Pro12Ala Sequence Variant of the PPARG Gene Is Associated with Postprandial Hypertriglyceridemia in Non-E3/E3 Patients with the Metabolic Syndrome

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Background: Postprandial hypertriglyceridemia, a component of the metabolic syndrome, has varied etiology and involves many genes related to triglyceride metabolism. Variations in these genes may affect postprandial hypertriglyceridemia in the context of the metabolic syndrome.

Methods: We orally administered 60 g of fat overload to 74 patients with the metabolic syndrome. We then measured baseline concentrations of cholesterol, triglycerides, HDL cholesterol, apolipoprotein AI, apolipoprotein B, uric acid, and uric acid excretion; we also performed homeostasis model assessments of insulin resistance and insulin sensitivity. At 3 h, we measured triglycerides, cholesterol, apolipoprotein AI, and apolipoprotein B. Patients were considered to have postprandial hypertriglyceridemia if the difference in plasma triglycerides between baseline and 3 h after the test was 1.71 mmol/L or more. We also measured anthropometrical variables and classified the patients according to their peroxisome proliferative activated receptor, gamma (PPARG) gene and apolipoprotein E (APOE) genotype.

Results: Postprandial hypertriglyceridemia occurred in 64.7% of patients with the Ala12 allele vs 19.9% of the Pro12Pro patients, (P = 0.00032; odds ratio, 7.6), and in 87.5% of the patients with both the Ala12 allele and the non-E3/E3 APOE genotype (odds ratio, 23.8). Logistic regression analysis showed that PPARG and APOE sequence variants were associated with the presence of postprandial hypertriglyceridemia.

Conclusion: The Pro12Ala PPARG sequence variant together with a non-E3/E3 APOE genotype is associated with a high risk for postprandial hypertriglyceridemia in patients with the metabolic syndrome, indicating a close association between these genes and the regulation of lipoproteinase clearance.

The metabolic syndrome is a complex disease marked by abdominal obesity, glucose intolerance, and dyslipidemia. Dyslipidemia associated with the metabolic syndrome is characterized by increased plasma concentrations of triglycerides, small, dense LDL particles, and decreased concentrations of HDL cholesterol (1). Postprandial hyperlipidemia is also associated with the metabolic syndrome (2).

Many studies have focused on genes associated with the metabolic syndrome. Peroxisome proliferator-activated receptor, gamma (PPARG) is a member of the steroid hormone receptor superfamily and is a critical transcriptional regulator of adipogenesis. In vivo ligands for PPARG are thought to include a variety of fatty acids, and it has been proposed that PPARG may be a mediator of physiological responses to lipids (3). Interestingly, human studies have shown that thiazolidinediones decrease insulin resistance and hypertriglyceridemia through their interaction with the PPAR receptor (4).
induction of lipoprotein lipase by PPARG promotes fatty
acid delivery, whereas induction of fatty acid transport
proteins and acyl-CoA synthetase leads to enhanced fatty
acid uptake. These actions contribute to increased triglyc-
eride storage in adipose tissue (5).

PPARG2, a variant of PPARG, contains an amino acid
substitution of proline for alanine at codon 12 (Pro12Ala)
(6). Functional differences observed for this Ala variant
are lower affinity for the response element and lower
capacity for activating target genes (7). Studies examining
the relationship between Pro12Ala and metabolic vari-
ables (8–11) have reported various results regarding
adiposity, with some reporting increased (8), some de-
creased (7, 9), and some neutral effects on body mass
index (BMI)5 (10).

The Pro12Ala sequence variant of the PPARG gene has
been associated with hypertriglyceridemia in obese per-
sons with familial combined hyperlipidemia. Increased
concentrations of triglycerides and decreased concentra-
tions of HDL cholesterol occur in persons with this
disease who carry the Ala12 allele (11), although no asso-
ciation has been found in the general population
between the Pro12Ala sequence variant of the PPARG
gene and fasting free fatty acids (12). The possibility of
different findings under other conditions (e.g., postpran-
dial) cannot be excluded. Therefore, we investigated the
influence of the Pro12Ala sequence variant of the PPARG
gene in postprandial hypertriglyceridemia in patients
with the metabolic syndrome, taking into consideration
the apolipoprotein (APO) E (APOE) genotype, because
our group has previously shown a close association
between this gene and postprandial hypertriglyceridemia
in the metabolic syndrome (13).

Patients and Methods
We studied 74 patients (57 men and 17 women) with
the metabolic syndrome as defined by the Adult Treatment
Panel III criteria; obesity (waist circumference >102 cm in
men or >88 cm in women), high blood pressure (systolic
blood pressure ≥130 or diastolic blood pressure ≥85 mm
Hg), fasting glucose ≥6.05 mmol/L, triglycerides ≥1.71
mmol/L, and HDL cholesterol (<1.04 mmol/L for men or
<1.3 mmol/L for women) (14). Patients with diabetes
(determined by a 75-g oral glucose tolerance test) were
excluded. For all patients, we performed baseline mea-
surements of cholesterol, triglycerides, APOAI, APOB,
inulin, uric acid, 24-h uric acid urinary excretion, HDL
cholesterol, waist-to-hip ratio, BMI, and age; and we
performed homeostasis model assessments of insulin re-
sistance (HOMA IR) and insulin sensitivity (HOMA IS).
The patients received a 60-g fat overload in a commercial
preparation (Supracal®). Only water was permitted dur-
ing the process, and no physical exercise was undertaken.
At 3 h after fat overload, we measured cholesterol, tri-
glycerides, HDL cholesterol, APOAI, and APOB. Patients
were classified as having postprandial hypertriglyceride-
mia if the difference in plasma triglycerides at baseline
and after 3 h was ≥1.71 mmol/L (Δ triglycerides). This
value was taken as the cutoff point because a previous
study of patients with the metabolic syndrome showed
that this value represented the 66th percentile (P66), and
patients above this percentile had a higher degree of
insulin resistance (15). The commercial preparation of 125
mL contained 60 g of fat (12 g saturated, 35.25 g mono-
unsaturated, and 12.75 g polyunsaturated). Each 100 mL
contained <1 g lauric acid, <1 g myristic acid, 4.8 g
palmitic acid, 1.4 g stearic acid, 27.7 g oleic acid, 9.6 g
linoleic acid, <1 g linolenic acid, 0.5 g arachidonic acid,
0.5 g eicosanoic acid, 1.4 g behenic acid, and 0.5 g
lignoceric acid.

All the patients gave informed consent, and the study
was approved by the Ethics Committee of Virgen de la
Victoria University Hospital.

DNA analysis
We used the salting out method of Miller, modified by
Queipo-Ortuño (16), to extract DNA from 200-µL samples
of whole blood. We used PCR and restriction-fragment
length variant enzyme digestion (17) to analyze of the
Pro12Ala sequence variant of the PPARG gene and a
lightcycler (13) to determine APOE genotype.

Statistical study
We used the Student t-test to compare biological vari-
ables. Data are presented as mean (SD) in the tables. We
used the χ2 test for analysis of the distribution of PPARG
and APOE genotypes and the presence of hypertriglycer-
idemia after the overload. We carried out logistic regres-
sion analysis with postprandial hypertriglyceridemia dif-
fERENCE of > or <1.71 mmol/L between fasting plasma
triglyceride concentrations and concentrations 3 h after an
oral fat overload test) as the dependent variable and age,
sex, genotypes, HOMA IR, HOMA IS, and baseline trig-
glycerides as the independent variables.

In all cases, the rejection degree for a null hypothesis
was a P <0.05 for 2 tails. Statistical analyses were per-
formed with SPSS 6.0 for Windows.

Results
The distribution of the Pro12Ala sequence variant in the
study patients was 77.8% Pro12Pro, 21% Pro12Ala, and
1.2% Ala12Ala. These frequencies were similar to those of
persons from the general population living in the same
area (data not shown). The frequency distributions of the
PPARG sequence variants and APOE were similar for
both sexes (58% of men with E3/E3 vs 42% non E3/E3
and 65% of women with E3/E3 vs 35% non-E3/E3; 80% of
men were Pro12Pro vs 20% Ala12; and 70% of women
were Pro12Pro vs 30% Ala12). The distribution of the 2

5 Nonstandard abbreviations: BMI, body mass index; APOE, apolipopro-
tein E; HOMA IR, homeostasis model assessment insulin resistance; HOMA IS,
homeostasis model assessment insulin sensibility.
sequence variants of these 2 genes was independent and also similar for both sexes (data not shown).

The distribution of the biological variables in the study patients is shown in Table 1, and the distribution of the presence or absence of the rare Ala12 allele of PPARG, according to the presence or absence of postprandial hypertriglyceridemia, is shown in Table 2. Most (64.7%) of the carriers of the Ala12 allele had a difference in triglyceride concentrations of $\geq$1.71 mmol/L ($P = 0.00032$). The odds ratio of having postprandial hypertriglyceridemia in carriers of this rare Ala12 allele was 7.6. The analysis shown in Table 3 includes the combination of the distribution of PPARG sequence variants and APOE genotypes, grouped according to the presence or absence of the Ala allele and the presence or absence of the E3/E3 genotype, respectively; 87.5% of the persons with the Ala12 allele and non-E3/E3 genotype had postprandial hypertriglyceridemia ($P < 0.00001$). The patients with these alleles (Ala12 and non-E3/E3), compared with the other groups (Pro12Pro with E3/E3; Pro12Pro with non E3/E3; Pro12Ala or Ala12Ala with E3/E3), had an odds ratio for postprandial hypertriglyceridemia occurrence of 23.8 vs the noncarriers of these alleles.

### Discussion

The association between the PPARG gene Pro12Ala sequence variant, with its lower capacity for activating target genes, and BMI remains unclear. We found no association between Pro12Ala and BMI in patients with the metabolic syndrome. Nor did we detect any differences in the various components used to define the metabolic syndrome (baseline triglyceride concentrations, HDL cholesterol, waist circumference, high blood pressure, and glucose concentrations). Furthermore, we also failed to detect any association between this sequence variant and insulin resistance or insulin sensitivity, an carriers did or did not have the E3/E3 genotype, is shown in Table 4. There were no substantial differences in any of the study variables in persons with the E3/E3 genotype who were carriers or noncarriers of the Ala12 allele. However, persons with the non-E3/E3 genotype who also carried the Ala12 sequence variant had a marked difference in $\Delta$ triglycerides [2.52 (1.15) vs 1.29 (0.80); $P = 0.004$]. No substantial differences were found in age, BMI, baseline triglyceride concentrations, or HDL cholesterol, although the carriers of the Ala12 allele had a lower waist-to-hip ratio [0.92 (0.03) vs 0.96 (0.04), $P = 0.035$] (Table 4).

After adjustment for age, sex, BMI, and baseline triglyceride concentrations, logistic regression analysis indicated that the only variables influencing the occurrence of postprandial hypertriglyceridemia after fat overload were the APOE and PPARG genotypes and the waist-to-hip ratio (Table 5). When we performed the same logistic regression analysis and included as an independent variable a term for the interaction between the sequence variants of the APOE and PPARG genes, the results were significant ($P = 0.0045$), indicating a possible interaction between the 2 genes. The low number of study patients, however, demands caution in interpreting the results.
association that is still open to debate. The Ala12 variant has decreased affinity for the response element and a lower capacity for activating target genes. Because glucose zones stimulate the activity of PPARγ and increase insulin sensitivity, it would follow that the presence of the Pro12Ala sequence variant is associated with insulin resistance. However, Koch et al (18) reported an association in 108 obese patients of the Pro12Ala sequence variant with improved insulin sensitivity estimated by an euglycemic hyperinsulinemic glucose clamp. Also, in nondiabetic overweight or obese Japanese patients, Hara et al (19) reported that carriers of the Ala12 allele had lower concentrations of fasting plasma insulin and higher insulin sensitivity, as measured by HOMA-IR, than non-carriers. On the other hand, in a Finnish study of 141 obese women (20), the Ala12Ala genotype was associated with increased BMI, fat mass, and waist and hip circumferences compared with obese women with the Pro12Ala or Pro12Pro genotypes. In a French study (n = 839), the Ala12 allele was associated with increased body weight, BMI, waist circumference, and an atherogenic lipid profile (21). Moreover, a prospective study showed that this polymorphic variant has a role in the etiology of type 2 diabetes mellitus and in the insulin resistance syndrome (22).

Our group found a close association between postprandial lipidemia and the Pro12Ala sequence variant of the PPARγ gene, with no differences in the baseline concentrations of triglycerides. No previous data are available concerning the association between this component of the metabolic syndrome (postprandial lipidemia) and the Pro12Ala sequence variant in question. This sequence variant, however, has been associated with baseline lipid concentrations, although this association is also controversial.

### Table 4. Distribution of the biological variables [mean (SD)] according to the Pro12Ala polymorphism of the PPARγ gene in the patients with the metabolic syndrome and in 2 subgroups of patients according to the APOE genotype.

<table>
<thead>
<tr>
<th>Variable</th>
<th>E3/3 (57)</th>
<th>Pro12Ala (17)</th>
<th>Non E3/3 (9)</th>
<th>Pro12Ala (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>43.72 (10.9)</td>
<td>43.6 (11.7)</td>
<td>43.6 (11.7)</td>
<td>43.72 (10.9)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.8 (3.9)</td>
<td>27.2 (2.91)</td>
<td>27.6 (1.6)</td>
<td>27.38 (3.78)</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.94 (0.05)</td>
<td>0.93 (0.06)</td>
<td>0.94 (0.05)</td>
<td>0.94 (0.06)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>100.30 (11.6)</td>
<td>96.7 (9.9)</td>
<td>100.30 (11.6)</td>
<td>96.7 (9.9)</td>
</tr>
<tr>
<td>Glycemia, mmol/L</td>
<td>5.47 (1.07)</td>
<td>5.67 (1.13)</td>
<td>5.42 (0.5)</td>
<td>5.37 (0.56)</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>6.33 (1.01)</td>
<td>6.52 (1.63)</td>
<td>6.39 (1.08)</td>
<td>5.26 (1.47)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>3.94 (3.380)</td>
<td>3.45 (2.23)</td>
<td>4.23 (4.05)</td>
<td>2.92 (2.24)</td>
</tr>
<tr>
<td>Uric acid, μmol/L</td>
<td>380.6 (95.16)</td>
<td>371.7 (66)</td>
<td>363.4 (95.76)</td>
<td>394.94 (54.3)</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.14 (0.04)</td>
<td>1.11 (0.23)</td>
<td>1.17 (0.46)</td>
<td>1.03 (0.19)</td>
</tr>
<tr>
<td>Triglycerides after 3 h, mmol/L</td>
<td>5.11 (3.84)</td>
<td>5.28 (3.12)</td>
<td>5.24 (4.62)</td>
<td>4.20 (2.64)</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>0.4863</td>
<td>0.3727</td>
<td>0.77–3.42</td>
<td>1.62</td>
</tr>
<tr>
<td>HOMA IS</td>
<td>157.6 (103.7)</td>
<td>156.2 (80.61)</td>
<td>167.47 (113.14)</td>
<td>167.9 (78.9)</td>
</tr>
<tr>
<td>Triglycerides baseline</td>
<td>0.0013</td>
<td>0.0012</td>
<td>0.99–1.00</td>
<td>1.0013</td>
</tr>
</tbody>
</table>

P (Student t-test). *P = 0.040; **P = 0.035; ***P = 0.035; ****P = 0.004.

### Table 5. Logistic regression model.

<table>
<thead>
<tr>
<th>Variable</th>
<th>β</th>
<th>SD β</th>
<th>95% CI</th>
<th>Odds ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.0036</td>
<td>0.0352</td>
<td>0.93–1.00</td>
<td>0.9964</td>
<td>0.74</td>
</tr>
<tr>
<td>Non E3 vs E3</td>
<td>2.1323</td>
<td>0.8618</td>
<td>1.50–46.99</td>
<td>8.4343</td>
<td>0.013</td>
</tr>
<tr>
<td>PPARγ (Ala12 vs non-Ala12)</td>
<td>2.3927</td>
<td>1.0206</td>
<td>1.42–84.2</td>
<td>10.9434</td>
<td>0.019</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>19.2411</td>
<td>9.0100</td>
<td>3.4–1.5 × 10¹⁶</td>
<td>2.27E + 08</td>
<td>0.032</td>
</tr>
<tr>
<td>Sex (2 vs 1)</td>
<td>1.7770</td>
<td>1.0329</td>
<td>0.74–46.65</td>
<td>5.9122</td>
<td>0.83</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.0133</td>
<td>0.0980</td>
<td>0.81–1.20</td>
<td>0.9868</td>
<td>0.07</td>
</tr>
<tr>
<td>Triglycerides baseline</td>
<td>0.0013</td>
<td>0.0012</td>
<td>0.99–1.00</td>
<td>1.0013</td>
<td>0.44</td>
</tr>
<tr>
<td>HOMA IR</td>
<td>0.4863</td>
<td>0.3727</td>
<td>0.77–3.42</td>
<td>1.62</td>
<td>0.19</td>
</tr>
<tr>
<td>HOMA IS</td>
<td>-0.0109</td>
<td>0.0074</td>
<td>0.974–1.003</td>
<td>0.98</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Dependent variable: difference in plasma triglyceride concentrations 3 h after an oral fat overload test and fasting concentrations, classified as a difference of more or less than 1.71 mmol/L. Independent variables: age, genotype, sex, HOMA IR, HOMA IS, BMI, uric acid and fasting plasma triglycerides. CI, confidence interval.
population study of 973 elderly Finnish patients (7) found that Ala/Ala individuals had considerably higher HDL cholesterol and lower triglyceride concentrations at follow-up than did Pro/Pro and Pro/Ala patients. Obese Pro/Ala and Ala/Ala patients had lower concentrations of HDL cholesterol and a trend toward higher concentrations of triglycerides than did obese Pro/Pro patients (23).

The association between postprandial lipemia and the Pro12Ala sequence variant has a plausible pathophysiological explanation. In vitro, the Ala12 variant of the PPARG gene has been less effective at activating target genes (7, 24), one of which is the lipoprotein lipase (LPL) gene (25). Lipoprotein lipase hydrolyzes triglycerides in circulating chylomicrons and VLDLs, releasing fatty acids, chylomicron remnants, and LDL cholesterol. Similar alterations in the blood lipid profile (increased triglycerides and decreased HDL) are seen in heterozygous lipoprotein lipase deficiency (26), an effect that is more pronounced in obese individuals (27). In addition, a PPARG agonist, rosiglitazone, has recently been reported to decrease postprandial lipemia in diabetic persons (28).

In our study, we also found that the association between postprandial lipemia and the Pro12Ala sequence variant was detected only in patients with the non-E3/E3 APOE genotype. Associations between apolipoproteins E2 and E4 and postprandial lipemia have been reported by many authors, and our group has previously shown that patients with the metabolic syndrome and a non-E3/E3 APOE genotype have a 6-fold greater risk of having postprandial lipemia than patients with the E3/E3 genotype (13). We now add to this finding by showing that non-E3/E3 patients with the metabolic syndrome who also have the Ala12 sequence variant have a substantially higher risk for postprandial lipemia, although this Pro12Ala sequence variant was not associated with postprandial lipemia in the E3/E3 patients. The APOE genotype plays an important role in the regulation of lipid metabolism. Apolipoprotein E (APOE) is a component of chylomicron remnants, VLDLs, and intermediate-density lipoproteins, and it plays critical protective roles in atherosclerosis, because recognition of APOE by LDL receptors facilitates hepatic uptake of lipoprotein remnants (29–30).

A previous study showed that PPARG produces an important reduction in the efflux of cholesterol in macrophages by inhibiting the expression of APOE and BACA1 (30). This Pro12Ala variant, which has a low capacity for activating target genes, might also affect the expression of APOE, but its clinical effects would be appreciable only in persons with the E2 and E4 genotypes who are less efficient at clearing lipoprotein remnants. Our study suggests a close cumulative effect of these 2 genes and the manifestation of postprandial lipemia. Others have also shown an interaction between APOE4 and other sequence variants of PPARG, such as C161T, and the risk for coronary heart disease (31).

Our study had several limitations, among which was the small sample size. Another limitation was the lack of use of a glucose clamp to calculate the insulin resistance index; we used the HOMA-IR, which is a well-established approximation. Furthermore, the study included only persons with the metabolic syndrome, because previous studies by our group have shown that persons without the metabolic syndrome do not have a >1.70 mmol/L difference in postprandial hypertriglyceridemia after a fat overload.

In conclusion, the Pro12Ala sequence variant together with a non-E3/E3 APOE genotype is associated with a high risk for postprandial lipemia in patients with the metabolic syndrome, suggesting a close association between these 2 genes in the regulation of the clearance of lipoprotein remnants.

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References


