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Genetic Variation at the PCSK9 Locus Moderately Lowers Low Density Lipoprotein Cholesterol Levels, But Does Not Significantly Lower Vascular Disease Risk in an Elderly Population

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Abstract

Caucasian carriers of the T allele at R46L in the proprotein convertase subtilisin/kexin type 9 (PCSK9) locus have been reported to have 15% lower low density lipoprotein (LDL) cholesterol (C) levels and 47% lower coronary heart disease (CHD) risk. Our objective was to examine two PCSK9 single nucleotide polymorphisms (SNPs), R46L and E670G, in 5,783 elderly participants in PROSPER (Prospective Study of Pravastatin in the Elderly at Risk), of whom 43% had a history of vascular disease at baseline, and who were randomized to pravastatin or placebo with followup. In this population 3.5% were carriers of the T allele at R46L, and these subjects had significantly ($p<0.001$) lower levels of LDL C (mean, −10%), no difference in LDL C lowering response to pravastatin, and a non-significant 19% unadjusted and 9% adjusted decreased risk of vascular disease at baseline, with no on trial effect. Moreover 6.0% were carriers of the G allele at E670G with no significant relationships with baseline LDL C, response to pravastatin, or vascular disease

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risk being observed. Our data support the concept that the rare allele of the R46L SNP at the PCSK9 locus significantly lowers LDL C, but does not greatly reduce CHD risk in an elderly population with a high prevalence of cardiovascular disease.

Keywords

Genetics; Statins; Low density lipoproteins (LDL); Coronary Heart Disease (CHD); Proprotein convertase subtilisin/kexin type 9 serine protease (PCSK9); Vascular Disease; Elderly

The proprotein convertase subtilisin/kexin type 9 serine protease gene (PCSK9) results in a gene product that is involved in the regulation of the number of low density lipoprotein (LDL) receptors (R) on the cell surface, and was first identified by Seidah et al[1] and subsequently by Abifadel et al and others. [2–4] Genetic variation at PCSK9 has been reported to significantly affect LDL cholesterol (C) levels in the plasma, LDL C lowering response to statins, and risk for premature coronary heart disease (CHD).[5–9] We sought to examine these issues further in the PROSPER study (Prospective Study of Pravastatin in the Elderly at Risk). 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.[10,11] The use of pravastatin in PROSPER was associated with a significant CHD risk reduction as compared to the placebo group. Our data are consistent with the concept that genetic variation at PCSK9 is significantly associated with baseline LDL C, but not to statin LDL lowering response or baseline or prospective CHD risk in an elderly population.

Materials and Methods

Study Subjects

The protocol of PROSPER has previously been published [10], as have the PROSPER results.[11] 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. 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 (T) C, HDL C, apolipoproteins (apo) A-I and B 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.[10-] DNA was isolated from cells from this cohort and we received DNA from 5783 subjects. Women in this study were significantly ($p<0.001$) different from the men in that they were older, had a higher body mass index, had less diabetes, had more hypertension, were less likely to smoke or consume alcohol. They also had higher triglyceride, TC, LDL C, apoB, HDL C, and apoA-I levels, and were less likely to have vascular disease.

ApoE phenotype was determined on plasma samples by western blotting, using the method of Havekes et al [12] 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. The gel phenotyping method has been shown to have 99% concordance with genotyping.[13] For DNA analysis we genotyped two single nucleotide polymorphisms (SNPs), R46L (rs11591147), and E670G (rs505151) of the PCSK9 gene using Taq Man® SNPs genotyping assays (Applied Biosystems, Foster City CA). The custom assays IDs are C_2018188_10 and C_998744_10 respectively. For reference the Genbank/EMBL accession numbers were NC 00001.9, NT 032977.9, mim 607786. 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, with subjects carrying the apoE4 allele having the greatest response in terms of LDL lowering and the highest CHD risk, and the fact that apoE2 and apoE4 phenotypes have opposite effects in this regard.[14–17] The subject characteristics for these individuals representing 2621 men and 2797 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 and in response to the treatment with pravastatin at 6 month and with the PCSK9 genotypes adjusted for gender, body mass index, age, alcohol, smoking, 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 disease (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), CHD death or non-fatal MI 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 PCSK9 genotypes using multivariable logistic regression analysis. All analyses were adjusted for age, sex, country, history of vascular disease, body mass index, history of diabetes, as well as history of hypertension, alcohol use, current smoking, and apoE phenotype. 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 2 SNPs of interest.[18] 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 R46L and E670G polymorphisms are shown in Table 1, along with the apoE phenotype distribution in this population. The distribution of genotypes in both PCSK9 SNPs were in Hardy–Weinberg equilibrium ($p>0.05$, data not shown). As can be seen from Table 2, 2.8% of the men carried the T allele at R46L, with none being homozygotes, while 4.2% of the women were heterozygotes for the GT genotype, and 0.07%, or two women, were homozygous for the T allele at the R46L locus. With regard to the E670G SNP, 6.3% of the men had the AG genotype, and none were homozygous for the G allele. For women this percentage was 5.6%, and 0.04%, or one of the women was a homozygote for the G allele.

Both the men and women who carried the GT genotype at the R46L locus had significantly lower levels of LDL C ($p<0.001$) (7.5 % or 10.1 mg/dl lower for men, and 11.9% or 17.3 mg/dl in women), and this was also the case for TC ($p<0.001$) and apoB levels ($p<0.001$) as compared to their GG counterparts (Table 2). For the two women who were homozygous at this locus (TT), similar differences as for heterozygotes were also observed. With regard to the E670G locus there were no statistically significant associations detected with TC, LDL C, or apoB (Table 2).

Haplotype analysis detected that the two SNPs under study were in linkage disequilibrium ($D'=0.717$) and revealed one common haplotype *R46L[G]-E670G[A]*, present in 95% of the participants, and two rare haplotypes *R46L[G]-E670G[G]* and *R46L [T]-E670G[A]*, present in 3% and 2% of participants, respectively. Carriers of *R46L[T]-E670G[A]* had statistically different TC and LDL C levels as compared to the *R46L [G]-E670G[A]* or *[G]* carriers ($p<0.001$). These data are consistent with the conclusion that it is only the T allele of R46L that has a significant effect on TC, LDL C and apoB levels.

In Table 3, data on response to pravastatin in terms of percent lowering of LDL C are provided. We can see that, not only are the alleles at the R46L related to baseline TC, LDL C, and apoB values, but also genetic variation at R46L was linked to LDL C lowering response ($p=0.032$). However, a difference in response to therapy in good compliers was driven by the one female homozygous carrier for the T allele (R46L TT). This particular subject had an LDL C reduction that was 63% (with a low baseline LDL C level of 93 mg/dl) versus an average of 36% for the other women (baseline LDL C in women with the

GG genotype was 145 mg/dl). With regard to E670G, variation at this locus was not related to either baseline values or to LDL C lowering response. These data indicate that genetic variation at these two loci within PCSK9 have little effect on LDL C lowering response, except for the one female on pravastatin who was homozygous at the R46L SNP. Haplotype analysis did not add further information. 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 odds ratios (OR) of having a history of 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, as well as hazard ratios (HR) on trial of the new onset of the primary endpoint (fatal and non-fatal MI or stroke), fatal or non-fatal MI, and all cardiovascular events (all of the above) are shown. With regard to all of these results at baseline or on trial none reached statistical significance for either of the rare alleles at R46L or E670G, and only the data for the R46L alleles are shown. With regard to baseline odds ratio for the rare R46L allele the value was 0.91 for all vascular disease after adjustment (p=0.58), while for MI the value was 1.25 after adjustment (p=0.31) as compared to wild type. For on trial data, the hazard ratios were 0.95 (p=0.81) for the primary endpoint, 0.97 (p=0.91) for fatal and non-fatal MI, and 0.88 (p=0.52) for all cardiovascular events after adjustment when comparing carriers of the rare allele with non-carriers.

We did observe that these carriers had a trend towards a lower history of coronary angioplasty or bypass surgery at baseline (OR 0.26 CI 0.06–1.06, p=0.06; 2/192 or 1.1% versus 220/5221 or 4.2%), however these difference did quite reach statistical significance. The E670G G allele carriers were significantly more likely to have a history of surgery for peripheral vascular disease at baseline (OR 1.92, CI 1.06–3.47, adjusted p value=0.03; 13/323 or 2.0% versus 106/5093 or 4.0%). No such difference were observed on trial, and these data should be assessed with caution because of small numbers. .

Discussion

Genetic variation at PCSK9 has been reported to significantly affect plasma LDL C concentrations, LDL C lowering response to statins, and coronary heart disease (CHD) risk.[5–9] We sought to examine these issues further in the PROSPER study. Our data indicate that the minor allele at the R46L locus in PCSK9 is significantly associated with decreased LDL C values, but does not affect statin response, or significantly reduce CHD or vascular disease risk. Moreover the rare allele at E670G was not associated with lipid levels, statin response, or CHD risk.

Chen et al sequenced PCSK9 exons and boundaries in 691 subjects, of whom 372 had established CHD as assessed by coronary angiography.[6] They reported that the SNP at E670G within exon 12 (replacement of glutamic acid with glycine at amino acid position 670), accounted for 3.5% of plasma LDL C variability. In their coronary disease population, 1.9% were homozygous for this allele (n=7), with LDL C levels that were 19% higher than non-carriers, and these subjects were significantly more likely to have a total coronary occlusion on angiography than non-carriers.[6] In this same group 11% were heterozygotes for

this allele, and their mean LDL C levels were 2.4% higher than non-carriers, with no difference in severity of coronary atherosclerosis.[6] Evans et al have similarly reported that the presence of the E670G allele at PCSK9 was associated with increased LDL-C in men (n=239), but not in women (n=267) attending a lipid clinic.[7] This reported gender difference may well have been driven by their finding of 3 homozygotes in the male group (1.3%) and none in the female group. In both these two studies the homozygotes for E670G clearly had increased LDL-C values. Kotowski et al also examined the impact of this allele on plasma lipid levels in 1,045 Caucasians and did not find any significant associations. [8]

In our elderly population, we found only one homozygote for the E670G allele, representing a prevalence of 0.018%, (whose LDL-C was slightly lower than wild type). This prevalence was much lower than that observed in the other two studies. We observed that 6% of the population were carriers for the E670G rare allele, with no significant differences in LDL C or CHD risk in carriers versus non-carriers. We did note that women who were heterozygotes had mean LDL C levels that were a non-significant 3.0% higher than non-carriers, but no such differences were seen in men.

Kotowski et al and Berge et al have reported a SNP within PCSK9, R46L (replacement of arginine at position 46 with leucine), that was associated with lower LDL C levels.[8,9] Cohen et al reported that 3.2% of a Caucasian population consisting of 9,524 male and female subjects of mean age 54 years were carriers of the R46L allele at PCSK9, and that the carrier state was associated with 15% lower LDL-C levels (116 mg/dl versus 137 mg/dl) and a 47% reduction in CHD risk as compared to non carriers.[5] This latter finding was based on the observation of 19 CHD cases out of 301 carriers (6.3%) versus 1089 cases out of 9,223 non-carriers (11.8%), with no significant differences in stroke or overall death rates. This observation has been heavily cited as providing evidence that lifelong lower LDL cholesterol levels markedly reduces CHD risk, but it is based on a very small number of cases in carriers of the R46L SNP. Cohen et al do mention that a limitation of their study was that they did not examine an elderly population.[5] Another limitation is the lack of power since the frequency of the R46L at PCSK9 is low. It is well known that most subjects develop CHD after the age of 65 years, and this is especially the case for women.

In our population we noted that 75 of 190 carriers of the rare allele had evidence of some form of vascular disease at baseline (39.5%) versus 2328 of 5221 non carriers (44.6%), and on trial we observed 27 new total cardiovascular events in 190 carriers (14.2%) versus 869 of 5221 non-carriers (16.6%). Therefore we did have more power to detect differences than Cohen et al, and this elderly do note that the presence of this allele does protect people from developing vascular disease. [5] Recently Scartezini et al determined the frequency of R46L as well as the E670G allele at the PCSK9 locus in 2444 healthy middle-aged men of whom 275 developed CHD events at followup. [19] They observed lower LDL C levels in carriers of the R46L rare allele, as well as lower CHD risk, but this latter finding was not significant. They saw no associations with the E670G allele. They concluded that the R46L allele at PCSK9 is unlikely to contribute significantly to CHD risk in the general population because of its low frequency. [19] We agree with their assessment.

In our study we examined a cohort of 5,783 elderly male and female subjects of mean age 75 years, with 43% (n=2,487) having a history of vascular disease (coronary, cerebral, or peripheral) or at least one vascular disease risk factor without evidence of vascular disease (57%, n=3,296, our control group). Similar to the findings of Cohen et al[5], we noted that 3.5% were carriers of the R46L allele, and these subjects had significantly lower LDL C levels versus non-carriers. Instead of a mean 15% difference in LDL-C values, we only noted a mean 11.4% difference, which normally would translate into a relative risk of about 0.90 similar to what we observed, in contrast to the 0.47 relative risk that Cohen et al observed.[5] According the National Cholesterol Education Program, as well as data from the Clinical Trialists Meta-Analysis, and the Framingham Study for every 1% reduction in LDL C values, there is an approximate 1% reduction in CHD risk . [20–22] LDL C differences, as shown in table 2, were 11.9% in heterozygous women, and 7.5% in men. Moreover we expected to find a higher frequency of this rare allele in an elderly population, especially in those without vascular disease, and this was not the case. We did find a trend towards decreased vascular disease prevalence at baseline and CHD risk on trial for those carrying the rare allele at this locus, but these differences did not reach statistical significance, and were commensurate with the degree of LDL C lowering that we observed.

Berge et al sequenced PCSK9 exons in 38 unrelated hypocholesterolemic subjects, 25 unrelated patients with heterozygous familial hypercholesterolemia (FH) with LDL receptor mutations, who were “hyper-responders” to statin therapy, and 441 hypercholesterolemic patients who did not have an LDL receptor mutation.[9] They report that 15.8% (6 of 38) of the hypocholesterolemic patients were heterozygotes for one of three PCSK9 variants, R46L, G106R, or R237W. They also noted that 8.8% (2 of 25) of the heterozygous hyper-responders were carriers of either the R46L or the N157K alleles. They found none of the rare alleles in the 441 hypercholesterolemic patients without LDL receptor mutations. They concluded that these PCSK9 variants are linked to hypocholesterolemia and possibly to hyper-response to statins. Rashid et al reported hypersensitivity to statin in mice lacking PCSK9 [23]. Dubuc et al reported that statins upregulate PCSK9 in HepG2 cells and in human primary hepatocytes [24]. The overall data do suggest that selected mutations in PCSK9 can affect LDL C levels and statin response due to effects on LDL receptor activity, recycling, and cell surface receptor number.

In our studies we observed no relationship of LDL C lowering response to pravastatin associated with the E670G allele, but we did observe a significant relationship with the R46L allele. However in heterozygotes the LDL C lowering was quite similar (36.1% in men, 36.8% in women) versus non-carriers (35.9% in men, 36.3% in women). In our studies the statistical relationship was driven by one female homozygote for R46L whose LDL C was already low at baseline at 93 mg/dl and was decreased by 63.0% on pravastatin down to 34 mg/dl. This problem was also noted in the haplotype analysis. Overall our data do not support the concept that the presence of either the R46L or the E670G rare alleles affects the LDL C lowering response to statins other than in homozygotes. However in younger patients with FH and decreased LDL receptor activity, PCSK9 variants may well affect statin response, as suggested by Berge et al.[9] Moreover markedly decreased PCSK9 activity as noted in homozygotes for the R46L allele as well as in mice lacking PCSK9 does result in statin hypersensitivity [23–24].

Our overall data indicate that the rare allele at R46L in the PCSK9 gene locus, but not E670G, is significantly associated with modest decreases in LDL C values, but that neither allele is associated with a statistically significant reduced CHD risk in the elderly, or in LDL C response to pravastatin.

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Table 1

Subject and Genetic Characteristics

Study characteristics Mean(SD)*	Men (N=2621)	Women (N=2797)
Age, years	74.99 (3.26)	75.64 (3.38)†
BMI, kg/m^2	26.56 (3.59)	27.12 (4.66)†
History Diabetes mellitus, N (%)	324 (12.36)	251 (8.97)†
History Hypertension, N (%)	1333 (50.86)	2027 (72.47)†
History Vascular Disease, N (%)	1371 (52.31)	1034 (36.97)†
Current smoking, N (%)	847 (32.32)	587 (20.99)†
Alcohol consumption, N (%)	1851 (70.62)	1166 (41.69)†
Total Cholesterol, mg/dl	207.0 (30.7)	231.8 (34.5)†
LDL Cholesterol, mg/dl	138.5 (27.8)	154.9 (31.4)†
HDL Cholesterol, mg/dl	45.6 (12.2)	53.0 (13.4)†
Triglyceride, mg/dl	132.4 (64.3)	140.5 (59.3)†
apoA-I, mg/dl	124.4 (22.2)	139.9 (24.1)†
apoB, mg/dl	110.6 (21.3)	119.1 (22.6)†
apoE 2/2 + 2/3, %	12.15	
apoE 3/3, %	64.45	
apoE 3/4 + 4/4, %	23.40	
R46L- rs11591147CGT (R-Arg) to CTT (L-Leu)	MAF T:0.018	
E670E -rs505151GAG (E-Glu) to GGG (G-Gly)	MAF G:0.03	

*unless specified otherwise

Differences between men and women were assessed using a t-test for continuous traits and χ^2 test for binary traits.

MAF – minor allele frequency.

†p<0.001, Mean values are presented

apoE 2/4 carriers were excluded (see Material and Methods section).

Table 2

Baseline Adjusted Mean Lipid Levels by Gender (mean ± SE, mg/dl).

SNP	N		TC		P	LDL-C		P	apoB		P
	Men	Women	Men	Women		Men	Women		Men	Women	
R46L											
GG	2546	2675	203.3±1.1	221.9±1.33		134.3±1.0	145.2±1.2		108.3±1.0	113.7±1.0	
GT	73	117	194.0±3.6	205.6±3.28	<0.001	124.2±3.2	127.9±2.9	<0.001	101.6±2.4	103.0±2.1	<0.001
TT	--	2	--	194.3±23.6		--	119.2±21.0		--	95.6±15.0	
E670G											
AA	2455	2638	203.01±1.1	220.8±1.3		134.0±1.0	144.2±1.2		108.1±1.0	113.1±1.0	
AG	165	157	203.4±2.5	226.4±2.9	0.27	134.3±2.2	148.6±2.6	0.34	108.6±1.7	114.9±1.8	0.69
GG	--	1	--	216.9±0.0		--	136.3±0.0		--	115.6±0.0	

Values for men and women combined; adjusted for sex, BMI, age, alcohol, smoking, diabetes, apoE phenotype, and country

Table 3

Low Density Lipoprotein Cholesterol Response to Pravastatin by Genotype

SNP	Adjusted Mean Percent LDL Cholesterol Reduction*				P†
	N	Men	N	Women	
R46L					
GG	924	−35.9 (−37.1- −34.7)	927	−36.3 (−37.7 - −34.8)	
GT	25	−36.1 (−41.2- −32.7)	38	−36.8(−40.5 - −33.1)	0.032≠
TT	0	--	1	−63.0 (--)	
E670G					
AA	887	−35.7 (−36.9- −34.5)	905	−36.1 (−37.6 - −34.7)	
AG	62	−38.2 (−40.9- 35.5)	61	−37.3 (−40.2 - −34.3)	
GG	0	--	1	−36.5 (--)	

*Values are provided as mean with the 95th percentile confidence intervals provided in parentheses

†P values for men and women combined; adjusted for sex, BMI, age, alcohol, smoking, diabetes, apoE phenotype, and country,

≠The p value is no longer significant if the homozygote is excluded.

Table 4

Analysis of Cardiovascular Diseases* at Baseline and Incidence of New Events* on Trial by PCSK9 R46L Genotype

Cases / total subjects (n=5413)	Number of case/ Total subjects (%)	Genotype	Unadjusted		Adjusted‡	
			OR†	P	OR†	P
	704/5221 (13.5)	GG	1		1	
	27/190 (14.2)	GT	1.05 (0.69–1.59)	0.81	1.25 (0.81–1.93)	0.31
Myocardial infarction* 731/5413	0/2 (--)	TT	--		--	
	2328/5221 (44.6)	GG	1		1	
	75/190 (39.5)	GT	0.81 (0.61–1.09)	0.17	0.91 (0.67–1.26)	0.58
Vascular disease* 2402/5413	0/2 (--)	TT	--		--	

New events / Total subjects (n=5413)	Number of new case/ total subjects (%)	Genotype	Unadjusted		Adjusted§	
			HR†	P	HR†	P
	781/5221 (14.9)	GG	1		1	
	26/190 (13.7)	GT	0.87 (0.59–1.29)	0.50	0.95 (0.64–1.41)	0.81
Primary endpoints * 807/5413	0/2 (--)	TT	--		--	
	578/5221 (11.1)	GG	1		1	
	20/190 (10.5)	GT	0.89 (0.57–1.41)	0.64	0.97 (0.62–1.53)	0.91
Fatal or non-fatal MI * 598/5413	0/2 (--)	TT	--		--	
	869/5221 (16.6)	GG	1		1	
	27/190 (14.2)	GT	0.81 (0.55–1.19)	0.28	0.88 (0.60–1.30)	0.52
Cardiovascular events * 896/5413	0/2 (--)	TT	--		--	

* Cardiovascular disease criteria are provided mentioned in the methods section.

† Odds ratios and Hazard ratios (95% Confidence Intervals) are presented.

‡ P values for GG vs GT for men and women combined: adjusted for sex, BMI, age, alcohol, smoking, diabetes, hypertension, apoE phenotype, and country. No significance was noted when men or women were separated.

§ P values for GG vs GT for men and women combined; adjusted for sex, BMI, age, alcohol, smoking, diabetes, hypertension, apoE phenotype, randomized treatment, and country. No significant differences were noted when men and women were separated