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Interactions between the SNPs in the homocysteine pathway (MTHFR 677C>T, MTHFR 1298 A>C and CBSins) and the efficacy of HMG-CoA reductase inhibitors in preventing cardiovascular disease in high-risk hypertensives: The GenHAT Study

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Abstract

Background—High homocysteine blood concentrations predispose to coronary artery disease and statins influence homocysteine levels.

Aim—To study whether genes that regulate homocysteine metabolism interact with statins to modify the risk of CHD and other cardiovascular outcomes.

Methods—GenHAT is an ancillary study of the Antihypertensive and Lipid Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). The genotyped population in the lipid lowering trial (LLT) of ALLHAT included 9,624 participants randomly assigned to pravastatin or to usual care. The efficacy of pravastatin in reducing risk of all-cause mortality and CHD was compared among genotype strata (MTHFR 677 CC, CT and TT, MTHFR 1298 AA, AC, and CC, CBSins DD and I^+) by examining an interaction term in a proportional hazards model.

Results—There was no evidence of a pharmacogenetic effect on statins with the MTHFR 1298 A>C genotype for CHD risk. However, in persons with the CC variant for the MTHFR 677 C>T genotype a significantly protective effect against CHD (0.71 (95% CI 0.58–0.87)) was shown. While in the CT (1.25 (95% CI 0.97–1.61) and TT groups (0.80 (95% CI 0.50–1.28) there were no such effects (interaction hazard ratio p=0.004) The CBSins, I+ variant was associated with a significantly reduced risk for CHD among those on statin treatment (0.58 (95% CI 0.44–0.78)) while the DD genotype showed no effect from statin therapy (1.01 (95% CI 0.84–1.20; p=0.002 for interaction). For the endpoint all-cause mortality, no significant differences in efficacy were noted.

Conclusions—Polymorphisms in genes in the homocysteine pathway (MTHFR 677 C>T and CBSins) appear to modify the efficacy of pravastatin in reducing risk of cardiovascular events.

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Introduction

The efficacy of cholesterol-lowering drug therapy in primary and secondary prevention has been firmly established. The combined results of 9 large long-term statin trials showed a 27% reduction in coronary heart disease (CHD) events and a 14% reduction in all-cause mortality (1–9). These reductions, however, were average effects of statin therapy for all patients included in the trials. Pharmacogenetic findings suggest that patients may differ in their response to statins because of their genetic constitution (10).

Homocysteine is an amino acid intermediate in the conversion of methionine to cysteine. Its level is dependent on dietary intake and on the activity of the methionine and homocysteine converting enzymes methylenetetrahydrofolate reductase (MTHFR) and cystathionine-beta-synthetase (CBS) (11). Homocysteine contributes to oxidative stress and endothelial damage (12), is considered to be a risk factor for coronary artery disease, and has been shown to be increased in persons with coronary artery disease (13–15). Both genetic differences in the CBS gene and the 677C>T polymorphism in the MTHFR gene have been associated with coronary artery disease (16,17). In a meta-analysis of 15 studies, statins were shown to cause small reductions (−3.5%) in homocysteine blood concentrations, but the clinical relevance of these small effects is not known (18). Since high homocysteine blood concentrations predispose to coronary artery disease and statins seem to influence homocysteine blood concentrations, there may be an interaction between single nucleotide polymorphisms (SNPs) in genes that regulate homocysteine and statin therapy in preventing coronary artery disease. Data from a large randomized clinical trial of pravastatin designed to show efficacy against cardiovascular disease was used to investigate whether the MTHFR 677C>T polymorphism, the MTHFR 1298 A>C polymorphism or the CBSins polymorphisms are associated with the efficacy of statins.

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 1 other 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. GenHAT is a post-hoc analysis of ALLHAT-LLT. Two outcomes: 1. CHD death [fatal CHD, coronary revascularization related mortality, previous angina or MI and no known potentially lethal non coronary disease process] and non-fatal MI, and 2. all-cause mortality were studied. The design of ALLHAT, including the LLT, and its participant and clinical site recruitment and selection have been reported elsewhere(8,19–21). Briefly, ALLHAT-LLT was a randomized, open-label, large simple trial conducted from February 1994 through March 2002 at 513 clinical centers in the United States, Puerto Rico, US Virgin Islands, and Canada. The intervention was open-label pravastatin (40 mg/d) versus usual care. Participants were drawn exclusively from the ALLHAT antihypertensive trial. The protocol of ALLHAT was approved by each participating center's institutional review board. The GenHAT study was approved by the institutional review boards of the University of Minnesota and the University of Texas Health Science Center at Houston.

Genotyping

DNA was isolated on FTA paper from blood samples. 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) as described previously (22).

Statistical methods

STATA© version 9.2 (STATA Corporation, College Station, Texas) was used for all analyses. Genotypes were modeled using indicator variables with three levels for MTHFR genotypes and two levels for the CBSins variant. Only two levels were required for CBSins because the Roche strip assay cannot distinguish between a homozygote for the CBS insertion (II) and a heterozygote (ID), therefore these are combined in one group.

For each SNP, goodness-of-fit to Hardy-Weinberg expectations was assessed for each race separately. An intension-to-treat analysis was used to analyse the trial data. 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 and the ratio of hazard ratios point estimates, respectively. Baseline adjustment variables included sex, race (black/non-black), smoking status (current smoker/non-smoker), type 2 diabetes status (yes/no), age, BMI, history of CHD, years of education, SBP, HDL cholesterol and LDL cholesterol.

Results

The genotyped population in the lipid lowering component of ALLHAT (ALLHAT-LLT) included 9624 participants. All SNPs were in Hardy Weinberg equilibrium when tested in separate race groups. The proportions and baseline characteristics of subjects assigned to each treatment (pravastatin versus usual care) were well-balanced (data not shown).

In Table 1 baseline characteristics according to a) the MTHFR 677C>T genotype, b) the MTHFR 1298 A>C genotype and c) the CBSins genotype for subjects randomized to pravastatin and subjects in the usual care group are shown. For all three SNPs there was a statistically significant difference in the frequency of the genotypes between black and non-blacks participants. Furthermore there were statistically significant differences for all three SNPs in BMI, history of coronary heart disease, and HDL cholesterol levels.

The total number of trial events and the cumulative 6-year Kaplan-Meier event rates per 100 participants of clinical outcomes by ACE genotype and treatment are shown in Table 2a (MTHFR 677C>T), 2b (CBSins) and 2c MTHFR 1298 A>C. In table 2a the largest difference in the Kaplan Meier risk estimate between subjects treated with pravastatin (8.3) and subjects (11.1) on usual care is found in the participants with the CC genotype. There is almost no difference in the CT group (pravastatin: 9.6 and UC 8.5), and a smaller difference in the TT group (pravastatin 9.9 and UC 12.6). In table 2b the Kaplan Meier Risk estimate was higher for participants with the I+ genotype that were not treated with pravastatin for both outcomes (MI 12.1 and all cause mortality 14.4) compared with subjects with the DD genotype. (MI 9.7 and all cause mortality 14.3).

For the MTHRF 1298 A>C variant in table 2c the difference between the Kaplan Meier estimates between pravastatin and usual care were largest in the AA group (8,0 vs 11,0). Hazard ratios of the effect of statin use are shown by genotype, in Table 3a (MTHFR 677C>T), Table 3b (CBSIns) and 3c (MTHFR 1298 A>C). Analyses of the homocysteine genotype, treatment and clinical outcome identified several differences in efficacy of pravastatin between patients for the MTHFR 677 C>T variant. In individuals with the CC genotype a significant protective effect of the statin against CHD (0.71 (95% CI 0.58–0.87)) was shown while in the CT (1.25

(95% CI 0.97–1.61) and TT group (0.80 (95% CI 0.50–1.28) there was no statistically significant effect (interaction hazard ratio (IHR) p=0.004) (Table 3). This interaction was not found for the endpoint all cause mortality (IHR p=0.77).

For the CBSins variant, we found that subjects with the I+ variant had a significantly reduced risk on fatal CHD and nonfatal MI (0.58 (95% CI 0.44–0.78)) while the DD subjects showed no benefit from statin therapy (1.01 (95% CI 0.84–1.20). The IHR was significantly different from 1 (IHR=0.58 and p=0.002). The IHR for the outcome all cause mortality was not significantly different from 1 (IHR 0.90, p=0.45) (table 3b). For the MTHFR 1298A>C there were no differences in efficacy between the different genotypes (table 3c).

Discussion and conclusion

The GenHAT-LLT study is a large pharmacogenetic trial of pravastatin versus usual care. Based on genotype data collected from almost 10,000 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 homocysteine regulation influenced the efficacy of pravastatin for preventing fatal CHD and nonfatal MI. For the CBSins gene variant there was a trend such that subjects with the I+ variant had a larger risk reduction compared with subjects with the DD genotype. The CBS 844 ins 68 polymorphism has been shown to increase plasma homocysteine blood concentrations. In our study, the cumulative 6 year Kaplan Meier risk estimate for subjects with I+ not treated with pravastatin was higher for both outcomes compared with subjects with the DD genotype (see table 2), but after the use of pravastatin this deleterious influence was no longer present (i.e., the efficacy of pravastatin was better in subjects with the I+ genotype). Because subjects with the II and ID levels are expected to have higher plasma homocysteine blood concentrations and therefore a higher risk on coronary artery disease this meets our expectations. Statins give a reduction of homocysteine blood concentrations and might therefore have a higher efficacy in patients with higher homocysteine blood concentrations.

While for the MTHFR1298 A>C genotype no difference was seen in the efficacy of statins, we found that the MTHFR 677 C>T genotype was associated with differential statin efficacy. Subjects with the CC genotype had a significantly protective effect from statins, while there was no significantly protective effect in the CT and TT groups. The IHR was significantly different from 1 (p=0.004). This is contrary to our expectation because subjects with the TT genotype are expected to have the highest homocysteine blood concentrations (23). This might be explained if the SNP is in linkage disequilibrium with other SNPs within this gene. It is also possible that in our study population homocysteine blood concentrations were not higher in subjects with the TT genotype. Unfortunately, we are not able to measure the homocysteine blood concentrations in the GENHAT population.

Furthermore it should be considered that the interaction might not mediated by homocysteine blood concentrations. Lowering homocysteine blood concentrations with B vitamins and folic acid did not lead to a reduction of coronary heart disease and stroke in patients with myocardial infarction (24), stroke (25) or vascular disease (26) in their medical history. This suggests that the interaction might be caused by another mechanism independently of homocysteine blood concentrations.

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

Study limitations

Small numbers in the MTHFR 677C>T TT group might have lead to non-significant results in that group. Because of the large number of tests performed some significant results might be due to chance. The GenHAT study selected individuals based on pre-existing coronary risk factors and hypertension. Therefore there is some uncertainty about the applicability of the findings of this study to the association in subjects without these risk factors. In the ALLHAT study no overall beneficial effect of pravastatin was demonstrated on the primary outcome. After 6 years of follow-up, 26% of subjects in the usual care arm used statins, and 16% in the pravastatin arm did not continue to use a lipid lowering drug.

Furthermore, within the GENHAT study it is not possible to obtain serum homocysteine levels. Therefore it is not possible to study the relationship between the polymorphisms and homocysteine serum blood concentrations in GenHAT.

Future research

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

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References

1. Anonymous. Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatine Survival Study. Lancet 1994;344:1383–1389. [PubMed: 7968073]
2. Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, McFarlane PW, McKillop JH, Packard CJ. Prevention of coronary heart disease with pravastatine in men with hypercholesterolemia. New Eng J Med 1995;333(20):1301–1307. [PubMed: 7566020]
3. Sacks F, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, Cole TG, Brown L, Warnica JW, Arnold JMO, Wun CC, Davis BR, Braunwals E. The effect of pravastatine on coronary events after myocardial infarction in patients with average cholesterol levels. N Eng J Med 1996;335(14):1001–1009.
4. Anonymous. Prevention of cardiovascular events and death with pravastatine in patients with coronary heart disease and a broad range of initial cholesterol levels. N Eng J Med 1998;339(19):1349–1357.
5. Downs JR, Clearfield M, Weis S, Whitney E, Shapiro DR, Beere PA, Langendorfer A, Stein EA, Kruyer W, Gotto AM. Primary prevention of acute coronary events with lovastatine in men and women with average cholesterol levels. Results of AFCAPS/TexCAPS. JAMA 1998;279(20):1615–1622. [PubMed: 9613910]
6. Anonymous. MRC/BHF Heart protection study of cholesterol lowering with simvastatin in 20,536 high risk individuals; a randomized placebo controlled trial. Lancet 2002;360:7–22. [PubMed: 12114036]
7. Serruys PW, de Feyter P, Macaya C, Kokott N, Puel J, Vrolix M, et al. Fluvastatin for prevention of cardiac events following successful first percutaneous coronary intervention: a randomized controlled trial. Jama 2002;287(24):3215–22. [PubMed: 12076217]
8. The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT-LLT). Major outcomes in moderately hypercholesterolemic, hypertensive patients randomized to pravastatin vs usual care. JAMA 2002;288(23):2998–3007. [PubMed: 12479764]
9. The Post Coronary Artery Bypass Graft Trial Investigators. The effect of aggressive lowering of low-density lipoprotein cholesterol levels and low-dose anticoagulation on obstructive changes in

saphenous-vein coronary-artery bypass grafts. N Engl J Med 1997;336(3):153–62. [PubMed: 8992351]

10. Maitland-van der Zee AH, Klungel OH, Stricker BCh, Verschuren W, Kastelein JJP, Leufkens HGM, et al. Genetic polymorphisms: importance for response to HMG-CoA reductase inhibitors. Atherosclerosis 2002;163:213–222. [PubMed: 12052467]
11. Doevendans PA, Jukema JW, Spiering W, Defesche JC, Kastelein JJP. Molecular genetics and gene expression in atherosclerosis. Int J Cardiol 2001;80(2–3):161–72. [PubMed: 11578709]
12. Schroecksnadel K, Frick B, Winkler C, Wirleitner B, Weiss G, Fuchs D. Atorvastatin suppresses homocysteine formation in stimulated human peripheral blood mononuclear cells. Clin Chem Lab Med 2005;43(12):1373–6. [PubMed: 16309375]
13. Taylor LM Jr, Moneta GL, Sexton GJ, Schuff RA, Porter JM. Prospective blinded study of the relationship between plasma homocysteine and progression of symptomatic peripheral arterial disease. J Vasc Surg 1999;29(1):8–19. 19–21. [PubMed: 9925456]
14. Rasouli ML, Nasir K, Blumenthal RS, Park R, Aziz DC, Budoff MJ. Plasma homocysteine predicts progression of atherosclerosis. Atherosclerosis 2005;181(1):159–65. [PubMed: 15939068]
15. Surdacki A, Stochmal E, Szurkowska M, Bode-Boger SM, Martens-Lobenhoffer J, Stochmal A, et al. Nontraditional atherosclerotic risk factors and extent of coronary atherosclerosis in patients with combined impaired fasting glucose and impaired glucose tolerance. Metabolism 2007;56(1):77–86. [PubMed: 17161229]
16. Urreizti R, Asteggiano C, Vilaseca MA, Corbella E, Pinto X, Grinberg D, et al. A CBS haplotype and a polymorphism at the MSR gene are associated with cardiovascular disease in a Spanish case-control study. Clin Biochem 2007;40(12):864–8. [PubMed: 17553479]
17. Guerzoni AR, Pavarino-Bertelli EC, Godoy MF, Graca CR, Biselli PM, Souza DR, et al. Methylenetetrahydrofolate reductase gene polymorphism and its association with coronary artery disease. Sao Paulo Med J 2007;125(1):4–8. [PubMed: 17505678]
18. Jankowski P, Kawecka-Jaszcz K. Lipid-lowering drugs and homocysteine. A comparison between statins and other lipid-lowering drugs. Atherosclerosis 2004;172(1):191–4. [PubMed: 14709376]
19. 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):342–60. [PubMed: 8722437]
20. Pressel S, Davis BR, Louis GT, Whelton P, Adrogue 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(6):674–86. [PubMed: 11738123]
21. 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(6):659–73. [PubMed: 11738122]
22. 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(10):936–49. [PubMed: 10523522]
23. Garcia-Pinilla JM, Espinosa-Caliani S, Jimenez-Navarro M, Gomez-Doblas JJ, Cabrera-Bueno F, Teresa-Galvan E. Influence of 677 C-T poymorphism on medium term prognosis after acute coronary syndrom. Tex Heart Inst J 2007;34:142–7. [PubMed: 17622358]
24. Bonaa KH, Njolstad I, Ueland PM, Schirmer H, Tverdal A, Steigen T, Wang H, Nodrehaug JE, Arnesen E, Rasmussen K. Homocysteine lowering and cardiovascular events after acute myocardial infarction. N Eng J Med 354(15):1578–88.
25. Toole JFT, Malinow MR, Chambless LE, Spence JD, Pettigrew LC, Howard VJ, Sides EG, Wang CH, Stampfer M. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and Death. The Vitamin Intervention for Stroke Prevention (VISP) Randomized Controlled Trial. JAMA 2004;291:565–575. [PubMed: 14762035]
26. Anonymous. Homocysteine lowering with folic acid and B vitamins in vascular disease. The Heart Outcomes Prevention Evaluation (HOPE) 2 investigators. N Eng J Med 2006;354:1567–1577.

Table 1

Table 1a. Baseline characteristics for participants by MTHFR 677 C>T genotype

Characteristic	CC	CT	TT	p-value*
Sample size, n	5310	3446	860	
Age (y), mean (sd)	66.2 (7.6)	66.7 (7.7)	66.1 (7.5)	0.007
Race, n (col %)				
White	2340 (44.1)	2456 (71.3)	707 (82.2)	
Black	2703 (50.9)	741 (21.5)	77 (9.0)	
Other	267 (5.0)	249 (7.2)	76 (8.8)	<0.001
Sex (male), n (col %)	2678 (50.4)	1776 (51.5)	462 (53.7)	0.17
Systolic Blood Pressure (mm Hg), mean (sd)	144.9 (14.1)	145.3 (13.7)	144.3 (13.9)	0.15
Diastolic Blood Pressure (mm Hg), mean (sd)	84.1 (10.0)	83.8 (9.6)	84.1 (9.7)	0.41
Body Mass Index (kg/m^2), mean (sd)	30.1 (6.2)	29.6 (5.9)	29.6 (6.0)	0.001
Smoker, n (%)	1266 (23.8)	791 (23.0)	202 (23.5)	0.63
History of type 2 diabetes, n (%)	1866 (35.1)	1167 (33.9)	300 (34.9)	0.47
History of MI or stroke, n (%)	882 (16.6)	636 (18.5)	142 (16.5)	0.07
History of Coronary Heart Disease, n (%)	707 (13.3)	543 (15.8)	137 (15.9)	0.003
Education (y), mean (sd)	10.6 (3.9)	10.8 (4.2)	10.9 (4.3)	0.04
Total cholesterol (mg/dL), mean (sd)	223.8 (26.7)	223.2 (26.9)	223.1 (26.1)	0.54
HDL cholesterol (mg/dL), mean (sd)	48.5 (13.8)	46.4 (13.0)	45.2 (12.1)	<0.001
LDL cholesterol (mg/dL), mean (sd)	145.7 (21.1)	145.0 (21.8)	144.7 (21.1)	0.17
Antihypertensive treatment group, n (col %)				
Doxazosin	1083 (20.4)	762 (22.1)	190 (22.1)	
Chlorthalidone	1950 (36.7)	1238 (35.9)	292 (34.0)	
Amlodipine	1159 (21.8)	725 (21.0)	191 (22.2)	
Lisinopril	1118 (21.1)	721 (20.9)	187 (21.7)	0.43

Table 1b. Baseline characteristics for participants by MTHFR 1298 A>C genotype

Characteristic	AA	AC	CC	p-value*
Sample size, n	5426	3503	623	
Age (y), mean (sd)	66.2 (7.6)	66.5 (7.6)	66.9 (7.3)	0.07
Race, n (col %)				
White	2699 (49.7)	2277 (65.0)	497 (79.8)	
Black	2387 (44.0)	1014 (29.0)	88 (14.1)	
Other	340 (6.3)	212 (6.1)	38 (6.1)	<0.001
Sex (male), n (col %)	2674 (49.3)	1874 (53.5)	345 (55.4)	<0.001
Systolic Blood Pressure (mm Hg), mean (sd)	145.0 (13.9)	145.0 (14.1)	144.8 (13.3)	0.95
Diastolic Blood Pressure (mm Hg), mean (sd)	84.2 (9.8)	83.9 (9.8)	82.9 (10.2)	0.004
Body Mass Index, mean (sd)	29.9 (6.1)	29.9 (6.0)	29.5 (6.1)	0.29
Smoker, n (%)	1245 (23.0)	848 (24.2)	146 (23.4)	0.39
History of type 2 diabetes, n (%)	1895 (34.9)	1194 (34.1)	215 (34.5)	0.72
History of MI or stroke, n (%)	884 (16.3)	631 (18.0)	131 (21.0)	0.004

Table 1b. Baseline characteristics for participants by MTHFR 1298 A>C genotype				
History of CHD, n (%)	747 (13.8)	518 (14.8)	115 (18.5)	0.005
Education (y), mean (sd)	10.6 (4.1)	10.9 (4.0)	11.3 (3.9)	<0.001
Total cholesterol (mg/dL), mean (sd)	223.8 (26.4)	223.4 (27.0)	222.1 (27.2)	0.29
HDL cholesterol (mg/dL), mean (sd)	48.0 (13.6)	46.8 (13.2)	45.6 (13.4)	<0.001
LDL cholesterol (mg/dL), mean (sd)	145.6 (21.1)	145.3 (21.7)	144.0 (21.1)	0.18
Antihypertensive treatment group, n (col %)				
Doxazosin	1168 (21.5)	706 (20.2)	144 (23.1)	
Chlorthalidone	1959 (36.1)	1282 (36.6)	222 (35.6)	
Amlodipine	1162 (21.4)	769 (22.0)	128 (20.6)	
Lisinopril	1137 (21.0)	746 (21.3)	129 (20.7)	0.66

Table 1c. Baseline characteristics for participants by CBSins genotype			
Characteristic	**DD**	**II or ID**	**p-value***
Sample size, n	7061	2543	
Age (y), mean (sd)	66.4 (7.6)	66.2 (7.8)	0.25
Race, n (col %)			
White	4566 (64.7)	931 (36.6)	
Black	1971 (27.9)	1544 (60.7)	
Other	524 (7.4)	68 (2.7)	<0.001
Sex (male), n (col %)	3654 (51.8)	1255 (49.4)	0.04
Systolic Blood Pressure (mm Hg), mean (sd)	144.9 (13.8)	145.3 (14.3)	0.21
Diastolic Blood Pressure (mm Hg), mean (sd)	83.9 (9.7)	84.3 (10.1)	0.07
Body Mass Index, mean (sd)	29.7 (6.0)	30.3 (6.3)	<0.001
Smoker, n (%)	1659 (23.5)	599 (23.6)	0.95
History of type 2 diabetes, n (%)	2413 (34.2)	913 (35.9)	0.12
History of MI or stroke, n (%)	1207 (17.1)	449 (17.7)	0.52
History of CHD, n (%)	1061 (15.0)	324 (12.7)	0.004
Education (y), mean (sd)	10.8 (4.1)	10.5 (3.9)	<0.001
Total cholesterol (mg/dL), mean (sd)	223.6 (26.9)	223.3 (26.1)	0.63
HDL cholesterol (mg/dL), mean (sd)	47.0 (13.3)	48.7 (13.7)	<0.001
LDL cholesterol (mg/dL), mean (sd)	145.3 (21.5)	145.5 (21.0)	0.61
Antihypertensive treatment group, n (col %)			
Doxazosin	1501 (21.3)	533 (21.0)	
Chlorthalidone	2573 (36.4)	905 (35.6)	
Amlodipine	1516 (21.5)	551 (21.7)	
Lisinopril	1471 (20.8)	554 (21.8)	0.73

* tests for differences between groups: ANOVA for continuous variables, chi-square for categorical variables

Table 2

Table 2a. Total number of trial events and cumulative 6-year Kaplan-Meier risk estimates of clinical outcomes for MTHFR 677C>T variant						
Genotype:	**CC**		**CT**		**TT**	
Treatment:	**Pravastatin**	**U.C.**	**Pravastatin**	**U.C.**	**Pravastatin**	**U.C.**
sample size	**2658**	**2652**	**1718**	**1728**	**425**	**435**
Fatal CHD + Nonfatal MI						
total number of events	171	239	140	119	37	41
cum. 6 yr KM risk est. per 100 participants	8.3	11.1	9.6	8.5	9.9	12.6
All cause mortality						
total number of events	342	335	203	196	47	51
cum. 6 yr KM risk est. per 100 participants	15.2	14.3	13.7	14.0	12.3	14.9

Table 2b. Total number of trial events and cumulative 6-year Kaplan-Meier risk estimates of clinical outcomes for CBSins variant				
Genotype:	DD		I+	
Treatment:	Pravastatin	U.C.	Pravastatin	U.C.
sample size	3533	3528	1265	1278
Fatal CHD + Nonfatal MI				
total number of events	269	267	78	131
cumulative 6 year KM risk est. per 100 participants	9.3	9.7	7.9	12.1
All cause mortality				
total number of events	424	407	167	174
cumulative 6 year KM risk est. per 100 participants	14.0	14.3	15.6	14.4

Table 2c. Total number of trial events and cumulative 6-year Kaplan-Meier risk estimates of clinical outcomes for MTHFR 1298A>C variant						
Genotype:	AA		AC		CC	
Treatment:	Pravastatin	U.C.	Pravastatin	U.C.	Pravastatin	U.C.
sample size	2687	2739	1774	1729	309	314
Fatal CHD + Nonfatal MI						
total number of events	187	231	137	136	24	30
cumulative 6 year KM risk est. per 100 participants	8.0	11.0	10.0	9.0	12.0	11.0
All cause mortality						
total number of events	322	351	224	192	42	38

Table 2c. Total number of trial events and cumulative 6-year Kaplan-Meier risk estimates of clinical outcomes for MTHFR 1298A>C variant						
cumulative 6 year KM risk est. per 100 participants	13.7	15.5	15.2	12.6	16.5	13.2

Table 3

Table 3a. Gene-treatment interaction: HRs and IHRs for MTHFR 677C>T variant						
Genotype:	**CC**		**CT**		**TT**	
Treatment:	**Pravastatin**	**U.C.**	**Pravastatin**	**U.C.**	**Pravastatin**	**U.C.**
Fatal CHD + Nonfatal MI						
adjusted HR (95% CI)	**0.71 (0.58–0.87)***		1.25 (0.97–1.61)		0.80 (0.50–1.28)	
interaction adjusted ratio of HRs (95% CI)	1.00		**1.73 (1.25–2.40)***		1.14 (0.69–1.89)	
Ho: interaction coefficients jointly equal zero	**p=0.004**					
All cause mortality						
adjusted HR (95% CI)	0.97 (0.83–1.13)		1.00 (0.82–1.12)		0.79 (0.52–1.21)	
interaction adjusted ratio of HRs (95% CI)	1.00		1.04 (0.81–1.35),		0.88 (0.57–1.37)	
Ho: interaction coefficients jointly equal zero	p=0.766					

Table 3b. Gene-treatment interaction: HRs and IHRs for CBSins variant				
Genotype:	DD		I+	
Treatment:	Pravastatin	U.C.	Pravastatin	U.C.
Fatal CHD + Nonfatal MI				
adjusted HR (95% CI)	1.01 (0.84–1.20)		**0.58 (0.44–0.78)***	
interaction adjusted ratio of HRs (95% CI)	1.00		**0.58 (0.42–0.82), p=0.002**	
All cause mortality				
adjusted HR (95% CI)	1.00 (0.87–1.15)		0.91 (0.72–1.13)	
interaction adjusted ratio of HRs (95% CI)	1.00		0.90 (0.69–1.18), p=0.448	

Table 3c. Gene-treatment interaction: HRs and IHRs for MTHFR 1298A>C variant						
Genotype:	AA		AC		CC	
Treatment:	Pravastatin	U.C.	Pravastatin	U.C.	Pravastatin	U.C.
Fatal CHD + Nonfatal MI						
adjusted HR (95% CI)	0.87 (0.71–1.06), p=0.179		0.93 (0.73–1.19), p=0.545		0.71 (0.40–1.26), p=0.240	
interaction adjusted ratio of HRs (95% CI)	1.00		1.07 (0.78–1.47), p=0.657		0.85 (0.47–1.56), p=0.610	
All cause mortality						
adjusted HR (95% CI)	0.92 (0.79–1.08), p=0.313		1.05 (0.86–1.29), p=0.612		1.04 (0.65–1.64), p=0.878	
interaction adjusted ratio of HRs (95% CI)	1.00		1.15 (0.89–1.49), p=0.277		1.10 (0.68–1.77), p=0.703	