SNP. The assumptions we used to determine the range of prior probabilities are described in the online supplement. The prior probability is directly

proportional to the assumptions: alternative false-positive report probability estimates can be calculated by choosing different prior probability assumptions.

Results

During 13 years of follow-up, 539 (12%) of the 4522 CHS participants in this analysis had an incident MI. We tested 74 SNPs separately in Whites and in African Americans for deviation from the genotype distribution expected under HWE and we found that 8 SNPs (5 in Whites and 3 in African Americans) deviated from HWE expectations ($P<0.05$, supplemental Table III). Had we adjusted the HWE test for multiple testing using a Bonferroni correction, none of the SNPs in African Americans, and only 3 of the SNPs in Whites would have deviated from HWE expectations. In Whites the 5 SNPs that nominally deviated from HWE expectations were rs3027309 in *ALOX12B*, rs11538264 in *BAT2*, rs11758242 in *LY6G5B*, rs402007 in *ADAMTS1*, and rs35690712 in *SLC39A7*. In African Americans the 3 SNPs were rs220479 in *ITGAE*, rs1804689 in *HPS1*, and rs3813135 in *PGLYRP2*. Because none of these SNPs deviated from HWE expectations in both Whites and African Americans, this deviation is unlikely to be attributable to genotyping error. Therefore we included all 74 SNPs in the analysis. Table 2 lists all 74 SNPs and the genes in which they are located.

In Whites, 8 SNPs in 7 genes were nominally associated ($P<0.05$) with incident MI after adjustment for age and sex (Table 3). The associations between all 74 SNPs and MI in Whites are available in the supplemental Table IV. The 8 nominally associated SNPs were in *KIF6*, *PGLYRP2* (2 SNPs), *LPA*, *MCM10, VAMP8*, *DCC,* and *TAS2R50*. We estimated the FDR for these 8 SNPs to be 0.43, indicating that about 4 of these SNPs are expected to be false-positives. When we considered the evidence for association with cardiovascular disease before testing in CHS, 4 of these 8 SNPs were among those with the strongest prior evidence (association in 3 studies after adjustment for multiple testing; supplemental Table I). The false-positive report probabilities for these 4 SNPs were all ≤0.01 (*KIF6* [0.0005; range 0.0005 to 0.08], *VAMP8* [0.005; range 0.002 to 0.31], *TAS2R50* [0.005; range 0.003 to 0.33], and *LPA* [0.01; range 0.01 to 0.66]), suggesting that they are unlikely to be false-positives. In contrast, 2 of the SNPs (in *MCM10* and *DCC*) had high false-positive report probability (> 0.9) indicating that they are likely to be false-positives, and the remaining 2 SNPs (both in *PGLYRP2*) had false-positive report probabilities that were intermediate (0.3). Adjustment for traditional risk factors did not appreciably change the risk estimates for these 8 SNPs although the association of the SNP in *LPA* was no longer nominally significant ($P=0.069$). Because we had previously observed that this *LPA* SNP was associated with plasma Lp(a) levels,[7] we investigated the association of the *LPA* SNP with Lp(a) and found that carriers of the risk allele had a higher median level of Lp(a) (63 mg/dL) than non carriers (42 mg/dL, $P<0.00005$). However, for the MI end point, adjustment of the risk estimate of the *LPA* SNP to account for Lp(a) levels did not appreciably change the hazard ratio (HR=1.64, 90%CI; 1.10 to 2.45).

In African Americans, 3 SNPs were nominally associated with incident MI after adjustment for age, sex, and traditional risk factors ($P<0.05$, Table 4). The association between all 74 SNPs and MI in African Americans are available in supplemental Table V. One of these 3 SNPs (rs2213948) is located in an intergenic region; the other 2 SNPs are located in *AQP10* and *FCAR*. This risk allele of the SNP in *FCAR* had been previously reported to be associated with increased risk of cardiovascular disease in the placebo arms of CARE and WOSCOPS.[8] The estimated FDR for this set of 3 SNPs was 0.67. For the SNPs in *VAMP8* and *KIF6*, which had the lowest false-positive report probabilities in White participants of CHS, the risk estimates in African Americans were high (1.71 [CI 0.92 to 3.19] for *VAMP8* and 4.14 [CI 0.79 to 21.77] for *KIF6*) but did not reach statistical significance ($P=0.08$ for both).

Discussion

We investigated the association between MI and 74 SNPs in CHS and found that 8 SNPs were nominally associated ($P<0.05$) with MI among White participants of CHS. The false discovery rate for these 8 SNPs was 0.43, suggesting that about 4 of these 8 are truly associated with MI.

Of these 8 SNPs, 4 had strong evidence for association with cardiovascular disease prior to testing in CHS. These 4 SNPs are located in *KIF6, TAS2R50*, *VAMP8*, and *LPA*. The strongest prior evidence for association with cardiovascular disease was for the SNPs in *KIF6* and *VAMP8*. The SNP in *KIF6* had been previously found to be associated with cardiovascular disease in the placebo arms of 2 statin trials, and the association remained significant after a Bonferroni correction for multiple testing.[9] The SNP in *VAMP8* had been found to be associated with MI in 3 case–control studies, with an FDR <0.1 in the third study.[6] The risk alleles of these 2 SNPs have also been found to be associated with increased risk of coronary heart disease in the Atherosclerosis Risk in Communities (ARIC) study,[19,20] a large population based prospective study of middle-aged Americans. Thus the SNPs in *KIF6* and *VAMP8* had been found to be consistently associated with cardiovascular disease prior to testing in CHS, and the associations found in CHS further strengthen the evidence for these associations. Furthermore, in African American participants of CHS, the risk estimates for the SNPs in *VAMP8* and *KIF6* were high (1.71 for *VAMP8* and 4.14 for *KIF6*), although they did not reach statistical significance ($P=0.08$ for both). However, there was a smaller number of African American participants in this study (673 African Americans compared with 3849 Whites), and consequently, the power to detect association was lower among African Americans than among Whites, which could partially account for the lack of statistical significance of these risk estimates.

The SNPs in TAS2R50 and LPA were not associated with MI among African American participants of CHS. However, there are considerable differences in the LD structure of the *LPA* and *TAS2R50* regions between Yoruba in Ibadan and CEPH (Utah residents with ancestry from Northern and Western Europe) populations.[21] Thus, different SNPs in these 2 genes should be explored in African American populations

Table 2. SNP2 Tested in CHS

Gene Symbol	dbSNP ID	Description
ABCG2	rs2231137	ATP-binding cassette, subfamily G (WHITE), member 2
ADAMTS1	rs428785	ADAM metallopeptidase with thrombospondin type 1 motif, 1
ADAMTS1	rs402007	ADAM metallopeptidase with thrombospondin type 1 motif, 1
ALOX12B	rs3027309	arachidonate 12-lipoxygenase, 12R type
AP3B1	rs6453373	adaptor-related protein complex 3, beta 1 subunit
AQP10	rs6685323	aquaporin 10
BAT2	rs11538264	HLA-B associated transcript 2
CALM1	rs3814843	calmodulin 1 (phosphorylase kinase, delta)
COG2	rs1051038	component of oligomeric golgi complex 2
CYBRD1	rs10455	cytochrome b reductase 1
CYP17A1	rs2486758	cytochrome P450, family 17, subfamily A, polypeptide 1
CYP2C8	rs10509681	cytochrome P450, family 2, subfamily C, polypeptide 8
DCC	rs1675225	deleted in colorectal carcinoma
EDG1	rs2038366	endothelial differentiation, sphingolipid G-protein-coupled receptor, 1
EIF2AK2	rs2307469	eukaryotic translation initiation factor 2-alpha kinase 2
F13A1	rs5985	coagulation factor XIII, A1 polypeptide
FABP2	rs1799883	fatty acid binding protein 2, intestinal
FCAR	rs11666735	Fc fragment of IgA, receptor for
FCRLM2	rs34868416	Fc receptor-like and mucin-like 2 isoform a
FSTL4	rs3749817	follistatin-like 4
FSTL4	rs13183672	follistatin-like 4
GJA4	rs1764391	gap junction protein, alpha 4, 37kDa
GRM8	rs3808117	glutamate receptor, metabotropic 8
HPS1	rs2296436	Hermansky-Pudlak syndrome 1
HPS1	rs1804689	Hermansky-Pudlak syndrome 1
IL1F10	rs6761276	interleukin 1 family, member 10 (theta)
IL1F5	rs2515401	interleukin 1 family, member 5 (delta)
ITGAE	rs220479	integrin, alpha E (antigen CD103)
K6IRS4	rs592720	keratin 74
KIAA1414	chr2:37081301	hypothetical protein LOC54497
KIF6	rs20455	kinesin family member 6
KRT5	rs89962	keratin 5
LGALS14	rs35541195	lectin, galactoside-binding, soluble, 14
LOC391102	rs943133	similar to 60S acidic ribosomal protein P0 (L10E)
LOC651870	rs3130210	similar to HLA class II histocompatibility antigen
LOC651870	rs3129196	similar to HLA class II histocompatibility antigen
LPA	rs3798220	lipoprotein, Lp(a)
LY6G5B	rs11758242	lymphocyte antigen 6 complex, locus G5B
MCM10	rs7905784	minichromosome maintenance complex component 10
MKI67	rs10082504	antigen identified by monoclonal antibody Ki-67
MKI67	rs11016076	antigen identified by monoclonal antibody Ki-67
MLF1	rs4875	myeloid leukemia factor 1
MYH15	rs3900940	myosin, heavy chain 15
MYOM3	rs12145360	myomesin family, member 3
None	rs2477037	
None	rs2213948	
OR13G1	rs1151640	olfactory receptor, family 13, subfamily G, member 1
OR2A25	rs2961135	olfactory receptor, family 2, subfamily A, member 25

(*Continued*)

Table 2. Continued

Gene Symbol	dbSNP ID	Description
P2RXL1	rs2277838	purinergic receptor P2X-like 1, orphan receptor
PALLD	rs12510359	palladin, cytoskeletal associated protein
PALLD	rs7439293	palladin, cytoskeletal associated protein
PGLYRP2	rs3813135	peptidoglycan recognition protein 2
PGLYRP2	rs892145	peptidoglycan recognition protein 2
PON1	rs662	paraoxonase 1
PRKG1	rs211070	protein kinase, cGMP-dependent, type I
DMXL2	rs12102203	Dmx-like 2
ROS1	rs619203	v-ros UR2 sarcoma virus oncogene homolog 1 (avian)
ROS1	rs529038	v-ros UR2 sarcoma virus oncogene homolog 1 (avian)
SERPINA9	rs17090921	serpin peptidase inhibitor, clade A (antitrypsin), member 9
SERPINB8	rs1944270	serpin peptidase inhibitor, clade B (ovalbumin), member 8
SGIP1	rs1325268	SH3-domain GRB2-like (endophilin) interacting protein 1
SLC26A8	rs2295852	solute carrier family 26, member 8
SLC39A7	rs35690712	solute carrier family 39 (zinc transporter), member 7
SNX19	rs2298566	sorting nexin 19
STRN	rs11685600	striatin, calmodulin binding protein
TAF3	rs4747647	TAF3 RNA polymerase II
TAS2R50	rs1376251	taste receptor, type 2, member 50
TMPRSS11B	rs12331141	transmembrane protease, serine 11B
TOX	rs2290526	thymocyte selection-associated high mobility group box
VAMP8	rs1010	vesicle-associated membrane protein 8 (endobrevin)
VTI1A	rs11814680	vesicle transport through interaction with t-SNAREs homolog 1A
WDR31	rs10817479	WD repeat domain 31
WDR55	rs2286394	WD repeat domain 55
ZNF132	rs1122955	zinc finger protein 132

to test whether other variants of these genes are associated with MI in this population.

For the SNP in *LPA*, the prior evidence was association with coronary stenosis in 3 case–control studies, association that remained significant after a Bonferroni correction for multiple testing in the third study.[7] For the SNP in *TAS2R50*, the prior evidence was association with MI in 3 case–control studies, with a false discovery rate of <0.1 in the third study.[5] However, these 2 SNPs (in *LPA* and *TAS2R50*) were not associated with coronary heart disease in ARIC.[19]

Table 3. SNPs Nominally Associated (*P*<0.05) With Incident MI in the White Participants of CHS

		Adjusted for Age and Sex				Fully Adjusted*	
Gene (SNP)	Prespecifed Model	HR (90% CI)	*P*	FDR†	FPRP† (range)	HR (90% CI)	*P*
KIF6 (rs20455)	Dom	1.29 (1.1–1.52)	0.004	0.20	0.0005 (0.0005–0.08)	1.29 (1.1–1.52)	0.005
PGLYRP2 (rs3813135)	Dom	1.28 (1.09–1.5)	0.006	0.20	0.28 (0.03–0.80)	1.28 (1.09–1.51)	0.006
PGLYRP2 (rs892145)	Dom	1.27 (1.09–1.49)	0.006	NA§	0.27 (0.03–0.80)	1.27 (1.08–1.49)	0.007
LPA (rs3798220)	Add	1.62 (1.09–2.42)	0.022	0.40	0.01 (0.01–0.66)	1.46 (0.96–2.24)	0.069
MCM10 (rs7905784)	Add	1.19 (1.02–1.37)	0.028	0.40	0.92 (0.53–0.99)	1.16 (1–1.35)	0.048
VAMP8 (rs1010)	Dom	1.2 (1.02–1.41)	0.032	0.40	0.005 (0.002–0.31)	1.21 (1.03–1.42)	0.029
DCC (rs1675225)	Add	1.22 (1.02–1.45)	0.036	0.40	0.95 (0.64–0.99)	1.24 (1.03–1.48)	0.026
TAS2R50 (rs1376251)	Add	1.13 (1–1.27)	0.046	0.43	0.005 (0.003–0.33)	1.14 (1.01–1.28)	0.038

Hazard ratios and P values were calculated using an additive inheritance model unless indicated otherwise. 1-sided *P* values for the HR using the prespecified risk allele.

*Adjusted for baseline age (continuous), sex, BMI (continuous), current smoking, diabetes or impaired fasting glucose, hypertension, LDL cholesterol (continuous), and HDL cholesterol (continuous).

†False discovery rate.

‡False positive report probability.

§For pairs of SNPs in the same gene, false discovery rate was calculated for the SNP with the higher (less significant) *P* value.

Table 4. 10 SNPs With Lowest *P* Values for Association With Incident MI in the African American Participants of CHS

		Adjusted for Age and Sex			Fully Adjusted*	
Gene (SNP)	Prespecified Model	HR (90% CI)	*P*	FDR†	HR (90% CI)	*P*
FCAR (rs11666735)	Dom	2.08 (1.23–3.53)	0.01	0.67	2.21 (1.29–3.79)	0.008
None (rs2213948)	Add	2.38 (1.04–5.43)	0.042	0.67	20.51 (1.08–50.82)	0.036
AQP10 (rs6685323)	Add	1.35 (1–1.82)	0.048	0.67	1.4 (1.03–1.91)	0.034
PALLD (rs12510359)	Rec	1.78 (0.98–3.22)	0.055	NA§	1.3 (0.67–20.54)	0.26
GJA4 (rs1764391)	Add	1.29 (0.97–1.71)	0.074	0.67	1.23 (0.91–1.65)	0.13
VAMP8 (rs1010)	Dom	1.71 (0.92–3.19)	0.078	0.67	1.81 (0.93–3.52)	0.07
TMPRSS11B (rs12331141)	Add	1.29 (0.96–1.72)	0.078	0.67	1.31 (0.97–1.77)	0.069
KIF6 (rs20455)	Dom	4.14 (0.79–21.77)	0.08	0.67	NA‡	
VTI1A (rs11814680)	Add	1.29 (0.95–1.73)	0.083	0.67	1.27 (0.93–1.73)	0.10
DCC (rs1675225)	Add	3.82 (0.73–20.1)	0.092	0.67	3.81 (0.72–20.2)	0.09

Hazard ratios and *P* values were calculated using an additive inheritance model unless indicated otherwise. 1-sided *P* values for the HR using the prespecified risk allele.

*Adjusted for baseline age (continuous), sex, BMI (continuous), current smoking, diabetes, or impaired fasting glucose, hypertension, LDL cholesterol (continuous), and HDL cholesterol (continuous).

†False discovery rate.

‡HR could not be estimated because there were no incident events in either the risk genotype or nonrisk genotype groups.

§For pairs of SNPs in the same gene, false discovery rate was calculated for the SNP with the higher (less significant) *P* value.

Because we tested 74 SNPs in CHS, we were concerned that multiple testing may have resulted in false-positive associations. To reduce the extent of multiple testing, we prespecified the risk allele and inheritance model for each SNP based on antecedent studies. Thus we tested a single hypothesis for each SNP. We used 2 different approaches to evaluate the extent to which multiple testing resulted in false-positives. The first method, FDR, is a frequentist approach that estimates the expected fraction of false-positives in a group of SNPs with probability values below a certain threshold.[16] The FDR is computed from the nominal probability values and the number of independent tests. The group of 8 SNPs that were nominally associated ($P<0.05$) with MI in White participants of CHS had an FDR of 0.43, suggesting that about 4 of these SNPs are expected to be false-positives. However, none of the SNPs we tested in the white participants of CHS had an FDR lower than 0.1.

The second method we used to account for multiple testing was a Bayesian approach—false-positive report probability—that takes into consideration not only the observed probability value but also the power of the study to detect association and the prior probability of the SNP being associated with disease.[18] We found that the false-positive report probabilities for the SNPs in *KIF6*, *VAMP8*, *LPA*, and *TAS2R50* were all ≤ 0.01, suggesting that these 4 SNPs are unlikely to be false-positives. For the SNP in *KIF6*, even the high-end of the false-positive report probability range (0.08) suggests a low probability of being a false-positive. However, the high-end of the false-positive report probability range of the SNPs in *VAMP8* (0.23), *TAS2R50* (0.31), and *LPA* (0.66) indicated an intermediate probability of being false-positives when the more conservative end of the prior-probability range was used to estimate the false-positive report probability.

We have previously discussed the potential role *LPA*, *VAMP8*, *TAS2R50*, and *KIF6* in cardiovascular disease,[5–7,9] however the mechanisms by which the variants of these genes influence the pathophysiology of disease is unknown. Briefly, the SNP in *LPA* encodes the apolipoprotein(a) protein portion of the Lp(a) particle, a known risk factor for cardiovascular disease.[22,23] We had previously reported that this SNP in *LPA* was associated with increased plasma levels of Lp(a).[7] We have now confirmed this finding in CHS Whites. We also found that in CHS, the risk associated with this *LPA* SNP remains unchanged after adjustment for Lp(a) levels. The protein encoded by the *VAMP8* gene plays a role in platelet degranulation.[24] The *TAS2R50* gene is a bitter taste receptor, and thus might be involved in food preference and diet.[25] *KIF6* encodes a member of the kinesin superfamily that plays a role in microtubule-mediated intracellular transport; however, its potential role in cardiovascular disease is unknown.

This study has several limitations. The antecedent studies that provided the prior evidence for the 74 SNPs were case–control studies, which might have resulted in selection and survival bias. Furthermore, because DNA limitations required the use of multiplexed assays for genotyping the CHS subjects, not all SNPs that were associated with disease in the antecedent studies were tested in CHS and some of the SNP included in the multiplexed assays had only been associated with cardiovascular disease in a single antecedent study. In this genetic study of CHS, we have not formally tested for population stratification, which could confound genetic association studies. However, because none of the 4 SNPs that are most likely to be true positives deviated from HWE expectations, these associations are unlikely to be confounded by population stratification. Additionally, participants in CHS were older than 65 at baseline (median 72 years); therefore, because cardiovascular disease heritability decreases with age,[1] it may be more difficult to identify genetic associations in this population. Furthermore, in this older population gene variants might be associated with MI because they affect disease pathways that are particularly important in older individuals.

In summary, we found that 4 gene variants that have strong prior evidence for their association with cardiovascular disease were also associated with incident MI in CHS. This study suggests that even in older adults, genetic variation may affect cardiovascular risk.

Acknowledgments

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Disclosures

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Arteriosclerosis, Thrombosis, and Vascular Biology

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Association of Gene Variants With Incident Myocardial Infarction in the Cardiovascular Health Study

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Contents

Online Only Supplementary Text: Genotype Determination

DNA isolation

DNA was isolated from peripheral blood leukocytes collected at baseline and stored as buffy coats at -80°C using a standard salt precipitation method[1]. In addition, some DNA was isolated using the Gentra Puregene DNA purification kit (Gentra Systems 13355 10th Ave North, Suite 120 Minneapolis, Minnesota 55441) from peripheral blood leukocytes collected at follow up examinations and stored as buffy coats at -80°C. DNA stock solutions were stored at -80°C.

Multiplex Genotyping

Genotypes of the CHS participants were determined using a genotyping method that included the following steps: multiplex PCR, allele-specific oligonucleotide ligation assays (OLA), hybridization to conjugated Luminex®100TM xMAP microspheres (Luminex, Austin, TX, USA), labeling with streptavidin-R-phycoerythrin, and detection on the Luminex®100TM flow cytometer instrument (Luminex, Austin, TX, USA).

Multiplex PCR

For each DNA sample, genomic regions (100bp to 150bp long) containing the target SNPs were simultaneously amplified using multiplex PCR[2]. 15μL PCR reactions were assembled using the following reagents: 2.5μL of pooled (1.2μM each) PCR primers (all oligonucleotides were obtained from Integrated DNA Technologies, Coralville, IA, USA), 1.5μL of 10X PCR buffer (150mM Tris-HCl pH 8.25; 500mM KCl; 40mM $MgCl_2$; 1mM dATP, dCTP, and dGTP; 0.5mM dTTP; 3mM dUTP; and 0.2% Triton X-

100), 0.5 units of AmpErase™ UNG (Applied Biosystems, Foster City, CA, USA), 5 units of Amplitaq Gold™ (Applied Biosystems, Foster City, CA, USA), and 3ng of genomic DNA. Thermalcycling was performed in a GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, CA, USA) as follows: first, incubation for 2 min at 50°C; second, incubation for 12 min at 95°C; third, 28 cycles (ramp setting 9600) of 15s at 95°C, 15s at 58°C, and 45s at 72°C; fourth, incubation for 7 min at 72°C; fifth, incubation for 30 min at 99°C and finally storage at 4°C.

Allele-specific oligonucleotide ligation

SNP alleles in each of the amplified regions were detected using multiplex OLA[3]. OLA reactions were assembled as follows: to each 15µl of multiplex PCR reaction from the previous step we added 5µL of OLA reaction mix, which contained 1µL of pooled (0.1µM each) allele-specific oligonucleotides (2 for each SNP), 1µL of pooled (0.2µM-0.8µM each) 3 prime biotinylated oligonucleotides that were to be ligated to the allele-specific oligonucleotides (1 for each SNP), 2µL of 10X OLA buffer (150mM Tris pH 6.7, 100mM $MgCl_2$, 750mM KCl, 100mM DTT, 10mM NAD, 1% Triton X-100), and 20 units of r*Tth* thermostable DNA ligase (Abbott Labs, Abbott Park, IL, USA). Thermalcycling was performed in a GeneAmp® PCR System 9700 as follows: 24 cycles (ramp setting 9600) of 5s at 95.5°C and 2 minutes at 50°C. After thermocycling, the reaction was incubated for 10 min at 99°C and finally stored at 4°C.

Hybridization to conjugated Luminex®100TM xMAP microspheres

The 5 prime end of each allele-specific oligonucleotide was designed to have a unique sequence that was complementary to one of 100 distinct types of capture oligonucleotides. Capture oligonucleotides were conjugated[4] to microspheres, such that each class of 100 spectrally distinct Luminex®100TM xMAP microsphere classes[5] was conjugated to one type of distinct capture oligonucleotides. The ligation products from the OLA step were hybridized with these conjugated Luminex®100TM xMAP microspheres by adding a 40μL of hybridization solution to the 20μL OLA reaction from the previous step. This 40μL of hybridization solution contained 1000 microspheres of each of the 100 different conjugated microsphere classes (a total of 100,000 microspheres) in hybridization buffer (20mM Tris-HCl pH 8, 20mM $MgCl_2$, 300mM NaCl, 0.1% Tween 20 with 0.09% (w/v) NaN_3). The hybridization reaction was carried out by incubation for 30 minutes at 55°C.

Labeling with streptavidin R-phycoerythrin

The ligation products that had been specifically captured in the previous hybridization step were fluorescently labeled by adding to the hybridization reaction from the previous step a 15μL solution containing 1μg PhycoLink® streptavidin-R-phycoerythrin (Prozyme, San Leandro, CA, USA), 10mM Tris-HCl pH 8, 10mM $MgCl_2$, 150mM NaCl, 0.05% Tween 20 with 0.09% (w/v) NaN_3 and incubating for 15 minutes at 45°C. The reaction was then stored at room temperature for up to 8 hours before data collection.

Detection on the Luminex®100TM flow cytometer instrument

Genotyping data was collected using the Luminex®100TM instrument[5] at 45°C. The Luminex®100TM instrument sampled 50μL per reaction well and analyzed a minimum of 100 microspheres of each microsphere class in the sample, and the median fluorescent intensity was reported.

Genotyping Accuracy

Genotyping accuracy of this methodology has been assessed in three previous studies by comparing genotype calls from the multiplex OLA assays to those from real time kinetic PCR assays for the same SNPs, and the overall concordance of the genotype calls from these two methods was >99% in each of these studies[6-8].

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Online Only Supplementary Text: Antecedent studies

The patient characteristics of the 2 case–control studies of MI are summarized in online Data Supplement Table II. One study of MI comprised 793 cases and 1000 controls that were enrolled between July 1989 and May 2005 by UCSF Genomic Resource in Arteriosclerosis. Participants included patients who underwent diagnostic or interventional cardiac catheterization, patients of the UCSF Lipid Clinic and healthy individuals. Cases had a history of MI. Controls had no history of MI, diabetes or symptomatic vascular disease. The other study of MI comprised 475 cases and 619 controls that were patients of CCF Heart Center and had undergone diagnostic or interventional cardiac catheterization between July 2001 and March 2003. Cases had a history of MI. Controls had no history of MI, and had less than 50% coronary luminal narrowing based on clinical angiography. This CCF study is similar to a study that was previously described (Study-2 in Shiffman et al.[1]).

All subjects in the antecedent studies were self–described non-Hispanic whites who had completed an Institutional Review Board approved questionnaire and given informed consent to participate in genetic studies.

The 65 SNPs with evidence for association in these 2 case–control studies were selected from a set of 16,339 SNPs that had been tested for association with MI.

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Online Only Supplementary Text: Prespecification of prior probabilities for SNPs investigated in CHS

The range of prior probabilities for the SNPs in *VAMP8*, *ROS1* (2 SNPs), TAS2R50, *OR13G1*, and *PALLD* (rs12510359) was based on previously published false discovery rates. These SNPs were associated with MI in 3 case–control studies, with a false discovery rate of <0.1 in the third study.[1, 2] Therefore, since the false discovery rate is <0.1, we used prior probabilities that ranged from 0.09 to 0.9. The *KIF6* SNP (rs20455) was associated with CHD in the placebo arms of two CHD prevention trials and the association remained significant after a Bonferroni correction for multiple testing.[3] Therefore, since the significance threshold was 0.05, we used prior probabilities that ranged from 0.095 to 0.95. Similarly, the SNP in *LPA* gene (rs3798220) was assigned a prior probability range of 0.095 to 0.95 because it was associated with CHD in three case–control studies and remained significant after a Bonferroni correction for multiple testing in the third study.[4] The remaining 65 SNPs fall into 3 categories corresponding to the number of studies in which they were found to be associated with CHD. For the SNPs in these 3 categories we assigned a range of prior probabilities based on point estimates of 0.003, 0.03 and 0.3 for SNPs that were found to be associated in 1, 2 or 3 studies respectively (counting the 2 antecedent MI case–control studies described here, and ARIC,[5] provided the same risk allele was found in all studies; none of these studies adjusted for multiple hypothesis testing). We arrived at this point estimate by assuming that 1 out of 3,000 functional SNPs (mainly non-synonymous in these studies) confers a measurable risk of CHD and that each of the studies resulted in a 10 fold enrichment of

true positive SNPs. We then assigned a range that extended ten fold higher and ten fold lower from this point estimate.

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Online Supplement Table I. Prior evidence for 74 SNPs tested in CHS

Gene	SNP	Prior evidence*	Prior model	Risk allele	Reference
Previously published Data					
KIF6	rs20455	A	dom	G	*J Amer Coll Cardiol.* 2007; in press.
LPA	rs3798220	A	add	C	*Arterioscler Thromb Vasc Biol.* 2007; online prior to publication.
OR13G1	rs1151640	A	dom	C	*Am J Hum Genet.* 2005;77:596-605
PALLD	rs12510359	A	rec	G	*Am J Hum Genet.* 2005;77:596-605
ROS1	rs619203	A	add	C	*Am J Hum Genet.* 2005;77:596-605
ROS1	rs529038	A	add	T	*Am J Hum Genet.* 2005;77:596-605
TAS2R50	rs1376251	A	add	C	*Am J Hum Genet.* 2005;77:596-605
VAMP8	rs1010	A	dom	C	*Arterioscler Thromb Vasc Biol.* 2006;26:1613-1618
FCAR	rs11666735	C	dom	A	*Arterioscler Thromb Vasc Biol.* 2006;26:2763-2768

					CCF				UCSF			
Gene	**SNP**	**Prior evidence***	**Prior model**	**Risk allele**	**OR**	***P***	**Strata**	**Model**	**OR**	***P***	**Strata**	**Model**
MYH15	rs3900940	B	rec	C	2.08	0.029	F	rec	1.71	0.03	F	rec
PALLD	rs7439293	B	add	A	1.21	0.042	ALL	add	1.22	0.006	ALL	add
SNX19	rs2298566	B	rec	C	1.24	0.093	ALL	rec	1.21	0.05	ALL	rec
ALOX12B	rs3027309	C	add	T	1.27	0.035	ALL	add	1.18	0.068	ALL	add
AP3B1	rs6453373	C	add	T	1.54	0.029	ALL	add	1.26	0.094	ALL	add
AQP10	rs6685323	C	add	T	1.33	0.003	ALL	add	1.19	0.02	ALL	add
BAT2	rs11538264	C	rec	G	2.92	0.061	F	rec	1.87	0.055	F	rec
CALM1	rs3814843	C	add	G	1.65	0.018	ALL	add	1.97	0.01	M	add
COG2	rs1051038	C	add	A	1.23	0.063	ALL	add	1.19	0.088	ALL	rec
CYBRD1	rs10455	C	add	A	1.18	0.086	ALL	add	1.13	0.079	ALL	add
CYP17A1	rs2486758	C	add	C	1.37	0.02	M	add	1.25	0.067	M	add
CYP2C8	rs10509681	C	rec	T	1.31	0.091	ALL	rec	1.37	0.09	F	rec

DMXL2	rs12102203	C	add	G	1.38	<0.001	ALL	add	1.18	0.014	ALL	add
EDG1	rs2038366	C	add	G	1.19	0.061	ALL	add	1.44	0.061	M	dom
EIF2AK2	rs2307469	C	add	C	1.48	0.086	ALL	add	1.71	0.006	ALL	add
F13A1	rs5985	C	add	A	1.23	0.04	ALL	add	1.21	0.013	ALL	add
FCRLM2	rs34868416	C	add	A	1.37	0.043	ALL	add	1.32	0.024	ALL	add
GRM8	rs3808117	C	add	T	1.58	<0.001	ALL	add	1.21	0.026	ALL	add
HPS1	rs2296436	C	add	T	1.36	0.054	ALL	add	1.32	0.095	M	add
HPS1	rs1804689	C	dom	T	1.26	0.065	ALL	dom	1.25	0.02	ALL	dom
IL1F10	rs6761276	C	add	T	1.25	0.021	ALL	add	1.23	0.004	ALL	add
IL1F5	rs2515401	C	add	T	1.26	0.012	ALL	add	1.13	0.077	ALL	add
ITGAE	rs220479	C	add	C	1.36	0.036	M	add	1.56	0.092	ALL	dom
KIAA1414	chr2:37081301	C	add	G	1.54	0.019	ALL	add	1.59	0.002	ALL	add
LGALS14	rs35541195	C	add	T	1.7	0.018	F	add	1.78	<0.001	F	add
LOC391102	rs943133	C	add	A	1.24	0.03	ALL	add	1.19	0.023	ALL	add
LOC651870	rs3130210	C	add	T	1.24	0.037	ALL	add	1.29	0.002	ALL	add
LOC651870	rs3129196	C	add	A	1.25	0.035	ALL	add	1.29	0.002	ALL	add
LY6G5B	rs11758242	C	rec	C	1.69	0.083	ALL	rec	1.51	0.034	ALL	rec
MKI67	rs10082504	C	add	C	1.38	0.034	ALL	add	1.26	0.043	ALL	add
MYOM3	rs12145360	C	add	A	1.36	0.069	M	add	1.59	0.001	M	add
None	rs2477037	C	add	G	1.31	0.021	M	add	1.31	0.006	M	add
None	rs2213948	C	add	G	1.61	0.019	F	add	1.43	0.013	F	add
P2RXL1	rs2277838	C	add	A	1.27	0.032	ALL	add	1.21	0.032	ALL	add
PGLYRP2	rs3813135	C	dom	C	1.25	0.095	ALL	dom	1.27	0.02	ALL	dom
PGLYRP2	rs892145	C	dom	T	1.29	0.048	ALL	dom	1.27	0.016	ALL	dom
PRKG1	rs211070	C	add	G	1.71	0.029	ALL	add	1.42	0.034	ALL	add
SERPINA9	rs17090921	C	rec	A	2.31	0.02	F	rec	1.5	0.087	F	rec
SLC39A7	rs35690712	C	add	G	1.55	0.06	ALL	add	1.42	0.024	ALL	add
STRN	rs11685600	C	add	C	1.78	0.014	ALL	add	1.39	0.061	ALL	add
TAF3	rs4747647	C	add	A	1.22	0.033	ALL	add	1.15	0.056	ALL	add
VTI1A	rs11814680	C	add	A	1.37	0.043	M	add	1.26	0.018	ALL	add
WDR31	rs10817479	C	add	G	1.44	0.068	ALL	add	1.39	0.016	ALL	add

WDR55	rs2286394	C	add	T	1.24	0.037	ALL	add	1.18	0.044	ALL	add
ABCG2	rs2231137	D	add	C	1.6	0.041	ALL	add	1.23	0.205	ALL	Add
ADAMTS1	rs428785	D	add	C	0.96	0.623	M	dom	1.28	0.096	M	Dom
ADAMTS1	rs402007	D	add	C	1.59	0.499	M	dom	1.29	0.083	M	Dom
DCC	rs1675225	D	add	C	0.78	0.763	ALL	dom	2.54	0.07	ALL	dom
FABP2	rs1799883	D	add	T	1.23	0.041	ALL	add	1.08	0.318	ALL	add
FSTL4	rs3749817	D	add	G	2.71	0.084	F	dom	1.1	0.68	ALL	dom
FSTL4	rs13183672	D	add	A	1.19	0.088	ALL	add	1.1	0.24	ALL	add
GJA4	rs1764391	D	add	C	1.21	0.056	ALL	add	1.05	0.499	ALL	add
K6IRS4	rs592720	D	add	G	0.65	0.953	ALL	dom	1.42	0.074	ALL	dom
KRT5	rs89962	D	add	T	1.2	0.04	ALL	add	1.03	0.629	ALL	add
MCM10	rs7905784	D	add	T	1.25	0.079	ALL	add	1.03	0.746	ALL	add
MKI67	rs11016076	D	add	C	1.2	0.111	ALL	add	1.17	0.087	ALL	add
MLF1	rs4875	D	add	C	1.19	0.051	ALL	add	1.07	0.291	ALL	add
OR2A25	rs2961135	D	add	G	1.37	0.03	ALL	dom	1.14	0.238	ALL	dom
PON1	rs662	D	add	C	1.33	0.078	F	add	0.83	0.96	F	add
SERPINB8	rs1944270	D	add	A	1.24	0.355	ALL	rec	1.59	0.009	ALL	rec
SGIP1	rs1325268	D	add	T	1.08	0.425	ALL	add	1.15	0.056	ALL	add
SLC26A8	rs2295852	D	add	C	1.1	0.284	ALL	add	1.15	0.051	ALL	add
TMPRSS11B	rs12331141	D	add	T	0.97	0.598	ALL	add	1.23	0.019	ALL	add
TOX	rs2290526	D	add	G	1.15	0.294	ALL	add	1.23	0.069	ALL	add
ZNF132	rs1122955	D	add	C	1.17	0.148	ALL	add	1.21	0.026	ALL	add

* A – published data in a manuscript that corrected for multiple testing; B – evidence from 2 MI studies reported in this table and from ARIC (Morrison et al. *Am J Epidemiol.* 2007;166:28-35); C – evidence from 2 studies; D – evidence from only one of two studies reported in this table.

Online Data Supplement Table II. Distribution of Traditional Risk Factors in Two Antecedent Case–Control Studies of MI

Data presented as percent of participants unless otherwise indicated.

	UCSF		CCF	
	Cases (n=793)	**Controls (n=1000)**	**Cases (n=475)**	**Controls (n=619)**
Male	61	43	61	62
Age at enrollment, median (range) ,y	62 (29–86)	70 (24–100)	60 (32–86)	58 (37–88)
Age at MI, median (range) ,y	52 (27–76)	NA	53 (29–77)*	NA
Smoking	64	45	73	54
Diabetes	20	0†	38	10
Dyslipidemia‡	84	53	95	56
Hypertension§	60	34	96	78
BMI mean ±SD, kg/m^2	28±5	26±4	31±6	30±7

Study participants were recruited at the University of California at San Francisco (UCSF) or the Cleveland Clinic Foundation (CCF).

NA indicates not applicable.

*Data available for 254 cases.

†Individuals with diabetes were excluded from control group.

‡Dyslipidemia was defined in CCF by the use of lipid lowering prescription drug(s), LDL cholesterol >129mg/dL, triglyceride >149 mg/dL or HDL cholesterol <45mg/dL and defined in UCSF by a self-reported history of a physician diagnosis of dyslipidemia or the use of lipid lowering prescription drugs.

§Hypertension was defined in CCF as the use of antihypertensive prescription drug(s), systolic blood pressure >160 mmHg, or diastolic blood pressure >90 mmHg and was defined in UCSF by a self–reported history of a physician diagnosis of hypertension or use of antihypertensive prescription drugs.

Online Data Supplement: Table III. 74 SNPs Tested in CHS

		African Americans		Whites	
Gene	**SNP***	**Allele freq.**	**HWE *P*-Value§**	**Allele freq.**	**HWE *P*-Value§**
ABCG2	rs2231137	0.95	1	0.95	0.15
ADAMTS1	rs428785	0.10	0.99	0.23	0.26
ADAMTS1	rs402007	0.10	0.51	0.23	0.01
ALOX12B	rs3027309	0.08	1	0.19	0.02
AP3B1	rs6453373	0.95	1	0.93	0.55
AQP10	rs6685323	0.30	0.46	0.29	0.36
BAT2	rs11538264	0.91	0.16	0.97	<0.0001
CALM1	rs3814843	0.02	0.18	0.04	0.19
COG2	rs1051038	0.79	0.56	0.79	0.15
CYBRD1	rs10455	0.88	0.27	0.67	0.11
CYP17A1	rs2486758	0.08	0.79	0.21	0.7
CYP2C8	rs10509681	0.98	1	0.89	0.5
DCC	rs1675225	0.97	1	0.89	0.05
DMXL2	rs12102203	0.47	0.47	0.51	0.49
EDG1	rs2038366	0.74	0.28	0.66	0.38
EIF2AK2	rs2307469	0.02	1	0.03	1
F13A1	rs5985	0.20	0.25	0.25	0.65

		African Americans		Whites	
Gene	**SNP***	**Allele freq.**	**HWE *P*-Value§**	**Allele freq.**	**HWE *P*-Value§**
FABP2	rs1799883	0.23	0.48	0.26	0.86
FCAR	rs11666735	0.05	1	0.08	0.54
FCRLM2	rs34868416	0.03	0.4	0.09	0.15
FSTL4	rs13183672	0.77	0.99	0.76	0.82
FSTL4	rs3749817	0.90	0.2	0.78	0.93
GJA4	rs1764391	0.47	0.04	0.69	0.92
GRM8	rs3808117	0.86	0.15	0.79	0.19
HPS1	rs2296436	0.87	0.36	0.91	0.74
HPS1	rs1804689	0.16	0.02	0.30	0.75
IL1F10	rs6761276	0.42	0.33	0.42	0.15
IL1F5	rs2515401	0.31	0.4	0.38	0.98
ITGAE	rs220479	0.95	0.04	0.82	0.17
K6IRS4	rs592720	0.95	0.7	0.74	0.72
KIAA1414	2:37139448	0.20	0.68	0.06	0.23
KIF6	rs20455	0.79	0.17	0.36	0.81
KRT5	rs89962	0.11	0.22	0.43	0.57
LGALS14	rs35541195	0.06	0.72	0.12	0.74
LOC391102	rs943133	0.09	0.05	0.27	0.22
LOC651870	rs3130210	0.39	0.46	0.22	0.79

		African Americans		Whites	
Gene	**SNP***	**Allele freq.**	**HWE *P*-Value§**	**Allele freq.**	**HWE *P*-Value§**
LOC651870	rs3129196	0.39	0.46	0.22	0.86
LPA	rs3798220	0.01	1	0.01	0.46
LY6G5B	rs11758242	0.89	0.38	0.96	<0.0001
MCM10	rs7905784	0.11	0.31	0.15	0.84
MKI67	rs11016076	0.67	0.59	0.81	0.76
MKI67	rs10082504	0.92	0.57	0.89	0.96
MLF1	rs4875	0.70	0.68	0.54	0.33
MYH15	rs3900940	0.17	0.54	0.29	0.46
MYOM3	rs12145360	0.84	0.17	0.85	0.32
OR13G1	rs1151640	0.18	0.61	0.45	0.8
OR2A25	rs2961135	0.33	0.12	0.51	0.26
P2RXL1	rs2277838	0.04	0.26	0.18	0.91
PALLD	rs12510359	0.28	0.54	0.63	0.29
PALLD	rs7439293	0.19	0.39	0.60	0.58
PGLYRP2	rs892145	0.42	0.21	0.36	0.63
PGLYRP2	rs3813135	0.37	0.005	0.37	0.29
PON1	rs662	0.67	0.34	0.30	0.91
PRKG1	rs211070	0.86	0.93	0.95	0.35
ROS1	rs529038	0.09	0.72	0.26	0.78

		African Americans		Whites	
Gene	**SNP***	**Allele freq.**	**HWE *P*-Value§**	**Allele freq.**	**HWE *P*-Value§**
ROS1	rs619203	0.10	0.72	0.26	0.74
SERPINA9	rs17090921	0.18	0.73	0.30	0.26
SERPINB8	rs1944270	0.57	0.3	0.29	0.51
SGIP1	rs1325268	0.26	0.81	0.29	0.48
SLC26A8	rs2295852	0.63	0.99	0.35	0.1
SLC39A7	rs35690712	0.99	1	0.95	0.0003
SNX19	rs2298566	0.92	0.59	0.74	0.79
STRN	rs11685600	0.15	0.5	0.03	1
TAF3	rs4747647	0.67	0.09	0.40	0.1
TAS2R50	rs1376251	0.88	0.49	0.67	0.88
TMPRSS11B	rs12331141	0.48	0.76	0.24	0.43
TOX	rs2290526	0.03	1	0.11	0.37
VAMP8	rs1010	0.55	0.57	0.41	0.27
VTI1A	rs11814680	0.52	0.92	0.15	0.16
WDR31	rs10817479	0.98	1	0.94	0.23
WDR55	rs2286394	0.08	0.78	0.22	0.98
ZNF132	rs1122955	0.88	0.28	0.79	0.35
None	rs2213948	0.93	0.22	0.84	0.26
None	rs2477037	0.76	0.14	0.61	0.16

*rs number or chromosome location (Build36)

§Hardy-Weinberg equilibrium: P-values from Pearson chi-square tests are shown. If either homozygote frequency was 5 or fewer, an exact test was used. Number of participants: African Americans, 673; whites, 3849.

Online Data Supplement: Table IV. Association of 74 SNPs with MI in the white participants of CHS.

Gene	Prespecified model	Adjusted for age and sex		Fully adjusted	
		HR (90% CI)	*P*	HR (90% CI)	*P*
KIF6 (rs20455)	dom	1.29 (1.1-1.52)	0.004	1.29 (1.1-1.52)	0.005
PGLYRP2 (rs3813135)	dom	1.28 (1.09-1.5)	0.006	1.28 (1.09-1.51)	0.006
PGLYRP2 (rs892145)	dom	1.27 (1.09-1.49)	0.006	1.27 (1.08-1.49)	0.007
LPA (rs3798220)	add	1.62 (1.09-2.42)	0.022	1.46 (0.96-2.24)	0.069
MCM10 (rs7905784)	add	1.19 (1.02-1.37)	0.028	1.16 (1-1.35)	0.048
VAMP8 (rs1010)	dom	1.2 (1.02-1.41)	0.032	1.21 (1.03-1.42)	0.029
DCC (rs1675225)	add	1.22 (1.02-1.45)	0.036	1.24 (1.03-1.48)	0.026
TAS2R50 (rs1376251)	add	1.13 (1-1.27)	0.046	1.14 (1.01-1.28)	0.038
GRM8 (rs3808117)	add	1.13 (0.99-1.3)	0.061	1.16 (1.01-1.33)	0.036
ROS1 (rs619203)	add	1.11 (0.99-1.25)	0.071	1.1 (0.98-1.25)	0.086
ROS1 (rs529038)	add	1.1 (0.98-1.24)	0.092	1.09 (0.97-1.23)	0.11
ALOX12B (rs3027309)	add	1.1 (0.97-1.26)	0.11	1.12 (0.99-1.28)	0.071
FABP2 (rs1799883)	add	1.09 (0.97-1.23)	0.12	1.12 (0.99-1.27)	0.061
MLF1 (rs4875)	add	1.07 (0.96-1.19)	0.15	1.07 (0.96-1.19)	0.17
IL1F5 (rs2515401)	add	1.07 (0.96-1.19)	0.17	1.06 (0.95-1.18)	0.21
EDG1 (rs2038366)	add	1.07 (0.95-1.2)	0.17	1.06 (0.94-1.19)	0.21
FCRLM2 (rs34868416)	add	1.11 (0.92-1.32)	0.18	1.1 (0.92-1.33)	0.19
STRN (rs11685600)	add	1.14 (0.86-1.52)	0.22	1.2 (0.9-1.59)	0.15
OR13G1	dom	1.08 (0.91-1.28)	0.22	1.11 (0.94-1.32)	0.16

Gene	Prespecified model	Adjusted for age and sex		Fully adjusted	
		HR (90% CI)	***P***	**HR (90% CI)**	***P***
(rs1151640)					
LOC391102 (rs943133)	add	1.05 (0.93-1.18)	0.25	1.04 (0.92-1.17)	0.29
P2RXL1 (rs2277838)	add	1.06 (0.92-1.21)	0.25	1.02 (0.89-1.18)	0.39
CYP17A1 (rs2486758)	add	1.05 (0.92-1.2)	0.26	1.09 (0.95-1.25)	0.14
BAT2 (rs11538264)	rec	1.13 (0.82-1.56)	0.26	1.18 (0.85-1.63)	0.20
WDR55 (rs2286394)	add	1.05 (0.92-1.19)	0.27	1.06 (0.93-1.2)	0.24
LY6G5B (rs11758242)	rec	1.12 (0.82-1.52)	0.28	1.17 (0.85-1.61)	0.21
KIAA1414 (chr2:37081301)	add	1.08 (0.86-1.36)	0.28	1.12 (0.89-1.41)	0.21
TAF3 (rs4747647)	add	1.03 (0.93-1.15)	0.30	1.02 (0.92-1.14)	0.36
ABCG2 (rs2231137)	add	1.08 (0.84-1.4)	0.31	1.07 (0.83-1.38)	0.34
PRKG1 (rs211070)	add	1.06 (0.82-1.38)	0.35	1.11 (0.85-1.45)	0.26
GJA4 (rs1764391)	add	1.03 (0.92-1.16)	0.35	1.03 (0.92-1.16)	0.33
MYOM3 (rs12145360)	add	1.04 (0.89-1.21)	0.35	1.04 (0.89-1.21)	0.35
HPS1 (rs2296436)	add	1.05 (0.86-1.27)	0.35	1.01 (0.83-1.23)	0.46
LGALS14 (rs35541195)	add	1.04 (0.88-1.22)	0.36	1.04 (0.88-1.23)	0.35
EIF2AK2 (rs2307469)	add	1.06 (0.79-1.43)	0.37	1.1 (0.82-1.49)	0.29
AQP10 (rs6685323)	add	1.02 (0.91-1.15)	0.38	1.03 (0.91-1.16)	0.35
ADAMTS1 (rs428785)	add	1.02 (0.9-1.16)	0.38	1.01 (0.89-1.15)	0.43
CYP2C8 (rs10509681)	rec	1.03 (0.85-1.25)	0.40	1 (0.82-1.21)	0.51
ZNF132 (rs1122955)	add	1.02 (0.89-1.16)	0.41	1.01 (0.89-1.16)	0.44
MKI67 (rs10082504)	add	1.02 (0.86-1.21)	0.43	1.03 (0.87-1.23)	0.38
DMXL2 (rs12102203)	add	1.01 (0.91-1.12)	0.44	1.03 (0.92-1.14)	0.34

Gene	Prespecified model	Adjusted for age and sex		Fully adjusted	
		HR (90% CI)	***P***	**HR (90% CI)**	***P***
PON1 (rs662)	add	1.01 (0.9-1.14)	0.45	1 (0.89-1.12)	0.52
SERPINB8 (rs1944270)	add	1 (0.89-1.13)	0.48	1.02 (0.9-1.15)	0.39
ADAMTS1 (rs402007)	add	1 (0.89-1.14)	0.48	0.99 (0.87-1.12)	0.55
KRT5 (rs89962)	add	1 (0.89-1.11)	0.51	0.99 (0.89-1.11)	0.56
TOX (rs2290526)	add	0.99 (0.83-1.18)	0.54	0.97 (0.81-1.16)	0.61
AP3B1 (rs6453373)	add	0.98 (0.79-1.21)	0.56	0.99 (0.8-1.22)	0.54
K6IRS4 (rs592720)	add	0.98 (0.86-1.1)	0.63	0.96 (0.85-1.09)	0.69
MYH15 (rs3900940)	rec	0.94 (0.71-1.25)	0.64	0.98 (0.74-1.29)	0.56
SNX19 (rs2298566)	rec	0.97 (0.83-1.13)	0.64	0.97 (0.83-1.13)	0.62
FCAR (rs11666735)	dom	0.95 (0.77-1.17)	0.67	0.92 (0.75-1.14)	0.74
None (rs2477037)	add	0.97 (0.87-1.08)	0.68	0.98 (0.88-1.1)	0.59
FSTL4 (rs3749817)	add	0.96 (0.84-1.09)	0.70	0.99 (0.87-1.13)	0.55
MKI67 (rs11016076)	add	0.96 (0.84-1.09)	0.71	0.96 (0.83-1.1)	0.71
ITGAE (rs220479)	add	0.95 (0.83-1.09)	0.73	0.97 (0.85-1.11)	0.64
COG2 (rs1051038)	add	0.95 (0.83-1.09)	0.73	0.93 (0.82-1.07)	0.81
OR2A25 (rs2961135)	add	0.95 (0.85-1.06)	0.78	0.95 (0.86-1.06)	0.77
VTI1A (rs11814680)	add	0.93 (0.79-1.08)	0.79	0.95 (0.81-1.11)	0.72
TMPRSS11B (rs12331141)	add	0.93 (0.82-1.06)	0.83	0.94 (0.83-1.07)	0.79
SGIP1 (rs1325268)	add	0.93 (0.83-1.05)	0.83	0.93 (0.82-1.05)	0.85
FSTL4 (rs13183672)	add	0.93 (0.82-1.05)	0.84	0.95 (0.84-1.08)	0.73
SLC39A7 (rs35690712)	add	0.86 (0.68-1.08)	0.86	0.89 (0.71-1.12)	0.80
WDR31	add	0.86 (0.7-1.07)	0.87	0.89 (0.71-1.1)	0.82

Gene	Prespecified model	Adjusted for age and sex		Fully adjusted	
		HR (90% CI)	***P***	**HR (90% CI)**	***P***
(rs10817479) SERPINA9 (rs17090921)	rec	0.83 (0.62-1.1)	0.87	0.8 (0.6-1.06)	0.90
None (rs2213948)	add	0.9 (0.78-1.03)	0.90	0.9 (0.78-1.04)	0.88
LOC651870 (rs3130210)	add	0.9 (0.79-1.03)	0.90	0.91 (0.79-1.04)	0.89
IL1F10 (rs6761276)	add	0.92 (0.83-1.03)	0.90	0.92 (0.83-1.03)	0.89
LOC651870 (rs3129196)	add	0.9 (0.79-1.03)	0.91	0.9 (0.79-1.03)	0.89
HPS1 (rs1804689)	dom	0.87 (0.75-1.02)	0.93	0.87 (0.75-1.02)	0.93
F13A1 (rs5985)	add	0.89 (0.78-1.01)	0.93	0.85 (0.75-0.97)	0.98
CALM1 (rs3814843)	add	0.77 (0.58-1.02)	0.94	0.8 (0.61-1.06)	0.91
PALLD (rs7439293)	add	0.9 (0.81-1.01)	0.94	0.91 (0.81-1.01)	0.93
PALLD (rs12510359)	rec	0.86 (0.74-1.01)	0.94	0.86 (0.74-1.01)	0.94
SLC26A8 (rs2295852)	add	0.89 (0.79-1)	0.95	0.89 (0.79-1)	0.95
CYBRD1 (rs10455)	add	0.88 (0.79-0.98)	0.97	0.88 (0.78-0.98)	0.98

Online supplement Table V. Association of 74 SNPs with MI in the African American participants of CHS.

	Adjusted for age and sex		Fully adjusted	
Gene (SNP)	**HR (90% CI)**	***P***	**HR (90% CI)**	***P***
FCAR (rs11666735)	2.08 (1.23-3.53)	0.01	2.21 (1.29-3.79)	0.008
None (rs2213948)	2.38 (1.04-5.43)	0.042	20.51 (1.08-50.82)	0.036
AQP10 (rs6685323)	1.35 (1-1.82)	0.048	1.4 (1.03-1.91)	0.034
PALLD (rs12510359)	1.78 (0.98-3.22)	0.055	1.3 (0.67-20.54)	0.26
GJA4 (rs1764391)	1.29 (0.97-1.71)	0.074	1.23 (0.91-1.65)	0.13
VAMP8 (rs1010)	1.71 (0.92-3.19)	0.078	1.81 (0.93-3.52)	0.07
TMPRSS11B (rs12331141)	1.29 (0.96-1.72)	0.078	1.31 (0.97-1.77)	0.069
KIF6 (rs20455)	4.14 (0.79-21.77)	0.08	NA*	
VTI1A (rs11814680)	1.29 (0.95-1.73)	0.083	1.27 (0.93-1.73)	0.10
DCC (rs1675225)	3.82 (0.73-20.1)	0.092	3.81 (0.72-20.2)	0.09
CALM1 (rs3814843)	1.77 (0.77-4.05)	0.13	1.55 (0.66-3.64)	0.20
COG2 (rs1051038)	1.28 (0.88-1.87)	0.14	1.31 (0.89-1.93)	0.13
TAF3 (rs4747647)	1.24 (0.89-1.73)	0.14	1.33 (0.94-1.87)	0.087
MYH15 (rs3900940)	1.87 (0.7-5.01)	0.15	2.24 (0.83-6.06)	0.091
SERPINB8 (rs1944270)	1.22 (0.89-1.65)	0.15	1.24 (0.91-1.7)	0.13
F13A1 (rs5985)	1.23 (0.87-1.74)	0.16	1.32 (0.92-1.88)	0.10
IL1F10 (rs6761276)	1.18 (0.89-1.57)	0.16	1.15 (0.86-1.53)	0.22
EDG1 (rs2038366)	1.2 (0.85-1.7)	0.19	1.23 (0.86-1.76)	0.18
ZNF132 (rs1122955)	1.24 (0.78-1.99)	0.22	1.25 (0.78-2)	0.22
GRM8 (rs3808117)	1.22 (0.78-1.9)	0.23	1.17 (0.72-1.88)	0.30

	Adjusted for age and sex		Fully adjusted	
Gene (SNP)	**HR (90% CI)**	***P***	**HR (90% CI)**	***P***
CYBRD1 (rs10455)	1.25 (0.76-2.05)	0.23	1.19 (0.72-1.97)	0.29
LOC391102 (rs943133)	1.22 (0.75-1.98)	0.25	1.28 (0.79-2.08)	0.20
FSTL4 (rs3749817)	1.24 (0.73-2.13)	0.25	1.43 (0.81-20.53)	0.15
MLF1 (rs4875)	1.14 (0.82-1.59)	0.26	1.11 (0.79-1.56)	0.30
PGLYRP2 (rs3813135)	1.18 (0.76-1.83)	0.27	1.4 (0.88-2.22)	0.12
FABP2 (rs1799883)	1.12 (0.81-1.55)	0.28	1.17 (0.84-1.63)	0.22
P2RXL1 (rs2277838)	1.24 (0.63-2.43)	0.30	1.21 (0.61-2.38)	0.33
FSTL4 (rs13183672)	1.12 (0.78-1.6)	0.31	1.13 (0.78-1.63)	0.30
LPA (rs3798220)	1.55 (0.29-8.21)	0.33	1.75 (0.33-9.36)	0.29
HPS1 (rs2296436)	1.11 (0.71-1.74)	0.35	1.03 (0.66-1.62)	0.46
K6IRS4 (rs592720)	1.16 (0.58-2.32)	0.36	1.17 (0.58-2.37)	0.36
SNX19 (rs2298566)	1.1 (0.63-1.94)	0.39	1.09 (0.62-1.93)	0.40
TAS2R50 (rs1376251)	1.04 (0.66-1.62)	0.45	0.99 (0.63-1.54)	0.52
MKI67 (rs11016076)	1.02 (0.74-1.4)	0.46	1.05 (0.76-1.46)	0.41
PRKG1 (rs211070)	1.02 (0.67-1.56)	0.47	0.92 (0.6-1.41)	0.62
IL1F5 (rs2515401)	1.01 (0.74-1.39)	0.47	0.98 (0.71-1.37)	0.53
CYP17A1 (rs2486758)	1.02 (0.59-1.76)	0.48	1.05 (0.61-1.82)	0.44
CYP2C8 (rs10509681)	NA*		NA*	
ITGAE (rs220479)	NA*		NA*	
SLC39A7 (rs35690712)	0.96 (0.18-5.04)	0.52	1.13 (0.21-6.07)	0.45
PON1 (rs662)	0.97 (0.72-1.31)	0.56	0.98 (0.72-1.34)	0.54
SGIP1 (rs1325268)	0.96 (0.69-1.35)	0.58	0.92 (0.65-1.31)	0.65

	Adjusted for age and sex		Fully adjusted	
Gene (SNP)	**HR (90% CI)**	***P***	**HR (90% CI)**	***P***
PGLYRP2 (rs892145)	0.95 (0.62-1.46)	0.58	1.14 (0.73-1.8)	0.31
WDR31 (rs10817479)	0.89 (0.34-2.35)	0.58	1.29 (0.39-4.26)	0.36
OR13G1 (rs1151640)	0.94 (0.6-1.46)	0.60	1.07 (0.68-1.68)	0.41
ABCG2 (rs2231137)	0.88 (0.46-1.67)	0.63	0.83 (0.43-1.58)	0.69
EIF2AK2 (rs2307469)	0.81 (0.31-2.14)	0.64	0.94 (0.35-20.5)	0.54
HPS1 (rs1804689)	0.9 (0.57-1.42)	0.65	0.92 (0.57-1.48)	0.61
AP3B1 (rs6453373)	0.84 (0.44-1.6)	0.68	0.76 (0.4-1.46)	0.75
FCRLM2 (rs34868416)	0.76 (0.29-1.96)	0.68	0.69 (0.26-1.8)	0.74
KRT5 (rs89962)	0.83 (0.52-1.33)	0.74	0.79 (0.49-1.29)	0.79
DMXL2 (rs12102203)	0.88 (0.66-1.19)	0.76	0.88 (0.65-1.2)	0.75
LGALS14 (rs35541195)	0.72 (0.34-1.54)	0.76	0.74 (0.35-1.59)	0.74
LOC651870 (rs3130210)	0.87 (0.64-1.19)	0.76	0.92 (0.67-1.27)	0.66
LOC651870 (rs3129196)	0.87 (0.64-1.19)	0.76	0.92 (0.67-1.27)	0.66
TOX (rs2290526)	0.59 (0.18-1.94)	0.77	0.63 (0.19-2.09)	0.74
ALOX12B (rs3027309)	0.76 (0.41-1.39)	0.77	0.77 (0.42-1.41)	0.76
WDR55 (rs2286394)	0.76 (0.42-1.37)	0.78	0.83 (0.45-1.51)	0.70
KIAA1414 (chr2:37081301)	0.83 (0.57-1.22)	0.78	0.85 (0.57-1.27)	0.74
SLC26A8 (rs2295852)	0.87 (0.64-1.16)	0.79	0.85 (0.62-1.15)	0.81
SERPINA9 (rs17090921)	0.44 (0.08-2.3)	0.79	0.47 (0.09-20.5)	0.77
LY6G5B (rs11758242)	0.78 (0.49-1.26)	0.80	0.81 (0.5-1.31)	0.77
MCM10 (rs7905784)	0.75 (0.45-1.24)	0.83	0.68 (0.4-1.16)	0.88
OR2A25 (rs2961135)	0.82 (0.59-1.14)	0.83	0.79 (0.56-1.11)	0.88

	Adjusted for age and sex		Fully adjusted	
Gene (SNP)	**HR (90% CI)**	***P***	**HR (90% CI)**	***P***
MYOM3 (rs12145360)	0.78 (0.53-1.15)	0.85	0.75 (0.51-1.1)	0.89
PALLD (rs7439293)	0.75 (0.51-1.12)	0.88	0.64 (0.41-0.98)	0.96
BAT2 (rs11538264)	0.68 (0.42-1.1)	0.91	0.69 (0.42-1.12)	0.90
ADAMTS1 (rs402007)	0.61 (0.34-1.12)	0.91	0.66 (0.36-1.22)	0.87
MKI67 (rs10082504)	0.66 (0.4-1.08)	0.92	0.64 (0.39-1.06)	0.93
STRN (rs11685600)	0.65 (0.4-1.06)	0.93	0.72 (0.44-1.18)	0.86
ROS1 (rs619203)	0.58 (0.32-1.05)	0.94	0.53 (0.28-1)	0.95
ROS1 (rs529038)	0.54 (0.28-1.02)	0.94	0.49 (0.25-0.98)	0.96
ADAMTS1 (rs428785)	0.51 (0.27-0.96)	0.96	0.55 (0.29-1.05)	0.94
None (rs2477037)	0.63 (0.46-0.88)	0.99	0.6 (0.43-0.84)	0.99

*HR could not be estimated because there were no incident events in the risk genotype or in the nonrisk genotype groups.