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FABP-2 and PPAR-γ Haplotype as Risk Factors for Dyslipidemia in a Type 2 Diabetes Mellitus Population of Santa Rosa del Conlara, San Luis, Argentina

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Abstract

Introduction: Type 2 Diabetes Mellitus (T2DM) is a complex disorder caused by the interaction between genetic predisposition and environmental factors. Genetics plays an important role on lipid homeostasis. Many genes are involved in the lipid metabolism, such as FABP-2 and PPAR-γ. Aim: To evaluate the association between specific SNPs and haplotypes of the FABP-2 and PPAR-γ genes with T2DM and lipid profile in an Argentinean population. Methods: The FABP-2 (rs1799883) and PPAR-γ (rs1801282) polymorphisms were genotyped and analyzed in association with lipid profile and T2DM, separately and also combined in haplotypes. Results: The frequency of the rare Thr54 allele of the FABP-2 polymorphism in control (0.33) was not different from the frequency in T2DM (0.27), whereas the frequency of the rare Ala12 allele of the PPAR-γ polymorphism in control was different from the frequency in T2DM (0.26 and 0.14, respectively; p = 0.0031). Frequencies of haplotypes for these two single-nucleotide polymorphisms differed significantly in control and T2DM. Haplotype association analysis showed the associations between ThrPro haplotype and TG levels (OR = 2.520; 95% CI = 1.139 - 5.575; p = 0.027) and between ThrPro haplotype and TC and LDL-c levels when compared to AlaPro haplotype (difference = 0.175, 95% CI = 0068 - 0.499, p < 0.0001; difference = 0.052, 95% CI = 0.017 - 0.158, p < 0.0001, respectively). Conclusions: These results from a haplotype analysis show for the first time that genetic combinations of alleles of the FABP-2 and PPAR-γ gene could play a role in the susceptibility to develop dyslipemia in T2DM.

Keywords

Type 2 Diabetes Mellitus, FABP-2, PPAR-γ, Polymorphism, Haplotypes, Dyslipemia

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Subject Areas: **Diabetes & Endocrinology, Medical Genetics**

1. Introduction

According to the World Health Organization (WHO), 346 million people worldwide have diabetes [1]. The nationwide prevalence of diabetes in Argentina now tops 8.5%, and is as high as 11.9% [2]. Type 2 diabetes mellitus (T2DM) accounts for almost 90% of all cases of diabetes in adults worldwide.

T2DM is characterized by a cluster of metabolic dysfunctions and cardiovascular risk factors, such as obesity, insulin resistance, dyslipidemia, atherosclerosis, hypertension, prothrombotic state, and endothelial dysfunction collectively known as the metabolic syndrome [3]. Environmental factors (e.g., obesity and sedentary lifestyles) give rise to T2DM [4].

It is well-known that high LDL-cholesterol (LDL-c) and triglycerides (TG) levels and low HDL-cholesterol (HDL-c) levels are strong predictable factors for cardiovascular events [5]. Thus, a dysregulation of metabolic homeostasis, together with an inadequate diet and lifestyle habits leads to alterations in lipid and lipoprotein profiles. The exact cause of dyslipidemia is not known. Genetics plays an important role on lipid homeostasis.

Many genes are involved in the regulation of exogenous and endogenous TGs [6] [7]. Some of the best described gene products act during intestinal absorption of dietary fat [fatty acid-binding protein-2 (FABP-2)] and the storage of excess free fatty acids [peroxisome proliferator-activated receptor γ (PPAR-γ)]. The occurrence of genetic polymorphisms in genes of molecules is strictly involved in regulation of fatty acid uptake and β-oxidation can have influence on lipid homeostasis, acting as risk factors for metabolic disturbances [8].

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors involved in the regulation of several biochemical pathways such as lipid and carbohydrate metabolism, lipoprotein synthesis, adipogenesis and insulin sensitivity [9] [10]. PPAR-γ is a transcription factor with a key role in adipogenesis and insulin sensitization [11]. Frequent mutations in the PPAR-γ gene have been described to be associated with obesity and diabetes-related phenotypes [12]-[14]. The common structural polymorphism with a proline (Pro) to alanine (Ala) substitution has been identified as a functional variant. Compared with the Pro allele, the Ala allele associates with reduced activity of PPAR-γ [12]. This polymorphism was extensively investigated for association with obesity and type 2 diabetes and is considered to be one of the best replicated genetic risk factors of common type 2 diabetes, carrying the Ala variant being protective against type 2 diabetes [15]-[17].

Absorption of fatty acids (FA) across the intestinal mucosa, especially long-chain FA, is carried out by the intestinal FA-binding protein (FABP) encoded by the FABP-2 gene [18]. A polymorphism at codon 54 of the FABP-2 gene, changing alanine to threonine (Ala54Thr), increases the affinity of intestinal FABP for long-chain FA [19]. In subjects without diabetes, the presence of the Ala54Thr polymorphism has been associated with increases in serum postprandial lipids [20]-[23]. Patients with type 2 diabetes, in addition to frequently exhibiting increased serum fasting triglycerides and decreased high-density lipoprotein cholesterol, also have increased postprandial serum triglycerides [24].

The purpose of the present study was to evaluate the association between specific SNPs and haplotypes of the FABP-2 and PPAR-γ genes with T2DM and lipid profile in the Santa Rosa del Conlara populations, San Luis, Argentina.

2. Materials and Methods

2.1. Study Population

The present study was carried out in accordance to the guidelines of the Helsinki Declaration. A total of 192 volunteers (100 patients with type 2 diabetes and 92 healthy age-matched controls) participated in this investigation. Criteria published by the Report of the Committee on the Classification and Diagnostic Criteria of Diabetes Mellitus, were used to diagnose Type 2 Diabetes Mellitus [25]. These patients reside in Santa Rosa del Conlara, San Luis, Argentina. The protocol for this study was approved by the local Institutional Review Board, and a written informed consent was obtained from each patient to be enrolled. Exclusion criteria included renal, hepatic or cerebrovascular disorders or endocrinal disorders, females on estrogen therapy and chronic disorders, as well as the use of lipid-lowering drugs, which can affect glucose metabolism and/or increase insulin resistance.

2.2. Anthropometric and Clinical Data

For each subject enrolled, were sized height (meters) and weight (Kg) measurements. Height and weight were measured to the nearest 0.5 cm and 0.1 Kg, respectively. The body mass index (BMI) was calculated as weight divided by height squared (Kg/m^2).

2.3. Blood Sampling

Blood samples were obtained from patients that had fasted overnight for a minimum of 12 h. Blood was collected in tubes containing 0.1% EDTA. Plasma and blood cells were separated by centrifugation at 2400 rpm for 20 min at room temperature. Plasma and packed blood cells were aliquoted and stored at −20˚C until use.

2.4. Biochemical Measurement

Fasting plasma glucose (FPG) was measured by using a glucose oxidase method with a commercial enzymatic kit (Wiener Laboratories, Rosario, Argentina). Glycated hemoglobin (HbA1c) concentration was measured with a coupled ionic-exchange chromatography/spectrophotometric assay (BioSystems, Barcelona, Spain). Total cholesterol (TC), triglycerides (TG) and HDL-c concentrations were measured using commercial kits by following manufacturer's instructions (Wiener Laboratories). Low density lipoprotein-cholesterol (LDL-c) was calculated with the Friedewald formula: LDL-c = total cholesterol (mg/dL) − HDL-c (mg/dL) − triglycerides (mg/dL)/5 [26].

2.5. Definitions

The criteria for lipid and lipoprotein levels were according to the National Cholesterol Education Program [27]. Participants were diagnosed with dyslipidemia if they had one or more of the following criteria: a plasma concentration of TC of ≥6.24 mmol/L (≥240 mg/dL), plasma concentration of TG ≥ 2.26 mmol/L (≥198 mg/dL); plasma concentration of HDL-c of <1.04 mmol/L (<40 mg/dL) for men or <1.30 mmol/L (<50 mg/dL) for women; and a plasma concentration of LDL-c ≥4.14 mmol/L (≥160 mg/dL).

2.6. Genotyping

DNA was extracted from packed blood cells using the Qiagen QiAmp Mini Kit (Valencia, CA). PCR amplifications and genotype determinations were conducted as follows:

FABP-2 (Ala54Thr). Ala54Thr (G/A) in exon 2 of FABP2 (rs1799883). DNA was amplified in a total volume of 20 µl containing 100 ng of genomic DNA, 20 pmol of each primer and 1 unit of AmpliTaq Gold DNA polymerase (Perkin-Elmer, Waltham, MA). These reactions were performed in a buffer containing 1.5 mmol/L $MgCl_2$, 50 mmol/L KCl, 20 mmol/L Tris-HCl (pH 8.4), and 200 pmol/L of each deoxynucleotide triphosphate. The primers were as follows: forward primer: 5'ACAGGTGTTAATATAGTGAAAAG3' and reverse primer: 5'TACCCTGAGTTCAGTTCCGTC3' [19]. The template DNA was denatured for 3 minutes at 95˚C before undergoing 30 cycles of amplification. Each amplification cycle included: denaturation for 30 seconds at 95˚C, primer annealing for 30 seconds at 55˚C, and extension for 45 seconds at 72˚C; followed by a final extension at 72˚C for 3 minutes. For Restriction Fragment Length Polymorphism (RFLP) analysis, 5 µl of PCR product were incubated with 0.4 µl of enzyme *CfoI* (10 U/µl, Promega, Madison, WI) in a final volume of 10 µl for 1 hour at 37˚C. The products were run on a 10% non-denaturing PAGE for 50 minutes at 110 V. Bands were observed after Ethidium bromide staining and UV light exposure. Visualization of two DNA fragments of the *CfoI* treated amplicon at 99 and 81 bp indicates a G allele (Ala54—presence of the restriction site), whereas an intact 180 bp indicates an A allele (Thr54—absence of the restriction site) (**Figure 1**).

PPAR-γ (Pro12Ala). The PPAR-γ polymorphism (rs1801282) Pro12Ala (C/G) was analyzed by Tetra Primer AMRS-PCR [28]. Two pairs of primers were used, one which amplifies a fragment of 553 bp, common to both alleles (outer primers: forward 5'AGACAGTGTGGCAATATTTTCCCTGTAA3' and reverse 5'GGTTC TGAACATGTTTTTAAATGAACGC3' and another pair specific for the SNP (inner primers): forward 5'GAAACTCTGGGAGATTCTCCTATTGTCC3' for the C allele (Pro12) and reverse 5'GTATCAGTGA AGGAATCGC TTTCAGC3' for the G allele (Ala12). Nucleotide sequence and SNP details were obtained from NCBI website (http://www.ncbi.nlm.nih.gob). The primers were designed "in silico" in a free access web

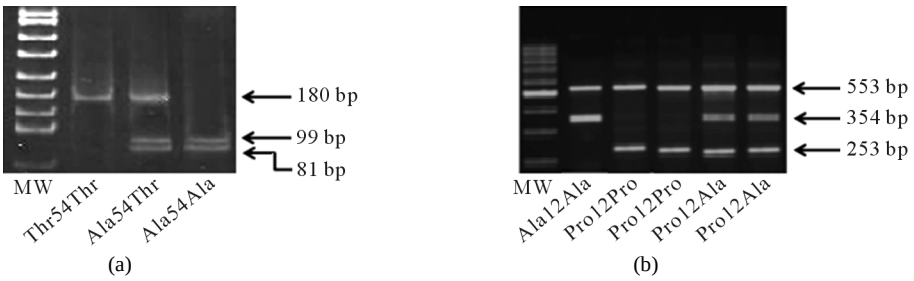

Figure 1. Representative gels of polymorphisms. (a) PCR-RFLP non-denaturing PAGE gel after digestion with *CfoI* enzyme. The 180 bp band corresponds to the G allele (Ala) and the 99 and 81 bp bands correspond to the A allele (Thr) of the FABP2 gene; (b) Results of PPAR-γ genotyping by Tetra Primer ARMS-PCR. The 553 bp band is the product of the outer primers, the 354 bp band, of an outer primer and the inner primer for allele G (Ala) and the 253 bp band, of the other outer primer and the inner primer for the C allele (Pro).

(http://cedar.genetics.soton.ac.uk) and then checked for specificity (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Each PCR reaction was carried out in a total volume of 35 µL, containing 200 ng of template DNA, 10 pmol of each inner primer, 1 pmol of each outer primer (1:10 ratio of outer to inner primer), 200 µM dNTPs, 2.5 mM $MgCl_2$, 1× buffer, and 1 unit of AmpliTaq Gold DNA polymerase (Perkin-Elmer). The template DNA was denatured for 3 minutes at 95˚C before undergoing 35 cycles of denaturation for 1 minute at 95˚C, primer annealing for 1 minute at 61˚C, and extension for 1 minute at 72˚C, and final extension at 72˚C for 3 minutes. The resultant products obtained after PCR were separated by electrophoresis on 2.5% agarose gel containing GelRed. The image was visualized and photographed under UV transillumination. This resulted in 3 DNA fragments: one of 553 bp, one of 253 bp for the C allele (Pro12) and one of 354 bp for the G allele (Ala12) (**Figure 1**).

Randomly selected 20% of samples were re-genotyped for cross validating initial genotypes. In case of unclear genotyping results, the samples were repeated again in duplicates till clear genotype was available. Unclear genotyping results, even after repetition was excluded from the study. No genotyping error was observed during cross validation.

2.7. Statistical Analysis

Allele frequencies for SNPs were calculated by allele counting. Chi square test was used to check adjustment of the data to the Hardy-Weinberg equilibrium and to compare the allelic frequencies between controls and diabetic subjects. Comparison of allele frequencies and genotype distributions between case and control samples were done by Pearson's chi-square test. To analyze the association between genotypes, clinical and biochemical parameters a Student *t*-test was used when variables were continuous, whereas a Fisher's exact test was used for the categorical variables. Tests for association of each SNP or haplotype with T2DM were performed by using the SNPStat software. A $p < 0.05$ was considered to be statically significant.

3. Results

3.1. Subject Characteristics

The anthropometric and clinical characteristics of the subjects in our study are shown in **Table 1**.

Table 2 provides detailed information of the selected SNPs, including their features, allelic variants, and the minor allele frequencies.

The frequencies of the Ala54Ala, Ala54Thr and Thr54Thr genotypes in the whole population of the FABP-2 polymorphism were 47.92%, 44.79% and 7.29%, respectively, while the frequencies of Pro12Pro, Pro12Ala and Ala12Ala genotypes of the PPAR-γ polymorphism were 66.67%, 27.08% and 6.25%, respectively. The distribution and the allele frequency of the 2 polymorphisms followed the Hardy-Weinberg equilibrium.

3.2. Inheritance Model

When genotypes of FABP-2 were associated with T2DM according to all possible genetic models, no association of FABP-2 Ala54Thr polymorphism was found with T2DM according to any genetic model used (**Table 3**).

Table 1. General characteristics of the studied subjects.

	Control subjects (n = 92)	Diabetic subjects (n = 100)	p
Age (years)	55.45 ± 12.16	59.12 ± 8.41	0.1808
BMI (kg/m^2)	27.11 ± 5.31	33.35 ± 8.70	<0.0001
FPG (mg/dL)	87.52 ± 15.65	209.13 ± 125.24	<0.0001
HbA1c (%)	5.41 ± 0.66	8.78 ± 2.95	<0.0001
TC (mg/dL)	197.93 ± 28.74	257.84 ± 61.18	<0.0001
HDL-c (mg/dL)	44.87 ± 5.49	39.34 ± 6.94	0.0093
LDL-c (mg/dL)	113.98 ± 31.27	140.12 ± 49.50	0.0023
TG (mg/dL)	154.52 ± 53.25	250.74 ± 86.89	0.0001

Data are shown as mean ± SD. Abbreviations used: BMI, body mass index; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triglycerides.

Table 2. Description of the selected SNPs for FABP-2 and PPAR-γ genes.

SNP ID	Chromosome	Position	Exon/Intron	Substitution	Functional Consequence	MAF[a]
FABP-2 rs1799883	4	120241902	Exon 2	G/A	Missense	0.30
PPAR-γ rs1801282	3	12393125	Exon A1	C/G	Missense	0.25

[a]MAF in the total group of this study. MAF, minor allele frequency; SNP, single nucleotide polymorphism.

Table 3. Frequencies of FABP2 genotypes according to Type 2 Diabetes Mellitus.

Model[a]	Genotype[b]	Control subjects n (%)[c]	Diabetic subjects n (%)[c]	p	Hom OR[d] (95% CI)
Codominant	AlaAla	42 (45.6)	50 (50.0)	0.31	1
	AlaThr	40 (43.5)	46 (46.0)		0.94 (0.41 - 2.20)
	ThrThr	10 (10.9)	4 (4.0)		0.27 (0.05 - 1.60)
Dominant	AlaAla	42 (45.6)	50 (50.0)	0.60	1
	AlaThr/ThrThr	50 (54.4)	50 (50.0)		0.80 (0.36 - 1.81)
Recessive	AlaAla/AlaThr	82 (89.19	96 (96.0)	0.12	1
	ThrThr	10 (10.9)	4 (4.0)		0.28 (0.05 - 1.56)
Overdominant	AlaAla/ThrThr	52 (56.5)	54 (54.0)	0.80	1
	AlaThr	40 (43.5)	46 (46.0)		1.11 (0.49 - 2.51)
Log-additive	-	-	-	0.29	0.70 (0.36 - 1.36)

[a]Inherintance models; [b]Genotypes and their groupings for the FABP-2 polymorfism (rs1799883); [c]Genotype frequency expressed as number of individuals, n-values in parentheses indicate percentage; [d]OR = odds ratio, 95% CI = 95% confidence interval.

For the PPAR-γ polymorphism, no association was found with T2DM according to any genetic model used (**Table 4**).

As shown in **Table 5**, the frequency of the rare Thr54 allele of the FABP-2 polymorphism in control (0.33) was not different from the frequency in T2DM (0.27), whereas the frequency of the rare Ala12 allele of the PPAR-γ polymorphism in control was different from the frequency in T2DM (0.26 and 0.14, respectively; p = 0.0031).

Due to very low frequencies of the homocygotes genotypes, the Thr54Thr of the FABP-2 and the Ala12Ala of

Table 4. Frequencies of PPAR-γ genotypes according to Type 2 Diabetes Mellitus.

Model[a]	Genotype[b]	Control subjects n (%)[c]	Diabetic subjects n (%)[c]	p	Hom OR[d] (95% CI)
Codominant	ProPro	52 (56.5)	76 (76.0)	0.19	1
	ProAla	32 (34.8)	20 (20.0)		0.46 (0.18 - 1.18)
	AlaAla	8 (8.7)	4 (4.0)		0.38 (0.06 - 2.25)
Dominant	ProPro	52 (56.5)	76 (76.0)	0.069	0.44 (0.18 - 1.08)
	ProAla/AlaAla	40 (43.5)	24 (24.0)		
Reccesive	ProPro/ProAla	84 (91.3)	96 (96.0)	0.41	0.48 (0.08 - 2.83)
	AlaAla	8 (8.7)	4 (4.0)		
Overdominant	ProPro/AlaAla	60 (65.2)	80 (80.0)	0.14	1
	ProAla	32 (34.8)	20 (20.0)		0.50 (0.20 - 1.28)
Log-additive	-	-	-	0.078	0.53 (0.26 - 1.09)

[a]Inherintance models; [b]Genotypes and their groupings for the PPAR-γ polymorfism (rs1801282); [c]Genotype frequency expressed as number of individuals, n-values in parentheses indicate percentage; [d]OR = odds ratio, 95% CI = 95% confidence interval.

Table 5. Genotypes and allele frequencies for the FABP-2 and PPAR-γ polymorphisms in control and Type 2 Diabetes Mellitus.

SNP	Allele	Control subjects F[a]	Duabetic subjects F[a]	Genotype	Control subjects n (%)[b]	Diabetic subjects n (%)[b]	Allelic OR[c] (95% CI)
rs1799883 (FABP-2)	Ala	0.67	0.73	AlaAla	42 (45.65)	50 (50.0)	p = 0.263
				AlaThr	40 (43.48)	46 (46.0)	0.76
	Thr	0.33	0.27	ThrThr	10 (10.87)	4 (4.0)	(0.76 - 1.18)
rs1801282 (PPAR-γ)	Pro	0.74	0.86	ProPro	52 (56.52)	76 (76.0)	p = 0.0031
				ProAla	32 (34.78)	20 (20.0)	0.45
	Ala	0.26	0.14	AlaAla	8 (8.69)	4 (4.0)	(0.27 - 0.76)

[a]F = Allele frequency; [b]Genotype frequency expressed as number of individuals, n-values in parentheses indicate percentage; [c]OR = odds ratio, 95% CI = 95% confidence interval.

the PPAR-γ were analyzed in the same category as the corresponding heterozygotic genotype, namely as non Ala54Ala (Thr carriers) and non Pro12Pro (Ala carriers), respectively.

The anthropometric and clinical characteristics of controls and T2DM for the Ala54Thr polymorphism (rs1799883) of the FABP-2 gene are shown in **Table 6**. There were no differences between control patients with Ala54Ala and non-Ala54Ala genotypes, whereas in T2DM the presence of the non-Ala54Ala genotype was associated with an increase in BMI compared with the Ala54Ala homocygotes.

The comparison of the anthropometric and clinical characteristics by the polymorphism PPAR-γ (rs1801282) in controls and T2DM are shown in **Table 7**. No statistically significant associations with anthropometric and clinical characteristics were observed for this polymorphism in controls. However, in diabetic subjects, there were statistically significant differences in total cholesterol (TC) values, with carriers of the Ala12 allele (non Pro12Pro) having higher TC values than Pro12 homozygotes (p = 0.0056).

3.3. Haplotype Analyses of PPAR-γ and FABP-2 SNPs

To further evaluate the role of these SNPs with T2DM, we sought to determine whether these SNPs demonstrated any evidence of association when examined together by performing haplotype analysis.

The two SNPs (rs1799883 and rs1801282) defined four haplotypes. Estimated frequencies of the four common haplotypes with respect to the presence of T2DM are shown in **Table 8**. The haplotypes that consisted of two common alleles at SNP rs1799883 and rs1801282 (Ala-Pro) were more frequent in T2DM subjects than in control subjects. Conversely, the haplotypes carrying one of the minor alleles at SNP rs7903146 or rs12255372

Table 6. Anthropometric and clinical characteristics by the FABP-2 polymorphism in controls and Type 2 Diabetes Mellitus.

	Control subjects (n = 92)		p	Diabetic subjects (n = 100)		p
	Ala54Ala	Non Ala54Ala		Ala54Ala	Non Ala54Ala	
BMI (kg/m^2)	27.67 ± 4.75	26.64 ± 5.79	0.2948	30.12 ± 5.28	36.59 ± 10.23	0.0161
FPG (mg/dL)	90.10 ± 15.01	85.36 ± 16.15	0.4203	218.84 ± 148.43	198.57 ± 96.19	0.9506
HbA1c (%)	5.29 ± 0.61	5.54 ± 0.72	0.3960	7.28 ± 2.06	9.13 ± 3.06	0.2263
TG (mg/dL)	167.14 ± 58.73	143.92 ± 46.76	0.1637	246.16 ± 80.18	255.32 ± 94.57	0.7123
TC (mg/dL)	214.14 ± 41.84	201.92 ± 33.95	0.3748	244.92 ± 78.87	235.92 ± 72.60	0.8158
HDL-c (mg/dL)	44.86 ± 5.31	44.88 ± 5.75	0.9824	38.12 ± 6.02	40.56 ± 7.21	0.346
LDL-c (mg/dL)	120.05 ± 35.97	108.88 ± 26.37	0.1402	147.28 ± 51.16	132.96 ± 47.73	0.1301

Data are shown as mean ± SD. Abbreviations used: BMI, body mass index; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triglycerides.

Table 7. Anthropometric and clinical characteristics by the PPAR-γ polymorphism in controls and Type 2 Diabetes Mellitus.

	Control subjects (n = 92)		p	Diabetic subjects (n = 100)		p
	Pro12Pro	Non Pro12Pro		Pro12Pro	Non Pro12Pro	
BMI (kg/m^2)	25.99 ± 4.86	28.56 ± 5.63	0.1106	33.59 ± 9.03	32.58 ± 7.85	0.8469
FPG (mg/dL)	82.92 ± 14.76	93.50 ± 15.06	0.0198	202.46 ± 126.12	231.55 ± 125.48	0.4692
HbA1c (%)	5.22 ± 0.60	5.66 ± 0.69	0.1642	9.32 ± 2.47	8.61 ± 3.14	0.3634
TG (mg/dL)	143.00 ± 44.56	169.50 ± 60.72	0.1457	243.55 ± 85.68	273.50 ± 90.55	0.2705
TC (mg/dL)	191.96 ± 24.70	205.70 ± 32.27	0.1702	244.92 ± 54.92	298.75 ± 64.21	0.0056
HDL-c (mg/dL)	44.08 ± 4.97	45.90 ± 6.08	0.2713	39.79 ± 7.15	37.92 ± 6.33	0.3627
LDL-c (mg/dL)	114.85 ± 35.10	112.85 ± 26.30	0.3847	133.21 ± 47.29	162.00 ± 52.00	0.0709

Data are shown as mean ± SD. Abbreviations used: BMI, body mass index; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; TC, total cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triglycerides.

Table 8. Estimated frequency of common haplotypes and their associations with Type 2 Diabetes Mellitus.

Haplotype		Control subjects F[a]	Diabetic subjects F[a]	OR (95% CI)[b]	p
Ala54Thr (rs1799883)	Pro12Ala (rs1801282)				
Ala	Pro	0.4712	0.6831	1	-
Ala	Ala	0.2028	0.0469	0.14 (0.04 - 0.53)	0.0045
Thr	Pro	0.268	0.1769	0.42 (0.18 - 0.97)	0.045
Thr	Ala	0.0581	0.0931	1.31 (0.30 - 5.73)	0.72

[a]F = haplotype frequency; [b]OR = odds ratio, 95% CI = 95% confidence interval were calculated by comparing each haplotype to the more common haplotype.

were more frequent in the control group.

We further examined the potential associations between dyslipidemia and haplotypes of rs1799883 and rs1801282 in T2DM patients. Haplotype association analysis showed the associations between ThrPro haplotype and TG levels (OR = 2.520; 95% CI = 1.139 - 5.575; p = 0.027) and between ThrPro haplotype and TC and LDL-c levels when compared to AlaPro haplotype (difference = 0.175, 95% CI = 0068 - 0.499, p < 0.0001; difference = 0.052, 95% CI = 0.017 - 0.158, p < 0.0001, respectively; **Table 9**).

Table 9. Haplotypes of FABP-2 rs1799883 and PPAR-γ rs1801282 polymorphisms and association with dyslipidemia in diabetic patients.

	Ala54Thr (rs1799883)	Pro12Ala (rs1801282)	Haplotype Frequency	OR (95% CI)	p
TC	Ala	Pro	0.570	1	
	Ala	Ala	0.055	0.272 (0.053 - 1.396)	0.146
	Thr	Pro	0.259	0.175 (0.068 - 0.499)	<0.0001
	Thr	Ala	0.111	0.544 (0.209 - 1.414)	0.2368
LDL-c	Ala	Pro	0.488	1	
	Ala	Ala	0.036	0.680 (0.155 - 2.983)	0.693
	Thr	Pro	0.360	0.052 (0.017 - 0.158)	<0.0001
	Thr	Ala	0.097	0.612 (0.233 - 1.611)	0.312
TG	Ala	Pro	0.701	1	
	Ala	Ala	0.052	0.209 (0.012 - 3.732)	0.207
	Thr	Pro	0.130	2.520 (1.139 - 5.575)	0.027
	Thr	Ala	0.017	0.400 (0.08 - 1.822)	0.371

OR = odds ratio, 95% CI = 95% confidence interval were calculated by comparing each haplotype to the more common haplotype.

4. Discussion

The FABP-2 gene has been proposed as a candidate gene for diabetes because the protein is involved in fatty acids absorption and metabolism and may, therefore, affect insulin sensitivity and glucose metabolism. The most extensively studied variant is the missense Ala54Thr variation, which is common in diverse populations and results in increased fatty acid absorption *in vivo*.

There are no previous reports about the prevalence of the Thr54 FABP-2 variant in our region. In the sample tested, the observed Thr54 frequency (0.30) is similar to that reported in most populations [29], but the Thr54 allele frequencies were slightly higher than those reported in different European countries (0.276) [30]. It must be stated that the study group is mostly of colonizing European populations origin, mainly from Italy and Spain, and a 2% of this group population have aboriginal ethnic ancestries suggesting that the original European allele frequency in this area has not conserved. The fact that the polymorphism is in Hardy Weinberg equilibrium suggests that there is no significant natural selection pressure acting against individuals with the Thr54 FABP-2 variant living in Santa Rosa del Conlara, San Luis, Argentina. Other South American frequency analysis of Thr54 were reported by an Argentinean group, who observed a Thr54 frequency of 0.277 in subjects belonged to an ongoing Regional Cardiovascular Prevention Program (RCP program), organized by the Public Health Care Program for Government Employees (OSEP) of Mendoza (Argentina) [31].

In our sample of patients with type 2 diabetes, the frequency of the Thr54 allele was 0.27 who was similar to a Brazilian study that reported a Thr54 frequency = 0.25 in diabetes type 2 individuals [32]. In the other hand, the frequency of the Thr54Thr genotype was 4.00% in our T2DM patients, whereas the frequency of the Thr54Thr genotype is 6.25% in Brazilian diabetic patients [32] and 4.00% in American patients with type 2 diabetes [33].

To investigate the hypothesis that defects in the FABP-2 gene could be associated with type 2 diabetes mellitus and their related metabolic traits, we studied the effects of Ala54Thr variant in Santa Rosa del Conlara population.

Studies examining the association of FABP-2 A54T polymorphism with T2DM are contradictory. Several studies have reported the association between the Ala54Thr polymorphism of FABP-2 with insulin resistance and diabetes [19] [34]-[39]. Other studies have reported no association between this polymorphism and T2DM [19] [31] [40]-[45]. There are differences among studies on the design as well as the genetic models used to assess associations of FABP-2 Ala54Thr polymorphism with this disease. We looked at the association of FABP-2 Ala54Thr polymorphism with T2DM according to all possible genetic models. No association of FABP-2

Ala54Thr polymorphism was found with T2DM according to any genetic model used, a finding shared by other studies that examined such an association [46].

In the present study we have not found differences in glycemia, HbA1c and serum lipids between FABP-2 genotype groups although significant differences in BMI were attained in Diabetic group carriers of the Thr allele. The results from studies published on the association of FABP-2 Ala54Thr polymorphism with body mass index (BMI) are conflicting, but the conclusions from the meta analysis carried out by Zhao *et al.* [47] showed no evidence that the FABP-2 Ala54Thr polymorphism is significantly associated with BMI in overall populations. It is possible that carriers of the Thr allele in our T2DM patients confers some degree of susceptibility to obesity, associated with an influence of gene/environment interactions such as diet, exercise, body composition and life style modification [46] [48].

In this study, the frequency of the Ala allele of the PPAR-γ polymorphism in participants with and without diabetes were 1.4% and 2.6%, respectively. The frequency of the Ala allele appears to vary greatly by the genetic background of the populations [49]. In general, the frequency of the Ala allele has been reported to be highest in Caucasians [50]. Variations in the allelic frequency of the Pro12Ala polymorphism across different ethnicities and regions could be attributed to genetic variations and to different environmental and lifestyle exposures [51].

We found that the Ala allele of the Pro12Ala polymorphism was associated with a significantly lower risk of type 2 diabetes in our population. Consistent with our results, Altshuler *et al.* [15] found a significant decrease in diabetes risk associated with the Ala allele in a Caucasian population. In Finnish subjects, the Ala12 variant of the PPAR-γ gene was associated with protection against T2DM [51]. Meta-analyses [4] [52] also showed a significant effect of the Ala allele on lower development of T2DM.

In the present study we have not found differences in glycemia, HbA1c, LDL-c, HDL-c, TG and BMI between PPAR-γ genotype groups although significant differences in TC were attained in Diabetic group carriers of the Ala allele. Mori *et al.* [53] found that carriers of the Ala12 allele had higher total cholesterol than those without the allele among diabetic subjects. One study in an Italian population [54] found that this polymorphism was not associated with anthropometrical and biochemical parameters among normoglycemic and diabetic subjects. Ethnic differences, study design, and effects of BMI may contribute to discrepancies in these results.

The substitution from proline to alanine at codon 12 has been shown to regulate transcriptional activity [12] [55]. Because this polymorphism is next to the amino-terminus of the protein in the ligand-independent activation domain, its activity is induced by insulin through phosphorylation. Alanine helps the formation of helices, but proline prevents it. Thus, it is possible that this amino acid change affects the structure and consequently the function of the protein [49]. The alanine isoform contributes to less efficient stimulation of PPAR-γ target genes and predisposes people to reduce levels of adipose tissue mass accumulation. This in turn may improve insulin sensitivity. It is known that decreased insulin sensitivity plays an important role in the pathogenesis of type 2 diabetes.

Although abundant data on individual gene variants affecting lipid and lipoprotein metabolism are present in the literature, their usefulness for identifying individual profiles for T2DM risk and its altered lipid profile is fairly limited. This is attributable to the small effect that a single gene has, which in addition can vary depending on factors such as life style, environmental stimuli, and other genes.

Further, we aimed to investigate the haplotype association of the variant at the FABP-2 (rs1799883 polymorphisms) and PPAR-γ (rs1801282) polymorphisms) locus with Type 2 Diabetes Mellitus and the characteristic dyslipidemia of this disease. Haplotype association analysis showed the associations between ThrPro haplotype and TG levels (OR = 2.520; 95% CI = 1.139 - 5.575; p = 0.027) and between ThrPro haplotype and TC and LDL-c levels when compared to AlaPro haplotype (difference = 0.175, 95% CI = 0068 - 0.499, $p < 0.0001$; difference = 0.052, 95% CI = 0.017 - 0.158, $p < 0.0001$, respectively; **Table 9**). There are no studies that associated haplotypes of these two polymorphisms with lipid metabolism alterations. So far, the haplotype analysis regarding rs1799883 and rs1801282 polymorphisms for dyslipidemia has not been reported yet.

Our study found that Thr-Pro haplotype of FABP-2 and PPAR-γ was associated with dyslipidemia. PPAR-γ plays an indispensible role in the regulation of adipocyte differentiation, lipid storage, glucose metabolism and the transcriptional regulation of a number of genes involved in these metabolic processes. The key target genes of PPAR-γ include the fat-specific AP2 gene, LPL (lipoprotein lipase), fatty acid transport protein, fatty acid binding protein, ABC-A1 [ATP-binding cassette, sub-family A (ABC1), member 1] and so on [56].

We thought that rs1801282 polymorphisms (Pro12 alelle) may influence the receptor activity, the ability to

transactivate responsive promoters and so on to regulate the key target genes of PPAR-γ which could influence the lipid metabolism. But the specific biological mechanism needs to be further studied.

In the other hand, FABP-2 is involved in the transport and metabolism of saturated and unsaturated long-chain fatty acids (FAs). The missense Ala54Thr variation results in increased activity. Thus, subjects with the Thr54 allele may increase intestinal absorption of cholesterol, and this is associated with the higher cholesterol and LDL cholesterol levels in those with Thr54 allele among diabetic.

5. Conclusions

In conclusion, our study has tested the gene-gene interaction between common polymorphisms within FABP-2 and PPAR-γ gene and dyslipidemia based on haplotype analyses. These results may help to evaluate their haplotypes as being characterized as genetic risk factors for dyslipidemia in Type 2 Diabetes Mellitus.

In conclusion, we report that in T2DM the additive effects of Thr-Pro haplotype of FABP-2 and PPAR-γ genes altered lipid metabolism.

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Conflict of Interests

The authors declare no conflict of interests with respect to the present paper.

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