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Polymorphism in the Apolipoprotein(a) Gene, Plasma Lipoprotein (a), Cardiovascular Disease, and Low-dose Aspirin Therapy

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Abstract

Objective—A minor allele variant (rs3798220) of apolipoprotein(a) has been reported to be associated with elevated plasma lipoprotein(a) [Lp(a)] and increased cardiovascular risk. We investigated whether this allele was associated with elevated Lp(a) and cardiovascular risk in Women's Health Study, a randomized trial of low-dose aspirin, and whether aspirin reduced cardiovascular risk in minor allele carriers.

Methods and Results—Genotypes of rs3798220 were determined for 25,131 initially healthy Caucasian participants. Median Lp(a) levels at baseline were 10.0, 79.5, and 153.9 mg/dL for major allele homozygotes, minor allele heterozygotes, and minor allele homozygotes, respectively ($P<0.0001$). During the 9.9 years of follow-up, minor allele carriers (3.7%) in the placebo group had two-fold higher risk of major cardiovascular events than non-carriers (age-adjusted hazard ratio (HR) = 2.21, 95% CI 1.39−3.52). Among carriers, risk was reduced more than two-fold by aspirin: for aspirin compared with placebo the age-adjusted HR was 0.44 (95% CI: 0.20−0.94); risk was not significantly reduced among non-carriers (age-adjusted HR=0.91, 95% CI: 0.77−1.08). This interaction between carrier status and aspirin allocation was significant ($P=0.048$).

Conclusions—In the Women's Health Study, carriers of an apolipoprotein(a) variant had elevated Lp(a), doubled cardiovascular risk, and appeared to benefit more from aspirin than non-carriers.

Keywords

cardiovascular disease; aspirin; lipoproteins; genetics; Lp(a)

Plasma lipoprotein(a) [Lp(a)] consists of a single apolipoprotein(a) [apo(a)] molecule covalently linked through a disulfide bond to a single apolipoprotein B-100 molecule together with cholesterol-rich lipid [1]. While the biological functions of Lp(a) remain uncertain [2,3],

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high levels of Lp(a) have been associated with increased cardiovascular risk, particularly when LDL-C is also elevated [4-6]. The cholesterol-bearing aspects of Lp(a) have largely focused investigation of associated cardiovascular risk on lipid-based biology [7-10]. However, apo(a) is also highly homologous to plasminogen, and in spite of the lack of recognized proteolytic activity in apo(a), this homology has focused alternative investigations into the role of Lp(a) in cardiovascular risk on hemostasis, platelet function, and thrombosis [11-17].

Recently, we and our collaborators described a non-synonymous single nucleotide polymorphism (SNP rs3798220) in apo(a) gene (*LPA*) that was a risk factor for cardiovascular disease. This SNP, encoding an isoleucine to methionine substitution in the protease-like domain of apo(a) at amino acid 4399 (I4399M), was associated with both elevated levels of Lp(a) and cardiovascular disease [18,19]. We had also found previously that plasma levels of Lp(a) were associated with increased vascular risk in the Women's Health Study (WHS), a randomized, placebo controlled trial of aspirin for primary prevention of cardiovascular disease [5,6]. Thus, we investigated whether the association of SNP rs3798220 with Lp(a) and cardiovascular risk could be confirmed in a large scale prospective cohort of Caucasian women and also whether allocation to aspirin could attenuate the SNP-based risk of incident cardiovascular events.

Methods

The primary study population derived from participants in the Women's Health Study (WHS), a randomized trial of aspirin (100 mg orally on alternate days) and placebo in the primary prevention of cardiovascular disease conducted among initially healthy women aged at least 45 at enrollment who were followed over a 10 year period for incident cardiovascular events [20,21]. At enrollment, participants provided baseline clinical and demographic information. Overall, 28,345 WHS participants, 25,815 of which were Caucasian, also provided adequate blood samples for both plasma and genetic analysis. Among these samples, baseline Lp(a) levels were measured with a turbidimetric assay that is not affected by the number of Kringle IV type-2 repeats on the Hitachi 917 analyzer (Roche Diagnostics, Indianapolis, ID) [6,22]. Baseline measurements of other lipid fractions, inflammation biomarkers, and other plasma components have been described previously [23]. Genotypes for rs3798220 in the WHS participants were determined by an oligonucleotide ligation procedure that combined PCR amplification of target sequences from 3ng of genomic DNA with subsequent allele-specific oligonucleotide ligation as previously described [19]. The ligation products of the two alleles were separated by hybridization to product-specific oligonucleotides, each coupled to spectrally distinct Luminex100 xMAP microspheres (Luminex, Austin, TX). The captured products were fluorescently labeled with streptavidin R-phycoerythrin (Prozyme, San Leandro, CA), sorted on the basis of microsphere spectrum, and detected by a Luminex100 instrument [19]. In total, genotype for the rs3798220 polymorphism was determined for 25,131 or 97.4% of the Caucasian women; and the 24,794 of these women had Lp(a) levels measured at baseline. The Caucasian women with rs3798220 genotype and Lp(a) level comprise the population for analysis in this study. Of these, 486 and 417 carriers of the minor allele, respectively, were randomly allocated to aspirin and placebo.

All women were followed prospectively for the occurrence of a first ever major cardiovascular event, a composite endpoint of myocardial infarction, ischemic stroke, and cardiovascular death. Medical records were obtained for all reported cardiovascular endpoints, and reviewed by an Endpoints Committee of physicians blinded to randomized treatment assignment. The occurrence of myocardial infarction was confirmed if symptoms met World Health Organization criteria and if the event was associated with abnormal levels of cardiac enzymes or diagnostic electrocardiograms. Stroke was confirmed if the participant had new neurologic deficits of sudden onset that persisted for more than 24 hours. Clinical information as well as

computed tomographic scans or magnetic resonance images were used to distinguish hemorrhagic from ischemic events. Cardiovascular deaths were confirmed by autopsy reports, death certificates, medical records, and information obtained from family members. Only confirmed endpoints are included in this report.

Deviation from Hardy-Weinberg equilibrium was evaluated using a log-likelihood ratio test. Differences in clinical covariates among the three genotype classes were assessed by ANOVA procedures for normally-distributed characteristics, by the Kruskal-Wallis test for non-normally distributed characteristics, and by a Chi-Squared test of proportions for categorical characteristics. Assessment of the association between rs3798220 and risk of major incident cardiovascular disease was performed with Cox proportional hazards models that estimated hazard ratios (HR) comparing the homozygotes of the major allele (non-carriers) to either heterozygotes alone or all carriers of the minor allele of rs3798220. None of the homozygotes of the minor allele experienced a major cardiovascular event. In addition, these models were adjusted for age and traditional risk factors including blood pressure coded by Framingham categories, history of diabetes, current smoking status at baseline, familial history of myocardial infarction, LDL-C, and HDL-C [5]. In all reported analysis, the assumption of proportionality in the Cox models could not be rejected ($P>0.05$). For detecting association between rs3798220 and major cardiovascular events in the Cox models, there was at least 80% power for detecting associations with HR at least 2.1 among the 12,379 Caucasians with rs3798220 genotype information who were allocated to placebo. Differences in risk due to rs3798220 carrier status according to treatment group were estimated from separate Cox models for the aspirin and placebo allocated participants. The significance of these differences was determined from a formal interaction term in Cox models that included all participants. Cumulative incidence of major cardiovascular events was estimated by the Kaplan-Meier procedure.

The study protocol was approved by the institutional review board of the Brigham and Women's Hospital, Boston, MA.

Results

Association between LPA polymorphism and Lp(a) levels

Among the 25,131 WHS Caucasian women with successful genotyping, the minor allele of rs3798220 in the *LPA* gene was carried by 906 (3.6%) heterozygotes and 15 (0.06%) homozygotes for a minor allele frequency of 1.9%. These genotypes represent slightly fewer heterozygotes (906) and slightly more homozygotes (15) of the minor allele than expected under Hardy-Weinberg equilibrium (917 and 9 expected, respectively; $P=0.05$). Age, BMI, smoking status, hormone replacement therapy use, and menopausal status as well as incidence of hypertension and diabetes were not different among the three genotypic classes (Table 1). However, median Lp(a) levels in heterozygous (79.5 mg/dL) and homozygous (153.9 mg/dL) carriers of the minor allele were respectively eight and 15 times higher than the Lp(a) levels in non-carriers (10.0 mg/dL, $P<<0.001$) (Table 1, Figure 1). There were also modest but significant elevations of total cholesterol, LDL-C, and apolipoprotein B among carriers as expected from the known contribution of Lp(a) to the measurement of these other lipid fractions [24]; and these differences were no longer significant after adjustment for Lp(a) levels. Other plasma biomarkers, including markers of inflammation, were not associated with rs3798220 genotype.

In marked contrast to the Lp(a) distribution among non-carriers of the rs3798220 minor allele, Lp(a) levels among heterozygotes were bimodally distributed (Figures 2A and 2B) with a split point at 30mg/dL. All homozygotes of the minor allele and 71% of the heterozygotes had Lp

(a) levels greater than the split point. The bimodal shape could not be explained by any of the baseline clinical characteristics (data not shown).

LPA polymorphism, major cardiovascular event rates, and aspirin therapy

During a median follow-up period of 9.9 years for the WHS randomized trial of aspirin, 510 Caucasian women in the current analysis suffered a first ever major cardiovascular event (myocardial infarction, ischemic stroke, or cardiovascular death). Since preliminary analysis had revealed an interaction between rs3798220 carrier status and aspirin allocation, we first investigated the risk associated with the rs3798220 in the placebo group (270 major cardiovascular events). In this group, women who carried one or more copies of the minor allele of rs3798220, compared with non-carriers, had an approximate 2-fold higher risk of major cardiovascular events (age-adjusted HR 2.22, 95% CI: 1.39−3.53, P=0.0008). For myocardial infarction and ischemic stroke the risk estimates were similar to the estimate for major cardiovascular events (Table 2). All of these risk estimates were essentially unchanged when further adjusted for traditional risk factors, including LDL-C (e.g. the HR for major events was 2.24, 95% CI: 1.37−3.68, P=0.0014), indicating that neither the small difference in LDL-C levels between carriers and non-carriers nor slight median elevations of Lp(a) levels (about 0.9 mg/dL, P=0.0005) associated with family history of myocardial infarction influence the risk estimate due to rs3798220 genotype. The risk estimates were attenuated but still significant in the whole study population, which included both placebo and aspirin allocated groups (age-adjusted HR for major events among carriers: 1.60, 95% CI:1.10−2.35, P=0.015).

Further analysis confirmed the effect of aspirin allocation in reducing risk in carriers of rs3798220 (Figure 3). Among rs379820 carriers, for aspirin compared with placebo, the age-adjusted hazard ratio for major cardiovascular events was 0.44 (95%CI: 0.20−0.94, P=0.033), a 56% reduction in risk of major cardiovascular events; and after the median 9.9 years of follow-up, the absolute risk was 2.14% (95%CI: 0.81%−3.45%) in the aspirin group compared with 4.83% (95%CI: 2.74%−6.87%) in the placebo group. In contrast, among non-carriers, for aspirin compared with placebo, the age-adjusted hazard ratio was 0.91 (95% CI: 0.77−1.08, P=0.30); and the absolute risk was 2.13% (95% CI: 1.87%−2.39% in the aspirin group compared with 2.25 (95% CI: 1.98%−2.51%) in the placebo group. This interaction between carrier status and aspirin allocation was significant ($P_{interaction}$=0.048). Similar differential effects of aspirin according to carrier status were also observed for the individual endpoints of myocardial infarction and ischemic stroke (Figure 3B and 3C).

Carriers of the minor allele of rs3798220 had both higher Lp(a) levels and a variant sequence in the protease-like domain of apo(a), either of which might explain the excess risk or the greater benefit from aspirin in carriers. Lp(a) levels in excess of the 90th percentile (65.1 mg/dL), had been shown previously to be associated with cardiovascular risk in the WHS [6]. However, we found that when aspirin was compared with placebo among those with Lp(a) levels higher than 65.1mg/dL, the age-adjusted hazard ratio was 0.75 (95% CI: 0.48−1.18, P=0.22), a risk reduction (25%) that was neither statistically significant nor as large as the risk reduction among rs3798220 carriers. Similarly, to investigate whether the excess risk of carriers could be explained solely by Lp(a) levels, we initially considered conducting a logistic regression analysis that compared carriers with a sample of non-carriers matched on Lp(a); however, the paucity of non-carriers with very high Lp(a) levels (Figure 2) would have resulted in small sample size and limited power. As an alternative, therefore, we examined event rates among carriers with high (>30 mg/dL), and low Lp(a) levels (<30mg/dL). The event rate among carriers with high Lp(a) levels (3.6 events/1000 person-years) was higher than the event rate among carriers with low Lp(a) levels (1.9 events/1000 person-years), a difference that was not statistically significant (log-rank P=0.19) but this analysis was limited by the small number of events (n=5) among carriers with low Lp(a) levels.

Discussion

In this large-scale study of initially healthy Caucasian women who participated in a randomized trial of low-dose aspirin, the 3.7% of the population that carried the minor allele of the rs3798220 polymorphism in the *LPA* gene had greatly elevated levels of Lp(a) as well as a doubling of the risk of major cardiovascular events. Among carriers randomly allocated to low-dose aspirin, this increased risk of future cardiovascular events was effectively abolished (relative risk reduction with aspirin was 56%, P=0.033), whereas among non-carriers, aspirin allocation did not provide significant benefit (relative risk reduction with aspirin 9%, P=0.30). The observed effects of rs3798220 on Lp(a) levels, future cardiovascular events, and outcome with aspirin allocation extend the preliminary data from the retrospective case-control cohorts of a previous study and a prospective assessment of cardiovascular risk among elderly North Americans [18,19].

While the differential benefit of aspirin therapy is intriguing, the number of events among rs3798220 minor allele carriers in the separate aspirin and placebo allocation groups was modest. Nevertheless, the gene based differential effects of aspirin are derived from a double-blind, placebo-controlled trial, a design that intrinsically eliminates many sources of bias that can affect pharmacogenetic analysis in observational cohorts. Further, the aspirin-by-gene interaction was significant and did not violate proportionality of hazards. The Kaplan-Meier survival curves demonstrate that the divergence of risk among carrier status by aspirin groups occurred early and was consistent and increasing over time. This differential, aspirin-by-gene interaction would have resulted in a number-needed-to-treat (NNT) to prevent one major cardiovascular event of 37 for carriers compared with 625 for non-carriers, with no difference in hemorrhage rates.

The interaction of rs3798220 SNP in *LPA* with aspirin, a drug with known antithrombotic activity, is consistent with a direct role of Lp(a) in hemostasis, thrombosis, and platelet function, perhaps through the plasminogen protease-like domain or lysine binding functions of the Kringle domains as has been suggested previously [12,18,25]. It is possible also that the interaction may be related to the reported modest reduction in apo(a) expression with aspirin treatment [26,27]. Whether or not this effect is large enough in the WHS to account for the diminished risk or may further interact with the rs3798220 minor allele remains unknown. However, pursuing these hypotheses in the WHS is hampered by the lack of plasma samples after randomization to aspirin or placebo.

The relationship between the non-synonymous isoleucine to methionine substitution encoded by rs3798220 (I4399M) and both Lp(a) levels and cardiovascular risk is unresolved. We do not know if the risk is solely related to increased Lp(a) levels or if it is related to intrinsic biological activity imparted by the amino acid substitution or other linked variation. Analytic attempts to distinguish between these two possibilities by resampling procedures as well as spline-based regression techniques were not sufficiently powered to account adequately for the marked differences in the Lp(a) distributions between carriers and non-carriers and the potential confounding due to colinearity of the polymorphism and Lp(a) levels. Similarly, potential linkage disequilibrium relationships between rs3798220 and other variation at the *LPA* locus may complicate understanding the molecular basis of the observed effects. Previous work likely excludes 18 additional SNPs at the *LPA* locus which were neither in LD with rs3789220 nor associated with incident cardiovascular disease [18]. However, the bimodal pattern of the Lp(a) distribution might be explained by the rs3798220 minor allele situated on two or more haplotypes, one of which expresses high levels of Lp(a) due to a small number of encoded type 2 Kringle IV domains [28-30], as previously published data suggests [18].

This study has several limitations. The data in this study are limited to initially healthy Caucasian women, and the number of major cardiovascular events among carriers of the rs3798220 minor allele was modest. Further, this study is not powered to test if the cardiovascular risk associated with the rs3798220 is explained by functional differences between the isoleucine and methionine variants at amino acid 4399 in apo(a), or if it is fully explained by differences in Lp(a) levels. However, the hazard ratio for association of rs3798220 with events in the placebo group was 2.2, a value greater then a hazard ratio of 2.1 for which there was 80% power (see methods).

Aside from providing clues about the biological properties of Lp(a), the associations of rs3798220 with Lp(a) levels, cardiovascular risk, and the effect of aspirin allocation illustrate the potential of genetic approaches in understanding and treating common complex disease. Thus, our results specifically suggest that future studies of the relationship between aspirin benefit, rs3798220 genotype, and Lp(a) levels are warranted, and more generally suggest the broader possibility of administering healthcare with higher precision through gene-based personalized medicine.

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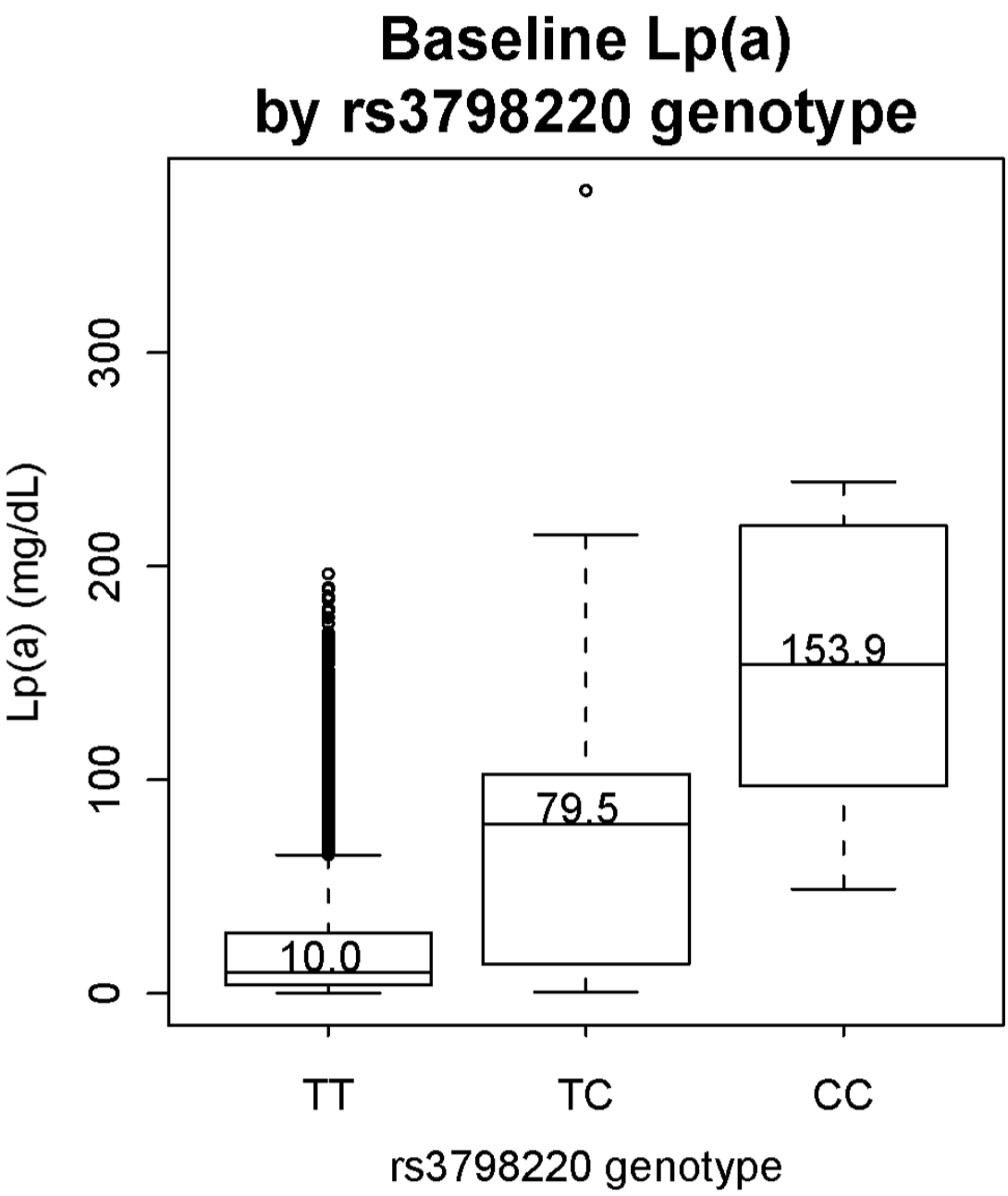

Figure 1.
Baseline plasma Lp(a) levels according to rs3798220 genotype among Caucasian participants from the Women's Health Study. The inter-quartile ranges (IQR) and medians for the three genotypes are indicated by the boxes and their midlines. The whiskers span the range of Lp(a) values as far as 1.5 times the IQR from the median, and extreme Lp(a) values beyond the whiskers are indicated by circles.

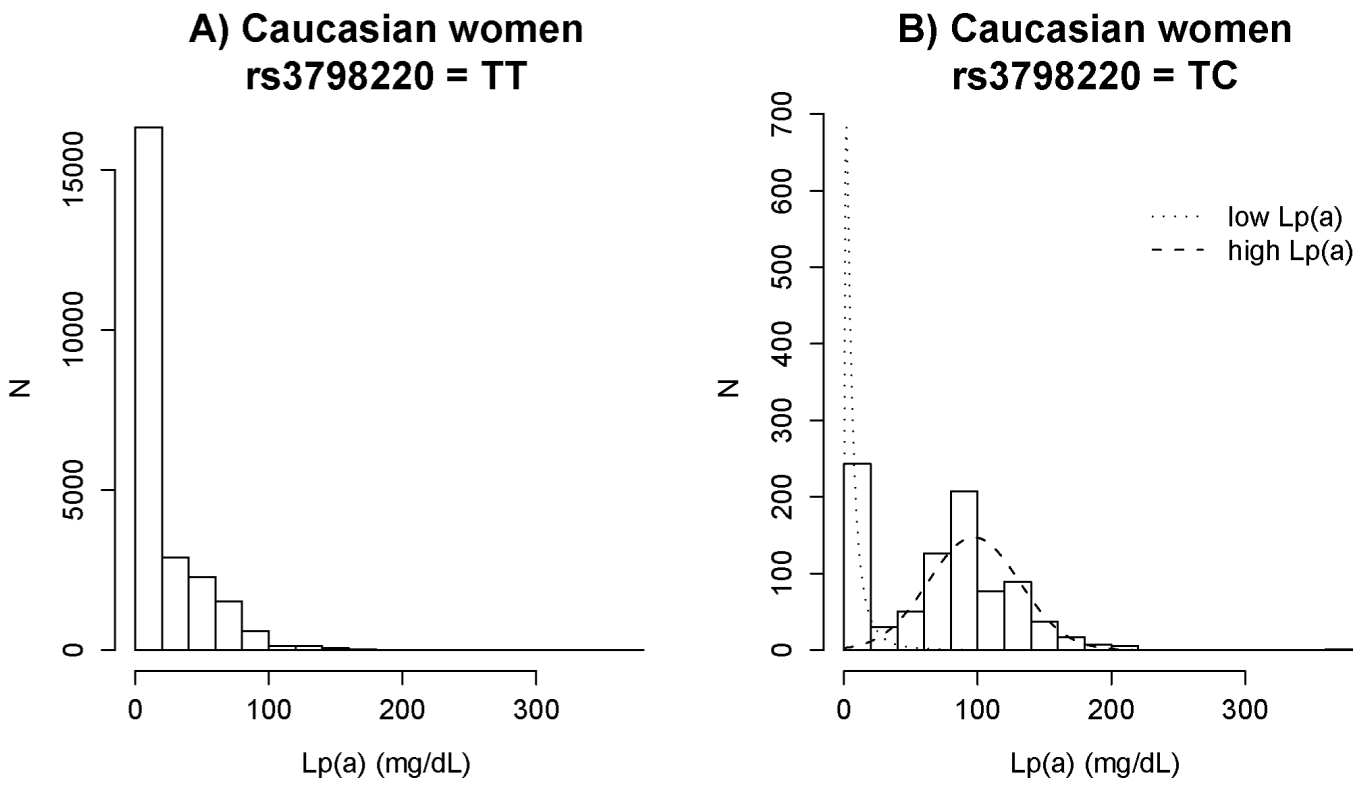

Figure 2.

Distribution of plasma Lp(a) levels among Caucasian study participants for non-carriers (TT, Panel A) and heterozygous carriers (TC, Panel B) of the rs3798220 minor allele. The bimodal split point of 30mg/dL was estimated by modeling the bimodal shape as the combination of two separate distributions (dotted and dashed lines).

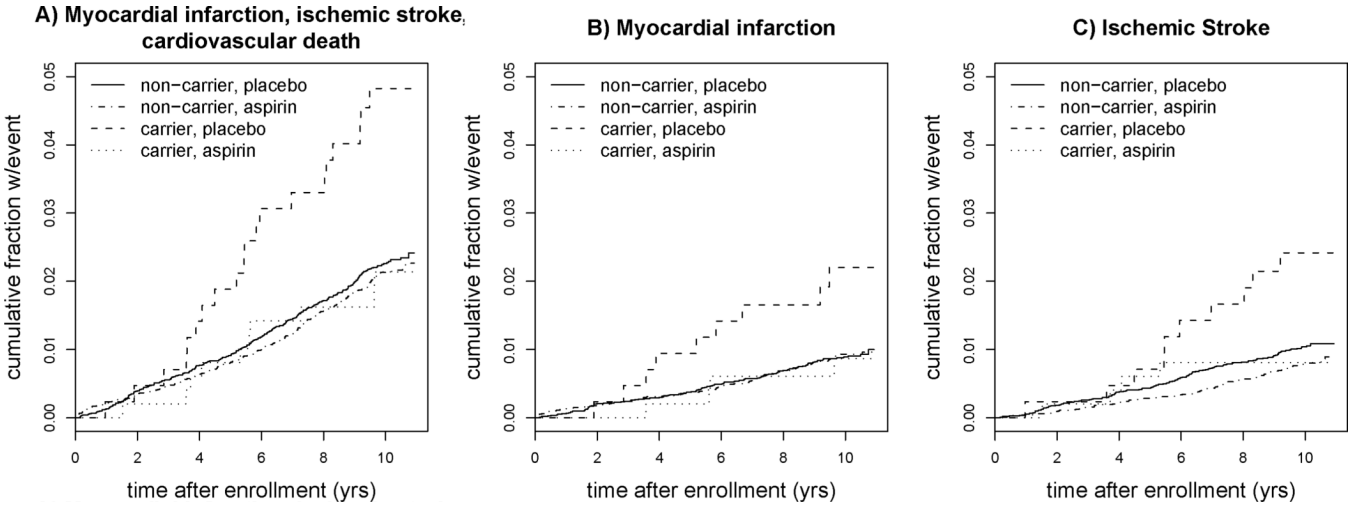

Figure 3.

Kaplan-Meier estimates of the cumulative fraction of Caucasian women with a first ever major CVD event (myocardial infarction, ischemic stroke, or cardiovascular death) according to rs3798220 carrier status and treatment group. A) composite endpoint; B) myocardial infarction; C) ischemic stroke.

Table 1

Baseline clinical profile for Caucasian WHS participants by genotype

	rs3798220 genotype*			
	TT (N=24210)	**TC (N=906)**	**CC (N=15)**	***P*-value**
Clinical characteristics (units)				
age (yrs.)	52.0 (48.0−59.0)	52.0 (48.2−58.0)	52 (49.5−55.0)	0.355
BMI (kg/m^2)	24.9 (22.5−28.3)	24.7 (22.4−28.2)	24.9 (22.1−28.9)	0.704
history hypertension	5972 (24.7)	237 (26.2)	3 (20.0)	0.526
blood pressure				0.346
<120/<75 mm Hg	8088 (33.8)	299 (33.1)	8 (53.3)	
<130/<85	7734 (32.3)	284 (31.5)	3 (20.0)	
<140/<90	4487 (18.8)	182 (20.2)	3 (20.0)	
<160/<95	3110 (13.0)	112 (12.4)	0 (0.0)	
>=160/>=95	508 (2.1)	25 (2.8)	1 (6.7)	
family history MI	2811 (12.9)	127 (15.5)	2 (15.4)	0.077
Current smoking	2809 (11.6)	109 (12.0)	2 (13.3)	0.920
Diabetes	618 (2.6)	22 (2.4)	0 (0.0)	0.885
HRT	10511 (43.5)	420 (46.5)	9 (60.0)	0.100
Menopause	13181 (54.5)	487 (53.8)	8 (53.3)	0.921
Lipid biomarkers (units)				
Total cholesterol (mg/dL)	208 (184.0−235.0)	215.0 (189.0−241.0)	229 (208.5−250.0)	0.0003†
LDL-C (mg/dL)	121.3 (100.4−144.2)	126.5 (105.6−149.3)	137.6 (118.8−157.4)	0.0002†
apolipoprotein B (mg/dL)	100.0 (83.9−121.3)	107.3 (87.1−125.1)	109.8 (100.4−127.0)	0.00001†
HDL-C (mg/dL)	51.8 (43.1−62.2)	51.8 (43.5−62.4)	51.7 (46.0−66.8)	0.588
apolipoprotein AI (mg/dL)	148.9 (132.3−167.8)	148.2 (133.3−166.4)	145.2 (135.9−168.3)	0.984
Triglycerides (mg/dL)	119.0 (84.0−176.0)	117.0 (83.0−177.0)	104 (90.0−149.0)	0.887
Lp(a) (mg/dL)	10.0 (4.2−28.5)	79.5 (13.8−102.8)	153.9 (105.6−215.0)	<<0.0001‡
Inflammation biomarkers (units)				
C-reactive protein (mg/L)	2.0 (0.8−4.4)	2.0 (0.8−4.2)	1.1 (0.9−2.4)	0.318
Soluble ICAM-1 (ng/ml)	342.9 (301.9−394.8)	343.7 (301.3−396.4)	349.4 (295.6−399.2)	0.946
fibrinogen (mg/L)	350.0 (307.1−401.4)	349.2 (307.6−402.2)	343.5 (318.4−382.7)	0.851
Other biomarkers (units)				
creatinine (mg/dL)	0.7 (0.6−0.8)	0.7 (0.6−0.8)	0.7 (0.7−0.8)	0.414
homocysteine (umol/dL)	10.5 (8.7−12.9)	10.5 (8.7−12.8)	11.6 (8.6−12.7)	0.433
HbA1c (%)	5.0 (4.8−5.2)	5.0 (4.8−5.2)	4.9 (4.8−5.2)	0.677

*Median (inter-quartile range) for quantitative characteristics or N (%) for categorical characteristics.

†Not significant after adjustment by Lp(a) levels

‡Analytic $P=5.6\times10^{-178}$

Table 2

Risk of major cardiovascular events among placebo allocated Caucasian WHS participants by rs3798220 minor allele carrier status in Cox proportional hazards models

Event	rs3798220 genotype	N*	N events*	Age-adjusted HR (95% CI)	Age-adjusted *P*	Fully-adjusted† HR (95% CI)	Fully-adjusted† *P*
Major CVD‡							
	TT (Ile/Ile) (reference)	11962	251	-	-	-	-
	TC (Ile/Met)	414	19	2.22 (1.39−3.53)	0.0008	2.24 (1.37−3.68)	0.0014
	CC (Met/Met)	3	0	NA	NA	NA	NA
	TC (Ile/Met) + CC (Met/Met)	417	19	2.21 (1.39−3.52)	0.0009	2.24 (1.36−3.68)	0.0014
Myocardial infarction‡							
	TT (Ile/Ile) (reference)	11962	99	-	-	-	-
	TC (Ile/Met) + CC (Met/Met)	417	8	2.35 (1.14−4.83)	0.020	1.97 (0.86−4.52)	0.11
Ischemic stroke‡							
	TT (Ile/Ile) (reference)	11962	114	-	-	-	-
	TC (Ile/Met) + CC (Met/Met)	417	9	2.26 (1.15−4.46)	0.018	2.49 (1.25−4.94)	0.0093

*Out of 12379 who had rs3798220 genotype, measured baseline Lp(a), and were allocated to placebo

†Adjusted for age, blood pressure, history of diabetes, smoking status, familial history of myocardial infarction, LDL-C, and HDL-C

‡First ever major CVD event (MI, ischemic stroke, or CV death), myocardial infarction, or ischemic stroke