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Genetic Variation at the LDL Receptor and HMG CoA Reductase Gene Loci, Lipid Levels, Statin Response, and Cardiovascular Disease Incidence in PROSPER

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Abstract

Our purpose was to evaluate associations of single nucleotide polymorphisms (SNPs) at the low density lipoprotein (LDL) receptor (LDLR C44857T, minor allele frequency (MAF) 0.26, and A44964G, MAF 0.25, both in the untranslated region) and HMG-CoA reductase (HMGCR i18T>G, MAF 0.019) gene loci with baseline lipid values, statin induced LDL- cholesterol (C) lowering response, and incident coronary heart disease (CHD) and cardiovascular disease on trial (CVD). Our population consisted of 5804 elderly men and women with vascular disease or one or more vascular disease risk factors, who were randomly allocated to pravastatin or placebo. Other risk factors and apolipoprotein (apo) E phenotype were controlled for in the analysis. Despite a prior report, no relationships with the HMGCR SNP were noted. For the LDLR SNPs C44857T and A44964G we noted significant associations of the rare alleles with baseline LDL-C and

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triglyceride levels, a modest association of the C44857T with LDL-C lowering to pravastatin in men, and significant associations with incident CHD and CVD of both SNPs, especially in men on pravastatin. Our data indicate that genetic variation at the LDLR locus can affect baseline lipids, response to pravastatin, and CVD risk in subjects placed on statin treatment.

Keywords

Genetics; Statins; Low density lipoproteins (LDL); Coronary Heart Disease (CHD); HMGCR gene; LDLR gene

Elevated levels of plasma low density lipoprotein (LDL) cholesterol (C) are a major independent risk factor for coronary heart disease (CHD) and cardiovascular disease (CVD, CHD plus stroke). The LDL receptor (R) plays a critical role in the catabolism of LDL¹. Moreover 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (HMGCR) is the rate limiting enzyme in cholesterol synthesis, and statins are competitive inhibitors of this process. Statin therapy has been shown to lower LDL levels because of enhanced fractional clearance related to upregulation of LDL receptor activity. Mutations at the LDLR gene locus are the cause of familial hypercholesterolemia^{2,3}, and these subjects have markedly elevated plasma LDL-C levels and premature death and disability from CHD⁴. Genetic variation at the LDLR gene locus in the general population can also affect LDL-C. Muallem et al. reported that single nucleotide polymorphisms (SNPs) at the 3'UTR significantly alter plasma LDL-C levels in Caucasian, but not in African American subjects⁵. Chasman et al. reported that the presence of two highly linked SNPs in HMGCR was related to pravastatin induced LDL-C lowering response⁶.

Our aim was to determine if SNPs at the 3'UTR LDLR region and in intron 18 at HMGCR could be related to baseline lipids, baseline vascular disease, lipid lowering response to pravastatin, and CHD and CVD risk in the PROSPER (Prospective Study of Pravastatin in the Elderly at Risk) Study. In this study 5804 male and female subjects, mean age 75.3 years, were selected for having a history of vascular disease or CHD risk factors (smoking, hypertension, or diabetes), and were randomized to either pravastatin 40 mg/day or placebo and were followed for a mean of 3.2 years.¹⁰ The use of pravastatin in PROSPER was associated with significant CHD risk reduction as compared to the placebo group.

Materials and Methods

Study Subjects

The protocol of PROSPER has previously been published⁷, as have the PROSPER results.⁸ In this study 2804 men and 3000 women, aged 70 to 82, with pre-existing vascular disease or at least one of three major vascular risk factors (diabetes, smoking, or hypertension) were randomized to pravastatin 40 mg/day (n = 2891) or placebo (n = 2913). The mean LDL-C reduction in this study in the active group was 32%, and the risk of developing coronary heart disease (CHD) was decreased by 19% over 3.2 years, which was statistically significant. This effect would translate into an estimated 30% risk reduction in CHD events over 5 years, consistent with other statin trials. Other changes in the treatment group were

that high density lipoprotein (HDL) C was increased by 5%, and triglycerides were decreased by 12% versus baseline in those placed on pravastatin. In those judged to have good compliance (i.e. taking medication more than 75% of the time), these alterations on the lipid levels were even greater at 34%, 5%, and 13%, respectively. No significant lipid changes were noted in the placebo group. Lipid values were virtually identical at onset of the study in subjects randomized to pravastatin or placebo.

Biochemical and DNA Analysis

Total cholesterol (TC), HDL-C, and triglycerides were assessed after an overnight fast, at baseline and at 6 months, and LDL-C was calculated by the Friedewald formula, as previously described.⁷⁻⁸ DNA was isolated from cells from this cohort and DNA samples from 5783 subjects were available to us. ApoE phenotype was determined on plasma samples by Western blotting, using the method of Havekes et al⁹ in the central laboratory of the Royal Infirmary in Scotland. Subjects were classified according to the presence of apoE2, apoE3, or apoE4 bands on gel blotting.

For DNA analysis we genotyped two single nucleotide polymorphisms (SNPs) in the 3'UTR of the LDLR gene, C44857T (rs1433099), and A44964G (rs2738466) and one SNP in the intron 18 T>G (rs17238540) of the HMGCR gene the using Taq Man® SNPs genotyping assays (Applied Biosystems, Foster City CA). The custom assays IDs are C_2018188_10, C_998744_10 and C__25652066_10 respectively. For reference the Genbank/EMBL accession numbers were NC 000019.8, mim 606945 for the LDLR gene and NC NC_000005.8, mim 142910. The end point was read after PCR amplification was performed using an Applied Biosystems 7900 HT Sequence Detection System. Genotypes with quality scores below the 95% were repeated and 5% blinded replicates for genotype determinations were performed. In addition, a total of 119 subjects or 2.2% who had the apoE4/2 phenotype were excluded from these analyses, as well as 246 subjects who had missing apoE phenotypes. These exclusions were carried out because apoE phenotype or genotype can affect statin induced LDL lowering response, as well as CHD risk. Subjects carrying the apoE4 allele have shown a lesser response in terms of LDL lowering and the highest CHD risk than in others, and apoE2 and apoE4 phenotypes have opposite effects in this regard.¹⁰⁻¹⁴ The subject characteristics for these individuals comprising 2621 men and 2796 women are shown in Table 1

Statistical Analysis

Observed genotype frequencies were compared with those expected under Hardy-Weinberg equilibrium using a χ^2 test. For data analysis, multivariable analysis of covariance (ANCOVA) was performed to detect associations between the lipoprotein levels at baseline as well as changes in response to the treatment with pravastatin at 6 month, and the LDLR and HMGCR genotypes adjusted for gender, body mass index, age, alcohol consumption, smoking status, diabetes, apoE phenotype, and country of origin, since subjects participating in PROSPER were either from Scotland, Ireland, or the Netherlands. Prevalence of both myocardial infarction (MI) and all vascular diseases (history of angina, claudication, MI, stroke, transient ischaemic attack, peripheral arterial disease surgery or amputation for vascular disease more than 6 months before study entry) at baseline, as well as incidence of

primary endpoints (CHD death or non-fatal MI or fatal or non-fatal stroke), and all cardiovascular events (primary endpoints and coronary artery bypass grafting, coronary angioplasty, and peripheral arterial surgery or angioplasty) was compared between carriers of different LDLR and HMGCR SNP genotypes using multivariable logistic regression analysis in all subjects and stratified by gender and treatment. All analyses were fully adjusted for the above covariates plus history of vascular disease, hypertension and randomized treatment. To evaluate the modifying effects of genotypes and gender on the response to treatment, gene-treatment and gene-gender interaction terms were added to the regression models. Lewontin's D value was calculated to assess the linkage disequilibrium (LD) between the two SNPs of interest.¹⁵ Haplotype analysis including both genotyped markers was carried out. All analyses were performed using SAS/STAT and SAS/Genetics [including proc haplotype procedure] (SAS version 9.1, SAS Institute, Inc., Cary, NC). A two-sided $p < 0.05$ was considered statistically significant.

Results

As can be seen from Table 1, as a group these subjects were elderly, with a mean age of 75 years (range 70–82 years at baseline). Their mean LDL-C levels were in the moderate-risk category (130–160 mg/dl), as defined by the United States National Cholesterol Education Program. More than 50% of the men and more than 30% of the women had a history of vascular disease at baseline. Data on allele frequencies for the C44857T and A44964G polymorphisms at LDLR and i18 T>G at the HMGCR gene are shown in Table 1, along with the apoE phenotype distribution in this population. The distribution of genotypes from all the SNPs were in Hardy – Weinberg equilibrium ($p > 0.05$, data not shown). There was no association with the presence of the minor allele i18 T>G at the HMGCR gene locus and baseline lipid, baseline vascular disease, LDL-C lowering response to pravastatin or on trial CHD or CVD outcomes (data not shown). For the GG genotype carriers, comprising 3.7% of the study population, the mean LDL-C lowering response (%) to pravastatin was -36.6% ($n=70$), while for non-carriers it was -36.0% ($n=1832$) in the fully adjusted model ($p=ns$) with subjects who reportedly had good compliance. Similar reductions were observed for the entire group of carriers ($n=102$) versus non-carriers ($n=2657$) placed on statins (ns).

Table 2 provides adjusted mean values stratified by sex. Our findings show that both men and women with the T allele at the C44857T locus had significantly lower levels of LDL-C (a difference of 2.7% or 4.0 mg/dl between TT and CC carriers in men and women combined, $p=0.039$), and this was also the case for total cholesterol ($p=0.031$). With regard to A44964G, the G allele was associated with lower TC (a difference of 1.2% or 2.5 mg/dl between GG and AA carriers, $p=0.002$), lower LDL-C (3.3% or 4.5 mg/dl, $p=0.001$), and higher TG which reached statistical significance in men only (Table 2). GG carriers also had significantly higher TG levels in a combined group of men and women (with a difference of 6.1% or 9.3 mg/dl compared to AA carriers, $p=0.009$, data not shown).

Haplotype analysis detected that the two SNPs in the 3'UTR LDLR region under study were in linkage disequilibrium ($D'=0.997$) and revealed three common haplotypes: C44857T[C]-A44964G[A], C44857T[C]-A44964G[G] and C44857T[T]-A44964G[A] present in 49%, 25% and 26%, respectively, and a very rare haplotype C44857T[T]-A44964G[G] present in

less than 0.1% of the participants. Carriers for the *C44857T[T]-A44964G[A]* had 2.2% lower TC levels and 3% lower LDL-C levels per each haplotype copy than those with the *C44857T[C]-A44964G[A]* haplotype ($p=0.001$), while individuals with the *C44857T[C]-A44964G[G]* haplotype had 1.74% lower TC levels ($p=0.013$) and 3.1% lower LDL-C levels ($p=0.0007$) than those with the *C44857T[C]-A44964G[A]* haplotype.

In Table 3, data on response to pravastatin in terms of percent LDL-C lowering are provided. Carriers for the minor allele at the C44857T SNP had a significantly, but modestly better LDL-C lowering response than non carriers ($p=0.03$). It should be noted that our analysis was based on people who reportedly had good compliance (i.e. taking their medication more than 75% of the time). However, the same effects were observed in the entire group (data not shown).

In Table 4, the hazard ratios (HR) on trial for new onset of the primary endpoint and all cardiovascular events are shown for the LDLR SNPs. Those with the TT genotype at C44857T had a HR of 0.66 (CI 0.48–0.92) for the primary endpoint in the fully adjusted model when compared to non-carriers. This association was mostly driven by males in the pravastatin group (Table 4). No such associations were noted in individuals on placebo or in women (data not shown). Regarding the A44964G for the primary endpoint and cardiovascular events, carriers of the GG genotype had a higher HR than non-carriers with the greatest risk in men on pravastatin. For primary endpoints, the adjusted HR was 1.32 (CI 1.02–1.71) in all individuals in the fully adjusted model and 2.23 (CI 1.37–3.63) in males on pravastatin. For cardiovascular events, the adjusted HR is 1.29 (CI 1.00–1.65) in the fully adjusted model and HR 1.97 (CI 1.23–3.16) in males on pravastatin. There was no significant difference in HR in all subjects on placebo or in women on pravastatin (Table 4). In haplotype analysis, those carrying the *C44857T[T]-A44964G[A]* haplotype had a lower risk for primary endpoints (HR 0.69, CI 0.52–0.90) and cardiovascular events (HR 0.74, CI 0.57–0.95) than the *C44857T[C]-A44964G[G]* haplotype carriers. The same was observed in subjects on pravastatin (HR 0.37, CI 0.21–0.66). In addition, in the men on pravastatin, carriers of *C44857T[C]-A44964G[A]* showed a trend toward lower risk of primary endpoints as compared to the *C44857T[C]-A44964G[G]* carriers (HR 0.59, CI 0.36–0.99) and lower risk of cardiovascular events (HR 0.62, CI, 0.38–1.00) (data not shown). The odds ratios (OR) of developing any form of vascular disease (angina, claudication, MI, stroke, transient ischemic attack, coronary angioplasty or bypass, or peripheral vascular surgery or angioplasty) or MI at baseline was not significantly associated with any of the SNPs (data not shown).

Discussion

Genetic variation at the HMGCR locus has been reported to affect LDL-C lowering response to pravastatin⁶. Chasman et al examined 148 SNPs at 10 candidate gene loci following 6 months of pravastatin therapy (ABCG5, ABCG8, APOB, APOE, CETP, CYP3A4), CYP3A5, FDFT1, HMGCR, and LDLR) in 1536 subjects and reported that the rare G allele at one of two tightly linked SNPs, (frequency 0.033) at the intron 18 SNP i18 T>G was related to a significantly less percent LDL-C lowering response (–27.2% for heterozygotes, $n=100$, versus –34.3% for non-carriers, $n=1,418$). In our study we noted a

lower frequency for the rare allele at this SNP (0.019) than that reported by Chasman et al, possibly because their population was more diverse. We also saw no differences with regard to the presence or absence of this rare allele at HMGCR and baseline lipids, baseline vascular disease, percent LDL-C lowering response to pravastatin, or on trial CHD or CVD events. Similar to our data, a recent study by Singer et al also found no associations between this SNP and response to fluvastatin in the ALERT study¹⁶. Chasman et al also examined SNPs at the LDLR locus including one (A44964G) that we have examined. The authors reported no significant differences with regard to A44964G and percentage LDL-C lowering response to pravastatin, consistent with our findings⁶.

Our collaborators H. Muallem and N. Maeda examined LDLR SNPs in the Atherosclerosis Risk in Communities (ARIC) study. They also noted that the T allele at C44857T was associated with significantly lower LDL-C levels, and these differences were statistically significant for the men, but not for women. In our studies we also noted lower LDL-C levels associated with this allele, and these differences were statistically significant in women, as well as in the entire group after adjustment. With regard the G allele at A44964G at the LDLR locus Muallem et al noted no significant effects on baseline lipids in ARIC, but they did not evaluate effects on TG levels. In our studies we found that this allele was associated with significant increases in triglyceride levels in the entire group as well as in men only, and significant decreases in LDL-C values in men only. In terms of haplotype analysis, Muallem et al reported that those with the *C44857T[T]- A44964G[A]* haplotype had lower LDL-C and TC levels, while those with the *C44857T[C]- A44964G[A]* haplotypes had higher LDL-C and TC. In our view these findings are driven by the C44857T allele, since this is the one that changes in these two haplotypes. We also found that those with *C44857T[T]- A44964G[A]* haplotype had lower levels of LDL-C (3.0%, $p=0.0014$) than subjects with the major haplotype *C44857T[C]- A44964G[A]*, and that those with the *C44857T[C]- A44964G[G]* haplotype had 3.1% lower levels of LDL-C ($p=0.0007$). In summary, carriers of haplotypes which include one of the alleles that are associated with lower LDL-C for any of the two SNPs, have a lower LDL-C levels than the major *C44857T[C]- A44964G[A]* haplotype.

Another novel feature of our study is that we have examined effects of these SNPs on cardiovascular endpoints. We report that the minor allele for the C44857T was associated with lower risk for CHD on trial, which is probably related to lower LDL-C levels¹⁷⁻¹⁹. Regarding the A44964G SNP, the minor allele is associated with higher risk of CHD on trial and in the haplotype analyses the HR is, in our view, driven by the A allele. These data suggest that carriers for the minor allele not only have higher TG levels, but also have higher CHD risk, especially in males on pravastatin. In conclusion, our overall data are consistent with the concepts that variation at the HMGCR_i15T>G SNP is not associated with baseline lipids, statin response, or CVD risk, but that variation at LDLR SNPs C44857T and A44964G can affect baseline lipids, statin response and CVD risk.

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Table 1

Subject and Genetic Characteristics N=5417

Study characteristics Mean(SD) ^a	Men (N=2621)	Women (N=2796)
Age, years	74.99 (3.26)	75.64 (3.38) ^b
BMI, kg/m ²	26.56 (3.59)	27.12 (4.66) ^b
History Diabetes mellitus, N (%)	324 (12.36)	251 (8.98) ^b
History Hypertension, N (%)	1333 (50.86)	2026 (72.46) ^b
History Vascular Disease, N (%)	1371 (52.31)	1033 (36.95) ^b
Current smoking, N (%)	847 (32.32)	586 (20.96) ^b
Alcohol consumption, N (%)	1851 (70.62)	1165 (41.67) ^b
Total Cholesterol, mg/dl	207.0 (30.7)	231.9 (34.5) ^b
LDL-Cholesterol, mg/dl	138.5 (27.8)	154.9 (35.3) ^b
HDL-Cholesterol, mg/dl	45.6 (12.2)	53.0 (13.4) ^b
VLDL-Cholesterol, mg/dl	23.0 (13.6)	24.0 (13.7)
Triglyceride, mg/dl	132.4 (64.3)	140.6 (59.4) ^b
apoA-I, mg/dl	124.4 (22.2)	139.9 (24.1) ^b
apoB, mg/dl	110.6 (21.3)	119.1 (22.6) ^b
apoE 2/2 + 2/3, %	342 (13.05)	315 (11.27)
apoE 3/3, %	1655 (63.14)	1837 (65.70)
apoE 3/4 + 4/4, %	624 (23.81)	644 (23.03)
LDLR_C44857T- rs1433099	MAF T:0.26	
LDLR_A44964G- rs2738466	MAF G:0.25	
HMGCR_i18 T>G- rs17238540	MAF G:0.019	

^a means (SD) unless otherwise specified; differences between men and women were assessed using a t-test for continuous variables, and χ^2 test for binary traits; MAF – minor allele frequency.

^b p<0.001, mean values are presented, apoE 2/4 carriers were excluded from the analysis (see Material and Methods section).

Table 2Adjusted Baseline Lipid Levels by Gender (mean $\pm$ SE, mg/dl) and LDLR gene SNPs.

LDLR SNPs	C44857T			P			A4964G			P
	CC	CT	TT				AA	AG	GG	
Men, N	1524	1081	183				1458	977	182	
TC	204 $\pm$ 1	203 $\pm$ 1	201 $\pm$ 2	0.60			205 $\pm$ 1	201 $\pm$ 1	202 $\pm$ 2	0.002
LDL-C	134 $\pm$ 1	134 $\pm$ 1	133 $\pm$ 2	0.87			136 $\pm$ 1	132 $\pm$ 1	131 $\pm$ 2	0.001
HDL-C	46 $\pm$ 1	46 $\pm$ 1	45 $\pm$ 1	0.61			46 $\pm$ 1	46 $\pm$ 1	45 $\pm$ 1	0.78
TG	140 $\pm$ 2	141 $\pm$ 3	140 $\pm$ 5	0.9			140 $\pm$ 2	139 $\pm$ 3	156 $\pm$ 5	0.003^a
Women, N	1430	1005	181				1621	1008	165	
TC	223 $\pm$ 2	220 $\pm$ 2	217 $\pm$ 3	0.02^a			221 $\pm$ 1	222 $\pm$ 2	220 $\pm$ 3	0.61
LDL-C	146 $\pm$ 1	144 $\pm$ 1	139 $\pm$ 2	0.009^a			144 $\pm$ 1	145 $\pm$ 1	143 $\pm$ 3	0.61
HDL-C	53 $\pm$ 1	54 $\pm$ 1	52 $\pm$ 1	0.60			53 $\pm$ 1	54 $\pm$ 1	53 $\pm$ 1	0.80
TG	149 $\pm$ 2	145 $\pm$ 3	150 $\pm$ 5	0.23			148 $\pm$ 3	146 $\pm$ 3	153 $\pm$ 3	0.56

Adjusted for BMI, age, alcohol, smoking, diabetes, apoE phenotype, and country.

^a p values also < 0.05 in the men and women combined and adjusted for gender

Table 3

Percent LDL-C Response to Pravastatin by LDLR SNP Genotype

SNP	Adjusted Mean Percent LDL-Cholesterol Reduction ^a				p ^b
	N	Men	N	Women	
C44857T					
CC	530	-35.5 ± 1	534	-35.8 ± 1	
CT	343	-36.8 ± 1	358	-36.7 ± 1	0.08
TT	67	-34.7 ± 1	66	-37.5 ± 1	0.03 ^c
A44964G					
AA	523	-36.1 ± 1	543	-35.9 ± 1	
AG	363	-35.9 ± 1	361	-36.6 ± 1	0.75
GG	54	-34.8 ± 1	56	-36.4 ± 1	

^a Values are provided as mean ± SE;

^b p values for data combining men and women, adjusted for gender, BMI, age, alcohol, smoking, diabetes, apoE phenotype, and country;

^c p value for CC vs CT + TT

Table 4

Analysis of Incidence of New Cardiovascular Events ^a on Trial by LDLR SNPs.

C44857T	Genotype	Primary endpoints ^a		Cardiovascular events ^a	
		New case/total subjects (%)	HR ^b	New case/total subjects (%)	HR ^b
Unadjusted	CC	463/2954 (15.7)	1	508/2954 (17.2)	1
	CT	303/2086 (14.5)	0.91 (0.79–1.05)	337/2086 (16.2)	0.92 (0.81–1.06)
	TT	39/364 (10.7)	0.67 (0.48–0.92)	49/364 (13.5)	0.76 (0.57–1.02)
Adjusted ^c	CC	463/2954 (15.7)	1	508/2954 (17.2)	1
	CT	303/2086 (14.5)	0.92 (0.80–1.07)	337/2086 (16.2)	0.93 (0.82–1.07)
	TT	39/364 (10.7)	0.66 (0.48–0.92)	49/364 (13.5)	0.76 (0.56–1.02)
On pravastatin ^d	CC	223/1492 (14.9)	1	244/1492 (16.6)	1
	CT	128/993 (12.9)	0.85 (0.68–1.06)	143/993 (14.4)	0.87 (0.70–1.07)
	TT	19/192 (9.90)	0.65 (0.41–1.03)	24/192 (12.5)	0.76 (0.50–1.15)
Males on pravastatin ^e	CC	133/729 (18.2)	1	145/729 (19.9)	1
	CT	60/475 (12.6)	0.66 (0.49–0.90)	72/475 (15.16)	0.73 (0.55–0.97)
	TT	11/95 (11.6)	0.65 (0.35–1.21)	16/95 (16.8)	0.88 (0.53–1.49)
A44964G					
Unadjusted	AA	436/3079 (14.2)	1	488/3079 (15.8)	1
	AG	303/1985 (15.3)	1.08 (0.93–1.25)	335/1985 (16.9)	1.07 (0.93–1.23)
	GG	66/347 (19.0)	1.38 (1.06–1.78)	71/347 (20.5)	1.33 (1.04–1.71)
Adjusted ^c	AA	436/3079 (14.2)	1	488/3079 (15.8)	1
	AG	303/1985 (15.3)	1.08 (0.94–1.26)	335/1985 (16.9)	1.07 (0.93–1.23)
	GG	66/347 (19.0)	1.32 (1.02–1.71)	71/347 (20.5)	1.29 (1.00–1.65)
On pravastatin ^d	AA	192/1504 (12.8)	1	215/1504 (14.3)	1
	AG	146/1028 (14.2)	1.11 (0.89–1.38)	161/1028 (15.7)	1.09 (0.89–1.34)
	GG	33/149 (22.1)	1.81 (1.25–2.62)	36/149 (24.2)	1.79 (1.26–2.55)
Males on pravastatin ^e	AA	97/718 (13.5)	1	114/718 (15.9)	1
			0.004		0.013

C44857T	Primary endpoints ^a			Cardiovascular events ^a		
	Genotype	New case/total subjects (%)		p	New case/total subjects (%)	
		HR ^b	p		HR ^b	p
AG	AG	87/512 (17.0)	1.27 (0.95–1.70)		98/512 (19.1)	1.22 (0.93–1.61)
GG	GG	20/70 (28.6)	2.23 (1.37–3.63)		21/70 (30)	1.97 (1.23–3.16)

^aCardiovascular disease characteristics are provided in Materials and Methods;

^b hazards ratio (95% Confidence Intervals) are presented;

^c p values for combined men and women data, adjusted for gender, BMI, age, alcohol, smoking, diabetes, hypertension, apoE phenotype, randomized treatment, and country;

^d p values for combined male and female data, adjusted for gender, BMI, age, alcohol, smoking, diabetes, hypertension, apoE phenotype, and country;

^e p values for men: adjusted for BMI, age, alcohol, smoking, diabetes, hypertension, apoE phenotype, and country.