Genetic Variants in the Apolipoprotein(a) Gene and Coronary Heart Disease

Yonghong Li, PhD; May M. Luke, PhD; Dov Shiffman, PhD; James J. Devlin, PhD

Two independent single-nucleotide polymorphisms (SNPs) in the gene encoding apolipoprotein(a) (LPA) have been shown to be associated with coronary heart disease (CHD) and lipoprotein(a) levels in Caucasians. For the missense SNP rs3798220 (Ile4399Met), carriers of 1 or 2 copies of the minor allele (Met) were at 57% increased risk of CHD. For the intronic SNP rs10455872, the risk of CHD increased by 42% for each copy of the minor allele. The rs3798220 SNP was also associated with differential response to aspirin therapy in the Women’s Health Study; carriers of the minor allele had a significant reduction in CHD events from aspirin therapy, whereas noncarriers did not have a significant reduction.

Lipoprotein(a) [Lp(a)] is a lipoprotein particle that consists of an apolipoprotein(a) [apo(a)] molecule covalently linked by a disulfide bond to the apolipoprotein B-100 (apoB-100) component of a low-density lipoprotein (LDL)-like particle (Figure 1, modified on the Albers et al figure). The LPA gene, which encodes apo(a), is thought to have been generated by a duplication of the neighboring plasminogen gene. Like the plasminogen protein, the apo(a) has a protease-like domain and multiple kringle domains. Different variants of the LPA gene can affect the plasma level of Lp(a), encode apo(a) size variants (ie, kringle repeat length variations), or both.

Lp(a) may contribute to cardiovascular disease through complex mechanisms that involve proatherogenic and prothrombotic pathways. Lp(a) accumulates in the arterial wall of patients with coronary heart disease [CHD] and contributes to cholesterol deposition, induction of endothelial adhesion molecules, chemotaxis of monocytes, foam cell formation, and also to smooth muscle cell proliferation and dedifferentiation. Lp(a) binds oxidized phospholipids that are capable of modulating inflammation and progression of atherosclerosis. In addition, the structural homology between apo(a) and plasminogen suggests that Lp(a) promotes thrombosis. For example, several studies have suggested that Lp(a) can interfere with plasminogen activation by competing for binding to fibrin and consequently attenuate fibrinolysis. Lp(a) may also promote thrombosis by a mechanism independent of plasminogen.

Apo(a) size, kringle repeat number, and Lp(a) plasma levels are all associated with CHD. For example, depending on the gene variant, the LPA gene can encode apo(a) that contains from 3 to >40 kringle IV type 2 (KIV-2) repeats, and the risk of CHD or stroke was 2-fold higher among individuals having small apo(a) molecules (<22 KIV-2 repeats) compared with those having larger apo(a) molecules (>22 repeats) in a recent meta-analysis of 40 studies involving >11 000 patients and >46 000 control subjects. Elevated Lp(a) plasma levels are also a risk factor for CHD: a recent meta-analysis of 36 prospective studies involving >126 000 individuals found "continuous, independent, and modest associations of Lp(a) concentration with risk of CHD and stroke." However, although Lp(a) plasma levels are inversely correlated with the size of the apo(a) protein, the relative contribution of apo(a) size (number of kringle repeats) and Lp(a) plasma levels to cardiovascular risk is not clear. In addition, Lp(a) levels are also associated with a pentanucleotide repeat polymorphism (TTTTA) in the promoter region of the LPA gene, a smaller number of the pentanucleotide repeats being associated with increased Lp(a) levels. A smaller number of the pentanucleotide repeats has also been associated with increased risk of CHD.

Previous studies of the LPA gene described variants that differed in the number of kringle repeats encoded and in the number of pentanucleotide repeats in the promoter region. Other genetic studies have included analyses of single-nucleotide polymorphisms (SNPs) in LPA. Several SNPs in LPA have been reported to affect Lp(a) levels, including SNPs in the promoter region (+93 C/T), in a splice site, and in exons. In a genome-wide association study, multiple SNPs in several genes in the LPA region on the long arm of chromosome 6 were reported to be associated with Lp(a) levels. However, whether these SNPs are also associated with CHD has not been well established. Other studies have reported that SNPs in the LPA region are associated with CHD or severe coronary stenosis. Most notably, among the SNPs in LPA that have been reported to be associated with CHD, the 2 SNPs with the largest effect sizes (rs3798220 and rs10455872) remained associated with CHD after adjustment for each other. Unlike other SNPs in the LPA region, both of these SNPs have been found to be associated with CHD in...
Caucasians in multiple studies; these 2 SNPs are also associated with Lp(a) levels. The rs3798220 SNP is in the region that encodes the protease-like domain of apo(a); this polymorphism results in an amino acid residue polymorphism at position 4399 of apo(a)—an isoleucine or methionine (residue numbering is based on the original cDNA sequence published by McLean et al). The other SNP, rs10455872, is intronic (Figure 1). In the present report, we review results from studies that have investigated the association of these 2 SNPs with apo(a) size, Lp(a) level, and cardiovascular disease risk, and we present a meta-analysis to summarize the available information on the magnitude of the CHD risk associated with these SNPs.

**Association of LPA Variants With Cardiovascular Disease**

Carriers (homozygotes or heterozygotes) of the 4399Met allele of rs3798220 (~3.2% of Caucasians; Table) are at greater risk of CHD than are noncarriers (isoleucine homozygotes). The Ile4399Met (rs3798220) SNP was reported to be associated with angiographically defined severe coronary stenosis in a case-control study involving 1806 subjects with

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**Table. Frequency (%) of the LPA rs3798220 (Ile4399Met) and rs10455872 Genotype by Ethnic Group**

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LPA indicates gene encoding apolipoprotein(a); SNP, single-nucleotide polymorphism.

*Risk genotypes; ND denotes not detected.

Genotype frequency was estimated for Caucasians from 319 individuals from throughout the United States obtained by Genomics Collaborative, Inc (GCI), 186 North Americans obtained by the National Institute of General Medical Science (NIHMS) Human Variation Panels (HVP), and 59 Utah residents from Northern and Western European ancestry from the CEPH collection obtained by HapMap (www.hapmap.org); for African Americans from 367 individuals from throughout the United States obtained by GCI, and 98 individuals obtained by NIHMS HVP; for East Asians from 99 NIHMS HVP Han People of Los Angeles, 90 Han Chinese in Beijing, China, collected as part of the National Human Genome Research Institute (NHGRI) International HapMap Project, 52 Japanese obtained by the NIHMS HVP and 91 individuals from Tokyo from the NHGRI International HapMap project; for Mexican Americans from 99 individuals from the NIHMS HVP from the Mexican-American community of Los Angeles and (for rs3798220) from 57 additional Mexican Americans from HapMap; and for Yorubans from 180 individuals from Ibadan, Nigeria, collected as part of the NHGRI International HapMap project. The genotypes for the 57 Mexican Americans were obtained from HapMap (http://hapmap.ncbi.nlm.nih.gov/cgi-perl/snp_details_phase3?name=rs3798220&source=hapmap26_B36&tmp1=snp_details_phase3); all other genotypes used to prepare this table were determined by allele-specific PCR in the Celera core genotyping laboratory, using DNA samples obtained from the Coriell Institute or GCI.
high stenosis scores and 1274 subjects with low stenosis scores.\textsuperscript{31} In a related study, this SNP was found to be associated with myocardial infarction (MI) in a case-control study (1816 cases and 2635 control subjects) in which the subjects partially overlapped with the coronary stenosis study.\textsuperscript{34} This SNP was also found to be associated with CHD in other case-control studies, one of 7991 cases and 7469 control subjects\textsuperscript{30} and another of 32 584 cases and control subjects.\textsuperscript{33}

In prospective studies, rs3798220 was associated (1) with incident MI among 3849 Caucasian men and women age 65 years or older in the Cardiovascular Health Study (CHS),\textsuperscript{36} (2) with incident major cardiovascular events (including MI, ischemic stroke, and cardiovascular death) among 25 131 initially healthy women age 45 years or older in the Women’s Health Study (WHS),\textsuperscript{29} and (3) with incident CHD (a composite of MI, CHD death, and coronary revascularization) among 5330 aspirin nonusers (but not among 1422 aspirin users) age 45 to 64 years in the Atherosclerosis Risk in Communities (ARIC) study.\textsuperscript{38} This SNP was not associated with incident CHD among 14 465 participants in the HPS study\textsuperscript{39} or with incident CHD among 1328 men and 1552 women in the Framingham Offspring study.\textsuperscript{40} In a meta-analysis that combined results from these case-control and prospective studies (see Methods and online-only Data Supplement Materials), carriers of the 4399Met allele had a 57% increased risk of CHD, compared with noncarriers ($P=1.04\times10^{-11}$, Figure 2A).

The intronic rs10455872 SNP was reported to be associated with CHD\textsuperscript{30} and with MI\textsuperscript{35} in case-control studies and with incident CHD in a prospective study.\textsuperscript{39} In a meta-analysis of these studies (see Methods and online-only Data Supplement Materials) the risk of CHD was 42% higher per copy of the minor allele (G) of rs10455872 (11% of the Caucasian populations carry at least 1 G allele; Table) ($P=3.80\times10^{-6}$, Figure 3).

These meta-analyses have several limitations. First, the analyses were limited to Caucasians, and therefore the conclusions cannot be extended to other ethnic groups. Second, the event definition was not uniform across all the studies in the meta-analyses: although MI events were part of the event (or case) definition in all the studies, some studies included additional endpoints; for example, coronary revascularization was included in the composite end point of the ARIC study. Thus, if these \textit{LPA} SNPs are associated only with MI, the inclusion of other endpoints could have diminished the risk
estimate of these SNPs. Last, these meta-analyses did not address the extent to which the associations between these SNPs and CHD are explained by Lp(a) levels—large studies with Lp(a) data available for all participants will be required to resolve this question.

The association of rs3798220 and rs10455872 with CHD was further confirmed by a haplotype analysis of more than 19,000 cases and control subjects. This haplotype analysis identified 2 haplotypes (or a particular combination of alleles) that were associated with CHD. These two 4-SNP haplotypes, CTTG and CCTC, were defined by different combinations of alleles from the same 4 SNPs (rs2048327, rs3127599, rs7767084 and rs10755578—not including rs3798220 or rs10455872) in the LPA region. Further analysis, however, suggested that the association between these 2 haplotypes and CHD could be fully explained by rs3798220 or rs10455872 because the CCTC haplotype was associated with CHD only among carriers of the 4399Met allele of rs3798220 and the CTTG haplotype was associated with CHD only among carriers of the G (risk) allele of rs10455872.

### Possible Explanations for the Association of SNPs in LPA and CHD

The rs3798220 (Ile4399Met) and rs10455872 variants are associated with CHD, Lp(a) levels, and apo(a) size (Figure 4A and Clarke et al). The association of the risk alleles with both high Lp(a) levels and increased risk of CHD has prompted efforts to determine the extent to which Lp(a) levels explain the association between these SNPs and CHD. However, these investigations have led to different conclusions. On the one hand, Clarke et al suggested that the association between these 2 LPA variants and CHD could be fully explained by their association with Lp(a) levels. They reported that a genotype score based on these 2 variants (the sum of the minor alleles of these 2 SNPs carried by an individual) was strongly associated with CHD and that this association was abolished by adjustment for Lp(a) levels. In contrast, adjustment for Lp(a) levels did not appreciably change the estimate of MI risk for 4399Met carriers in the CHS study. In fact, Chasman et al suggested that adjustment for Lp(a) levels may not be appropriate for an analysis of the association between rs3798220 and cardiovascular disease because of the colinearity between Lp(a) level and rs3798220 genotype.

The association between LPA SNPs and CHD may be explained by mechanisms other than Lp(a) level alone. For example, Luke et al reported that 4399Met allele carriers had high Lp(a) levels (Figure 4A) and were more likely to have a small number (a median of 17) of KIV-2 repeats (Figure 4B), suggesting linkage disequilibrium between the 4399Met allele and a kringle repeat length polymorphism that encodes about 17 KIV-2 repeats; noncarriers (≈97% of Caucasians) have a kringle repeat distribution similar to the general population. A small number of KIV-2 repeats has been shown to be associated with increased risk of CHD after adjusting for Lp(a) levels. Carriers of the 4399Met allele also have more oxidized phospholipids on their apoB-100 particles (Figure 4C), and oxidized phospholipids on apoB particles have been shown to be associated with CHD. Although both LDL and Lp(a) particles contain apoB-100, oxidized phospholipids are predominantly associated with Lp(a) rather than with LDL particles, and these oxidized phospholipids may mediate the atherogenicity of Lp(a). The higher levels of oxidized phospholipids in carriers of the 4399Met allele might result if the plasma half-life of the 4399Met Lp(a) were longer than that of the common Ile4399 variant—a longer half-life might permit more time for the oxidation of phospholipids. Thus the effect of the Ile4399Met polymorphism on the clearance and degradation of Lp(a) particles should be explored. Last, it is possible that oxidation of the methionine residue encoded by the minor allele of rs3798220 could alter Lp(a) catabolism, similar to what has been reported for the methionine residue in apolipoprotein A-I.

Biochemical characterization of the 4399Met and
Ile4399 variants of apo(a) is needed to examine this possibility.

**rs3798220 (Ile4399Met) and Cardiovascular Event Reduction From Aspirin Therapy**

Cardiovascular event reduction by aspirin therapy differed significantly according to rs3798220 (Ile4399Met) genotype in a post hoc analysis of 25,131 Caucasian women in WHS, a randomized trial of aspirin and Vitamin E for primary prevention of cardiovascular disease and cancer among initially healthy women. Among carriers of the 4399Met allele, the absolute risk of a major cardiovascular event after 9.9 years of follow-up was 2.14% in the aspirin group and 4.83% in the placebo group, a 56% relative risk reduction from aspirin therapy (Figure 5). In contrast, among noncarriers of the 4399Met allele there was no significant difference in the absolute risk of major cardiovascular events between the aspirin and the placebo groups (2.13% and 2.25%, respectively). Thus, in WHS the number needed to treat (NNT) to prevent 1 major cardiovascular event was 37 for 4399Met carriers and 625 for noncarriers, with no difference in hemorrhage rates between carriers and noncarriers (Figure 6). The somewhat larger NNT for those at the 75th and 90th percentile of Lp(a) levels, compared with 4399Met carriers, suggests that the Ile4399Met variant has greater clinical relevance than other SNPs associated with Lp(a) levels when considering aspirin therapy for primary prevention. Similarly, for the individual components of the cardiovascular end point (MI and ischemic stroke), event reduction from aspirin therapy was also greater among carriers of the 4399Met allele than among noncarriers, although these differences between carriers and noncarriers did not reach statistical significance.

Aspirin use also appeared to have reduced the risk of CHD among carriers of the 4399Met allele in ARIC, a prospective, population-based observational study. In ARIC, when the association between 4399Met carrier status and CHD was investigated separately among aspirin users and nonusers, carriers of the 4399Met allele, compared with noncarriers, were at increased risk among nonusers: the odds ratio was 1.57 (95% confidence interval [CI], 0.92–2.69; \(P=0.098\)). In contrast, among aspirin users in ARIC, carriers of the 4399Met allele were not at increased risk: the hazard ratio was 0.86 (95% CI, 0.38–1.95; \(P=0.73\)). Although the difference in the risk estimates between the users and nonusers of aspirin in ARIC did not reach significance (\(P=0.22\)), the direction of the effect was similar to that observed in the WHS study, in which the difference was significant (\(P=0.048\)). In the WHS study, carriers of the 4399Met allele, compared with noncarriers, were at increased risk in the placebo group (the hazard ratio was 2.21 [95% CI, 1.39–3.52]) but not in the aspirin group (the hazard ratio was 0.84 [95% CI, 0.43–1.62]) (see online-only Data Supplement Materials). Thus, aspirin use may explain why the Ile4399Met polymorphism was not associated with CHD in the ARIC study when aspirin users and nonusers were combined. Aspirin use was also common (used by 62% of the patients) in the Heart Protection Study (HPS), which might have attenuated the association between rs3798220 and CHD in that study. The hazard ratio for CHD for 4399Met,

**Figure 5.** Kaplan-Meier estimates of the cumulative fraction of women with a first-ever major cardiovascular disease (CVD), myocardial infarction (MI), or ischemic stroke event, according to rs3798220 genotype and treatment group in the WHS cohort. Major cardiovascular events include MI, ischemic stroke, or cardiovascular death. The probability values of the log-rank tests for the Kaplan-Meier comparing carriers in the placebo group with carriers in the aspirin group were 0.02 (for major CVD event), 0.09 (for MI), and 0.06 (for ischemic stroke). There were 12,124 noncarriers in the placebo group, 12,086 noncarriers in the aspirin group, 425 carriers in the placebo group, and 496 carriers in the aspirin group.

**Figure 6.** Number needed to treat (NNT) to prevent 1 major cardiovascular event in the genetic study of the WHS cohort. The NNT was estimated for carriers of the 4399Met allele, noncarriers, and subgroups by the percentile of the plasma lipoprotein(a) [Lp(a)] levels.
compared with the Ile4399, was reported to be 1.17 (95% CI, 0.96–1.43) in HPS, a risk estimate that is somewhat lower than that observed in nonusers of aspirin in WHS and ARIC (see Figure 2). However, the risk estimate for rs10455872 was also lower in HPS than has been observed in other studies: in HPS, the hazard ratio was 1.18 (95% CI, 1.07–1.30) for the G allele, compared with the A allele. When studies in which a substantial proportion of the participants used aspirin were excluded from a meta-analysis and carriers of the 4399Met allele were then compared with noncarriers, the odds ratio for CHD was 1.69 (95% CI, 1.49–1.92) (Figure 2B).

**Potential Clinical Value of LPA Genotype Information**

Given the relatively high level of risk associated with the rs3798220 and rs10455872 SNPs, LPA genotype information could be used as an aid in making clinical recommendations for aspirin use. For example, the US Preventive Services Task Force (USPSTF) guidelines regarding aspirin use for the prevention of cardiovascular disease suggest that only patients at a high 10-year risk of cardiovascular events should use aspirin to ensure that the benefit of aspirin use (ie, reduction of cardiovascular events) outweighs the potential harm caused by aspirin use (ie, major bleeding events). These guidelines suggest that patients whose 10-year risk of cardiovascular events (MI in men; stroke in women) is below age and sex specific thresholds should not use aspirin for primary prevention. However, among these nominally low-risk individuals, LPA genotype information could be used to identify people whose 10-year risk of events actually exceeds the threshold for aspirin use.

**Future Directions**

Given that Lp(a) level is a risk factor of CHD and that Lp(a) level is largely genetically determined, a comprehensive analysis of the LPA region and other loci that modulate Lp(a) level for association with CHD is warranted. Genetic variation in the LPA locus explains a substantial portion of the variation in Lp(a) levels; however, the genetic variants known to be associated with CHD and Lp(a) levels do not account for all the genetic variability of Lp(a) levels. For example, the KIV-2 repeat polymorphism explained only 21% to 27% of the variation in Lp(a) levels in a European population and rs3798220 (Ile4399Met) and rs10455872 in LPA are somewhat correlated with KIV-2 repeat size in European populations, suggesting that additional genetic variants may contribute to the variation in Lp(a) levels. Indeed, several genome-wide linkage analyses have reported that loci on several chromosomes are associated with Lp(a) level (see, for example, Broeckel et al34), and a genome-wide association study has shown that multiple SNPs in multiple genes on the long arm of chromosome 6 may be associated with Lp(a) levels. In addition to rs3798220 (Ile4399Met) and rs10455872, 5 other SNPs in the LPA region also independently but modestly contribute to the variation in Lp(a) levels.30 Investigations of the association between LPA variants and CHD have been carried out mostly in Caucasian populations.

Similar studies should be extended to other ethnic groups because both Lp(a) levels and the genetic regulation of Lp(a) level differ between ethnicities—for example, the average Lp(a) level is approximately 2 times higher in African Americans than in Caucasians, and Lp(a) levels are less strongly associated with apo(a) size polymorphism in African Americans than in Caucasians. The difference in Lp(a) levels among ethnicities may be explained in part by SNPs in the LPA gene. The allele frequencies of SNPs in the LPA region have been characterized in several ethnic groups. Based on our investigations, the risk allele frequency of rs3798220 (4399Met) is low in African Americans and Caucasians, higher in Asians, and higher still in Mexican Americans; rs3798220 was not detected in Yorubans. It is noteworthy that approximately 40% of Mexican Americans carry the 4399Met allele; thus, whether this SNP is also associated with CHD in this population may be of particular interest. For rs10455872, the risk allele (G) frequency is low in African Americans and Mexican Americans and higher in Caucasians; rs10455872 was not detected in Asians or Yorubans (Table, also see HapMap [www.hapmap.org]).

Finally, pharmacogenetic analysis of the rs3798220 (Ile4399Met) and rs10455872 LPA SNPs in additional randomized studies of aspirin would be warranted, given the association between event reduction from aspirin therapy and carrier status of the 4399Met allele that was observed in WHS.

**Methods**

**Literature Search**

Publications for meta-analysis were identified through searches of PubMed, National Human Genome Research Institute (NGRI) Catalog of Published Genome-Wide Association Studies (www.genome.gov/gwastudies), and Google Scholar. The search terms were coronary disease OR cardiovascular disease OR myocardial infarction AND (“apolipoprotein(a)” OR LPA) AND (allele OR polymorphism OR variant) for PubMed; coronary artery disease or myocardial infarction for NGRI; and (rs3798220 OR rs10455872) for Google Scholar. Bibliographies in the publications that were selected for meta-analysis were also examined to identify relevant studies.

**Study Selection**

Included studies met all following criteria: (1) the study was an original report with an abstract in citation; (2) the study used a case-control or a prospective design; (3) the study was conducted in the Caucasian populations; (4) the phenotype investigated was CHD, coronary artery disease, MI, or a combined end point of major cardiovascular events; and (5) the study reported odds ratios or hazard ratios for rs3798220 and/or rs10455872 (judged possible during examination of abstracts and definite after review of full publication). Studies were excluded if they reported association results only for an LPA haplotype, results for ethnically mixed populations, association with coronary stenosis but not coronary events, or when studies used samples that overlapped with a previously reported study that had been included in the meta-analysis.

**Data Extraction**

Data extraction was performed independently by 2 authors, with identical results.
Statistical Analysis

The effect sizes of SNPs when combined across studies were calculated using a random-effects model. Homogeneity of the effect of the SNP across studies was tested with the Q-test, and we report $I^2$—the proportion of total variation in the estimates of effect that is due to heterogeneity between studies. Funnel plots of the risk ratio on the x-axis versus the standard error of the log risk ratio on the y-axis were inspected visually for evidence of heterogeneity in the effect of the SNP by study size, and the symmetry of the distribution of effect sizes around the common effect size was formally evaluated using the method proposed by Egger et al. Sensitivity analyses were performed to assess the influence of individual studies on the overall estimate of the effect of the SNP by recalculating the overall effect of omitting one study at a time. Statistical analyses were performed using R.

For the meta-analysis of rs3798220 (Ile4399Met), we assumed that the risk among carriers of the 4399Met allele was the same as among heterozygotes. This assumption is likely to have a small effect on the risk estimate because 4399Met homozygotes are rare in the Caucasian populations (see Table).

For rs3798220 (Ile4399Met), the hazard ratio for major cardiovascular events in the aspirin group in the genetic study of the WHS cohort was not reported in the Chasman et al publication. To estimate this hazard ratio we assumed that the natural log of the hazard ratio in the combined aspirin and placebo groups was a weighted sum of the natural logs of the hazard ratios in the aspirin and placebo groups with inverse-variance weighting.

Conclusions

In Caucasians, 2 SNPs in the LPA gene, rs3798220 (Ile4399Met) and rs10455872, are independently associated with Lp(a) levels and rs3798220 may also predict the reduction of cardiovascular events by aspirin therapy.

Acknowledgments

We thank Dr Daniel I. Chasman for his contribution to Figure 5 and for sharing unpublished data (Figure 6). For brevity, we were unable to cite many of the primary papers in the vast field of Lp(a) research.

Disclosures

Drs Li, Luke, Shiffman, and Devlin are employees of Celera and hold stock interest in the company.

References


Key Words: aspirin ■ cardiovascular disease ■ coronary disease ■ genetics ■ lipoproteins
SUPPLEMENTARY MATERIALS

Supplementary Results

Summary of Literature Search
We identified 9 publications\(^1-9\) that met our criteria for meta-analysis from three sources (Supplementary Figure 1). From these 9 publications, we extracted association results from 16 studies for rs3798220 and 6 studies for rs10455872 (Supplementary Table 1). The case-control study of prevalent CHD in Hopewell \textit{et al.}\(^3\) was excluded because it used the same controls that were used in the earlier ISIS study, which is included in the meta-analysis.

Meta-analysis, Publication Bias Assessment and Sensitivity Analysis
Since there was variability among the specific endpoints and sampling designs of the individual studies, we conducted meta-analyses for rs3798220 and rs10455872 using the random effects model to incorporate between study heterogeneity. The results are presented in Figures 2 and 3 in the main text.

To assess publication bias, we generated Begg's funnel plots for each of the 3 meta-analyses and tested asymmetry by Egger's method. Neither visual inspection of funnel plots nor Egger's test of symmetry provided obvious indications of publication bias for rs3798220 (Supplementary Figures 2 and 3). For rs10455872 there were not enough studies to adequately evaluate the plots (Supplementary Figure 4). However, while there was no obvious evidence of publication bias, we cannot rule out potential bias of the effect size due to publication bias or other causes since inspection of the plot is subjective and the number of available studies is likely to result in a low power to detect asymmetry with Egger's test.
A sensitivity analysis omitting one study at a time found modest changes in the overall estimate of effect (Supplementary Figures 5, 6 and 7).

**Risk Estimation in the Aspirin Group in the Genetic Study of the WHS Cohort**

For rs3798220, the hazard ratio for CHD was reported in the aspirin users and nonusers of the genetic study of the ARIC cohort\(^6\) and that for major vascular events was reported in the placebo group but not in the aspirin group of the genetic study of the WHS cohort.\(^1\) To facilitate comparison between these studies, we estimated the hazard ratio for major vascular events in the aspirin group in the genetic study of WHS cohort. In the combined aspirin and placebo groups of the genetic study of the WHS cohort, carriers of the 4399Met allele, compared with noncarriers, had a hazard ratio for major vascular events of 1.60 (95% CI: 1.10-2.35). This hazard ratio in the placebo group was 2.21 (95% CI: 1.39-3.52).\(^2\) We calculated that the hazard ratio was 0.84 (95% CI: 0.43-1.62) in the aspirin group.
## Supplementary Table: Summary of studies included in meta-analysis

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**MI**: myocardial infarction; **CVD**: cardiovascular disease; **ACS**: acute coronary syndrome; **CAD**: coronary artery disease; **CHD**: coronary heart disease

**estimated from the original publication assuming event rate was the same among Caucasian and African American participants
Supplementary Figure 1. Literature search results

572 publications identified from PubMed

561 publications excluded based on review of abstracts

11 potential relevant publications underwent full review

4 publications excluded
*2 did not report rs13798220 or rs10455872
*1 reported risk in mixed populations
*1 reported coronary stenosis not events

8 publications identified from National Human Genome Research Institute GWAS Catalog

7 publications included in meta-analysis

1 additional publication included in meta-analysis

8 publications included in meta-analysis

60 citations identified from Google Scholar

1 additional publication included in meta-analysis

9 publications included in meta-analysis
Supplementary Figure 2. Funnel plot (top panel) and Egger’s test of symmetry (bottom panel): rs3798220 in all studies.
Supplementary Figure 3. Funnel plot (top panel) and Egger’s test of symmetry (bottom panel): rs3798220 in studies that did not involve substantial use of aspirin
Supplementary Figure 4. Funnel plot (top panel) and Egger’s test of symmetry (bottom panel):
rs10455872 in all studies
Supplementary Figure 5. Sensitivity analysis: rs3798220 in all studies

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Overall (random effects)                    | 1.57| [1.38; 1.79] |
Supplementary Figure 6. Sensitivity analysis: rs3798220 in studies that did not involve substantial use of aspirin

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**Overall (random effects)**  
1.69 [1.49; 1.92]
Supplementary Figure 7. Sensitivity analysis: rs10455872 in all studies

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Supplementary References


6. Shiffman D, Chasman DI, Ballantyne CM, Nambi V, Devlin JJ, Boerwinkle E. Coronary heart disease risk, aspirin use, and apolipoprotein(a) 4399Met allele in the

