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ADIPOCYTE "FATTY ACID BINDING PROTEIN" GENE POLYMORPHISMS (*rs1054135, rs16909196* AND *rs16909187*) IN JORDANIANS WITH OBESITY AND TYPE 2 DIABETES MELLITUS

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ABSTRACT

Background: Obesity, type 2 diabetes mellitus (T2DM), and dyslipidemia may result from the interactions of genetic and environmental factors. There are controversial reports concerning the association of polymorphisms (*rs1054135, rs16909196* and *rs16909187*) in the gene of adipocyte fatty acid binding protein (FABP4) with obesity and T2DM. Therefore, we designed this study to determine the association of these polymorphisms with obesity, T2DM, and dyslipidemia among Jordanian subjects.

Methods: The study was approved by the National Center for Diabetes, Endocrinology, and Genetics (NCDEG) Institutional Review Board (IRB). A total of 397 subjects were enrolled in the study and divided into four groups as described in materials and methods section. The fatty acid binding protein 4 (*FABP4)* gene containing (*rs1054135, rs16909196* and *rs16909187*) single nucleotide polymorphisms (SNP) was amplified by polymerase chain reaction (PCR) followed by Sanger DNA sequencing of the PCR product.

Results: None of the three SNPs were associated with T2DM ($p > 0.05$). The *rs16909187* and *rs16909196* were significantly associated with obesity. The wild type (CC) of *rs16909187* was significantly higher among the overweight and obese group compared with normal weight controls (OD = 2.17, 95% CI = 1.18 - 3.96, $p = 0.01$). The wild type of *rs16909196* (AA) was significantly higher among the overweight and obese group compared to controls, (OD = 2.26, 95% CI = 1.24 - 4.14, $p = 0.01$). These results may indicate that the wild-type may be a risk factor for obesity.

Only the *rs1054135* SNP was significantly associated with increased low density lipoprotein (LDL) levels in the overweight and obese group compared with the controls ($p = 0.03$).

Conclusions: The wild-type genotypes of *rs16909196* and *rs16909187* may be risk factors for obesity but not T2DM. None of the three SNPs was associated with T2DM.

Key words: Diabetes mellitus; *FABP4* gene polymorphism; Obesity; *rs1054135*; *rs16909196*; *rs16909187*.

INTRODUCTION

Obesity is a growing health problem worldwide. An increase in visceral adiposity is a considerable risk factor for many metabolic and cardiovascular disorders. Several studies provide evidence that obesity and weight gain are associated with an increased risk of developing diabetes mellitus, high blood pressure, and high cholesterol, while weight loss decreases this risk [1].

Fatty acid binding proteins (FABPs) are intracellular lipid chaperones that regulate lipid trafficking and responses in cells and facilitate lipolysis in adipocytes. At least nine different isoforms have been identified in mammals [2]. FABP4 (A-FABP) is mainly expressed in adipocytes and macrophages and have a significant role in the development of insulin resistance and atherosclerosis. FABP4 plays an important role as an adipokine, and its increased circulating levels were associated with metabolic syndrome, obesity, type 2 diabetes mellitus, insulin resistance, hypertension, cardiovascular disease, atherosclerosis, cardiovascular events, alcoholic steatohepatitis, adipose tissue inflamma-

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tion, diabetic nephropathy, adverse renal outcomes, mortality, and elevated low-density lipoprotein cholesterol, and reduced high-density lipoprotein cholesterol [2 - 4].

One prospective study investigated the association between circulating FABP4 levels and the development of subclinical atherosclerosis in type 2 diabetes patients over 8 years. They concluded that FABP4 levels predict the development of subclinical atherosclerosis in type 2 diabetic patients [5]. Another prospective study over 10 years showed that high FABP4 levels at baseline independently predicted the development of type 2 diabetes [6]. Both of these studies were performed on Chinese subjects.

FABP4 levels are substantially increased by lipolytic stimulation. High amounts of circulating fatty acids contribute to the development of insulin resistance. Long-term elevation of free fatty acids predispose for inhibition of insulin-stimulated glucose uptake and glycogen synthesis and β-cell death [7]. FABP4 deficiency in diet-induced and genetic obesity mice models resulted in increasing sensitivity to insulin and reducing hyperinsulinemia. FABP4-deficient adipocytes have attenuated lipolysis and fatty acid mobilization both *in vitro* and *in vivo* [8]. FABP4 has been shown to bind to and inhibit insulin receptor signaling [7].

Hundreds of compounds were synthesized in the past years to serve as FABP4 inhibitors. The purpose was to find drugs effective in the treatment of atherosclerosis and diabetes [9]. In preclinical studies using genetic mouse models, a potent and selective human and murine FABP4 inhibitor (BMS309403) was among them. This inhibitor has been shown to reduce inflammation and atherosclerosis, to improve lipid profiles and glucose homeostasis, and inhibit tumor progression and metastasis [7, 10, 11]. Some studies provided evidence that FABP4 might be a potential target for some drugs, and inhibitors of FABP4 may serve as therapeutic agents to treat some components of the metabolic syndrome. Anagliptin, a DPP-4 inhibitor, was found to reduce FABP4 concentration in patients with type 2 diabetes and dyslipidemia treated with statins. The effect was not related to hemoglobin A_{1c} or LDL-cholesterol levels [12]. Metformin was found to inhibit the intracellular accumulation of lipids in macrophages and to reduce the expression of FABP4 [13]. A number of structurally different angiotensin II receptor blockers given to hypertensive patients reduced circulating FABP4 levels. This effect was not due to blocking their receptors on adipocytes [13, 14]. Atorvastatin, sitagliptin, and omega-3 fatty acids were found to decrease circulating FABP4 concentrations [14].

The *aP2* (*FABP4*) gene locus was mapped to chromosome *8q21*. It consists of 4 exons and encodes a 132-amino acid protein [15]. A functionally significant genetic variation at the *aP2* locus was found to be associated with decreased adipose tissue expression of the *aP2* gene. Subjects having the *T-87C* polymorphism had lower serum triglycerides and reduced risk of coronary heart disease and type 2 diabetes than individuals with the wild-type allele [14, 16]. Genomic DNA sequence of the promoter and coding regions identified 5 distinct SNPs. Two of these variants (*C2600T* and *G4356C*) were previously identified as *rs8192688* and *rs1051252*, respectively. All of the SNPs were outside the coding region except the *G4356C*, which has been described as a silent variant on exon 4 [16]. Another missense SNP (*rs1054135*) of *FABP4* gene is also located on chromosome 8 [3]. One study genotyped 7 SNPs near the *FABP4* gene and measured FABP4 levels in older adults aged 65 years and older [17]. The authors concluded that there is an association between *FABP4* gene SNPs and fasting glucose levels, but not fasting insulin or body mass index (BMI). The SNPs *rs1054135*, *rs16909196*, and *rs16909187* were among the genotyped SNPs. The *rs16909187* polymorphism was found to have no effect on FAPB4 concentration in Sorbs from Germany [18].

Few publications are available regarding the association of *FABP4* gene polymorphisms with the development of type 2 diabetes mellitus and obesity. Such information is not available among Jordanians. Therefore, we designed this study to investigate the association of 3 SNPs in *FABP4* gene (*rs1054135*, *rs16909196* and *rs16909187*) with type 2 diabetes and obesity in Jordanians.

MATERIALS AND METHODS

Subjects:

A total of 397 Jordanians were enrolled in the study. They were recruited from the National Center for Diabetes, Endocrinology and Genetics (NCDEG), Amman, Jordan. The study protocol was approved by The NCDEG Institutional Review Board. A written and signed consent form had been obtained from each participant before blood sampling and data collection.

Study design:

This study was a cross sectional study where the study population has been divided into four groups: Group 1 constituted type 2 adult diabetic patients who are either obese or over-weight (BMI>25 kg/m²). Group 2 patients were type 2 adult diabetic patients with normal body weight (BMI<25 kg/m²). Group 3 patients were overweight and obese adults (BMI>25 kg/m²) with normal serum glucose and HBA1c < 5.7. Group 4 patients were normal weight adults (BMI<25 kg/m²) with normal serum glucose and (HBA1c < 5.7) and served as the control group.

Every subject donated 3-5 mL of venous blood in EDTA tubes.

Genomic DNA Extraction:

Genomic DNA was extracted in the same day of blood withdrawal using Promega-Wizard (USA) genomic purification kit, according to the manufacturer recommendations. The absorbance was measured at a 260 nm wavelength (A260) by using Nano Drop Spectrophotometer (Thermo Fisher, USA) in the NCDEG and the ratio of the absorbance at A260/A280 nm wavelengths was measured. DNA extraction was considered adequate when the ratio was between 1.6-1.8 [19]. The concentration of all DNA samples were also measured. The precise length of genomic DNA was determined by gel electrophoresis using 1% agarose gel.

Genomic amplification by PCR:

The FABP4 sequence containing (*rs1054135*, *rs16909196* and *rs16909187*) SNPs was amplified by PCR followed by sequencing of the PCR product. The primers sequences were: *CACGAGAGTTTATGAGAGAGC* for forward primer and *GCAACGCACTAAGACAGAG* for reverse primer. Primers were designed by DNA-MAN program (www.lynnon.com/dnaman.html) and both selectivity and specificity were checked.

The PCR protocol constituted initial denaturation at 95ºC for 5 minutes followed by 39 cycles of denaturation at 95ºC for 30 seconds, annealing at 55ºC for 30 seconds, extension at 72ºC for 45 seconds and final extension at 72ºC for 7 minutes.

PCR products were visualized using agarose gel run in a tris-borate-EDTA (TBE) buffer. The agarose gel was stained using RedSafe™ dye, and PCR products' bands were detected by loading them in the stained agarose gel that was placed under UV light. A 100-1500 base pair (bp) ladder (New England Biolabs, USA) was used as a marker to estimate the size of the amplified product. PCR products were sent for DNA Sanger Sequencing Analysis to Macrogen, South Korea. PCR product size was 530 bp.

Statistical analysis:

χ^2 was calculated using an online calculator for 2x2 contingency tables at http://www.socscistatistics.com/tests/chisquare/Default2.aspx. The odds ratios were calculated using the online statistical software available at https://www.medcalc.org/calc/odds_ratio.php. The Hardy-Weinberg equilibrium was assessed using the online calculator at https://www.wolframalpha.com/widgets/view.jsp?id=2fefa8b126607e29fe2990c722ee6cae. This final site calculates the parameter X. When X is greater or equal to zero, then there are significant differences between the observed and expected genotype frequencies, and the genotypes are not in Hardy-Weinberg equilibrium. The linkage disequilibrium (LD) analysis and estimation of haplotype diversity among the various groups were performed using Haploview 4.2 population genetic analysis software at https://haploview.software.informer.com/4.2/. The LD between the genetic variants was measured using D′and correlation coefficient (r^2).

RESULTS

The study was performed on 397 subjects who were divided into 4 groups. Males were more than females (252 subjects, 63.5%). The age range was 18-80 years. The number of subjects in each group, the demographics, and clinical laboratory findings of subjects enrolled in the study are presented in (Table 1). The control group (group 4) has the lowest values for triglycerides, total cholesterol,

Table 1. Demographic and clinical laboratory characteristics of subjects enrolled in the study.

Characteristic		Group 1 (Obese or overweight diabetics) (n=98)	Group 2 (Diabetics with normal weight) (n=103)	Group 3 (Nondiabetic but overweight or obese) (n=102)	Group 4 (Normal weight and nondiabetics) (n=94)	p-value
Mean age ± SD[a] (years)		57.6 ± 9.8	52.5 ±16.1	47.8 ± 12.4	34.5 ± 14.8	0.00[b]
Gender	Male	46	64	83	59	0.00[c]
	Female	52	39	19	35	
Mean TG ± SD (mg/dL)		184.7 ± 158.9	174.5 ± 136.0	223.05 ± 184.1	110.1 ± 66.1	0.00[b]
Mean TC ± SD (mg/dL)		187.3 ± 43.3	189.1 ± 44.6	197.6 ± 36.8	181.9 ± 44.4	0.00[b]
Mean LDL ± SD (mg/dL)		123.3 ± 36.4	121.8 ± 37.8	128.5 ± 33.8	108.2 ± 36.2	0.00[b]
Mean HDL ± SD (mg/dL)		43.5 ± 12.3	45.6 ± 19.4	39.3 ± 13.8	44.6 ± 11.4	0.016[b]
Mean Hb_{A1C} ± SD (%)		8.8 ± 8.8	7.8 ± 2.1	5.07 ± 0.39	4.8 ± 0.44	0.00[b]
Mean BMI ± SD (Kg/m^2)		33.8 ± 6.5	22.1 ± 2.1	31.3 ± 4.6	21.5 ± 2.09	0.00[b]

TG (triglycerides), TC (total cholesterol), LDL (low density lipoprotein), HDL (high density lipoprotein), HB_{A1C} (hemoglobin A1C), BMI (body mass index), m (height in meters), SD[a] (standard deviation), [b] (ANOVA test), [c] (Pearson Chi-square test).

LDL-cholesterol, hemoglobin A_{1C} and body mass index. HDL-cholesterol was lowest in group 3 (Non-diabetic but overweight or obese). Concerning age, group 4 subjects were the youngest.

The genotype and allele frequencies of the three SNPs (*rs1054135*, *rs16909196* and *rs16909187*) in the whole sample studied are presented in (Table 2). The minor allele frequencies were 0.068, 0.202, and 0.199 for *rs1054135*, *rs16909196* and *rs16909187*, respectively.

The Hardy-Weinberg equilibrium was calculated in all the studied subjects together and in the control group alone for each of the 3 SNPs. The results showed that the 3 SNPs followed the Hardy-Weinberg equilibrium in the control group who were non-obese and non-diabetic, and that equilibrium was not affected by the disease states. The results are presented in (Table 3).

The distribution of the genotypes of the 3 SNPs among the 4 groups studied is presented in (Table 4). The genotype distribution was similar for the 3 SNPs in group 1 (Obese or overweight diabetics) compared to group 4 (normal weight non-diabetics) and in group 2 (diabetics with normal weight) compared to group 4. The distribution of *rs1054135* genotypes were also similar in group 3 (Non-diabetic but overweight or obese) compared to group 4. The distribution of the genotypes of *rs16909196* and *rs16909187* was similar in group 2 compared to group 4. However, the distribution of the genotypes in group 3 was significantly different from that in group 4. Regarding

Table 2. Genotypes and allele frequencies of *rs1054135, rs16909196* and *rs16909187* SNPs among all groups combined.

rs1054135			
Genotype		**Number of subjects**	**Frequency**
Homozygous major allele	*GG*	348	0.877
Heterozygous	*GA*	44	0.111
Homozygous minor allele	*AA*	5	0.013
	Total	397	1.0
	Allele	Number of copies	Frequency
	G	740	0.932
	A	54	0.068
	Total	794	1.0
rs16909196			
Genotype		**Number of subjects**	**Frequency**
Homozygous major allele	*AA*	259	0.652
Heterozygous	*AT*	116	0.292
Homozygous minor allele	*TT*	22	0.055
	Total	397	1.0
	Allele	Number of copies	Frequency
	A	634	0.798
	T	160	0.202
	Total	794	1.0
rs16909187			
Genotype		**Number of subjects**	**Frequency**
Homozygous major allele	*CC*	261	0.657
Heterozygous	*CT*	114	0.287
Homozygous minor allele	*TT*	22	0.055
	Total	397	1.0
	Allele	Number of copies	Frequency
	C	636	0.801
	T	158	0.199
	Total	794	

Table 3. Assessment of the Hardy-Weinberg equilibrium of the three SNPs in the study sample as a whole and in the control group (group 4) alone.

SNP	Genotypes	The whole studied group (n=397)		X* parameter	Control group (n=94)		X* parameter
		Observed	Expected		Observed	Expected	
rs1054135	*GG*	0.8770	0.8687		0.8400	0.8263	
	GA	0.1110	0.1276	- 3.8245	0.1380	0.1654	- 3.8140
	AA	0.0130	0.0047		0.0220	0.0083	
rs16909196	*AA*	0.6520	0.6374		0.5740	0.5700	
	AT	0.2920	0.3211	- 3.8333	0.3620	0.3670	- 3.8410
	TT	0.0550	0.0404		0.0640	0.0600	
rs16909187	*CC*	0.6570	0.6414		0.5640	0.5625	
	CT	0.2870	0.3181	- 3.8319	0.3720	0.3750	- 3.8414
	TT	0.0550	0.0394		0.064	0.0625	

***** According to the online calculator used, when X is greater than or equal to zero, then there are significant changes between the observed and expected genotype frequencies, and the genotypes are not in Hardy-Weinberg equilibrium.

Table 4: Genotype distribution of FABP4 SNPs *rs1054135, rs16909196*, and *rs16909187* among the four study groups.

SNPs		***rs1054135***			***rs16909196***			***rs16909187***		
Genotypes		***GG***	***GA***	***AA***	***AA***	***AT***	***TT***	***CC***	***CT***	***TT***
Group 1 n=98		88	8	2	62	29	7	63	28	7
Group 2 n=103		90	12	1	68	28	7	68	28	7
Group 3 n=102		91	11	0	76	24	2	76	24	2
Group 4 n=94		79	13	2	53	35	6	54	34	6
Groups 1 *vs* 4	Z score	1.176	1.245	0.042	0.972	1.123	0.21	0.971	1.124	0.210
	P value	0.24	0.21	0.97	0.33	0.26	0.83	0.33	0.2	0.83
	Odds ratio	1.67 (0.71-3.93)	0.55 (0.22-1.40)	0.96 (0.13-6.95)	1.33 (0.75-2.38)	0.71 (0.39-1.29)	1.13 (0.36-3.49)	1.33 (0.75-2.38)	0.71 (0.38-1.30)	1.13 (0.36-3.49)
Groups 2 *vs* 4	Z score	0.668	0.458	0.646	1.385	1.506	0.117	1.236	1.353	0.117
	P value	0.50	0.65	0.52	0.17	0.13	0.91	0.22	0.18	0.91
	Odds ratio	1.31 (0.59-2.93)	0.82 (0.36-1.90	0.45 (0.04-5.06)	1.5 (0.84-2.68)	0.63 (0.34-1.15)	1.07 (0.35-3.3)	1.44 (0.81-2.56)	0.66 (0.36-1.20)	1.07 (0.35-3.30)
Groups 3 *vs* 4	Z score	1.061	0.650	1.100	2.649	2.080	1.479	2.505	1.926	1.479
	P value	0.29	0.52	0.27	0.01	0.04	0.14	0.01	0.05	0.14
	Odds ratio	1.57 (0.68-3.62)	0.75 (0.32-1.77)	0.18 (0.01-3.81)	2.26 (1.24-4.14)	0.52 (0.28-0.96)	0.29 (0.06-1.49)	2.17 (1.18-3.96)	0.54 (0.29-1.01)	0.29 (0.06-1.49)

rs16909196, the homozygous wild type genotype (*AA*), was significantly higher in group 3 than in group 4 (OD = 2.26, 95% CI 1.24-4.14, z score 2.649 and p-value 0.01). There was no difference in the homozygous minor allele genotype (*TT*) between the two groups (OD = 0.29, 95% CI 0.06-1.49, z score 1.479 and p-value 0.14). The heterozygous genotype (*AT*) was higher in the control group (OD = 0.52, 95% CI 0.28-0.96, z score 2.08 and p-value 0.04). This result may indicate that the wild type homozygous genotype *AA* of *rs16909196* SNP may be a risk factor for obesity, while the heterozygous *AT* may be protective. With regard to *rs16909187*, the homozygous wild type genotype *CC* was significantly higher in group 3 than in group 4 (OD = 2.17, 95% confidence interval 1.18-3.96, z score 2.505 and p-value 0.01). There was no difference in the homozygous minor allele genotype (*TT*) between

the two groups (OD = 0.29, 95% CI 0.06-1.49, z score 1.479 and p-value 0.14). Concerning the heterozygous genotype (*CT*), it was higher in the control group (OD = 0.54, 95% CI 0.29-1.01, z score 1.926 and p-value 0.05), but it did not each statistical significance. This result may indicate that the wild type homozygous genotype *CC* of *rs16909187* SNP may be a risk factor for obesity, while the heterozygous may be protective. The association between (*rs1054135*, *rs16909196* and *rs16909187*) haplotypes and obesity is presented in (Table 5). The haplotype of the wild type allele of the 3 SNPs (*GAC*) is significantly associated with obesity or overweight (p-value = 0.02). A higher proportion of the obese or overweight subjects had this haplotype than control group. While the haplotype composed of the wild type allele of *rs1054135* and the recessive alleles of *rs16909196* and *rs16909187* (*GTT*) was found at higher proportion in the control group when compared to the obese or overweight group, but the difference did not reach statistical significance (p = 0.05). The haplotypes *AAC* was not significantly associated with obesity and overweight and diabetes (p = 0.33).

The linkage disequilibrium (LD) analysis, estimation of haplotype diversity among the four groups was performed using Haploview 4.2 population genetic analysis software (Table 6). The D′ and r^2 values obtained were used to assess linkage disequilibrium. The results show that *rs16909196*, and *rs16909187* SNPs have a strong linkage disequilibrium in the control group (D′ = 1, r^2=0.97). These 2 SNPs remained in strong linkage disequilibrium in the other 3 groups as well. The SNP *rs1054135* was not in linkage disequilibrium with either *rs16909187* (D′ = 1, r^2=0.03) or *rs16909196* (D′ = 1, r^2=0.03) SNPs.

Concerning the association of *FABP4* gene polymorphisms (*rs1054135*, *rs16909196* and *rs16909187*) with

Table 5. Comparison of (*rs1054135, rs16909196,* and *rs16909187*) haplotypes between control group and other groups.

***rs1054135*, *rs16909196*, and *rs16909187* haplotypes**		***GAC***	***GTT***	***AAC***
Group 1 (Obese or overweight diabetics)		0.732	0.202	0.049
Group 2 (Diabetics with normal weight)		0.723	0.199	0.068
Group 3 (Non-diabetic but overweight or obese)		0.809	0.137	0.054
Group 4 (Control) (Normal weight nondiabetics)		0.660	0.245	0.090
Groups 1 *vs* 4	z score	1.085	-0.716	-1.12
	p value	0.28	0.47	0.26
Groups 2 *vs* 4	z score	0.958	-0.777	-0.574
	p value	0.34	0.44	0.57
Groups 3 *vs* 4	z score	2.369	-1.93	-0.979
	p value	**0.02**	**0.05**	0.33

Table 6. Linkage disequilibrium of FABP4 genetic variants (*rs1054135, rs16909187,* and *rs16909196*) in the four study groups using the Haploview program.

Study Group	**FABP4 Gene Variants**	**D′ (Linkage disequilibrium parameter)**	**R^2 (Correlation Coefficient)**
Group 1: (Obese or overweight diabetics)	*rs1054135* and *rs16909187*	0.046	0.0
	rs1054135 and *rs16909196*	0.084	0.0
	rs16909187 and *rs16909196*	1.0	0.97
Group 2: (Diabetics with normal weight)	*rs1054135* and *rs16909187*	1.0	0.019
	rs1054135 and *rs16909196*	1.0	0.019
	rs16909187 and *rs16909196*	0.97	0.94
Group 3: Nondiabetic but overweight or obese)	*rs1054135* and *rs16909187*	1.0	0.009
	rs1054135 and *rs16909196*	1.0	0.009
	rs16909187 and *rs16909196*	1.0	1.0
Group 4: (Control) (Normal weight nondiabetics)	*rs1054135* and *rs16909187*	1.0	0.03
	rs1054135 and *rs16909196*	1.0	0.03
	rs16909187 and *rs16909196*	1.0	0.97

serum concentrations of LDL, HDL, TC and TG among the studied groups, only the *rs1054135* SNP showed significant association with higher LDL levels in the third group subjects who are overweight or obese (p- value=0.03). However, there was no significant association between *rs1054135* SNP and HDL, TC and TG levels. The rest of the results in this regard are not presented.

DISCUSSION

This study examined the association of (*rs1054135*, *rs16909196* and *rs16909187*) polymorphisms in the *FABP4* gene with the susceptibility to T2DM, obesity, and the relation of these variants with serum concentrations of total cholesterol, LDL, HDL, and triglycerides.

The observed minor allele frequency of *rs1054135* of 0.09 in the control group is not statistically different from that of the whole studied sample (0.068).

The genotypes of the three SNPs are in Hardy-Weinberg equilibrium in our control group and remained so in the whole studied sample.

Our findings, showing no association between *rs1054135* and both T2DM and obesity, are consistent with what was reported among postmenopausal women, 50-79 years old of multiple ethnicities (Whites, African Americans, Hispanic/Latino Americans, Asian, American-Asian Pacific Islanders) who were enrolled in the "Women's Health Initiative" study [20]. The study examined 11 SNPs including *rs1054135*, and the results did not support the association of these SNPs with increased risk of type 2 diabetes mellitus. On the other hand, our results were not in agreement with those of two other studies. A study was performed on 309 children, between 5-7 years of age, in the United States. The children were divided into obese and non-obese groups. Most of the children were white, but the study also included African-Americans. Four SNPs were tested (*rs1051231*, *rs2303519*, *rs16909233* and *rs1054135*) and fasting plasma glucose, lipids, insulin, hsCRP, and FABP4 levels were measured. HOMA (homeostasis model assessment) was used as a measure of insulin sensitivity. The frequency of *rs1054135* allelic variant was increased in obese children (3). A second study, performed among Chinese teenagers in Shanghai, concluded that the *rs1054135* minor allele frequency was significantly lower among obese females compared with normal weight females [21].

Concerning *rs16909187* and *rs16909196*, we found that the homozygous wild type genotype (*CC*) of *rs16909187* and the homozygous wild type genotype (*AA*) of *rs16909196* were significantly associated with obesity. However, *rs16909187* and *rs16909196* minor allele (*T*) may be protective against obesity. The number of subjects with the homozygous minor allele genotypes is too small to draw conclusions in this regard. The heterozygous minor allele genotypes hint to such protection. The alleles of these two SNPs were not associated with type 2 diabetes mellitus.

The haplotypes' frequencies of *rs1054135*, *rs16909196*, and *rs16909187* were compared among the various studied groups. Only haplotypes *GAC* and *GTT* were related to obesity but not with T2DM. The frequency of the *GAC* haplotype was higher in the overweight and obese group, while that of *GTT* haplotype was higher in the control group.

The present study showed a significant association between homozygous (*GG*) and heterozygous (*GA*) wild type genotypes of rs1054135 and high level of LDL among group 3 (non-diabetic but obese) in comparison with controls (p-value = 0.03). However, there was no significant association between *rs1054135* SNP and the serum levels of total cholesterol, HDL and triglycerides among all groups. The rs*16909187* and *rs16909196* SNPs were not found to be associated with total cholesterol, LDL, HDL, or triglycerides in any of the groups studied.

The *rs16909187* and *rs16909196* SNPs were in linkage disequilibrium among all studied groups. However, none of these two SNPs were in linkage disequilibrium with *rs1054135*. This agrees with what has been previously reported: *rs1054135* has no linkage disequilibrium with other SNPs in *FABP4* gene [20].

The limitations of our study are the small sample size studied (397 subjects in the four groups) and the reduced number of females compared to males in all of the groups studied.

In conclusion, the 3 SNPs studied followed Hardy-Weinberg equilibrium. There was no association between (*rs1054135*, *rs16909196* and *rs16909187*) polymorphisms and T2DM. Both *rs16909196* and *rs16909187* SNPs homozygous wild type genotypes were associated with obesity, while the minor allele genotypes may be protective against obesity. The *rs1054135* SNP was not associated with obesity. The *rs1054135* SNP was significantly associated with an increase in LDL serum level among obese subjects, but the other two SNPs (*rs16909196* and *rs16909187*) were not associated with any significant changes in lipid profile parameters. There was linkage disequilibrium between the *rs16909187* and *rs16909196* SNPs in the control and the other studied groups. The *rs1054135* SNP was not in linkage disequilibrium with either of the two SNPs.

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