

ARTICLE

# Effect of the *APOC3* Sst I SNP on fasting triglyceride levels in men heterozygous for the *LPL* P207L deficiency

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Lipoprotein lipase (LPL) plays a major role in triglyceride (TG)-rich lipoprotein catabolism. A mutation at codon 207 (P207L) in the exon 5 of the *LPL* gene has been associated with 50% reduction in postheparin plasma LPL activity and significant increase in plasma TG levels in heterozygous individuals with low HDL. However, heterogeneity in fasting TG concentrations among these carriers suggests that other factors may be involved in the expression of this hypertriglyceridemic state. Indeed, previous studies have shown that the rare S2 allele of the *APOC3* Sst I polymorphism was associated with higher concentrations of TG levels in noncarriers of *LPL* defect. Therefore, we investigated the association of the *APOC3* Sst I variant on fasting lipoprotein–lipid levels in a sample of 35 heterozygous men bearing the *LPL* P207L mutation. Genetic association analyses were performed using the two-genotype groups S1/S1 and S1/S2. The genotype S1/S2 group was characterized by greater plasma cholesterol (plasma-C,  $P=0.02$ ), plasma-TG ( $P=0.04$ ), very low-density lipoproteins (VLDL)-C ( $P=0.004$ ), VLDL-TG ( $P=0.01$ ), VLDL-apolipoprotein B (apoB) ( $P=0.001$ ) levels and cholesterol/HDL-C ratio ( $P=0.008$ ), as well as lower VLDL-TG/VLDL-apoB ratio compared to the S1/S1 genotype group. These results support an exacerbating effect of the *APOC3* Sst I single-nucleotide polymorphism on fasting TG levels since a large number of smaller VLDL particles are observed in *LPL*-deficient men bearing the *APOC3* S2 allele.

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## Introduction

Lipoprotein lipase (LPL; E.C. 3.3.1.34) is a homodimeric enzyme that hydrolyses triglycerides (TG) transported by chylomicrons and very low-density lipoproteins (VLDL). Several mutations in the coding region of the *LPL* gene have been identified, some of these resulting in complete

loss of postheparin LPL activity, which is clinically described as familial *LPL* deficiency.<sup>1</sup> The missense mutation at codon 207 (P207L) located in the exon 5 of the *LPL* gene leads to a complete or a 50% loss of plasma postheparin LPL activity in homozygous and heterozygous, respectively.<sup>2</sup> This mutation is the most prevalent *LPL* genetic defect that leads to *LPL* deficiency in the French-Canadian population since it is present in approximately 70% of homozygotes.<sup>3</sup> In carriers, this mutation causes an altered lipoprotein profile characterized by slight to moderate elevation of fasting TG, low HDL cholesterol and the presence of small dense LDL particles, but normal apolipoprotein B (apoB) levels.<sup>2</sup> However, the

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heterogeneous expression in TG levels among carriers of the *LPL* P207L mutation suggests that several factors may be involved in the phenotypic expression of this dyslipidemic state. In this regard, moderately obese carriers have TG levels that are partly explained by hyperinsulinemia (~8% of the total variance) or abdominal obesity (~4% of the total variance).<sup>4</sup> However, an important fraction of the total variance of TG levels (~64%) is not explained by the *LPL* mutations that lead to *LPL* deficiency.

The apolipoprotein CIII (apoC-III), synthesized by the liver, is a constituent of chylomicrons, VLDL and HDL,<sup>5</sup> and plays a central role in TG metabolism as a noncompetitive inhibitor of plasma LPL activity.<sup>6</sup> The apolipoprotein E (apoE) synthesized also by the liver is a constituent of chylomicrons and VLDL and plays a central role in cholesterol and TG metabolism.<sup>7</sup> The metabolism of remnant lipoproteins is influenced by three apoE isoforms (apoE2, apoE3 and apoE4) translated from the respective  $\epsilon 2$ ,  $\epsilon 3$  and  $\epsilon 4$  alleles.<sup>8</sup> Several association studies with TG have reported some gene–gene interaction effects between *LPL* and *APOE* genes as well as between *LPL* polymorphisms and *APOC3* genotypes.<sup>9</sup>

A single-nucleotide polymorphism (SNP) located in the 3'-untranslated region of the *APOC3* gene, the C3238G also called Sst I, has been associated with HTG<sup>10</sup> and with elevated plasma apoC-III and TG levels.<sup>11</sup> A combined effect of hyperinsulinemia and the presence of the S2 allele of the *APOC3* Sst I SNP have been associated with the expression of hyperlipidemia in *APOE*  $\epsilon 2$  homozygotes.<sup>12</sup> Two *LPL* deficiency mutations (G188E and P207L) have also been shown to induce the expression of type III dyslipidemia in all subjects carrying the  $\epsilon 2/\epsilon 2$  genotype of the *APOE* gene. A recent report has demonstrated that the hypertriglyceridemia observed in mice carriers of apoE2 requires the carboxy-terminal residues of the apoE2, which impairs the interaction between the LDL receptor and the apoE-enriched lipoprotein remnants.<sup>13</sup> In the Chinese population, the combination of obesity with the presence of the S2 allele of the *APOC3* Sst I SNP and the homozygosity for the *LPL* Hind III variant has more predictive value for hypertriglyceridemia than any single factor.<sup>14</sup> Thus, the principal aim of this study was to investigate whether the *APOC3* Sst I polymorphism may modulate fasting lipoprotein–lipid levels in men bearing one allele of the P207L *LPL* deficiency mutation. Also, we evaluated whether this SNP could affect the relationship between TG concentrations and obesity-related phenotypes.

## Materials and methods

### Subjects

A total of 35 men heterozygous for the *LPL* P207L mutation were recruited among patients attending our lipid clinic. All subjects were screened for other common *LPL* muta-

tions (D9N, G188E and N291S) known to be present in Québec. None of them were carriers of these *LPL* gene mutations. All subjects were under their usual diet. The whole study has been approved by the ethic committee of the CHUQ. Written informed consent was approved by the ethic committee of the CHUQ and was obtained from each participant.

### Phenotype measurements

**Anthropometric measurements** Body weight, height as well as waist and hip circumferences were measured using standard procedures,<sup>15</sup> and the waist-to-hip ratio (WHR) was derived. The body mass index (BMI) was calculated as weight (kg)/height (m)<sup>2</sup>.

**Fasting plasma lipoprotein concentrations** Blood samples were collected from the antecubital vein into vacutainer tubes containing EDTA. Samples were taken in the morning after at least a 12-h overnight fast while subjects were in the supine position. Blood was drawn and prepared according to a standard protocol. Cholesterol and TG levels were determined in plasma and lipoprotein fractions by enzymatic methods (Randox Co., Crumlin, UK) using an RA-500 analyzer (Bayer Corporation Inc., Tarrytown, NY, USA), as described previously.<sup>16</sup> The chylomicron fraction ( $d = 0.95$  g/ml) was separated from plasma by ultracentrifugation (30 000 r.p.m., 30 min). Then, plasma VLDL ( $d < 1.006$  g/ml) was isolated by ultracentrifugation. In the infranatant, the HDL fraction was obtained after precipitation of apoB containing lipoproteins ( $d > 1.006$  g/ml) with heparin and  $MnCl_2$ .<sup>17</sup> Cholesterol and TG levels in the chylomicron fraction were obtained by the following computation: levels in total plasma minus delipidized plasma. TG and cholesterol levels were measured in total plasma, delipidized plasma, VLDL and HDL fractions. Cholesterol and TG levels in the infranatant fraction measured before and after the precipitation step were used for LDL estimation. apoB concentrations were measured by the rocket immunoelectrophoretic method of Laurel<sup>18</sup> as described previously<sup>16</sup> in the plasma and infranatant fraction ( $d > 1.006$  g/l). VLDL-apoB concentrations were computed using the following formula: plasma-apoB minus infranatant-apoB concentrations.

### Genotype determination

The *LPL* P207L mutation was assessed using a mismatch PCR-based approach method followed by digestion with *DdeI* restriction enzyme.<sup>19</sup> The *APOC3* Sst I polymorphism and the *APOE* genotype were assessed with the PCR technique followed by digestion with the *SstI*<sup>10</sup> and *HhaI*<sup>20</sup> restriction enzymes, respectively. *LPL* D9N, G188E and N291S mutations were assessed using *AvaII* and *RsaI* restriction enzyme as described previously.<sup>21,22</sup>

### Statistical analyses

All statistical analyses were performed using StatView (SAS Institute Inc.). Two genotype groups were considered: carriers (genotype S1/S2) and noncarriers (genotype S1/S1) of the APOC3 S2 allele. Associations between the APOC3 Sst I polymorphism and each phenotype were investigated using ANCOVA (general linear model) procedure. Pearson's correlation coefficients were calculated between fasting lipid profile and anthropometric measures to determine whether these relationships were different according to the APOC3 Sst I SNP. A Fisher z-test was performed to test whether these correlation coefficients were different in carriers and noncarriers of the S2 allele. Finally, a set of multivariate analysis was performed in LPL-deficient men (ANCOVA) and in men bearing or not the APOC3 S2 allele (ANOVA) to determine the percent contribution of age, BMI and WHR on VLDL-TG levels and the VLDL-TG/VLDL-apoB ratio.

### Results

Men bearing the LPL P207L mutation were between 21 and 68 years old (mean age  $\pm$ SD: 41.6  $\pm$  10.1 years) with a BMI ranging from 20.2 to 34.8 kg/m<sup>2</sup> (mean BMI  $\pm$ SD: 27.8  $\pm$  3.6 kg/m<sup>2</sup>). Subjects exhibited normal (0.74 mmol/l) to elevated (27.5 mmol/l) plasma-TG concentrations (mean  $\pm$ SD: 7.47  $\pm$  6.64 mmol/l) as well as low HDL-C (mean  $\pm$ SD: 0.66  $\pm$  0.17 mmol/l) ranging from 0.42 to 1.1 mmol/l. Furthermore, patients presented near normal plasma-apoB concentration (mean  $\pm$ SD: 1.17  $\pm$  0.43 g/l)

ranging from 0.69 to 2.8 g/l. The S2 allele frequency of the APOC3 gene was relatively high (0.2) and was significantly different to the frequency already observed among French-Canadian in the metropolitan Québec city area (0.081) ( $P < 0.005$ ).<sup>23</sup> The APOE genotype frequency was similar in the APOC3 S2 allele carriers (E2/E2 = 0.07, E2/E3 = 0.36, E2/E4 = 0.07, E3/E3 = 0.22, E3/E4 = 0.14, E4/E4 = 0.14) and noncarriers (E2/E2 = 0.05, E2/E3 = 0.29, E2/E4 = 0.05, E3/E3 = 0.33, E3/E4 = 0.19, E4/E4 = 0.09) ( $\chi^2 = 0.997$ , ddl = 5,  $P = 0.96$ ).

Association analyses showed that the APOC3 Sst I SNP was not associated with any anthropometric phenotype in men heterozygous for the LPL deficiency (Table 1). However, several significant associations were observed between the APOC3 Sst I SNP and fasting plasma lipoprotein-lipid levels measured in men bearing the LPL P207L mutation (Table 2). Carriers of the S2 allele had greater

**Table 1** Anthropometric measurements in men bearing the LPL P207L mutation classified on the basis of APOC3 Sst I SNP

Variables	Sst I polymorphism		P-value
	S1/S1	S1/S2	
Number of subjects	21	14	
Age (years)	39 $\pm$ 9	43 $\pm$ 11	NS
Body weight (kg)	80.5 $\pm$ 13.2	79 $\pm$ 12.6	NS
BMI (kg/m <sup>2</sup> )	27.9 $\pm$ 3.8	27.8 $\pm$ 3.5	NS
Waist circumference (cm)	94.7 $\pm$ 10.6	92.1 $\pm$ 7.8	NS
WHR	0.95 $\pm$ 0.08	0.93 $\pm$ 0.05	NS

Values are mean  $\pm$  SD, NS = not significant ( $P > 0.05$ ).

**Table 2** Fasting lipid profile in men carrying the LPL P207L mutation classified on the basis of APOC3 Sst I SNP

Variables	Sst I polymorphism		P-value
	S1/S1	S1/S2	
<b>Cholesterol (mmol/l)</b>			
Plasma	5.62 $\pm$ 1.78 (21)	8.04 $\pm$ 3.82 (14)	0.0162
Chylomicrons	1.89 $\pm$ 1.99 (9)	4.99 $\pm$ 4.80 (6)	NS
VLDL	1.59 $\pm$ 1.10 (21)	2.96 $\pm$ 1.51 (14)	0.0040
LDL	2.53 $\pm$ 1.48 (21)	2.31 $\pm$ 1.21 (14)	NS
HDL	0.69 $\pm$ 0.18 (21)	0.63 $\pm$ 0.15 (14)	NS
Plasma-C/HDL-C	8.5 $\pm$ 2.9 (21)	13.8 $\pm$ 7.7 (14)	0.0077
<b>TG (mmol/l)</b>			
Plasma	5.58 $\pm$ 5.01 (21)	10.31 $\pm$ 7.90 (14)	0.0370
Chylomicrons	4.95 $\pm$ 6.22 (9)	10.20 $\pm$ 9.23 (6)	NS
VLDL	2.72 $\pm$ 2.11 (21)	4.99 $\pm$ 3.06 (14)	0.0138
LDL	0.40 $\pm$ 0.15 (21)	0.55 $\pm$ 0.15 (14)	0.0052
HDL	0.34 $\pm$ 0.20 (21)	0.41 $\pm$ 0.13 (14)	NS
<b>apoB (g/l)</b>			
Plasma	1.06 $\pm$ 0.33 (21)	1.33 $\pm$ 0.51 (14)	NS
VLDL	0.22 $\pm$ 0.12 (21)	0.40 $\pm$ 0.19 (14)	0.0014
LDL	0.84 $\pm$ 0.32 (21)	0.94 $\pm$ 0.44 (14)	NS
LDL-C/LDL-apoB (mmol/g)	2.87 $\pm$ 0.67 (21)	2.57 $\pm$ 0.84 (14)	NS
VLDL-TG/VLDL-apoB (mmol/g)	2.38 $\pm$ 1.07 (21)	1.63 $\pm$ 1.03 (14)	0.0481

Values are mean  $\pm$  SD (number of subjects), NS = not significant ( $P > 0.05$ ).

concentrations of cholesterol in plasma (+43%,  $P=0.02$ ) and in VLDL particles (+86%,  $P=0.004$ ) as well as greater TG content in plasma (+85%,  $P=0.04$ ), VLDL (+83%,  $P=0.01$ ) and LDL (+37%,  $P=0.005$ ) particles compared to S1 homozygous subjects. The S2 allele was also associated with elevated apoB concentrations only in VLDL particles (+82%,  $P=0.001$ ) and a greater plasma-C/HDL-C ratio (61%,  $P=0.008$ ). The VLDL-TG/VLDL-apoB ratio was smaller in S2 carriers (-21%,  $P=0.05$ ) than in S1 homozygous men. Testing only the effect of the APOC3 Sst I polymorphism on different lipid variables, it was accounting for by an important portion of the plasma-C (16%), VLDL-C (22%), plasma-TG (12%), VLDL-TG (17%), VLDL-apoB (27%) and plasma-C/HDL-C (20%) and VLDL-TG/VLDL-apoB (11%) ratios total variance.

Using all lipid traits that presented significant association (Table 2), correlations have been found between weight, BMI and waist circumference with VLDL-TG concentrations in men bearing the LPL P202L mutation and the APOC3 S2 allele (Table 3). In the same genotype group, only the BMI and the waist circumference were significantly correlated with the VLDL-TG/VLDL-apoB ratio. According to the Fisher z-test, these correlations differ significantly from those obtained in noncarriers of the S2 allele, except for the correlation between weight and the VLDL-TG/VLDL-apoB ratio. In men heterozygous for the S1 allele, no statistical significant correlation was observed.

A set of multivariate analyses was performed in the attempt to better understand the contribution of age, anthropometric indexes (BMI and WHR) and APOC3 Sst I SNP to the total variance of VLDL-TG levels and the VLDL-TG/VLDL-apoB ratio (Table 4). In LPL-deficient men, the

**Table 3** Correlation between anthropometric measures and fasting VLDL-TG concentration and VLDL-TG/VLDL-apoB ratio in men carrying the LPL P207L mutation separated on the basis of the APOC3 Sst I polymorphism

	Sst I polymorphism		
	S1/S1 (21)	S1/S2 (14)	P-value
<b>VLDL-TG</b>			
Weight (kg)	0.25	0.65**	<0.001
BMI (kg/m <sup>2</sup> )	0.22	0.69***	<0.001
Waist circumference (cm)	0.29	0.69***	<0.001
WHR	0.27	0.18	NS
<b>VLDL-TG/VLDL-apoB</b>			
Weight (kg)	0.18	0.53	NS
BMI (kg/m <sup>2</sup> )	0.37	0.54*	NS
Waist circumference (cm)	0.25	0.75***	0.002
WHR	0.20	0.49	NS

\* $P<0.05$  the Spearman correlation coefficient, \*\* $P<0.01$  the Spearman correlation coefficient, \*\*\* $P<0.005$  the Spearman correlation coefficient, NS = not significant ( $P>0.05$ ). P-values are obtained following the Fisher z-test to determine whether these correlations were different between genotypic groups.

most important factors contributing to these plasma lipid traits were the BMI and the APOC3 Sst I SNP. However, these effects did not reach the significance level, and the age and the WHR had null contributions. Performing the same analyses separately in each APOC3 genotype groups, we observed that in the APOC3 S1/S1 genotype group, all the independent variables tested had no significant contribution to either VLDL-TG levels or the VLDL-TG/VLDL-apoB ratio. In contrast, in the APOC3 S1/S2 genotype group, we found a large contribution of BMI ( $R^2=46%$ ,  $P=0.01$  and  $39%$ ,  $P=0.02$ ) to the VLDL-TG levels and the VLDL-TG/VLDL-apoB ratio, respectively. Finally, adding the APOE genotype groups such as E2 (E2/E2 + E2/E3 and E2/E4), E3 (E3/E3) and E4 (E3/E4 and E4/E4) carriers in the multivariate analysis had no significant effect on the results.

## Discussion

Previous studies on LPL deficiency have shown that patients with this genetic defect exhibited a broad heterogeneity in their LPL mass and activity.<sup>2</sup> The partial loss of LPL activity in the heterozygous state induces significant alterations in TG-rich lipoprotein metabolism. The partly active plasma LPL results in a delayed hydrolysis of TG-rich lipoproteins. For instance, as compared to normal subjects with a chylomicrons clearance time of 5–10 min and a

**Table 4** Percentage contribution of age, BMI, WHR and APOC3 Sst I SNP in LPL P207 deficiency on VLDL-TG levels and the VLDL-TG/VLDL-apoB ratio

	VLDL-TG (%)	VLDL-TG/VLDL-apoB (%)
<b>All subjects</b>		
Unexplained	69	83
Age (years)	<1	<1
BMI (kg/m <sup>2</sup> )	12	6
WHR	<1	<1
APOC3 Sst I SNP	18	11
<b>S1/S1 carriers</b>		
Unexplained	94	98
Age (years)	1	<1
BMI (kg/m <sup>2</sup> )	2	<1
WHR	3	<1
<b>S1/S2 carriers</b>		
Unexplained	48	53
Age (years)	5	6
BMI (kg/m <sup>2</sup> )	46*	39*
WHR	<1	2

Multivariate analysis using age, BMI, WHR and APOC3 Sst I SNP to determine their influence on VLDL-TG levels and the VLDL-TG/VLDL-apoB ratio. Analyses were also performed separately in men carriers of the S1/S1 or the S1/S2 genotypes.

\*Only percent contribution of BMI on the VLDL-TG levels ( $P=0.01$ ) and the VLDL-TG/VLDL-apoB ratio ( $P=0.02$ ) in men bearing the S1/S2 genotype were significant.

VLDL clearance of 15 min to 1 h, heterozygous *LPL*-deficient patients are characterized by delayed catabolism of their TG-rich lipoproteins. Thus, in *LPL*-deficient patients, these lipoprotein profile may induce the production of atherogenic particles,<sup>2</sup> increasing their risk to develop cardiovascular diseases.

In the present study, plasma-TG levels were strongly elevated in men bearing the S2 allele, reflecting an exacerbating effect of the *APOC3* Sst I SNP on the delayed TG catabolism. Indeed, an elevated VLDL-TG level is caused by a significant increase in the number of VLDL particles as shown by the VLDL-apoB values that are greater in S2 allele carriers. Moreover, the lower VLDL-TG/VLDL-apoB ratio of men bearing the S2 allele demonstrated that VLDL particles tended to be smaller than those from S1/S1 carriers.

In *LPL* deficiency, it has been shown that the cholesteryl esters transfer from HDL to VLDL particles by the cholesteryl ester transfer protein (CETP) was increased.<sup>24</sup> However, enhanced cholesteryl ester transfer observed in *LPL* deficiency seems rather to be the result of altered HDL and VLDL particle compositions and concentrations than enhanced CETP enzyme activity.<sup>24</sup> The additional presence of the *APOC3* S2 allele in *LPL* deficiency could favor subtract availability for CETP by diminished VLDL-TG catabolism and then provide more opportunities to CETP to transfer TG molecules from TG-rich lipoproteins toward HDL particles in exchange of esterified cholesterol. In the present study, the higher VLDL-C, LDL-TG and a tendency for greater HDL-TG levels in men bearing the S2 allele could reflect an increased CETP-induced transfer of lipids between lipoprotein particles. The smaller VLDL particles (lower VLDL-TG/VLDL-apoB ratio) may also reflect this CETP transfer activity. Finally, *in vitro* studies have shown that apoC-III inhibits lecithin-cholesterol acyltransferase activity<sup>25</sup> but activates CETP activity.<sup>26</sup> Unfortunately, our study did not allow us to address the role of the CETP in our observations.

Since its first description in 1983,<sup>27</sup> the rare allele (S2 allele) of Sst I SNP located in the 3'-untranslated region of the *APOC3* gene was associated with elevated plasma apoC-III concentrations in healthy subjects<sup>10</sup> and in relatives of patients developing familial combined hyperlipidemia.<sup>28</sup> This *APOC3* SNP has been shown to be in linkage disequilibrium with the two T-455C and C-482T SNPs located in the insulin response element (IRE) of the promoter of the gene.<sup>10</sup> These IRE SNPs were proposed to induce a loss of an insulin downregulation mechanism and may represent a major contributing factor to the development of hypertriglyceridemia, presumably by elevating plasma apoC-III levels.<sup>10</sup> Recently, it was reported that only women carrying the mutant allele of either T-455C or C-482T SNPs exhibited an insulin-dependent increase in TG-rich lipoprotein levels, while functional alleles did not.<sup>29</sup> However, two studies failed to demonstrate any

association between the IRE SNPs and plasma apoC-III levels or TG levels in a population of Italian school-children<sup>11</sup> as well as in participants of the ARIC study.<sup>30</sup> Even if the IRE SNPs were in linkage disequilibrium with the Sst I SNP, most of the results in TG levels were mainly attributable to the Sst I SNP. The molecular mechanisms inducing such elevation of the plasma apoC-III concentration are not already elucidated, but an *APOC3* mRNA-stabilizing effect has been proposed for subjects bearing the S2 allele.<sup>10</sup> Furthermore, a significant 13% increase in plasma apoC-III concentrations has been demonstrated in a large cohort of patients presenting angiographically documented coronary atherosclerosis and bearing the S2 allele of the *APOC3* Sst I SNP.<sup>31</sup> Finally, several reports support strong associations between *APOC3* SNPs and atherogenic lipoprotein profiles<sup>32</sup> or hypertriglyceridemia.<sup>33</sup> Thus, the mechanism by which the S2 allele exacerbates plasma TG profile can result from a greater mRNA stabilization,<sup>10</sup> leading to greater apoC-III production and LPL activity inhibition. Interestingly, the frequency of the S2 genotype seems to be more prevalent in *LPL* P207L-deficient patients from the present study than in a random population representing the metropolitan Québec City area.<sup>23</sup> However, it is not obvious to consider that patients bearing the *LPL* P207L mutation as well as the *APOC3* S2 allele are those exhibiting a high lipid profile alteration that conduct them to the lipid clinic and this should be one reason that may explain the high *APOC3* S2 allele frequency observed in *LPL* P20L patients.

In the present study, the most intriguing results were observed with VLDL particles. In heterozygous, we can speculate that (1) VLDL particles enriched in apoC-III would remain longer in the blood compartment, increasing their ability to interact with other circulating lipoproteins and favoring apolipoprotein and lipid exchanges mediated by the CETP activity, (2) the elevated apoC-III concentrations in VLDL subclass of the *APOC3* S2 allele carriers<sup>10</sup> is believed to be particularly atherogenic since it would delay the clearance of these particles by inhibiting LPL activity and reducing the clearance of remnants lipoproteins via LDL and VLDL receptors (for review),<sup>5</sup> (3) finally, additive effects may occur as the LPL binding activity (responsible for the TG-rich lipoprotein internalization by LDL and VLDL receptors via the LPL-mediated binding of particles to heparin sulfate proteoglycans) is altered in *LPL*-deficient heterozygotes since they lack the carboxyl-terminal part of the LPL protein (at least in half of the protein) that is essential for the binding activity.<sup>34</sup> As observed in the present association analysis, the relative risk of having an atherogenic VLDL profile is considerably increased in patients bearing the S2 allele since they exhibited a greater number of small VLDL particles. Furthermore, correlation data provided us with some evidence that the concentrations of VLDL-TG are strongly correlated with the waist circumference only in carriers of

the *APOC3* S2 allele. Taking into account other variables such as age- and obesity-related phenotype, we observed that BMI appeared to contribute strongly and significantly on the VLDL-TG levels and the VLDL-TG/VLDL-apoB ratio only in men bearing the S2 allele. We should point out also that, in our models, the *APOE* genotype did not contribute to the VLDL-TG levels. All together, management of abdominal obesity (waist circumference) as well as the presence of the *APOC3* S2 allele are suggested as important factors that must be taken into account for efficient clinical control of TG levels in *LPL* deficiency patients. Furthermore, these results are of great importance regarding the fibrate treatment of heterozygous *LPL* deficiency patients bearing the S2 allele. Indeed, fibrate treatment has been shown to inhibit the hepatic expression of the *APOC3* gene,<sup>35</sup> the impact of fibrate on the expression of this gene has not been investigated in cell models bearing the *APOC3* Sst I SNP. Thus, it would be interesting to verify whether the presence of this mutation may have an impact on the *APOC3* gene expression response to fibrate as compared to the wild-type allele.

In conclusion, although elevated in subjects bearing the *LPL* P207L mutation, fasting TG levels were higher in men carrying the S2 allele of the *APOC3* gene. This was principally expressed by a greater amount of small VLDL particles. Also, BMI is of great importance in the heterogeneity TG levels observed in heterozygous for the *LPL* P207L mutation and should be managed carefully in patients bearing the S2 allele. The *APOC3* Sst I SNP should be used in clinical genetic screening of *LPL*-deficient men.

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