Research Article

Interaction Effects of the Leu162Val PPARα and Pro12Ala PPARγ2 Gene Variants with Renal Function in Metabolic Syndrome Population

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Leu162Val PPARα and Pro12Ala PPARγ2 were investigated for their individual and their interactive impact on MS and renal functionality (RF). 522 subjects were investigated for biochemical and anthropometric measurements. The diagnosis of MS was based on the IDF definition (2009). The HOMA 2 was used to determine HOMA-β, HOMA-S and HOMA-IR from FPG and FPI concentrations. RF was assessed by estimating the GFR. PCR-RFLP was performed for DNA genotyping. Allele frequencies were 0.845 for Pro and 0.155 for Ala, and were 0.915 for Leu and 0.085 for Val. We showed that carriers of the PPARα Val 162 allele had lower urea, UA and higher GFR compared to those homozygous for the Leu162 allele. Subjects carried by PPARγ2Ala allele had similar results. They also had reduced FPG, FPI and HOMA-IR, and elevated HOMA-β and HOMA-S compared to those homozygous for the Pro allele. Subjects were divided into 4 groups according to the combinations of genetic alleles of the 2 polymorphisms. Subjects carrying the Leu/Val with an Ala allele had lower FPG, PPI, HOMA-IR, urea, UA levels, higher HOMA-β, HOMA-S and GFR than different genotype combinations. Leu162Val PPARα and Pro12Ala PPARγ2 can interact with each other to modulate glucose and insulin homeostasis and expand their association with overall better RF.

1. Introduction

Metabolic syndrome (MS) is a complex disorder characterized by the clustering of several metabolic diseases such as abdominal obesity, insulin resistance (IR), elevated plasma triglycerides level (TG), low high density lipoprotein cholesterol (cHDL), high blood pressure, and altered glucose homeostasis [1]. Environmental factors such as low physical activity and inappropriate dietary habits are strong determinants of the MS. In addition, genetic factors also contribute to the individual susceptibility to MS [2].

All components of the MS have individually been associated with the incidence and progression of chronic kidney diseases (CKDs). The mechanisms and impacts of hypertensive and diabetic injuries, the two major etiologies of CKD in the world, have been well studied and described [3–5]. Several observational studies found that individuals with the MS are at increased risk for presenting renal manifestations, namely, microalbuminuria and decreased glomerular filtration rate (GFR). In fact, epidemiological studies have linked MS with an increased risk for microalbuminuria, an early marker of glomerular injury and endothelial dysfunction [6–8].

Peroxisome proliferator-activated receptors (PPARs) are nuclear hormone receptors. They are ligand-dependent intracellular proteins that stimulate transcription of specific genes by binding to specific DNA sequences [9]. There are three PPAR subtypes, products of the distinct genes commonly designated as PPARα, PPARγ, and PPAR β/δ and expressed in various tissues [10–13]. In humans, renal PPARα and PPARγ isotypes are abundantly expressed [14, 15].

In this regard, two common polymorphisms affecting the amino acid sequence of the PPARα and PPARγ2 gene are relevant candidates, the Leu162Val PPARα and Pro12Ala PPARγ2. We, therefore, assessed the potential relationships of these polymorphisms variants for their individual effect as well as their interactive impact on MS and renal injuries.

2. Materials and Methods

2.1. Study Population. 522 subjects undergoing routine control were investigated for biochemical, anthropometric, and clinical examination at the occupational medicine of the University Hospital of Monastir, Tunisia. All the subjects enrolled in this study were coming from central Tunisia and there were no consanguinity relationships among them. Participants gave their written informed consent prior to their participation. The study was approved by the ethical committee of the hospital.

2.2. Diagnostic Criteria for Metabolic Syndrome. The diagnosis of MS was based on the IDF and AHA/NHLBI definition, which requires the presence of at least three of the following criteria: the central (abdominal) obesity (defined as waist circumference (WC) $\geq$94 cm in men and $\geq$80 cm in women), the raised TG $\geq$1.70 mmol/L (drug treatment for elevated triglycerides is an alternate indicator), the reduced cHDL <1.04 mmol/L in men and <1.29 mmol/L in women (or specific treatment for this lipid abnormality), the elevated systolic blood pressure (SBP) $\geq$130 mmHg and/or diastolic blood pressure (DBP) $\geq$85 mmHg (antihypertensive drug treatment in a patient with a history of hypertension was an alternate indicator), and the elevated FPG $\geq$5.56 mmol/L or previously diagnosed type 2 diabetes [16].

2.3. Anthropometric Measurements. Height and weight were measured according to a standardized protocol in the study population, with subjects wearing light clothing and no shoes. Body mass index (BMI) was calculated by dividing weight in kilograms by height in square meters (kg/m^2). The waist circumference was measured in the horizontal plane at the midpoint between the lowest rib and the iliac crest. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured to the nearest 5 mmHg with a mercury sphygmomanometer, with subjects in a supine position and having relaxed for 5 minutes.

2.4. Biochemical Analysis. The blood samples of the study population were collected in the morning after a 12-hour fasting period, heparinazed serum was immediately obtained by blood centrifugation at 4°C at 3000 rpm for 15 min. All analyses were carried out in biochemistry and toxicology laboratory of the Hospital using a Cobas 6000TM analyzer (Roche Diagnostics Mannheim, Germany). Serum total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (cHDL), low density lipoprotein cholesterol (cLDL), and Uric acid (UA) serum levels and fasting plasma

TABLE 1: Anthropometric and biochemical characteristics of study population.

Variables	MS$^-$ ($n = 264$)	MS$^+$ ($n = 258$)	P
Age (years)	41.0 (29.0–51.7)	38.0 (29.0–54.0)	0.695
Gender M/F (%)	137/127 (51.9/48.1)	128/130 (49.6/50.4)	0.602
Diabetes (n (%))	31 (11.7)	71 (27.9)	<0.001
Hypertension (n (%))	36 (13.6)	101 (39.7)	<0.001
SBP (mmHg)	120 (115–125)	140 (130–150)	<0.001
DBP (mmHg)	80 (75–80)	85 (80–90)	<0.001
BMI (kg/m^2)	24.5 (23.7–25.9)	27.7 (26.4–29.4)	<0.001
Men WC (cm)	93 (89–95)	98 (96–101)	<0.001
Women WC (cm)	79 (77–86)	93 (90–98)	<0.001
TG (mmol/L)	1.07 ± 0.44	1.91 ± 0.71	<0.001
TC (mmol/L)	4.51 (4.12–4.99)	5.15 (4.24–5.78)	<0.001
cLDL (mmol/L)	2.92 (2.52–3.31)	3.36 (2.76–3.95)	<0.001
Men cHDL (mmol/L)	1.10 (1.02–1.16)	0.84 (0.74–0.92)	<0.001
Women cHDL (mmol/L)	1.45 (1.34–1.53)	1.10 (0.89–1.26)	<0.001
FPG (mmol/L)	4.94 ± 0.95	6.96 ± 1.78	<0.001
FPI (mIU/L)	7.12 (5.97–8.61)	12.73 (9.59–13.22)	<0.001
HOMA-β%	97 (88–109)	72 (52–94)	<0.001
HOMA-S%	110 (88–130)	56 (53–78)	<0.001
HOMA-IR	0.9 (0.8–1.1)	1.8 (1.3–1.9)	<0.001
Cr (μmol/L)	87.5 ± 31	111.4 ± 46.4	<0.001
Urea (mmol/L)	4.46 ± 2.63	7.72 ± 4.82	<0.001
UA (μmol/L)	267.5 (213.5–307.7)	358 (285–4.28)	<0.001
GFR (mL/min)	92.9 (78.8–107.4)	85.6 (61.9–106.3)	0.004

MS$^-$: without metabolic syndrome; MS$^+$: with metabolic syndrome, SBP: systolic blood pressure, DBP: diastolic blood pressure; BMI: body mass index; WC: waist circumference; TG: triglycerides; TC: total cholesterol; cHDL: high density lipoprotein cholesterol; cLDL: low density lipoprotein cholesterol; FPG: fasting plasma glucose; FPI: fasting plasma insulin; HOMA-β%: % β-cell function; HOMA-S%: % cell insulin sensitivity; HOMA-IR: insulin resistance; Cr: creatinine; UA: uric acid; GFR: glomerular filtration rate.

glucose (FPG) were determined by using enzymatic techniques. Fasting plasma insulin (FPI) was measured by electrochemiluminescence immuno assay (ECLIA), and serum creatinine concentration was determined by the kinetic Jaffe method.

The computer model HOMA 2 was used to determine β-cell function (HOMA-β%), insulin sensitivity (HOMA-S%), and insulin resistance (HOMA-IR) from paired fasting glucose (mmol/L) and insulin (mIU/L) concentrations [17].

Renal function was assessed by estimating the GFR, with the Cockcroft and Gault formula:

$$\text{GFR (mL/min)} = \frac{[140 - \text{age (years)}] \times \text{BM (kg)}}{0.814 \times \text{creatinine } (\mu\text{mol/L})}. \quad (1)$$

In female subjects, the result was multiplied by 0.85 [18].

TABLE 2: Leu162Val PPARα and Pro12Ala PPARγ2 genotype and allele distribution.

	Total (522)	MS⁻ (264)	MS⁺ (258)	P	OR (95% CI)
		Pro12 Ala PPARγ2			
		Allele			
Pro (n (%))	882 (84.5)[a]	430 (81.4)	452 (87.6)	0.006	1.610 (1.144–2.265)
Ala (n (%))	162 (15.5)	98 (18.6)	64 (12.4)	0.006	0.621 (0.442–0.874)
		Genotype			
Pro/Pro (n (%))	369 (70.69)	174 (65.91)	195 (75.58)	0.015	1.601 (1.093–2.344)
Pro/Ala (n (%))	144 (27.59)	82 (31.06)	62 (24.03)	0.046	0.675 (0.458–0.995)
Ala/Ala (n (%))	9 (1.72)	8 (3.03)	1(0.39)	0.013	0.112 (0.014–0.901)
		Leu162Val PPARα			
		Allele			
Leu (n (%))	956 (91.6)	471 (89.2)	485 (93.9)	0.005	1.893 (1.201–2.985)
Val (n (%))	88 (8.4)	57 (10.8)	31 (6.1)	0.005	0.528 (0.335–0.833)
		Genotype			
Leu/Leu (n (%))	441 (84.48)	213 (80.68)	228 (88.37)	0.015	1.820 (1.117–2.985)
Leu/Val (n (%))	74 (14.17)	45 (17.05)	29 (11.24)	0.046	0.602 (0.364–0.995)
Val/Val (n (%))	7 (1.35)	6 (2.27)	1 (0.39)	0.049	0.156 (0.019–1.304)

[a]Number (% of total); SM⁻: without metabolic syndrome; SM⁺: with metabolic syndrome; OR: odds ratio; CI: confidence interval.

TABLE 3: Anthropometric, HOMA index, and biochemical characteristics of subjects with different groups of genotype of the Leu162Val PPARα and Pro12Ala PPARγ2 polymorphisms.

Parameters	Pro12Ala PPARγ2			Leu162Val PPARα		
	P/P (n = 369)	X/A (n =153)	P	L/L (n = 441)	X/V (n = 81)	P
Age (years)	41 (29–53)	38 (28–49)	0.088	39 (29–53)	38 (29–48)	0.177
SBP (mmHg)	125 (120–140)	125 (120–135)	0.102	125 (120–140)	130 (120–135)	0.854
DBP (mmHg)	80 (80–85)	80 (80–85)	0.127	80 (80–85)	80 (80–85)	0.465
BMI (kg/m²)	26.4 (24.4–28.3)	25.6 (24.1–27.9)	0.108	25.9 (24.3–28.2)	26.6 (24.4–29.1)	0.201
WC (cm)	93.0 (85.0–98.0)	93.0 (81.5–97)	0.404	93.0 (83.0–97.0)	94.0 (86.5–98.5)	0.076
TG (mmol/L)	1.51 ± 0.71	1.43 ± 0.76	0.186	1.50 ± 0.72	1.44 ± 0.72	0.497
TC (mmol/L)	4.73 (4.19–5.49)	4.71 (4.11–5.27)	0.271	4.72 (4.19–5.45)	4.71 (4.12–5.41)	0.526
cLDL (mmol/L)	3.09 (2.61–3.71)	3.00 (2.62–3.67)	0.513	3.07 (2.62–3.73)	3.09 (2.57–3.63)	0.410
cHDL (mmol/L)	1.12 (0.88–1.27)	1.09 (0.90–1.34)	0.368	1.11 (0.88–1.29)	1.12 (0.92–1.33)	0.475
FPG (mmol/L)	6.14 ± 1.77	5.45 ± 1.57	<0.001	6.05 ± 1.79	5.36 ± 1.30	0.001
FPI (mIU/L)	9.83 (7.12–12.88)	7.85 (6.16–11.22)	<0.001	9.23 (6.92–12.83)	8.91 (6.65–11.95)	0.139
HOMA-β%	86 (64–100)	95 (82–105)	<0.001	88 (67–99)	95 (80–119)	<0.001
HOMA-S%	77 (54–109)	102 (68–126)	<0.001	80 (55–112)	85 (62–118)	0.043
HOMA-IR	1.3 (0.9–1.8)	1.0 (0.8–1.5)	<0.001	1.2 (0.9–1.8)	1.2 (0.9–1.6)	0.067
Cr (μmol/L)	102.1 ± 45.8	92.5 ± 25.2	0.016	99.6 ± 41.6	97.9 ± 38.2	0.749
Urea (mmol/L)	6.45 ± 4.33	5.17 ± 2.34	0.001	6.29 ± 4.13	4.89 ± 1.83	0.003
UA (μmol/L)	307 (248–385)	292 (234–350)	0.036	306 (247–382)	292 (233–341)	0.042
GFR (mL/min)	89.5 (72.7–105.1)	93.8 (71.6–111.3)	0.004	89.0 (71.5–104.9)	102.6 (76.1–113.5)	0.002

P: Pro; A: Ala; L: Leu; V: Val; X/A: Pro/Ala and Ala/Ala; X/V: Leu/Val and Val/Val.
SBP: systolic blood pressure, DBP: diastolic blood pressure; BMI: body mass index; WC: waist circumference; TG: triglycerides; TC: total cholesterol; cHDL: high density lipoprotein cholesterol; cLDL: low density lipoprotein cholesterol; FPG: fasting plasma glucose; FPI: fasting plasma insulin; HOMA-β%: % β-cell function; HOMA-S%: % cell insulin sensitivity; HOMA-IR: insulin resistance; Cr: creatinine; UA: uric acid; GFR: glomerular filtration rate.

2.5. Genetic Analysis.

Genotyping was carried out on genomic DNA extracted from subjects' blood samples by salt fractionation. The primers used for polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) from the PPAR α Leu 162 Val SNP were 5′-GACTCAAGCTGGTGTATGACAAGT-3′ as the forward primer and the reverse-mismatch primer 5′CGTTGT-GTGACATCCCGACAGAAT-3′ with the mismatch nucleotide in the reverse primer underlined. A mixture of Taq polymerase 10X buffer 2.5 μL, 0.2 μL Taq DNA

TABLE 4: Comparison of anthropometric, HOMA index, and biochemical characteristics of subjects between different groups of Pro12Ala PPARγ2 genotypes in two subdivided groups according to metabolic syndrome.

Parameters	SM⁻ Pro12Ala PPARγ2			SM⁺ Pro12Ala PPARγ2		
	P/P ($n = 174$)	X/A ($n = 90$)	P	P/P ($n = 195$)	X/A ($n = 63$)	P
Age (years)	42 (29–53)	38 (28–49)	0.455	39 (29–56)	37 (28–49)	0.103
SBP (mmHg)	120 (115–125)	120 (115–125)	0.708	140 (130–150)	140 (130–145)	0.675
DBP (mmHg)	80 (75–80)	80 (70–80)	0.277	85 (80–90)	85 (80–90)	0.965
BMI (kg/m^2)	24.5 (23.8–25.9)	24.4 (23.6–25.7)	0.228	27.7 (26.3–29.4)	27.6 (26.5–29.3)	0.940
WC (cm)	87 (79–93)	90 (79–93)	0.758	96 (92–99)	96 (93–99)	0.997
TG (mmol/L)	1.07 ± 0.43	1.07 ± 0.43	0.942	1.91 ± 0.67	1.92 ± 0.84	0.964
TC (mmol/L)	4.51 (4.15–4.85)	4.62 (4.02–5.17)	0.633	5.18 (4.27–5.78)	4.81 (4.19–5.59)	0.190
cLDL (mmol/L)	2.90 (2.54–3.29)	2.98 (2.40–3.53)	0.729	3.48 (2.76–4.10)	3.24 (2.85–3.85)	0.499
cHDL (mmol/L)	1.18 (1.11–1.43)	1.19 (1.04–1.46)	0.868	0.91 (0.79–1.17)	0.92 (0.77–1.14)	0.925
FPG (mmol/L)	4.94 ± 1.09	4.95 ± 0.87	0.946	7.21 ± 1.67	6.16 ± 1.86	<0.001
FPI (mIU/L)	7.13 (6.14–8.65)	6.93 (5.61–8.58)	0.277	12.83 (10.76–13.29)	10.58 (7.92–12.98)	<0.001
HOMA-β%	96 (87–111)	97 (88–106)	0.698	68 (52–88)	86 (71–101)	<0.001
HOMA-S%	108 (87–128)	113 (89–140)	0.270	56 (53–66)	70 (55–103)	<0.001
HOMA-IR	0.9 (0.8–1.2)	0.9 (0.7–1.1)	0.282	1.8 (1.5–1.9)	1.4 (1.0–1.8)	<0.001
Cr (μmol/L)	87.29 ± 36.16	87.87 ± 25.2	0.579	115.35 ± 49.51	99.30 ± 32.42	0.017
Urea (mmol/L)	4.52 ± 3.05	4.35 ± 17.24	0.619	6.33 ± 2.78	8.16 ± 4.58	0.003
UA (μmol/L)	268 (210–313)	266 (215–306)	0.996	358 (285–437)	348 (278–396)	0.062
GFR (mL/min)	93.4 (82.3–106.4)	91.5 (70.5–109.1)	0.192	84.0 (58.8–104.5)	98.8 (78.8–113.8)	0.001

P: Pro; A: Ala; X/A: Pro/Ala and Ala/Ala.
SBP: systolic blood pressure, DBP: diastolic blood pressure; BMI: body mass index; WC: waist circumference; TG: triglycerides; TC: total cholesterol; cHDL: high density lipoprotein cholesterol; cLDL: low density lipoprotein cholesterol; FPG: fasting plasma glucose; FPI: fasting plasma insulin; HOMA-β%: % β-cell function; HOMA-S%: % cell insulin sensitivity; HOMA-IR: insulin resistance; Cr: creatinine; UA: uric acid; GFR: glomerular filtration rate.

polymerase, 2 μL dNTP, 10 pmol of each primer, and 6 μL of template DNA was used at a total volume of 25 μL, and the mixture was amplified in PCR equipment (TECHNE TC-312). The reaction was carried out using 30 cycles of predenaturation at 94°C for 3 min, denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and elongation at 72°C for 5 min. Electrophoresis was conducted in 1% agarose gel to confirm the 117 bp PCR product, and the restriction was performed by using 8 U of a Hinf I enzyme at 37°C overnight. The restriction site was confirmed at 3% agarose gel. The Leu/Leu homozygote produced 1 fragment at 117 bp, the Leu/Val heterozygote produced 3 fragments at 117, 93, and 24 bp, and the Val/Val homozygote produced 2 fragments at 93 and 24 bp. The primers used for PCR-RFLP from the PPARγ2 Pro 12 Ala SNP were 5′-CAAGCCCAGGTCCTTTCTGTG-3′ as the forward primer and 5′-AGTGAAGGAATCGCTTTCCG-3′ as the reverse primer. A mixture of Taq polymerase 10X buffer 2.5 μL, 0.2 μL Taq DNA polymerase, 2 μL dNTP, 10 pmol of each primer, and 6 μL of template DNA was used at a total volume of 25 μL, and the mixture was amplified in PCR equipment (TECHNE TC-312). The reaction was carried out using 30 cycles of predenaturation at 94°C for 3 min, denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and elongation at 72°C for 5 min. Electrophoresis was conducted in 1% agarose gel to confirm the 237 bp PCR product, and the restriction was performed by using 8 U of a HpaII enzyme at 37°C overnight. The restriction site was confirmed at 3% agarose

gel. The Pro/Pro homozygote produced 2 fragments at 217 and 20 bp, the Pro/Ala heterozygote produced 3 fragments at 237, 217, and 20 bp, and the Ala/Ala homozygote produced 1 fragment at 237 bp, which did not splice when the HpaII restriction enzyme was used.

2.6. Statistical Analysis. Data were analyzed by SPSS 17.0 for Windows. Continuous results that satisfied a normal distribution are expressed as mean ± standard deviation (SD). Those results that provided abnormal distribution data are expressed as median and quartile and frequencies for qualitative variables. Comparisons among groups were assessed using the independent-sample t-test for quantitative variables and Pearson's chi-square test for qualitative variables. The one-way analysis of variance (ANOVA) method was used to compare differences between genotype groups. Pearson's chi-square test (χ^2) was used to compare the genotype prevalence between different groups. The Hardy-Weinberg equilibrium was performed using the χ^2 test. A two-sided $P < 0.05$ was considered as statistically significant.

3. Results

Among the 522 subjects who were enrolled, 258 presented metabolic syndrome (SM⁺) and 264 were without (MS⁻). Table 1 shows that SM⁺ subjects have higher BMI, WC, SBP, DBP, TG, cLDL, TC, FPG, and FPI and reduced cHDL

TABLE 5: Comparison of anthropometric, HOMA index, and biochemical characteristics of subjects between different groups of Leu162Val PPARα genotypes in two subdivided groups according to metabolic syndrome.

Parameters	SM⁻ Leu162Val PPARα			SM⁺ Leu162Val PPARα		
	L/L (n = 213)	X/V (n = 51)	P	L/L (n = 228)	X/V (n = 30)	P
Age (years)	42 (29–52)	38 (32–49)	0.566	39 (29–56)	36 (29–45)	0.898
SBP (mmHg)	120 (115–120)	120 (120–130)	0.073	140 (130–150)	135 (130–150)	0.300
DBP (mmHg)	80 (75–80)	80 (75–80)	0.260	85 (80–90)	85 (80–90)	0.090
BMI (kg/m²)	24.5 (23.7–25.8)	25.0 (23.9–28.1)	0.172	27.7 (26.4–29.4)	27.6 (26.5–29.4)	0.400
WC (cm)	86 (79–93)	93 (84–97)	0.658	96 (92–99)	96 (93–100)	0.615
TG (mmol/L)	1.06 ± 0.44	1.09 ± 0.42	0.691	1.90 ± 0.70	2.02 ± 0.77	0.382
TC (mmol/L)	4.51 (4.14–4.93)	4.53 (3.90–5.06)	0.659	5.15 (4.22–5.78)	5.12 (4.23–5.78)	0.330
cLDL (mmol/L)	2.92 (2.52–3.31)	2.97 (2.31–3.40)	0.717	3.42 (2.76–4.03)	3.30 (2.83–3.85)	0.351
cHDL (mmol/L)	1.19 (1.10–1.46)	1.16 (1.04–1.39)	0.777	0.90 (0.79–1.14)	0.95 (0.82–1.28)	0.935
FPG (mmol/L)	4.94 ± 0.96	4.96 ± 0.88	0.846	7.08 ± 1.76	6.02 ± 1.60	0.002
FPI (mIU/L)	7.06 (5.86–8.25)	7.48 (6.17–10.04)	0.626	12.77 (10.09–13.25)	11.26 (7.85–13.03)	0.879
HOMA-β%	97 (88–105)	100 (86–121)	0.283	69 (52–93)	85 (66–106)	0.219
HOMA-S%	111 (95–134)	103 (75–126)	0.602	56 (53–73)	64 (56–99)	0.564
HOMA-IR	0.9 (0.8–1.1)	1.0 (0.8–1.3)	0.761	1.8 (1.4–1.9)	1.6 (1.0–1.8)	0.457
Cr (μmol/L)	86.3 ± 32.6	92.3 ± 22.5	0.216	111.9 ± 45.2	107.6 ± 54.7	0.631
Urea (mmol/L)	4.55 ± 2.87	4.12 ± 1.17	0.303	7.92 ± 4.46	6.02 ± 2.01	0.039
UA (μmol/L)	268 (206–313)	250 (223–302)	0.752	358 (285–433)	348 (288–398)	0.847
GFR (mL/min)	91.7 (79.4–105.8)	99.1 (71.6–109.4)	0.302	84.5 (60.4–104.7)	108.8 (82.1–124.5)	0.679

L, Leu; V, Val; X/V: Leu/Val and Val/Val.
SBP: systolic blood pressure, DBP: diastolic blood pressure; BMI: body mass index; WC: waist circumference; TG: triglycerides; TC: total cholesterol; cHDL: high density lipoprotein cholesterol; cLDL: low density lipoprotein cholesterol; FPG: fasting plasma glucose; FPI: fasting plasma insulin; HOMA-β%: % β-cell function; HOMA-S%: % cell insulin sensitivity; HOMA-IR: insulin resistance; Cr: creatinine; UA: uric acid; GFR: glomerular filtration rate.

compared to subjects MS⁻. We also noted elevated IR and reduced HOMA β and HOMA S and higher creatinine, urea, and UA plasma level and reduced GFR in SM⁺ group.

The Pro/Pro genotype was present in 70.69% (369 subjects); Pro/Ala genotype in 27.59% (144 subjects) and Ala/Ala genotype was present in 1.72% (9 subjects) of 522 subjects. Allele frequencies were 0.845 for Pro allele and 0.155 for Ala allele. Allele frequency of the two genotypes satisfied the Hardy-Weinberg equilibrium. The Leu/Leu genotype was present in 84.48% (441 subjects), Leu/Val genotype in 14.17% (74 subjects), and Val/Val genotype in 1.35% (7 subjects) of 522 subjects. Allele frequencies were 0.916 for Leu allele and 0.084 for Val allele. Allele frequency of the two genotypes satisfied the Hardy-Weinberg equilibrium. The allelic frequency of Ala allele was significantly ($P = 0.005$) lower in MS⁺ group (0.124%) than in MS⁻ group (0.186). Also, the frequency of Val allele was significantly ($P = 0.006$) lower in MS⁺ group (0.061) than in MS⁻ group (0.108). Subjects with the Ala allele had a decreased risk for MS (odds ratio (OR) = 0.621, 95% CI (0.442–0.874)). The Val allele decreases the risk of MS (OR = 0.528, 95% CI (0.335–0.833)) (Table 2).

The independent effects of each polymorphism on anthropometric and biochemical characteristics-related variables are presented in Table 3. There were no differences among the genotypes in terms of age, BMI, blood pressure, TG, TC, cHDL, and cLDL either in X/Ala (Pro/Ala and Ala/Ala) PPARγ2 or X/Val (Leu/Val and Val/Val) PPARα. Subjects with Pro/Pro had a significantly higher FPG, FPI,

and HOMA-IR. In parallel, creatinine, urea, and uric acid serum levels were found to be elevated in these Pro/Pro subjects. In addition, Pro/Pro subjects also display reduced HOMA-β and HOMA-S together with decreased GFR compared to X/Ala (Pro/Ala and Ala/Ala) subjects. Furthermore, subjects with Leu/Leu have only a significantly reduced HOMA-β and elevated creatinine, urea, and uric acid serum levels and a decreased GFR compared to X/Val (Leu/Val and Val/Val) subjects.

To check if the effects of the SNPs are associated with MS, subjects were divided into people displaying metabolic syndrome (SM⁺) or not (MS⁻) (Tables 4 and 5). For Pro12Ala PPARγ2 gene in the SM⁺ group, Ala allele carriers had higher HOMA-β, HOMA-S and GFR, and decreased FPG, FPI, HOMA-IR, creatinine, and urea serum levels compared to non-Ala allele carriers. For Leu162Val PPARα gene, both groups did not show any significant difference on the measures.

To check if the effects of the SNPs are associated with age, we divided the subjects into two groups. The first group had subjects who were younger than 60 years old, and the second group had subjects that were 60 years old and more; here we compared the parameters related to MS between these two groups (Tables 6 and 7). For Pro12Ala PPARγ2 gene, in the group aged <60 years old, Ala allele carriers had higher HOMA-β and HOMA-S and decreased FPG, FPI, HOMA-IR, creatinine, and urea serum levels than non-Ala allele carriers. For Pro12Ala PPARγ2 gene, in the group aged ≥60 years

TABLE 6: Comparison of anthropometric, HOMA index, and biochemical characteristics of subjects between different groups of Pro12Ala PPARγ2 genotypes in two subdivided groups according to age.

Parameters	Age < 60 years Pro12Ala PPARγ2			Age ≥ 60 years Pro12Ala PPARγ2		
	P/P ($n = 289$)	X/A ($n = 125$)	P	P/P ($n = 80$)	X/A ($n = 28$)	P
Age (years)	36 (29–46)	35 (28–41)	0.094	62 (59–66)	64 (59–69)	0.287
SBP (mmHg)	125 (120–140)	125 (120–130)	0.110	130 (120–140)	130 (120–140)	0.845
DBP (mmHg)	80 (80–85)	80 (80–80)	0.065	80 (80–85)	80 (75–90)	0.709
BMI (kg/m^2)	26.0 (24.3–28.3)	25.4 (24.2–27.9)	0.276	26.9 (24.7–27.8)	25.9 (23.7–28.1)	0.237
WC (cm)	93.0 (84.0–98.0)	93.0 (82.5–97)	0.568	93.0 (86.0–98.0)	91.0 (79.0–97.5)	0.448
TG (mmol/L)	1.48 ± 0.71	1.44 ± 0.78	0.577	1.63 ± 0.70	1.34 ± 0.67	0.058
TC (mmol/L)	4.68 (4.11–5.45)	4.68 (4.10–5.25)	0.665	5.03 (4.46–5.68)	4.74 (4.10–5.57)	0.185
cLDL (mmol/L)	3.09 (2.61–3.71)	3.00 (2.54–3.66)	0.918	3.31 (2.92–3.84)	3.09 (2.82–3.84)	0.308
cHDL (mmol/L)	1.13 (0.88–1.28)	1.13 (0.91–1.34)	0.425	1.03 (0.88–1.24)	1.05 (0.87–1.41)	0.596
FPG (mmol/L)	6.08 ± 1.75	5.42 ± 1.49	<0.001	6.36 ± 1.84	5.58 ± 1.91	0.057
FPI (mIU/L)	9.74 (7.12–12.88)	7.92 (6.18–11.09)	<0.001	10.47 (7.30–12.89)	7.52 (6.01–11.49)	0.049
HOMA-β%	88 (66–101)	96 (82–106)	0.002	81 (55–99)	91 (82–103)	0.056
HOMA-S%	79 (55–110)	102 (68–126)	<0.001	73 (53–104)	103 (67–131)	0.030
HOMA-IR	1.3 (0.9–1.8)	1.0 (0.8–1.5)	<0.001	1.4 (1.0–1.9)	1.0 (0.8–1.5)	0.038
Cr (μmol/L)	100.0 ± 45.4	89.3 ± 19.7	0.012	109.6± 47.1	107.0 ± 39.0	0.377
Urea (mmol/L)	6.15 ± 4.05	5.05 ± 2.19	0.005	7.54 ± 5.08	5.70 ± 2.90	0.073
UA (μmol/L)	302 (247–387)	294 (241–353)	0.143	319 (248–383)	279 (211–347)	0.083
GFR (mL/min)	93.5 (83.3–109.4)	101.9 (85.2–112.9)	0.054	66.4 (48.9–77.6)	60.2 (52.7–71.6)	0.002

P: Pro; A: Ala; L: Leu; X/A: Pro/Ala and Ala/Ala.
SBP: systolic blood pressure, DBP: diastolic blood pressure; BMI: body mass index; WC: waist circumference; TG: triglycerides; TC: total cholesterol; cHDL: high density lipoprotein cholesterol; cLDL: low density lipoprotein cholesterol; FPG: fasting plasma glucose; FPI: fasting plasma insulin; HOMA-β%: % β-cell function; HOMA-S%: % cell insulin sensitivity; HOMA-IR: insulin resistance; Cr: creatinine; UA: uric acid; GFR: glomerular filtration rate.

old, Ala allele carriers had higher HOMA-S and GFR and decreased FPI and HOMA-IR than non-Ala allele carriers. For Leu162Val PPARα gene, both groups showed significantly higher HOMA-β and reduced FPG levels in Val-carriers than Pro-carriers.

To check if the effects of the SNPs are associated with BMI, we divided the subjects into three groups, a lean group (BMI < 25 kg/m^2), an overweight group (BMI ≥ 25 kg/m^2 and BMI ≤ 30 kg/m^2), and obese group (BMI > 30 kg/m^2). We compared the genotype and allele frequency among the three groups by using Pearson's chi-square test (χ^2). There was no difference among the three groups in terms of both polymorphisms ($P = 0.063, 0.902$). Further analysis by gender was conducted and same result was achieved by both males and females. There was no difference found in terms of Leu162Val and Pro 12 Ala PPARγ2 in genotype of both polymorphisms ($P = 0.224, 0.889$).

In order to evaluate the interaction between Leu162Val PPARα and Pro12Ala PPARγ2 polymorphisms, we divided the subjects into four groups according to the combination of the 2 genotypes from each SNP, that is, Leu/Leu PPARα with Pro/Pro PPARγ2 genotype ($n = 329$), Leu/Leu PPARα with an Ala allele ($n = 112$), Leu/Val PPARα with Pro/Pro PPARγ2 ($n = 40$), and Leu/Val PPARα with an Ala allele ($n = 41$), and compared the study parameters among the groups. There were no differences in the anthropometric measurements and lipid profile

variables among the different combination groups of the polymorphisms. The Leu/Val genotype carries with an Ala allele group had significantly reduced FPG, FBI, HOMA-IR, urea, and UA levels and elevated HOMA-S, HOMA-β, and GFR compared to the different combination groups (Table 8).

4. Discussion

MS is a complex disorder resulting from the interaction between genetic and environmental factors. A major part of our study has focused on the genetics of PPARα and PPARγ2 polymorphisms. Understanding the genetics of these polymorphisms is not only important because it is associated with the MS, but also it has been recently recognized to be related to renal function [19–22]. The present study investigated the independent effect of Leu162Val PPARα and Pro12Ala PPARγ2 polymorphisms as well as their impact on glucose, insulin, HOMA index, urea, UA, and GFR.

We showed that carriers of the PPARα Val162 allele had lower urea, UA, and raised GFR compared to those homozygous for the Leu162 allele. Also, subjects carrying the PPARγ2 Ala allele had the same results. In addition, they had reduced FPG, FPI, and HOMA-IR and elevated HOMA-β and HOMA-S. The Leu/Val genotype carriers with an Ala allele group had lower FPG, PPI, HOMA-IR, urea, and UA levels and higher HOMA-β, HOMA-S, and GFR than other

TABLE 7: Comparison of anthropometric, HOMA index, and biochemical characteristics of subjects between different groups of Leu162Val PPARα genotypes in two subdivided groups according to age.

Parameters	Age < 60 years Leu162Val PPARα			Age ≥ 60 years Leu162Val PPARα		
	L/L ($n = 345$)	X/V ($n = 69$)	P	L/L ($n = 96$)	X/V ($n = 12$)	P
Age (years)	36 (28–45)	36 (28–43)	0.622	62 (60–67)	62 (60–69)	0.957
SBP (mmHg)	125 (120–140)	130 (120–135)	0.389	130 (120–140)	125 (120–135)	0.178
DBP (mmHg)	80 (80–85)	80 (80–85)	0.859	80 (80–85)	80 (75–80)	0.159
BMI (kg/m^2)	25.7 (24.3–28.3)	26.6 (24.3–29.4)	0.147	26.9 (24.5–27.7)	26.8 (24.5–28.6)	0.965
WC (cm)	93.0 (82.0–97.0)	94.0 (87.5–98.5)	0.060	93.0 (85.5–97.5)	92.0 (81.5–98.5)	0.934
TG (mmol/L)	1.47 ± 0.73	1.47 ± 0.73	0.985	1.59 ± 0.69	1.24 ± 0.72	0.095
TC (mmol/L)	4.73 (4.19–5.49)	4.72 (4.14–5.42)	0.870	4.89 (4.48–5.68)	4.63 (3.95–5.27)	0.127
cLDL (mmol/L)	3.09 (2.61–3.71)	3.17 (2.57–3.64)	0.847	3.31 (2.91–3.84)	3.06 (2.39–3.79)	0.261
cHDL (mmol/L)	1.13 (0.88–1.29)	1.14 (0.92–1.34)	0.305	1.03 (0.88–1.27)	1.07 (0.76–1.33)	0.663
FPG (mmol/L)	5.96 ± 1.75	5.46 ± 1.33	0.026	6.34 ± 1.90	4.73 ± 0.84	0.005
FPI (mIU/L)	9.83 (7.12–12.88)	9.05 (6.78–12.28)	0.510	9.68 (7.12–12.89)	7.38 (5.54–11.04)	0.039
HOMA-β%	86 (64–100)	94 (77–111)	0.017	83 (56–99)	110 (84–131)	0.003
HOMA-S%	77 (54–109)	84 (61–118)	0.262	79 (53–111)	106 (72–142)	0.025
HOMA-IR	1.2 (0.9–1.8)	1.2 (0.9–1.7)	0.322	1.3 (0.9–1.9)	1.0 (0.8–1.4)	0.037
Cr (μmol/L)	97.1 ± 40.7	95.3 ± 34.5	0.731	108.4 ± 44.0	113.3 ± 54.1	0.722
Urea (mmol/L)	5.99 ± 3.87	4.94 ± 1.82	0.028	7.36 ± 4.83	4.65 ± 1.94	0.058
UA (μmol/L)	307 (248–385)	292 (233–341)	0.090	319 (237–377)	265 (217–352)	0.247
GFR (mL/min)	89.5 (72.7–105.1)	105.5 (91.8–117.2)	0.004	64.2 (47.1–75.1)	68.2 (56.9–82.1)	0.475

L, Leu; V, Val; X/V: Leu/Val and Val/Val.
SBP: systolic blood pressure, DBP: diastolic blood pressure; BMI: body mass index; WC: waist circumference; TG: triglycerides; TC: total cholesterol; cHDL: high density lipoprotein cholesterol; cLDL: low density lipoprotein cholesterol; FPG: fasting plasma glucose; FPI: fasting plasma insulin; HOMA-β%: % β-cell function; HOMA-S%: % cell insulin sensitivity; HOMA-IR: insulin resistance; Cr: creatinine; UA: uric acid; GFR: glomerular filtration rate.

TABLE 8: Anthropometric and biochemical characteristics of subjects within different groups according to the simultaneous existence of different genotypes of the Leu162Val PPARα and Pro12Ala PPARγ2 polymorphisms.

	L/L P/P ($n = 329$)	L/L X/A ($n = 112$)	P/P X/V ($n = 40$)	X/A X/V ($n = 41$)	P
Age (years)	41 (29–54)	38 (28–49)	38 (29–47)	37 (28–49)	0.298
SBP (mmHg)	125 (120–140)	125 (120–140)	125 (120–140)	130 (120–135)	0.284
DBP (mmHg)	80 (80–85)	80 (80–85)	80 (80–90)	80 (75–80)	0.216
BMI (kg/m^2)	26.4 (24.4–28.2)	24.9 (23.9–27.3)	26.0 (24.5–29.1)	27.1 (24.2–29.1)	0.053
WC (cm)	93 (85–98)	93 (79–96)	93 (85–98)	95 (88–98)	0.052
TG (mmol/L)	1.53 ± 0.71	1.40 ± 0.75	1.38 ± 0.66	1.49 ± 0.79	0.289
TC (mmol/L)	4.73 (4.19–5.49)	4.71 (4.12–5.25)	4.73 (4.13–5.43)	4.71 (4.03–5.35)	0.645
cLDL (mmol/L)	3.13 (2.61–3.74)	3.00 (2.62–3.65)	3.06 (2.53–3.43)	3.20 (2.53–3.72)	0.578
cHDL (mmol/L)	1.09 (0.88–1.26)	1.13 (0.92–1.39)	1.17 (1.03–1.38)	1.05 (0.84–1.32)	0.028
FPG (mmol/L)	6.22 ± 1.80	5.55 ± 1.67	5.54 ± 1.32	5.18 ± 1.27	<0.001
FPI (mIU/L)	10.36 (7.12–12.89)	7.49 (6.02–10.93)	8.92 (6.95–12.44)	8.91 (6.16–11.26)	<0.001
HOMA-β%	84 (59–99)	94 (80–101)	89 (74–115)	99 (88–120)	<0.001
HOMA-S%	73 (54–108)	82 (58–117)	85 (59–115)	87 (67–128)	<0.001
HOMA-IR	1.4 (0.9–1.8)	1.2 (0.8–1.7)	1.2 (0.9–1.7)	1.1 (0.8–1.5)	<0.001
Cr (μmol/L)	102.7 ± 46.2	90.4 ± 21.2	97.5 ± 42.9	98.4 ± 33.5	0.056
Urea (mmol/L)	6.61 ± 4.51	5.36 ± 4.54	5.15 ± 2.04	4.65 ± 1.59	<0.001
UA (μmol/L)	313.0 (248.0–399.0)	301.5 (237.5–360.2)	304.0 (236.5–346.0)	266.0 (225.0–331.0)	0.017
GFR (mL/min)	87.9 (71.7–104.5)	91.5 (71.5–109.4)	101.5 (84.0–112.8)	102.6 (72.6–118.2)	0.011

P: Pro; A: Ala; L: Leu; V: Val; X/A: Pro/Ala and Ala/Ala; X/V: Leu/Val and Val/Val.
SBP: systolic blood pressure, DBP: diastolic blood pressure; BMI: body mass index; WC: waist circumference; TG: triglycerides; TC: total cholesterol; cHDL: high density lipoprotein cholesterol; cLDL: low density lipoprotein cholesterol; FPG: fasting plasma glucose; FPI: fasting plasma insulin; HOMA-β%: % β-cell function; HOMA-S%: % cell insulin sensitivity; HOMA-IR: insulin resistance; Cr: creatinine; UA: uric acid; GFR: glomerular filtration rate.

different genotype combinations. Thus, the effect of one allele in one gene seems to depend upon the presence of another allele in a second gene.

The Leu162Val PPARα and Pro12Ala PPARγ2 polymorphisms have opposite effects on the transcriptional activity of their respective receptors. Indeed, the Ala12 allele results in a less active form of PPARγ2, while the Val162 allele results in a more active form of PPARα [23–25]. However, the observation that the PPARγ2 Ala12 allele mediates its lowering effect only on a PPARα genetic background complicates the explanation. It demonstrated that the PPARγ2 Ala12 allele was associated with greater insulin sensitivity [25]. Similarly, the Ala12 allele in PPARγ2 attenuates the effect of the PPARα Val162 allele on glucose and insulin homeostasis [26]. Genetic variation in PPARγ coactivator-1, which also coactivates PPARα, influences the insulin secretory response [27].

Many studies found a significant association between MS and CKD and consistently demonstrated an increased risk parallel to the number of MS traits [28–30]. The association between MS and renal damage is, in part, explained by hypertension and impaired glucose metabolism. However, the underlying mechanisms include an increasing body mass, insulin resistance, inflammation, renal endothelial dysfunction, oxidative stress, and altered renal haemodynamics, activation of the renin-angiotensin-aldosterone system and sympathetic nervous system, and dietary factors [31].

Urine analysis and blood biochemistry have been of great help in the assessment of renal function. Uric acid is an end product of purine (a component of nucleic acids and nucleoproteins) metabolism; urea is an end product of protein metabolism and the creatinine is derived from the creatine and is a waste product. The major cause of increased levels of plasma creatinine, urea, and uric acid is the poor clearance of these substances by the kidneys rather than excessive production. Insulin may induce renal fibrosis by stimulating mesangial cells and proximal tubule cells to produce tumor growth factor $β$ (TGF-$β$) [32, 33].

Insulin stimulates the production of insulin-like growth factory 1 (IGF-1) by vascular smooth muscle cells and other cell types, which have been implicated in the development of diabetic kidney disease [34]. IGF-1 increases the activity of connective tissue growth factor, a cytokine that has profibrogenic actions on renal tubular cells and interstitial fibroblasts. In addition, IGF-1 decreases the activity of matrix metalloproteinase-2, an enzyme responsible for extracellular matrix degradation, thereby promoting extracellular matrix expansion and renal fibrosis [35, 36]. Additionally, IR promotes sodium and UA reabsorption resulting in salt-sensitive hypertension and hyperuricemia [37].

Insulin resistance and the release of inflammatory cytokines induce mesangial expansion, basement membrane thickening, podocytopathy, and loss of slit pore diaphragm integrity leading to the so-called obesity-related glomerulopathy [38, 39]. In accordance with the results of other studies, expression of PPARα in glomerular mesangial cells has also been reported. Thus, it is likely that PPARα activation in mesangial cells could block TGF-$β$ signalling pathway by attenuating glomerular matrix proliferation. Therefore, it is likely that PPARα activation may facilitate albumin reabsorption and degradation in the nephron segment [40, 41]. Bossé et al. observed a deleterious effect of the PPARα Val162 allele on glucose and insulin levels during a glucose challenge but suggested that Leu162Val PPARα and Pro12Ala PPARγ2 polymorphisms interact with each other to modulate some features of glucose and insulin homeostasis [42]. Moreover, it was found that the Ala-allele is associated with enhanced decline in GFR and predicts end-stage renal disease (ESRD) and all-cause mortality in patients with nephropathy [43].

Taken together, these observations may partly explain the synergetic effect of Leu162Val PPARα and Pro12Ala PPARγ2 polymorphisms on MS and renal injuries.

5. Conclusion

We suggest that Leu162Val PPARα and Pro12Ala PPARγ2 polymorphisms can interact with each other to modulate glucose and insulin homeostasis and expand their association with the overall renal function. However, a replication of this study is required before a firm conclusion can be reached.

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