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CETP genotypes and HDL-cholesterol phenotypes in the HERITAGE Family Study

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Spielmann N, Leon AS, Rao DC, Rice T, Skinner JS, Bouchard C, Rankinen T. CETP genotypes and HDL-cholesterol phenotypes in the HERITAGE Family Study. *Physiol Genomics* 31: 25–31, 2007. First published May 22, 2007; doi:10.1152/physiolgenomics.00281.2006.—Associations between cholesteryl ester transfer protein (CETP) polymorphisms and high-density lipoprotein cholesterol (HDL-c) levels before and after 20 wk of endurance training were investigated in the HERITAGE Family Study. Plasma HDL-c, HDL_2-c, HDL_3-c, and apolipoprotein (apo)A1 levels were measured, and 13 CETP single nucleotide polymorphisms (SNPs) were genotyped in 265 blacks and 486 whites. Three haplotypes defined by SNPs at the −1337, −971, and −629 sites were strongly associated with baseline HDL-c levels in whites. Both C−1337T and C−629A were associated with baseline HDL-c ($P < 0.001$) and apoA1 ($P < 0.01$) when tested separately. However, only C−629A remained significant in a combined model. G−971A was not associated with HDL phenotypes, but showed significant interactions with C−629A ($P = 0.002$) on baseline traits. Genotype-by-sex interactions were observed at the −629 locus for HDL_3-c ($P = 0.004$) and apoA1 ($P = 0.02$) training responses in whites. In women, the −629 A/A homozygotes showed greater increases in HDL_3-c ($P = 0.02$) and apoA1 ($P = 0.02$) levels than the other genotypes. Finally, apolipoprotein E (APOE) genotype and the CETP C−629A locus contributed independently and in additive fashion to the HDL traits, explaining 6.0–8.8% of the variance. The CETP −1337T and −629A alleles are associated with higher baseline HDL-c and apoA1 levels. The beneficial effects of endurance training on plasma HDL_3-c and apoA1 levels are evident in white women homozygous for the −629A allele. The CETP and APOE genotypes account for up to 9% of the variance in HDL-c phenotypes in the HERITAGE Family Study.

single nucleotide polymorphism; high-density lipoprotein-cholesterol; exercise training; family study

CHOLESTERYL ESTER (CE) transfer protein (CETP) plays a central role in human cholesterol metabolism and transport. Plasma CETP protein shuttles CE from apolipoprotein (apo)A1 containing high-density lipoproteins (HDL) to very low (VLDL) and low-density (LDL) lipoproteins in exchange for triglycerides (31). CETP facilitates the removal of excess cholesterol from the body via LDL receptor-mediated uptake in the liver. CETP is a key enzyme in reverse cholesterol transport (17). Human CETP deficiencies caused by mutations in the CETP gene are characterized by very high HDL-cholesterol (HDL-c) and very low LDL-cholesterol (LDL-c) levels (18, 28, 34). This observation supports the notion that CETP activity modifies plasma lipoprotein levels, although data regarding the consequent effects on cardiovascular disease risk are still inconclusive. Recent reviews have addressed these issues in detail (4, 7, 10).

Regular physical activity reduces the risk of coronary heart disease by multiple mechanisms, including an increase in plasma HDL-c levels (11, 16, 25, 27). It is widely recognized that regular physical activity is associated with a less atherogenic lipid and lipoprotein profile. However, considerable heterogeneity in the responsiveness of plasma HDL-c levels to exercise training has been reported (6, 24). For instance, in the HERITAGE Family Study, marked interindividual variability was found in the HDL-c response to a fully standardized endurance training program (23).

Several polymorphisms have been identified in the human CETP gene. Most correspond to single nucleotide polymorphisms (SNPs) located in the promoter region of the gene, in introns, and in exons (21). In vitro studies discovered three functional CETP promoter polymorphisms (C−1337T, G−971A, C−629A) affecting plasma CETP activity (9, 12, 22). These findings suggest that DNA sequence variation at the CETP gene locus may affect plasma CETP activity and lipoprotein levels in humans (19, 32). We hypothesized that DNA sequence variation in the CETP promoter region is associated with lipid and lipoprotein levels in the sedentary state and in response to 20 wk of endurance exercise training in the HERITAGE Family Study cohort.

MATERIALS AND METHODS

Subjects. The study cohort consists of 265 black subjects (89 men and 176 women) from 114 family units and 486 white subjects (231 men and 255 women) from 99 nuclear families. The study design has been described previously (5). Subjects ranged in age from 17 to 65 yr, were healthy and sedentary, and met a number of inclusion and exclusion criteria (5). Briefly, any chronic disease or condition preventing participation in the training program was defined as an exclusion criterion. The study protocol was approved by the Institutional Review Board of each of the HERITAGE Family Study research consortium centers. All subjects gave written informed consent.

Exercise training program. The 20-wk exercise training program was standardized for each participant based on the heart rate (HR)-oxygen consumption ($\dot{V}O_2$) relationship measured at baseline (30). During the first 2 wk, subjects trained at a HR corresponding to 55% of baseline maximal $\dot{V}O_2$ ($\dot{V}O_{2max}$) for 30 min per session. Duration and intensity of the training sessions were gradually increased to 50 min and the HR associated with 75% of baseline $\dot{V}O_{2max}$, which were

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then sustained for the last 6 wk. Training frequency was three times per week, and all training sessions were performed on cycle ergometers in the laboratory. Trained exercise specialists supervised each exercise session.

Body mass index. Stature and body mass were measured with a standardized protocol, and body mass index (BMI) was calculated by dividing body mass (kg) by stature squared (m^2) (33).

Plasma lipid and lipoprotein determinations. Plasma lipids were determined as previously described (24). Briefly, blood samples were obtained with participants in a semirecumbent position from an antecubital vein into Vacutainer tubes containing EDTA in the morning after a 12-h fast. Samples were taken twice at baseline and 24 and 72 h after the last exercise training session. Cholesterol and triglyceride levels were determined in plasma and lipoproteins by enzymatic methods with a Technicon RA-500 analyzer (Bayer, Tarrytown, NY). Plasma VLDL was isolated by ultracentrifugation (15). The HDL fraction was obtained after precipitation of LDL in the infranatant by the heparin-manganese chloride method (8). Selective precipitation was used to isolate HDL fractions with dextran sulfate (14). apoA1 and apoB concentrations were measured in plasma by the rocket immunoelectrophoretic method of Laurell (20). Extensive quality control procedures were implemented to ensure high-quality lipid assays and other study data (13).

Determination of genotypes. The CETP SNPs were selected from the Seattle SNP database (**http://pga.mbt.washington.edu**). Supplemental Table I gives an overview of all 13 CETP polymorphisms defined by the nomenclature, the location, and the allelic variation.[1] All SNPs were genotyped by a primer extension method with fluorescence polarization detection (FP-TDI). Changes in fluorescence polarization after excitation of the samples by plane-polarized light were measured with a Victor2 plate reader (Perkin Elmer Life Sciences). The allele calling was done with SNP scorer genotyping software (Perkin Elmer Life Sciences).

Statistical analyses. A χ^2-test was used to verify whether the observed genotype frequencies were in Hardy-Weinberg equilibrium (HWE). The pairwise linkage disequilibrium (LD) between the SNPs was assessed with the ldmax program available in the GOLD software package (3). Associations between the CETP SNPs and plasma lipid and lipoprotein phenotypes were analyzed with two complementary methods: a variance components and likelihood ratio test-based procedure in the QTDT software package (1) and a MIXED model-based procedure in the SAS software package. The QTDT uses all the family data and provides a genetic model-based approach. On the other hand, the MIXED model, while using all the family data, provides a simple and yet powerful approach. Haplotypes were constructed with the MERLIN software package (2). Associations with the haplotypes were analyzed with the QTDT association model.

The total association model of the QTDT software utilizes a variance-components framework to combine a phenotypic means model and the estimates of additive genetic, residual genetic, and residual environmental variances from a variance-covariance matrix into a single likelihood model (1). The evidence of association is evaluated by maximizing the likelihoods under two conditions: the null hypothesis (L0) restricts the additive genetic effect of the marker locus to zero ($\beta a = 0$), whereas the alternative hypothesis (L1) does not impose any restrictions to βa. The quantity of twice the difference of the log likelihoods between the null and the alternative hypotheses $\{2[\ln (L1) - \ln (L0)]\}$ is distributed as χ^2 with 1 df (difference in number of parameters estimated). In the MIXED model, nonindependence among family members was adjusted for with a "sandwich estimator," which asymptotically yields the same parameter estimates as ordinary least squares or regression methods. The standard errors and consequently the hypothesis tests are adjusted for the dependencies. The method is general, assuming the same degree of dependence among all members within a family. Baseline plasma lipid and lipoprotein phenotypes were adjusted for age, sex, and BMI. The training response phenotypes were adjusted for age, sex, BMI, and the baseline value of the phenotype. The phenotypes HDL-c, HDL_2-c, and HDL_3-c in blacks and HDL_2-c in whites had to be logarithmically transformed before analysis to normalize their distributions.

Since multiple SNPs were used for the association studies, we applied a multiple testing correction proposed by Nyholt (29). Briefly, the method utilizes spectral decomposition of matrices of pairwise linkage disequilibriums (r) to estimate variance of eigenvalues. The effective number of independent SNPs can be calculated based on the ratio of observed eigenvalue variance and its maximum. The effective number of SNPs can then be used to adjust the standard α-level (e.g., 5%). In our study, the corrected threshold for statistical significance was set to $P < 0.0051$.

RESULTS

The baseline and training response characteristics of the subjects are presented in Table 1. The minor allele frequencies and the pairwise linkage disequilibrium (r^2 and D') for all markers are shown in Supplemental Table II. All SNPs were tested for HWE among unrelated individuals (parents and randomly selected offspring) of the HERITAGE families. In whites, the genotype frequencies of C−1337T, rs12708968, G−971A, rs17245715, rs4783962, C−631A, rs1532624, and rs5882 (I422V) were in HWE both in parents and in offspring. In white parents, a slight excess of heterozygotes was observed at C−629A and rs708272. However, the genotype frequencies were in HWE in offspring. All genotype frequencies were in HWE in blacks.

Haplotype construction using all 13 polymorphisms resulted in 26 haplotypes in whites (Table 2) and 31 haplotypes in blacks (data not shown). In whites, *haplotypes 4* and *8* were strongly associated with baseline HDL-c ($P < 0.001$ for both), HDL_2-c ($P = 0.01$ and $P < 0.001$, respectively), HDL_3-c ($P < 0.001$ and $P = 0.02$, respectively), and apoA1 ($P = 0.02$ for both) levels. *Haplotype 18* showed suggestive associations with total HDL-c, HDL_2-c, and apoA1 levels. *Haplotype 4* was associated with higher HDL phenotype levels, whereas *allele 8* carriers had significantly lower HDL levels than noncarriers. The differences between *haplotypes 4* and *8* were defined by SNPs C−1337T, C−629A and markers rs708272 and rs1532624, which are in relatively strong LD ($r^2 > 0.8$) with C−629A. *Haplotype 18* was identical to *haplotype 8*, except for SNP G−971A, which had A and G in *haplotypes 8* and *18*, respectively. On the basis of the haplotype results, we focused next on the independent contributions and potential interactions of C−1337T, G−971A, and C−629A.

Both C−1337T and C−629A were significantly associated with the age-, sex-, and BMI-adjusted baseline HDL phenotypes when tested in separate models. In both sites, the minor allele homozygotes had considerably higher plasma levels than the C/C homozygotes (Table 3). However, when both markers were entered in the same model, only C−629A remained significant. The G−971A variant was not associated with any of the baseline HDL phenotypes. However, we found significant interactions between G−971A and C−629A on baseline HDL traits (Fig. 1). The strong association between the C−629A locus and plasma HDL was particularly evident among the −971A allele carriers but not the −971 G/G homozygotes.

[1] The online version of this article contains supplemental material.

Table 1. *Basic characteristics of subjects*

Variables	Black		White	
	Men	Women	Men	Women
n (baseline/training response)	89/85	176/159	231/219	255/245
Age, yr	32.7±12.3	35.9±14.9	33.1±11.3	34.9±14.1
BMI, kg/m^2				
Baseline	27.5±5.6	26.6±4.9	28.3±6.6	25.1±5.0
Training response	−0.23±0.9	−0.13±0.7	−0.16±1.1	−0.04±0.8
Plasma cholesterol, mmol/l				
Baseline	4.4±1.0	4.5±1.0	4.2±0.8	4.4±0.9
Training response	+0.04±0.4	−0.02±0.4	+0.04±0.4	+0.08±0.4
HDL-c, mmol/l				
Baseline	1.0±0.3	0.9±0.2	1.1±0.3	1.1±0.3
Training response	+0.03±0.1	+0.03±0.1	+0.03±0.1	+0.05±0.1
HDL_2-c, mmol/l				
Baseline	0.3±0.2	0.3±0.1	0.4±0.2	0.4±0.2
Training response	+0.03±0.1	+0.01±0.1	+0.05±0.1	+0.03±0.1
HDL_3-c, mmol/l				
Baseline	0.7±0.1	0.7±0.1	0.7±0.2	0.7±0.1
Training response	−0.01±0.1	+0.02±0.1	−0.01±0.1	+0.02±0.1
apoA1, g/l				
Baseline	1.1±0.2	1.1±0.2	1.2±0.2	1.2±0.2
Training response	+0.02±0.1	+0.03±0.1	+0.01±0.1	+0.04±0.1
Plasma triglyceride, mmol/l				
Baseline	1.2±0.8	1.6±0.9	0.9±0.4	1.2±0.6
Training response	+0.01±0.6	−0.07±0.5	−0.05±0.3	+0.03±0.4
LDL-c, mmol/l				
Baseline	2.9±0.8	3.1±0.9	2.8±0.7	2.9±0.8
Training response	+0.00±0.4	−0.02±0.4	+0.02±0.4	+0.01±0.4
apoB, g/l				
Baseline	0.8±0.3	0.9±0.3	0.8±0.2	0.8±0.2
Training response	+0.01±0.1	−0.01±0.1	+0.01±0.1	+0.02±0.1

Data are presented as means ± SD for *n* subjects. BMI, body mass index; HDL-c, high-density lipoprotein-cholesterol, apo, apolipoprotein; LDL-c, low-density lipoprotein-cholesterol.

In black HERITAGE subjects, C−629A showed similar associations with HDL-c and HDL_2-c as in white subjects: the A/A homozygotes had 5% and 21% higher HDL-c and HDL_2-c levels, respectively, than the C/C homozygotes. Plasma HDL_3-c and apoA1 levels did not differ among genotypes (Table 3). One of the black men (−629 C/A heterozygote) had very high HDL-c levels both at baseline (3.54 mmol/l) and after training (3.79 mmol/l). When the analyses were repeated without this outlier, the associations with HDL-c ($P = 0.017$) and HDL_2-c ($P = 0.0016$) remained unchanged.

In whites, the CETP haplotype construct showed a global association with HDL_3-c training response ($P = 0.005$), which was due to associations with *haplotypes 4* and *8* ($P = 0.021$ for both). *Haplotype 4* was also associated with the apoA1 training response ($P = 0.011$). In single marker analyses, the C−629A marker showed the strongest associations with plasma HDL_2-c and apoA1 training responses, whereas total HDL-c and HDL_2-c responses were not associated with any of the markers. Further analyses revealed marked sex differences in the associations between the C−629A genotype and HDL_3-c and apoA1 training responses: no associations were found in men, whereas in women the −629 A/A homozygotes showed considerably greater increases in HDL_3-c and apoA1 levels than the C/C and C/A genotypes (Fig. 2). Similar analyses were performed for total cholesterol, triglycerides, LDL-c, and apoB phenotypes across CETP genotypes, and no significant associations were observed.

We previously reported (26) strong associations between the apolipoprotein E (APOE) genotype and plasma HDL levels in the HERITAGE Family Study. When both the APOE genotype and CETP C−629A were modeled simultaneously, the results indicated that the two gene loci contribute independently and in an additive fashion, explaining 6.2–7.3% and 6.0–8.8% of the variance in the HDL phenotype levels at baseline and after training, respectively (Table 4). Interestingly, in the sedentary state (baseline), the CETP locus had a slightly stronger effect on total HDL-c and HDL_2-c levels, whereas the APOE locus contributed more to the HDL_3-c and apoA1 levels than CETP. However, after training, the contribution of each locus was approximately equal across all four phenotypes.

DISCUSSION

The main findings of the present study are *1*) the significant associations between the C−1337T and C−629A variants and plasma HDL-c and apoA1 levels, *2*) the G−973A-by-C−629A interaction effects on HDL-c phenotypes, *3*) the associations between the C−629A variant and plasma HDL_3-c and apoA1 training responses in white women, and *4*) the additive contributions of the CETP and APOE gene loci to plasma HDL-related phenotypes.

Our initial haplotype analysis identified two haplotypes that were strongly associated with baseline HDL-c levels in whites. These two haplotypes were defined by sequence variations at the −1337 and −629 sites. A third haplotype, defined by variant −971, showed moderate associations with the HDL phenotypes. When analyzed separately, both the −1337 and −629 variants were strongly associated with the HDL pheno-

Table 2. *Associations between CETP haplotypes and baseline HDL-related phenotypes in white subjects*

		CETP Polymorphisms													*P* Values			
Haplotypes	Frequency, %	C−1337T	rs12708968	rs13338602	G−971A	rs17245715	rs4783962	rs17237883	rs1800776	C−629A	rs17231520	rs708272	rs1532624	rs5882	HDL-c	HDL_2-c	HDL_3-c	apo A1
Global															5×10^{-6}	1×10^{-6}	0.0024	0.0075
1	0.12	T	A	C	A	C	G	C	A	C	C	C	C	T	1.00	1.00	1.00	1.00
2	1.59	T	A	C	A	C	G	C	C	A	C	C	A	C	0.603	0.202	0.689	0.413
3	10.0	T	A	C	A	C	G	C	C	A	C	T	A	C	0.181	0.320	0.386	0.286
4	22.2	T	A	C	A	C	G	C	C	A	C	T	A	T	0.00005	0.006	0.0002	0.017
5	0.37	T	A	C	A	C	G	C	C	A	C	T	C	T	0.380	0.680	0.252	0.639
6	0.12	T	G	C	A	C	G	C	C	A	C	T	A	C	0.920	1.00	0.764	1.00
7	0.85	C	A	C	A	C	G	C	C	C	C	C	C	C	0.039	0.032	0.292	0.252
8	11.0	C	A	C	A	C	G	C	C	C	C	C	C	T	0.0002	0.00002	0.020	0.022
9	2.56	C	A	C	A	C	G	C	C	A	C	C	C	C	0.671	0.026	0.194	0.502
10	1.46	C	A	C	A	C	G	C	C	A	C	C	C	T	0.920	0.584	0.337	0.560
11	2.68	C	A	C	A	C	G	C	C	A	C	C	C	T	0.777	0.740	1.00	0.345
12	0.24	C	A	C	A	C	G	C	C	A	C	T	A	T	0.192	0.232	0.752	0.718
13	3.41	C	A	C	G	C	A	C	C	C	C	C	A	C	0.438	0.517	0.232	0.887
14	17.1	C	A	C	G	C	A	C	C	C	C	C	C	C	1.00	0.791	0.806	0.671
15	1.83	C	A	C	G	C	A	C	C	C	C	C	C	T	0.199	0.073	0.862	0.823
16	9.27	C	A	C	G	C	G	C	A	C	C	C	C	C	0.181	0.325	0.406	0.467
17	1.83	C	A	C	G	C	G	C	C	C	C	C	C	C	0.823	0.752	0.493	0.095
18	5.37	C	A	C	G	C	G	C	C	C	C	C	C	T	0.064	0.025	0.624	0.018
19	0.37	C	A	C	G	T	G	C	C	A	C	T	A	C	0.698	0.791	0.294	0.256
20	0.12	C	G	C	G	C	A	C	C	C	C	C	C	T	0.689	0.920	0.399	0.777
21	0.24	C	G	C	G	T	G	C	C	C	C	T	A	C	0.267	0.409	0.590	0.228
22	0.12	C	G	C	G	T	G	C	C	C	C	T	A	T	0.320	0.330	0.663	0.164
23	0.12	C	G	C	G	T	G	C	C	A	C	C	A	T	1.00	1.00	1.00	1.00
24	4.88	C	G	C	G	T	G	C	C	A	C	T	A	C	0.170	0.136	0.458	0.107
25	1.71	C	G	C	G	T	G	C	C	A	C	T	A	T	0.427	1.00	0.269	0.098
26	0.24	C	G	G	G	C	G	C	C	A	C	C	A	C	0.632	1.00	0.343	0.162

CETP, cholesteryl ester transfer protein. rs5882 = I422V.

Table 3. *Associations between C−1337T and C−629A and HDL-c, HDL_2-c, HDL_3-c, and apoA1 levels at baseline and in response to training*

	C−1337T					C−629A				
	C/C	C/T	T/T	*P* Value MIXED	*P* Value QTDT	C/C	C/A	A/A	*P* Value MIXED	*P* Value QTDT
					Black					
n	200/183	63/59	5/3			64/58	112/102	82/77		
HDL-c										
Baseline	1.08 (0.02)	1.09 (0.03)	1.03 (0.04)	0.92	0.48	1.08 (0.04)	1.07 (0.03)	1.13 (0.03)	0.30	0.01
Training	+0.02 (0.01)	+0.06 (0.02)	−0.002 (0.04)	0.01	0.20	+0.02 (0.02)	+0.03 (0.01)	+0.04 (0.01)	0.77	0.70
HDL_2-c										
Baseline	0.30 (0.03)	0.34 (0.03)	0.27 (0.02)	0.32	0.14	0.28 (0.02)	0.30 (0.03)	0.35 (0.03)	0.003	0.01
Training	+0.04 (0.01)	+0.05 (0.02)	+0.10 (0.05)	0.48	0.40	+0.05 (0.02)	+0.04 (0.01)	+0.04 (0.01)	0.53	0.82
HDL_3-c										
Baseline	0.72 (0.01)	0.71 (0.01)	0.76 (0.06)	0.40	0.86	0.71 (0.03)	0.70 (0.03)	0.72 (0.03)	0.61	0.27
Training	−0.02 (0.01)	+0.01 (0.01)	−0.10 (0.03)	0.03	0.42	−0.03 (0.02)	−0.01 (0.01)	−0.01 (0.01)	0.50	0.92
apoA1										
Baseline	1.15 (0.01)	1.15 (0.02)	1.25 (0.09)	0.38	0.58	1.14 (0.02)	1.15 (0.14)	1.17 (0.02)	0.83	0.81
Training	+0.01 (0.01)	+0.03 (0.01)	−0.06 (0.05)	0.15	0.46	+0.00 (0.02)	+0.02 (0.01)	+0.01 (0.01)	0.59	0.55
					White					
n	213/204	221/211	54/51			121/115	263/251	94/90		
HDL-c										
Baseline	1.00 (0.02)	1.07 (0.02)	1.13 (0.03)	<0.001	<0.001	0.96 (0.02)	1.00 (0.02)	1.11 (0.02)	<0.001	<0.001
Training	+0.04 (0.01)	+0.05 (0.01)	+0.04 (0.02)	0.24	0.02	+0.04 (0.01)	+0.05 (0.01)	+0.05 (0.02)	0.14	0.12
HDL_2-c										
Baseline	0.29 (0.02)	0.32 (0.03)	0.36 (0.03)	<0.001	<0.001	0.26 (0.02)	0.31 (0.03)	0.36 (0.03)	<0.001	<0.001
Training	+0.02 (0.01)	+0.02 (0.01)	+0.02 (0.02)	0.29	0.14	+0.02 (0.01)	+0.03 (0.01)	+0.01 (0.01)	0.13	0.65
HDL_3-c										
Baseline	0.67 (0.01)	0.70 (0.01)	0.73 (0.02)	0.001	<0.001	0.66 (0.01)	0.70 (0.01)	0.71 (0.01)	0.01	0.01
Training	+0.02 (0.01)	+0.02 (0.01)	+0.02 (0.02)	0.09	0.02	+0.01 (0.01)	+0.02 (0.01)	+0.04 (0.01)	0.004	<0.001
apoA1										
Baseline	1.16 (0.01)	1.19 (0.01)	1.21 (0.02)	0.008	0.003	1.13 (0.02)	1.19 (0.01)	1.21 (0.02)	0.001	<0.001
Training	+0.03 (0.01)	+0.03 (0.01)	+0.06 (0.02)	0.05	0.02	+0.04 (0.01)	+0.03 (0.01)	+0.06 (0.02)	0.02	0.001

Data are presented as means (SE) for *n* subjects (baseline/after training).

types, whereas the −971 locus showed no associations. However, when analyzed in the same model, only the −629 polymorphism remained significant. Our data are in agreement with previous studies reporting that the −629 locus is the strongest CETP gene variant predictor of plasma HDL-cholesterol levels in Caucasians (12, 17).

Although the −971 locus per se was not associated with HDL traits, our data strongly suggest that the −971 variant modifies the effect of the −629 locus on plasma HDL levels. The strong association between HDL-c and the −629 locus was even more pronounced among subjects who carried at least one copy of the A allele at the −971 site, whereas the HDL levels did not differ among the −629 genotypes at the −971 G/G homozygotes.

The novel finding of the present study is the association between the −629 genotypes and endurance training-induced changes in plasma HDL_3-c and apoA1 levels. In white HERITAGE subjects, the −629 A/A homozygotes showed significantly greater HDL_3-c and apoA1 training responses than the other −629 genotypes. In addition, we observed significant sex-by-genotype interactions at the −629 locus for HDL_3-c and apoA1 training responses in white subjects. The associations between the −629 genotypes and the HDL_3-c and apoA1 training responses were evident in women, while no

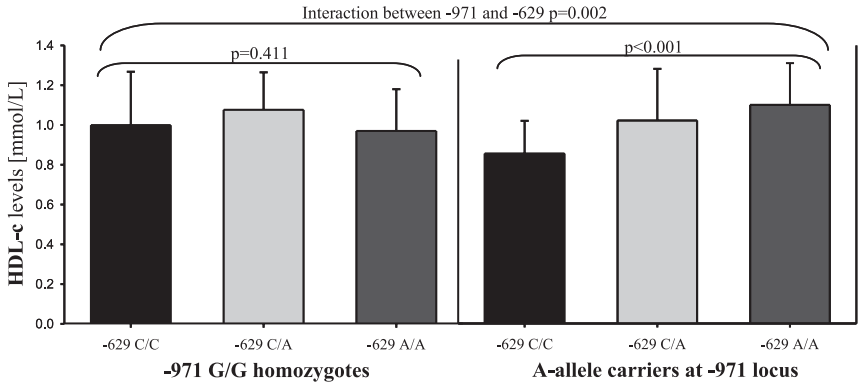

Fig. 1. Baseline plasma high-density lipoprotein cholesterol (HDL-c) levels in the C−629A genotypes stratified by −971A allele carrier status.

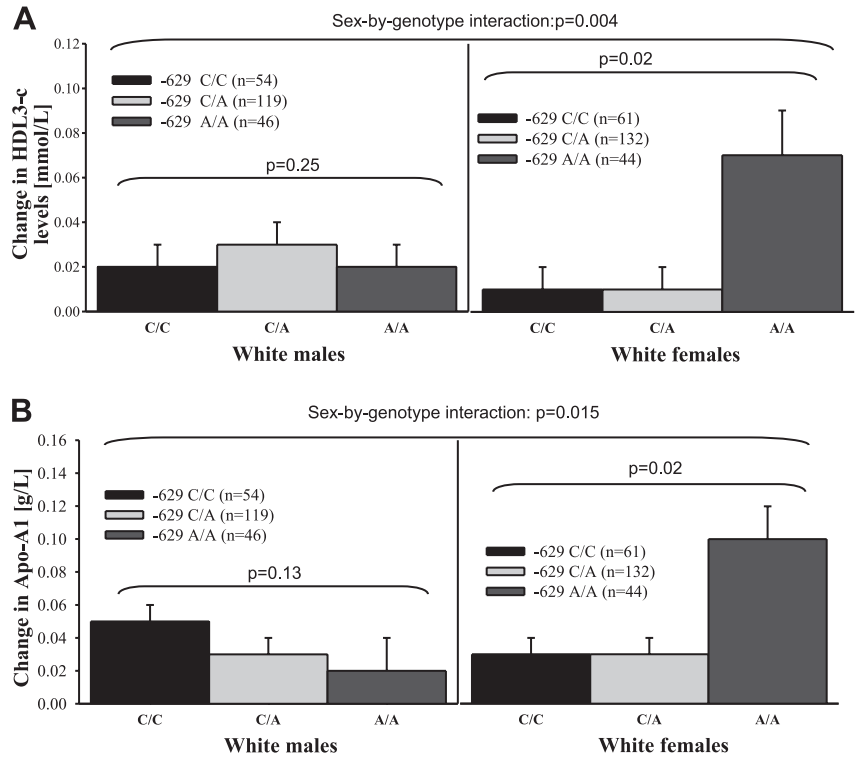

Fig. 2. Plasma HDL_3-c (*A*) and apolipoprotein (apo)A1 (*B*) training response as a function of the C−629A genotype in white men and women.

differences among the genotypes were observed in men. The greater training response in the −629 A/A homozygote women translates into significantly higher plasma HDL_3-c and apoA1 levels in the trained state. Interestingly, this pattern was evident in men already in the sedentary state (baseline) but emerged in women only after 20 wk of regular exercise.

Plasma HDL-c is a typical multifactorial phenotype affected by genetic and nongenetic factors and their interactions. Furthermore, the genetic component is characterized by oligo-/polygenic effects. Our data from the HERITAGE Family Study provide an excellent example of the oligogenic background of HDL-c-related phenotypes. We previously reported (26) a strong association between baseline HDL-c levels and APOE genotypes. We incorporated the previous APOE results with those of the present CETP analyses and were able to show that both loci contribute independently and in additive fashion to plasma HDL-c, HDL_2-c, HDL_3-c, and apoA1 levels in the sedentary (baseline) and physically active (posttraining) states. The APOE and CETP informative polymorphism contributions to the variance in plasma HDL-c phenotypes ranged from 5% to 9%.

In summary, the CETP −1337A and −629A alleles are strongly associated with higher baseline HDL-c and apoA1 levels. Haplotype analysis identified an interaction between G−971A and C−629A affecting plasma HDL-c levels and revealed that the −971 locus modifies the association between −629 genotypes and HDL-c levels. Furthermore, the beneficial effect of endurance training on plasma HDL_3-c and apoA1 levels was particularly evident in white −629 A/A homozygous women. The CETP locus variants together with the common polymorphism in the APOE gene account for as much

Table 4. *Contribution of CETP C−629A and APOE polymorphisms and covariates age, sex, and BMI on HDL-c and apoA1 phenotypes at baseline and after training in white subjects*

	HDL-c		HDL_2-c		HDL_3-c		apoA1	
	R^2	*P* Value	R^2	*P* Value	R^2	*P* Value	R^2	*P* Value
Baseline								
Age	0.03	<0.0001	0.02	0.0002	0.01	0.03	0.06	<0.0001
Sex	0.15	<0.0001	0.16	<0.0001	0.03	<0.0001	0.04	<0.0001
BMI	0.08	<0.0001	0.10	<0.0001	0.02	0.002	0.02	0.002
C−629A	0.04	<0.0001	0.05	<0.0001	0.02	0.02	0.03	0.001
APOE	0.03	0.001	0.01	0.32	0.05	0.0001	0.04	0.0004
After training								
Age	0.02	0.0001	0.02	0.0004	0.01	0.01	0.05	<0.0001
Sex	0.15	<0.0001	0.19	<0.0001	0.03	0.0001	0.05	<0.0001
BMI	0.08	<0.0001	0.09	<0.0001	0.02	0.002	0.02	0.005
C−629A	0.04	<0.0001	0.03	0.0001	0.04	0.0001	0.04	<0.0001
APOE	0.04	<0.0001	0.03	0.001	0.05	0.0001	0.05	<0.0001

APOE, apolipoprotein E.

as 5–9% of the variance in plasma HDL-c phenotypes in the sedentary state, as well as after training.

Studies are needed to define the molecular mechanisms modulating the expression of the CETP gene among various genotypes, their role in HDL metabolism, and how they influence the response to regular exercise.

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