Association of the A-204C polymorphism in the cholesterol 7α-hydroxylase gene with variations in plasma low density lipoprotein cholesterol levels in the Framingham Offspring Study

Patrick Couture,* James D. Otvos,† L. Adrienne Cupples,§ Peter W. F. Wilson, Ernst J. Schaefer,* Jose M. Ordovas[1,*]**

Lipid Metabolism Laboratory,* Jean Mayer-USDA Human Nutrition Center on Aging at Tufts University, Boston, MA 02111; Department of Biochemistry,† North Carolina State University, Raleigh, NC 27695; Boston University School of Public Health,§ Boston, MA 02118; and The Framingham Heart Study,** National Heart, Lung, and Blood Institute, Framingham, MA 01701

Abstract The first reaction of the catabolic pathway of cholesterol is catalyzed by CYP7 and serves as the rate-limiting step and major site of regulation of bile acid synthesis in the liver. A common A to C substitution at position −204 of the promoter of CYP7 gene has been associated with variations in plasma LDL-cholesterol concentrations but the effect of this polymorphism is unknown in the general population. The aim of the present study was therefore to investigate the association of this polymorphism to lipoprotein levels in a population-based sample of 1139 male and 1191 female Framingham Offspring participants. In men, the C variant was associated with higher plasma concentrations of LDL-cholesterol and this association remained significant after adjustment for familial relationship, age, BMI, smoking, alcohol intake, the use of beta-blockers, and apoE genotype. The C variant was also associated with an increased TC/HDL ratio in men. Variance components analysis indicated that allelic variability at nucleotide −204 of the CYP7 gene and polymorphism of the apoE gene accounted for 1 and 5% of the variation of plasma LDL-cholesterol concentrations, respectively. In women, however, there was no relationship between LDL-cholesterol and the A-204C polymorphism but subjects homozygous for the CC genotype had significantly lower triglyceride levels than heterozygotes. Moreover, no significant relationship was found between the A-204C variants and lipoprotein particle diameter or the prevalence of coronary heart disease in both genders. Thus, our results show that the A-204C polymorphism in the CYP7 gene is associated with statistically significant variations in LDL-C and triglyceride concentrations in men and women, respectively, but the cumulative effects of these variations on atherosclerotic risk remain uncertain.—Couture, P., J. D. Otvos, L. A. Cupples, P. W. F. Wilson, E. J. Schaefer, and J. M. Ordovas. **Association of the A-204C polymorphism in the cholesterol 7α-hydroxylase gene with variations in plasma low density lipoprotein cholesterol levels in the Framingham Offspring Study. *J. Lipid Res.* 1999.** 40: **1883–1889.**

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Evidence from epidemiologic studies clearly indicates that high plasma concentrations of LDL-cholesterol are associated with an increased risk of developing CHD (1). Other major CHD risk factors include age, male gender, arterial hypertension, diabetes, smoking, a familial history of premature CHD disease, and a decreased plasma HDL-cholesterol (2–4). Data from family and twin studies have indicated that genetic factors play a major role in the susceptibility to atherosclerosis and that these influences are thought to result from variability in multiple genes, which act in concert to increase susceptibility (5). The influence of genetic variation on lipoprotein levels primarily manifests as decreased HDL-cholesterol and elevated LDL-cholesterol (6, 7). The molecular mechanisms responsible for genetic variation in plasma LDL-cholesterol levels have been most clearly elucidated in Mendelian disorders of LDL metabolism. Familial hypercholesterolemia is a common autosomal codominant disorder caused by mutations in the LDL receptor gene and is characterized by markedly elevated plasma LDL-cholesterol concentrations, tendinous xanthomatosis, and premature CHD (8). Moreover, familial defective apoB and familial hypocholesterolemia in which individuals have abnormal plasma levels of LDL-cholesterol are caused by mutations in the gene encoding

Abbreviations: apo, apolipoprotein; CHD, coronary heart disease; CYP7, cholesterol 7α-hydroxylase; HDL, high density lipoprotein; LDL, low density lipoprotein; NMR, nuclear magnetic resonance; OR, odds ratio; TC, total cholesterol.

[1] To whom correspondence should be addressed.

apoB (9, 10). These disorders, however, are uncommon and account for a small fraction of the genetically determined variation in plasma LDL-cholesterol levels (11).

In the general population, associations between genetic polymorphisms and interindividual variations of plasma LDL-cholesterol concentrations have been reported, but these relationships seem to be inconsistent or at least influenced by other factors, such as age, gender, diet, and ethnic origin. Specific genetic polymorphisms in LDL receptor, apoB, and microsomal triglyceride transfer protein gene have been studied but their contribution to the population variance in LDL-cholesterol remains controversial (12–17). In fact, only the polymorphism in apoE has been shown to be associated consistently with genetically determined variations in plasma LDL-cholesterol levels (18-20). ApoE acts as a ligand for apoB/E receptor uptake and thus plays an essential role in the catabolism of triglyceride-rich lipoproteins such as VLDL and remnants (21). The molecular basis of apoE polymorphism can be traced to two amino acid changes and, taken together, the three common isoforms of apoE (E2, E3, and E4) would account for 5–20% of the genetically determined variation in plasma LDL-cholesterol concentrations. Thus, the major genetic determinants of plasma LDL-cholesterol concentrations still remain to be determined.

In a recent study, the common functional polymorphism in the promoter region of the CYP7 gene has been shown to be associated with significant variations in plasma LDL-cholesterol levels (22). This transversion referred to as C-278A (22) is located 204 bp upstream of the transcription start site (23, 24). CYP7 plays a major role in cholesterol metabolism by catalyzing the first and rate-limiting step in bile acid synthesis in the liver. However, data from the general population with regard to the effect of this common CYP7 variant on lipid and lipoprotein levels, as well as heterogeneity and possibly atherogenecity of the three major lipoprotein classes, are clearly missing. The purpose of the current study, therefore, was to examine the frequency, phenotypic effect on lipoprotein levels, and lipoprotein subclass profiles as well as potential modulation of CHD risk in the Framingham Offspring Study by the A-204C polymorphism in the CYP7 gene.

METHODS

Population subjects

Subjects were participants in the Framingham Offspring Study, a long-term prospective evaluation of risk factors of cardiovascular disease in which participants are the offspring of the subjects of the Framingham Heart Study and their spouses. The details of the design and methods of the Framingham Offspring Study have been presented elsewhere (25). Starting in 1971, a total of 5124 subjects were enrolled (26). Lipid, lipoprotein, and apoprotein measurements as well as DNA, and information on CHD risk factors were available for 1139 men and 1191 women who attended the 4th and 5th examination visits of the Framingham Offspring Study conducted between 1987 and 1995. Nearly all subjects were Caucasians. Data on smoking, blood pressure, height, weight, and diabetes were obtained on these subjects as previously described (26, 27). CHD cases were adjudicated up to 1994, by criteria established for the analysis of Framingham Offspring Study, as described elsewhere (28). CHD included the presence of myocardial infarction, angina pectoris, coronary insufficiency, and coronary death. Subjects taking a lipid-lowering medication were included for the calculation of the CHD prevalence at exam 5.

Plasma lipid, lipoprotein, and apolipoprotein measurements

Plasma was isolated from blood drawn into EDTA tubes after a 12–14 h fast and stored at −70°C for later determination of apolipoproteins and lipoprotein subspecies. Plasma total cholesterol, HDL-cholesterol, and triglyceride levels were measured as previously described (29). LDL-cholesterol concentrations were estimated with the equation of Friedewald, Levy, and Fredrickson (30). The within and between run coefficients of variation for lipid measurements were all less than 5% (31). Plasma levels of apoA-I and apoB were measured by non-competitive enzyme-linked immunosorbent assay, using affinity-purified polyclonal antibodies (32, 33).

Plasma lipoprotein concentrations and subclasses distributions were also determined by proton NMR spectroscopy as described elsewhere (34). The 10 lipoprotein subclass categories used were the following: large VLDL and remnants (40–220 nm), intermediate VLDL (31–40 nm), small VLDL (27–31 nm), large LDL (21.3–27.0 nm), intermediate LDL (19.8–21.2), small LDL (18.3–19.7 nm), large HDL (8.8–13.0 nm), intermediate HDL (7.8–8.8 nm), and small HDL (7.3–7.7 nm). Levels of VLDL subclasses are expressed in units of triglyceride (mg/dL), and those of LDL and HDL subclasses in units of cholesterol (mg/dL). LDL and HDL subclass distributions determined by gradient gel electrophoresis and NMR have also been shown to be closely correlated (35).

DNA analysis

Genomic DNA was isolated from peripheral blood leucocytes by standard methods (36). CYP7 genotyping was performed as previously described (22).

Statistical analyses

To compare men and women who participated in the study, we used chi-square tests for categorical measures and two-sample *t* test for continuous measures. We estimated the allele frequency of the C allele and apoE alleles with the chromosome counting method and used a chi-square test to compare it in men and women. To evaluate the relationship between the CYP7 genotypes (AA, AC, and CC) and lipid levels, we used analysis of covariance techniques which accounted for the familial relationships among the members of the study (mostly siblings and cousins). We used two approaches to accomplish these analyses. First, we used a repeated measures approach which assumed an exchangeable correlation structure among all members of a family, using PROC MIXED in SAS. As this approach does not accurately represent the true correlation structure within these pedigrees, we also used a measured genotype approach (37) as implemented in SOLAR, a variance component analysis computer package for quantitative traits measured in pedigrees of arbitrary size (38). The latter approach fully accounts for the different types of relationships within a pedigree in performing an analysis of variance on the defined genotypes. In these analyses, we used several different models to adjust for potential confounders. First, we obtained essentially crude results which accounted only for the family structure; second, we adjusted for age, BMI, smoking, alcohol consumption, beta-blockers, and menopausal status and hormonal replacement therapy in women. In our final analysis, we

added apoE genotypes to the model with E2/E2 and E2/E3 in one group, E3/E4 and E4/E4 in a second group, and E3/E3 as the reference group. Subjects with apoE2/E4 genotypes, of which there were very few, were excluded.

RESULTS

Demographic, genotypic, and biochemical characteristics

We analyzed a total of 2330 subjects (1139 males and 1191 females) who participated in the Framingham Offspring Study and who had lipid values available off lipid lowering medication. The demographic, genotypic, and biochemical characteristics of the participants according to gender are presented in **Table 1**. There was no significant difference in the frequency of the C allele between men and women and the distribution of alleles was consistent with Hardy-Weinberg equilibrium. The mean age of men and women at examination was 52.1 and 51.4 years, respectively. BMI, alcohol consumption, plasma LDL-cholesterol, total apoB, triglycerides as well as glucose levels were significantly higher in men compared with women and HDL, HDL_2, and HDL_3-cholesterol as well as apoA-I concentrations were significantly higher in female participants. Although a similar proportion of men and women were smokers, male subjects smoked more cigarettes per day than the female subjects and over half of the female participants (54.9%) were postmenopausal. Moreover, the apoE genotype distribution was similar in men and women ($P = 0.3570$).

TABLE 1. Demographic, genotypic, and biochemical characteristics of FOS participants according to gender

Variable	Men	Women	*P*
n (Total)	1139	1191	
AA (%)	434 (38.1)	411 (34.5)	
AC (%)	535 (47.0)	589 (49.5)	
CC (%)	170 (14.9)	191 (16.0)	
C Allele frequency	0.384	0.408	0.1940
ApoE alleles	2232	2294	0.3570
E2 (%)	154 (6.9)	184 (8.0)	
E3 (%)	1811 (81.1)	1839 (80.2)	
E4 (%)	267 (12.0)	271 (11.8)	
Age (years)	52.1 ± 10.1	51.4 ± 9.8	0.1033
BMI (kg/m^2)	27.7 ± 3.9	26.0 ± 5.3	0.0001
TC (mg/dL)	202 ± 37	203 ± 39	0.7521
LDL-C (mg/dL)	133 ± 33	126 ± 35	<0.0001
HDL-C (mg/dL)	43.6 ± 11.4	55.6 ± 14.9	0.0001
HDL_2-C (mg/dL)	5.3 ± 3.7	9.7 ± 5.8	0.0001
HDL_3-C (mg/dL)	38.4 ± 8.9	46.0 ± 11.0	0.0001
TG (mg/dL)	135 ± 99	108 ± 83	0.0001
ApoA-I (g/L)	135 ± 24	154 ± 31	0.0001
ApoB (g/L)	102 ± 24	95 ± 25	<0.0001
TC/HDL-C	4.94 ± 1.53	3.92 ± 1.41	0.0001
Glucose (mg/dL)	98 ± 27	93 ± 24	0.0001
Alcohol (ounces/week)	4.0 ± 5.2	1.8 ± 2.6	0.0001
Cigarette/day	6.0 ± 12.8	4.7 ± 10.3	0.0070
Post-menopausal (%)	—	54.9	
On estrogen Rx (%)[a]	—	12.4	

Results are listed as means ± SD.

[a] Includes hormonal replacement therapy and the use of oral contraceptives.

Association of the CYP7 promoter genotypes with lipid, lipoprotein, and apolipoprotein levels

Table 2 shows that, in men and women, the three genotypic groups were equivalent with respect to age and BMI. A total of 18 linear regressions were performed to test for potential associations of the CYP7 promoter polymorphism with lipid and apoprotein profiles. In men, the C variant was associated with higher plasma concentrations of total and LDL-cholesterol and this association remained significant after adjustment for familial relationship, age, BMI, smoking, alcohol intake, the use of beta-blockers, and apoE genotype. The C variant was also associated with an increased TC/HDL ratio in men. Moreover, there were no significant associations between the CYP7 polymorphism and variations in HDL-cholesterol and its subfractions, triglycerides, apoA-I, and apoB. In women, no relationship was found between LDL-cholesterol and the A-204C polymorphism but subjects homozygous for the CC genotype had significantly lower triglyceride levels compared with heterozygotes. This association remained significant after adjustment for covariates.

Association of the CYP7 promoter genotypes with lipoprotein subclass profiles

Potential associations of the CYP7 promoter polymorphism with variations in lipoprotein subclass profiles and lipoprotein particle size were also investigated. Lipoprotein subclass profiles were characterized using automated NMR spectroscopy. As shown in **Table 3**, after adjustment for familial relationship and other covariables, female subjects homozygous for the CC genotype have significantly lower levels of intermediate VLDL and LDL than women carrying the AC genotype. In men, no significant relationships have been found between the CYP7 polymorphism and lipoprotein subfraction profiles. **Table 4** shows the lipoprotein particle size according to CYP7 genotype. In both genders, there was no significant relationship between genotype of the A-204C polymorphism and VLDL, LDL, and HDL particle diameter.

CYP7 promoter polymorphism and risk of CHD

CHD was present in 133 men (12.5%) and 50 women (4.2%). Of the 443 male homozygous for the AA genotype, 44 (9.9%) had a history of CHD compared with 57 (10.1%) of 566 subjects with the AC genotype and 32 (17.2%) of the 186 homozygotes for the CC genotype ($P = 0.0160$). However, this association between the CYP7 polymorphism and CHD did not reach statistical significance in a logistic regression model adjusted for age, BMI, smoking, alcohol intake, use of beta-blocker, systolic blood pressure, and diabetes mellitus (OR = 0.8910; $P = 0.6708$ for the AC genotype and OR = 1.3300; $P = 0.3937$ for the CC genotype).

In women, 11 (2.6%) of the 419 subjects homozygous for the AA genotype had a history of CHD compared with 26 (4.3%) of the 608 heterozygotes and 13 (6.4%) of the 202 homozygotes for the CC genotype. ($P = 0.0740$). As observed in men, this association was not significant after adjustment for covariates including menopausal status and

TABLE 2. Plasma levels of lipids, lipoproteins, and apolipoproteins of FOS subjects according to CYP7 genotypes at nucleotide −204

Variable	AA	AC	CC	P^a	P^b	P^c
Men						
n	434	535	170			
Age (years)	51.4 ± 10.2	52.3 ± 9.9	53.1 ± 10.8	0.1291		
BMI (kg/m^2)	27.8 ± 3.9	27.6 ± 3.9	27.5 ± 3.9	0.6964		
TC (mg/dL)	199 ± 33	204 ± 39	204 ± 38	0.0630	0.0914	0.0496
LDL-C (mg/dL)	129 ± 30	135 ± 36	135 ± 33	0.0292[d]	0.0314[d]	0.0125[d]
HDL-C (mg/dL)	43.6 ± 10.3	43.3 ± 11.7	44.6 ± 13.0	0.4799	0.2602	0.2383
HDL_2-C (mg/dL)	5.4 ± 3.5	5.2 ± 3.7	5.6 ± 4.2	0.3706	0.2140	0.1881
HDL_3-C (mg/dL)	38.3 ± 7.9	38.2 ± 9.3	39.0 ± 10.1	0.2964	0.3436	0.3281
TG (mg/dL)	136 ± 96	137 ± 103	130 ± 95	0.5158	0.2633	0.2327
ApoA-I (g/L)	136 ± 22	134 ± 25	136 ± 26	0.2504	0.1227	0.2070
ApoB (g/L)	101 ± 23	102 ± 25	103 ± 25	0.4518	0.4783	0.2024
TC/HDL-C	4.81 ± 1.33	5.05 ± 1.68	4.91 ± 1.49	0.0219[d]	0.0104[d]	0.0054[d]
Women						
n	411	589	191			
Age (years)	50.9 ± 10.0	51.4 ± 9.6	52.4 ± 10.1	0.2180		
BMI (kg/m^2)	26.0 ± 5.5	26.0 ± 5.2	25.7 ± 5.0	0.6819		
TC (mg/dL)	203 ± 38	203 ± 39	202 ± 41	0.7937	0.5423	0.2619
LDL-C (mg/dL)	126 ± 36	126 ± 34	125 ± 37	0.9065	0.7150	0.5063
HDL-C (mg/dL)	55.3 ± 14.4	55.3 ± 15.0	57.5 ± 15.2	0.1652	0.4258	0.6166
HDL_2-C (mg/dL)	9.4 ± 5.5	9.7 ± 5.9	10.1 ± 5.9	0.3666	0.5725	0.7931
HDL_3-C (mg/dL)	45.9 ± 10.5	45.6 ± 11.0	47.4 ± 11.7	0.1569	0.3737	0.5080
TG (mg/dL)	107 ± 68	113 ± 98	96 ± 53	0.0363[e]	0.0145[e]	0.0218[e]
ApoA-I (g/L)	153 ± 30	153 ± 29	157 ± 36	0.2818	0.5795	0.9030
ApoB (g/L)	96 ± 26	95 ± 25	94 ± 26	0.7732	0.5890	0.5654
TC/HDL-C	3.92 ± 1.35	3.97 ± 1.49	3.75 ± 1.24	0.1517	0.1676	0.1769

Results are listed as means ± SD.

[a] After adjustment for familial relationship.

[b] After adjustment for familial relationship, age, BMI, smoking, alcohol intake and the use of beta-blockers (menopausal status and estrogen therapy in women).

[c] After adjustment for familial relationship, age, BMI, smoking, alcohol intake, use of beta-blockers (menopausal status and estrogen therapy in women) and apoE.

[d] Significant difference between the AC and AA groups.

[e] Significant difference between the AC and CC groups.

hormonal replacement therapy (OR = 1.643; $P = 0.2178$ for the AC genotype and OR = 2.096; $P = 0.1342$ for the CC genotype). Furthermore, in both genders, no significant difference in the age of onset of CHD was observed among the three genotypic groups (data not shown).

DISCUSSION

Due to the high incidence of CHD in developed countries, numerous efforts have been aimed at identifying both environmental and genetic factors related to its pathogenesis. Several epidemiologic studies (6, 7, 39) have suggested that approximately 50% of the variability in plasma LDL-cholesterol concentrations in the general population can be attributed to genetic factors but the number of genes involved and the magnitude of their contribution remain to be determined. To date, only the polymorphism of the apoE gene (ε2, ε3, and ε4) has consistently been shown to be associated with heritable variations in LDL-C concentrations in several populations (40). Recently, genetic variability at CYP7 locus has been linked to significant variations in plasma LDL-cholesterol concentrations. The aim of the present study was therefore to investigate the association between the A-204C polymorphism in the CYP7 promoter gene and variations in lipid and apolipoprotein levels, VLDL, LDL, and HDL subclass profiles, and the risk of developing CHD in 2330 participants of the Framingham Offspring Study. We report here that approximately 60% of North Americans of Caucasian descent may be carriers of the C variant of the A-204C polymorphism. The carrier frequency for the C-204 variant in the Framingham Offspring Study was comparable to the prevalence reported earlier in the American population (22).

The association between the A-204C polymorphism in the CYP7 promoter gene and plasma LDL-cholesterol levels that we have observed in the Framingham Offspring Study male participants is in agreement with a previous study in which Wang et al. (22) showed that allelic variability at CYP7 locus accounted for 15% of the overall variation in plasma LDL-cholesterol levels in 150 nuclear families. In this study, the A-204C polymorphism was also found to be associated with significant variations in LDL-cholesterol levels in unrelated white male and female Americans. Compared with carriers of the AA genotype, male and female carriers of the CC genotype had a 9 and 15% increase in plasma LDL-cholesterol levels, respectively. In contrast to these results, however, the variance components analysis performed in the Framingham Offspring Study participants indicated that the A-204C polymorphism in the CYP7 gene and the polymorphism of apoE

TABLE 3. Lipoprotein subclass distributions of FOS subjects according to CYP7 genotypes at nucleotide −204

Variable	AA	AC	CC	P^a	P^b	P^c
Men						
VLDL						
Large	10.0 ± 16.5	10.7 ± 19.0	10.2 ± 18.9	0.9015	0.6809	0.6208
Intermediate	73.3 ± 60.7	75.2 ± 64.9	67.5 ± 61.9	0.3736	0.1998	0.2063
Small	20.3 ± 12.3	20.7 ± 13.4	20.8 ± 14.5	0.8179	0.8313	0.7109
LDL						
Large	69.2 ± 33.6	69.7 ± 33.7	70.6 ± 32.6	0.5192	0.6812	0.6355
Intermediate	36.3 ± 25.1	37.1 ± 23.8	37.2 ± 26.2	0.4038	0.4304	0.2741
Small	31.7 ± 26.1	33.6 ± 25.5	32.7 ± 23.4	0.7413	0.6709	0.6953
HDL						
Large	14.5 ± 11.3	14.8 ± 12.4	15.4 ± 13.0	0.6317	0.5620	0.5362
Intermediate	21.5 ± 6.5	21.2 ± 6.7	21.2 ± 6.8	0.4205	0.3383	0.2093
Small	8.6 ± 5.1	8.6 ± 5.3	8.6 ± 5.4	0.9446	0.9183	0.9060
Women						
VLDL						
Large	5.5 ± 13.3	6.6 ± 18.3	4.3 ± 7.4	0.7627	0.8441	0.8820
Intermediate	48.3 ± 44.6	53.5 ± 54.8	42.3 ± 44.3	0.0112[d]	0.0103[d]	0.0081[d]
Small	21.9 ± 13.7	22.4 ± 13.0	21.3 ± 12.3	0.6394	0.5889	0.5289
LDL						
Large	85.6 ± 34.7	82.0 ± 31.9	85.7 ± 36.3	0.7706	0.7606	0.5164
Intermediate	30.7 ± 24.0	32.5 ± 24.2	28.6 ± 23.8	0.0449[d]	0.0318[d]	0.0218[d]
Small	18.8 ± 19.2	17.9 ± 18.0	22.1 ± 18.5	0.5263	0.6133	0.4911
HDL						
Large	30.7 ± 16.2	30.8 ± 16.7	32.5 ± 16.7	0.5694	0.8730	0.9277
Intermediate	20.7 ± 6.4	20.9 ± 6.9	20.1 ± 7.2	0.2954	0.2342	0.2061
Small	5.4 ± 4.6	5.6 ± 4.8	5.5 ± 4.5	0.9465	0.9452	0.8724

Results are listed as means ± SD.
[a] After adjustment for familial relationship.
[b] After adjustment for familial relationship, age, BMI, smoking, alcohol intake, and the use of beta-blockers (menopausal status and estrogen therapy in women).
[c] After adjustment for familial relationship, age, BMI, smoking, alcohol intake, use of beta-blockers (menopausal status and estrogen therapy in women) and apoE.
[d] Significant difference between the AC and CC groups.

gene accounted for 1 and 5% of the variation of LDL-cholesterol concentrations, respectively (19). Furthermore, the overall effect of the CYP7 polymorphism on LDL-cholesterol levels was significant in men only. In fact, males with the CC genotype had a 4.7% higher plasma LDL-cholesterol concentrations than subjects carrying the AA genotype. Interestingly, plasma triglyceride concentrations were lower in women with the CC genotype. The significance of this finding remains to be established as the CYP7 genotype did not significantly affect triglyceride levels in men. Several lines of evidence demonstrate that the expression of CYP7 is hormonally regulated and its regulation is gender-dependent. Sex steroids have been shown to increase CYP7 mRNA in nonhuman primates. Therefore, one will expect a higher biliary cholesterol secretion and a depletion of hepatic cholesterol which, in turn, may decrease the synthesis and secretion of VLDL and consequently plasma triglyceride levels as demonstrated by Spady et al. (41). Furthermore, some authors (42) have proposed that plasma triglyceride, but not cholesterol lev-

TABLE 4. Lipoprotein diameters of FOS subjects according to CYP7 genotypes at nucleotide −204

Variable	AA	AC	CC	P^a	P^b	P^c
Men						
VLDL	48.44 ± 9.41	48.25 ± 9.47	48.12 ± 9.20	0.9337	0.8563	0.7687
LDL	20.73 ± 0.56	20.69 ± 0.57	20.71 ± 0.54	0.7410	0.5906	0.6073
HDL	8.92 ± 0.37	8.93 ± 0.41	8.95 ± 0.46	0.6658	0.8025	0.8433
Women						
VLDL	44.34 ± 8.50	44.46 ± 8.72	44.21 ± 8.39	0.8819	0.9490	0.9658
LDL	21.07 ± 0.43	21.06 ± 0.49	21.10 ± 0.47	0.5099	0.6040	0.5560
HDL	9.38 ± 0.42	9.38 ± 0.44	9.46 ± 0.46	0.0720	0.1446	0.2126

Results are listed as means ± SD.
[a] After adjustment for familial relationship.
[b] After adjustment for familial relationship, age, BMI, smoking, alcohol intake, and the use of beta-blockers (menopausal status and estrogen therapy in women).
[c] After adjustment for familial relationship, age, BMI, smoking, alcohol intake, use of beta-blockers (menopausal status and estrogen therapy in women) and apoE.

els, were directly correlated with CYP7 activity in humans. Further studies will be required to assess the gender-dependent relationship between plasma triglyceride levels and CYP7 genotype. At least, we can speculate that the CYP7 genotype may influence the metabolic relationships determining how triglyceride-rich lipoproteins are secreted in women. The differences in the phenotypic expression of the CYP7 variants could also be related to factors such as sample size, admixture or population stratification, differences in environmental factors, and/or differences in genetic background of the populations studied. It should be noted that dietary fats modulate the regulatory effect of dietary cholesterol on CYP7 (43). Therefore, different dietary habits across populations may influence the genotype/lipid associations. Moreover, the older age of participants in our study may be responsible, at least in part, for these discrepancies because, as previously indicated, the hormonal profile in women might modulate the effect of CYP7 polymorphism on LDL-cholesterol and triglyceride levels.

A number of arguments suggest that genetic polymorphism of the CYP7 gene might influence lipid and lipoprotein profiles. The cholesterol catabolic pathway, which is exclusive to the liver, is comprised of several enzymatic reactions which convert cholesterol into bile acids. The first reaction of this pathway is catalyzed by CYP7 and serves as the rate-limiting step and major site of regulation of bile acid synthesis. Previous studies have shown that CYP7 is regulated by bile acid feedback, cholesterol and hormonal factors (44) and, in humans, administration of cholestyramine, a bile acid-binding resin, has also led to a 5-fold increase in CYP7 activity that was associated with a 15–20% decrease in plasma LDL-cholesterol levels (45). Thus, the CYP7 activity seems to be inversely correlated with plasma LDL-cholesterol concentrations. Moreover, the study of the molecular mechanisms of the transcriptional regulation of CYP7 by sterols and bile acids has revealed that the promoter region between −432 and −220 contains several cell-specific enhancer elements whose activity is controlled, in part, by hepatocyte nuclear factor 3 (46). Therefore, it is conceivable that the A-204C polymorphism might modulate transcription of the CYP7 gene and, consequently, the rate of cholesterol catabolism. However, as we did not measure CYP7 activity directly in the Framingham Offspring Study participants, we can only speculate on the mechanisms underlying the association between the A-204C polymorphism and variations in plasma LDL-cholesterol concentrations. In fact, it is possible that this polymorphism does not directly modulate transcriptional regulation of CYP7, but that another linked polymorphism in CYP7 gene affects gene expression. Finally, we cannot exclude that the A-204C polymorphism is in linkage disequilibrium with another unidentified gene responsible for variations in plasma LDL-cholesterol concentrations but, so far, no other genes affecting plasma LDL-cholesterol levels have been identified in close proximity to the CYP7 locus. Even in the event that this variant affects or is associated with different expression of the CYP7, the evidence from the transgenic mice model indicates that we should not expect dramatic changes in plasma lipid levels. In this regard, Schwarz et al. (47) have demonstrated that marked reduction in bile acid synthesis in CYP7-deficient mice did not lead to diminished tissue cholesterol turnover or to hypercholesterolemia. This study clearly shows the complex interactions between bile acid and cholesterol metabolism to maintain cholesterol balance. These data are consistent with the mild effect of this genetic variant reported in our study. In contrast, apoE-deficient mice develop severe hypercholesterolemia, which also agrees with the greater role of the apoE gene in determining plasma cholesterol variability in humans.

Thus, the results of the present study clearly show that the A-204C polymorphism in the CYP7 gene is associated with significant variations in plasma LDL-cholesterol concentrations but the cumulative effects of these variations on atherosclerotic risk remain uncertain, probably due to the small numbers of CHD patients in this young cohort. Polymorphism of the CYP7 gene accounts for a small but significant proportion of the genetic variability in plasma LDL-cholesterol concentrations in the general population. This finding indicates that genetically determined variability in plasma LDL-cholesterol levels is most likely due to allelic variation in a relatively large number of genes, each of which may have small or moderate influences on plasma LDL-cholesterol levels.

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REFERENCES

1. National Cholesterol Education Program. 1993. Summary of the second report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). *J. Am. Med. Assoc.* **269:** 3015–3023.
2. Miller, G. J., and N. E. Miller. 1975. Plasma-high-density-lipoprotein concentration and development of ischaemic heart-disease. *Lancet.* **1:** 16–19.
3. Assmann, G., and H. Schulte. 1992. Relation of high-density lipoprotein cholesterol and triglycerides to incidence of atherosclerotic coronary artery disease (the PROCAM experience). Prospective Cardiovascular Munster study. *Am. J. Cardiol.* **70:** 733–737.
4. Wilson, P. W., R. B. D'Agostino, D. Levy, A. M. Belanger, H. Silbershatz, and W. B. Kannel. 1998. Prediction of coronary heart disease using risk factor categories. *Circulation.* **97:** 1837–1847.
5. Marenberg, M. E., N. Risch, L. F. Berkman, B. Floderus, and U. de Faire. 1994. Genetic susceptibility to death from coronary heart disease in a study of twins. *N. Engl. J. Med.* **330:** 1041–1046.
6. Pérusse, L., J. P. Després, A. Tremblay, C. Leblanc, J. Talbot, C. Allard, and C. Bouchard. 1989. Genetic and environmental determinants of serum lipids and lipoproteins in French Canadian families. *Arteriosclerosis.* **9:** 308–318.
7. Rice, T., G. P. Vogler, T. S. Perry, P. M. Laskarzewski, and D. C. Rao. 1991. Familial aggregation of lipids and lipoproteins in families ascertained through random and nonrandom probands in the Iowa Lipid Research Clinics family study. *Hum. Hered.* **41:** 107–121.
8. Goldstein, J. L., H. H. Hobbs, and M. S. Brown. 1995. Familial hypercholesterolemia. *In* The Metabolic Basis of Inherited Diseases.

C. R. Scriver, A. L. Beaudet, W. S. Sly and D. Valle, editors. McGraw-Hill Publishing Co., New York. 1981–2030.
9. Myant, N. B. 1993. Familial defective apolipoprotein B-100: a review, including some comparisons with familial hypercholesterolaemia. *Atherosclerosis.* **104:** 1–18.
10. Welty, F. K., J. Ordovas, E. J. Schaefer, P. W. F. Wilson, and S. G. Young. 1995. Identification and molecular analysis of two apoB gene mutations causing low plasma cholesterol levels. *Circulation.* **92:** 2036–2040.
11. Welty, F. K., C. Lahoz, K. L. Tucker, J. M. Ordovas, P. W. Wilson, and E. J. Schaefer. 1998. Frequency of apoB and apoE gene mutations as causes of hypobetalipoproteinemia in the Framingham Offspring Population. *Arterioscler. Thromb. Vasc. Biol.* **18:** 1745–1751.
12. Humphries, S., D. A. Coviello, P. Masturzo, R. Balestreri, G. Orecchini, and S. Bertolini. 1991. Variation in the low density lipoprotein receptor gene is associated with differences in plasma low density lipoprotein cholesterol levels in young and old normal individuals from Italy. *Arterioscler Thromb.* **11:** 509–516.
13. Klausen, I. C., P. S. Hansen, L. U. Gerdes, N. Rudiger, N. Gregersen, and O. Faergeman. 1993. A PvuII polymorphism of the low density lipoprotein receptor gene is not associated with plasma concentrations of low density lipoproteins including Lp(a). *Hum. Genet.* **91:** 193–195.
14. Hansen, P. S., L. U. Gerdes, I. C. Klausen, N. Gregersen, and O. Faergeman. 1993. Polymorphisms in the apolipoprotein B-100 gene contributes to normal variation in plasma lipids in 464 Danish men born in 1948. *Hum. Genet.* **91:** 45–50.
15. Gaffney, D., D. J. Freeman, J. Shepherd, and C. J. Packard. 1993. The ins/del polymorphism in the signal sequence of apolipoprotein B has no effect on lipid parameters. *Clin. Chim. Acta.* **218:** 131–138.
16. Karpe, F., B. Lundahl, E. Ehrenborg, P. Eriksson, and A. Hamsten. 1998. A common functional polymorphism in the promoter region of the microsomal triglyceride transfer protein gene influences plasma LDL levels. *Arterioscler. Thromb. Vasc. Biol.* **18:** 756–761.
17. Herrmann, S. M., O. Poirier, V. Nicaud, A. Evans, J. B. Ruidavets, G. Luc, D. Arveiler, C. Bao-Sheng, and F. Cambien. 1998. Identification of two polymorphisms in the promoter of the microsomal triglyceride transfer protein (MTP) gene: lack of association with lipoprotein profiles. *J. Lipid Res.* **39:** 2432–2435.
18. de Knijff, P., A. M. van den Maagdenberg, R. R. Frants, and L. M. Havekes. 1994. Genetic heterogeneity of apolipoprotein E and its influence on plasma lipid and lipoprotein levels. *Hum. Mutat.* **4:** 178–194.
19. Schaefer, E. J., S. Lamon-Fava, S. Johnson, J. M. Ordovas, M. M. Schaefer, W. P. Castelli, and P. W. Wilson. 1994. Effects of gender and menopausal status on the association of apolipoprotein E phenotype with plasma lipoprotein levels. Results from the Framingham Offspring Study. *Arterioscler. Thromb.* **14:** 1105–1113.
20. Dallongeville, J., C. S. Lussier, and J. Davignon. 1992. Modulation of plasma triglyceride levels by apoE phenotype: a meta-analysis. *J. Lipid Res.* **33:** 447–454.
21. Utermann, G., M. Hees, and A. Steinmetz. 1977. Polymorphism of apolipoprotein E and occurrence of dysbetalipoproteinaemia in man. *Nature.* **269:** 604–607.
22. Wang, J., D. J. Freeman, S. M. Grundy, D. M. Levine, R. Guerra, and J. C. Cohen. 1998. Linkage between cholesterol 7 alpha-hydroxylase and high plasma low-density lipoprotein cholesterol concentrations. *J Clin Invest.* **101:** 1283–1291.
23. Wang, D. P., and J. Y. Chiang. 1994. Structure and nucleotide sequences of the human cholesterol 7 alpha-hydroxylase gene (CYP7). *Genomics.* **20:** 320–323.
24. Nishimoto, M., M. Noshiro, and K. Okuda. 1993. Structure of the gene encoding human liver cholesterol 7 alpha-hydroxylase. *Biochim. Biophys. Acta.* **1172:** 147–150.
25. Feinleib, M., W. B. Kannel, R. J. Garrison, P. M. McNamara, and W. P. Castelli. 1975. The Framingham Offspring Study. Design and preliminary data. *Prev. Med.* **4:** 518–525.
26. Kannel, W. B., M. Feinleib, P. M. McNamara, R. J. Garrison, and W. P. Castelli. 1979. An investigation of coronary heart disease in families. The Framingham offspring study. *Am. J. Epidemiol.* **110:** 281–290.
27. Dawber, T. R., G. F. Meadors, and R. Moore. 1951. Epidemiological approach to heart disease: The Framingham Study. *Am. J. Public Health.* **41:** 279–286.
28. Cupples, L. A., D. R. Gagnon, and W. B. Kannel. 1992. Long- and short-term risk of sudden coronary death. *Circulation.* **85:** I11–I18.
29. Lipid Research Clinic Program. 1979. The Coronary Primary Prevention Trial: design and implementation. *J. Chronic Dis.* **14:** 1250–1257.
30. Friedewald, W. T., R. I. Levy, and D. S. Fredrickson. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **18:** 499–502.
31. McNamara, J., and E. Schaefer. 1987. Automated enzymatic standardized lipid analyses for plasma and lipoprotein fractions. *Clin. Chim. Acta.* **166:** 1–8.
32. Schaefer, E., and J. Ordovas. 1986. Metabolism of apolipoproteins A-I, A-II, and A-IV. *Methods Enzymol.* **129:** 420–443.
33. Ordovas, J. M., J. P. Peterson, P. Santaniello, J. S. Cohn, P. W. Wilson, and E. J. Schaefer. 1987. Enzyme-linked immunosorbent assay for human plasma apolipoprotein B. *J. Lipid Res.* **28:** 1216–1224.
34. Otvos, J. D., E. J. Jeyarajah, D. W. Bennett, and R. M. Krauss. 1992. Development of a proton nuclear magnetic resonance spectroscopic method for determining plasma lipoprotein concentrations and subspecies distributions from a single, rapid measurement. *Clin. Chem.* **38:** 1632–1638.
35. Otvos, J. D. 1997. Measurement of lipoprotein subclass profile by nuclear magnetic resonance. *In* Handbook of Lipoprotein Testing. N. Rifai, G. R. Warnick and M. H. Dominiczak, editors. AACC press, Washington, DC. 497–508.
36. Miller, S. A., D. D. Dykes, and H. F. Polesky. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* **16:** 1215.
37. Boerwinkle, E., and G. Utermann. 1988. Simultaneous effects of the apolipoprotein E polymorphism on apolipoprotein E, apolipoprotein B, and cholesterol metabolism. *Am. J. Hum. Genet.* **42:** 104–112.
38. Almasy, L., and J. Blangero. 1998. Multipoint quantitative-trait linkage analysis in general pedigrees. *Am. J. Hum. Genet.* **62:** 1198–1211.
39. Austin, M. A., M. C. King, R. D. Bawol, S. B. Hulley, and G. D. Friedman. 1987. Risk factors for coronary heart disease in adult female twins. Genetic heritability and shared environmental influences. *Am. J. Epidemiol.* **125:** 308–318.
40. Boerwinkle, E., S. Visvikis, D. Welsh, J. Steinmetz, S. M. Hanash, and C. F. Sing. 1987. The use of measured genotype information in the analysis of quantitative phenotypes in man. II. The role of the apolipoprotein E polymorphism in determining levels, variability, and covariability of cholesterol, betalipoprotein, and triglycerides in a sample of unrelated individuals. *Am. J. Med. Genet.* **27:** 567–582.
41. Spady, D. K., J. A. Cuthbert, M. N. Willard, and R. S. Meidell. 1998. Overexpression of cholesterol 7 alpha-hydroxylase (CYP7A) in mice lacking the low density lipoprotein (LDL) receptor gene. LDL transport and plasma LDL concentrations are reduced. *J. Biol. Chem.* **273:** 126–132.
42. Bertolotti, M., N. Abate, S. Bertolotti, P. Loria, M. Concari, R. Messora, F. Carubbi, A. Pinetti, and N. Carulli. 1993. Effect of aging on cholesterol 7α-hydroxylation in humans. *J. Lipid Res.* **34:** 1001–1007.
43. Cheema, S. K., D. Cikaluk, and L. B. Agellon. 1997. Dietary fats modulate the regulatory potential of dietary cholesterol on cholesterol 7α-hydroxylase gene expression. *J. Lipid Res.* **38:** 315–323.
44. Russell, D. W., and K. D. Setchell. 1992. Bile acid biosynthesis. *Biochemistry.* **31:** 4737–4749.
45. Reihner, E., I. Bjorkhem, B. Angelin, S. Ewerth, and K. Einarsson. 1989. Bile acid synthesis in humans: regulation of hepatic microsomal cholesterol 7 alpha-hydroxylase activity. *Gastroenterology.* **97:** 1498–1505.
46. Molowa, D. T., W. S. Chen, G. M. Cimis, and C. P. Tan. 1992. Transcriptional regulation of the human cholesterol 7 alpha-hydroxylase gene. *Biochemistry.* **31:** 2539–2544.
47. Schwarz, M., D. W. Russell, J. M. Dietschy, and S. D. Turley. 1998. Marked reduction in bile acid synthesis in cholesterol 7α-hydroxylase-deficient mice does not lead to diminished tissue cholesterol turnover or to hypercholesterolemia. *J. Lipid Res.* **39:** 1833–1843.