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ORIGINAL ARTICLE

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Effect of liver fatty acid binding protein (FABP) T94A missense mutation on plasma lipoprotein responsiveness to treatment with fenofibrate

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Abstract Fenofibrate, a peroxisome proliferated activated receptor alpha (PPARα) agonist, has been shown to decrease plasma triglyceride (TG) and increase plasma high-density lipoprotein (HDL) cholesterol levels despite a large interindividual variation in the response. Fenofibrate-activated PPARα binds to a DNA sequence element termed PPAR response element (PPRE) present in regulatory regions of target genes. A PPRE has been identified in the proximal 5′ flanking region of the gene encoding the liver fatty acid binding protein (LFABP). LFABP is a small cytosolic protein of 14 kDa present in the liver and the intestine and is a member of the superfamily of the fatty acid binding proteins (FABPs). FABPs play a role in the solubilization of long-chain fatty acids (LCFAs) and their CoA-ester to various intracellular organelles. FABPs serves as intracellular acceptors of LCFAs, and they may also have an impact in ligand-dependent transactivation of PPARs in trafficking LCFAs to the nucleus. Since PPARs are known to regulate the transcription of many genes involved in lipid metabolism, the importance of LFABP in fatty acid uptake has to be considered. The aim of this study was to verify whether genetic variations in the LFABP gene may impact on plasma lipoprotein/lipid levels in the fasting state as well as on the response to a lipid-lowering therapy with fenofibrate on plasma lipids and obesity variables. We also wanted to verify whether the presence of the PPARα L162V mutation interacts with genetic variants in LFABP gene. To achieve this goal, we first determined the genomic structure of the human LFABP gene and then designed intronic primers to sequence the coding regions, all exon-intron splicing boundaries, and the promoter region of the gene in 24 patients showing divergent plasma lipoprotein/lipid response to fenofibrate. Sequence analysis revealed the presence of a T94A missense mutation in exon 3. Interspecies comparison revealed that threonine 94 is conserved among species. We subsequently screened another sample of 130 French Canadian subjects treated with fenofibrate for the presence of the LFABP T94A mutation. Carriers of the A94 allele were at increased risk to exhibit plasma TG levels above 2.00 mmol/l after treatment with fenofibrate [2.75 (1.03–7.34); OR 95% confidence interval (CI)]. In addition, carriers of the A94 allele were characterized by higher baseline plasma-free fatty acid levels (FFA) ($p = 0.01$) and by a lower body mass index (BMI) ($p = 0.05$) and waist circumference ($p = 0.005$) than T94 homozygotes. Moreover, PPARα L162V and LFABP T94A showed to have a synergistic effect on BMI (p interaction = 0.03). These results suggest that the LFABP T94A missense mutation could influence obesity indices as well as the risk to exhibit residual hypertriglyceridmia following a lipid-lowering therapy with fenofibrate.

Keywords Fibrates · Fenofibrate · Fatty acid binding proteins · PPAR · Lipid response · Blood lipids · Triglyceride

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Introduction

In hypertriglyceridemic subjects of all causes, except those with type 1 familial hyperchylomicronemia, fibrates are

excellent therapeutic choices when pharmacological treatment is required. Fenofibrate, a peroxisome proliferated activated receptor alpha (PPARα) agonist, has been shown to significantly increase plasma high-density lipoprotein cholesterol (HDL-C) concentrations while decreasing plasma triglyceride (TG) levels and has a marginal impact on plasma low-density lipoprotein cholesterol (LDL-C) levels (Zhu et al. 2003). Fenofibrate regulates gene expression by acting as a ligand for PPARα, which subsequently binds to a DNA sequence element termed PPAR response element (PPRE) present in the regulatory region of target genes (Forman et al. 1996).

A PPRE has been identified in the 5′ flanking region of the gene encoding the liver fatty acid binding protein (LFABP) (Issemann et al. 1992; Schoonjans et al. 1997). In vitro, this PPRE proved to be efficient in permitting the binding of PPARs (α, γ, δ) to the LFABP gene (Brandes et al. 1990; Carlsson et al. 2001). LFABP is a small cytosolic protein (14 kDa) present in the liver as well as in the intestine and is a member of the superfamily of the fatty acid binding proteins (FABPs). FABPs play a role in solubilization of long-chain fatty acids (LCFAs) and their CoA-ester to various intracellular organelles (Storch et al. 2000; Zimmerman et al. 2002). LFABP serves as an intracellular acceptor of LCFAs, which can enhance both the cellular uptake and the intracellular diffusion of these fatty acids. FABPs are also suspected to play a role in mitochondrial and peroxisomal β-oxidation, gene transcription and cell growth/differentiation, as well as in incorporation of fatty acids into TG (Veerkamp and van Moerkerk 1993). This later function of LFABP may be reflected in the observation that the protein stimulates enzyme activities and processes that are fatty-acid dependent, including the biosynthesis of phospholipids and TG (Bass 1988; Prows et al. 1995). LFABP may thus have an important role in ligand-dependent transactivation of PPARs in trafficking LCFAs to the nucleus (Wolfrum et al. 2001). The latter can be done via direct protein–protein interaction with PPARs in the nucleus (Lawrence et al. 2000). The importance of LFABP in fatty-acid uptake deserves attention, since PPARs are known to be implicated in activation and inhibition of many genes regulating lipid metabolism. The objective of the present study was to verify whether genetic variations in the LFABP gene may be associated with plasma lipid levels in the fasting state as well as in response to a lipid-lowering therapy with fenofibrate. We also wanted to verify whether the PPARα L162V mutation known to influence plasma lipoprotein/lipid responsiveness to fibric acid derivatives (Bosse et al. 2002; Brisson et al. 2002) interacts with LFABP genetic variants to modulate plasma lipoprotein/lipid levels.

Methods

Study sample Subjects were all hypertriglyceridemic (fasting TG >2.0 mmol/l) and were followed at the Chicoutimi Hospital lipid clinic (Québec, Canada). All 130 subjects underwent a 3-month lipid-lowering treatment with fenofibrate (200 mg/daily). Mean age was of 49.6 ± 9.8 (range 20–72) years. Subjects with hyperchylomicronemia (familial type I dyslipidemia) or subjects taking hypocholesterolemic drugs and/or drugs affecting plasma glucose levels, including PPARγ agonists, were excluded. During treatment, every subject followed dietary counseling from a dietitian and were assigned to the American Heart Association phase 1 diet. They also gave informed consent to participate in a study on genetic determinants of type 2 diabetes mellitus or coronary artery disease and were assigned a code to classify all clinical data (Gaudet et al. 1999a). This study combined a genome-wide scan and candidate gene strategies and received the approval of the ethics committee (Gaudet 1999a; Gaudet et al. 2000; Vohl et al. 2000). Body mass index (BMI) was calculated (kilograms per meter squared) and waist circumference measured according to the procedure recommended by the Airlie Conference (Standardization of Anthropometric Measurements 1988; Gaudet et al. 1999a; Gaudet et al. 1999b; Vohl et al. 2000). Blood samples were taken from the antecubital vein into vacutainer tubes containing EDTA after a 12-h overnight fast. LDL ($d > 1.006$ g/ml) were precipitated with heparin, and $MnCl_2$ and HDL were obtained from the infranatant (Standardization of Anthropometric Measurements 1988; Gaudet et al. 1999a; Gaudet et al. 1999b; Havel et al. 1955; Vohl et al. 2000). Plasma glucose, cholesterol, and TG levels were enzymatically measured on a Multiparity Analyser CX7 (Beckman, Fullerton, CA, USA). This study received the approval of the Chicoutimi Hospital ethics committee.

Genomic structure of the LFABP gene In order to design intronic primers for the amplification of each exon, genomic sequences were sought for the intronic regions surrounding all LFABP exons and for the promoter region. To do so, we compared the cDNA (Ac: NM_001443) to the sequence of a clone from GeneBank (Ac: AC118140). Intronic primers were then designed using the Primer 3.0 software available on the Whitehead Institute/MIT Center for Genome Research server (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi).

Polymerase chain reaction (PCR) amplification of LFABP exons and sequencing All exons and exon-intron boundaries were amplified from genomic DNA by use of specific primers derived from all 5′ and 3′ end of intronic sequences (Table 1). The annealing temperature for all pairs was 60°C. Polymerase chain reaction (PCR) conditions were 50 ng genomic DNA, 1.25 U Quiagen *Amplitaq* (Quiagen, Mississauga, Ontario, Canada), 1.5 mmol/l $MgCl_2$, 0.2 mmol/l dNTPs, and 0.4 μmol/l primers in a 50-μl reaction. PCR products

Table 1 Polymerase chain reaction (PCR) primers for genomic amplification of liver fatty acid binding protein (LFABP) exons

Exons	Oligonucleotides	Product size (bp)	Annealing temperature (°C)
1 and promotor	LFABP.R 5′-CCTAAATAGCCACTGCTGGTAGAG-3′ LFABP.L 5′-CATACCTGCATATACACACATTTCG-3′	458	60
2	LFABP.R 5′-GTATCTGTGGGTGGAATTGTGAG-3′ LFABP.L 5′-CCTTTTTATAAATTGACTGGCAAGA-3′	505	60
3	LFABP.R 5′-CAGATACTGACCACAGGAAAGAAGT-3′ LFABP.L 5′-CTTAGGTGTTCCATGCTCATTTCTA-3′	488	60
4	LFABP.R 5′-TTTTGTGTTCAGGGAATTACTCTTC-3′ LFABP.L 5′-GTTTGGATGGTCTTGCTATAACAGT-3′	546	60

were purified (Multiscreen; Millipore, Bedford, MA). Sequencing reactions were performed using BigDye Terminator v3.0 cycle sequencing (ABI Prism, Applied Biosystems, Foster city, CA, USA), and the products were analyzed on ABI 3700 automated DNA sequencer (PE Applied Biosystems). The gel files were processed using the ABI Prism 3700 data collection software applied biosystem version 1.1 and ABI Prism DNA sequencing analysis software (PE Applied Biosystems) then assembled and analyzed using the STADEN pre-Gap4 and Gap4 programs.

Genotyping The LFABP T94A mutation does not alter any restriction site; thus, the mismatch PCR method was performed using primers LFABPex3.L-mismatch (5′-CAGTTGGAAGGTGACAATAAACTGGTGAAA-3′, mismatch is underlined) and LFABPex3.R (5′-ATACTGACCACAGGAAAGAAGTTTGGGG-3′). PCR conditions were as described above, but annealing temperature was of 66°C. PCR products were digested with *HindIII*. Digestion products were electrophoresed through 4% agarose gel and stained with ethidium bromide.

The presence of the L162V mutation in the PPARα gene was determined using a mismatch PCR-restriction fragment-length polymorphism-based method. PCR products were digested with *Hinf I* restriction enzyme, as previously described (Vohl et al. 2000). Apoprotein E (ApoE) genotype was determined by PCR amplification, as previously described (Hixson et al. 1990), followed by digestion with *Hha* I restriction enzyme.

Statistical analyses Plasma lipid concentrations measured before and after treatment with fenofibrate were compared using a paired Student's *t* test. Some variables were $\log_{10}$-transformed because of their skewed distribution. Multilogistic regression models were built in order to evaluate the association between the LFABP T94A genotype and the probability to exhibit plasma TG concentrations, HDL-C, LDL-C, and total-cholesterol (TC)/HDL-C) ratio below or above the therapeutic targets of 2.0, 0.9, 3.0, and 5 mmol/l, respectively, following treatment with fibrates. The cutoffs were established based on primary prevention therapeutic target values of Canadian, European, or NCEP-ATPII guidelines (Prevention of Coronary Heart Disease in Clinical Practice 1998; Cleeman et al. 1997; Fodor et al. 2000). Effects related to age, gender, BMI, alcohol consumption, smoking, TG levels, baseline data of each variables, and the ApoE genotype were considered in this model. In the different analyses, odds ratios (OR) with 95% CI of reaching TG, HDL-C, LDL-C, and TC/HDL-C ratio target values associated with LFABP T94A genotype were reported following fibrate treatment. Analyses of covariance were also conducted to evaluate the mean phenotypic differences at baseline and in response to the treatment therapy between carriers and noncarriers of the LFABP T94A mutation. A 2×2 ANOVA was used to evaluate the possible interaction between LFABP T94A and PPARα L162V genotype on plasma lipid variables. Models were adjusted for age, gender, ApoE genotype, smoking, alcohol, and BMI. A significance level of 5% was employed. Statistical analyses were performed using SAS (SAS institute, 8th version).

Results

Structure of the human LFABP gene

The structural organization of the gene was determined by comparing the cDNA (ac: NM_001443) with the genomic sequence of the gene (Ac: AC118140). All the intron-exon splicing boundaries were confirmed by sequencing of each exon using primers derived from flanking intronic sequences. All 5′ donor and the 3′ acceptor splice sites were found to follow the gt···ag rule. The human LFABP gene spans 5.1 kb. Exon 1 consists of 111 bp and has a 42-bp untranslated region in 5′. Exons 2 and 3 are, respectively, 174 bp and 95 bp long. Exon 4 consists of 114 bp and is terminated by an untranslated 3′ region of 67 bp (Fig. 1).

Identification of genetic variants in the human LFABP

The sequencing of all exons and intron-exon boundaries of 24 subjects who underwent fibrate treatment exhibited the expected lengths, consistent with absence of deletion, duplication, or rearrangement, for all four amplified fragments. A sequence variation was discovered in exon 3. Based on the predicted amino acid

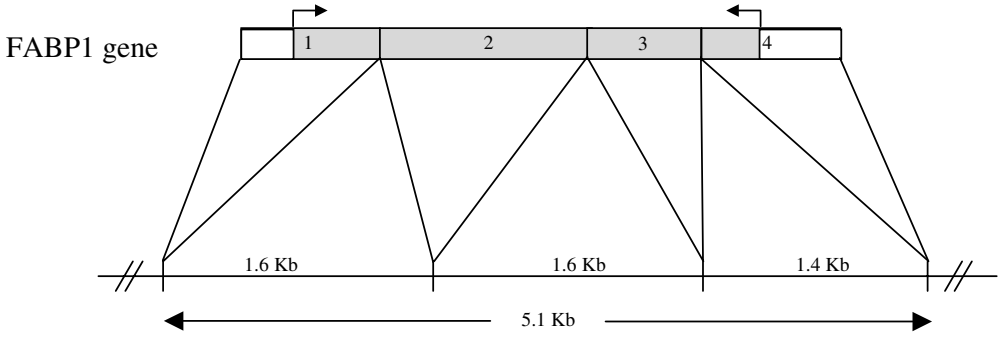

Fig. 1 Schematic representation of the human liver fatty acid binding protein (LFABP) gene. *Upper panel* a representation of the human messenger RNA. The coding region is *shaded*, and *arrows* indicate translation start (met) and stop codon (UAA). The spatial localization of exons within the gene and the intron size is shown in the *lower panel*. The gene spans 5.1 kb of genomic DNA

sequence for this gene, the polymorphism results in a T94A missense mutation. This mutation leads to an A to T change in the first position of codon 94 and corresponds to a substitution of a threonine for an alanine. The threonine at codon 94 (T) is conserved among two species, *Bos Taurus* and *Rattus Norvegicus*, which have, respectively, 81 and 82% homology with the human LFABP gene.

Genotype determination of the LFABP T94A mutation

A rapid screening test was developed to genotype the LFABP T94A mutation. We used a mismatch PCR method using primers LFABPex3.L-mismatch and LFABPex3.R, as described above. The PCR product was designed to contain an *HindIII* restriction site in the presence of the C at position 322, which is the mutated allele. The PCR products of the normal allele yielded to a fragment of 221 bp. The mutant allele's product is cleaved by *HindIII* into fragments of 33 bp and 188 bp. Genotypes obtained by the mismatch PCR approach corresponded to those obtained by sequencing. The reproducibility rate was 100%.

Association between the LFABP T94A missense mutation and plasma lipid concentrations

As shown in Table 2, genotyping of the LFABP T94A mutation resulted in 57 carriers of the A94 allele. The frequency of the A94 mutant allele was 32.3%. The genotype distribution was found to be in Hardy-Weinberg equilibrium (χ^2, $p = 0.92$). No significant association between LFABP T94A and the change (Δ) during treatment with fenofibrate was discovered, even after adjustments for confounding factors including age, gender, ApoE genotype, alcohol, smoking, pretreatment lipid data, and/or BMI (Table 2). Plasma TG tended to be higher for carriers of the A94 allele compared to T94 homozygotes, but these differences did not reach statistical significance. However posttreatment plasma TG levels did reach statistical significance, with carriers of the A94 allele having the highest plasma TG levels

Table 2 Subjects characteristics according to the liver fatty acid binding protein (LFABP) T94A genotype. Values are means ± SD. *BMI* body mass index, *TG* triglyceride, *FFA* plasma-free fatty acids, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *TC* total cholesterol

	T/T (62)	T/A and A/A (68)	ρ value[a]
Age (years)	49.47 ± 1.19 (62)	48.87 ± 1.14 (68)	0.72
Male/female	34/28	47/21	
Body composition			
BMI (kg/m^2)	28.90 ± 7.72 (53)	27.27 ± 7.92 (58)	0.05
ΔBMI (kg/m^2)	−1.61 ± 2.48 (19)	−1.80 ± 2.59 (20)	0.59
Waist girth (cm)	94.80 ± 18.75 (52)	88.87 ± 19.16 (56)	0.005
ΔWaist girth (cm)	−6.23 ± 15.04 (19)	−6.51 ± 15.43 (20)	0.89
Lipid levels			
TG (mmol/l)	8.68 ± 20.75 (53)	12.8 ± 20.70 (52)	0.14
ΔTG (mmol/l)	−5.48 ± 18.93 (53)	−8.34 ± 19.88 (58)	0.87
FFA (mmol/l)	0.53 ± 0.70 (49)	0.69 ± 0.76 (48)	0.01
ΔFFA (mmol/l)	−0.05 ± 0.60 (14)	−0.18 ± 0.66 (15)	0.29
LDL-C (mmol/l)	3.39 ± 3.01 (41)	3.10 ± 2.95 (43)	0.44
ΔLDL-C (mmol/l)	0.07 ± 2.96 (38)	0.35 ± 2.74 (34)	0.36
HDL-C (mmol/l)	0.71 ± 0.50 (50)	0.74 ± 0.52 (56)	0.71
ΔHDL-C (mmol/l)	0.32 ± 0.55 (48)	0.23 ± 0.51 (53)	0.12
TC (mmol/l)	9.16 ± 6.99 (53)	9.95 ± 7.31 (58)	0.20
ΔTC (mmol/l)	−3.90 ± 6.48 (53)	−4.05 ± 6.78 (58)	0.84
Glycerol (mmol/l)	0.08 ± 0.14 (50)	0.09 ± 0.14 (50)	0.26
Δglycerol (mmol/l)	−0.01 ± 0.26 (14)	−0.02 ± 0.35 (19)	0.62
TC/HDL-C	15.94 ± 21.35 (50)	18.09 ± 22.00 (56)	0.36
ΔTC/HDL-C	−12.51 ± 17.04 (48)	−12.08 ± 17.25 (53)	0.32

Δ Change occurs with fenofibrate

[a] Adjusted for age, gender, ApoE, alcohol, smoking, and BMI (except for BMI and waist girth variables)

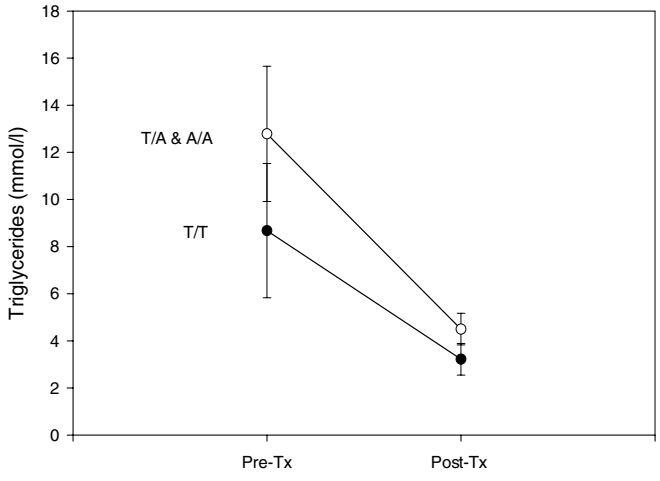

Fig. 2 Plasma triglyceride (TG) concentrations before treatment (pre-Tx) and after treatment (post-Tx) with fibrate, among LFABP T94 HMZ and carriers of liver fatty acid binding protein (LFABP) A94 allele. Data are mean ± SE. All values were adjusted for age, gender, BMI, ApoE, alcohol, and smoking

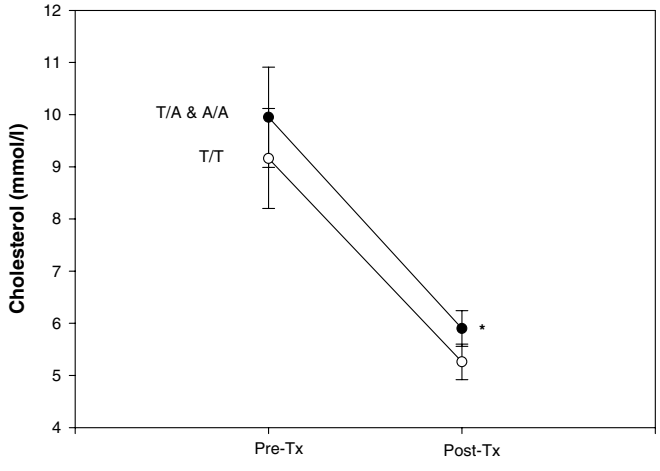

Fig. 3 Plasma cholesterol concentrations before treatment (pre-Tx) and after treatment (post-Tx) with fibrate, among liver fatty acid binding protein (LFABP) T94 HMZ and carriers of the LFABP A94 allele. Data are mean ± SE. All values were adjusted for age, gender, BMI, ApoE genotype, alcohol, and smoking. *Asterisk* is allowed for significant difference

($p = 0.02$) (Fig. 2). Similar results were observed with TC levels, with carriers of the A94 allele exhibiting significantly higher plasma TC levels following treatment with fenofibrate (p value = 0.03, Fig. 3). Moreover, plasma-free fatty acids (FFA) concentrations before treatment were higher in carriers of the A94 allele ($p = 0.01$) (Table 2). Surprisingly, BMI and waist girth were significantly lower in carriers of the A94 allele (Table 2).

Multivariate logistic regression analyses were performed to determine the risk to exhibit elevated plasma TG (> 2.00 mmol/l), LDL-C (> 3.00 mmol/l), HDL-C (> 0.9 mmol/l), TC (> 5.00 mmol/l), TC/HDL-C (> 5.00) following the treatment with fenofibrate, depending on the LFABP T94A genotype. Three models were constructed to test the influence of covariates such as pretreatment lipid data, BMI, and pretreatment TG. We observed that, after adjustments for age, gender, alcohol, smoking, ApoE and baseline TG levels, and/or BMI, carriers of the A94 allele were at increased risk to exhibit posttreatment plasma TG levels above the therapeutic target (OD: 2.75 (1.03–7.34) (Table 3). No other significant difference between genotypes in reaching the target values were observed.

Interaction between LFABP T94A et PPARα L162V

To evaluate the interaction between LFABP T94A and PPARα L162V polymorphisms, subjects were divided into four genotype groups based on the presence or absence of the two variants. No difference was observed between the four genotype groups for waist girth, TG, FFA, LDL-C, HDL-C, TC, and fasting glycerol levels as well as for the TC/HDL-C ratio (Table 4). However, carriers of both mutations tended to have a lower BMI, and the gene-gene interaction effect was found to be significant ($p = 0.03$).

Discussion

The human LFABP gene has been mapped on chromosome 2p11. Using information from GeneBank, we have determined the exon-intron structure of the human LFABP gene. Direct sequencing of the entire coding

Table 3 Multivariate estimation of the risk to exhibit plasma HDL < 0.9 mmol/l, LDL > 3.0 mmol/l, TG > 2.0 mmol/l, TC > 5.0 mmol/l, and TC/HDL-C ratio > 5 for fatty acid binding protein (LFABP) A94 allele carriers following fibrates treatment. All three models included the following covariates: age, gender, alcohol, smoking, data from each covariate before fibrates treatment, and ApoE. The effect of BMI was added to Model 2. TG before fibrates treatment were considered in Model 3. *BMI* body mass index, *HDL* high-density lipoprotein cholesterol, *LDL* low-density lipoprotein cholesterol, *TG* triglycerides, *TC* total cholesterol

Variables	Model 1, OR (95% CI)	Model 2 (model 1 + BMI), OR (95% CI)	Model 3 (Model 2 + TG pretreatment) OR (95% CI)
HDL	1.16 (0.44–3.03)	1.35 (0.50–3.65)	1.42 (0.48–4.20)
LDL	1.32 (0.42–4.18)	1.28 (0.40–4.12)	1.23 (0.34–4.51)
TG	2.02 (0.81–5.04)	2.75 (1.03–7.34)	
TC/HDL	0.90 (0.39–2.06)	1.37 (0.54–3.47)	1.43 (0.55–3.72)

Table 4 Effects of liver fatty acid binding protein (LFABP) T94A, peroxisome proliferated activated receptor alpha (PPARα) L162V and their interaction. Values are means ± SD. Values are adjusted for age, gender, ApoE, alcohol, smoking and BMI (excepted for the BMI and the waist girth variables). *BMI* body mass index, *TG* triglyceride, *FFA* plasma-free fatty acids, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *TC* total cholesterol

	LFABP T94A/PPARα				ρ-Value		
	LFABP T/T PPARα L/L (38)	LFABP T/T PPARα X/V (15)	LFABP X/A PPARα L/L (38)	LFABP A/A PPARα V/V (20)	PPARα L162V	LFABP T94A	Interaction
BMI (kg/m^2)	29.17 ± 7.09 (n = 38)	31.08 ± 5.54 (n = 15)	28.26 ± 7.15 (n = 38)	26.21 ± 5.95 (n = 20)	0.85	0.05	0.03
ΔBMI	−1.80 ± 2. (n = 12)	−0.79 ± 2.14 (n = 7)	−1.74 ± 2.18 (n = 8)	−1.98 ± 2.18 (n = 12)	0.50 (n = 39)	0.59 (n = 39)	0.14
Waist girth	94.80 ± 17.76 (n = 37)	100.05 ± 13.90 (n = 15)	89.78 ± 17.76 (n = 36)	89.24 ± 14.76 (n = 20)	0.32 (n = 108)	0.005 (n = 108)	0.20
ΔWaist girth	−5.51 ± 14.34 (n = 12)	−8.55 ± 12.94 (n = 7)	−7.65 ± 12.42 (n = 8)	−7.25 ± 12.51 (n = 12)	0.62 (n = 39)	0.89 (n = 39)	0.49
Lipid levels							
TG (mmol/l)	7.90 ± 14.24 (n = 38)	8.14 ± 11.15 (n = 15)	7.99 ± 13.84 (n = 33)	9.64 ± 11.81 (n = 19)	0.86 (n = 105)	0.14 (n = 105)	0.86
ΔTG (mmol/l)	−4.48 ± 12.64 (n = 38)	−3.47 ± 15.72 (n = 15)	−3.55 ± 13.19 (n = 38)	−5.15 ± 10.42 (n = 19)	0.39 (n = 105)	0.87 (n = 105)	0.24
FFA (mmol/l)	0.55 ± 0.45 (n = 35)	0.49 ± 0.56 (n = 14)	0.71 ± 0.71 (n = 30)	0.62 ± 0.59 (n = 18)	0.44 (n = 97)	0.01 (n = 97)	0.45
ΔFFA (mmol/l)	0.01 ± 0.06 (n = 10)	0.06 ± 0.70 (n = 4)	−0.02 ± 0.63 (n = 10)	−0.17 ± 0.54 (n = 5)	0.81 (n = 29)	0.29 (n = 29)	0.46
LDL-C (mmol/l)	3.67 ± 2.75 (n = 28)	3.04 ± 2.24 (n = 13)	3.18 ± 2.85 (n = 30)	3.51 ± 2.27 (n = 13)	0.75 (n = 84)	0.44 (n = 84)	0.25
ΔLDL-C (mmol/l)	−0.08 ± 2.81 (n = 27)	0.14 ± 1.89 (n = 11)	0.15 ± 2.54 (n = 23)	0.47 ± 1.99 (n = 11)	0.44 (n = 72)	0.36 (n = 72)	0.90
HDL-C (mmol/l)	0.71 ± 0.47 (n = 35)	0.62 ± 0.35 (n = 15)	0.72 ± 0.44 (n = 30)	0.68 ± 0.40 (n = 20)	0.47 (n = 106)	0.71 (n = 106)	0.67
ΔHDL-C (mmol/l)	0.32 ± 0.52 (n = 34)	0.26 ± 0.37 (n = 14)	0.25 ± 0.47 (n = 34)	0.23 ± 0.44 (n = 19)	0.59 (n = 101)	0.12 (n = 101)	0.72
TC (mmol/l)	9.37 ± 0.51 (n = 38)	8.20 ± 4.03 (n = 15)	9.80 ± 5.18 (n = 38)	8.62 ± 4.38 (n = 20)	0.03 (n = 111)	0.20 (n = 111)	0.92
ΔTC (mmol/l)	−3.84 ± 4.68 (n = 38)	−3.06 ± 3.68 (n = 15)	−3.65 ± 4.30 (n = 38)	−2.55 ± 3.98 (n = 20)	0.10 (n = 111)	(n = 111)	0.79
Glycerol (mmol/l)	0.08 ± 0.12 (n = 35)	0.08 ± 0.08 (n = 15)	0.10 ± 0.11 (n = 32)	0.08 ± 0.08 (n = 18)	0.43 (n = 100)	0.26 (n = 100)	0.76
ΔGlycerol (mmol/l)	0.03 ± 0.25 (n = 10)	0.03 ± 0.20 (n = 4)	−0.001 ± 0.31 (n = 12)	0.03 ± 0.26 (n = 7)	0.62 (n = 33)	0.62 (n = 33)	0.79
TC/HDL-C (mmol/l)	16.48 ± 20.82 (n = 35)	18.05 ± 16.00 (n = 15)	19.92 ± 20.46 (n = 36)	17.78 ± 17.49 (n = 20)	0.68 (n = 108)	0.36 (n = 108)	0.57
ΔTC/HDL-C (mmol/l)	−13.23 ± 16.56 (n = 34)	−14.75 ± 12.50 (n = 14)	−13.70 ± 15.80 (n = 34)	−11.34 ± 13.78 (n = 19)	0.31 (n = 101)	0.32 (n = 101)	0.36

region of the human LFABP gene as well as exon-intron boundaries revealed the presence of an A to T substitution in exon 3. This substitution leads to a T94A missense mutation. Comparison with other species revealed that T94 is well conserved among species like *Rattus Norvegicus* and *Mus Musculus*. This suggests that the threonine 94 could be important to assure the physiological role of the protein.

We investigated the implication of this mutation on the effectiveness of a fenofibrate treatment. Although most current guidelines recognize the contribution of genetic factors to lipid-profile-associated risk, few actually allow integration of genetic information in risk evaluation or individualization of a dyslipidemia treatment plan. As observed in the present study, the pharmacological treatment is effective in hypertriglyceridemia, no matter what the underlying LFABP genotype was. However, this study also demonstrated that the LFABP genotype may affect reaching target values for TG levels. The LFABP T94A mutation was associated with an increased risk of exhibiting residual TG beyond the upper limit of 2.0 mmol/l after 3 months of fenofibrate treatment. This association was significant after adjustment for BMI. Interestingly, the association remains significant after adjustment for plasma TG levels measured at baseline, suggesting that LFABP modulate the response to fibrates. The possible modulation of TG response to fenofibrate treatment by LFABP genotype is supported by other studies showing that this gene is implicated in the esterification of microsomal fatty acids to glycerides (Jefferson et al. 1990; Jolly et al. 2000; Murphy et al. 1996). Following this finding, for a defective mutation we would expect lower TG levels. Surprisingly, carriers of the mutation had 29% higher plasma TG levels than normal homozygotes after treatment with fenofibrate. This controversial effect was also observed with LFABP null mice (Martin et al. 2003). Against all expectation, no significant modification of TG levels were observed with LFABP−/−mice (Martin et al. 2003). The explication of the maintenance of TG levels among LFABP null mice remains unclear. Moreover, the effect of fenofibrate on plasma TG levels could be mediated via the activation of PPARα within the nucleus. In fact, fenofibrate enters the liver cells with the help of transporters such as LFABP and is transferred to PPARα. The PPARα then binds to its response element in the target gene and increases the transcriptional rate of genes such as LFABP (Issemann et al. 1992; Linden et al. 2002; Schoonjans et al. 1997). Unfortunately, no interaction between LFABP T94A and PPARα L162V genotypes were found to affect plasma lipid levels. Finally, the effect of the LFABP T94A genotype on the reaching of therapeutic target value for plasma TG levels is the only result showing an implication of LFABP in the plasma lipoprotein/lipid responsiveness to fenofibrate. This result should be interpreted with caution, because certain combinations of genotypes may have different effects on triglyceride-lowering drugs (Brisson et al. 2002). Therefore, the reaching of the target value could be facilitated. In this study, the effect of ApoE and LPL P207L genotypes were controlled. LPL P207L has already proven to modulate response to triglyceride-lowering treatment and has been reported to enhance HDL-C response to fibric-acid-derivative treatment (Brisson et al. 2002).

In this study sample, LFABP seems to play a role in the transport and metabolism of FFA. In fact, carriers of the A94 allele exhibited 23% more FFA levels in circulation than T94 homozygotes. This result seems to be in contradiction with the previous finding about TG levels, which was found to be 29% higher for the A94 allele carriers. LFABP carries FFA inside the cell and mediates the transformation in TG; therefore, we would expect to have lower TG levels if the mutation affects FFA transport into the liver cell. We also observed that the mutation was associated with higher plasma cholesterol levels (11%) after the treatment. No difference was observed for plasma cholesterol at baseline. The lack of association with baseline values could be the result of a wider range of cholesterol data (Fig. 3). A study on intestinal LFABP reported similar results for cholesterol levels in women subjects (Galluzzi et al. 2001). Similarly, LFABP null mice had higher liver cholesterol (Martin et al. 2003). Surprisingly, carriers of the mutation showed lower BMI and waist girth than T94 homozygotes (Fig. 4). Interaction analyses between LFABP T94A and PPARα L162V demonstrated a synergetic action of these variants. Carriers of both mutated alleles tended to have a lower BMI than the normal genotype carriers (Fig. 4). The PPARα V162 allele has already been associated with lower BMI (Bosse et al. 2003). PPARα is known to modulate the expression of many genes implicated in lipid metabolism, and LFABP is a transporter that brings ligands needed for the transactivation of genes by PPARα. We can speculate

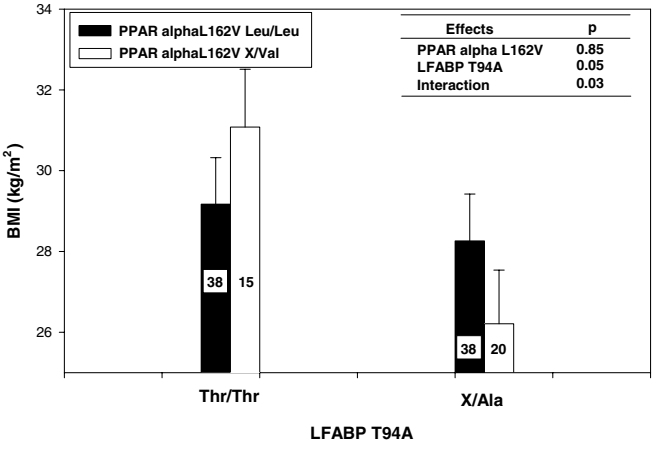

Fig. 4 Effect of peroxisome proliferated activated receptor alpha (PPARα) L162V, liver fatty acid binding protein (LFABP) T94A, and their interaction on BMI (kilograms per meter squared). Data are mean ± SE. All values were adjusted for age, gender, ApoE genotype, alcohol, and smoking

that carriers of the LFABP T94A mutation are unable to sustain adequate transport of fatty acids into cells, so FFA and cholesterol therefore stay in circulation, reducing levels of potential ligands for PPARα. Moreover, the BMI of carriers of the LFABP T94A mutation could be lower due to deficient fatty acid transport to the liver. Other studies are needed to confirm the present findings as well as to elucidate the role of LFABP in lipid metabolism.

In summary, sequencing of the entire coding region allowed us to identify a T94A missense mutation in the LFABP gene. Carriers of the A94 allele were at increased risk to exhibit residual hypertriglyceridemia above the therapeutic target following therapy with fenofibrate. The A94 allele is also associated with a lower BMI and waist girth and higher fasting FFA concentrations. Mutations LFABP T94A and PPARα L162V interact together to modulate BMI. Functional studies as well as longitudinal and prospective studies will be required to elucidate how the LFABP T94A mutation can lead to the observed effects.

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