Brief Genetics Report

The Proline 12 Alanine Substitution in the Peroxisome Proliferator–Activated Receptor-γ2 Gene Is Associated With Lower Lipoprotein Lipase Activity in Vivo

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Lipoprotein lipase (LPL) plays a key role in lipid metabolism by hydrolyzing triglycerides in circulating lipoproteins. Low LPL activity has been linked to coronary artery disease (CAD), but the factors influencing LPL expression are not completely understood. Peroxisome proliferator–activated receptor (PPAR)-γ is a nuclear receptor regulating lipid and glucose metabolism, and a PPAR-responsive element is present in the LPL promoter. We determined the Pro12Ala polymorphism in the PPAR-γ2 gene in 194 male CAD patients because this allele is associated with decreased PPAR activity and reduced LPL promoter activity in vitro. Presence of 12Ala was associated with 20% lower LPL activity in postheparin plasma (141 ± 58 vs. 177 ± 77 nmol · ml^{-1} · min^{-1}, $P < 0.005$). Remarkably, the influence of 12Ala on LPL was greater than that of the frequent polymorphisms (*Hin*dIII +9%, *Pvu*II ±0%, 447stop +12%) in the LPL gene itself. To confirm these results in a different group of patients, we analyzed 100 diabetic patients in whom the 12Ala allele was also associated with lower LPL activity (12Ala: 132 ± 88 vs. 190 ± 129 nmol · ml^{-1} · min^{-1}, $P < 0.05$). Our data demonstrate that the Pro12Ala substitution in PPAR-γ2 is associated with lower LPL activity in vivo and provides a new target for the analysis of genetic influences on LPL activity and CAD risk. *Diabetes* 51:867–870, 2002

Lipoprotein lipase (LPL) is a key enzyme in lipid metabolism. It hydrolyzes core triglycerides of circulating chylomicrons and VLDL (1). The diabetic dyslipidemia of low HDL and high triglycerides has been attributed, in part, to low LPL activity (2). LPL activity varies greatly between individuals, but the factors influencing LPL expression are not completely understood. Recently, a peroxisome proliferator–activated receptor (PPAR) response element was identified in the human LPL promoter (3).

PPAR-γ is a transcription factor that belongs to the nuclear receptor family. In humans, alternate use of promoters and differential splicing of PPAR-γ results in three different isoforms, PPAR-γ1, PPAR-γ2, and PPAR-γ3. The three isoforms differ in their tissue distribution, but all of them are expressed in adipose tissue, where PPAR-γ2 predominates. The PPARs have been implicated in the regulation of adipocyte differentiation and lipid and fatty acid metabolism. They also play a role in glucose homeostasis as molecular targets for thiazolidinediones (4).

PPAR-γ induces the transcription of target genes after ligand-dependent activation (5) or in a ligand-independent manner (6). A rare mutation (Pro115Glu) in the ligand-independent activation domain suggested a possibly distinct and essential role of the PPAR-γ2 isoform in obesity, insulin resistance, and diabetes and, moreover, a possible explanation of this effect by functional differences in the NH_2 terminus (7).

Recently, a Pro12Ala substitution in exon B of PPAR-γ2 was reported to be associated with lower BMI, improved insulin sensitivity, and higher HDL cholesterol in a Finnish population of middle-aged men and women (8). In a multiple-sample study on genetic associations of diabetes, the 12Ala allele was less frequent among patients with type 2 diabetes (9). PPAR-γ function appears to be a key factor in mediating conditions such as dyslipidemia, obesity, and insulin resistance and has also been implicated in the development of coronary artery disease (CAD).

Because the LPL promoter was shown to be ~30% less efficiently transactivated in the presence of the Ala allele in PPAR-γ2 in vitro (8), we hypothesized that the Pro12Ala substitution may lead to decreased LPL activity in vivo.

To test this hypothesis, we studied the effect of the Pro12Ala substitution on common metabolic variables and LPL activity in 194 male patients who underwent elective coronary angiography. The allele frequency of the Pro12Ala substitution in PPAR-γ was 0.103 and therefore within the range of earlier publications. Table 1 shows the anthropometric and metabolic parameters of the 194 male patients according to PPAR-γ2 genotype. 12Ala was associated with a highly significant, 20% lower LPL activity (141 ± 58 vs. 177 ± 77 nmol · ml^{-1} · min^{-1}, $P < 0.005$) (Fig. 1). Of this group, 32 patients had type 2 diabetes. When these patients were omitted from the analysis, those in

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CAD, coronary artery disease; LPL, lipoprotein lipase; PPAR, peroxisome proliferator–activated receptor.

TABLE 1
Anthropometric and biochemical variables of the 194 male study subjects

Variable	12Ala absent	12Ala present	*P*
n	156	38	
Age (years)	62.0 ± 9.3	61.0 ± 11.7	0.57
BMI (kg/m^2)	27.5 ± 3.4	27.7 ± 3.3	0.74
Total cholesterol (mmol/l)	5.4 ± 1.3	5.2 ± 1.2	0.38
LDL cholesterol (mmol/l)	3.8 ± 1.1	3.6 ± 1.0	0.30
HDL cholesterol (mmol/l)	1.0 ± 0.3	1.0 ± 0.3	1.00
VLDL cholesterol (mmol/l)	0.7 ± 0.9	0.6 ± 0.5	0.51
Triglycerides (mmol/l)	1.8 ± 1.5	1.6 ± 0.9	0.43
Insulin (pmol/l)	173.3 ± 89.0	159.3 ± 58.5	0.35

Data are means ± SE.

whom the polymorphism was present also had significantly lower LPL activity (n = 36, 135 ± 49 vs. 172 ± 70 nmol · ml^{-1} · min^{-1}, P = 0.001).

To further evaluate the effect of the Pro12Ala polymorphism on LPL activity, we compared it with the role of known polymorphisms within the LPL gene (Fig. 2). The presence of the rare allele for the *Hin*dIII polymorphism, which has been suggested to modulate lipid values and even atherosclerotic risk (10), led to a trend toward higher LPL activity (162 vs. 177 nmol · ml^{-1} · min^{-1}, P = 0.09). In addition, the presence of the 447Stop polymorphism in the LPL gene, which has previously been suggested to be associated with higher LPL activity (11), also showed a trend toward higher LPL activity (167 vs. 191 nmol · ml^{-1} · min^{-1}, P = 0.09). The PPAR-γ2 polymorphism was not in linkage dysequilibrium with the LPL polymorphisms. To simultaneously quantitate the effect of the four polymorphisms, we performed a multivariate regression analysis with LPL activity as the dependent variable. Of the four tested polymorphisms, only Pro12Ala was significantly associated with LPL activity (P = 0.005), and it was the only variable with a value >2.0 in the T statistics. These data demonstrate that the influence of the Pro12Ala polymorphism in the PPAR-γ2 gene on LPL activity is significantly greater than that of the polymorphisms in the LPL gene itself.

To confirm the association of the Pro12Ala polymorphism with LPL activity in a separate group of patients, we measured both parameters in 100 patients with type 2 diabetes. Frequency of the 12Ala allele in the diabetic

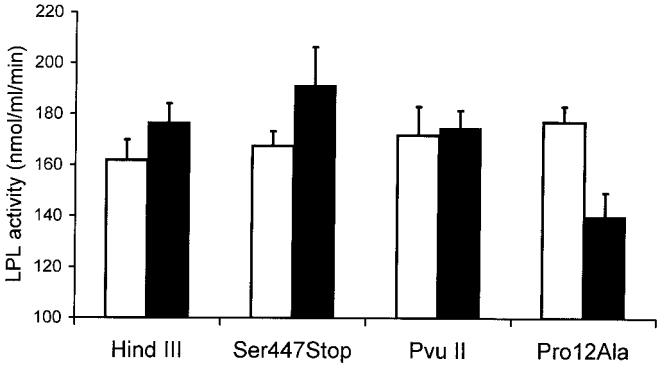

FIG. 2. Genetic influences on LPL activity found in the 194 male patients who underwent elective coronary angiography. □, Wild-type LPL; ■, rare allele. Data are means ± SE. P = 0.09 for Hind III; P = 0.09 for Ser447Stop; P = 0.83 for Pvu II; and P < 0.005 for Pro12Ala.

patients was 0.075 and therefore lower than in the CAD patients. LPL activity was 35% lower in presence of the 12Ala allele (132 ± 88 vs. 190 ± 129 nmol · ml^{-1} · min^{-1}, P < 0.05) (Fig. 3), confirming the association of the Pro12Ala polymorphism with LPL activity in vivo. There was no significant influence of the Pro12Ala polymorphism on hepatic lipase activity in either group (nondiabetic patients: 287 ± 106 vs. 288 ± 112, P = 0.93; diabetic patients: 246 ± 98 vs. 269 ± 98, P = 0.41).

The observed influence of the PPAR-γ2 polymorphism on LPL gene expression is most likely due to an effect on a functional PPAR response element in the LPL promoter (3), and the lower transactivation capacity of the PPAR-γ2 isoform on the LPL gene seen in vitro (8). The effect of the Pro12Ala polymorphism on LPL activity that we found in vivo was greater than that of the common polymorphisms in the LPL gene itself, and it was independent of age, BMI, lipid levels, diabetic control, and insulin levels (Tables 1 and 2) as well as medication (data not shown). Stepwise multiple regression analysis confirmed that the association of the Pro12Ala polymorphism with LPL activity was independent of potential confounding factors such as age, BMI, insulin levels, hypertension, and pack-years (data not shown). Our data of the association of a PPAR-γ2 polymorphism that is characterized by lower transcriptional activity with lower LPL activity in vivo supports the recent observation that treatment with troglitazone, a PPAR-γ agonist, increases LPL concentration in postheparin plasma (12).

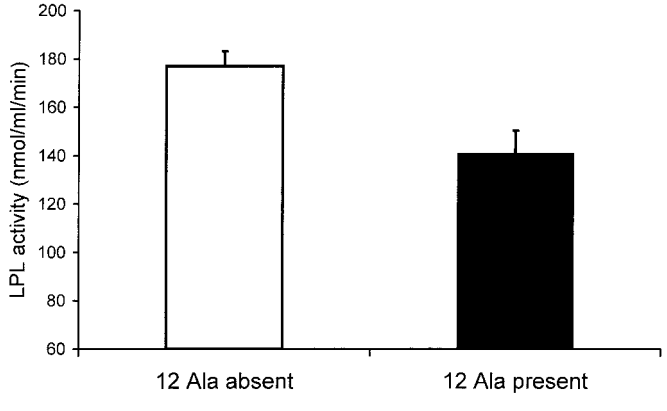

FIG. 1. Lower LPL activity in 194 male patients with at least one allele of the rare isoform 12Ala. Data are means ± SE. P < 0.005.

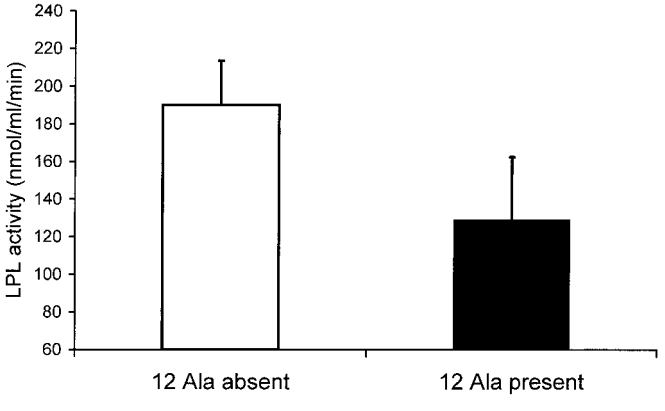

FIG. 3. Lower LPL activity in 100 diabetic patients with at least one allele of the rare isoform 12Ala. Data are means ± SE. P < 0.05.

TABLE 2
Anthropometric and biochemical variables of the diabetic subjects

Variable	12Ala absent	12Ala present	*P*
n	87	13	
Age (years)	63.3 ± 8.0	62.7 ± 6.6	0.70
BMI (kg/m^2)	28.7 ± 4.1	27.6 ± 3.4	0.26
Total cholesterol (mmol/l)	5.8 ± 1.6	5.1 ± 1.2	0.06
LDL cholesterol (mmol/l)	3.8 ± 1.3	3.5 ± 0.8	0.32
HDL cholesterol (mmol/l)	1.1 ± 0.4	1.0 ± 0.4	0.31
VLDL cholesterol (mmol/l)	1.0 ± 1.5	0.7 ± 0.5	0.37
Triglycerides (mmol/l)	2.3 ± 2.2	1.7 ± 1.0	0.22
Fasting plasma glucose (mmol/l)	8.7 ± 3.0	8.2 ± 3.2	0.50
Insulin (pmol/l)	247.6 ± 190.0	211.7 ± 118.2	0.41
HbA_{1c} (%)	7.7 ± 1.2	7.7 ± 0.8	1

Data are means ± SE.

LPL is mainly synthesized in adipose tissue, myocytes, and macrophages. The source of LPL found in postheparin plasma is not known, although analysis of mice deficient for macrophage LPL revealed that macrophage LPL did not significantly affect postheparin LPL activity (13). PPAR-γ2 is mainly expressed in adipose tissue, and the effect of the PPAR-γ2 polymorphism we observed on LPL activity in vivo was only slightly smaller than that seen in vitro (8). This suggests that a significant portion, perhaps even the majority of postheparin LPL activity, is derived from adipose tissue.

Lower plasma LPL activity as well as mutations and polymorphisms in the LPL gene have been suggested to be proatherogenic (14-16) and associated with endothelial dysfunction (17). LPL activity in postheparin plasma is difficult to measure and appears to be an insensitive measure of CAD risk (18). The identification of a novel genetic factor modulating LPL activity in vivo will therefore help to elucidate the influence of LPL on CAD risk.

In summary, our data show that the Pro12Ala polymorphism in the PPAR-γ2 gene significantly modulates LPL activity in patients, and that the influence on LPL activity is greater than that of the frequent polymorphisms within the LPL gene itself. These findings provide a new target for the analysis of genetic influences on LPL activity and CAD risk.

RESEARCH DESIGN AND METHODS

A total of 194 consecutive male patients undergoing elective coronary angiography were studied. Exclusion criteria were intravenous or subcutaneous treatment with heparin in the 72 h before study, severe kidney or liver disease, treatment with drugs known to affect LPL activity (such as fibrates), and a fasting triglyceride level >11.4 mmol/l (1,000 mg/dl). Furthermore, 100 patients (71 male and 29 female subjects) with known type 2 diabetes were studied. Exclusion criteria for the diabetic patient group were the same as noted above for the coronary angiography group. Each patient gave written informed consent. Key demographic characteristics were recorded from the patients' charts, including age, sex, BMI, history of disease, and present medication.

Determination of LPL activity. EDTA blood was obtained before and 10 min after a bolus injection of heparin (60 IU/kg body wt). The samples were immediately chilled to 4°C, centrifuged, divided into aliquots, flash-frozen on dry ice, and stored at −70°C until assayed. Pre- and postheparin LPL activity was quantified using a triolein-phosphatidylcholine emulsion. Lipase activity is expressed as nanomoles of free fatty acids released per milliliter per minute.

Genotyping. Genomic DNA was isolated from whole blood, using the QIAmp Blood kit (Qiagen). The Pro12Ala genotype in exon B of PPARγ2 was determined by modifying PCR, followed by digestion with the restriction endonuclease *BstUI* (New England Biolabs, Beverly, MA) and agarose gel electrophoresis, as described elsewhere (19). Additionally, three common polymorphisms within the gene for LPL were determined in the 194 male patients, as described elsewhere (20).

Statistical analysis. Data are means ± SD. Statistical analyses were performed with SPSS for Windows, release 10.05 (SPSS, Chicago, IL). A χ^2 analysis was used to evaluate the allelic and genotypic frequencies that were calculated from the observed genotypic counts and to assess Hardy-Weinberg expectations. Two-tailed bivariate correlations were determined by the Pearson coefficient for parameters with normal distribution, and with the Spearman coefficient for parameters with other distributions. Stepwise multiple regression analysis was performed to assess the influence of independent variables on LPL activity. Because of the small number of Ala/Ala homozygous subjects, they were combined with the Pro12Ala heterozygous group for statistical analysis. Comparisons of quantitative variables between the two sets of patients were performed by the unpaired *t* test and the Mann-Whitney *U* test as appropriate. Statistical significance was accepted at $P < 0.05$.

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