

Gender-Specific Association of a Perilipin Gene Haplotype with Obesity Risk in a White Population

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Abstract

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Objective: Perilipin is a class of protein-coating lipid droplets in adipocytes and steroidogenic cells. Our purpose was to examine the association between common single-nucleotide polymorphisms (SNPs) at the perilipin (*PLIN*) locus and obesity, as well as related phenotypes, in unrelated American adults.

Research Methods and Procedures: Four *PLIN* SNPs (*PLIN* 6209T>C, 11482G>A, 13041A>G, and 14995A>T) were typed in 734 white subjects (373 men and 361 women) attending a residential lifestyle intervention program. The baseline anthropometric and biochemical measures were used. Obesity was defined as BMI \geq 30 kg/m².

Results: Multivariate analysis demonstrated that, in women, two of the SNPs (13041A>G, and 14995A>T) were significantly associated with percentage body fat ($p = 0.016$ for 13041A>G and $p = 0.010$ for 14995A>T) and waist

circumference ($p = 0.020$ for 13041A>G and $p = 0.045$ for 14995A>T). Moreover, haplotype analysis using these two SNPs indicated that haplotypes A/T and G/T were both associated with significantly increased obesity risk (odds ratio = 1.76, 95% confidence interval 1.07 to 2.90 for haplotype A/T, and odds ratio = 1.73, 95% confidence interval 1.06 to 2.82 for haplotype G/T) when compared with haplotype A/A. No significant associations between *PLIN* variations and obesity were found in men.

Discussion: Our data support the hypothesis that the *PLIN* locus may be a significant genetic determinant for obesity risk in whites and that women are more sensitive to the genetic effects of perilipin than men.

Key words: adipose, lipolysis, triacylglycerol, polymorphism, haplotype

Introduction

Current evidence has supported the view that genetic factors play a critical role in the etiology of human obesity (1). However, less is known about the specific genes involved in human obesity and their specific contribution to the current epidemic of obesity. The metabolic status of the adipocyte is tightly related to energy homeostasis and the development of obesity (2). Several genes expressed in the adipocyte have been studied, and their variants have been associated with body fat and obesity risk (3–6), supporting the notion that these genes contribute to variability in measures of obesity in the general population (7, 8).

Perilipins are a family of proteins that coat the intracellular lipid droplets. The expression of perilipin appears to be primarily in adipocytes and steroidogenic cells (9–12), and its major demonstrated role is in the regulation of intracellular lipolysis in adipocytes (13,14). In experimental animal models, the absence of perilipin has resulted in lean phenotypes and has counterbalanced both genetic- and dietary-induced obesity in mice (15,16). In contrast, elevated ex-

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pression of perilipin has correlated with increased adiposity in humans (17). Moreover, the perilipin (*PLIN*)¹ 11482 G>A polymorphism in the human *PLIN* gene has been found to be associated with decreased perilipin content and increased lipolytic activity in women (18). The overall evidence has supported the notion that *PLIN* may be a candidate gene for human obesity. In this study, we examined the potential associations among four single-nucleotide polymorphisms (SNPs) at the *PLIN* locus and the risk of obesity. We also examined whether the associations extended to other phenotypes related to lipid and glucose metabolism in a white population.

Research Methods and Procedures

Subjects and Study Design

A total of 734 white subjects, 373 men (mean age 58.6 years) and 361 women (mean age 56.1 years) attending a residential lifestyle intervention program (The Pritikin Longevity Center, Santa Monica, CA) (19) were included in this study. In this population, current smoking was reported by 10.2% and alcohol consumption (>1 drink/week) by 46.8% of the subjects. Medication use was as follows: 10.1% were taking hypoglycemic agents, 16.1% were on cholesterol-lowering drugs, 14.9% were on thyroid medication, and 35.7% of women subjects were on hormone replacement therapy. Due to limitations in DNA availability, genotypes were successfully obtained from 706 subjects for *PLIN* 6209T>C and 13041A>G and from 705 subjects for *PLIN* 11482G>A and 14995A>T. Obesity was defined as BMI \geq 30 kg/m². Percentage body fat was estimated using DXA by a highly trained technical staff. There were no significant differences in the anthropometric and biochemical measures between the individuals with or without genotype information.

Biochemical Measurements

Fasting blood samples were drawn from all subjects at entry into the program (baseline). The blood samples were placed into tubes containing either SST clot-activating gel (BD Biosciences vacutainer system, BD Biosciences, San Jose, CA) for lipid and glucose measurements or 0.1% EDTA for apolipoprotein measurements. The samples for lipid and glucose measurements were allowed to clot, and serum was separated by centrifugation for 15 minutes at 2500 rpm. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), and glucose levels were measured by standardized automated enzymatic methods (GlaxoSmithKline, Welwyn Garden City, Hertford-

shire, UK), whereas low-density lipoprotein-cholesterol (LDL-C) was calculated as described previously (20).

DNA Isolation and Genotyping

Genomic DNA was isolated from whole blood using the QIA amp Blood Kit (QIAGEN, Valencia, CA). First, the DNA fragments containing target SNPs were amplified by multiplex polymerase chain reaction (PCR). The primers used are displayed in Table 1. PCR reactions were carried out in a 10- μ l reaction volume containing 0.2 mM of each dNTP, 0.2 μ M of each primer, 3.0 mM magnesium chloride, and 0.8 U of Qiagen Hotstar Taq polymerase. PCR cycling conditions were 95 °C for 10 minutes followed by seven cycles of 95 °C for 30 seconds, 70 °C for 30 seconds, and 72 °C for 1 minute, then followed by 41 cycles of 95 °C for 30 seconds, 65 °C for 30 seconds, and 72 °C for 1 minute. A final extension phase of 5 minutes at 72 °C was included at the end of the protocol. The PCR products were incubated for 60 minutes at 37 °C with 2.5 U each of Exonuclease I (New England Biolabs, Inc., Beverly, MA) and calf intestinal phosphatase (New England Biolabs, Inc.) to remove unincorporated dNTPs and primers, followed by 15-minute incubation at 75 °C to inactivate the enzymes. Single nucleotide extension was subsequently carried out using the ABI Prism SnaPshot system (Applied Biosystems, Foster City, CA). Probes used are presented in Table 1. The reaction mixture for the extension reaction contained 1.5 μ L of the Snapshot Ready Reaction Mastermix (Applied Biosystems), 1.0 μ L of water, and 1.5 μ L of multiplex PCR products and 1.0 μ L of the probe mixture (2 μ M for each probe). The reaction conditions were 35 cycles of 96 °C for 30 seconds, 50 °C for 30 seconds, and 60 °C for 30 seconds. Products were incubated for 60 minutes at 37 °C with 3 U of calf intestinal phosphatase to remove unincorporated dNTPs, followed by incubation for 15 minutes at 75 °C to inactivate the enzymes. Finally, genotyping was carried out on an ABI Prism 3100 genetic analyzer (Applied Biosystems) using Genotyper version 3.7 (Applied Biosystems).

Statistical Analyses

Multivariate linear regression analysis was used to test the null hypotheses of no association between genetic variants and phenotypic outcomes adjusting for covariates (age, BMI, tobacco smoking, alcohol consumption, and medication status). Analysis of covariance (Tukey test) was employed to compare phenotypic outcomes between genotypic groups with multiple adjustments for covariates. An additive genetic model (grouping was based on the number of variant allele at each polymorphic site) was finally used according to the observed allelic effect. Interactions between gender and *PLIN* genotypes were tested by introduction of the corresponding product terms into the models. The SAS 8.0 statistical package (SAS Institute Inc., Cary, NC) was used to carry out hypothesis testing. A statistical *p*

¹ Nonstandard abbreviations: *PLIN*, perilipin; SNP, single-nucleotide polymorphism; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein-cholesterol; PCR, polymerase chain reaction; FG, fasting glucose; LD, linkage disequilibrium.

Table 1. Description of *PLIN* SNPs, primers, and probes

SNPs	Primers and probes
6209*T>C rs2289487†	Forward: CTCTGTTCTCCAGGGACCAAGTCAGAT Reverse: CCTACACTCTGGGGATGCGGAGAT Probe: GACTGACTGACTGACTGACTGACCCCACTGCCTAGAA
11482G>A rs894160	Forward: AAGTGTGCCCCTGCAGGAAT Reverse: GAGTGGAAGTCTGGGCCATA Probe: GACTGACTGACTGACTGACTGACTGACTGACTGACTGACTTGTGGGGCTCCCTAGA
13041A>G rs2304795	Forward: CTCACCGGCACGTAATGCAC Reverse: CCCTCCAGACCACCATCTCG Probe: GACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACCTTGGTTGAGGAGACAGC
14995A>T rs1052700	Forward: AAGCAGCTGGCTCTACAAAGCA Reverse: AGCATCCTTTGGGGCTTCA Probe: GACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGCCTG CTGGGAGCCT

* The coding number is the number of bases from the variants and the A of ATG of the initiator methionine codon that is denoted as nucleotide +1.

† dbSNP cluster identification.

value <0.05 was considered as a significant boundary. Fasting glucose (FG) and TGs were logarithmically transformed to achieve a normal distribution before statistical testing. The THESIAS program was used to calculate allele frequency, to test pair-wise linkage disequilibrium (LD), and to infer haplotypes. This computer program is based on the maximum likelihood model described by Tregouet et al. (21). We estimated LD for all two-way comparisons of individual SNPs using two common measures: The r^2 (the square of the standardized correlation coefficient δ) and the Lewontin D' ($D' = D/\text{maximum value of } D \text{ if } D > 0 \text{ or } D' = D/\text{minimum value of } D \text{ if } D < 0$). Haplotype association with obesity risk was examined with multiple adjustments for the covariates described above.

Results

The identification of common polymorphisms at the *PLIN* locus was carried out by resequencing of conserved regions between humans and mice in 40 unrelated subjects and by searching one of the public SNP databases (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=5346). Four common polymorphisms, *PLIN* 6209T>C, 11482G>A, 13041A>G, and 14995A>T, were identified and selected for this study. The numbering of these SNPs reflects their relative position to the A of the ATG of the initiator Methionine codon of *PLIN*, which was numbered as “+1” (at position 157,157 on the reference sequence, accession no. GI21431190). Genotype distributions did not deviate from Hardy-Weinberg expectations. Minor allele

frequencies for the SNPs examined were 0.453 for 6209T, 0.299 for 11482A, 0.336 for 13041G, and 0.360 for 14995T. Examination of pair-wise LD indicated that both *PLIN* 6209T>C and 11482G>A were in strong LD ($D' = 0.92$, $p < 0.001$). No significant LD was detected between these SNPs and the 13,041 A>G SNPs ($D' = 0.04$, $p = 0.224$ for 6209T>C/13041A>G pair, and $D' = 0.05$, $p = 0.110$ for 11482G>A/13041A>G pair). Finally, the *PLIN* 14995A>T showed different levels of LD as shown in Figure 1.

We found significant interactions between *PLIN* genotypes and gender for the outcome variables. Therefore, we carried out the analyses for men and women separately.

	6209 T>C	11482 G>A	13041 A>G	14995 A>T
6209 T>C		0.92 (0.66)	0.04 (0.03)	0.47 (0.39)
11482 G>A	<0.001		0.05 (0.04)	0.50 (0.43)
13041 A>G	0.224	0.110		0.20 (0.19)
14995 A>T	<0.001	<0.001	<0.001	

Figure 1: LD matrix in the study population. Pair-wise LD measures: D' and R^2 among the four genotyped *PLIN* SNPs (6209C>T, 11482G>A, 13041A>G, and, 14995A>T) are displayed above the diagonal, whereas the corresponding p values are presented below the diagonal.

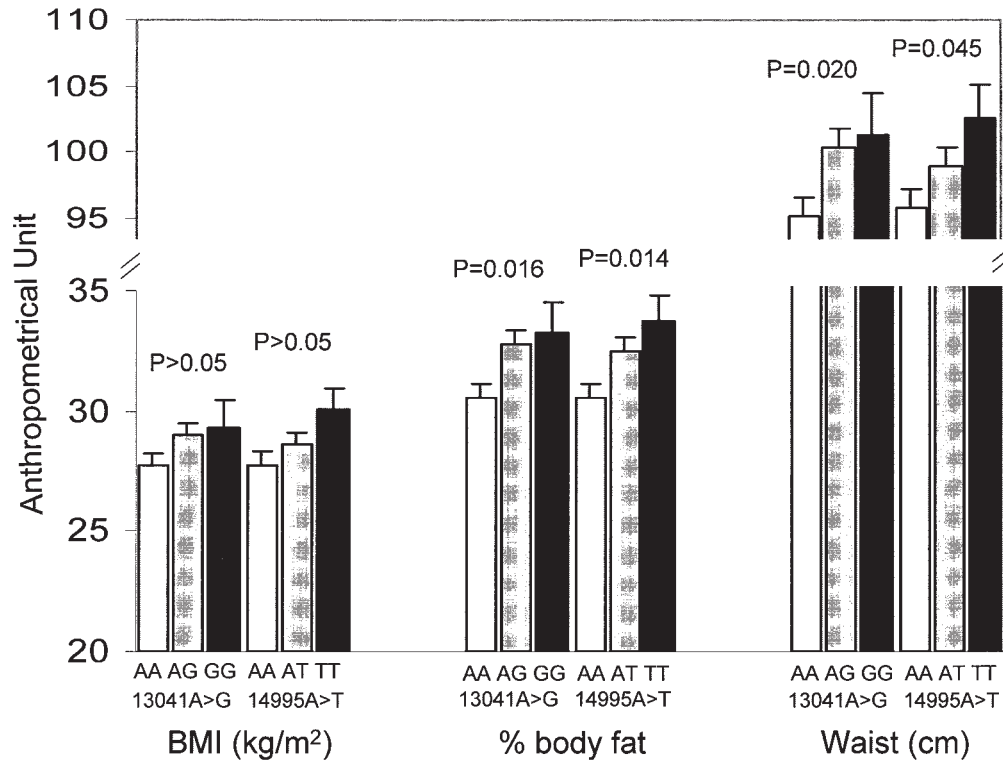


Figure 2: Differences in body fatness measures (BMI, percentage body fat, and waist circumference) and SEs among genotypes at the *PLIN* 13041A>G and 14995A>T SNPs in women. For each polymorphism, *p* values indicate the statistical significance for the global comparison among genotypes for each one of the anthropometric measures examined. The number of subjects for each one of the genotype groups was as follows: for the *PLIN* 13041A>G SNP, AA = 151, AG = 164, and GG = 36; and for the 14995A>T SNP, AA = 138, AT = 159, and TT = 55.

First, we examined the allelic associations for each of the SNPs with body fat measures, including BMI, percentage body fat, and waist circumference. In women, we found significant allelic differences in percentage body fat and waist circumference. For *PLIN* 13041A>G, the mean percentage body fat values for the AA, AG, and GG groups were 30.6%, 32.7%, and 33.3%, respectively ($p = 0.016$). A similar association was observed for mean waist circumference: 95.1, 96.9, and 105.1 cm for AA, AG, and GG subjects, respectively ($p = 0.020$). We observed similar associations for the *PLIN* 14995A>T SNP. Mean percentage body fat in the AA, AT, and TT subjects was 30.5%, 32.5%, and 33.7%, respectively ($p = 0.0104$); and mean waist circumference was 95.7, 98.9, and 102.6 cm, respectively ($p = 0.0453$). Subjects carrying the G/A and the G/G genotypes at the *PLIN* 13041A>G had BMI values 1.25 and 1.60 kg/m² higher than AA subjects. Similarly, for the *PLIN* 14995A>T SNP, AT and TT subjects had 0.87 kg/m² and 2.32 kg/m², respectively, higher BMI than AA subjects (Figure 2). No significant association was found between *PLIN* 6209T>C and *PLIN* 11482G>A genotypes and body fat measures in women. In men, there were no significant genotype-related differences for any of the variables examined (data not shown)

We also examined the association between *PLIN* variations and the risk of obesity. We inferred haplotypes from the four SNPs, and we found that haplotypes containing the minor alleles at SNPs 13,041 or/and 14,995 tended to had increased obesity risk, whereas haplotypes containing the minor alleles at the 6209 and/or 11,482 tended to have decreased obesity risk in women. Among them, haplotype T/G/G/T was associated with the highest obesity risk [odds ratio (OR) = 2.09, 95% confidence interval 0.83 to 5.23] and haplotype C/G/A/A was associated with the highest obesity protection (OR = 0.58, 95% confidence interval 0.25 to 1.34) after adjusting for covariates as previously described. However, none of these associations reached statistical significance due to limitations in sample size. Therefore, to improve the study power, we also analyzed the haplotypic association based on either 6209T>C/11482G>A or 13041A>G/14995A>T haplotypes. We did not find any significant association between haplotypes inferred from 6209T>C/11482G>A in both men and women. When haplotypes inferred from 13041A>G/14995A>T were examined, both haplotype A/T (OR = 1.76, 95% confidence interval 1.07 to 2.90) and haplotype G/T (OR = 1.73, 95% confidence interval 1.06 to 2.82) were significantly as-

Table 2. Frequencies of *PLIN* haplotypes according to the obese/nonobese status and haplotypic OR estimates in women

6209	<i>PLIN</i> SNP*			Nonobese (n = 237)	Obese (n = 122)	OR†	95% CI	
	11482	13041	14995				Lower	Upper
X	X	A	A	0.49	0.37	1 ‡		
X	X	A	T	0.20	0.25	1.76	1.07	2.90
X	X	G	A	0.16	0.19	1.44	0.81	2.55
X	X	G	T	0.15	0.19	1.73	1.06	2.82

* Likelihood ratio test (LRT) a global haplotype effect: LRT statistic = 8.60, with 3 *df*, *p* = 0.035.

† Multiple adjustment for age, smoking, alcohol consumption, and medication status.

‡ Haplotype treated as reference.

sociated with increased risk of obesity as compared with haplotype A/A in women (Table 2). We did not find significant association between 13041A>G/14995A>T haplotypes and the risk of obesity in men.

Because of the tight relationship between body fatness and energy homeostasis, we then analyzed the association between *PLIN* genotypes and some metabolic measures

related to energy homeostasis. In the women subjects, although associated with increased body fatness, *PLIN* 13041A>G and 14995A>T were not significantly associated with the metabolic measures examined (Table 3). In contrast, *PLIN* 6209T>C and 11482G>A were associated with LDL-C level (*p* = 0.006 for *PLIN* 6209T>C and *p* = 0.021 for *PLIN* 11482G>A; Table 3). Unlike the additive

Table 3. Plasma lipid and glucose measures* by *PLIN* genotypes in women

	Genotypes: <i>PLIN</i> 6209 T>C			<i>p</i> †	Genotypes <i>PLIN</i> 11482 G>A			<i>p</i> †
	TT (n = 103)	TC (n = 168)	CC (n = 80)		GG (n = 163)	GA (n = 154)	AA (n = 34)	
FG (mg/dL)	94.2 (2.7)	97.0 (2.1)	95.8 (3.1)	0.831	96.3 (2.2)	95.4 (2.2)	96.5 (4.7)	0.998
TG (mg/dL)	153 (7)	155 (6)	148 (9)	0.914	148 (6)	160 (6)	151 (13)	0.186
TC (mg/dL)	215 (4)	211 (3)	220 (4)	0.223	211 (3)	214 (3)	228 (7)	0.090
LDL-C (mg/dL)	124 (3)	116 (3)	130 (4)	0.006	121 (3)	119 (3)	136 (6)	0.021
HDL-C (mg/dL)	61.0 (1.4)	63.5 (1.1)	60.7 (1.6)	0.190	61.0 (1.1)	63.5 (1.1)	61.8 (2.3)	0.265
TC/HDL-C	3.73 (0.10)	3.48 (0.08)	3.71 (0.11)	0.078	3.63 (0.08)	3.56 (0.08)	3.69 (0.17)	0.705
	<i>PLIN</i> 13041 A>G)			<i>p</i> †	<i>PLIN</i> 14995 A>T			<i>p</i> †
	AA (n = 151)	AG (n = 164)	GG (n = 36)		AA (n = 138)	AT (n = 159)	TT (n = 55)	
FG (mg/dL)	93.9 (2.2)	96.7 (2.2)	101.2 (4.7)	0.410	93.5 (2.3)	98.2 (2.2)	95.2 (3.7)	0.487
TG (mg/dL)	148 (6)	155 (6)	170 (13)	0.172	145 (6)	156 (6)	164 (10)	0.155
TC (mg/dL)	211 (3)	214 (3)	227 (7)	0.081	212 (3)	215 (3)	217 (5)	0.756
LDL-C (mg/dL)	120 (3)	120 (3)	129 (6)	0.342	120 (3)	121 (3)	124 (5)	0.814
HDL-C (mg/dL)	61.0 (1.1)	62.6 (1.1)	64.8 (2.4)	0.298	63.1 (1.2)	61.8 (1.1)	60.7 (1.9)	0.504
TC/HDL-C	3.60 (0.08)	3.57 (0.08)	3.84 (0.17)	0.333	3.56 (0.08)	3.65 (0.08)	3.61 (0.13)	0.742

* Presented as mean (SE).

† Test of homogeneity, with multiple adjustment for age, BMI, tobacco smoking, alcohol consumption, and medication status.

Table 4. Plasma lipid and glucose measures* by *PLIN* genotypes in men

	Genotypes: <i>PLIN</i> 6209 T>C			<i>p</i> †	Genotypes: <i>PLIN</i> 11482 G>A			<i>p</i> †
	TT (<i>n</i> = 118)	TC (<i>n</i> = 162)	CC (<i>n</i> = 75)		GG (<i>n</i> = 189)	GA (<i>n</i> = 131)	AA (<i>n</i> = 34)	
FG (mg/dL)	107 (3)	107 (3)	108 (4)	0.992	109 (3)	105 (3)	103 (6)	0.487
TG (mg/dL)	187 (10)	190 (9)	193 (13)	0.791	190 (8)	189 (10)	182 (19)	0.860
TC (mg/dL)	211 (4)	206 (3)	209 (5)	0.675	207 (3)	210 (4)	212 (8)	0.686
LDL-C (mg/dL)	125 (3)	123 (3)	125 (4)	0.938	121 (3)	128 (3)	127 (6)	0.197
HDL-C (mg/dL)	48.0 (1.1)	47.3 (0.9)	46.2 (1.4)	0.585	47.9 (0.9)	46.0 (1.1)	49.2 (2.1)	0.245
TC/HDL-C	4.65 (0.12)	4.57 (0.11)	4.77 (0.16)	0.582	4.56 (0.10)	4.74 (0.12)	4.61 (0.23)	0.530

	<i>PLIN</i> 13041 A>G			<i>p</i> †	<i>PLIN</i> 14995 A>T			<i>p</i> †
	AA (<i>n</i> = 160)	AG (<i>n</i> = 151)	GG (<i>n</i> = 44)		AA (<i>n</i> = 158)	AT (<i>n</i> = 151)	TT (<i>n</i> = 44)	
FG (mg/dL)	110 (3)	105 (3)	106 (5)	0.476	110 (3)	105 (3)	106 (5)	0.654
TG (mg/dL)	191 (9)	196 (9)	158 (17)	0.153	198 (9)	183 (9)	181 (17)	0.747
TC (mg/dL)	215 (3)	203 (4)	204 (7)	0.051	209 (3)	207 (4)	214 (7)	0.669
LDL-C (mg/dL)	129 (3)	120 (3)	121 (5)	0.049	123 (3)	125 (3)	129 (5)	0.605
HDL-C (mg/dL)	47.6 (0.9)	45.9 (1.0)	50.9 (1.8)	0.047	46.7 (1.0)	47.5 (1.0)	49.3 (1.8)	0.438
TC/HDL-C	4.75 (0.11)	4.62 (0.11)	4.30 (0.21)	0.158	4.71 (0.11)	4.60 (0.11)	4.52 (0.21)	0.625

* Presented as mean (SE).

† Test of homogeneity, with multiple adjustment for age, BMI, tobacco smoking, alcohol consumption, and medication status.

allele effects shown by *PLIN* 13041A>G/14995A>T on body fatness, only the carriers with homozygous variations of *PLIN* 6209T>C/11482G>A tended to have higher LDL-C, whereas carriers of other genotypes had comparable levels in these measures. In the men, we found that the study subjects who carried *PLIN* 13041G tended to have lower LDL-C levels in comparison with those carrying wild-type homozygotes ($p = 0.049$ for LDL-C). It was noticed that such associations were all marginal. In addition, we also observed an association between the *PLIN* 13041A>G SNP and HDL-C levels ($p = 0.047$). The genotypes of *PLIN* 6209T>C, 11482G>A, and 14995A>T were not associated with any metabolic measures examined in men (Table 4). However, we cannot discard the possibility that some of these significant associations demonstrated for plasma lipid variables were a result of chance due to the fact that we analyzed four SNPs and six variables.

Discussion

First reported in the early 1990s, perilipin has been emerging as a key regulator of lipolysis in adipocytes and body fat accumulation (14–17,22–24). More recently, genetic variation at the *PLIN* locus has been associated with

decreased perilipin content and increased lipolytic activity in human adipocytes (18), supporting the role of *PLIN* as a locus for obesity in the general population. In the present study, we examined the association between variability at the *PLIN* locus and anthropometric and metabolic variables in a white population with elevated mean BMI. Among four common SNPs identified and genotyped in this population, we found that two SNPs (*PLIN* 13041A>G and 14995A>T) located in the 3' region were significantly associated with increased percentage body fat and waist circumference and marginally associated with increased BMI in women subjects. Moreover, analyses of inferred haplotypes using the *PLIN* 13041A>G and 14995A>T SNPs demonstrated an increased risk of obesity for the A/T and G/T haplotypes. Conversely, in men, *PLIN* polymorphisms were not significantly associated with any of the measured parameters of body fatness.

Perilipins are expressed mostly in adipose cells and stereogenic cells. Because of their physical location within fat depots, perilipins have been examined for their roles in regulating the mobilization of fat reserves and body fat accumulation, and several in vitro studies have supported this notion (13,23,25). Further in vivo evidence for these roles has come from the knockout mice models (15,16). Our

current findings in relation to human *PLIN* gene variants were also consistent with the results derived from the experimental models, suggesting a conserved role of perilipin in lipolysis across different species.

Several perilipin isoforms have been identified resulting from alternative splicing (9,26), and these isoforms may be functionally different (24). Both *PLIN* 13041A>G and 14995A>T are located in the 3' region, where alternative splicing occurs during transcription. These polymorphisms may alter the transcription product by affecting splicing. *PLIN* 13041A>G and 14995A>T are in significant LD with each other. Therefore, we postulate that the observed associations between these two polymorphisms and body fat measures may be pointing to the same causal mutation, and, considering that the 14995T allele was consistently present in haplotypes associated with increased obesity risk, we hypothesize that this allele may be more closely associated with the causal mutation.

In our study, we examined several anthropometric measures (BMI, percentage body fat, and waist circumference). Although they are significantly correlated, these measurements are not identical in representing body fatness. Thus, BMI does not distinguish fat from lean mass. Moreover, these correlations are age dependent (27,28). On the other hand, waist circumference has been proposed as a more precise measurement to identify those at higher risk for metabolic syndrome (29). Despite those differences, it is reassuring that we have found consistent associations between *PLIN* polymorphisms and several indices of obesity.

Measures of obesity are usually correlated with abnormalities in glucose and lipid metabolism. However, in our study, we did not find significant associations between the *PLIN* 13041A>G and the 14995A>T SNPs and glucose or lipid-related measures. Similar findings have been observed in experimental models. Thus, the *PLIN* knockout mice appear to adapt to the constitutively activated lipolysis caused by *PLIN* gene ablation by activating mechanisms to dispose of these lipolytic products through up-regulation of oxidative catabolic pathways and down-regulation of lipid/sterol synthetic pathways (30). We suggest that such compensatory mechanisms may also take place when lipolysis is repressed.

The other two SNPs examined (*PLIN* 6209T>C and 11482G>A) were not associated with body adiposity in this study. *PLIN* 11482G>A has been previously reported by Mottagui-Tabar et al. (18) in association with decreased perilipin contents and increased lipolysis rate in obese women. Therefore, we expected that *PLIN* 11482A would be associated with leanness phenotypes. Several reasons may account for the null association between this polymorphism and body fat measures in our study. First, our study population consisted of more obese subjects than the general population (mean BMI = 29.6 kg/m²). It is possible that these subjects were genetically predisposed to obesity

due to the influence of other loci and that the expression of the protective effect of *PLIN* 11482A may be repressed under these conditions. Moreover, the *PLIN* 11482G>A polymorphism reported by Mottagui-Tabar et al. is an intronic SNP probably in LD with a functional mutation. As such, the association between *PLIN* 11482G>A and phenotypic variables could be affected by population-specific genetic structure, in which the magnitude of pair-wise LD between *PLIN* 11482G>A and the functional variation may be diminished in our population.

The finding that women who carried *PLIN* 11482AA genotype appeared to have higher LDL-C was in line with Mottagui-Tabar et al.'s study (18) in which AA genotype was associated with increased adipose lipolysis rate. The elevated fatty acid in circulation would increase their flux into the liver resulting in altered lipid metabolism and promoting cholesterol production (31). Because *PLIN* 6209T>C and 11482G>A were in almost complete LD, we postulated that the observed association between *PLIN* 6209 and LDL-C concentrations may have the same genetic basis as the *PLIN* 11482G>A SNP. However, in the current study, we did not correct the *p* value for multiple comparisons. Therefore, we cannot rule out the presence of false positives, even at higher levels of significance (i.e., *p* = 0.01), and further studies will be required to confirm the observed association between this locus and plasma lipid-related variables. However, when it comes to our findings regarding anthropometric variables, false positives are not a major issue. It should be noted that these contrasts were our primary hypotheses, and our findings support previous evidence implicating this locus with obesity in humans.

The *PLIN* locus was not associated with obesity-related measures in men. It has been proposed that men and women may have different sets of obesity susceptibility genes (7). In addition, twin studies have suggested that obesity may be more inheritable in women than in men (32). However, larger studies are needed before we conclude that *PLIN* is not a locus for obesity-related phenotypes in men. The differential expression levels of perilipin in men and women (33) may account for their different sensitivity to the genetic effects of *PLIN*.

In summary, we found significant associations between two SNPs (*PLIN* 13041A>G and 14995A>T) at the 3' region of the *PLIN* locus and obesity risk in white women. Carriers of the variant alleles at these two SNPs had increased mean body fat content, waist circumference, and BMI as compared with the carriers of the wild-type genotypes. Conversely, no significant associations were found between *PLIN* polymorphisms and body fatness measures in men. Our findings support a significant role of *PLIN* as a locus for obesity risk in women.

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