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Polymorphisms in the Peroxisome Proliferator Activated Receptor α Gene Are Associated with Levels of Apolipoprotein CIII and Triglyceride in African-Americans But Not Caucasians

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Abstract

Background—We tested whether single nucleotide polymorphisms (SNPs) in the *PPAR α* gene (*PPARA*) are associated with variations in levels of plasma apolipoprotein CIII (apoCIII) levels, as well as other lipids and lipoproteins in African-Americans and Caucasians.

Methods and Results—We initially identified an intronic SNP (rs4253728) in *PPARA* that was associated with plasma apoCIII level ($p < 0.05$) in a subset of 435 individuals from the total study population ($n = 944$; 335 African Americans and 609 Caucasians). This SNP was then genotyped in a second subset of 476 individuals (total 911 subjects with available data), and a previously described *PPARA* coding SNP (L162V) which was shown to be in moderate linkage disequilibrium with the intronic SNP ($r^2 = 0.18$) was genotyped in 928 subjects from the same study population. The minor allele frequencies for both SNPs were significantly lower in African-Americans compared with Caucasians (7.2% vs. 27.3% for rs4253728, 1.5% vs. 6.1% for L162V, both $p < 0.0001$). African-Americans had significantly lower levels of TG and apoCIII compared with Caucasians after adjusting for age, sex, body mass index (BMI), waist circumference and other baseline characteristics. However, racial differences in TG levels were attenuated after adjusting for apoCIII levels. The minor alleles for both *PPARA* SNPs were associated with higher TG and apoCIII levels. Race modified the associations of L162V with TG (p for interaction = 0.0056) and apoCIII (p for interaction = 0.0011). Levels of both TG and apoCIII were lower in African-American but not Caucasian homozygotes for the major allele compared with carriers of the minor allele. Similar results were obtained for the intronic SNP, but the findings were no longer significant in a model that also contained L162V.

Conclusions—Two *PPARA* SNPs, L162V and rs4253728 (intronic), are less prevalent in African-Americans than in Caucasians and in African-Americans only are associated with higher apoCIII and TG levels.

Keywords

PPAR α ; gene polymorphism; race; apolipoprotein CIII; triglyceride

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INTRODUCTION

Peroxisome proliferator activated receptor α (PPAR α) regulates the transcription of multiple genes involved in lipid metabolism [1–2]. In vitro, activation of PPAR- α has been associated with increased lipoprotein lipase (LPL) gene transcription [3] and reduced apolipoprotein CIII (apoCIII) gene expression that can lead to increased LPL-mediated catabolism and plasma clearance of very low density lipoproteins (VLDLs) [4–6]. Fibrate drugs, which are agonists for PPAR α , lower levels of plasma triglyceride (TG) and increase HDL-cholesterol concentrations in humans [7–10]. Fibrate-induced reductions in levels of apoCIII, mediated at the transcriptional level by a PPAR α response element in the apoCIII gene, have been implicated in mediating the TG-lowering response to fibrates [11]. This effect is likely due to inhibition by apoCIII of both VLDL TG lipolysis by lipoprotein lipase and receptor-mediated clearance of VLDL lipolytic remnants [12].

There have been reports that *PPARA* single nucleotide polymorphisms (SNPs) are associated with blood lipid levels and with progression of coronary artery disease and type 2 diabetes mellitus, but the findings have been inconsistent [13–17]. Moreover, to date most of the studies have been conducted in Caucasian populations. There are no published reports of associations of *PPARA* SNPs with blood lipids and lipoproteins in black subjects.

In the present study, we sought to determine the existence of *PPARA* genetic polymorphisms associated with variations in plasma apoCIII levels, as well as other lipids and lipoproteins, in a group of 944 Caucasian and African-American subjects.

RESEARCH DESIGN AND METHODS

Study population

The total study population consisted of 609 Caucasians and 335 African-Americans aged 30 years or older, with baseline total cholesterol 160 to 400 mg/dL and no use of drugs known to affect lipoprotein metabolism who participated in a trial of simvastatin therapy in the Cholesterol and Atherosclerosis Pharmacogenetics (CAP) substudy of the NIH-funded Pharmacogenomics and Risk of Cardiovascular Disease project [18]. The data for the present report were obtained after a two-week run-in period on placebo. Participants self-reported race/ethnicity. We required that three or more grandparents be reported as African-American or Caucasian for a participant to be classified as African American or Caucasian, respectively. Potential subjects from other racial/ethnic groups were not enrolled. Subjects were recruited from clinical centers in Los Angeles, CA and San Francisco, CA, and informed consent was obtained. The study was approved by local Institutional Review Boards as well as the University of California, San Francisco, University of California, Los Angeles, and Children's Hospital and Research Center of Oakland. Baseline demographic data included self-reported age, sex, and ethnicity. Participants were asked about smoking status, alcohol consumption, level of physical activity, history of diabetes, hypertension, or coronary heart disease and use of oral estrogen preparations. They underwent a brief physical examination for measurement of seated blood pressure using an electronic manometer. Weight was measured using a standard balance beam scale or an electronic scale and height was measured using a height rod of a standard beam scale or a wall-mounted stadiometer. Body mass index (BMI) was calculated as weight in kg divided by height in meters squared. Waist circumference was measured twice to the nearest 0.1 cm with a flexible tape measure at the level of the minimum circumference, usually at the level of the navel. Blood was collected after overnight fast into tubes containing EDTA, and the plasma was separated and stored at -80°C .

Measurements

Plasma total cholesterol and triglyceride (TG) levels were determined by enzymatic procedures on an Express 550 Plus analyzer (Ciba Corning, Oberlin, OH). These measurements were consistently in control as monitored by the CDC-NHLBI standardization program. HDL cholesterol was measured after dextran sulfate precipitation of plasma [19], and LDL-C was calculated from the formula of Friedewald et al. [20]. Apolipoproteins AI (apoAI) and B (apoB) were measured by immunoturbidometric assay (Express 550 Plus analyzer). Plasma apoCIII concentrations were determined in triplicate by sandwich assay using two polyclonal antibodies (International Immunology Corp., Murrieta, CA). Fasting plasma glucose and insulin were measured and the homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated as the product of [fasting plasma insulin ($\mu\text{U/L}$) $\times$ fasting plasma glucose (mmol/L)] divided by 22.5 [21].

SNP identification and genetic analysis

SNPs within *PPARA* were identified by genomic sequencing of the entire *PPARA* gene, with an additional 2.5 Kb upstream and 1.5 Kb downstream, in 24 African-American and 23 Caucasian (specifically European-American) individuals. The sequences from the 47 individuals were assembled with the Phred, Phrap and Consed, and PolyPhred was used to identify sequence variants. The University of California at Santa Cruz Golden Path human genome assembly was used as a reference sequence to identify gene architecture and map identified SNPs. One hundred ninety two polymorphic sites were identified in the *PPARA* gene and 24 tag SNPs were selected for genotyping using the LD-select algorithm. Genotypes for these SNPs were obtained in 435 of the first 458 subjects enrolled in CAP (179 African Americans and 256 Caucasians) by Illumina (San Diego, CA) using BeadArray™ technology. Among these SNPs, one intronic SNP (rs4253728) was selected to test for replication in the remaining 486 subjects in CAP because of its association ($p < 0.05$) with apoCIII after adjustment for age, sex, BMI and race (See supplemental data). Another previously described non-synonymous SNP (L162V, rs1800206) was also genotyped in the entire CAP population. After exclusion of subjects with missing data for these two SNPs, there were a total of 911 individuals with results for rs 4253728, and 928 with results for L162V. The two genotypes were then tested for associations with apoCIII, as well as triglyceride, total and LDL cholesterol, HDL cholesterol, apoB and apoAI in the full study population.

Statistical analysis

We used JMP version 5.0 for Windows (SAS Ins., Chicago, IL, USA) for all statistical analyses. Each variable was examined for normality, and non-normally distributed variables (TG, apoCIII, and HOMA-IR) were log-transformed. Differences in blood lipids and apolipoproteins between racial groups and genotypes were compared using t-tests or Kruskal-Wallis test as appropriate. Chi-squared test was used to compare differences in allele frequency between African-Americans and Caucasians. General linear model and multiple linear regression analysis were used to examine the *PPARα* genotype effect and interaction effect between genotype and race on blood lipids and apolipoproteins. Tests for Hardy-Weinberg equilibrium and standardized linkage disequilibrium (D') were determined by using Haploview 3.2 (<http://www.broad.mit.edu/mpg/haploview>). A p value of less than 0.05 was considered to be statistically significant.

RESULTS

Table 1 displays the baseline characteristics of the full population studied here. Age and sex distribution were similar between African-Americans and Caucasians. As described previously [18], African-Americans had significantly higher BMI and waist circumference, were more

likely to be current smokers, and had higher prevalence of hypertension and type 2 diabetes mellitus compared with Caucasians.

As shown in Table 2, the African-Americans had significantly lower levels of TG, total cholesterol, apoB and apoCIII than the Caucasians. After adjusting for age, sex, BMI, waist circumference and other baseline characteristics including current smoking, exercise level, alcohol consumption, estrogen use, and presence of hypertension and type 2 diabetes mellitus, the African-Americans had significantly higher levels of HDL-cholesterol and apoAI than the Caucasians. After adjusting for plasma apoCIII, the racial differences in apoB and TG levels were no longer significant. However, levels of HDL-cholesterol and apoAI remained significantly higher in the African-Americans compared with the Caucasians after adjusting for plasma apoCIII levels (Table 2).

Table 3 presents the genotype distributions of the two *PPARA* SNPs. The minor allele frequencies for both SNPs were significantly lower in the African-Americans compared with the Caucasians (1.5% vs. 6.1% for L162V, 7.2% vs. 27.3% for rs4253728, $p < 0.001$). The L162V SNP was not in Hardy-Weinberg equilibrium in the full study population (91.6% CC, 7.7% GC and 0.6% GG) or in the Caucasians (88.7% CC, 10.3% GC and 1.0% GG). However, the genotype distributions were very similar to those reported in earlier studies [14–17]. In the African-Americans, the genotype distribution was in Hardy-Weinberg equilibrium (96.9% CC, 3.1% GC). The intronic SNP was in Hardy-Weinberg equilibrium in the whole study population (64.5% GG, 30.5% GA and 4.9% AA) and in Caucasians (52.4% GG, 40.7% GA and 6.9% AA). However, in the African-Americans, it was not in Hardy-Weinberg equilibrium (86.9% GG, 11.8% GA and 1.3% AA, $p = 0.03908$). The intronic and coding SNPs were in linkage disequilibrium ($D' = 0.965$, $r^2 = 0.18$, $p = 0.003$).

Table 4 displays the associations between the L162V SNP/intronic SNP and blood lipids, lipoproteins, and apoCIII in the entire study population. Plasma levels of apoCIII and TG were significantly lower in L162 homozygotes than in carriers of the V162 allele after adjusting for age, BMI, sex, race, interaction of race with genotype and other baseline characteristics. Levels of apoAI and HDL-cholesterol tended to be higher in the carriers of the V162 allele compared with L162 homozygotes, although the differences did not reach statistical significance. Race modified the genotype effects with TG (p for interaction = 0.0056) and apoCIII (p for interaction = 0.0011). In African-Americans, the carriers of the V162 allele had higher levels of plasma apoCIII ($p = 0.0005$) and TG ($p = 0.009$) than L162 homozygotes, whereas there were no differences in plasma apoCIII and TG between the genotypes in Caucasians (Fig. 1).

For the intronic SNP (rs4253728), plasma levels of apoCIII and TG were significantly lower in GG homozygotes than in the carriers of the A allele after adjusting for age, BMI, sex, race, interaction of race with genotype and other baseline characteristics (Table 4). Again, the association between genotype with TG and apoCIII varied by race (p for interaction = 0.01 for TG and 0.03 for apoCIII). In the African-Americans, plasma levels of apoCIII and TG were significantly higher in carriers of the A allele than GG homozygotes (apoCIII: 13.5 ± 0.5 vs. 11.5 ± 0.5 $\mu\text{g/mL}$, $p = 0.03$; TG: 135.0 ± 7.5 vs. 104.8 ± 7.3 mg/dL , $p = 0.02$), whereas there were no differences in plasma apoCIII and TG among the genotypes in the Caucasians. The associations with the intronic SNP, however, were no longer significant in a model including the L162V SNP. After further adjusting for apoCIII, neither the L162V SNP nor the intronic SNP were associated with plasma levels of TG in the African-Americans.

DISCUSSION

We have found significant associations of plasma levels of apoCIII and TG with two SNPs in the gene for *PPAR α* , a functional leucine 162 to valine (L162V) variant and a novel intronic

SNP. These associations however varied by race, suggesting differential regulation by *PPARα* of apoCIII and TG levels between African-Americans and Caucasians. Specifically, in African-Americans, both L162 homozygotes and GG homozygotes for the intronic SNP had lower levels of plasma apoCIII and TG than carriers of the 162V allele and the A allele of the intronic SNP. On the other hand, there were no differences in plasma apoCIII and TG levels between carriers of these alleles in Caucasians.

The *PPARA* L162V polymorphism has been reported to alter transcriptional activation of *PPARA* associated with fibrate treatment in vitro [15,22]. Flavell et al. [15] reported that V162 *PPARα* showed greater transactivation of a reporter gene construct compared with L162 *PPARα*. In that study, however, protein concentrations of both variants were not different after transient transfection as assessed by western blot analysis. There is also evidence that the effect of the L162V polymorphism on the transcriptional activation of *PPARα* depends on the concentration of the ligand to which it is exposed [22]. It has been reported that *PPARα* genetic variations contribute to inter-individual variability in total cholesterol [15,23], LDL-cholesterol [13,23], HDL-cholesterol [15], apoB [13,23] and apoAI [15]. Despite the prominent role of *PPARα* in the regulation of proteins involved in TG-rich lipoprotein metabolism, reports of associations between genetic variations in the *PPARA* and plasma TG and apoCIII concentrations have been inconsistent. In particular, some have found no associations between the L162V polymorphism and plasma levels of TG [13,15–16]. However, a study by Nielsen et al. [24] found that the V162 allele of *PPARα* was associated with a decreased level of TG in glucose tolerant white subjects. Tai et al. [25] reported that apoCIII levels were higher in Caucasian carriers of the V162 allele, with a significant interaction between the *PPARA* L162V polymorphism and polyunsaturated fat (PUFA) intake with respect to plasma levels of apoCIII and TG. As a result, the L162 allele was associated with lower TG and apoCIII levels only in subjects with PUFA fat intake less than 6% of energy. The possibility might therefore be considered that the results of the current study could reflect lower PUFA intake in African-Americans than Caucasians. There is no evidence, however, from the National Health and Nutrition Evaluation Survey (NHANES) that this is the case (Mangravite LM and Krauss RM, unpublished), and detailed dietary information is not available for participants in the present study.

Another possible explanation for our findings is that *PPARA* effects on apoCIII in the African-American population can be influenced by variations in other genes whose prevalence may differ from those in Caucasians. For example, there is evidence that the *PPARA* L162V SNP interacts with the G3238G in the apoCIII gene and with the *APOE* locus in modulating plasma apoCIII concentrations [23]. Also, *PPARα* has been reported to be regulated by hepatocyte nuclear factor 4α [26] and retinoid X receptor α [27]. It is possible that differing combinations of SNPs in these or other genes in African-Americans vs. Caucasians may be responsible for the differences in the *PPARA* SNP associations that have been shown here for these two populations. It is also possible that there are differences between African-American and Caucasians in linkage disequilibrium of these SNPs with unknown SNPs that affect *PPARα* function.

With regard to the intronic SNP, there is no evidence that this SNP is itself functional. This SNP was in linkage disequilibrium with the functional L162V variant, and thus it is possible that the intronic SNP might be simply tagging the functional SNP. However, the r^2 for the linkage disequilibrium was relatively small, and the two SNPs were not within the same LD block (Supplementary Figure). Thus, the possibility cannot be excluded that the intronic SNP may affect the expression level of the gene via an unknown mechanism, similar, for example, to the actions of intronic SNPs in *Calpain10* [28] and *COL1A1* [29].

ApoCIII can modulate plasma TG levels by inhibiting LPL and reducing apoE mediated hepatic uptake of TG rich lipoprotein remnants [12]. Recently, Florez et al. reported that African-Americans had significantly lower levels of apoCIII and TG compared with Hispanics and White non-Hispanics independently of gender and type 2 diabetes mellitus. However, in that study, multiple linear regression revealed that apoCIII was the most important contributor to ethnicity-adjusted TG levels, suggesting that ethnic differences in apoCIII levels partially contribute to differences in TG levels [30]. In the present study, our results showed that the effects of both the L162V and intronic *PPARA* SNPs on plasma TG were no longer significant when adjusting for apoCIII. These results raise the possibility that plasma apoCIII levels modulated by *PPARA* SNPs may be involved in regulation of plasma TG levels. On the other hand, plasma apoCIII and TG levels are strongly correlated ($r^2=0.48$ in the present study population), and it is possible that the primary associations of the SNPs with apoCIII vs. TG reflect greater analytic variability in the TG measurement rather than a causal relationship. Testing of the present findings in larger population groups, as well as studies designed to test the functional effects of these SNPs on TG metabolism, would be required to address this issue.

Limitations of the study include that participants were volunteers in a clinical trial, and hence may not be representative of the general population, and their ethnicity was defined using self-reported information rather than genetic markers.

In conclusion, a functional variant (L162V) and an intronic SNP in the *PPARA* gene were associated with the plasma levels of apoCIII and TG in African-Americans, while there was no association between these two SNPs and plasma levels of apoCIII and TG in Caucasians. These results indicate that ethnicity should be considered in evaluating the associations of *PPARA* SNPs with plasma lipoprotein metabolism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Plasma apo-CIII and TG levels in Caucasians and African-Americans according to the PPARα L162V SNP

Means ± S.E. Means were adjusted for age, sex, BMI, waist, current smoking, alcohol consumption, exercise levels, estrogen use and presence of hypertension and type 2 diabetes mellitus

Table 1

Baseline characteristics of the study population

	Caucasians (n=609)	African-Americans (n=335)	p value
Male/Female	323/286	162/173	NS
Age(years)	54.6±0.5	54.3±0.7	NS
BMI(kg/m ²)	27.7±0.2	30.1±0.4	<0.001
Waist (cm)	94.5±0.6	98.7±0.8	<0.001
SBP (mmHg)	123.2±0.7	128.5±0.9	<0.001
DBP (mmHg)	70.6±0.4	73.9±0.5	<0.001
Hypertension (%)	70	81	<0.001
Type 2 diabetes mellitus (%)	2	8	<0.001
Current smoker (%)	14	31	<0.001
Estrogen use (%) ^I	19	10	0.02
Physical activity level			0.04
More active than peers	43	42	
Less active than peers	15	21	
About the same	43	37	
Alcohol intake			<0.001
None	27	45	
<=2/wk	45	42	
>=3wk	27	13	

Means ± S.E. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure

^I Based on the responses from 459 women

Comparisons of blood lipids and apolipoproteins between Caucasians and African-Americans

Table 2

	C (n=609)	AA (n=335)	p value	p value ¹	p value ²
Triglyceride (mg/dL)	128.3 ± 2.8	109.6 ± 3.7	<0.001	<0.001	NS
Total cholesterol (mg/dL)	210.7 ± 1.5	205.9 ± 1.9	0.045	NS	NS
LDL-cholesterol (mg/dL)	131.9 ± 1.3	129.4 ± 1.9	NS	NS	NS
HDL-cholesterol (mg/dL)	53.2 ± 0.7	54.8 ± 0.9	NS	<0.001	<0.001
ApoAI(mg/dL)	126.8 ± 1.1	129.4 ± 1.5	NS	<0.001	<0.001
ApoB (mg/dL)	95.9 ± 0.9	91.7 ± 1.1	0.004	<0.001	NS
HOMA-IR	3.2 ± 0.1	3.7 ± 0.2	0.027	NS	NS
ApoCIII (µg/mL)	13.5 ± 0.2	11.4 ± 0.2	<0.001	<0.001	-

¹ After adjusting for age, sex, BMI, waist, current smoking, exercise, drink, estrogen use and presence of hypertension/type 2 diabetes mellitus

² After adjusting age, sex, BMI, waist, current smoking, exercise, drink, estrogen use, presence of hypertension/type 2 diabetes mellitus and apoCIII

Mean ± S.E. C: Caucasians, AA: African-Americans

Table 3

PPARα genotype distribution of study population

	Whole population (n=928)			Caucasians (n=602)			African-Americans (n=326)			p-value ^I
L162V (rs1800206)	CC	850		CC	534		CC	316		
	GC	72		GC	62		GC	10		
	GG	6		GG	6		GG	0		
	Whole population (n=911)			Caucasians (n=590)			African-Americans (n=321)			
Intronic SNP (rs4253728)	GG	588		GG	309		GG	279		<0.001
	GA	278		GA	240		GA	38		
	AA	45		AA	41		AA	4		

^I comparison of minor allele frequency between Caucasians and African-Americans using chi-squared test

Table 4
Comparisons of blood lipids and apolipoproteins among PPARA genotypes

	SNP L162V			SNP rs4253728		
	CC (n=850)	GC+GG (n=78)	p value	GG (n=588)	GA+AA (n=323)	p value
Triglyceride (mg/dL)	120.9 ± 6.7	139.7 ± 13.2	0.024	119.9 ± 6.8	134.8 ± 8.5	0.016
Total cholesterol (mg/dL)	203.6 ± 3.9	210.0 ± 7.9	NS	204.3 ± 4.0	208.1 ± 4.9	NS
LDL-cholesterol (mg/dL)	124.5 ± 3.8	122.5 ± 7.4	NS	125.1 ± 3.8	125.8 ± 4.7	NS
HDL-cholesterol (mg/dL)	55.0 ± 1.5	59.6 ± 2.9	NS	55.3 ± 1.5	55.2 ± 1.9	NS
ApoA1(mg/dL)	135.2 ± 2.4	143.1 ± 4.7	NS	135.2 ± 2.5	136.7 ± 3.0	NS
ApoB (mg/dL)	92.1 ± 2.3	94.1 ± 4.5	NS	92.5 ± 2.3	94.9 ± 2.9	NS
ApoCIII (μ/mL)	12.7 ± 0.5	15.1 ± 0.9	0.0008	12.5 ± 0.5	13.7 ± 0.6	0.0077

Mean ± S.E.

Means were adjusted for age, sex, BMI, waist, race, HOMA-IR, current smoking, exercise, drink, estrogen use, presence of hypertension/type 2 diabetes mellitus and interaction between race and the PPARα SNP