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Association of Perilipin and Insulin Receptor Substrate-1 Genes Polymorphism with Lipid Profiles, Central Obesity, and Type 2 Diabetes in a Sample of an Iranian Population

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Abstract

Background: Since insulin receptor substrate-1 (*IRS-1*) is the main substrate of the insulin receptor tyrosine kinase and has been detected to activate phosphatidylinositol (PI) 3-kinase and promote GLUT4 translocation, the *IRS-1* gene is a possible candidate for development of type2 diabetes, insulin resistance, and obesity. Preilipin (*PLIN*) coats intracellular lipid droplets and modulates adipocyte lipolysis.
Objectives: In this study, we investigated whether insulin receptor substrate-1 (*IRS-1*) and Perilipin (*PLIN*) genes polymorphism were associated with Type 2 diabetes (T2D), obesity, and lipid profiles in a sample of the Iranian population (Southeast of Iran).
Methods: In this randomized case-control study (Feb, 2016 to Sep, 2016), we genotyped 200 patients with T2D and the same number (200) of controls by using the combined nested-polymerase chain reaction (PCR) as well as the amplification refractory mutation system (ARMS)-PCR for *IRS-1* variant and Tetra-ARMS-PCR for *PLIN* variant in southeast Iran (Zahedan).
Results: The polymorphism of 4578621 in the *PLIN* gene was associated with T2D. GG genotype and G allele at rs4578621of *PLIN* gene were significantly higher in patients than in controls (for the G allele: OR = 2.31, 95%CI = 1.55 - 3.46, P < 0.0001; for the GG genotype: OR = OR = 2.67, 95%CI = 1.71-4.19, P<0.0001). Furthermore, women with fast blood glucose (FBG) < 110 mg/dL are in protection of diabetes (P < 0.001, OR = 0.561 (0.430 - 0.733), for women, (P = 0.002, OR = 0.481 95%CI: 0.305 - 0.756), for FBG, (P = 0.002, OR = 0.591, 95%CI = 0.422 - 0.829), for triglyceride (TG) < 130 mg/dL, and (P = 0.001, OR. = 0.563, 95%CI = 0.407 - 0.780) for TG $\geq$ 130. On the other hand, the polymorphism of 2943641 in *IRS-1* gene was not associated with T2D (P = 0.08). However, the frequencies of the risk A allele at rs29436411of *IRS-1* was significantly higher in patients than in controls (G allele: OR = 0.55, 95% CI = 0.36 - 0.848, P = 0.0086). Also detected, BMI $\geq$ 25 is a risk factor for diabetes in rs29436411of *IRS-1* gene (P = 0.024, OR = 1.604 95%CI = 1.063 - 2.420).
Conclusions: The rs2943641 polymorphism of the *IRS-1* gene is a major genetic determinant of obesity but not Type 2 diabetes and lipid profiles. The rs4578621 polymorphism of the *PLIN* gene related with T2D and TG but not obesity.

Keywords: Type 2 Diabetes, Lipid Profiles, *PLIN*, *IRS-1*, Gene Polymorphism, Obesity

1. Background

Type 2 diabetes (T2D) is detected by insulin resistance in tissues dependent to insulin and diminished insulin secretion from pancreatic beta cells (1-3). Insulin receptor substrate 1 (*IRS-1*) gene has been suggested significantly by playing a role in susceptibility to T2D (4). Offered that the polymorphism in the *IRS-1* gene have been contributed in obesity and T2D (5). *IRS-1* could have a central role in the insulin-signaling pathway (4). The *IRS-1* is a cytoplasm protein existed in insulin-dependent tissues, which has a central role in regulating the effects of insulin (6). Rung and colleagues (4) showed rs2943641 in locus 500 kb of downstream the gene (IRS1), is as a risk locus for susceptibility to T2D. Although the diabetes genetics replication and meta-analysis consortium (DIAGRAM) did not recognize this single nucleotide polymorphism (SNP) as a risk factor for T2D (7), there is this possibility that insulin resistance might cause by obesity, and also insulin resistance could cause the expansion of obesity (8). In this study, we investigated the association between polymorphism in The *IRS-1* gene (rs2943641) with T2D, metabolic traits (e.g. lipid profiles) and obesity. We assumed the *IRS-1* variant through body mass index (BMI) would be related with metabolic traits and change in metabolic traits may predispose a person to T2D. However, the effects of the *IRS-1* genotype on pre-diabetic mediated features were not known. Recently the researchers showed that this gene is not only associated

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with Type 2 diabetes in different populations but also it may have a significant correlation with diabetic complications, particularly with coronary artery disease (CAD) and obesity (9-11). Another gene that was investigated in this study was perilipin (*PLIN*) (rs4578621). *PLIN*s are phosphoproteins that cover intracellular lipid droplets (12). Recent studies have shown that these proteins are necessary in the adjustment of Triacylglycerols (TAG's) content and locomotion (13). In humans, the *PLIN* gene has been located in chromosomal 15q26.1 (14) in the proximity of already stated susceptibility loci for obesity, diabetes, and hypertriglyceridemia (15). *PLIN* is an objective of protein kinase A (PKA), and non-phosphorylated *PLIN* may operate as an obstacle for the hormone sensitive lipase (HSL) that intercede lipolysis of TAG's in lipid droplets (16). Furthermore, with phosphorylation, *PLIN* may cause HSL function (17). In addition, the arrangement of this function may play a role in obesity and changes in lipid metabolism. Several studies recognized the level of *PLIN* expression relating with the obesity situation. In a survey done in 2003 detected obese individuals indicated lower levels of *PLIN* than thin individuals (18). However, another study demonstrated that *PLIN*, mRNA, and protein were elevated in obese individuals (19). In the recent investigation (2016), Nicolaidou et al. demonstrated that the *PLIN* gene was associated with obesity and obesity-Related diseases (20). The purpose of this study was the investigation of the genetic variability of the *IRS-1* and perilipin genes and them feasible relation with obesity, Type 2 diabetes, lipid profiles, and related phenotypes in a random sample of the southeast Iranian population.

2. Methods

The protocol of this case-control study was approved by the local ethics committee of the Medical University of Zahedan, Iran (ethical code: 7179) and informed consent was obtained from both T2D patients and controls before the study entry. Subjects were asked to fast for 12 hours before their morning clinic appointments. This study was performed on diabetic patients referred to the diabetes center of Ali Asghar hospital in Zahedan (southeast Iran) during 7 months, from Feb. 2016 to Sep. 2016 (including Zahedan and near regions e.g. Mirjaveh, Nosrat Abad, etc.), and clinically analyzed the study population, which included 200 patients with Type 2 diabetes and the same number of control group. Demographic information was collected through face-to-face interviews including age, gender, and BMI (the weight (kilograms) divided by the square of the height (meters)). T2D was diagnosed in accordance to their medical records and fasting plasma glucose levels using the American diabetic association (ADA) criteria (21) taken as fasting blood glucose values $\geq$ 126 mg/dL or current treatment with oral hypoglycemic agents, our previous studies (3, 22), and confirmed by at least two endocrinologists. Patients with Type 1 diabetes, gestational diabetes, and T2D with any diabetes complications such as cardiovascular disease, diabetic nephropathy, diabetic neuropathy, and diabetic retinopathy were excluded from the study. In our hospitals Routine Laboratory, the control group, which included 200 individuals, underwent a medical checkup. The exclusion criteria for the control group included fasting blood glucose (FBG) levels of more than 100 mg/dl or a family history of T2D.

2.1. Sample Collection

Peripheral blood (5 mL) samples from an antecubital vein with minimal trauma were taken from all subjects (both case (NO. 200) and control (NO. 200) groups) and collected in separator tubes 2 mL in CBC tubes (Tajhiz-Gostar, Tehran, Iran) (contain EDTA, 0.5 M) for DNA extraction and remain in Serum Separator Tubes (Tajhiz-Gostar, Tehran, Iran) to collect serum for measuring lipid profiles then both kinds of tubes stored at -70°C until analysis. The samples were analyzed within 4 weeks of receipt at the Cellular and Molecular Research Center, Ali-Asghar hospital, Zahedan, Iran.

2.2. DNA Extraction and Genotyping

Genomic DNA was extracted from the CBC tubes of 200 subjects with T2D and 200 healthy controls by using the salting out extraction method as previously described (23) with some minor modifications. Briefly, 0.5 mL of blood from the CBC tube was transferred to 1.5-mL of micro-tubes (Eppendorf, Hamburg, Germany) and then was added to 800 μL Cell lysis buffer-I (including: Tris-HCl (10 mM) (Merck, Germany), Sucrose (11%w/v) (Merck, Germany), Triton X-100 (11%v/v) (Merck, Germany), and $MgCl_2$ (5 mM) (Merck, Germany)). The micro-tubes were vortexed (Vortex, RAZI CO., Iran) for 2 minutes at room temperature (RT) and centrifuged at 3400 gravity (g) (Eppendorf, Hamburg, Germany) then removed supernatant and then repeated twice more. Afterwards, 400 μL Cell lysis buffer-II (including: Tris-HCl (10 mM), Sodium citrate (10 mM) (Merck, Germany), and EDTA (10mM) (Merck, Germany)) was added to 60 μL SDS (10%) (Merck, Germany) and incubated for 15-minutes in RT (in the meantime, micro-tubes were vortex (Vortex, RAZI CO., Iran) 3 times). In the next stage, 500 μL Chloroform (Atlas shimi Co., Tehran, Iran) and 150 μL saturated salt solution were added to prior micro-tubes, vortexed (Vortex, RAZI CO., Iran), and centrifuged for 2 minutes at 3,400 g then, the supernatant was transferred to a

new RNase/DNase-free tube (Invitrogen Life Technologies, Gaithersburg, MD, USA) and was added 800μL cold Isopropanol (Atlas shimi Co., Tehran , Iran) and stored at 4°C for 1 hour. The micro-tubes were centrifuged (13,000 g) (Eppendorf, Hamburg, Germany) for 2 minutes at refrigerator centrifuge (at 4°C). After removing the supernatant, 800 μL cold 70% Ethanol (Atlas shimi Co., Tehran , Iran) was added and gently mixed, and finally, the micro-tubes centrifuged for 2 minutes at 13,000 g at refrigerator centrifuge (at 4°C) (Eppendorf, Hamburg, Germany). After discarding the supernatant, the micro-tubes that were incubated in 37°C for 20 minutes to dry remain Ethanol. In the end, DNA pellets were dissolved in 100 μL of distilled water (SinaClon BioScience Co., Tehran, Iran) and then micro-tubes were stored in -20°C. The quality of DNA extracts were analyzed by electrophoresis. By Nano- Drop (Nano-Drop Technologies, Wilmington, DE, USA) DNA concentrations about 60ng/ml was obtained and ratio of 260/280 nm around 1.7 to 1.9 was accepted.

2.3. A/G polymorphism of *IRS-1* rs2943641

A modified method of Nested-PCR and ARMS-PCR in combination (Nested-ARMS-PCR) was setup for the evaluation of polymorphism between T2D patients and healthy controls. In the first step (NESTED-PCR stage), for amplification of *IRS-1* rs2943641 product in (20 μL volume), 1.5 μL template DNA (~ 80 - 100 ng/μL), 1 μL of each primers (forward and reverse) (10 pmol/μL) (Pishgam company, Tehran, Iran), 10 μL master-mix (Ampliqon Taq 2x master-mix, Denmark), and 6.5 μL DNase-free water (SinaClon BioScience Co., Tehran, Iran) were added. The PCR conditions were set as follow (in Biorad thermal cycler (Bio-Rad Laboratories, Hercules CA)): 96°C for 5 minutes, 30 cycles of 95°C for 30 seconds, 58°C for 30 seconds and 72°C for 30 seconds, and the final extension at 72°C for 4 minutes. In the second step of the method (ARMS-PCR stage), the PCR product resulting from the NESTED stage was used as the template for the next stage (ARMS-PCR). According to this template, an amount of 1:20 was diluted (1 volume PCR product in 19-volume DNase-free water (SinaClon BioScience Co., Tehran, Iran)). Then, ARMS-PCR was used to detect the variant. PCR was performed for 20 μL volume: 2 μL template (1:20 dilution), 1 μL of each primer (10-pmol/μL) (Pishgam company, Tehran, Iran), 10 μL master-mix (Ampliqon Taq 2x master-mix, Denmark), and 6 μL DNase-free water (SinaClon BioScience Co., Tehran, Iran) were added. The PCR conditions were set (in Biorad thermal cycler (Bio-Rad Laboratories, Hercules CA)) as follow: 96°C for 6 minutes, 30 cycles of 96°C for 30 seconds, 52°C for 30 seconds, 72°C for 30 seconds, and a final extension at 72°C for 6 min. PCR products were detected by electrophoresis on a 1.5% agarose gel (Invitrogen Life Technologies, Gaithersburg, MD, USA) staining by Ethidium Bromide (Sigma-Aldrich, Steinheim, Germany).

The primers were designed to amplify this gene polymorphism according to the modified methods of Nested-PCR and ARMS-PCR in combination (Nested-ARMS-PCR) showed in Table 1.

Table 1. Allele-Specific Polymerase Chain Reaction (ASP)

Primer 5'-3'	Method
***IRS-1* rs2943641**	Nested-ARMS-PCR
F; GCAGTAGTATGATAGAATAGTG	
R_{common}[a]; TCTGAGTTGAAGTAGCCATCT	
F_{wild}; TAGTTGGAAATGAGAGGAAAC	
F_{Mutant}; TAGTTGGAAATGAGAGGAAAT	
Perilipin rs4578621	ARMS-PCR
R_{wild}: TGGACATCTCACTGTATTGCTC	
R_{Mutant}: TGGACATCTCACTGTATTGCTG	
F_{common}: AAATGCAGGTAGCCATAAGA	

Abbreviations: ARMS; amplification refractory mutation system; F; forward; *IRS-1*; insulin receptor substance-1; PCR; polymerase chain reaction; R; reverse.
[a]R; reverse primer is common between Nested-PCR and ARMS-PCR methods.

2.4. C/G Polymorphism PLIN rs4578621

To amplify the region of *PLIN* gene in (20 μL volume), 1 μL template DNA (~ 80 - 100 ng/μL), 1 μL of each primer (10 pmol/μL) (Pishgam company, Tehran, Iran), 10 μL mastermix (Ampliqon Taq 2x mastermix, Denmark), and 7 μL DNase-free water (SinaClon BioScience Co., Tehran, Iran) were added in the following condition (in Biorad thermal cycler (Bio-Rad Laboratories, Hercules CA)): an initial denaturation 96°C for 6 minutes and following that 30 cycles with this condition: denaturation 96°C for 30s, anealing 47°C for 30 seconds, extension 72°C for 29 seconds, and the final extension was 72°C for 6 min. The product size of this SNP was verified on 1.5% agarose gel (Invitrogen Life Technologies, Gaithersburg, MD, USA) staining Ethidium Bromide (Sigma-Aldrich, Steinheim, Germany) by electrophoresis after the PCR product was observed under UV light.

The primers (Pishgam company, Tehran, Iran) were designed for amplification of gene polymorphism according to the Tetra-ARMS method shown in Table 1.

2.5. Measurement of Lipid Profile

Serum separator tubes centrifuged (Eppendorf, Hamburg, Germany) at 3,500 - 4,000 rpm for 5 minutes to getting serum, then, serum total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), and Triglycerides

(TG) were measured by enzymatic methods (24-26) (Pars Azmoon-co, Iran; TC = 5 - 500 mg/dL, HDL-C = 1 - 150 mg/dL, and TG = 5 - 700 mg/dL) on Autoanalyzer BT 1500 (Biotecnica, Italy). The low- density lipoprotein-cholesterol (LDL-C) level was determined using the Friedewald formula (27).

2.6. Statistical Analysis

SPSS version 21.0 (SPSS, Chicago, IL, USA) and SNPStats version 1.14.0 were used for all the statistical analyses. Normal assumption was checked. Mean ± Standard deviation was used for quantitative data then the Student's t-test was used for comparison of quantitative variants of both of the 2 groups (T2D and healthy). The Fisher's exact test or χ^2-test was used for analysis genotypes and frequencies of data that accessed in this study. The calculation frequency of alleles was done by the gene counting method. The 95% confidence intervals (95% CI) and odds ratio were estimated by Logistic regression. Statistical significance for all data was considered with P < 0.05.

Table 2. Characteristics of Case and Control Subjects[a]

Characteristics	Control (n = 200)	Case (n = 200)	P Value
Age, y	48.75 ± 10.24	54.40 ± 9.90	0.593
Gender			
Female	142 (71)	143 (73)	0.37
TC, mg/dL	180 ± 35.90	184 ± 44.66	0.345
TG, mg/dL	124 (93)	149 (103)	0.001
HDL, mg/dL	53 (18)	50 (22)	0.530
LDL, mg/dL	100 (37)	93 (40)	0.030
FBG, mg/dL	93 (13)	173 (106)	< 0.001
BMI, mg/dL	21.87 (3.42)	26.24 (5.31)	< 0.001

Abbreviations: BMI, body mass index; FBG, fasting blood glucose; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; TC, total Cholesterol; TG: triglycerides.

[a]Values are expressed as No, (%) or mean ± SD, median, and interquartile range for normally and non-normally distributed variables, respectively. Comparisons were performed by one-way ANOVA and Kruskal-Wallis test. Also, the post hoc test and Mann-Whitney U test were used for comparison between groups. χ^2 of test results for categorical data.

3. Results

3.1. The Clinical Characteristics of the Study Population

A total of 400 (200 T2D patients and 200 health controls) individuals were contributed at this investigation. Data showed no significant difference in age (mean ± SD of (case group) 54.40 ± 9.90 and (control group) 48.75 ± 10.24 years) between the patients and control group (P = 0.593). The levels of BMI, FBG, LDL-C, and TG were significantly higher in patients with T2D than controls (P < 0.05), whereas about TC and HDL-C not seen any significant difference between patients and healthy individuals (P > 0.05) (Table 2).

3.2. Allele and Genotype Distribution

As shown in Table 3, the frequencies of the risk G allele (OR = 2.31, 95%CI = 1.55 - 3.46, P < 0.0001) and the GG genotype (OR = 2.67, 95%CI = 1.71 - 4.19, P < 0.0001) at rs4578621 of *PLIN* gene were significantly higher in patients than in the controls. As shown in Table 4 a significant association between T2D and rs2943641 was observed (for the G allele: OR = 0.55, 95% CI = 0.36 - 0.848, P = 0.0086). However, were no significant differences between *IRS-1* (rs2943641) genotypes with susceptibility to T2D (P > 0.05).

3.3. The Association Between the Genotypes of PLIN rs4578621and IRS-1 rs2943641with the Clinical Characteristics

As shown in Table 5 individuals with BMI ≥ 25 and age > 50 among genotypes of *IRS-1* rs2943641 years are at higher risk for T2D (P = 0.024, OR = 1.604 95%CI = 1.063 - 2.420, P = 0.006, OR = 1.886 95%CI = 1.201 - 2.962, respectively). Although, there was no relation seen between FBG, TG, HDL-C, LDL-C, TC, and gender with T2D among genotypes of *IRS-1* rs2943641. As shown in Table 6, there were no association between genotypes of *PLIN* rs4578621 with BMI, TG, HDL-C, LDL-C, TC, and age. Although women with FBG < 110 were considered as factors with protective roles for diabetes among genotypes of *PLIN* rs4578621 P < 0.001, (OR = 0.561, 95%CI = 0.430 - 0.733) for women, P = 0.002, (OR = 0.481, 9%CI = 0.305 - 0.756) for FBG.

4. Discussion

In this study, we explored the effect of *IRS-1*(rs2943641) and *PLIN* (rs4578621) polymorphism in a sample of Iranian T2D patients (Southeast of Iran). Our result offered that diabetes risk allele (A) in *IRS-1* rs2943641 polymorphism was related with age > 50 years and BMI ≥ 25 in Southeast Iran type 2 diabetes patients. *PLIN* (rs4578621) gene was significantly related with Type 2 diabetes. Furthermore, various studies showing variations in genes regulating adipose metabolism have been associated with diabetes risk (28, 29). Although, in the French population, there was no relation between rs4578621 polymorphism and T2D found (30). Significant associations were not seen between *PLIN* (rs4578621) SNP and the risk of obesity, plasma triglyceride, cholesterol, and glucose concentrations. However, individuals that have FBG < 110 mg/dL and BMI < 25 were considered as a protective factor for diabetes (P = 0.002, OR = 0.481, 95%CI = 0.305 - 0.756, P < 0.001, OR = 0.391, 95%CI =

Table 3. Genotype and Allele Frequencies of the Perilipin Gene rs4578621 Polymorphism in T2D (Case) and Control Subjects

rs4578621	Case	Control	OR (95%CI)	P Value	Study Power, %
Genotype					
CC	60 (30)	87(43.5)	1.0	-	77
CG	22 (11)	49 (24.5)	0.65 (0.36 - 1.89)	0.180	92
GG	118 (59)	64 (32)	2.67 (1.71 - 4.19)	< 0.0001	100
Allele					
C	142 (35.5)	223 (55.8)	1.0	-	100
G	258 (64.5)	177 (44.2)	2.31 (1.55 - 3.46)	< 0.0001	100

Table 4. Genotype and Allele Frequencies of the *IRS-1* rs2943641 Polymorphism in T2D (Case) and Control Subjects

Rs2943641	Case	Control	OR (95%CI)	P Value	Study Power, %
Genotype					
AA	105 (52.5)	87 (43.5)	1.0	-	40
AG	78 (39)	91 (45.5)	0.71 (0.47 - 1.08)	0.114	23
GG	17 (8.5)	22 (11)	0.64 (0.32 - 1.28)	0.222	13
Allele					
A	288 (72)	265 (66.3)	1.0	-	96
G	112 (28)	135 (33.8)	0.76 (0.57-1.03)	0.092	96

0.245 - 0.626, respectively). According to the our results, in a study done in US Women, detected *PLIN* polymorphism was not significantly related with obesity risk (31). However the role of obesity with diabetes and related genes remains obscure. Affect of the *PLIN* polymorphism was done in other diseases including polycystic ovary syndrome (PCOS) and ischemic stroke. The result detected in PCOS individuals was the variation in the *PLIN* gene affects glucose and lipid metabolism (32). However, there was no association between *PLIN* polymorphism with ischemic stroke that was observed (33). Another gene that surveyed in this study was *IRS-1*. As aforementioned, Insulin receptor substrate 1 (*IRS-1*) have a major role in the insulin-stimulated signal transduction pathway (4). In various investigations including the Saudi population (34), French population (35), US men and women (35), and Chinese Han population (36), it was detected that there was a significant association between *IRS-1* rs2943641 and diabetes risk elevation. However, in a study done in European detected *IRS-1* rs2943641, polymorphism had a protective role in the occurrence of diabetes (4). In the present survey, we did not observe any significant relation between *IRS-1* rs2943641genotypes and T2D occurrence. However G allele carriers of *IRS-1* rs2943641 have a relative risk of 4.336 (95% CI 1.592 - 6.43, P = 0.006) for diabetes. Consistent with our results, in Turkish individuals there was no existed relation between this gene and T2D (37). Interestingly, in *IRS-1* rs2943641 genotypes, it was observed that the individuals with BMI $\geq$ 25 (Kg/m^2) and aged > 50 years are at greater risk for developing diabetes. It is assumed that BMI $\geq$ 25 might cause insulin resistance and finally diabetes occurrence. In various studies, it was detected that obese individuals are insulin resistance than individual with a normal weight (8, 38). In obese individuals, detected adipose tissue releases elevated content of non-esterified fatty acids, glycerol, hormones, and other agents that are involved in the increase of insulin resistance. When insulin resistance is associated with dysfunction of adipose tissue, the cells that release insulin in tissues dependent to insulin -failure to control blood fatty acids levels are therefore, critical in defining the risk and expansion of obesity and relative disorders (39). Other studies showing inflammation in fat has an important role in the expansion of obesity-induced insulin resistance (40, 41). In our previous studies, detected IL-6R polymorphism was related with obesity and risk diabetes (42). Therefore, we assumed that various genes were involved in the development of obesity, some lipid profiles, and diabetes. In order to achieve more accurate results must done on different genes, and also done comparisons between these genes with each other in disease development.

Table 5. Association Between the *IRS-1* rs2943641 Polymorphism and Clinical Characteristics

Variable	*IRS-1* rs2943641						P Value	OR
	AA		AG		GG			
	Case	Control	Case	Control	Case	Control		
Age, y								
≤ 50	37 (48.7)	62 (48.8)	31 (40.8)	53 (41.7)	8 (10.5)	12 (9.4)	0.899	0.973 (0.632 - 1.496)
> 50	68 (54.8)	25 (34.2)	47 (37.9)	38 (52.1)	9 (7.3)	10 (13.7)	0.006	1.886 (1.201 - 2.962)
Gender								
Female	75 (51.4)	61 (43)	60 (41.1)	66 (46.4)	11 (7.5)	15 (10.6)	0.134	1.318 (0.919 - 1.891)
Male	30 (55.6)	26 (44.8)	18 (33.3)	25 (43.1)	6 (11.1)	7 (12.1)	0.368	1.286 (0.74 - 2.22)
BMI, kg/m^2								
< 25	54 (49.1)	>10 (45.5)	44 (40)	10 (45.5)	12 (10.9)	2 (9.1)	0.908	1.041 (0.527 - 2.057)
≥ 25	51 (56.7)	77 (43.3)	34 (37.8)	81 (45.5)	5 (5.6)	20 (11.2)	0.024	1.604 (1.063 - 2.420)
TG, mg/dL								
< 130	37 (46.8)	47 (42)	36 (45.6)	49 (43.8)	6 (7.6)	16 (14.3)	0.242	1.298 (0.829 - 2.007)
≥ 130	68 (57.1)	37 (46.2)	41 (34.5)	37 (46.2)	10 (8.4)	5 (6.3)	0.339	1.244 (0.795 - 1.945)
FBG, mg/dL								
≥ 110	88 (54)	10 (58.8)	63 (38.7)	5 (29.4)	12 (7.4)	2 (11.8)	0.979	0.989 (0.450 - 2.174)
< 110	17 (48.6)	75 (43.1)	14 (40)	81 (46.6)	4 (11.4)	18 (10.3)	0.719	1.108 (0.634 - 1.936)
HDL-C, mg/dL	52.5 ± 21	55.1 ± 14.1	55.9 ± 18.3	51.7 ± 14	54 ± 20.01	53.6 ± 14	0.552	1.004 (0.990 - 1.019)
LDL-C, mg/dL	97.8 ± 34.7	>94.7 ± 21.9	97.75 ± 27	112.3 ± 35	99 ± 34.8	103.5 ± 29	0.670	1.01(0.999 - 1.020)
TC, mg/dL	183 ± 40	174 ± 32.7	181 ± 38.8	185.4 ± 39	184 ± 44.6	180 ± 35.9	0.1	0.993 (0.984 - 1.001)

Abbreviations: BMI, body mass index; FBS, fast blood sugar; HDL-C, High-density lipoprotein- cholesterol; LDL-C, low-density lipoprotein-cholesterol; TC, total cholesterol; TG, triglyceride.

Our study has some limitations; 1, based on the published data, several Perilipin and insulin receptor substrate-1 genes polymorphism have been identified in humans; however, we investigated only 1 polymorphism from each gen; 2, we did not consider different indexes related obesity such as: Waist circumference, Hip circumference, Waist-to-hip ratio, etc. Third, the study had a relatively small sample size for reasons, such as the exclusion criteria. Nevertheless, we think that the present study makes a significant contribution to the debate concerning the clinical relevance of the investigated variants.

In summary, our findings showed that the *PLIN* gene polymorphism rs4578621 is a risk factor for T2D but not obesity. On the other hand, *IRS-1* gene polymorphism rs2943641 is a risk factor for obesity but not lipid profiles. Furthermore, there was no significant association between clinical and basic characteristics (TG, TC, FBG, HDL-C, LDL-C, age, and gender) with susceptibility to diabetes about polymorphism of the 2 genes that was seen. However, in regards to the *PLIN* gene, individuals with FBG < 110 mg/dL are in protection for diabetes. These results may be useful for directed treatment of Type 2 diabetes and obesity. To the best of our knowledge, this is the first study to indicate the plausibility of an association of Perilipin and insulin receptor substrate-1 with the development of Type 2 diabetes in our county. Nevertheless, more studies, including studies with a large sample size, more strict clinical experiment, are needed in the future.

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Footnotes

Authors' Contribution: Ramin Saravani and Hamid Reza Galavi conceived and designed the experiments; Ramin Saravani and Hamid Reza Galavi analyzed the data; Nafiseh

Table 6. Association Between the Perilipin rs4578621 Polymorphism and Clinical Characteristics

Variable	Perilipin rs4578621						P Value	OR
	CC		CG		GG			
	Case	Control	Case	Control	Case	Control		
Age, y								
≤ 50	25 (32.9)	51 (40.2)	6 (7.9)	35 (27.6)	45 (59.2)	41 (32.3)	0.009	0.644 (0.464 - 0.895)
> 50	35 (28.2)	36 (49.3)	16 (12.9)	14 (19.2)	73 (58.9)	23 (31.5)	< 0.001	0.553 (0.399 - 0.768)
Gender								
Female	42 (28.8)	63 (44.4)	16 (11)	36 (25.4)	88 (60.3)	43 (30.2)	< 0.001	0.561 (0.430 - 0.733)
Male	18 (33.3)	24 (41.4)	6 (11.1)	13 (22.4)	30 (55.6)	21 (36.2)	0.112	0.715 (0.472 - 1.082)
BMI, kg/m^2								
< 25	37 (27)	19 (61.3)	17 (12.4)	6 (19.4)	83 (60.6)	6 (19.4)	< 0.001	0.391 (0.245 - 0.626)
≥ 25	23 (36.5)	68 (40.2)	5 (7.9)	43 (25.4)	35 (55.6)	58 (34.3)	0.059	0.727 (0.522 - 1.012)
TG, mg/dL								
< 130	21 (26.6)	44 (39.3)	7 (8.9)	27 (24.1)	51 (64.6)	41 (36.6)	0. 002	0.591 (0.422 - 0.829)
≥ 130	38 (31.9)	39 (48.8)	15 (12.6)	20 (25)	66 (55.5)	21 (26.2)	0.001	0.563 (0.407 - 0.780)
FBG, mg/dL								
≥ 110	51 (31.3)	5 (29.4)	17 (10.4)	3 (17.6)	95 (58.3)	9 (52.9)	0.874	1.093 (0.366 - 3.265)
< 110	8 (22.9)	77 (44.3)	5 (14.3)	43 (24.7)	22 (62.9)	54 (31)	0.002	0.481 (0.305 - 0.756)
HDL-C, mg/dL	54 ± 20.0	53 ± 12.7	51.7 ± 15.9	55 ± 15.73	50.7 ± 18.2	52.9 ± 14.8	0.369	1.007 (0.992 - 1.022)
LDL-C, mg/dL	94.7 ± 30.78	101.1 ± 33	103.7 ± 23	106.9 ± 28	97.17 ± 29	104.7 ± 21	0.052	1.011 (1.000 - 1.022)
TC, mg/dL	175.9 ± 38.8	176.6 ± 38	185 ± 25.2	104.7 ± 22	178.4 ± 36	183.5 ± 34	0.154	0.993 (0.984 - 1.003)

Abbreviations: BMI, body mass index; FBS: fast blood sugar; HDL-C; high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; TC, total cholesterol; TG: Triglyceride.

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