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Association of polymorphisms in genes involved in lipoprotein metabolism with plasma concentrations of remnant lipoproteins and HDL subpopulations before and after hormone therapy in postmenopausal women

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SUMMARY

Objective—A high degree of inter-individual variability in plasma lipid level response to hormone therapy (HT) has been reported. Variations in the oestrogen receptor α gene (*ESR1*) and in genes involved in lipid metabolism may explain some of the variability in response to HT.

Subjects—Postmenopausal Caucasian women (N=208) participating in a placebo-controlled randomized trial of 3.2 years of hormone therapy (HT).

Methods—Plasma triglycerides (TG), remnant lipoprotein cholesterol (RLP-C), and high-density lipoprotein cholesterol (HDL-C) levels and HDL subpopulations were assessed at baseline and at follow-up. Single nucleotide polymorphisms (SNPs) in *ESR1* and in the ATP binding cassette A1 (*ABCA1*), cholesteryl ester transfer protein (*CETP*), hepatic lipase (*LIPC*), lipoprotein lipase (*LPL*), and scavenger receptor class B type I (*SRBI*) genes were assessed for their association with baseline plasma levels and HT-related changes in levels of RLP-C and HDL subpopulations.

Results—Carriers of the *ESR1* PvuII or IVS1-1505 variants had lower plasma TG concentrations and higher plasma HDL-C and α -1 and pre α -1 HDL particle levels at baseline and showed greater increases in HDL-C, apo A-I and α -1 particle levels after HT than wild-type carriers. Carriers of the N291S and D9N variants in the *LPL* gene had significantly higher remnant lipoproteins and lower α -2 HDL particle levels at baseline. The *CETP* TaqIB SNP was a significant determinant of baseline plasma HDL-C and HDL subpopulation profile.

Conclusions—SNPs in *ESR1*, *CETP* and *LPL* had significant effects on baseline plasma levels of TG-rich and HDL subpopulations. With the exception of *ESR1* SNPs, variation in genes involved in lipid metabolism has a very modest effect on lipoprotein response to HT.

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Keywords

high-density lipoprotein; remnant lipoproteins; single nucleotide polymorphism hormone therapy

INTRODUCTION

Environmental factors, such as body weight, smoking, use of medications, and dietary fat intake contribute significantly to the inter-individual variability in plasma lipid levels (1). Also, polymorphisms of genes involved in lipoprotein metabolism explain part of the inter-individual variability in plasma lipid levels (2). Triglyceride (TG) -rich lipoproteins, such as very low-density lipoproteins and chylomicrons, are secreted by the liver and the intestine, respectively, and undergo rapid catabolism to remnant lipoproteins. Lipoprotein lipase (LPL), hepatic lipase (HL), and apolipoprotein (apo) C-III are among the most important factors in the catabolism of TG-rich lipoproteins (3). Apo A-I, the major protein component of high-density lipoproteins (HDL), is secreted by the liver and intestine as a lipid-poor HDL particle which acquires free cholesterol from peripheral tissues by interacting with the ATP-binding cassette A1 (ABCA1) and G1 (ABCG1) receptors (4). Free cholesterol is converted to cholesteryl ester by the enzyme lecithin:cholesterol acyl transferase (LCAT). Cholesteryl ester in HDL is selectively uptaken by the liver via the scavenger receptor class B type 1 (SR-BI) or is exchanged for TG via the cholesteryl ester transfer protein (CETP). Lipolytic enzymes such as LPL, HL, and endothelial lipase also participate in the plasma HDL metabolism (4).

Hormone therapy (HT) is known to affect plasma lipid levels in postmenopausal women (5;6). Individual variability in plasma lipid response to HT has been described and has been attributed to genetic variation (7). Variability at the oestrogen receptor α (*ESR1*) locus has been shown to explain part of the inter-individual variability in HDL cholesterol response to HT in some (8), but not all studies (9;10). Due to the fact that oestrogen regulates the expression of some of the genes involved in lipid metabolism (11;12), variations in these genes may also affect plasma lipid response to HT. By studying the effect of genotypes on the response of different lipoprotein subclasses to HT, we can derive information on the specific metabolic effects of the candidate gene involved. To date, very few studies have examined the effect of variants of lipid metabolism genes on HT response.

SUBJECTS AND METHODS

Subjects

Subjects were postmenopausal women participating in the Oestrogen Replacement and Atherosclerosis (ERA) trial (13;14). All participants had established coronary heart disease (CHD) as assessed by quantitative coronary angiography. Women with a previous history of thromboembolism, symptomatic gallstones, uncontrolled diabetes or hypertension, or with plasma TG levels >4.8 mmol/L were excluded from participation in the study. Also, women on hormonal replacement therapy were asked to stop treatment for at least three months before starting participation. A total of 309 women were enrolled and were randomized to three parallel treatment arms: placebo, conjugated equine oestrogen (0.625 mg/day), and conjugated equine oestrogen plus medroxyprogesterone acetate (0.625 mg/day + 2.5 mg/day, respectively). Of these subjects, 83% (N=256) were Caucasian. In the current study, 208 Caucasian women with complete lipid measurements at baseline and follow-up and genotyping were included (73 were randomized to placebo, and 135 were randomized to either hormonal treatment). Only Caucasian subjects were included in the analysis, due to previous observations of a different effects size of polymorphisms of genes involved in lipid metabolism on plasma lipid levels in Americans with African or European ancestry (15).

Approval of the study protocol was obtained from the Institutional Review Boards of the different sites involved in patient recruitment. Study candidates provided informed consent.

Determinations of plasma lipids, HDL subpopulations and lipoprotein remnants

Plasma total cholesterol (TC) and TG levels were measured by automated enzymatic assays (16). Plasma HDL-C levels were measured after heparin-manganese precipitation of apo B-containing lipoproteins (17).

Plasma remnant-like particle cholesterol (RLP-C) levels were measured using an immunoseparation technique (Polymedco, Cortlandt Manor, NY) (18–20).

Plasma apo A-I and apo C-III concentrations were measured on a Hitachi 911 autoanalyzer (Hitachi, Inc.) using assays and calibrators from Wako Diagnostics (Richmond, VA) (21). Apo A-I-containing HDL subpopulations in plasma were measured by non-denaturing two-dimensional gel electrophoresis methodology, which separates HDL particles according to size and composition into pre β -1, pre β -2, α -1, α -2, α -3, pre α -1, pre α -2, and pre α -3 (22).

DNA analysis

Genomic DNA was isolated from stored buffy coats using a standard guanidine thiocyanate procedure. Genotyping of single nucleotide polymorphisms (SNPs) was performed using the MassARRAY SNP genotyping system (Sequenom, Inc.). The genes and SNPs of interest were: oestrogen receptor α [*ESR1*; rs2234693 (PvuII, intron 1), rs9340799 (XbaI, intron 1), rs4870056 (IVS1-1505, intron 1), rs9340894 (intron 3), rs985192 (intron 4), rs926777 (intron 4), rs3020325 (intron 4), and rs3020368 (intron 5)], *ABCA1* [(G3456C (E1112D) and rs2230806 (R219K)], *CETP* [rs 708272 (TaqIB)], hepatic lipase [*LIPC*; rs1800588 (C-514T)], *LPL* [rs1800590 (T-93G), rs1801177 (D9N), rs268 (9N291S), and rs328 (S447X)], and SR-BI [*SCARB1*; rs4238001 (G2S)]. PCR and extension primers were designed using SpectroDesigner software (Sequenom, Inc.) and reactions were performed according to the manufacturer's instructions.

Statistical analysis

Hardy-Weinberg equilibrium was assessed using a chi-square goodness of fit test. Variables that were not normally distributed were log-transformed prior to analysis. Associations between variables and genotypes were assessed using linear regression models, using both dominant (patients carrying one or two alleles being compared to those homozygous for the wild-type allele) and additive (comparison among the three different genotypes) genetic models. Recessive models (comparison of patients homozygous for the rare allele with those carrying one or two wild-type alleles) were not used because the number of subjects homozygous for the minor allele of the SNPs studied were too few to support the use of recessive models. Models were adjusted for age, BMI, smoking, diabetes, and use of lipid-lowering medications. In the analysis of the effect of the interaction between treatment and SNP on HT-related plasma lipid changes, the oestrogen and oestrogen plus progestin groups were combined (N=133), since we have previously shown that the plasma lipid response to HT in this population is similar in these two groups (20). A P value ≤ 0.05 was considered significant.

RESULTS

At baseline, characteristics of postmenopausal Caucasian women randomized to placebo or HT were similar, with the exception of HDL-C levels, which were higher in subjects randomized to HT (Table 1). Only eight women reported niacin use and sixteen reported gemfibrozil use at baseline. Statins were the most common lipid-lowering medication.

SNPs were tested for associations with baseline plasma lipid levels and the HT-related changes in lipid levels. Only the SNPs found to have significant associations are shown in Table 2. All SNPs tested were in Hardy-Weinberg equilibrium. The *ESR1* PvuII and IVS1-1505 SNPs were in strong linkage disequilibrium ($D=0.98$, $P<0.001$).

Baseline plasma TG levels were associated with the *ESR1* PvuII and IVS1-1505 SNPs, with a significant lowering effect of the minor alleles (Table 3). *ESR1* SNPs did not significantly affect baseline plasma levels of remnant lipoproteins and of apo C-III. Baseline plasma HDL-C levels, but not apo A-I levels, were significantly affected by the PvuII and IVS1-1505 SNPs, with subjects carrying the minor alleles having higher plasma HDL-C levels (Table 3). This was accompanied by a significant increase in pre α -1 HDL particles and a trend toward an increase in α -1 particles in carriers of the minor allele compared to carriers of wild-type allele. The *CETP* TaqIB polymorphism had a strong dominant effect on baseline plasma HDL-C levels and α -1, α -2, pre α -1, and pre α -2 particles (Table 3). Only trend associations were observed for the polymorphism located in the promoter region of *LIPC*, with higher pre β -1 and α -1 particle levels in subjects carrying the rare allele. Both the N291S and the D9N SNPs of the *LPL* gene were associated with significantly higher baseline remnant lipoprotein levels, and a trend toward higher baseline TG levels. In addition, the N291S minor allele was associated with significantly lower α -2 particle levels, while the minor D9N allele was associated with significantly higher pre α -3 and a trend toward higher α -3 particle levels (Table 3).

A significant interaction between treatment and the *ESR1* PvuII SNP was observed for apo C-III, with subjects carrying the minor allele showing significantly greater increases in apo C-III levels following HT (Table 4). Both the PvuII and IVS1-1505 minor alleles were associated with greater increases in HDL-C and apo A-I levels during HT (Table 4). These changes were accompanied by greater increases in α -1 particle levels. The *ABCA1* E1112D SNP -was associated with greater reductions in HDL pre α 3 levels following HT. The *CETP* TaqIB and the *LIPC* C-514 T SNPs were not associated with HT-related changes in HDL parameters in this population, but a significantly greater increase in apo C-III following HT was observed in carriers of the minor *CETP* TaqIB allele (Table 4).

DISCUSSION

The assessment of lipoprotein subpopulations in plasma provides a more detailed characterization of possible defective steps in lipoprotein metabolism than the standard lipid measurements. In the current report, we investigated the effect of SNPs in genes involved in lipoprotein metabolism both on baseline plasma lipoprotein subfractions, and on the HT-related changes of these subfractions.

The oestrogen in HT has been shown to increase plasma TG levels by increasing the secretion of hepatic TG-rich lipoproteins (23), but also to reduce plasma levels of the remnants of these lipoproteins, possibly through an increase in the hepatic expression of the LDL receptor (24;25). In addition, oestrogen is known to increase plasma levels of apo A-I and HDL-C (24;25), both by increasing the expression of apo A-I in the liver (19;26) and by modulating the expression of proteins involved in the metabolism of HDL, such as SR-BI and HL (11;12). There is a large inter-individual variability in plasma lipid response to HT, and the objective of this study was to evaluate the contribution of genetic polymorphism at the *ESR1* gene locus or at loci involved in lipoprotein metabolism to this variability.

The PvuII and XbaI polymorphisms are among the most studied SNPs of the *ESR1* gene (27). While a previous report in the ERA population (including all races and ethnic groups) had shown a significant effect of the minor PvuII allele on plasma HDL-C and apo A-I

levels (8). other studies have not found a significant association of this SNP with plasma HDL-C (9;10;28). Our current study, focusing only on Caucasian women in the ERA study, indicates that carriers of the minor allele had significantly higher baseline HDL-C levels, accompanied by significantly lower baseline plasma TG levels. The metabolism of TG-rich lipoprotein is tightly bound to that of HDL lipoproteins, and plasma TG and HDL-C levels are inversely associated (29). Thus, it is likely that the PvuII SNP affects a common step in the TG-HDL metabolism. Carriers of the minor PvuII allele had significantly higher pre α -1 levels and a trend toward higher α -1, compared to wild-type carriers. While HT resulted in similar changes in plasma TG and remnant levels in carriers and non carriers of the PvuII rare allele, a significantly greater increase in HDL-C, apo A-I, and α -1 particle levels was observed in the carriers of the minor allele. Very similar associations were observed for the *ESR1* ISV1-1505 SNP, which is in high linkage disequilibrium with the PvuII SNP. In addition, carriers of the minor allele for the PvuII or the ISV1-1505 SNP had greater increases in apo C-III following HT, which could lead to greater inhibition of LPL activity. This could explain the lack of effect of these SNPs on the change in TG and lipoprotein remnant after HT, in spite of the fact that these subjects had lower TG at baseline.

The TaqIB variant of the CETP gene is a common mutation located in the first intron of the gene and is in linkage disequilibrium with a functional mutation in the promoter region (30;31). This variant is associated with lower CETP activity in plasma (31) and is an important determinant of plasma HDL-C levels and CHD risk (32). Our results are in agreement with previous studies showing a significant effect of the *CETP* TaqIB SNP on HDL-C levels and furthers previous observations by showing that the increase in HDL-C levels with the minor allele is selectively affecting the large HDL particles α -1, α -2, pre α -1, and pre α -2 levels. This effect is expected, based on observations in subjects with heterozygous CETP deficiency, who have significantly increased levels of the large HDL particles (33). The TaqIB SNP was not associated with the HT-related changes in lipid or lipoprotein levels, in agreement with a previous study conducted in Korean women (34).

In our population, the *ABCA1* E1112D SNP was not associated with baseline plasma lipoprotein levels. Similarly, no association between this SNP and HDL-C levels was observed in other larger healthy or CHD populations (35). We found a significant interaction of this SNP with the HT-related change in HDL pre α -3 particles. However, the clinical significance of this observation is not clear.

HL, an enzyme secreted from hepatocytes and bound to the surface of hepatocytes and liver sinusoid capillaries, participates in the hydrolysis of TG and phospholipids in remnant lipoproteins and HDL (36). The C-518T polymorphism, located in the promoter region of the gene, is associated with a reduced enzymatic activity and higher levels of both HDL-C and large HDL particles in the general population (37;38). In our study, subjects carrying the minor allele had higher HDL-C, apo A-I, α -1, α -2, pre α -1, and pre α -2 concentrations, but these differences did not reach statistical significance, possibly due to the smaller sample size of our population. No significant interaction between treatment assignment and this polymorphism was observed for changes in plasma lipid levels following HT treatment, in agreement with previous observations (39;40),.

The N291S and D9N are relatively rare mutations (<5% allele frequency) affecting the amino acid sequence of LPL and resulting in reduced enzymatic activity (41–43). A significant effect of these SNPs was observed on baseline remnant lipoprotein levels, with carriers of the minor alleles having significantly greater levels than wild-type homozygotes. These results are consistent with the concept that reduced LPL activity leads to delayed clearance of TG-rich lipoproteins and increased concentrations of remnants. There was a trend toward lower HDL-C and apo A-I levels and significantly lower α -2 levels in carriers

of the minor allele for the N291S SNP, and higher α -3 and pre α -3 particles in carriers of the minor allele for the D9N SNP. Several studies have documented a significant effect of both N291S and D9N polymorphisms on plasma TG and HDL-C levels (44;45). The efficiency of lipolysis plays an important role in the association between TG and HDL-C levels (46). Our results emphasize the importance of LPL not only in the clearance of remnant lipoproteins, but also in the formation of large HDL particles.

In conclusion, our study confirms previous observations of the impact of variations at gene loci involved in lipid metabolism on baseline TG and HDL-C levels and further extends these observations to the subpopulations of TG-rich and HDL lipoproteins. In addition, our study clearly indicates that polymorphisms of genes involved in lipoprotein metabolism do not explain most of the variability in lipid and lipoprotein subfraction response to HT.

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Table 1

Baseline characteristics of postmenopausal Caucasian women, according to randomization to treatment.

	Placebo (N=73)	HT (N=135)	P
Age, years	66±7	66±7	0.91
BMI, kg/m ²	30.3±9.2	28.8±7.2	0.23
Systolic blood pressure, mmHg	135±17	134±17	0.64
Diastolic blood pressure, mmHg	74±8	73±8	0.67
Smoking, %	21	22	0.48
Diabetes, %	27	20	0.14
Lipid-lowering medications, %	34	35	0.52
Plasma lipids, mmol/L			
TC	5.56±1.06	5.59±0.96	0.64
TG	2.51±1.41	2.12±1.10	0.09
LDL-C	3.49±0.93	3.47±0.83	0.93
HDL-C	1.06±0.26	1.14±0.33	0.03

Mean±SD

P value, Mann-Whitney test for continuous variables, and Fisher's exact test for categorical variables.

Table 2

Genotype frequencies of selected SNPs among postmenopausal Caucasian women.

Gene	SNP name	Mutation	Major allele	Heterozygote	Minor allele
			(Percent)		
<i>ESR1</i>	PvuII	T→C	31	51	18
	XbaI	A→G	40	51	9
	IVS1-1505	A→G	31	52	17
<i>ABCA1</i>	E1112D	C→G	96	4	0
<i>CETP</i>	TaqIB	C→T	33	44	23
<i>LIPC</i>	C-514T	C→T	62	35	3
<i>LPL</i>	N291S	A→G	95	5	0
	D9N	G→A	96	4	0

Table 3

Effects of selected SNPs on baseline plasma lipid and lipoprotein subclasses concentrations in postmenopausal Caucasian women (N=208).

SNP	TG* mmol/L	RLP-C* mmol/L	apoC-III g/L	HDL-C mmol/L	apoA-I g/L	preβ1 g/L	preβ2* g/L	a1 g/L	a2 g/L	a3 g/L	preα1* g/L	preα2 g/L	preα3* g/L
ESR1													
PvuII TT	2.20	0.29	0.15	1.06	1.23	0.23	0.015	0.11	0.38	0.38	0.014	0.042	0.048
TC	1.94	0.28	0.15	1.14	1.27	0.24	0.016	0.12	0.38	0.39	0.017	0.044	0.045
CC	1.76	0.24	0.13	1.19	1.25	0.22	0.018	0.14	0.38	0.38	0.021	0.046	0.044
P (dominant)	0.11	0.54	0.30	0.06	0.26	0.56	0.13	0.19	0.83	0.75	0.06	0.27	0.36
P (additive)	0.04	0.19	0.18	0.04	0.40	0.76	0.08	0.09	0.90	0.83	0.02	0.11	0.52
XbaI AA	2.16	0.30	0.15	1.09	1.25	0.23	0.015	0.12	0.38	0.39	0.015	0.043	0.048
AG	1.85	0.26	0.14	1.16	1.26	0.23	0.017	0.12	0.38	0.39	0.018	0.043	0.044
GG	1.99	0.26	0.15	1.11	1.28	0.23	0.017	0.13	0.40	0.39	0.020	0.048	0.048
P (dominant)	0.08	0.21	0.54	0.16	0.66	0.84	0.41	0.63	0.69	0.90	0.24	0.63	0.26
P (additive)	0.19	0.27	0.48	0.33	0.54	0.95	0.42	0.48	0.42	0.88	0.16	0.35	0.66
IVS1-1505 GG	2.22	0.29	0.15	1.09	1.24	0.23	0.015	0.11	0.39	0.39	0.013	0.043	0.048
GA	1.95	0.28	0.15	1.14	1.26	0.24	0.016	0.12	0.38	0.39	0.017	0.044	0.045
AA	1.70	0.24	0.13	1.22	1.26	0.22	0.018	0.14	0.38	0.38	0.022	0.048	0.046
P (dominant)	0.05	0.30	0.14	0.13	0.31	0.48	0.15	0.20	0.80	0.76	0.008	0.22	0.59
P (additive)	0.01	0.10	0.09	0.05	0.42	0.97	0.10	0.10	0.84	0.96	0.003	0.07	0.95
ABCA1													
E1112D CC	2.09	0.28	0.14	1.14	1.25	0.23	0.016	0.12	0.38	0.39	0.017	0.044	0.046
CG	1.87	0.27	0.14	1.22	1.30	0.26	0.015	0.11	0.40	0.40	0.015	0.038	0.045
P (dominant)	0.85	0.88	0.94	0.82	0.86	0.61	0.71	0.42	0.97	0.31	0.51	0.22	0.87
P (additive)	0.85	0.88	0.94	0.82	0.86	0.61	0.71	0.42	0.97	0.31	0.51	0.22	0.87
CETP													
TaqIB CC	2.21	0.29	0.15	1.03	1.22	0.23	0.015	0.10	0.35	0.40	0.013	0.039	0.047
CT	1.87	0.27	0.14	1.16	1.27	0.23	0.018	0.13	0.39	0.38	0.019	0.047	0.049
TT	1.92	0.26	0.15	1.19	1.28	0.23	0.015	0.13	0.40	0.38	0.019	0.044	0.041
P (dominant)	0.11	0.31	0.61	0.008	0.13	0.52	0.36	0.05	0.02	0.09	0.01	0.03	0.72
P (additive)	0.38	0.38	0.68	0.03	0.19	0.55	0.96	0.23	0.03	0.32	0.16	0.23	0.14

SNP	TG* mmol/L	RLP-C* mmol/L	apoC-III g/L	HDL-C mmol/L	apoA-I g/L	preβ1 g/L	preβ2* g/L	α1 g/L	α2 g/L	α3 g/L	preα1* g/L	preα2 g/L	preα3* g/L
LIPC													
C-514T CC	2.08	0.28	0.14	1.11	1.24	0.22	0.016	0.11	0.38	0.40	0.016	0.043	0.046
CT	2.15	0.28	0.15	1.14	1.27	0.24	0.016	0.13	0.38	0.38	0.019	0.046	0.048
TT	1.63	0.21	0.14	1.34	1.40	0.27	0.019	0.19	0.47	0.35	0.029	0.042	0.036
P (dominant)	0.64	0.96	0.84	0.61	0.21	0.11	0.64	0.15	0.54	0.19	0.51	0.45	0.32
P (additive)	0.81	0.48	0.61	0.55	0.14	0.09	0.50	0.08	0.34	0.16	0.51	0.45	0.48
LPL													
N291S AA	1.95	0.27	0.14	1.14	1.26	0.23	0.016	0.12	0.38	0.39	0.017	0.044	0.046
AG	2.64	0.41	0.17	1.00	1.15	0.23	0.021	0.09	0.31	0.38	0.011	0.039	0.051
P (dominant)	0.07	0.04	0.21	0.15	0.07	0.95	0.15	0.40	0.05	0.75	0.18	0.41	0.57
P (additive)	0.07	0.04	0.21	0.15	0.07	0.95	0.15	0.40	0.05	0.75	0.18	0.41	0.57
D9N GG	2.01	0.27	0.14	1.14	1.26	0.23	0.016	0.12	0.38	0.39	0.017	0.044	0.046
GA	2.53	0.40	0.17	1.00	1.19	0.21	0.014	0.10	0.33	0.43	0.013	0.042	0.061
P (dominant)	0.07	0.03	0.07	0.17	0.27	0.36	0.54	0.40	0.57	0.06	0.31	0.56	0.03
P (additive)	0.07	0.03	0.07	0.17	0.27	0.36	0.54	0.40	0.57	0.06	0.31	0.56	0.03

Values shown are means, with the exception of *, where values are geometric means. HDL subpopulations expressed as apo A-I g/L.

Models for the effect of SNPs on baseline levels were adjusted for age, BMI, diabetes, smoking, and use of lipid-lowering medications.

Table 4

Effects of selected SNPs on the change in plasma lipid and lipoprotein subclasses concentrations following HT in postmenopausal Caucasian women (N=135).

SNP	TG*	RLP-C*	apoC-III	HDL-C	apoA-I	preβ1	preβ2*	α1	α2	α3	preα1*	preα2	preα3*
<i>ESR1</i>													
PvuII TT	0.03	-0.16	-0.003	0.13	0.14	0.014	0.002	0.042	0.047	0.024	0.005	0.004	-0.007
TC	0.07	-0.21	0.002	0.16	0.18	0.017	0.000	0.058	0.055	0.029	0.004	0.006	0.000
CC	0.05	-0.25	0.007	0.34	0.30	0.022	0.002	0.092	0.013	0.032	0.004	0.018	-0.001
P (additive)	0.76	0.90	0.04	0.007	0.03	0.18	0.60	0.06	0.14	0.67	0.76	0.14	0.27
XbaI AA	0.03	-0.23	-0.005	0.16	0.15	0.009	0.002	0.051	0.059	0.018	0.004	0.004	-0.001
AG	0.10	-0.17	0.006	0.18	0.20	0.024	0.000	0.057	0.057	0.038	0.004	0.008	0.001
GG	-0.07	-0.26	-0.000	0.31	0.25	0.008	0.002	0.082	0.011	0.014	0.006	0.016	-0.001
P (additive)	0.92	0.58	0.06	0.11	0.08	0.10	0.38	0.51	0.57	0.18	0.92	0.78	0.57
IVS1-1505 GG	0.01	-0.16	-0.002	0.16	0.15	0.014	0.002	0.042	0.050	0.030	0.005	0.004	-0.001
GA	0.07	-0.23	0.001	0.13	0.16	0.016	0.000	0.055	0.049	0.025	0.003	0.006	0.000
AA	0.05	-0.25	0.007	0.34	0.30	0.022	0.002	0.092	0.013	0.032	0.004	0.018	-0.001
P (additive)	0.81	0.65	0.07	0.004	0.01	0.15	0.83	0.02	0.12	0.63	0.96	0.09	0.38
<i>ABCA1</i>													
E1112D CC	0.04	-0.21	0.002	0.18	0.17	0.017	0.001	0.058	0.056	0.028	0.004	0.007	0.000
CG	0.19	-0.12	-0.027	0.21	0.23	0.012	0.00	0.059	0.095	0.056	0.004	0.006	-0.001
P (additive)	0.53	0.22	0.35	0.46	0.27	0.83	0.29	0.99	0.20	0.27	0.91	0.14	0.03
<i>CETP</i>													
TaqIB CC	-0.02	-0.26	-0.007	0.18	0.17	0.011	0.001	0.055	0.061	0.024	0.004	0.006	-0.00
CT	0.07	-0.22	0.003	0.18	0.18	0.024	0.001	0.052	0.058	0.030	0.005	0.008	-0.001
TT	0.14	-0.10	0.012	0.16	0.20	0.017	0.001	0.067	0.061	0.033	0.003	0.007	0.001
P (additive)	0.25	0.42	0.02	0.87	0.37	0.73	0.34	0.37	0.56	0.85	0.23	0.54	0.43
<i>LIPC</i>													
C-514T CC	0.04	-0.21	0.003	0.18	0.17	0.017	0.001	0.053	0.056	0.029	0.004	0.008	0.000
CT	0.06	-0.22	-0.003	0.18	0.19	0.013	0.001	0.063	0.070	0.025	0.004	0.007	-0.000
TT	0.24	-0.15	0.017	0.16	0.16	0.005	0.003	0.051	-0.026	0.066	0.002	0.002	0.000

SNP	TG*	RLP-C*	apoC-III	HDL-C	apoA-I	preβ1	preβ2*	α1	α2	α3	preα1*	preα2	preα3*
P (additive)	0.69	0.66	0.58	0.61	0.30	0.77	0.22	0.31	0.48	0.95	0.33	0.78	0.67
<i>LDL</i>													
N291S AA	0.04	-0.22	0.000	0.18	0.18	0.015	0.001	0.055	0.060	0.027	0.004	0.007	0.000
AG	-0.08	-0.47	-0.003	0.05	0.14	0.015	0.000	0.049	0.071	0.018	0.007	0.005	-0.001
P (additive)	0.28	0.13	0.10	0.82	0.96	0.69	0.21	0.98	0.80	0.90	0.92	0.11	0.03
D9N GG	0.05	-0.21	0.000	0.16	0.18	0.016	0.001	0.057	0.061	0.027	0.004	0.007	0.000
GA	0.19	-0.13	0.018	0.03	0.20	0.037	0.000	0.033	0.061	0.061	0.002	0.006	-0.001
P (additive)	0.28	0.07	0.19	0.24	0.79	0.56	0.08	0.54	0.93	0.32	0.35	0.77	0.97

Values shown are mean changes, with the exception of *, where values are the difference of log-transformed values. Change in HDL subpopulations expressed as change in apo A-I g/L.
P value for the interaction between treatment (placebo or HT) and SNP.