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Genetic Variants of the *ENPP1/PC-1* Gene Are Associated With Hypertriglyceridemia in Male Subjects

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Abstract

Background: Hypertriglyceridemia is associated with insulin resistance, type 2 diabetes, and the metabolic syndrome. Membrane glycoprotein PC-1 (also termed ENPP1) is a direct insulin receptor inhibitor, and certain polymorphisms of the *ENPP1/PC-1* gene have been associated with insulin resistance, type 2 diabetes, obesity, and diabetic complications.
Methods: We examined the effect of 3 *ENPP1/PC-1* variants (K121Q, rs1044498, and IVS20delT-11, rs1799774, and A→G+1044TGA, rs7754561) on plasma triglyceride levels in 1112 subjects of non-Hispanic American white European ancestry.
Results: Two of the *ENPP1/PC-1* variants—A→G+1044TGA (odds ratio [OR] 1.48, 95% confidence interval [CI], 1.54–1.82, $P = 0.002$) and IVS20delT-11 (OR 1.41, 95% CI, 1.08–1.84, $P = 0.012$)—were significantly associated with hypertriglyceridemia. Haplotype analyses also revealed an association with hypertriglyceridemia. In the variant analyses and in the haplotype analysis, the associations with hypertriglyceridemia were observed in male but not female subjects. Interestingly, the more widely studied K121Q *ENPP1/PC-1* variant was not associated with hypertriglyceridemia in any group or subgroup analysis.
Conclusion: In the present study, we find that genetic variants of the *ENPP1/PC-1* gene are associated with hypertriglyceridemia in male subjects, and may contribute to the development of the insulin resistance/metabolic syndrome in this population.

Introduction

Hypertriglyceridemia is a component of the insulin resistance/metabolic syndrome and is a common finding in individuals with insulin resistance and type 2 diabetes.[1] A causative role for insulin resistance in the development of hypertriglyceridemia has been postulated. In patients with insulin resistance and hypertriglyceridemia, decreased activity of lipoprotein lipase and elevated apolipoprotein CIII content causes triglyceride-rich lipoproteins to accumulate in the circulation.[2]

The membrane glycoprotein PC-1 (also termed ectonucleotide pyrophosphatase phosphodiesterase 1 [ENPP1]) is a class II transmembrane glycoprotein and a direct inhibitor of the insulin receptor.[3] By interacting with the connecting domain(s) on the α-subunit region of the insulin receptor, PC-1 inhibits the movement of the β-subunits after the binding of insulin, thereby inhibiting insulin receptor autophosphorylation and downstream insulin signaling events. Thus, PC-1 is a strong candidate molecule in the pathogenesis of insulin resistance.[4,5]

The *ENPP1/PC-1* gene is located on the long arm of chromosome 6 (6q23.2),[6] and several polymorphisms in both coding and noncoding regions of the gene have been identified. Furthermore, human genetic studies,[7–9] *in vitro* studies in cultured cells,[5] and *in vivo* studies in experimental animals[10] have demonstrated strong associations of increased

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ENPP1/PC-1 expression and/or the presence of specific genetic variants with insulin resistance and its associated co-morbidities.

A specific *ENPP1/PC-1* polymorphism in exon 4 (K121Q [rs1044498]) has been widely studied and encodes an *ENPP1/PC-1* variant (Q121 allele) in the coding region that both interacts more strongly with the insulin receptor and inhibits its insulin receptor signaling more effectively than the common protein (K121 allele).[11] The Q variant has been associated with insulin resistance in subjects without type 2 diabetes in many,[4,12–14] but not all, populations.[4,12,15] Similarly, the Q allele has been associated with type 2 diabetes in many,[9,12,14,16–18] but not all, populations,[13,15,19–21] as well as with obesity in some,[22] but not all, populations.[20,23] The largest meta-analysis thus far carried out demonstrated an association with type 2 diabetes consistent with a recessive model of inheritance.[17]

In a study involving over 6000 patients, Meyre et al.[7] reported a unique *ENPP1/PC-1* "at risk" haplotype. This haplotype consists of: (1) the aforementioned Q121 allele (rs1044498); (2) a T deletion in an intervening sequence IVS20delT-11 (rs1799774); and (3) a G allele in the 3′ untranslated region (UTR) sequence A→G+1044TGA (rs7754561). This haplotype was associated with obesity and increased risk for type 2 diabetes; however, the independent role of obesity in the risk for type 2 diabetes in that study was unclear. These investigators, in a study involving 5153 middle-aged French individuals,[24] then reported that the Q121 allele was associated with severe adult obesity and the risk of developing hyperglycemia or type 2 diabetes in those subjects with a family history of type 2 diabetes. Bottcher et al.[22] also reported that the "at risk" haplotype was characterized by impaired glucose metabolism in obese German children. These 3 studies indicate therefore, that variants other than K121Q are associated with insulin resistance.

The aim of this study was to determine whether variants of the *ENPP1/PC-1* gene are related to elevated triglyceride levels. A previous study reported that the *ENPP1/PC-1* K121Q variant was not associated with dyslipidemia and hypertriglyceridemia.[14] However, in that study, other *ENPP1/PC-1* variants, such as those in the "at risk" haplotype, were not analyzed. Accordingly, in the present study, we analyzed the association of the *ENPP1/PC-1* variants of the "at risk" haplotype with hypertriglyceridemia.

Research Design and Methods

Study subjects

This report is a retrospective study on the frequency of *ENPP1/PC-1* gene variants among population samples from the University of California, San Francisco (UCSF) Genomic Resource in Arteriosclerosis (GRA). UCSF institutional review board approval was obtained for these studies. A total of 1112 subjects of white European ancestry from the UCSF Lipid Clinic, living either in northern California or Utah, were selected for analyses in a case–control design. Detailed disease and medication history, anthropometric measurements, and plasma levels of total cholesterol (TC) and triglyceride (TG) were available for all subjects. For most subjects, lipoprotein lipid measurements were also available. These measurements were obtained from the subjects' first visit when they were not taking any lipid-lowering medications. Subjects in the case group were selected by having TG levels greater than 4.52 mmol/L. This group ($n = 817$) had a mean TG level of 10.2 ± 0.4 mmol/L. A total of 108 subjects in the case group had type 2 diabetes according to World Health Organization (WHO) criteria. The control group was selected from Bay Area Health Fairs, UCSF Employees, Huntsman World Senior Games in Utah, and patients' spouses. Lipid criteria for men were TG ≤ 1.69 mmol/L, low-density lipoprotein cholesterol (LDL-C) ≤3.37 mmol/L and high-density lipoprotein cholesterol (HDL-C) ≥1.04 mmol/L; for women, TG ≤1.69 mmol/L, LDL-C ≤3.37 mmol/L, and HDL-C ≥1.17 mmol/L.[25] The control group ($n = 295$) had a mean TG level of 0.98 ± 0.02 mmol/L, and none had type 2 diabetes.

Genotyping of ENPP1/PC-1 variants

Three variants were selected for genotyping purposes: rs1044498 (K121Q), rs1799774 (IVS20delT-11), and rs7754561 (A→G+1044TGA)]. Genotyping was performed using the TaqMan allelic discrimination assay (Applied Biosystems, Inc., Foster City, CA). The TaqMan genotyping reaction was amplified on a GeneAmp PCR system 9700 (95°C for 10 min, 92°C for 15 s, and 60°C for 1 min, for 40 cycles). Fluorescence was detected on an ABI PRISM 7700 sequence detector (Applied Biosystems, Inc.). To assess genotyping reproducibility, approximately 5% of the samples were regenotyped for all single nucleotide polymorphisms; all repeated genotypes matched their initial designation. Haploview software was used to identify haplotypes (http://www.broad.mit.edu/mpg/haploview/index.php).

Statistics and data analysis

Data were analyzed using SPSS 10.0. Body mass index (BMI) is expressed as kg/m^2; lipid parameters are in mmol/L. All values are reported as mean ± standard error of the mean (SEM). Mean values were compared with *t*-tests according to genotype or haplotype; $P < 0.05$ (2-tailed) was considered significant. Chi-squared analyses were used to evaluate differences between hypertriglyceridemic and normolipidemic groups for allele or haplotype frequencies after stratification by gender.

Logistic regression analysis was performed to assess the effect of the variants on the hypertriglyceridemic group versus the normolipidemic group. Genotype data were the initial covariates employed; age, gender, and BMI were added subsequently. The results obtained with this comprehensive logistic regression model are presented. When variant effects were analyzed by gender, age, and BMI, only age and BMI were included in these analyses. A general linear model was used to test gender and single-nucleotide polymorphism (SNP) interaction for levels of TG. In this model, univariate analysis was performed; genotype information and gender were added as fixed factors, and type III sum of squares was used.

Results

The anthropometric and biochemical characteristics of the 1112 cases and control subjects are listed in Table 1. When the hypertriglyceridemic subjects were compared to normolipidemic controls, they were younger (51.8 vs. 59.9 years, $P < 0.001$) and had higher BMI values (28.7 vs. 25.1 kg/m^2,

Table 1. Clinical Characteristics and Lipid Analyses of the Study Populations

	Hypertriglyceridemic total group	*Normolipidemic total group*	*P value*
n	817	295	
Gender (male/female)	575/242	153/142	<0.001
Age (years)	51.8 ± 0.4	59.9 ± 0.8	<0.001
BMI (kg/m^2)	28.7 ± 0.1	25.1 ± 0.2	<0.001
Total cholesterol (mmol/L)	8.87 ± 0.13	4.72 ± 0.04	<0.001
Triglycerides (mmol/L)	10.2 ± 0.4	0.98 ± 0.02[a]	
HDL-C (mmol/L)	0.93 ± 0.02	1.67 ± 0.01	<0.001
LDL-C (mmol/L)	3.5 ± 0.07	2.6 ± 0.03	<0.001
LDL-TG (mmol/L)	2.2 ± 0.1	0.6 ± 0.03	<0.001
VLDL-C (mmol/L)	1.98 ± 0.01	0.1 ± 0.01	<0.001
VLDL-TG (mmol/L)	8.9 ± 0.4	0.38 ± 0.05	<0.001
TG/HDL-C	39.7 ± 3	1.4 ± 0.01	<0.001

With the exception of gender, the mean ± SEM is reported.

[a]Use of this variable as a case selection criterion precludes reporting of a statistical significance.

Abbreviations: BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very-low-density lipoprotein cholesterol; VLDL-TG, very-low-density lipoprotein triglyceride.

$P < 0.001$). In addition, the hypertriglyceridemic subjects had higher fasting total cholesterol (8.87 vs. 4.72 mmol/L, $P < 0.001$), and lower HDL-C (0.93 vs. 1.67 mmol/L, $P < 0.001$). Moreover, they had higher levels of LDL-TG, LDL-C, very-low-density lipoprotein (VLDL), and a higher TG/HDL-C ratio. Higher TC levels are due in part to elevated VLDL-C levels. Lower HDL-C levels result from the hyperbolic relationship between HDL-C and TG due to transfer of cholesterol esters from HDL to TG-rich lipoproteins.[26] We found similar associations between plasma lipid parameters and PC-1 variants. Only the TG parameter was presented in the results.

The reason the levels of LDL-C are higher in the case group could, in part, reflect recruitment in a tertiary lipid clinic. Many patients with elevated TG have combined hyperlipidemia. Importantly, the genotypic distributions of all 3 alleles were in Hardy–Weinberg equilibrium ($P > 0.1$). The minor allele frequency (MAF) values for the 3 variants (K121Q, IVS20delT-11, and A→G+1044TGA) were 15.1%, 22.2%, and 28.6%, respectively, and similar to other white European populations[19,20,24]and the International HapMap CEU population.

K121Q genotype in the white European case–control population

The MAF of the K121Q variant (rs1044498) was higher in the hypertriglyceridemic case population than in the normolipidemic control population, but this was not statistically different (15.8% vs. 14.4%, odds ratio [OR] 1.121, $P = 0.3$). The MAF of the K121Q variant was also not statistically different between the hypertriglyceridemic and normolipidemic subjects after gender stratification (male subjects, 16.4% vs. 14.4%, respectively; female subjects, 14.3% vs. 14.4%, respectively). Using a logistic regression analysis with an additive inheritance model, no statistically significant association was found between the K121Q variant and hypertriglyceridemia (OR 1.121, 95% confidence interval [CI], 0.822–1.528; $P = 0.472$) (Table 2).

IVS20delT-11 (rs1799774) genotype in the white European case–control population

The MAF of the delT (IVS20delT-11) variant (rs1799774) was also not statistically different between the entire hypertriglyceridemic case population and the normolipidemic control population, although there was a trend. (23.2% vs. 19.5%, $P = 0.063$). We then performed gender stratification. The MAF of the delT (IVS20delT-11) variant was statistically different between the hypertriglyceridemic and normolipidemic male subjects (24.3% vs. 19.0%, $P = 0.048$); in contrast, there was no significant difference between the hypertriglyceridemic and normolipidemic female subjects (20.7% vs. 20.1%, $P = 0.09$). When male and female subjects were analyzed together, logistic regression analyses revealed a positive association between the delT (IVS20delT-11) variant and hypertriglyceridemia (OR 1.408, 95% CI, 1.077–1.841; $P = 0.012$). The odds ratio was significant in male (OR 1.819, 95% CI, 1.212–2.729; $P = 0.004$) but not female subjects (OR 1.061, 95% CI, 0.707–1.592; $P =$ not significant [N.S.]) (Table 2).

3 UTR variant (A→G+1044TGA, rs7754561) genotype in the white European case–control population

The MAF of the G allele (A→G+1044TGA) (rs7754561) was statistically different between the entire hypertriglyceridemic case group and the normolipidemic control group (30.4% vs. 23.2%, $P < 0.001$). However, similar to that of the delT (IVS20delT-11) variant, the MAF of the G allele (A→G+1044TGA) was statistically different in male subjects (33.2% vs. 23.2%, $P < 0.001$); but not in females (23.6% vs. 23.2%, $P = 0.09$). When both male and female subjects were analyzed together, logistic regression analysis revealed a positive association between the G allele (A→G+1044TGA) and hypertriglyceridemia (OR 1.478, 95% CI, 1.154–1.893; $P = 0.002$). As with the analyses of the delT (IVS20delT-11) variant, the odds ratio was significant in male (OR 1.864, 95% CI,

Table 2. *ENPP1/PC-1* Variant Minor Allele Frequencies and Odds Ratios According to Triglyceride Status

Variants	*MAF%*		*P value*	*Odds ratio (95% CI)*	*P value*
K121Q (rs1044498)	Case[a]	Control[b]			
Total	15.8	14.4	NS	1.121 (0.822–1.528)	NS
Male	16.4	14.4	NS	1.230 (0.766–1.977)	NS
Female	14.4	14.3	NS	1.125 (0.719–1.761)	NS
IVS20delT-11 (rs1799774)					
Total	23.2	19.5	0.063	1.408 (1.077–1.841)	0.012
Male	24.3	19.0	0.048	1.819 (1.212–2.729)	0.004
Female	20.7	20.1	NS	1.061 (0.707–1.592)	NS
A→G+1044TGA (rs7754561)					
Total	30.4	23.2	0.001	1.478 (1.154–1.893)	0.002
Male	33.2	23.2	0.001	1.864 (1.294–2.686)	0.001
Female	23.6	23.2	NS	1.097 (0.747–1.612)	NS

Odds ratios for hypertriglyceridemic cases are given in comparison with the control normolipidemic controls with the 95% CI and *P* values after age and BMI adjusted.

[a]Plasma triglycerides level >4.52 mmol/L.

[b]Plasma triglycerides level <1.69 mmol/L and no other lipid parameter abnormalities.

Abbreviations: MAF, minor allele frequency; NS, not significant; CI, confidence interval; BMI, body mass index.

1.294–2.686; $P = 0.001$) but not female subjects (OR 1.097, 95% CI, 0.747–1.612; $P =$ N.S.) (Table 2).

The relationship between ENPP1/PC-1 variants and type 2 diabetes

There was no major relationship between the 3 variants and the presence of type 2 diabetes. Only in the case of the (A→G+1044TGA) (rs7754561) variant, and in the K-delT-G haplotype, was there a weak but significant association with type 2 diabetes (Table 2).

ENPP1/PC-1 haplotype analysis in the study population

The 3 variants of the "at risk" haplotype (K121Q, IVS20delT-11 [delT], and A→G+1044TGA [G]) were employed in an *ENPP1/PC-1* haplotype analysis of the association of these variants with hypertriglyceridemia. In the entire population (males and females), 4 haplotypes with a frequency >5% accounted for 89.8% of all haplotypes. In the study population, the K121-T-G haplotype was significantly higher in the hypertriglyceridemic group than in the normolipidemic group (10.2% vs. 7.3%, $P = 0.037$), and the K121-delT-G haplotype showed a trend toward significance (9.9% vs. 7.3%, $P = 0.059$).

After gender stratification (Table 3), the frequency of only the K121-delT-G haplotype was significantly higher in the hypertriglyceridemic group than in the normolipidemic group (11.2% vs. 6.7%, $P = 0.019$), and only in male subjects; no specific haplotype frequencies were significantly higher in the hypertriglyceridemic and normolipidemic women.

Discussion

In this study, we examined the association between *ENPP1/PC-1* genetic variants and hypertriglyceridemia in a population of white European descendents. Three specific *ENPP1/PC-1* variants were evaluated (K121Q, IVS20delT-11, and A→G+1044TGA). Two of these, the noncoding variants G allele (A→G+1044TGA) (rs7754561) and delT (IVS20delT-11) (rs1799774), were found to be significantly associated with hypertriglyceridemia in male, but not in female subjects. Moreover, the K121-T-G haplotype showed a significant association with the hypertriglyceridemic group.

Keene et al. recently investigated type 2 diabetes and diabetic nephropathy in an African-American population.[27] Interestingly, as in the present study of hypertriglyceridemia, they observed that both the 3′ UTR A→G+1044TGA and the delT (IVS20delT-11) *ENPP1/PC-1* noncoding variants, but not the K121Q coding variant, were associated with nephropathy. Moreover, in their analysis the K121-T-G haplotype also showed a significant association with nephropathy. The G allele (A→G+1044TGA) and the delT (IVS20delT-11) *ENPP1/PC-1* variants are in noncoding regions of the gene, and the mechanism(s) by which they influence the *ENPP1/PC-1* gene are unknown.

Frittitta et al. found that a cluster of 3 alleles in the 3′ UTR of *ENPP1/PC-1* (rs1044548 A, rs11964389 C, and rs104455 T) increased *ENPP1/PC-1* mRNA stability and was associated with *ENPP1/PC-1* overexpression.[28] It was previously reported that the 3′ UTR A→G+1044TGA variant was highly expressed in 3 insulin-sensitive tissues: liver, adipocytes, and pancreatic β-cells.[7] Moreover, these investigators found that *ENPP1/PC-1* levels in serum were increased in subjects with the "at risk" haplotype. Thus, noncoding *ENPP1/PC-1*

Table 3. Haplotype Distribution in the Study Populations

Haplotype			Frequency (%)	Case vs. control (%)	P value
Male ($n = 728$)					
K121	T	A	60.5	58.6 vs. 66.8	0.008
K121	T	G	10.2	10.5 vs. 8.2	NS
K121	delT	G	9.9	11.2 vs. 6.7	0.019
Q121	delT	G	9.4	9.9 vs. 8.1	NS
Female ($n = 384$)					
K121	T	A	65.9	65 vs. 67.4	NS
K121	T	G	7.7	8.4 vs. 6.6	NS
K121	delT	G	8.4	8.4 vs. 8.3	NS
Q121	delT	G	8.7	9.1 vs. 8.1	NS

The K121Q (rs1044498), IVS20delT-11 (rs1799774), and A→G+1044 (rs7754561) single-nucleotide polymorphisms were used to determine haplotypes. Hypertriglyceridemic cases and normolipidemic, healthy controls were evaluated. The Haploview program was used to compare the haplotype frequency differences between the case and control groups.

variants may either modulate gene transcription or affect mRNA stability.

Gender differences in association studies with the *ENPP1/PC-1* gene have been observed previously.[29] Recently, we observed a gender difference with obesity in a Turkish population. We found that both the Q121 variant and the "at risk" haplotype were associated with obesity in males but not in females.[30] Wan et al. reported a gender difference in the association between the Q121 variant and the risk of obesity in a Chinese population.[29] In contrast to our previous study,[30] they found that female subjects with the Q allele were at higher risk for obesity than males. We did not find any gender–SNP(s) interaction on TG levels in the present study. The reasons for these divergent observations are unknown, but most likely reflect differences in the genetic and environmental backgrounds of the study populations and in recruitment and evaluation factors. Relatively few female subjects were analyzed in our study, and there may not have enough power to reach significance.

Interestingly, the widely-studied K121Q coding variant (rs1044498) was not associated with hypertriglyceridemia in our population, consistent with findings from a previous study evaluating a white Finnish population.[14] Unlike the 3′ UTR G allele (A→G+1044TGA) and the intron 20 delT (IVS20delT-11) variants, the K121Q variant is the only common variant in the *ENPP1/PC-1* gene that results in an amino acid change; furthermore, its encoded protein (Q121 variant) binds more strongly to the insulin receptor and more effectively inhibits insulin receptor function and downstream signaling.[11] Thus, the Q121 variant has been a logical candidate underlying the association of the *ENPP1/PC-1* locus with metabolic perturbations. However, as has been observed with prior studies evaluating the association between the Q121 variant and insulin resistance, diabetes, and obesity, discordant results in genotype–phenotype associations are not uncommon, especially in the evaluation of complex disorders such as insulin resistance and hyperlipidemia. Thus, although the Q121 MAF was higher in the case group than the control group, the reported lack of association between the Q121 variant and hypertriglyceridemia may not be a negative result. Therefore, larger studies may be needed to evaluate the role of *ENPP1/PC-1* variants in hypertriglyceridemia.[23]

In the present study, we found strong association with PC-1 variants and dyslipidemia. It is worth noting that patients were selected from a lipid clinic and may have other genetic defects. Extreme lipid abnormalities, such as lipoprotein lipase deficiency, or defects in apolipoproteins C-II or A-V in addition to insulin resistance may dilute the association. If such patients were excluded from the study, the association between *PC-1* variants and hypertriglyceridemia may be even stronger than we report here.

In conclusion, we report that 2 *ENPP1/PC-1* gene variants, G allele (A→G+1044TGA) (rs7754561) and delT (IVS20delT-11) (rs1799774), are significantly associated with hypertriglyceridemia in white European males. In this population, these polymorphisms may thus serve as markers to identify individuals at risk for hypertriglyceridemia and, potentially, the metabolic syndrome. Moreover, an understanding of the biological function of these variants may provide novel insights into the pathogenesis of insulin resistance-associated dyslipidemia.

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Author Disclosure Statement

No competing financial interests exist.

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