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Gene 529 (2013) 326–331

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Short Communication

Peroxisome proliferator-activated receptors alpha and gamma2 polymorphisms in nonalcoholic fatty liver disease: A study in Brazilian patients

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ARTICLE INFO

Article history:
Accepted 27 June 2013
Available online 24 July 2013

Keywords:
Nonalcoholic fatty liver disease
NASH
PPAR
Polymorphism

ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) refers to the accumulation of hepatic steatosis in the absence of excess alcohol consumption. The pathogenesis of fatty liver disease and steatohepatitis (NASH) is not fully elucidated, but the common association with visceral obesity, hyperlipidemia, hypertension and type 2 diabetes mellitus (T2DM) suggests that it is the hepatic manifestation of metabolic syndrome. Peroxisome proliferator-activated receptor PPARα and PPARγ are members of a family of nuclear receptors involved in the metabolism of lipids and carbohydrates, adipogenesis and sensitivity to insulin. The objective of this study was to analyze the polymorphisms Leu162Val of *PPARα* and Pro12Ala of *PPARγ* as genetic risk factors for the development and progression of NAFLD.
Methods: One hundred and three NAFLD patients (89 NASH, 14 pure steatosis) and 103 healthy volunteers were included. Single nucleotide polymorphisms (SNPs) Leu162Val and Pro12Ala were analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP).
Results: NASH patients presented higher BMI, AST and prevalence of T2DM than patients with pure steatosis. A higher prevalence of 12Ala allele was observed in the NASH Subgroup when compared to Control Group. When we grouped NASH and Steatosis Subgroups (NAFLD), we found lower serum glucose and more advanced fibrosis in the Leu162Val SNP. On the other hand, there was no statistical difference in clinical, laboratorial and histological parameters according to the Pro12Ala SNP.
Conclusions: We documented a lower prevalence of 12Ala allele of gene *PPARγ* in the NASH Subgroup when compared to Control Group. In NAFLD patients, there were no associations among the occurrence of Pro12Ala SNP with clinical, laboratorial and histological parameters. We also documented more advanced fibrosis in the Leu162Val SNP. The obtained data suggest that Pro12Ala SNP may result in protection against liver injury and that Leu162Val SNP may be involved in the progression of NAFLD.

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1. Introduction

Nonalcoholic fatty liver disease (NAFLD) includes a simple steatosis (fat accumulation in hepatocytes without concomitant inflammation or fibrosis) and non-alcoholic steatohepatitis (NASH), with a necroinflammatory component (Matteoni et al., 1999). The diagnosis of NAFLD requires detection of steatosis by liver histology or imaging modalities, and the exclusion of other liver diseases, such as alcoholic liver disease (AFLD) and viral hepatitis (Bedogni et al., 2005). NAFLD is a condition which cause concern because it may progress to cirrhosis, liver failure and hepatocellular carcinoma (de Alwis and Day, 2008).

NAFLD occurs worldwide and it is present in various ethnic groups, making it the a common liver condition (Das et al., 2010; Fan et al., 2005; Zelber-Sagi et al., 2006). In Brazilian studies, the most common histological finding was NASH with fibrosis in asymptomatic males, and a significant number of cases already presented cirrhosis at the moment of diagnosis (Abdelmalek et al., 2001; Cotrim et al., 2011).

Obesity and insulin resistance (IR) are factors related to NAFLD (Petta et al., 2009; Vuppalanchi and Chalasani, 2009). Others causes include parenteral nutrition, use of hepatotoxic drugs, gastric bypass surgery and disorders associated with fatty acid metabolism (Brunt, 2009; Paschos and Paletas, 2009). Currently, the pathogenesis of NAFLD has been attributed to the "multiparallel hits" hypothesis, which includes

Abbreviations: NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PPAR, Peroxisome proliferator-activated receptor; SNPs, single nucleotide polymorphisms.

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http://dx.doi.org/10.1016/j.gene.2013.06.091

the presence of IR, adipocytokines, lipotoxicity, endoplasmic reticulum stress, interaction between adipose tissue with hepatocytes and genetic factors (Smith and Adams, 2011).

Various single nucleotide polymorphisms (SNPs) have been implicated in the predisposition to NAFLD or NASH, including genes encoding adiponutrin/patatin-like phospholipase-3 (PNPLA3), adiponectin, TNFα, microsomal triglyceride transfer protein and peroxisome proliferator-activated receptor (*PPAR*). The *PPARα* gene is located on chromosome 22q13.3. Some SNPs in this gene were associated with dyslipidemia, IR, type 2 diabetes mellitus (T2DM) and cardiovascular disease (Yong et al., 2008).

The Leu162Val *PPARα* SNP (rs1800206) represents a nitrogen base substitution from cytosine (C) to guanine (G), leading to a leucine to valine amino acid exchange in codon 162 (Flavell et al., 2000; Sapone et al., 2000). For this SNP, Leu162Leu characterizes the wild homozygous genotype. The substitution of a single nitrogen base characterizes the heterozygous genotype (Leu162Val), and the substitution of two basis points to the mutated homozygous (Val162Val).

The *PPARγ* gene is located on chromosome 3p25 and encodes a nuclear transcription factor involved in the expression of hundreds of genes. In the Pro12Ala *PPARγ* SNP (rs1801282), the substitution of one nitrogen base alters the coded amino acid (proline to alanine) in codon 12 in exon 2 (Fajas et al., 1997). The association between the 12Ala variant and IR, T2DM, higher BMI, and obesity has been described in several studies (Altshuler et al., 2000; Mori et al., 2001). The role of this SNP in the pathogenesis and progression of fatty liver disease is still debated.

The purpose of this study was to analyze the SNPs Leu162Val and Pro12Ala of *PPARα* and *PPARγ* as genetic risk factors for the development and progression of the NAFLD.

2. Materials and methods

2.1. Subjects

This study included 103 outpatients with NAFLD, diagnosed according to the histopathological findings from the liver biopsy. The Control Group consisted of 103 healthy subjects without laboratorial signs of hepatitis or other liver dysfunctions, and presenting normal liver ultrasound. All volunteers were registered in a Brazilian university hospital and provided informed consent according to the institutional research ethics committee (Proc#7697/2007).

2.2. Liver histology

The grading and staging of all liver biopsy specimens were conducted by one pathologist specialized in hepatology, and followed Brunt's criteria. Liver samples were prepared with hematoxylin-eosin, Masson's trichrome and silver reticulin stains. The presence of more than 5% steatotic hepatocytes in a liver tissue section was accepted as the minimum criterion for the histological diagnosis of NAFLD (Brunt, 2010; Brunt et al., 2011). Briefly, histological activity was graded based on steatosis [grade 0 (no steatosis), grade 1 (5-33%), grade 2 (33-60%) and grade 3 (>60%)]; lobular inflammation (0-3); portal chronic inflammation (0-2); ballooning (0-2) and fibrosis scores (0-3). The NAFLD activity score (NAS) was used to classify NAFLD into Steato7sis (NAS $\leq$ 4) and NASH (NAS $\geq$ 5) Subgroups. For the present study, the grading of steatosis, lobular inflammation, ballooning and fibrosis was used to rank the histological findings into moderate (0 $\leq$ score $\leq$ 1) or severe (score $\geq$ 2).

3. Methods

3.1. Biochemical analyses

Venous blood samples were obtained after a 12-hour overnight fast. Plasma glucose, serum aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase, iron, ferritin, tryglicerides, total and HDL-cholesterol were measured in a conventional automated analyzer. Low-density lipoprotein cholesterol (LDL-c) was calculated according to the equation of Friedewald et al. (1972). Serum adiponectin, leptin and insulin levels were measured using enzyme linked immunosorbent assays (Millipore, Missouri, USA).

3.2. Clinical and nutritional assessment

The subjects' weight and height were measured with standard techniques. The body mass index (BMI) was calculated by the formula: body weight (kg) / [height (m)]^2. A BMI over 27 kg/m^2 defined obesity (Bray, 1992). We selected 30 individuals of each group randomly and they answered a semi-quantitative food consumption frequency questionnaire, which described food consumption within 6 months prior to the study. Diabetes mellitus was established in patients with a prior diagnosis of T2DM and those with glucose concentrations above 100 mg/dL (Deurenberg et al., 1998; Genuth et al., 2003). Dyslipidemia was defined when total cholesterol was above 240 mg/dL or triglycerides above or equal to 150 mg/dL or LDL-c above or equal to 160 mg/dL or HDL-c below 40 mg/dL for men and below 50 mg/dL for women (JAMA, 2001). IR was calculated according to the homeostasis model assessment of the insulin resistance index (HOMA-IR), computed through the formula: [fasting serum insulin (μU/mL) × fasting serum glucose (mg/dL)] / 405 (Matthews et al., 1985). HOMA above 2.5 defined IR (Guidorizzi de Siqueira et al., 2005).

3.3. Genotyping of Leu162Val of PPAR-α and Pro12Ala of PPARγ

DNA was isolated from peripheral blood leukocytes by the salting-out method (Miller et al., 1988). The Leu162Val and Pro12ALA SNPs were detected through polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) following previously described protocols (Al-Shali et al., 2004; Vohl et al., 2000). PCR products were digested overnight at 37 °C with 2 U of *Hinf I* and 1 U of *Hae III* restriction enzymes to analyze Leu162Val and Pro12ALA SNPs, respectively. In both cases, the DNA fragments were visualized on 10% polyacrylamide gels followed by silver staining (Sanguinetti et al., 1994).

3.4. Statistical analysis

Statistical analysis was performed using the software SAS 9.2 (SAS Institute Inc, Cary, USA). Continuous variables were presented as means ± standard deviation. Simple comparison of the clinical data between NAFLD and Control Groups was performed using the Mann-Whitney U test. The comparison of the clinical data among the different genotypes was carried out using the analysis of variance test (ANOVA) after logarithmic transformation. Genotype and allele distributions in NAFLD and Control Groups were analyzed by Fisher's exact test. Concordance to the frequency predicted by the Hardy-Weinberg equilibrium was assessed by the chi-square test. Logistic regression analyses were used to assess the association between these SNPs with disease using sex, age and BMI as covariates at the model. Odds ratio (OR) and the 95% confidence interval (CI) were estimated. Moreover, a logistic regression analysis was included for the evaluation of the association between genotypes and histological disease severity. To verify the association between SNPs and comorbidities, Fisher's exact test was used and the variables were expressed as percentages. The level of significance was set at $p \leq 0.05$ in all analyses.

4. Results

The energy (1838.8 ± 281.1 vs 2013.3 ± 539.3 kcal/day, $p = 0.14$) and carbohydrate (261.5 ± 54.8 vs 230.7 ± 1;66.3 g/day, $p = 0.06$) consumption was similar between the volunteers of NAFLD and Control Group,

Table 1
Demographic and clinical features of NAFLD and Control Groups.

	NAFLD Group (n = 103)	Control Group (n = 103)	p-Values*
Gender (male/female)	47/56	28/75	<0.01
Age (years)	44.5 ± 2.5	31.9 ± 9.6	<0.01
BMI (kg/m^2)	31.3 ± 5.2	23.6 ± 3.0	<0.01
Aspartate aminotransferase (U/L)	46.9 ± 36.3	22.1 ± 4.8	<0.01
Alanine aminotransferase (U/L)	67.9 ± 43.6	18.8 ± 7.5	<0.01
Gamma glutamyltransferase (U/L)	95.1 ± 93.5	24.1 ± 7.6	<0.01
Alkaline phosphatase (U/L)	206.1 ± 80.3	170.1 ± 28.6	<0.01
Iron (ug/dL)	90.2 ± 29.1	116.3 ± 34.2	<0.01
Ferritin (ng/mL)	308.4 ± 270.4	101.2 ± 101.1	<0.01
Total cholesterol (mg/dL)	199.2 ± 41.6	183.0 ± 34.2	<0.01
HDL-c (mg/dL)	43.2 ± 10.0	58.3 ± 13.0	<0.01
LDL-c (mg/dL)	123.9 ± 42.1	107.0 ± 28.5	<0.01
Tryglicerides (mg/dL)	180.8 ± 102.9	88.6 ± 38.2	<0.01
Glucose (mg/dL)	111.9 ± 50.8	77.6 ± 7.9	<0.01
Insulin (μU/mL)	22.2 ± 14.7	7.6 ± 3.4	<0.01
HOMA-IR	6.2 ± 5.0	1.4 ± 0.6	<0.01
Leptin (ng/mL)	25.7 ± 18.9	19.9 ± 17.0	<0.01
Adiponectin (μg/mL)	1.8 ± 1.8	10.9 ± 4.9	<0.01

*Mann-Whitney U test.

respectively. The subjects of the NAFLD Group present lower lipid (59.4 ± 17.8 vs 76.9 ± 24.4 g/day, p = 0.004) and protein (74.7 ± 15.8 vs 95.5 ± 34.4 g/day, p = 0.006) intake. Demographic and clinical features of NAFLD and control subjects are available in Table 1. Obesity was documented in 87 patients (84%), dyslipidemia in 87 (84%), T2DM in 41 (40%) and IR in 80 (82%) among all individuals with NAFLD. Demographic and clinical features of NAFLD patients, classified according to the histological diagnosis are shown in Table 2.

4.1. Polymorphisms frequency and correlation with biochemical and histological features of NAFLD

The allelic and genotypic frequencies of the Leu162Val *PPARα* and Pro12Ala *PPARγ* SNPs in patients with NAFLD and controls are shown in Table 3. Both SNPs were in accordance with the Hardy-Weinberg equilibrium. There were no statistical differences in the genotypic and allelic frequencies of Leu162Val *PPARα* and Pro12Ala *PPARγ* SNPs between NAFLD and Control Groups. However, a statistical difference was observed in the allelic frequency regarding the Pro12Ala SNP, with the 12Ala allele being more prevalent in the Control Group when compared to the NASH Group.

Table 2
Demographic and clinical features of NAFLD patients, classified according to the histological diagnosis of NASH or steatosis.

	NASH Subgroup (n = 89)	Steatosis Subgroup (n = 14)	p-Values*
Gender (male/female)	39/50	8/6	0.93
Age (years)	44.6 ± 12.3	43.7 ± 13.9	0.82
BMI (kg/m^2)	31.9 ± 5.3	27.7 ± 2.7	<0.01
Aspartate aminotransferase (U/L)	49.5 ± 37.7	30.9 ± 20.9	0.01
Alanine aminotransferase (U/L)	70.2 ± 43.2	53.6 ± 45.0	0.07
Gamma glutamyl transferase (U/L)	92.8 ± 81.9	108.7 ± 151.0	0.97
Alkaline phosphatase (U/L)	211.6 ± 81.1	172.3 ± 67.8	0.21
Iron (ug/dL)	90.6 ± 29.0	88.2 ± 31.3	0.89
Ferritin (ng/mL)	320.0 ± 282.5	230.0 ± 151.8	0.39
Total Cholesterol (mg/dL)	197.3 ± 42.4	210.9 ± 35.0	0.28
Tryglicerides (mg/dL)	185.5 ± 107.0	151.4 ± 67.5	0.31
HDL-c (mg/dL)	43.0 ± 10.1	44.5 ± 9.4	0.52
LDL-c (mg/dL)	121.8 ± 43.7	138.0 ± 27.1	0.10
Glucose (mg/dL)	115.1 ± 53.3	88.8 ± 8.9	0.08
Insulin (μU/mL)	22.2 ± 14.0	22.1 ± 19.6	0.69
HOMA-IR	6.4 ± 5.1	4.9 ± 4.6	0.17
Leptin (ng/mL)	26.7 ± 19.4	18.7 ± 12.9	0.19
Adiponectin (μg/mL)	1.7 ± 1.8	2.1 ± 1.7	0.18
Obesity [n, (%)]	75 (84)	12 (86)	1.00
T2DM [n, (%)]	40 (45)	1 (7)	<0.01
Dyslipidemia [n, (%)]	76 (85)	11 (79)	0.45
IR [n, (%)]	70 (81)	10 (83)	1.00

* Mann-Whitney U test.

Table 3
Allelic and genotypic frequencies of Leu162Val (rs1800206) and Pro12Ala (rs1801282) SNPs in NAFLD and Control Groups.

	Allele/genotype	NAFLD Group (n = 103)		Control Group* (n = 103)
		NASH Subgroup (n = 89)	Steatosis Subgroup (n = 14)	
SNP Leu162Val	Leu-carriers	0.94	1.00	0.94
	Val-carriers	0.06	0	0.06
	Leu162Leu [n, (%)]	79 (89)	14 (100)	92 (89)
	Leu162Val [n, (%)]	10 (11)	0 (0)	11 (11)
	Val162Val [n, (%)]	0 (0)	0 (0)	0 (0)
SNP Pro12Ala	Pro-carriers	0.93	0.93	0.86
	Ala-carriers	0.07	0.07	0.14*
	Pro12Pro [n, (%)]	76 (85)	11 (79)	77 (75)
	Pro12Ala [n, (%)]	13 (15)	3 (21)	23 (22)
	Ala12Ala [n, (%)]	0 (0)	0 (0)	3 (3)

* Fisher exact test (p = 0.03) for NASH Subgroup *vs.* Control Group [OR: 0.48 (0.24-0.96)].

In NAFLD subjects, for the Leu162Val SNP we found 93 subjects grouped with the wild homozygous genotype, from which 50 were female, 45.2 ± 12.4 years and a BMI of 31.0 ± 4.8 kg/m^2. Among the 10 individuals with heterozygous genotype, 6 were female, 37.7 ± 11.8 years and a BMI of 34.5 ± 7.7 kg/m^2. The wild homozygous genotype (Pro12Pro) was documented in 87 individuals, from which 46 were female, 44.6 ± 12.6 years and a BMI of 31.3 ± 5.3 kg/m^2. Among the 16 subjects with heterozygous genotype, 10 were female, 44.1 ± 12.3 years and a BMI of 31.9 ± 5.1 kg/m^2. We show the laboratory data (Table 4) and clinical/liver histopathological findings (Table 5) of NAFLD patients according to genotype Leu162Val and Pro12Ala.

5. Discussion

This study underlies the existence of several clinical and laboratorial differences between NAFLD subjects and healthy controls. Moreover, it evidences similarities between the NASH and the Steatosis Subgroups, except for higher values of BMI, serum AST and the prevalence of T2DM in NASH patients. The NASH Subgroup presented a lower prevalence of the *PPARγ* 12Ala allele when compared to controls. When we analyzed the NAFLD Group according to *PPARα* SNPs, lower serum glucose levels and a higher percentage of severe fibrosis were documented in the Leu162Val. On the other hand, there was no statistical difference in clinical, laboratorial and histologic data according to the *PPARγ* SNPs.

In this study, the prevalence of 12Ala allele in the *PPARγ* SNP was lower in the NASH Subgroup than in controls. Dongiovanni et al. (2010) showed that the frequency distributions of Leu162Val and Pro12Ala SNPs were not different between Italian subjects with NAFLD and controls (Dongiovanni et al., 2010). In several populations from different ethnic origins, the frequency of 12Ala ranged from 8-20% (Radha et al., 2006; Yen et al., 1997). Among Brazilians of European descent, the frequency of 12Ala allele was the same as described for European and European-derived population (Mattevi et al., 2007).

Different from our findings, studies of *PPARγ* in different ethnic populations suggest a complex interaction among the Pro12Ala SNP, environmental factors and SNPs in other genes. The prevalence of the Pro12Ala genotype was significantly higher in the NAFLD patients, whose body mass index was higher than 25 kg/m^2 (Gupta et al., 2010). The presence of the 12Ala allele may result in higher sensitivity to insulin than individuals homozygous for Pro12 (Tavares et al., 2005). Among indigenous from Brazil there was association between the Pro12Ala variant and features of metabolic syndrome (Vieira-Filho

Table 4
Laboratorial data of NAFLD patients according to genotype of Leu162Val and Pro12Ala SNPs.

	SNP Leu162Val (n = 103)			SNP Pro12Ala (n = 103)		
	Homozygote Leu162Leu (n = 93)	Heterozygote Leu162Val (n = 10)	p-Values*	Homozygote Pro12Pro (n = 87)	Heterozygote Pro12Ala (n = 16)	p-Values*
Laboratory data						
Aspartate aminotransferase(U/L)	44.9 ± 26.5	65.7 ± 85.7	0.72	45.4 ± 27.1	55.3 ± 68.4	0.93
Alanine aminotransferase (U/L)	68.5 ± 42.7	62.3 ± 53.6	0.08	67.6 ± 42.3	69.6 ± 51.9	0.99
Gamma glutamyl transferase (U/L)	96.0 ± 95.9	85.7 ± 70.8	0.58	93.9 ± 93.7	101.0 ± 95.1	0.70
Alkaline phosphatase (U/L)	205.8 ± 79.3	209.2 ± 94.9	0.57	203.2 ± 80.4	223.1 ± 80.0	0.18
Iron (ug/dL)	90.9 ± 28.7	84.3 ± 33.5	0.28	90.0 ± 29.1	91.6 ± 30.2	0.67
Ferritin (ng/mL)	306.9 ± 266.5	322.6 ± 319.9	0.66	287.9 ± 239.9	417.8 ± 387.5	0.17
Total Cholesterol (mg/dL)	199.7 ± 41.2	194.3 ± 47.5	0.92	196.9 ± 40.8	211.7 ± 45.2	0.23
Tryglicerides (mg/dL)	178.1 ± 103.4	205.6 ± 99.7	0.69	181.7 ± 107.3	176.2 ± 77.6	0.96
HDL-cholesterol (mg/dL)	43.1 ± 10.2	43.8 ± 8.1	0.63	42.8 ± 10.2	44.9 ± 9.3	0.50
LDL-cholesterol(mg/dL)	125.6 ± 43.2	109.5 ± 28.4	0.65	146.5 ± 68.9	119.5 ± 33.4	0.05
Glucose (mg/dL)	113.9 ± 52.4	91.8 ± 22.9	0.05	112.6 ± 51.1	108.6 ± 50.4	0.53
Insulin (μU/mL)	22.0 ± 15.1	24.3 ± 10.3	0.74	22.7 ± 15.4	19.1 ± 9.4	0.39
HOMA-IR	6.2 ± 5.1	6.3 ± 3.5	0.76	6.4 ± 5.3	5.1 ± 2.8	0.38
Leptin (ng/mL)	24.8 ± 18.6	34.9 ± 19.8	0.69	24.4 ± 18.9	32.9 ± 17.3	0.14
Adiponectin (μg/mL)	1.8 ± 1.9	1.5 ± 1.0	0.64	1.8 ± 1.9	1.6 ± 0.9	0.68

* Adjusted for age, gender and BMI.

et al., 2004). Associations of Leu162Val SNP with obesity, T2DM and lipid serum levels were not documented in the Brazilian population (Chen et al., 2010). However, there was an association between allele frequencies for 162Val and dyslipidemia, even after adjustment for alcohol consumption and waist-to-rip ratio (Mazzotti et al., 2010).

Our results showed lower fasting glucose in carriers of 162Val allele and there was no association between thise SNP with obesity, T2DM, insulin resistance and dyslipidemia. Some authors did not find any associations between genotype Leu162Val SNP and T2DM (Verdi et al., 2005; Silbernagel et al., 2009). On the other hand, the meta-analysis conducted by the Human Genome Epidemiology (HuGE) showed association between 12Ala allele and a reduction in the risk of T2DM (odds ratio = 0.86, 95% CI: 0.81, 0.90) (Gouda et al., 2010), which agrees with previous studies (Lohmueller et al., 2003; Ludovico et al., 2007; Parikh and Groop, 2004). There was no documented association between the Leu162Val SNP and body fat (Verdi et al., 2005; Silbernagel et al., 2009), steatosis (Silbernagel et al., 2009) and blood lipid profile (Verdi et al., 2005). The Leu162Val SNP may be associated with some specific blood lipid alterations in diabetic patients (Flavell et al., 2000; Robitaille et al., 2004; Vohl et al., 2000) as well as in healthy subjects (Jamshidi et al., 2002; Nielsen et al., 2003; Robitaille et al., 2004).

In this study, we observed an association between fibrosis stage with the presence of Leu162Val, but not of Pro12Ala. Contrary to our finding, Dongiovanni et al. (2010) did not establish association between PPARα 162Val allele with severity of steatosis, necroinflammation and fibrose liver damage in NAFLD (Dongiovanni et al., 2010). Regarding the Pro12Ala SNP, the presence of the 12Ala allele was not associated with the progression of NAFLD, although increased anti-inflammatory activity was associated with this SNP in AFLD patients (Rey et al., 2010).

One study limitation was the relatively small sample, especially when compared to population studies on the subject. However, the number of patients in the present study was adequate to analyze the influence of *PPARα* and *PPARγ* SNPs on the susceptibility to and progression of NAFLD. The inclusion of a Control Group in genotyping is one of the study's strengths, apart from the simultaneous evaluation of several laboratorial variables. On the other hand, we can't rule out the possibility that a higher BMI and older age in NAFLD patients may have contributed to the development of the disease. Liver biopsy remains the gold standard for the diagnosis of NAFLD, but its usage is strictly clinical and depends on patient consent (Brunt et al., 2011; Cheung and Sanyal, 2009). In this context, a thorough and updated protocol of liver biopsy histological parameters was used to rank NASH and

Table 5
Clinical and liver histopathologic findings of NAFLD patients according to genotype of Leu162Val and Pro12Ala SNPs.

	SNP Leu162Val (n = 103)			SNP Pro12Ala (n = 103)		
	Homozygote Leu162Leu (n = 93)	Heterozygote Leu162Val (n = 10)	p-Values	Homozygote Pro12Pro (n = 87)	Heterozygote Pro12Ala (n = 16)	p-Values
Frequency of comorbidities [n, (%)]						
Obesity	79 (85)	8 (80)	0.68	74 (85)	13 (81)	0.71
T2DM	37 (49)	4 (40)	0.99	35 (40)	6 (38)	1.00
Dyslipidemia	78 (84)	9 (90)	0.61	67 (77)	16 (100)	0.07
IR	74 (82)	6 (75)	0.61	67 (77)	12 (75)	1.00
Histologic classification [n, (%)]						
Moderate steatosis	31 (33)	5 (50)	0.31	29 (33)	7 (44)	0.57
Severe steatosis	62 (67)	5 (50)		58 (67)	9 (56)	
Moderate lobular inflammation	32 (34)	2 (20)	0.49	28 (32)	6 (37)	0.77
Severe lobular inflammation	61 (66)	8 (80)		59 (68)	10 (62)	
Moderate ballooning	38 (41)	3 (30)	0.74	34 (39)	7 (44)	0.78
Severe ballooning	55 (59)	7 (70)		53 (61)	9 (56)	
Moderate fibrosis	41 (45)	1 (10)	0.04	35 (40)	7 (44)	0.79
Severe fibrosis	52 (56)	9 (90)		52 (60)	9 (56)	

steatosis, thus allowing the analysis in patient subgroups. Other SNPs of *PPARα* and *PPARγ*, apart from other genes, could be involved in the susceptibility (Chen et al., 2008) and progression (Rotman et al., 2010) of NAFLD. Gawrieh et al. (2012) did not find any individual effects of Pro12Ala and C1431T SNPs on the risk of developing NAFLD and NASH, though the simultaneous occurrence of these SNPs raised the susceptibility to liver disease. In this same study, it was shown that the GT haplotype, which comprises both minor alleles, was associated with inflammatory and fibrotic alterations, which in turn indicate more advanced NAFLD (Gawrieh et al., 2012).

In conclusion, we showed that 12Ala allele of *PPARγ* was less prevalent among NASH patients than the Control Group. There were no associations among *PPARγ* SNPs (Pro12Ala) and clinical, laboratorial and histological parameters in NAFLD patients. However, we documented more advanced fibrosis in carriers of the Leu162Val *PPARα* SNP. The obtained data suggest that the Pro12Ala SNP may result in protection against liver injury and that the Leu162Val SNP may be involved in the progression of NAFLD.

Disclosure

The authors declared no conflict of interest.

Acknowledgments

Financial support from CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) is acknowledged.

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