RESEARCH ARTICLE

Association of ADIPOQ, leptin, LEPR, and resistin polymorphisms with obesity parameters in Hammam Sousse Sahloul Heart Study

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Background: Adipose tissue is an important endocrine organ that secretes a number of adipokines, such as adiponectin (ADIPOQ), leptin (LEP), leptin receptor (LEPR), and resistin (RETN) which may be implicated in obesity. Some adipokines' polymorphisms of genes might influence their concentrations and/or activities. Our aim was to study the relationship between seven SNPs in ADIPOQ (+45T<G (rs2241766); +276G<T (rs1501299); -4255C<T (rs822393); -395G<T (rs17366568)), LEP (2548G<A (rs7799039)), LEPR (223Q<R (rs1137101)), and RETN (-420C<G (rs1862513)) and obesity in Hammam Sousse Sahloul Heart Study (HSHS).

Methods: The study, carried out between February and June 2009, is mainly focused on 1121 respondents in HSHS which is a population-based epidemiological study of type "community-based" on cardiovascular risk. Genotyping was performed using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). Serum lipids and anthropometric parameters were measured. Statistic analysis was performed on SPSSv19.

Results: The polymorphisms of ADIPOQ 4522C<T and 276G<T, LEP 2548G<A, and RETN 420C<G seem to contribute to obesity. In fact, adjusted odds ratios (ORs) of obesity associated with mutated genotypes of each polymorphism were respectively OR=1.38, $P=.037$; OR=0.608, $P<.001$; OR=2.23, $P=.034$; and OR=2.18, $P<.001$. The 276G<T, 4522C<T, and 420C<G were associated with increased BMI ($P=.010$, $P=.028$, and $P<.001$). A significant association was found between the 276G<T; 4522C<T, LEP 2548G<A and 420C<G, and the waist circumference and hip measurements.

Conclusion: ADIPOQ, LEP, and RETN gene polymorphisms were associated with obesity parameters in HSHS population.

KEYWORDS

ADIPOQ, LEP, LEPR, polymorphism, RETN

Abbreviations: ACDC, C1q and collagen domain-containing; ADIPOQ, Adiponectin; BMI, Body mass index; CAD, Coronary artery disease; CRH1, Cytokine receptor homologous 1; GWAS, Several genome wide association studies; H, Haplotype; HDL-C, High-density lipoprotein-cholesterol; HM, Hip measurement; HSHS, Hammam Sousse Sahloul Heart Study; LD, Linkage disequilibrium; LDL-C, Low-density lipoprotein-cholesterol; LEP, Leptin; LEPR, Leptin receptor; MetS, Metabolic Syndrome; mRNA, Messenger ribonucleic acid; PCR-RFLP, Polymerase chain reaction restriction fragment length polymorphism; RETN, Resistin; SNP, Single nucleotide polymorphism; Sp, Factor of transcription protein; TC, Total cholesterol; TG, Triglycerides; TNF α , Tumor necrosis factor α ; WC, Waist circumference; α -MSH, α Melanocyte-stimulating hormone.

1 | INTRODUCTION

Obesity is a major influential factor in the development of complex diseases such as type 2 diabetes, hypertension, gout, and cardiovascular disease. Familial studies in humans indicate that obesity is related to genes and environmental factors,¹ and indeed, it shows a high-risk presence in relatives.² The mode of inheritance for obesity, however, remains unclear. Adipose tissue is an important endocrine organ that secretes a number of factors, adipocytokines, or adipokines,³ and several proteins produced by adipose tissue have been discovered which may provide the link between insulin resistance, obesity, and development of diabetes.^{4,5}

Adiponectin (ADIPOQ) is encoded by adipocyte, C1q, and collagen domain-containing (ACDC), which is located on chromosome 3 at q27, where genomewide scans have revealed a susceptibility locus for type 2 diabetes, coronary artery disease (CAD), and obesity.^{6,7}

Adiponectin levels have a strong genetic component, with heritability estimated between 30% and 50%.⁸ Several genomewide association studies (GWAS) among European and Asian populations identified ADIPOQ locus as the major gene for variation in the serum adiponectin levels.^{9,10}

The leptin (LEP) protein is produced by the leptin gene from adipose tissue, through binding with a specific receptor in the hypothalamus.¹¹ It has a critical role in the pathway of food intake, regulation of body weight, and energy homeostasis.¹² The leptin receptor (LEPR), also identified as the diabetes gene product, is a single transmembrane protein that is established in many tissues and has several alternatively spliced isoforms.¹³

Resistin is a macrophage-derived signaling polypeptide hormone¹⁴ which belongs to cysteine-rich protein family.¹⁵

In mice, the highest expression of this protein is found in adipocytes.^{16,17} However, in humans it has become increasingly clear that resistin is mostly expressed by monocytes and macrophages.^{18,19} Hence, besides its involvement in obesity and type 2 diabetes, resistin has also been linked to inflammation. Not only does this protein stimulate expression of pro-inflammatory cytokines, its own expression is also under regulation of inflammatory factors.^{20,21}

Several association studies have reported associations between RETN polymorphisms and obesity,^{22,23} type 2 diabetes,²⁴ and insulin resistance.^{25,26} However, not all of the initially reported associations have been replicated in other studies and results have been conflicting.

Based on conflicting results of ADIPOQ, LEP, LEPR, and RETN polymorphisms' association with obesity in different populations, we aimed to study the relationship between seven SNPs in ADIPOQ (+45T<G (rs2241766); +276G<T (rs1501299); -4255C<T (rs822393); -395G<T (rs17366568)), LEP (2548G<A(rs7799039)), LEPR (223Q<R(rs1137101)), and RETN (-420C<G (rs1862513)) and obesity in Hammam Sousse Sahloul Heart Study (HSLS).

2 | METHODS

2.1 | Study population

As expressed previously by Boumaiza et al.,²⁷ the HSLS is an epidemiological population study type "community-based" on cardiovascular

risks with broad research aims. Hammam Sousse city (the governorate of Sousse, Tunisia), located in the east of Tunisia, included a population of 35 000 people. The HSLS study was led on a sample of 1000 households (33 clusters—33 households) of the city of Hammam Sousse, from February to June 2009, and drawn lots by the technique of sampling in clusters in two degrees and in proportional probability. All people present at home the day of the investigation, 20 or more years old, were included in the study.

The main purpose of the HSLS study is to evaluate the impact of lifestyle factors and metabolic perturbations on aspect of health, such as life quality, MetS prevalence, obesity, hypertension, type 2 diabetes, and chronic disease incidence.

A structured questionnaire was completed by the entire members of the household. Anthropometric, demographic, and socioeconomic data were obtained for each subject including age, gender, marital status, education level, occupation, household income, reproductive history for women, and family and personal history of obesity, diabetes, hypertension, and CVD. Also data on lifestyle, including physical exercise, dietary intake, smoking habits, and frequency of alcohol consumption, were collected.

We calculated the size of the sample by reference to the following hypothesis:

1. A confidence level of 95%
2. An estimated prevalence of 50%
3. A precision of 3%

So, an optimal sample size of 1111 subjects using the equation $n = [z^2(pq/\Delta^2)k]$ has been determined (z =reduced gap; p =prevalence; $q=1-p$; Δ =precision; k =cluster coefficient).

The study was approved by the Hospital Medical Ethics Committee, and informed consent was obtained from all the study subjects.

2.2 | Anthropometric parameters and blood pressure measurements

Weight and height were measured on the subjects barefooted and lightly clothed. Waist circumference (WC) was measured by trained examiner from the narrowest point between the lower borders of the rib cage and the iliac crest. Body mass index (BMI) was calculated as body weight (kg)/height² (m²), and obesity was defined as BMI ≥ 30 kg/m².²⁸ Blood pressure was measured three times from the left arm of seated subjects with a blood pressure monitor after 20 minutes of rest. The average of the two last measurements was recorded for each subject.

2.3 | Biochemical measurements

Blood samples were collected from subjects after 12-hour overnight fast. Serum total cholesterol (TC) and triglycerides (TG) were determined by standard assays. HDL-C was measured by direct assay. Low-density lipoprotein-cholesterol (LDL-C) concentrations

were calculated with the Friedewald formula²⁹ if TG<4 mmol/L. If not, LDL-C concentrations were measured by direct assay. Fasting glucose was measured by the glucose oxidase method. All biochemical parameters were measured on the Synchron CX7 Clinical System using the Beckman reagents (Beckman Coulter, Fullerton, CA, USA).

2.4 | Definitions of risk factors

Diabetes mellitus was defined as fasting glucose more than 7 mmol/L or currently receiving antidiabetic medication.³⁰ Hypertension was defined as greater than 140/90 mmHg or currently receiving antihypertensive medication.³¹ Dyslipidemia was defined as LDL-C concentration ≥ 4.1 mmol/L and/or HDL-C concentration ≤ 1 mmol/L and/or TG concentration 1.71 mmol/L.³²

2.5 | DNA analysis

Genomic DNA was isolated from peripheral blood leukocytes by the salting-out method.³³ The genotypes for each ADIPOQ (+45T<G; +276G<T; -4255C<T; -395G<T), LEP (2548G<A), LEPR (223Q<R), and RETN (-420C<G) were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as described by Kyriakou et al.,³⁴ Constantin et al.,³⁵ and Cao et al.³⁶ PCR products and the digest product were resolved by 2% agarose gel electrophoresis and visualized by ethidium bromide staining.

2.6 | Statistical analysis

Statistical analyses were performed by SPSS19.0. The biological parameter values were reported as mean $\pm$ SD and were compared by Student's t-test if they were in Gaussian distribution and reported as median [min-max] and compared by nonparametric Mann-Whitney U-test. Categorical variables were analyzed by the chi-square test or by the Fisher exact test for small number. We used SNPAnalyzer 2 program to test genotype frequencies for Hardy-Weinberg equilibrium, and to determine haplotype frequencies.³⁷ Odds ratios (ORs), two-tailed *P*-values, and 95% confidence interval (CI) were calculated as a measure of the association of the SNPs with presence of obesity. ORs were adjusted to confounding parameters (all parameters that show a *P* < .25 between the two groups) by logistic binary regression. A *P*-value of <.05 was considered statistically significant for all tests.

3 | RESULTS

3.1 | Patient characteristics

A total of 1441 subjects completed the interview. We excluded 320 people who did not complete physical examination and/or biological specimen collection. We thus included 1121 participants with complete data. The sociodemographic and clinical characteristics of the study population are presented in Table 1.

TABLE 1 Sociodemographic and clinical characteristics of the study subjects, n (%)

Variables	Results
Age tranches	
20-29	174 (15.5)
30-39	198 (17.7)
40-49	272 (24.3)
50-59	211 (18.8)
60-69	137 (12.2)
≥ 70	129 (11.5)
Sex ratio M/W	0.48
Men	364 (32.5)
Women	757 (67.5)
Tabac	139 (12.4)
Obesity disease $\geq 30\text{Kg/m}^2$	
Obese	721 (69.5)
Nonobese	400 (30.5)
Civil status	
Married	785 (70)
Single	211 (18.8)
Divorcee	21 (1.9)
Widower	102 (9.1)
Imprecise	2 (0.2)
Academic level	
Illiterate	199 (17.8)
Primary school	404 (36)
Middle school (college)	73 (6.5)
High school	257 (22.9)
Superior (higher education)	147 (13.1)
Diploma after universities	41 (3.7)
Professional activity	
Senior official of the state	18 (1.6)
Employee of the state	85 (7.6)
High executive in the private	27 (2.4)
Employee on the private	147 (13.1)
Independent	63 (5.6)
Worker	83 (7.4)
Student/high school student	63 (5.6)
Retired man	486 (43.4)
Unemployed person	36 (3.2)
Disabled person	18 (1.6)
Imprecise	6 (0.5)
Known diseases	
Diabetes	145 (12.9)
Hypertension	232 (20.7)
Dyslipidemia	100 (8.9)
Dysthyroidism	50 (4.5)
CVD	122 (10.9)

This study included 1121 subjects (364 men and 757 women) with a mean age of 47.5 ± 16.25 years and a sex ratio of 0.48. Hypertension was the most frequent known pathology in our population (20.7%) followed by type 2 diabetes (12.9%), CVD (10.9%), and dyslipidemia (8.9%).

3.2 | ADIPOQ, LEP, LEPR, and RETN gene SNP association with clinical and metabolic parameters

Genotype distribution of different SNPs was in Hardy-Weinberg equilibrium and is illustrated in Table 2.

The clinical and metabolic characteristics were analyzed according to different genotypes in all the studied subjects (Table 3).

No association was found with the 45T<G, 395G<A, and LEPR 223 Q<R and any clinical parameters.

The 276G<T, 4522C<T, and 420C<G were associated with increased BMI ($P=.010$, $P=.028$, and $P<.001$).

A significant association was found between the 276G<T and 4522C<T, LEP 2548G<A and 420C<G, and the WC and HM.

3.3 | ADIPOQ, LEP, LEPR, and RETN genotypes, genotype frequencies, and the risk of obesity

Genotype distributions of different SNPs were in Hardy-Weinberg equilibrium in obese and nonobese groups. The frequencies of the ADIPOQ (+276G<T; 4522C<T), LEP (2548G<A), and RETN (420C<G)

polymorphisms differed significantly between the two groups ($P<.05$) (Table 4).

Comparison of population characteristics in obese and nonobese groups showed a difference with $P<.05$ for age ($P=.070$), gender ($P<.001$), alcohol consumption ($P=.03$), and physical activity ($P=.020$). We considered these parameters as confounding factors for further analyses.

According to dominant model and after adjustment for factors age, gender, smoking status physical activity, and coffee consumption, the OR of obesity associated with mutated genotypes of 4522C<T, 276G<T, 420C<G, and 2548G<A seemed to be significantly associated with higher risk of obesity in comparison with their normal genotypes.

On the other hand, no statistical differences were observed for the 45T<G, 395G<A, and 223Q<R (Table 5).

3.4 | Haplotype frequency distribution

SNP analysis showed 175 haplotypes (H) and only 28 haplotypes had a frequency >1% in our study population, and comparison of the haplotype frequencies between obese and nonobese groups showed a significant difference for seven haplotypes (Table 6).

Haplotypes follow this order: 45T<G, 395G<A, 4522C<T, 2548G<A, LEP 223Q<R, 276G<T, 420C<G.

H1, H8, and H23 were more frequent in the nonobese group, and H3, H11, H12, and H15 were more frequent in the obese group. The more protective haplotype seemed to be H8 "TGCGAGG", OR=0.253 ($P=.002$), and the highest risk was associated with H15 "TGCAAGC", OR=9.06 ($P=.010$).

TABLE 2 Genotype frequency in general population HSHS

	SNPs	Genotypes	n (%)
ADIPOQ	45T/G	TT	694 (62)
		TG	367 (32.7)
		GG	60 (5.3)
	276G/T	GG	357 (31.85)
		GT	537 (47.9)
		TT	227 (20.25)
	4522C/T	CC	567 (50.6)
		CT	447 (39.8)
		TT	107 (9.6)
	395G/A	GG	654 (58)
		GA	395 (35)
		AA	79 (7)
LEP	G2548A	GG	234 (20.87)
		GA	566 (50.49)
		AA	321 (28.64)
LEPR	Q223R	QQ	585 (52.2)
		QR	435 (38.8)
		RR	101 (9)
RETN	420C/G	CC	223 (19.7)
		CG	520 (47.3)
		GG	638 (33.1)

4 | DISCUSSION

Obesity has present like a global epidemic and represents a major public health problem in adults and children.^{38,39} Obesity is a multifactorial condition influenced by the combined effects of genes and environment and also their interactions.⁴⁰

Recently, in Tunisian population Gannar et al.⁴¹ found that abdominal obesity was significantly more frequent in women than in men, with higher prevalence of MetS in women with 93%. Although the number of candidate genes has increased and several polymorphisms have been studied in human populations, knowledge about genetic factors underlying the susceptibility to obesity remains incomplete.

However, research has also established that several adipokines, such as leptin, adiponectin, and resistin, might influence both adipose and bone tissues.^{42,43}

One of the most controversial adipokines is resistin (RETN); it was suggested to be the link between obesity, associated insulin resistances, and type 2 diabetes mellitus risks. These discrepancies could be attributed to differences in sample size, ethnicity, and disease status.

RETN is four times more highly expressed in human omental and abdominal subcutaneous white adipocytes than in adipocytes from

TABLE 3 Clinical characteristics according to the genotypes of SNPs

Genotypes	TG (mmol/L)	CT (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	Glycemia (mmol/L)	BMI (kg/m ²)	WC (cm)	HM (cm)	
45T/G	TT	0.79 [0.16-14.3]	4.92±0.99	1.16±0.35	3.31±0.80	5.74±1.99	27.8 [15.7-48.2]	94.68±13.82	104.66±11.97
	TG	0.76 [0.2-5.01]	4.85±0.96	1.13±0.35	3.27±0.82	5.72±1.89	28 [16.3-44.2]	94.87±13.45	104.70±11.08
	GG	0.87 [0.35-2.32]	5.07±0.94	1.09±0.31	3.48±0.84	5.64±1.61	27.7 [19.1-46.4]	98.35±16.92	106.29±10.59
	P	.408	.265	.220	.281	.932	.576	.154	.589
276G/T	GG	0.76 [0.24-14.7]	4.94±1.00	1.16±0.34	3.36±0.81	5.79±2.17	28.7 [18.1-48.2]	96.22±14.9	106.05±11.72
	GT	0.75 [0.16-3.87]	4.86±0.99	1.15±0.34	3.28±0.807	5.64±1.91	27.0 [16.3-46.1]	103.1±11.2	103.15±11.29
	TT	0.83 [0.19-14.31]	4.9±1.01	1.14±0.32	3.27±0.83	5.88±2	29.1 [17.1-45.5]	106.48±11.3	106.48±11.36
	P	.041	.514	.771	.369	.290	.010	<.001	<.001
4522C/T	CC	0.7 [0.2-5.5]	4.89±1.01	1.15±0.338	3.31±0.38	5.74±1.98	27.5 [15.7-44.3]	94.36±13.9	103.96±11.17
	CT	0.82 [0.19-14.31]	4.97±0.96	1.13±0.337	3.36±0.786	5.74±2.06	29.1 [16.8-48.2]	96.69±14.2	106.29±11.96
	TT	0.68 [0.31-3.5]	4.97±0.87	1.23±0.35	3.17±0.805	5.49±1.41	29.1 [16.3-39.4]	92.07±3.55	102.79±10.03
	P	.046	.196	.045	.148	.455	.028	.002	.001
395G/A	GG	0.78 [0.19-14.3]	4.92±1.01	1.16±0.33	3.30±0.82	5.64±1.77	27.8 [16.3-48.2]	94.94±13.8	104.78±11.79
	GA	0.76 [0.16-5.57]	4.89±1	1.16±0.36	3.31±0.84	5.71±1.92	27.6 [27.6-46.8]	94.57±14.9	104.32±11.75
	AA	0.84 [0.28-3.5]	4.84±1.04	1.108±0.37	3.31±0.86	5.85±2.03	28.4 [19.2-41.6]	96.19±13.3	106.05±12.38
	P	.523	.768	.510	.985	.610	.729	.646	.484
420C/G	CC	0.75 [0.2-5.8]	4.83±1.01	1.13±0.32	3.27±0.83	5.75±1.98	27.6 [17.4-42.4]	92.82±13.58	103.17±10.25
	CG	0.86 [0.16-14.7]	4.99±0.99	1.16±0.36	3.34±0.8	5.84±1.98	30.2 [15.7-46.1]	98.74±14.23	107.406±12.18
	GG	0.72 [0.2-3.52]	4.76±0.95	1.15±0.32	3.21±0.79	5.59±2	26.7 [16.8-48.2]	91.77±0.56	103.48±10.94
	P	<.001	.002	.665	.111	.189	<.001	<.001	<.001
LEP 2548G/A	GG	0.77 [0.16-5.5]	4.91±1.09	1.14±0.30	3.33±0.87	5.67±1.94	26.9 [16.8-48.2]	91.71±13.42	103.05±10.35
	GA	0.76 [0.23-14.3]	4.89±0.97	1.15±0.34	3.30±0.82	5.71±1.88	27.8 [15.7-46.8]	94.99±14.54	104.29±11.77
	AA	0.24 [0.24-5.82]	4.89±0.94	3.27±0.75	3.27±0.75	5.63±1.72	29.36 [16.4-44]	96.35±0.43	106.32±11.90
	P	.937	.976	.347	.794	.299	<.001	<.001	.021
LEPR Q223R	GG	0.78 [0.25-5.57]	4.90±0.97	1.16±0.33	3.29±0.79	5.67±1.97	27.9 [15.7-48.2]	95.42±14.9	105.02±11.42
	GA	0.78 [0.16-5.01]	4.98±1.02	1.13±0.33	3.32±0.84	5.67±1.81	27.7 [17.4-46.1]	94.54±13.47	104.67±11.41
	AA	0.68 [0.2-14.31]	4.86±1	1.19±0.41	3.19±0.82	5.88±2.22	29.3 [17.6-43.3]	92.55±13.03	102.35±0.60
	P	.492	.945	.346	.470	.663	.246	.151	.098

BMI, body mass index; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein-cholesterol; LDL, low-density lipoprotein-cholesterol; WC, waist circumference; HM, hip measurement. Means±standard deviation or n (%).

TABLE 4 Genotype frequencies in HSHS population

	SNPs	Genotypes	Non-obese (n=721) (n%)	Obese (n=400) (n%)	P
ADIPOQ	45T/G	TT	443 (61.5)	251 (62.8)	.379
		TG	244 (33.8)	123 (30.8)	
		GG	34 (4.8)	26 (6.4)	
	276G/T	GG	201 (27.9)	156 (39.1)	<.001
		GT	397 (55.1)	136 (34)	
		TT	123 (17)	108 (26.9)	
	4522C/T	CC	394 (54.6)	173 (43.2)	<.001
		CT	252 (34.9)	195 (48.8)	
		TT	75 (10.5)	32 (8)	
LEP	395 G/A	GG	426 (59.1)	228 (19.9)	.250
		GA	251 (34.8)	144 (12.6)	
		AA	44 (6.1)	35 (3.1)	
	G2548A	GG	183 (25.4)	51 (12.7)	<.001
		GA	370 (51.3)	196 (49)	
		AA	168 (23.3)	153 (38.25)	
LEPR	Q223R	QQ	369 (51.2)	216 (53.9)	.126
		QR	278 (38.6)	157 (39.4)	
		RR	74 (10.2)	27 (6.7)	
RETN	420C/G	CC	159 (22)	64 (16)	<.001
		CG	260 (36.1)	260 (65)	
		GG	302 (41.9)	76 (19)	

p, comparison between obese and nonobese groups.

The bold values are significant ($P < .05$).

the thigh, suggesting that human RETN could play a major role in obesity-related insulin resistance.⁵

The frequencies of 420C<G genotypes in our study were similar to those found in different populations.^{44,45} However, there have been reports of differences in the frequencies of the 420C<G in other ethnic groups.^{46,47} RETN 420C<G has been studied extensively in regard to obesity risk but with conflicting reports. Individuals with the GG genotype have been reported to have a higher prevalence of obesity and MetS^{48,49} and the G allele has been associated with increased BMI, weight, body fat mass, and waist circumference,²² similar to our study results.

The effect of the 420C<G SNP on serum resistin levels has been measured. Many studies demonstrated that the G allele results in increased serum RETN. This increase in serum resistin is likely the result of increased resistin promoter activity observed with the G allele.^{24,50} In fact, the DNA element of SNP -420C and SNP-420G of RETN gene had different binding affinities for stimulatory protein 1 (Sp1) and stimulatory protein 3 (Sp3), Sp1 and Sp3 transcription factors specifically bound to the DNA element of SNP-420G with high affinity and enhanced expression of the RETN gene.^{24,51} In another wise in the liver, higher expression of RETN using adenovirus in mice shows enhanced insulin resistance, low serum HDL-C, and high TG levels; all these consequences resemble MetS and obesity parameters in humans.⁵²

We have interested too in LEP which is considered like a gold element that decreases the food intake by stimulating the appearance

of melanocortin, leading to an increase in the hypothalamic hormone α -melanocyte-stimulating hormone (α -MSH). The α -MSH, while binding to its hypothalamic receptor (melanocortin 4) MC4, decreases the food intake. Subsequently, the LEP inhibits the activity of the orexigenic neurons type hypothalamic neuropeptide Y/agouti-related protein. The low level of LEP causes some damage in this mechanism, leading to an increase in the food intake, thus obesity.⁵³

Data in the literature concerning the association between the LEP 2548G/A polymorphism and obesity-related phenotypes are controversial. A lack of association between this polymorphism and obesity has been reported in different populations.⁵⁴⁻⁵⁶ But, negative results have also been reported for African and Romanian populations.^{35,57} These different results may arise from a strong environmental influence, genotype is only one factor in the causal pathway to the disease, and gene-gene and gene-environment interactions can influence the final association genotype/disease and the genetic mode of action.

Our results showed significant differences in genotypic distribution and allele frequencies of the LEP 2548G<A polymorphism between obese patients and normal-weight subjects.

This significant association may be explained by the fact that LEP 2548G<A is a substitution G to A at nucleotide -2548 upstream of the ATG start site in the LEP gene promoter. So, this polymorphism is located at the 5' end of the promoter region of LEP, and it is suggested that this region may contain inhibitory elements from transcription in adipocytes.⁵⁸

TABLE 5 Associations between ADIPOQ, LEP, LEPR, and RETN genotypes and the risk of obesity

	Polymorphisms	OR crude	CI	P	OR adjusted ^a	CI	P
ADIPOQ	45T/G GG+TG/TT	0.943	0.721-1.216	.649	0.831	0.350-1.975	.675
	4522C/T CT+TT/CC	1.52	1.230-1.994	<.001	1.38	1.104-9.252	.037
	395G/A AA+GA/GG	1.135	0.88-1.44	.312	1.994	0.354-11.23	.434
	276G/T TT+GT/GG	0.603	0.467-0.779	<.001	0.608	0.465-0.794	<.001
RETN	420C/G CG+GG/CC	1.48	1.09-2	.011	2.18	1.59-2.98	<.001
LEPR	Q223R GA+AA/GG	0.896	0.703-1.142	.376	1	0.995-1.005	.979
LEP	G2548A AA+GA/GG	2.36	1.75-3.18	<.001	2.23	1.393-12.73	.034

The bold values are significant ($P < .05$).

^aAfter adjustments for age, smoking, gender, physical activity, and coffee consumption.

TABLE 6 Haplotypes associated with obesity

Haplotypes (H)		OR	CI	Frequency (Total population)	Obesity		P
					Frequency (Nonobese)	Frequency (Obese)	
H1	TGCGGGG	0.579	0.393-0.854	0.06952	0.08573	0.03685	.004
H3	TGCAGGC	1.848	1.288-2.650	0.05158	0.04195	0.06281	<.001
H8	TGCGAGG	0.253	0.10-0.72	0.03346	0.02599	0.02115	.002
H11	TGTAGGC	2.03	1.06-3.86	0.02210	0.01384	0.03974	.031
H12	GGCAGTG	1.91	1.04-3.50	0.02179	0.02120	0.02881	.0036
H15	TGCAAGC	9.06	1.05-77.72	0.01653	0.01597	0.03894	.010
H23	TGTGAGG	0.28	0.10-0.82	0.01326	0.01675	0.00280	.007

p, comparison between obese and nonobese groups.

Haplotypes follow this order: 45T<G, 395G<A, 4522C<T, 2548G<A, LEP 223Q<R, 276G<T, 420C<G.

Gong et al. described two repetitive sequences (MER11 and Alu) located on the promoter of the LEP (−2514 to 1545) susceptible to regulate the expression of the gene.⁵⁸ The later identification of the factor of transcription Sp1 at the level of the repetitive sequence suggested that the expression of the LEP is the result of the insertion of this element at the level of the sequence MER11.⁵⁹ Besides, the location of the polymorphism 2548G<A near a site known for connection of the Sp1 (2539) suggests its possible implication in the regulation of the transcription of the LEP via an imbalance of connection with another functional polymorphism places at the level of the sequence MER11 still not identify.

The studied LEPR polymorphism was 223Q<R, and data in the literature concerning its association with obesity are controversial. It has been significant in different Caucasian populations,^{60–62} but not in our population nor in a Greek population.⁶³

The missing of 223Q<R polymorphism effects may be explained by its location in the cytokine receptor homologous 1 (CRH1) domain in LEPR, which was reported to be probably not involved in LEP binding by functional studies.⁶⁴

However, in a previous study, a deletion of CRH1 resulted in a lower LEP response, and it seems that CRH1 contributes to optimal LEPR activation.⁶⁵

The third adipokine studied was ADIPOQ. ADIPOQ has been shown in animal model to be a potent insulin enhancer with an effect on glucose tolerance and regulating energy homeostasis.⁶⁶ In fact, mice fed a high-fat diet experienced a profound weight loss when chronically treated with a proteolytic fragment of ADIPOQ.⁶⁷

In the present study, we examined the obesity association with four common and widely studied SNPs (+45T<G; +276G<T; −4255C<T; −395G<T) of ADIPOQ gene in HSHS population.

We noted that only the 276G<T (localized in exon 2) was associated with obesity ($P < .001$) and suggest a protective role. Our results was confirmed by Al-Daghri et al.,⁶⁸ Boumaiza et al.,⁶⁹ and Ramya et al.⁷⁰ but not by Gui et al.⁷¹ This SNP 276G<T is unlikely to exert a direct biological effect and may not be independently associated with the metabolic phenotypes.

The found association with obesity may be explained by linkage disequilibrium (LD) with another functional variant.

This LD of the ADIPOQ gene is moderate, but there are two small LD blocks, one including SNPs in the promoter region and another one spanning the boundary of exon 2–intron 2.⁷²

In fact, Vasseur et al.⁷³ reported an LD with –11377C<G and –11391G<A SNPs of the 5' promoter region and Menzaghi et al.⁷⁴ found that the 276G<T is present like a marker for functional variant in 3' UTR region through LD.

In the same context, Boumaiza et al.⁶⁹ found that 276G<T was in LD with +2019delA localized in the 3' UTR, a region playing a critical role in the control of gene expression by binding proteins that regulate mRNA processing, translation, and/or degradation.

It has also been reported that LD structures may vary between the populations,⁸ and this may be explained this divergence in results.

Finally, we have performed haplotype analysis for all studied polymorphisms (haplotypes follow this order: 45T<G, 395G<A, 4522C<T, 2548G<A, LEP 223Q<R, 276G<T, 420C<G).

The TGCAGGG, TGCAGG, and TGTGAGG seem to be protective against obesity, but H3 (TGCAGGC), H11 (TGTAGGC), H12 (GGCAGTG), and H15 (TGCAAGC) seem to be risk factors.

The variation in our results of the several studied polymorphisms and obesity may be explained by different genetic backgrounds, environmental conditions, and ethnic and lifestyle characteristics of distinct population groups rather than by the SNP itself. Also, in a polygenic disease such as obesity, with a strong environmental influence, genotype is only one factor in the causal pathway to the disease, and gene–gene and gene–environment interactions can influence the final association between genotype and disease.

Our study has some limitations: The serum level was not measured, and thus, the pathophysiologic mechanism underlying the association could not be further dissected, but the presence of multimers in human serum has been suggested, and this may affect the assay results.

5 | CONCLUSION

In conclusion, this study showed that ADIPOQ 4522C<T and 276G<T, LEP 2548G<A, and RETN 420C<G polymorphisms were associated with obesity risk in HSHS population.

These studied polymorphisms seem to have a synergic effect when combined in haplotypes:

The TGCAGGG, TGCAGG, and TGTGAGG with a potential protective effect and H3 (TGCAGGC), H11 (TGTAGGC), H12 (GGCAGTG), and H15 (TGCAAGC) with a potential pro-obesity effect.

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AUTHORS' CONTRIBUTIONS

Zayani Nesrine carried out the molecular genetic studies, designed the study, and drafted the manuscript. Asma Omezzine carried out the molecular genetic studies, designed the study, and drafted the manuscript. Imen Boumaiza recruited patients and collected clinical and demographic data. Ons Achour, Ahmed Ben Abdelaziz, and Ali Bouslama conceived of the study and participated in design and coordination. Lamia Rebhi, Jihen Rejeb, and Nabila Ben REJEB conceived of the study. All authors read and approved the final manuscript.

ETHICS

The participants underwent physical examinations and laboratory tests. The examiners undertook training in the questionnaire collections and measures. The patients provide informed consent to the molecular diagnosis, in agreement with the guidelines approved by the Hospital Medical Ethics Committee, and informed consent was obtained from all study subjects.

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