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The effect of nine common polymorphisms in coagulation factor genes (F2, F5, F7, F12 and F13) on the effectiveness of statins: the GenHAT study

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Abstract

Background—Pharmacogenetic research has shown that genetic variation may influence statin responsiveness. Statins exert a variety of beneficial effects beyond lipid lowering, including antithrombotic effects, which contribute to the risk reduction of cardiovascular disease. Statins have been shown to influence the expression of coagulation factors II, V, VII, XII and XIII.

AimData from a large randomized clinical trial of pravastatin, designed to show efficacy relative to usual care, were used to investigate whether a pharmacogenetic effect of polymorphisms in genes coding for coagulation factors II, V, VII, XII and XIII is associated with reduced fatal coronary heart disease (CHD) and nonfatal myocardial infarction, combined CHD and all-cause mortality.

Methods—The Genetics of Hypertension Associated Treatment is an ancillary study of the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial. The genotyped population in the lipid-lowering trial of Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial included 9624 participants randomly assigned to pravastatin or to usual care. The efficacy of pravastatin in reducing risk of all-cause mortality, CHD plus nonfatal myocardial infarction and combined CHD, was compared among genotype strata by examining an interaction term in a proportional hazards model.

Results—None of the polymorphisms were associated with the clinical outcomes. For the F7 (−323) del/ins polymorphism there was no interaction with pravastatin for either outcome. For both the F5 Arg506Gln G>A (rs6025) polymorphism and F7 Arg353Gln G>A (rs6046) polymorphism there were no interactions with pravastatin in relation to all-cause mortality, but there were significant interactions with combined CHD [interaction hazard ratio $\lambda=1.33$, 95% confidence interval (1.01–1.76) and interaction hazard ratio $\lambda=1.92$, 95% confidence interval (1.00–3.65), respectively]. There were no interactions between the polymorphisms in the other coagulation genes and pravastatin in relation to any outcome.

Conclusion—Polymorphisms in anticoagulation genes (F5 and F7) seem to modify the efficacy of pravastatin in reducing risk of cardiovascular events.

Keywords

anticoagulation factor; pharmacogenetics; statin

Introduction

The coagulation system plays a pivotal role in the development of arterial thrombosis. Rupture of an atherosclerotic plaque exposes the lipid core, smooth-muscle cells, macrophages and collagen to the bloodstream resulting in the activation of the coagulation cascade. Ultimately, a thrombus is formed that can either completely or incompletely occlude an artery, resulting in clinical events such as myocardial infarction (MI) and stroke [1].

Statins are mainstay drugs in cardiovascular risk management. The efficacy of statin therapy has been well established. Average risk reductions of 27 and 15% have been shown for major coronary events and all-cause mortality, respectively [2]. Importantly, these risk reductions are averages where there is individual variation in response to statins. Pharmacogenetic research has shown that genetic variation may influence statin responsiveness [3].

Statins exert a variety of other beneficial effects beyond lipid lowering, which may contribute to the risk reduction of cardiovascular disease, known as pleiotropic effects [4]. These include antithrombotic effects. Among other coagulation factors, statins have been shown to influence the expression of coagulation factors II, V, VII, XII and XIII [5,6].

Genetic variability within these coagulation genes has been shown to alter activity or expression of the coagulation factors and therefore the coagulation state. In addition, a range of polymorphisms in the aforementioned genes have previously been associated with coronary artery disease [7,8].

The effects of statins on coagulation haemostasis to reduce clot formation or reduce stability of fibrin clots may be affected by polymorphisms in these genes. In this study, data from a large randomized clinical trial of pravastatin designed to show efficacy against cardiovascular disease were used to investigate whether the F13 Pro564Leu C>T (rs5982), F13 Val35Leu C>T (rs5985), F12 -46 C>T, F7 -401 G>T (rs7981123), F7 -402 G>A (rs762637), F7 (-323) del/ins (rs742910), A4 F7 arg353gln G>A (rs6046), F5 arg506gln G>A (rs6025) and F2 20210 G>A (rs1799963) polymorphisms are associated with the efficacy of statins with respect to clinical outcomes.

Methods

Study population and design

The Genetics of Hypertension Associated Treatment (GenHAT) study is an ancillary study of the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). The Lipid-Lowering Treatment (LLT) component of ALLHAT was designed to evaluate the impact of large sustained cholesterol reductions on all-cause mortality in a hypertensive cohort with at least one other coronary heart disease (CHD) risk factor and to assess CHD reduction and other benefits in populations that had been excluded or underrepresented in previous trials, particularly older persons, women, racial and ethnic minority groups and persons with diabetes. The GenHAT is a post-hoc analysis of ALLHAT-LLT. The genotyped population in the lipid-lowering component of ALLHAT (ALLHAT-LLT) included 9624 participants. The primary outcome was all-cause mortality. Furthermore, two a priori secondary outcomes were analyzed:

(i) a combination of CHD death (fatal CHD, coronary revascularization-related mortality, previous angina or MI and unknown potentially lethal noncoronary disease process) and nonfatal MI and (ii) combined CHD (CHD death, coronary revascularization, hospitalized angina). The design of ALLHAT, including the LLT, and its participants and clinical site recruitment and selection have been reported elsewhere [9–12]. Briefly, ALLHAT-LLT was a randomized, open-label, large simple trial conducted from February 1994 to March 2002 at 513 clinical centres in the United States, Puerto Rico, US Virgin Islands and Canada. The intervention was open-label pravastatin (40 mg/day) versus usual care. Participants were selected exclusively from the ALLHAT antihypertensive trial AQ5. The protocol of ALLHAT was approved by each participating centre's institutional review board. The GenHAT study was approved by the institutional review boards of the University of Alabama at Birmingham, the University of Minnesota and the University of Texas Health Science Centre at Houston.

Genotyping

DNA was isolated on Flinders technology associates filter paper (Whatman Bioscience, Cambridge, UK) from blood samples. AQ6 Genotyping was performed using the amplified DNA products of a multiplex PCR and detected using a colorimetric reaction of allele-specific oligonucleotide probes hybridized to a nylon membrane (i.e. Roche strip; Roche Molecular Systems, Inc., Alameda, California, USA) AQ7 as described earlier [13].

Statistical methods

STATA version 9.2 (STATA Corporation, College Station, Texas, USA) was used for all analyses. Tests for differences between treatment groups for baseline characteristics were carried out using analysis of variance for continuous variables and the χ^2 tests for categorical variables (Table 1). AQ8. Hardy–Weinberg equilibrium (HWE) was assessed using the χ^2 tests. As a result of the small number of minor allele homozygotes, genotypes were modelled dominantly by collapsing the minor homozygotes with the heterozygotes, resulting in two categories for each variant. For each outcome, Cox proportional-hazards regression was used for testing the main effect of pravastatin within genotype-specific groups, and the genotype-by-treatment interactions, resulting in hazard ratios (HR) and ratio of HR (interaction hazard ratio, IHR) point estimates, respectively. Adjustment variables included sex, ethnicity (black/nonblack), smoking status (current smoker/nonsmoker), type 2 diabetes status (yes/no), age, BMI, history of CHD, years of education, as well as baseline values of systolic blood pressure, HDL cholesterol and LDL cholesterol. The Kaplan–Meier risk estimates were evaluated at 6-year follow-up, the Cox regression models used the full follow-up time available. A value of P less than 0.05 was used for statistical significance. However, given the many genotype, multivariate and interaction analyses performed, statistical significance at this level should be interpreted with caution. To account for multiple statistical testing, q values were calculated [14]. The q value of a test gives the proportion of false positives incurred (false discovery rate) when that particular test is considered significant [15]. Calculations were carried out using the R package implemented QValue software available at <http://genomics.princeton.edu/storeylab/qvalue/> [14]. To adjust for multiple testing, P values of 27 pharmacogenetic interactions (nine gene–treatment interactions with three outcomes) were used to calculate the q values.

Results

Hardy–Weinberg equilibrium

The F7 (–323) del/ins, F5 arg506gln G>A, F7 –401 G>T, F7 –402 G>A and F13 Val35Leu C>T variants were in HWE when tested in an ethnicity-specific manner, whereas the F2 20210 G>A variant was not. The F7 arg353gln G>A was not in HWE for blacks or nonblacks ($P\lambda=0.01$ and $P\lambda=0.003$, respectively). However, when further stratified by self-identified

Hispanic status, both Hispanic black and Hispanic nonblack participants were in HWE ($P\lambda=0.52$ and $P\lambda=0.72$, respectively), whereas non-Hispanic black and non-Hispanic nonblack participants were not ($P\lambda=0.03$ and $P<0.001$, respectively). Furthermore, the HWE was violated by the nonblack non-Hispanic group for the F12 -46 C>T polymorphism ($P<0.001$) and by the non-Hispanic black participants for the F13 Pro564Leu C>T polymorphism ($P\lambda=0.01$).

Effect of genotype on outcomes

There were no statistically significant effects from the genotypes on the outcomes (Table 2).

Gene-treatment interactions

There were no statistically significant interactions detected between the F13 Pro564Leu C>T, F13 Val35Leu C>T, F12 -46 C>T, F7 -401 G>T, F7 -402 G>A, F7 (-323) del/ins and F2 20210 G>A polymorphisms and pravastatin on CHD or all-cause mortality (Table 3). There was a significant interaction for F5 Arg506Gln G>A on combined CHD [HR $\lambda=1.92$, 95% confidence interval (CI) (1.00–3.65)]. For the F7 Arg353Gln G>A polymorphism, there was no interaction with pravastatin in preventing all-cause mortality, but there was a significant interaction with fatal CHD plus nonfatal MI, and with combined CHD.

When randomized to pravastatin, participants with the GA or AA genotype had a higher risk of fatal CHD plus nonfatal MI [HR $\lambda=1.23$, 95% CI (0.89–1.70)] than those randomized to usual care, whereas the more common GG genotype group had a decreased risk of this outcome [HR $\lambda=0.80$, 95% CI (0.67–0.95)]. This difference between the genotype groups (the pharmacogenetic effect) was statistically significant [IHR $\lambda=1.53$, 95% CI (1.06–2.20)] with a q value of 0.45. There was a similar pharmacogenetic effect on combined CHD: GA or AA participants randomized to pravastatin had increased risk of combined CHD [HR $\lambda=1.23$, 95% CI (0.95–1.57)] compared with the usual care group, whereas the GG group on pravastatin had a decreased risk [HR $\lambda=0.91$, 95% CI (0.81–1.04)] compared with the usual care group, and again the pharmacogenetic effect was significant [IHR $\lambda=1.33$, 95% CI (1.01–1.76)] with a q value of 0.45 (Table 4).AQ9

For the F5 arg506gln G>A polymorphism, there was no interaction with pravastatin in preventing all-cause mortality. There was a trend towards an interaction with pravastatin on fatal CHD plus nonfatal MI: the minor allele carriers (GA and AA genotypes) had an increased risk when randomized to pravastatin versus usual care [HR $\lambda=2.24$, 95% CI (0.91–5.55)], whereas the more common GG genotype group had decreased risk when randomized to pravastatin versus usual care [HR $\lambda=0.85$, 95% CI (0.73–0.99)], although this difference between genotype groups was not statistically significant [IHR $\lambda=2.12$, 95% CI (0.88–5.12)]. There was a significant pharmacogenetic effect of pravastatin in preventing combined CHD: when randomized to pravastatin, the GA and AA group had increased risk of combined CHD outcomes compared with the usual care group [HR $\lambda=1.87$, 95% CI (0.97–3.59)], whereas the GG genotype group had slightly reduced risk [HR $\lambda=0.95$, 95% CI (0.85–1.06)] and this pharmacogenetic effect was statistically significant [IHR $\lambda=1.92$, 95% CI (1.00–3.65)] with a q value of 0.45 (Table 5).

Discussion

The GenHAT-LLT study is a large pharmacogenetic trial of pravastatin versus usual care. On the basis of genotype data collected from almost 100,000 individuals who were followed for cardiovascular events using standard, well-defined definitions for cardiovascular outcomes, we found that polymorphisms in two genes that are involved in anticoagulation [the F7

arg353gln G>A polymorphism and the F5 arg506gln G>A polymorphism (also known as Factor V Leiden)] changed the efficacy of pravastatin in preventing CHD.

For the F7 arg353gln G>A variant, that is located in exon 8, there was a trend towards risk reduction in participants with the GG genotype (wild type), whereas participants with the GA or AA genotype had an increased risk of CHD events when randomized to pravastatin versus usual care. The variant allele has been associated with low plasma levels of Factor VIIa and also with a decreased risk of MI [16]. In our study, there was no statistically significant relationship between the polymorphisms in F7 and the outcomes, but as expected the variant allele seemed to be protective compared with the wild type. Statin treatment might decrease Factor VII production and activation [5]. The carriers of the wild-type allele have a higher Factor VIIa baseline level and this explains our result that wild-type carriers have a larger risk reduction with statin therapy.

The other polymorphisms that were studied in the same gene [F7 (–323) del/ins, F7 –401 G>T, F7 –402 G>A polymorphism] are located in the promoter region of the gene. There were no associations with outcomes and no interactions with pravastatin found.

The F5 arg506gln G>A polymorphism changes the cleavage site for activated protein C. The mutation prevents efficient inactivation of Factor V. When Factor V remains active, it facilitates overproduction of thrombin leading to excess fibrin generation and excess clotting. Therefore, carriers of Factor V Leiden have a well-established increased risk of venous thrombosis. Two large meta-analyses have reported an odds ratio of approximately 1.3 on MI in carriers of the Factor V Leiden mutation [17,18]. In another meta-analysis, no association with MI was found [19]. In our study, there was also no association found. Statins possibly decrease Factor V activation and increase inactivation of FVa [5]. In our study, carriers of the mutation do not seem to benefit from statin therapy, and in fact have increased risk of CHD events when randomized to pravastatin versus usual care, although the association does not quite reach statistical significance ($HR\lambda=2.24$, $P\lambda=0.08$). The fact that the interaction term is not statistically significant for fatal CHD and nonfatal MI is probably because of the small numbers in the variant group. This seems especially true because the value of the interaction term ($IHR\lambda=2.12$) is similar to the statistically significant interaction term for combined CHD ($IHR\lambda=1.92$). We hypothesize that statins are not able to inactivate Factor V Leiden in these patients because of the mutation.

The F2 gene encodes for prothrombin. The F2 20210 G>A polymorphism is located in the 3'-untranslated region. The polymorphism is associated with a 25% increase in plasma thrombin activity and has been associated with a higher risk of MI [20]. The variant allele of the –46 C>T polymorphism in the promoter region of the F12 gene has been strongly associated with lower plasma levels of activated Factor XII, but no association with coronary artery disease has been shown so far [21]. The two polymorphisms in the F13 gene (F13 Pro564Leu C>T and F13 Val35Leu C>T) have been shown to affect the activity of Factor XIII [22,23], but only for the variant allele of F13 Val35Leu C>T has been reported to be protective against MI [7]. In our study, there were no differences in the risk on CHD, and even though statins are more or less likely to decrease the generation of these coagulation factors [5] there was also no interaction with statins on preventing CHDQA10.

There were no differences for the outcome total mortality for all genotypes. Total mortality includes noncardiac causes of death, and therefore the effect in this endpoint might be diluted.

Study limitations

Small numbers in the mutation groups forced us to combine homozygous and heterozygous carriers. Therefore, we were not able to distinguish between these groups. Furthermore, this

might have led to nonsignificant results in those groups. As a result of the large number of tests performed, our significant results might be because of chance. Therefore, we accounted for multiple testing by calculating q values. All of the significant interactions had a q value of 0.45. This means that we have to conclude that there is a fair chance that our findings were chance findings. Therefore, the pharmacogenetic interactions that were found should be considered hypothesis generating and replication in other populations is of major importance. Currently, there are no clinical implications of this study.

The GenHAT study selected individuals based on preexisting coronary risk factors and hypertension. Therefore, there is some uncertainty about the applicability of the findings of this study to the association in participants without these risk factors. In the ALLHAT study, no overall beneficial effect of pravastatin was shown on the primary outcome. After 6 years of follow-up, 26% of participants in the usual care arm used statins, and 16% in the pravastatin arm did not use a lipid-lowering drug anymore. This might have also led to a dilution of the interaction effect that was found.

Furthermore, for some population strata the polymorphisms were not in HWE. This might be because of nonrandom sampling, because all the participants from this study were selected from a hypertensive cohort and not from the general population. Deviations from HWE can also be because of inbreeding, population stratification or selection. They can also signify a disease association. All of these causes are unlikely in our trial. Apparent deviations from HWE can arise in the presence of a common deletion polymorphism, because of a mutant PCR-primer site or because of a tendency to miscall heterozygotes as homozygotes. Quality control in a subset for two single nucleotide polymorphisms (SNPs) AQ12 showed for F2 20210 G>A, that only two (0.3%) out of 722 results disagreed. For F7 arg353gln, six (0.8%) out of 729 comparisons were not in agreement. These quality control results suggest that genotyping errors are an unlikely explanation for why these SNPs are out of HWE. However, the results of these two polymorphisms should be interpreted with caution [24].

In the GenHAT trial, black and white participants (both groups including self-identified Hispanics) were included. This could affect the estimate of the main effect of a gene–outcome relationship, however, it is unlikely that it influences the main pharmacogenetic effects, because it is assumed that in a clinical trial participants from different races are randomly distributed among the treatment groups. However, there could be a difference in pharmacogenetic effects in different races. Therefore, we stratified the population by race (data not shown), and no statistically significant differences in pharmacogenetic interactions were found.

Future research

This is a first report to show that genes involved in anticoagulation might be important for the pharmacogenetics of pravastatin. Replication studies with genes involved in the blood coagulation pathway, and with more SNPs in these genes are necessary to clarify if differences in those genes might be clinically relevant.

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References

1. Yeghiazarians Y, Braunstein J, Askari A, Stone P. Unstable angina pectoris. *N Engl J Med* 2000;342:101–114. [PubMed: 10631280]
2. Cheung BM, Laufer IJ, Lau CP, Kumana CR. Meta-analysis of large randomized controlled trials to evaluate the impact of statins on cardiovascular outcomes. *Br J Clin Pharmacol* 2004;57:640–651. [PubMed: 15089818]
3. Mangravite LM, Thorn CF, Krauss RM. Clinical implications of pharmacogenomics of statin treatment. *Pharmacogenomics J* 2006;6:360–374. [PubMed: 16550210]
4. Liao JK, Laufs U. Pleiotropic effects of statins. *Annu Rev Pharmacol Toxicol* 2005;45:89–118. [PubMed: 15822172]
5. Undas A, Brummel-Ziedins KE, Mann KG. Statins and blood coagulation. *Arterioscler Thromb Vasc Biol* 2005;25:287–294. [PubMed: 15569822]
6. Dick J, McClaren M, Ford R. The effect of atorvastatin on activated factor XII, fibrinogen and lipid profile. *Haemostasis* 2000;30:42.
7. Endler G, Mannhalter C. Polymorphisms in coagulation factor genes and their impact on arterial and venous thrombosis. *Clin Chim Acta* 2003;330:31–55. [PubMed: 12636925]
8. Ye Z, Liu EH, Higgins JP, Keavney BD, Lowe GD, Collins R, et al. Seven haemostatic gene polymorphisms in coronary disease: meta-analysis of 66,155 cases and 91,307 controls. *Lancet* 2006;367:651–658. [PubMed: 16503463]
9. ALLHAT Research Group. Major outcomes in moderately hypercholesterolemic, hypertensive patients randomized to pravastatin vs. usual care: the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT-LLT). *JAMA* 2002;288:2998–3007. [PubMed: 12479764]
10. Davis BR, Cutler JA, Gordon DJ, Furberg CD, Wright JT Jr, Cushman WC, et al. Rationale and design for the Antihypertensive and Lipid Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). ALLHAT Research Group. *Am J Hypertens* 1996;9(4 Pt 1):342–360. [PubMed: 8722437]
11. Pressel S, Davis BR, Louis GT, Whelton P, Adroge H, Egan D, et al. Participant recruitment in the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). *Control Clin Trials* 2001;22:674–686. [PubMed: 11738123]
12. Wright JT Jr, Cushman WC, Davis BR, Barzilay J, Colon P, Egan D, et al. The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT): clinical center recruitment experience. *Control Clin Trials* 2001;22:659–673. [PubMed: 11738122]
13. Cheng S, Grow MA, Pallaud C, Klitz W, Erlich HA, Visvikis S, et al. A multilocus genotyping assay for candidate markers of cardiovascular disease risk. *Genome Res* 1999;9:936–949. [PubMed: 10523522]
14. Storey JD. A direct approach to false discovery rates. *J R Stat Soc B* 2002;64:479–498.
15. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B* 1995;57:289–300.
16. Girelli D, Russo C, Ferraresi P, Olivieri O, Pinotti M, Friso S, et al. Polymorphisms in the factor VII gene and the risk of myocardial infarction in patients with coronary artery disease. *N Engl J Med* 2000;343:774–780. [PubMed: 10984565]
17. Juul K, Tybjaerg-Hansen A, Steffensen R, Kofoed S, Jensen G, Nordestgaard BG. Factor V Leiden: the Copenhagen City Heart Study and 2 meta-analyses. *Blood* 2002;100:3–10. [PubMed: 12070000]
18. Boekholdt SM, Bijsterveld NR, Moons AH, Levi M, Buller HR, Peters RJ. Genetic variation in coagulation and fibrinolytic proteins and their relation with acute myocardial infarction: a systematic review. *Circulation* 2001;104:3063–3068. [PubMed: 11748101]
19. Wu AH, Tsongalis GJ. Correlation of polymorphisms to coagulation and biochemical risk factors for cardiovascular diseases. *Am J Cardiol* 2001;87:1361–1366. [PubMed: 11397354]
20. Franco RF, Reitsma PH. Gene polymorphisms of the haemostatic system and the risk of arterial thrombotic disease. *Br J Haematol* 2001;115:491–506. [PubMed: 11736929]

21. Kohler HP, Futers TS, Grant PJ. FXII (46C-->T) polymorphism and in vivo generation of FXII activity--gene frequencies and relationship in patients with coronary artery disease. *Thromb Haemost* 1999;81:745–747. [PubMed: 10365748]
22. Ariens RA, Kohler HP, Mansfield MW, Grant PJ. Subunit antigen and activity levels of blood coagulation factor XIII in healthy individuals. Relation to sex, age, smoking, and hypertension. *Arterioscler Thromb Vasc Biol* 1999;19:2012–2016. [PubMed: 10446086]
23. Kohler HP, Futers TS, Grant PJ. Prevalence of three common polymorphisms in the A-subunit gene of factor XIII in patients with coronary artery disease. *Thromb Haemost* 1999;81:511–515. [PubMed: 10235430]
24. Balding DJ. A tutorial on statistical methods for population association studies. *Nat Rev Genet* 2006;7:781–791. [PubMed: 16983374]

Table 1
Baseline characteristics for participants by treatment group

Characteristics	Pravastatin	Usual care	<i>P</i> value ^a
λSample size, <i>n</i> (%) by treatment	4808 (50.0)	4818 (50.1)	
Age (years), mean (SD)	66.4 (7.7)	66.3 (7.5)	0.35
Race, <i>n</i> (col %)			0.17
vWhite	2719 (56.6)	2787 (57.9)	
vBlack	1804 (37.5)	1723 (35.8)	
vOther	285 (5.9)	308 (6.4)	
Sex (male), <i>n</i> (%)	2469 (51.4)	2450 (50.9)	0.62
Systolic blood pressure (mmHg), mean (SD)	144.9 (13.9)	145.0 (14.0)	0.72
Diastolic blood pressure (mmHg), mean (SD)	84.1 (9.8)	83.9 (9.8)	0.37
Body mass index, mean (SD)	29.8 (5.9)	29.9 (6.2)	0.55
Smoker, <i>n</i> (%)	1128 (23.5)	1133 (23.5)	0.95
History of type 2 diabetes, <i>n</i> (%)	1697 (35.3)	1639 (34.0)	0.19
History of MI or stroke, <i>n</i> (%)	818 (17.0)	842 (17.5)	0.55
History of CHD, <i>n</i> (%)	654 (13.6)	733 (15.2)	0.02
Education (years), mean (SD)	10.7 (4.0)	10.8 (4.1)	0.80
Total cholesterol (mg/dl), mean (SD)	223.5 (26.9)	223.6 (26.5)	0.92
HDL cholesterol (mg/dl), mean (SD)	47.5 (13.4)	47.3 (13.5)	0.41
LDL cholesterol (mg/dl), mean (SD)	145.4 (21.4)	145.3 (21.3)	0.84
Antihypertensive treatment group, <i>n</i> (col %)			0.97
vDoxazosin	1008 (21.0)	1029 (21.4)	
vChlorthalidone	1743 (36.3)	1741 (36.1)	
vAmlodipine	1039 (21.6)	1037 (21.5)	
vLisinopril	1018 (21.2)	1011 (21.0)	
F7 (−323) del/ins, <i>n</i> (%)			0.89
vDD	3158 (65.7)	3172 (65.9)	
vDI	1432 (29.8)	1418 (29.5)	
vII	215 (4.5)	223 (4.6)	
F7 arg353gln G>A, <i>n</i> (%)			0.72
vGG	3705 (77.4)	3728 (77.8)	
vGA	994 (20.8)	969 (20.2)	
vAA	87 (1.8)	94 (2.0)	
F5 arg506gln G>A, <i>n</i> (%)			0.02
vGG	4675 (97.4)	4673 (97.1)	
vGA	122 (2.5)	138 (2.9)	
vAA	5 (0.1)	0 (0.0)	
F2 20210 G>A, <i>n</i> (%)			0.34
vGG	4633 (97.3)	4673 (97.6)	
vGA	126 (2.6)	106 (2.2)	
vAA	5 (0.1)	7 (0.2)	
F12 −46 C>T, <i>n</i> (%)			0.82
vCC	2252 (47.3)	2285 (48.0)	

Characteristics	Pravastatin	Usual care	<i>P</i> value ^a
vCT	1947 (40.9)	1926 (40.4)	0.98
vTT	561 (11.8)	554 (11.6)	
F13 Val35Leu C>T, <i>n</i> (%)			
vCC	2898 (60.9)	2913 (61.1)	0.07
vCT	1626 (34.2)	1620 (34.0)	
vTT	235 (4.9)	235 (4.9)	
F13 Pro564Leu C>T, <i>n</i> (%)			0.33
vCC	3239 (68.1)	3166 (66.4)	
vCT	1368 (28.8)	1422 (29.8)	
vTT	150 (3.2)	184 (3.9)	0.88
F7 -402 G>A, <i>n</i> (%)			
vGG	3056 (64.1)	3049 (63.8)	
vGA	1532 (32.1)	1517 (31.8)	0.88
vAA	182 (3.8)	211 (4.4)	
F7 -401 G>T, <i>n</i> (%)			
vGG	3145 (65.9)	3170 (66.4)	
vGT	1408 (29.5)	1388 (29.1)	
vTT	217 (4.6)	219 (4.6)	

CHD, coronary heart disease; del/ins, deletion/insertion; MI, myocardial infarction.

^aTests for differences between groups: analysis of variance for continuous variables, χ^2 test for categorical variables.AQ14

Table 2Main effects of genotypes on outcomes^a

	Fatal CHD plus nonfatal MI	Combined CHD	All-cause mortality
λF7 (−323) del/ins, <i>n</i> (col %)			
vDD (<i>n</i> λ=λ6330)	1.00	1.00	1.00
vDI+II (<i>n</i> λ=λ3288)	0.89 (0.75–1.05)	0.93 (0.82–1.06)	0.92 (0.80–1.04)
<i>vP</i> value	0.16	0.27	0.19
F7 arg353gln G>A, <i>n</i> (col %)			
vGG (<i>n</i> λ=λ7433)	1.00	1.00	1.00
vGA+AA (<i>n</i> =2144)	0.97 (0.80–1.16)	0.90 (0.78–1.04)	0.89 (0.77–1.03)
<i>vP</i> value	0.71	0.14	0.12
F5 arg506gln G>A, <i>n</i> (col %)			
vGG (<i>n</i> λ=λ9348)	1.00	1.00	1.00
vGA+AA (<i>n</i> λ=λ265)	1.00 (0.65–1.53)	1.02 (0.74–1.39)	0.87 (0.59–1.27)
<i>vP</i> value	0.99	0.92	0.46
F2 20210 G>A, <i>n</i> (col %)			
vGG (<i>n</i> λ=λ9306)	1.00	1.00	1.00
vGA+AA (<i>n</i> λ=λ244)	1.07 (0.67–1.71)	0.80 (0.54–1.18)	0.95 (0.63–1.44)
<i>vP</i> value	0.78	0.26	0.82
F12 −46 C>T, <i>n</i> (col %)			
vCC (<i>n</i> λ=λ4537)	1.00	1.00	1.00
vCT+TT (<i>n</i> λ=λ4988)	1.00 (0.86–1.17)	1.05 (0.93–1.17)	0.99 (0.87–1.11)
<i>vP</i> value	0.98	0.44	0.82
F13 Val35Leu C>T, <i>n</i> (col %)			
vCC (<i>n</i> λ=λ5811)	1.00	1.00	1.00
vCT+TT (<i>n</i> λ=λ3716)	0.97 (0.83–1.13)	1.00 (0.89–1.12)	0.95 (0.84–1.07)
<i>vP</i> value	0.69	0.98	0.38
F13 Pro564Leu C>T, <i>n</i> (col %)			
vCC (<i>n</i> λ=λ6405)	1.00	1.00	1.00
vCT+TT (<i>n</i> λ=λ3124)	1.07 (0.92–1.25)	1.11 (0.99–1.25)	1.03 (0.91–1.17)
<i>vP</i> value	0.39	0.08	0.65
F7 −402 G>A, <i>n</i> (col %)			
vGG (<i>n</i> λ=λ6105)	1.00	1.00	1.00
vGA+AA (<i>n</i> λ=λ3442)	0.92 (0.79–1.08)	0.99 (0.88–1.11)	0.97 (0.85–1.10)
<i>vP</i> value	0.33	0.87	0.58
F7 −401 G>T, <i>n</i> (col %)			
vGG (<i>n</i> λ=λ6315)	1.00	1.00	1.00
vGT+TT (<i>n</i> λ=λ3232)	0.87 (0.73–1.03)	0.93 (0.82–1.06)	0.90 (0.79–1.03)
<i>vP</i> value	0.10	0.29	0.12

CHD, coronary heart disease; MI, myocardial infarction; SBP, systolic blood pressure.

^a Adjusted variables for sex, ethnicity (black/non-black), smoking status (current smoker/non-smoker), type 2 diabetes status (yes/no), age, BMI, history of CHD, years of education, as well as baseline values of SBP, HDL cholesterol and LDL cholesterol.

Table 3Interaction adjusted ratio of HRs (95% CI) pravastatin versus UC on outcomes^a

	Fatal CHD plus nonfatal MI	Combined CHD	All-cause mortality
λF2 20210 G>A			
vGG ($n\lambda=\lambda 9306$)	1	1	1
vGA+AA ($n\lambda=\lambda 244$)	1.21 (0.47–3.11)	0.72 (0.33–1.56)	0.62 (0.27–1.43)
F5 arg506gln G>A			
vGG ($n\lambda=\lambda 9348$)	1	1	1
vGA+AA ($n\lambda=\lambda 265$)	2.12 (0.88–5.12)	1.92 (1.00–3.65) ^b	1.15 (0.54–2.48)
F7 arg353gln G>A			
vGG ($n\lambda=\lambda 7433$)	1	1	1
vGA+AA ($n\lambda=\lambda 2144$)	1.53 (1.06–2.20) ^b	1.33 (1.01–1.76) ^b	1.19 (0.89–1.60)
F7 (–323) del/ins			
vDD ($n\lambda=\lambda 6330$)	1	1	1
vDI+II ($n\lambda=\lambda 3288$)	1.08 (0.78–1.50)	1.04 (0.81–1.32)	1.05 (0.81–1.35)
F7 –402 G>A			
vGG ($n\lambda=\lambda 6105$)	1	1	1
vGA+AA ($n\lambda=\lambda 3442$)	1.00 (0.73–1.36)	1.09 (0.87–1.38)	1.00 (0.78–1.28)
F7 –401 G>T			
vGG ($n\lambda=\lambda 6315$)	1	1	1
vGT+TT ($n\lambda=\lambda 3232$)	1.04 (0.75–1.44)	1.02 (0.80–1.30)	1.06 (0.83–1.37)
F12 –46 C>T			
vCC ($n\lambda=\lambda 4537$)	1	1	1
vCT+TT ($n\lambda=\lambda 4988$)	1.11 (0.83–1.50)	1.03 (0.82–1.29)	1.19 (0.94–1.51)
F13 Val35Leu C>T			
vCC ($n\lambda=\lambda 5811$)	1	1	1
vCT+TT ($n\lambda=\lambda 3716$)	0.88 (0.65–1.20)	0.91 (0.72–1.14)	0.92 (0.72–1.17)
F13 Pro564Leu C>T			
vCC ($n\lambda=\lambda 6405$)	1	1	1
vCT+TT ($n\lambda=\lambda 3124$)	0.99 (0.72–1.36)	0.95 (0.75–1.20)	1.12 (0.87–1.44)

CHD, coronary heart disease; CI, confidence interval; HRs, hazard ratios; MI, myocardial infarction; SBP, systolic blood pressure; UC, usual care.

^a Adjusted variables for sex, ethnicity (black/nonblack), smoking status (current smoker/nonsmoker), type 2 diabetes status (yes/no), age, BMI, history of CHD, years of education, as well as baseline values of SBP, HDL cholesterol and LDL cholesterol^b Interaction ratios $\lambda=\lambda P$ value < 0.05.

Table 4
F7 arg353gln G>A gene–treatment interaction: outcome frequency, risk, HRs and IHRsAQ15

Genotype	GG		GA+AA	
Treatment	Pravastatin	UC	Pravastatin	UC
Sample size	3705	3728	1081	1063
λ Fatal CHD+nonfatal MI				
vTotal number of events	255	324	90	70
vAdjusted HR (95% CI) for prav vs. UC by genotype	0.80 (0.67–0.95)	$P\lambda=\lambda 0.01$	1.23 (0.89–1.70)	$P\lambda=\lambda 0.22$
vInteraction adjusted ratio of HRs (95% CI) prav vs. UC Combined CHD	1.00		1.53 (1.06–2.20) ^a	$P\lambda=\lambda 0.02^a$
vTotal number of events	490	548	150	124
vAdjusted HR (95% CI) for prav vs. UC by genotype	0.91 (0.81–1.04)	$P\lambda=\lambda 0.16$	1.23 (0.95–1.57)	$P\lambda=\lambda 0.11$
vInteraction adjusted ratio of HRs (95% CI) prav vs. UC All cause mortality	1.00		1.33 (1.01–1.76) ^a	$P<0.05^a$
vTotal number of events	457	465	134	113
vAdjusted HR (95% CI) for prav vs. UC by genotype	0.94 (0.82–1.08)	$P\lambda=\lambda 0.38$	1.10 (0.85–1.44)	$P\lambda=\lambda 0.46$
vInteraction adjusted ratio of HRs (95% CI) prav vs. UC	1.00		1.19 (0.89–1.60)	$P\lambda=\lambda 0.25$

CHD, coronary heart disease; CI, confidence interval; HR, hazard ratio; IHR, interaction hazard ratio; MI, myocardial infarction; Prav, pravastatin; UC, usual care.

^a Interaction ratios $\lambda=\lambda P$ value <0.05 .

Table 5
F5 arg506gln G>A gene–treatment interaction: outcome frequency, risk, HRs and IHRs

Genotype	GG		GA+AA	
Treatment	Pravastatin	UC	Pravastatin	UC
Sample size	4675	4673	127	138
λFatal CHD+nonfatal MI				
vTotal number of events	332	390	16	8
vAdjusted HR (95% CI) for prav vs. UC by genotype	0.85 (0.73–0.99)	$P_{\lambda}=0.04$	2.24 (0.91–5.55)	$P_{\lambda}=\lambda 0.08$
vInteraction adjusted ratio of HRs (95% CI) prav vs. UC Combined CHD	1.00		2.12 (0.88–5.12)	$P_{\lambda}=\lambda 0.10$
vTotal number of events	615	662	29	16
vAdjusted HR (95% CI) for prav vs. UC by genotype	0.95 (0.85–1.06)	$P_{\lambda}=\lambda 0.35$	1.87 (0.97–3.59)	$P_{\lambda}=\lambda 0.06$
vInteraction adjusted ratio of HRs (95% CI) prav vs. UC All-cause mortality	1.00		1.92 (1.00–3.65)	$P_{\lambda}=\lambda 0.05^a$
vTotal number of events	576	568	15	14
vadjusted HR (95% CI) for prav vs. UC by genotype	0.96 (0.85–1.09)	$P_{\lambda}=\lambda 0.56$	1.06 (0.48–2.32)	$P_{\lambda}=\lambda 0.89$
vInteraction adjusted ratio of HRs (95% CI) prav vs. UC	1.00		1.15 (0.54–2.48)	$P_{\lambda}=\lambda 0.71$

Smoking status taken out of model – unstable because of small numbers

CHD, coronary heart disease; CI, confidence interval; HR, hazard ratio; IHR, interaction HR; MI, myocardial infarction; Prav, pravastatin; UC, usual care.

^aInteraction ratio $\lambda = \lambda P$ value < 0.05. A Q16