Molecular scanning of the human PPARα gene: association of the L162V mutation with hyperapobetalipoproteinemia

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Abstract **Peroxisome proliferator-activated receptor alpha (PPARα) is a member of the steroid hormone receptor super family involved in the control of cellular lipid utilization. This makes PPARα a candidate gene for type 2 diabetes and dyslipidemia. The aim of this study was to investigate whether genetic variation in the human PPARα gene can influence the risk of type 2 diabetes and dyslipidemia among French Canadians. We therefore first determined the genomic structure of human PPARα, and then designed intronic primers to sequence the coding region and the exon–intron boundaries of the gene in 12 patients with type 2 diabetes and in 2 nondiabetic subjects. Sequence analysis revealed the presence of a L162V missense mutation in exon 5 of one diabetic patient. Leucine 162 is contained within the DNA binding domain of the human PPARα gene, and is conserved among humans, mice, rats, and guinea pigs. We subsequently screened a sample of 121 patients newly diagnosed with type 2 diabetes and their age and sex-matched nondiabetic controls, recruited from the Saguenay-Lac-St-Jean region of Northeastern Quebec, for the presence of the L162V mutation by a PCR-RFLP based method. There was no difference in L162 homozygote or V162 carrier frequencies between diabetics and nondiabetics. However, whether diabetic or not, carriers of the V162 allele had higher plasma apolipoprotein B levels compared to noncarriers ($P = 0.05$). To further this association, we screened another sample of 193 nondiabetic subjects recruited in the greater Quebec City area. Carriers of the V162 allele compared with homozygotes of the L162 allele had significantly higher concentrations of plasma total and LDL-apolipoprotein B as well as LDL cholesterol ($P \leq 0.02$). These results suggest an association between the PPARα V162 allele and the atherogenic/hyperapolipoprotein B dyslipidemia.**—Vohl, M-C., P. Lepage, D. Gaudet, C. G. Brewer, C. Bétard, P. Perron, G. Houde, C. Cellier, J. M. Faith, J-P. Després, K. Morgan, and T. J. Hudson. **Molecular scanning of the human PPARα gene: association of the L162V mutation with hyperapobetalipoproteinemia.** ***J. Lipid Res.*** **2000.** 41: **945–952.**

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Peroxisome proliferator-activated receptors (PPARs) are members of a large family of ligand-inducible transcription factors that include receptors for retinoids and vitamin D, thyroid, and steroid hormones (1). PPARs regulate the expression of target genes by binding to DNA sequence elements, termed PPAR response elements (PPRE) (2, 3). PPREs have been identified in the regulatory regions of a variety of genes involved in lipid and energy balance. Three different PPAR genes have been identified (α, δ, and γ) (2, 3). PPARγ, expressed mainly in adipose tissue, regulates adipocyte differentiation (4, 5). PPARα, the first one to be identified, is expressed mainly in tissues that have a high ratio of fatty acid (FA) oxidation such as the liver, kidney, heart, and muscle (6, 7). Numerous studies have demonstrated that several genes encoding enzymes involved in β and ω-oxidation are modulated by PPARα (2, 3). PPARα is activated by medium and very long chain FA, polyunsaturated FA, such as eicosapentaenoic acid (8). In addition to its role in FA oxidation, PPARα mediates the action of hypolipidemic drugs of the fibrate class on lipid and lipoprotein metabolism, it alters the synthesis of lipoprotein lipase and regulates the transcription of apolipoprotein C-III and other lipid-related genes (9, 10).

Fatty acids are essential biological components, used as metabolic fuels, covalent regulators of signaling molecules, and essential components of cellular membranes. Thus, FA must be kept within a physiological window. Altered levels of FA are linked to a variety of metabolic diseases including atherosclerosis, hyperlipidemia, obesity, insulin resistance, and type 2 diabetes (11, 12). The findings that PPARα modulates gene expression of enzymes controlling FA oxidation suggest that PPARα may be a

Abbreviations: PPARα, peroxisome proliferator-activated receptor alpha; PPRE, peroxisome proliferator response elements; FA, fatty acid; SLSJ, Saugenay-Lac-St-Jean; FH, familial hypercholesterolemia; BMI, body mass index; HREs, hormone response elements.

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candidate gene for type 2 diabetes and dyslipidemia. To investigate whether genetic variations in PPARα could be associated with the development of these metabolic disorders, we determined the genomic structure of PPARα, and screened all exons and exon–intron boundaries in French-Canadian subjects. We identified a missense mutation (L162V) and investigated its association with type 2 diabetes and plasma lipoprotein-lipid levels in two independently recruited French-Canadian samples. We showed that the heterozygosity for the L162V mutation is unlikely to be a major risk factor for the development of type 2 diabetes but is associated with increased plasma apolipoprotein B levels.

METHODS

Subjects

Two independently recruited samples of unrelated adult Caucasian subjects from the Quebec City area and the Saguenay-Lac-St-Jean (SLSJ) region of Quebec were studied to investigate the hypothesis that genetic variation in PPARα may be associated with the development of type 2 diabetes and dyslipidemia.

Study sample 1 of type 2 diabetic/nondiabetic patients

All individuals included in this study sample were unrelated adults (above 18 years of age) and of French-Canadian descent. Between January and December 1998, they were seen at the Lipid and Diabetes Research Unit of the Chicoutimi Hospital, and recruited on the basis of having a family history of dyslipidemia or type 2 diabetes. These participants were assigned a code which systematically denominalizes all clinical data (13). One hundred and twenty one patients (67 men and 54 women; diabetic group) newly diagnosed with type 2 diabetes following a 75 g oral glucose load were age and sex-matched with an individual having a normal glucose tolerance (nondiabetic group). Type 2 diabetes was defined according to the World Health Organization criteria as a 2-h glucose concentration $\geqslant$11.1 mmol/L after a standard 75 g oral glucose load, whereas subjects were included in the nondiabetic group when their 2-h glucose concentration was below 7.8 mmol/L (14). Subjects with type I hyperlipoproteinemia were excluded from the present study as well as those with LDL-receptor gene mutations causing familial hypercholesterolemia (FH) among French-Canadians. FH screening included two deletions (5 kb and >15 kb) and five point mutations W66G, E207K, C646Y, Y268X, and R329X (15–17). Waist and hip circumferences were assessed according to the procedures recommended by the Airlie Conference (18). Body weight and height were also recorded, and the body mass index (BMI) was calculated in kg/m^2. A medication-free lipoprotein profile was obtained for each subject enrolled in this study. Blood samples were obtained the morning after a 12-h fast from an antecubital vein into vacutainer tubes containing EDTA. Plasma total cholesterol, triglyceride, and HDL-cholesterol levels were measured using enzymatic assays (19–21). Total cholesterol was determined in plasma and HDL-cholesterol was measured in the supernatant after precipitation of VLDL and LDL with dextran sulphate and magnesium chloride (21). The variation coefficients of these various lipid measurements were of 2.1% for high cholesterol values, 2.7% for low cholesterol, and 3.3% for HDL-cholesterol. Plasma LDL-cholesterol levels were estimated with the Friedewald formula (22) when triglyceride levels were <5 mmol/L. Plasma apolipoprotein B levels were measured according to the rocket immunoelectrophoretic method of Laurell (23). Biological and lifestyle variables as well as medical and nutritional histories were obtained through questionnaires and physical exams performed by trained nurses, dieticians, and physicians. Smoking habits were classified as never smoked versus ever smoked. Alcohol consumption was classified as regular drinkers (>5 ounces of absolute alcohol/week) versus nonregular drinkers. A subject was considered to be hypertensive when diagnosis of essential hypertension had been previously established or when three values of systolic $\geqslant$140 mm Hg or diastolic $\geqslant$90 mm Hg blood pressure had been recorded in the patient's medical chart. This study was approved by the Chicoutimi Hospital Ethics Committee.

Study sample 2 of nondiabetic subjects

This cohort included 193 men who were recruited from the Quebec City area by solicitation through the media and were selected to cover a wide range of body fatness. All subjects were sedentary, nonsmokers, and free from metabolic disorders requiring treatment such as diabetes, hypertension, severe dyslipidemia, and coronary artery disease. A high alcohol consumption was also an exclusion criteria. BMI and waist circumference were measured as described above. Blood samples were obtained from an antecubital vein after an overnight fast. Cholesterol and triglyceride concentrations in plasma and lipoprotein fractions were enzymatically measured on an Analyzer Technicon RA-500 (Bayer Corporation, Tarrytown, NY). VLDL ($d < 1.006$ g/ml) were isolated by ultracentrifugation (24) and the HDL fraction was obtained after precipitation of LDL in the infranatant ($d > 1.006$ g/ml) with heparin and $MnCl_2$ (25). The cholesterol content of HDL_2 and HDL_3 subfractions prepared by a precipitation method was also determined (26). Apolipoprotein B concentrations were measured in plasma and infranatant (LDL-apolipoprotein B; $d > 1.006$ g/ml) by the rocket immunoelectrophoretic method of Laurell (23), as previously described (27). Serum standards were prepared in laboratory, calibrated against reference sera from the Centers for Disease Control (Atlanta, GA), lyophilized, and stored at −85°C until use. Participants gave their written consent to participate in this study which received the approval of the Medical Ethics Committee of Laval University.

Genomic structure of the PPARα gene

In order to design intronic primers for the amplification of each exon, genomic sequences were sought for the intronic regions surrounding all PPARα exons. To do so, we compared the mRNA sequence of PPARα (28) with a contiguous genomic DNA region taken from sequences of three overlapping clones from GenBank (Accession numbers: Z94161.5, AL049856, AL078611). 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).

PCR amplification of PPARα exons and sequencing

All exons and exon-intron boundaries were amplified from genomic DNA by use of specific primers derived from the 5′ and 3′ ends of intronic sequence (**Table 1**). The annealing temperature for all primer pairs was 59°C. PCR conditions were as follows: reaction volume was 50 μl, 1.25 unit AmpliTaq Gold polymerase (Perkin-Elmer Cetus) in the buffer recommended by the manufacturer, 2.5 mm $MgCl_2$, 0.2 mm dNTPs, primers at a final concentration of 0.5 μm and 100 ng of template genomic DNA. PCR products were purified with BioMag DNA Sep magnetic beads (PerSeptive Biosystems, Framingham, MA). Sequencing reactions were performed using BigDye Primer Cycle sequencing ready reactions −21M13 kit (PE Applied BioSystems, Foster City, CA) and the products were analyzed on ABI 377 automated DNA

TABLE 1. PCR primers for genomic amplification of PPARα exons

Exons	Oligonucleotides	Product Size
1	PPARαex1.F 5′-**TGTAAAACGACGGCCAGT**CCATCTGGAAACAGTAAATTAAACC-3′	169 bp
	PPARαex1.R 5′-GCATCCAGAGAACAACCGTAA-3′	
2	PPARαex2.F 5′-**TGTAAAACGACGGCCAGT**TAATGATAACAGAATTCATCCACCA-3′	213 bp
	PPARαex2.R 5′-TCCATTCAAGCTGCTATAACAAAAT-3′	
3	PPARαex3.F 5′-**TGTAAAACGACGGCCAGT**CGTCAGTCTTACCAATTGTTCCT-3′	311 bp
	PPARαex3.R 5′-AAACTTTCTAGGAAACGGCACA-3′	
4	PPARαex4.F 5′-**TGTAAAACGACGGCCAGT**CGTATGCGAAATCACATCCTC-3′	300 bp
	PPARαex4.R 5′-TAAGTAGTTGATGGTGGCGG-3′	
5	PPARαex5.F 5′-**TGTAAAACGACGGCCAGT**AGTAAAGCAAGTGCGCTGGT-3′	243 bp
	PPARαex5.R 5′-AAGGAAGGGGAACTGAGGAA-3′	
6	PPARαex6.F 5′-**TGTAAAACGACGGCCAGT**CTCACTGCTCATGCCTGTGT-3′	274 bp
	PPARαex6.R 5′-CCAAGAGAACCCAGAACAGC-3′	
7	PPARαex7.F 5′-**TGTAAAACGACGGCCAGT**GCATCCCACATCACCTGAC-3′	530 bp
	PPARαex7.R 5′-TCAGTGACATGATACCAGCAGA-3′	
8	PPARαex8.F 5′-**TGTAAAACGACGGCCAGT**TGATAAGCAGTTCTTGGGTGA-3′	566 bp
	PPARgex8.R 5′-ACCATTGAGCATAATTCGCC-3′	

M13 tail inserted into forward primers is indicated in bold.

sequencers (PE Applied BioSystems, Foster City, CA). The gel files were processed using the Sequencing Analysis Software (PE Applied BioSystems, Foster City, CA), and then assembled and analyzed using Autoassembler 2.0 (PE Applied BioSystems, Foster City, CA).

Detection of the L162V mutation by polymerase chain reaction, restriction fragment length polymorphism (PCR-RFLP)

The L162V mutation is caused by a C→G transversion at nucleotide 484 in exon 5. It does not alter any restriction site; thus, mismatch PCR method was performed with the following primers Ex5.F(2) 5′-GACTCAAGCTGGTGTATGACAAGT-3′ and Ex5.R-mismatch 5′-CGTTGTGTGACATCCCGACAGAAT-3′ (note: the mismatch nucleotide in the reverse primer is underlined). PCR conditions were as described above. PCR products were digested with *Hinf*I, electrophoresed through either 12% acrylamide or 4% agarose gel, and stained with ethidium bromide.

Statistical analysis

Genotype and allele frequencies of the diabetic and nondiabetic SLSJ subjects were compared with CLUMP (29), a program that uses Monte Carlo simulation to evaluate the statistical significance of the Pearson X^2 statistic for a 2× m contingency table; 10,000 random tables with the same marginal counts as the observed table were generated. Prior to statistical analysis of lipid related traits, 27 heterozygous FH subjects from study sample 1 of type 2 diabetic/nondiabetic patients from SLSJ were excluded. A chi-squared test was used to compare confounding factors such as smoking, alcohol consumption, hypertension, use of beta-blockers, and use of diuretics. Student's *t*-test was used to test the statistical significance of the continuous variables. A log-transform was applied to specific variables in order to normalize their distributions (**Table 2** and **Table 3**). An analysis of variance of lipid and lipoprotein variables was performed after adjusting for age, age and BMI or waist girth, and age and gender. Fisher's exact test was used to assess the difference in the L162 homozygous status between the SLSJ and Quebec City samples. A significance level of 5% was employed. Statistical analyses were performed using SPSS (SPSS Inc.) or JMP (SAS Institute).

RESULTS

Structure of the human PPARα gene

The structural organization of the gene was determined by comparing the human PPARα mRNA sequence with genomic sequences comprising three overlapping clones (**Fig. 1**). All 5′ donor and 3′ acceptor splice sites were found to follow the gt . . . ag rule (**Table 4**). Furthermore,

TABLE 2. Subjects' characteristics according to the L162V genotype in the study sample 1 from SLSJ

Variable	L/L[1]	L/V[2b]	*P*-value
Age, years	53.1 ± 7.6 (161)	52.9 ± 7.0 (54)	0.87
BMI,[a] kg/m^2	28.9 ± 4.1 (161)	28.8 ± 4.5 (54)	0.94
Waist circumference, cm	95.7 ± 12.3 (156)	95.6 ± 11.9 (54)	0.93
Fasting glucose,[a] mmol/L	5.89 ± 1.61 (161)	5.73 ± 1.11 (54)	0.59
Fasting insulin,[a] pmol/L	134.5 ± 115.7 (92)	133.2 ± 100.4 (33)	0.81
Total-cholesterol,[a] mmol/L	6.55 ± 1.55 (161)	6.74 ± 1.40 (54)	0.38
LDL-cholesterol,[a] mmol/L	4.17 ± 1.18 (127)	4.55 ± 1.19 (42)	0.09
HDL-cholesterol,[a] mmol/L	0.98 ± 0.35 (155)	1.00 ± 0.33 (53)	0.63
Triglycerides,[a] mmol/L	3.58 ± 3.23 (161)	3.30 ± 2.65 (54)	0.60
Total apolipoprotein B, g/L	1.19 ± 0.26 (61)	1.28 ± 0.27 (39)	0.05

[a] Log_{10}-transformed variables. Data are means ± SD.
[b] One homozygote V/V included in the L/V group. Number of subjects is shown in parenthesis. A total of 27 FH patients were excluded prior to statistical analyses. The male/female ratio was 89/72 and 32/22, respectively, for groups 1 and 2; group numbers are identified by superscripts.

TABLE 3. Subjects' characteristics according to the L162V genotype in the study sample 2 of nondiabetic men

Variable	L/L	L/V	*P*-value
Age, years	41.7 ± 8.1 (172)	44.7 ± 7.8 (26)	0.08
BMI, kg/m^2	29.9 ± 4.3 (171)	29.1 ± 2.9 (25)	0.36
Waist circumference, cm	101.0 ± 10.7 (171)	99.7 ± 8.9 (26)	0.54
Fasting glucose,[a] mmol/L	5.41 ± 0.56 (170)	5.33 ± 0.33 (26)	0.55
Fasting insulin,[a] pmol/L	109.9 ± 83.7 (170)	85.8 ± 42.5 (26)	0.14
Total cholesterol, mmol/L	5.21 ± 0.79 (169)	5.50 ± 0.58 (25)	0.08
LDL cholesterol, mmol/L	3.49 ± 0.74 (169)	3.86 ± 0.56 (25)	0.02
HDL cholesterol,[a] mmol/L	0.93 ± 0.20 (169)	0.91 ± 0.17 (25)	0.70
HDL_2 cholesterol, mmol/L	0.26 ± 0.16 (166)	0.27 ± 0.17 (25)	0.78
Triglycerides,[a] mmol/L	2.17 ± 1.16 (169)	1.98 ± 0.80 (25)	0.66
Total apolipoprotein B,[a] g/L	1.07 ± 0.24 (166)	1.18 ± 0.16 (25)	0.02
LDL apolipoprotein B, g/L	0.94 ± 0.20 (166)	1.05 ± 0.13 (25)	0.01

[a] Log_{10}-transformed variables. Data are means ± SD. Number of subjects is shown in parenthesis.

all exon–intron boundaries were confirmed by direct sequencing of each exon using primers derived from flanking intronic sequences. The human PPARα gene spans 83.7 kb. As in the mouse, human PPARα gene mRNA is composed of 8 exons, with a 5′ untranslated region encoded by exons 1, 2, and part of exon 3. The coding region of PPARα comprises the remainder of exon 3 and exons 4–8. The 3′ untranslated region consists of the last 232 bp of exon 8. Intron lengths vary from 1.4 to 24.8 kb as indicated in Fig. 1.

Identification of polymorphisms in the human PPARα gene

All exons and the exon–intron splicing boundaries of PPARα were screened by sequencing 12 patients, who had been diagnosed with type 2 diabetes, and 2 nondiabetic patients, all from SLSJ. All amplified fragments of the PPARα gene exhibited the expected lengths, consistent with the absence of deletions, duplications, or rearrangements within these fragments. Two polymorphisms were discovered in the exons. Based on the predicted amino acid sequence for this gene, the polymorphism in exon 7 is silent with a G to A substitution, which does not alter the predicted threonine at codon 253. A new polymorphism was discovered in exon 5 of one diabetic patient, which results in a L162V missense mutation. Specifically, the C to G transversion in the first position of codon 162 leads to a substitution of a leucine for a valine residue.

Genotype determination of the L162V mutation

We developed a rapid screening test using the mismatch PCR method to genotype the L162V polymorphism. Using primers Ex5R-mismatch and Ex5.F(2) described in Methods, the PCR product of the mutant allele was engineered to contain an *Hinf*I restriction site that is abolished in the presence of the C at position 484. The PCR product of the normal allele yields a 117 bp fragment whereas the mutant allele's product is cleaved by *Hinf*I into 93 bp and 24 bp fragments. Genotypes obtained by the mismatch PCR method and by direct sequencing of exon 5 were in perfect agreement (data not shown). Moreover, genotypes obtained by the mismatch PCR approach were repeated for a minimum of 60 randomly chosen individuals, with a reproducibility of 100%.

Lack of association of the L162V polymorphism with type 2 diabetes

In order to assess the contribution of the L162V polymorphism to the development of type 2 diabetes, we com-

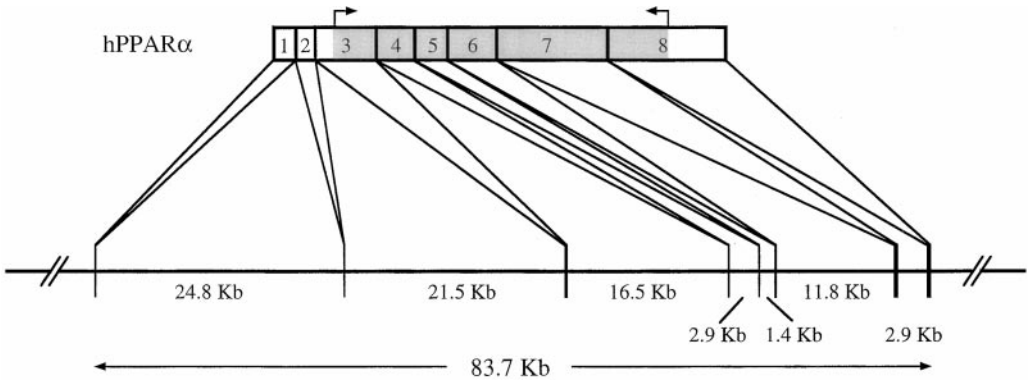

Fig. 1. Schematic representation of the human PPARα gene. Upper panel: a representation of the human PPARα messenger RNA. The coding region is shaded, and arrows indicate translation start (methionine) and stop codons. The spatial localization of exons within the gene is shown in the lower panel. Intron sizes are also indicated. The gene spans 83.7 kb of genomic DNA.

TABLE 4. Exon–intron splicing boundaries

Exon	Donor . . . . . Acceptor	Exon
bp		
exon 1 (87)	CAG:gtaa . . . . . acag:TTC	exon 2
exon 2 (84)	GAG:gtac . . . . . ccag:TAG	exon 3
exon 3 (250)	CGG:gtaa . . . . . ccag:ACA	exon 4
exon 4 (161)	AAG:gtag . . . . . acag:GGC	exon 5
exon 5 (139)	ACG:gtag . . . . . ctag:CGA	exon 6
exon 6 (203)	CCA:gtag . . . . . gtag:CCT	exon 7
exon 7 (448)	GAG:gtga . . . . . ctag:ATC	exon 8
exon 8 (480)	TACTGAgttc	

Nucleotides in exons are indicated in uppercase letters, whereas the flanking nucleotides in introns are in lower case. The exact length in base pairs of the exons is indicated in parenthesis.

pared allele frequencies of that variant in the study sample 1 of diabetics and nondiabetics recruited from the SLSJ area. In this sample, L162 homozygotes were the most common genotype group, and all but one of the remainder were heterozygous for the minor V162 variant. As shown in **Table 5**, no difference in allele frequencies was observed between diabetics and nondiabetics, the V162 allele had frequencies of 12.8% and 12.0% among nondiabetics and diabetics, respectively. The single V162 homozygote was diabetic.

Association between the L162V missense mutation and plasma lipoprotein-lipid concentrations

Chi-square analysis performed independently within each of the SLSJ diabetic and nondiabetic groups revealed that the prevalence of confounding factors such as the smoking status, alcohol consumption, and the use of beta-blockers or diuretic drugs as well as hypertension were similar between the two PPARα genotypic groups (not shown). Since a medication-free lipoprotein-lipid profile as well as fasting blood glucose and insulin levels were also obtained from SLSJ individuals, we compared the lipoprotein-lipid profile according to the L162V genotype independently of their diabetic status (Table 2). Prior to statistical analyses, we excluded 27 subjects bearing a mutation in the LDL-receptor gene that causes FH, a metabolic disorder associated with severe plasma LDL-cholesterol increase and premature atherosclerosis. Carriers of the V162 allele and L162 homozygotes had similar fasting glucose and insulin levels as well as plasma triglyceride and HDL-cholesterol concentrations. No difference was observed even after adjustments for covariates including gender, age, and/or BMI. However, carriers of the V162 allele had higher plasma apolipoprotein B levels ($P = 0.05$) than L162 homozygotes and a trend toward higher plasma LDL-cholesterol levels was observed. When the plasma lipoprotein-lipid profile of carriers versus noncarriers of the V162 allele was compared within the diabetic and the nondiabetic groups separately, the difference in plasma apolipoprotein B levels did not reach statistical significance although carriers of the V162 allele had higher levels (not shown). The 7.5% increase in plasma apolipoprotein B levels observed among V162 carriers encouraged us to further investigate the effect of the missense mutation in an alternative sample. We subsequently screened a sample of 193 nondiabetic men who were recruited in the greater Quebec City area and for whom a detailed lipoprotein-lipid profile was obtained. These subjects were free of metabolic disorders requiring treatment including type 2 diabetes, hypertension, or coronary artery disease. In this sample of nondiabetic men from the Quebec City area, the frequency of the V162 allele was 6.6% (Table 5), significantly less than observed in the subjects from the SLSJ region. Indeed, the prevalence of L162 homozygotes versus V162 carriers (L/V and V/V) was significantly higher in the SLSJ study sample (54.1%) than in nondiabetics from the greater Quebec City area (45.9%) ($P < 0.001$, Fisher's exact test). Comparison of the lipoprotein-lipid profile between the two PPARα genotypes in this sample showed that subjects carrying the V162 allele had higher LDL-cholesterol, LDL-apolipoprotein B, and total apolipoprotein B levels than L162 homozygotes, and these differences were statistically significant (Table 3). Notably, these differences remained significant after adjustments for age and BMI or for age and waist circumference.

TABLE 5. PPARα allele frequencies

Population	L162	V162
Study sample 1 from SLSJ		
Diabetic patients	213 (88.0%)	29 (12.0%)
Nondiabetic patients	211 (87.2%)	31 (12.8%)
Study sample # 2	360 (93.4%)	26 (6.6%)
Quebec city nondiabetic men		

Number of alleles and percentage in parenthesis.

DISCUSSION

The human PPARα gene has been mapped on chromosome 22 slightly telomeric to a linkage group of six genes and genetic markers located in the region 22q12-q13.1 (28). Using information available from GenBank, we have determined the exon–intron structure of the human PPARα gene. This information should facilitate genetic studies evaluating the role of variation in this gene in insulin resistance, type 2 diabetes and dyslipidemia. The human PPARα gene spans 83.7 kb and is composed of 8 exons. The 5′ donor and 3′ acceptor splice sites were found to be in agreement with the consensus splice donor and acceptor sequences. The position of introns separating coding exons is conserved between the human and mouse genes (30). However, the sequence of the noncoding portion of the gene shows no significant homology between the two species (28, 30). It is not clear whether this divergence causes any difference in gene regulation between the two species. Notably, it has been observed that liver PPARα mRNA levels differ between species, with lower transcript levels in human than mouse (31, 32).

Direct sequencing of the entire coding region of the human PPARα gene as well as exon–intron boundaries revealed the presence of a silent G to A substitution in exon 7. This substitution does not lead to an amino acid change

and was not further pursued. A L162V missense mutation was also identified. To investigate the contribution of this newly identified mutation to the risk of type 2 diabetes, we compared the allele frequency of the L162V mutation in the study sample 1 of diabetic/nondiabetic patients from SLSJ. There was no difference in the frequency of the L162V variant between diabetic patients and their age and sex-matched nondiabetic subjects suggesting that this mutation, in the heterozygous state, is unlikely to be a major contributor to type 2 diabetes. Furthermore, our results do not support a major role for the L162V mutation in the development of obesity as no significant difference in BMI and waist circumference was observed between the two genotype groups, either in diabetic or nondiabetic patients.

The contribution of the L162V missense mutation to the modulation of the plasma lipoprotein-lipid profile was also examined. In the study sample 1, higher plasma apolipoprotein B levels were observed among carriers of the V162 allele. The study of nondiabetic men in sample 2 confirmed these results: carriers of the V162 allele had higher total and LDL-apolipoprotein B concentrations as well as greater LDL-cholesterol levels. The presence of the V162 allele was associated with 11.7, 10.3, and 10.7% increases in plasma total apolipoprotein B, LDL-apolipoprotein B, and LDL-cholesterol levels, respectively. These results suggest that the PPARα L162V missense mutation contributes to an increase in the concentration of apolipoprotein B-containing lipoproteins and as a consequence, carriers of the V162 allele may be more prone to develop atherosclerosis. Apolipoprotein B and LDL-cholesterol levels are well-known risk factors for coronary artery disease (33–36). According to data from the Quebec Cardiovascular Study, a 10% increase in plasma total cholesterol or apolipoprotein B levels is associated with a 27 and 17% increase in the risk of ischemic heart disease, respectively (37). An elevation of apolipoprotein B levels is also a common feature of familial combined hyperlipidemia and the insulin resistance-dyslipidemic syndrome of visceral obesity, two common diseases associated with an increased risk of coronary artery disease (38, 39).

Several studies have clearly demonstrated that PPARα is a key regulator of lipid metabolism. Djouadi and colleagues reported that in mice lacking PPARα, the inhibition of cellular FA flux caused massive hepatic and cardiac lipid accumulation resulting in the death of 100% of males but only 25% of females (40). Estradiol pretreatment reduced lethality in male PPARα −/− mice. An additional study showed significantly higher basal cholesterol levels in PPARα (−/−) mice as compared to PPARα (+/+) mice (41). The most striking difference was the increase in serum HDL-cholesterol levels in (−/−) mice. Accordingly, the regulatory effect of fibrate lipid-lowering drugs normally observed in PPARα (+/+) mice, was absent in (−/−) mice. Administration of fibrates, which are known activators of PPARα in humans, results in increased plasma HDL-cholesterol levels and its apolipoprotein constituents A-I and A-II (42). It has also been proven that the administration of fibrates retards the progression of atherosclerosis in angiographic trials (43–45). However, the effect of fibric acid derivatives on LDL-cholesterol levels is variable. Although an important decrease occurs in the majority of individuals, a paradoxical increase is also noted in a subset of nonresponders (46, 47). Thus, it is possible that the response to lipid-lowering fibrate drugs could be influenced by mutations in the PPARα gene or that changes in PPARα activity could contribute to the observed lipoprotein-lipid variability in the population. Results presented in this study suggest that the L162V mutation is associated with a moderate increase in the concentration of apolipoprotein B-containing lipoproteins. This missense mutation results in a nonconservative amino acid substitution located within the DNA binding domain or C domain of the protein (31). This domain, which targets the receptor to specific DNA sequences known as hormone response elements (HREs), is the most conserved among nuclear receptor domains (2, 3, 31). It is also important to note that leucine 162 is highly conserved among species including mice, rats, guinea pigs, and humans (30, 48). Taken together, these observations suggest that leucine 162 may be important for PPARα activity and that the valine substitution may possibly alter its activity. At this point, however, we cannot rule out the possibility that the L162V mutation might be in linkage disequilibrium with a functional mutation in a nearby gene which also regulates lipid metabolism and apolipoprotein B concentrations.

The observation of a nearly almost two-fold increase in the V162 variant in the SLSJ sample compared to the Quebec City sample could be related to differences in the coronary artery disease risk profile between these samples. Indeed, diabetic and nondiabetic subjects from SLSJ were recruited through a clinic specializing in the evaluation of cardiovascular and metabolic disease risk. Thus, the SLSJ sample probably includes individuals with increased genetic and nongenetic risk factors for coronary artery disease (the former evidenced by the elevated frequency of FH patients from the pool of potential subjects for this study), whereas subjects from the Quebec City sample were recruited through the media, and metabolic disorders such as hypertension and diabetes were exclusion criteria. Alternatively, the differences between these samples in V162 variant frequencies may be due to population genetic variation. Founder effects for various Mendelian diseases have been documented in the SLSJ population (49–51) which may account for this two-fold variation in the frequency of the V162 allele. There are many examples of allele frequency differences between both populations, including mutations in the LDL-receptor or lipoprotein lipase genes which are more common in the SLSJ population than in the population of Quebec city area (52–54). Further studies will be required to better understand the allele frequency differences between samples.

In summary, sequencing of the entire coding region allowed us to identify a missense mutation in PPARα, L162V. Carriers of this mutation do not appear to be at increased risk of developing type 2 diabetes in the French-Canadian population studied. The V162 allele is associated with an increase in plasma LDL-cholesterol, total and LDL-apolipoprotein B levels. Functional studies (includ-

ing longitudinal prospective studies) will be required to elucidate how the L162V mutation can lead to hyperapobetalipoproteinemia and possibly increase the risk of coronary artery disease.

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