

# Polymorphisms in *PLIN* and Hypertension Combined with Obesity and Lipid Profiles in Han Chinese

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## Abstract

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The current study investigated the association between *PLIN* polymorphisms and the combination of hypertension and obesity (HO) and the related clinical features. The polymorphisms 1237 (T/C), 1243 (C/T), and 1323 (C/G) were genotyped in 503 cases with HO and 511 unrelated controls. No associations between polymorphism 1237 (T/C) or 1243 (C/T) and HO were found. However, total cholesterol (TC) levels were significantly different among genotypes of polymorphism 1243 ( $p = 0.023$ , power = 0.55). In male cases, 1243T carriers (TT + CT) had higher TC, high-density lipoprotein-cholesterol, and low-density lipoprotein-cholesterol levels compared with CC homozygote carriers ( $5.23 \pm 0.88$  vs.  $4.98 \pm 0.90$ ,  $p = 0.024$ ;  $1.13 \pm 0.23$  vs.  $1.07 \pm 0.22$  mM,  $p = 0.034$ ;  $3.3 \pm 0.78$  vs.  $3.11 \pm 0.80$ ,  $p = 0.03$ , respectively). Additionally, 1243T allele carriers were more prevalent among the subjects with both HO and elevated TC levels ( $\geq 5.2$  mM) than those with HO and optimal TC levels ( $< 5.2$  mM) ( $\chi^2 = 8.53$ ;  $p < 0.003$ ; odds ratio, 1.69; 95% confidence interval, 1.19–2.42). Multiple logistic regression analysis suggested a significant contribution of polymorphism 1243 to the elevated TC levels after controlling for conventional risk

factors (odds ratio, 1.48; 95% confidence interval, 1.14–1.91;  $p = 0.003$ ). Polymorphism 1243 in the *PLIN* gene did not seem to be associated with HO but with TC levels in Chinese. The *PLIN* gene may be involved in human lipid metabolism.

**Key words:** perilipin, adipocytes, combination of hypertension and obesity, case control study, lipolysis

The perilipins are the most abundant proteins at the surface of lipid droplets in adipocytes. They are encoded by a single-copy gene called *PLIN*. *PLIN* gives rise to multiple mRNA by alternative and tissue-specific splicing; thus, it can yield three protein isoforms (1). Perilipin A is the most abundant isoform expressed in both adipose and steroidogenic cells; the B and C isoforms are expressed in adipocytes and steroidogenic cells, respectively (2). Perilipin A coats the lipid droplets of adipocytes. The role of perilipin A in regulating triacylglycerol hydrolysis has been confirmed in several studies (3,4) to be associated with hormone-sensitive lipase (HSL)<sup>1</sup> and tumor necrosis factor- $\alpha$ . A recent study has shown that the absence of perilipin results in leanness and reverses obesity in *Lepr* (*db/db*) mice (5). Therefore, the perilipins have been considered a potential target of antiobesity medications (5,6).

The human *PLIN* gene has been localized to 15q26 by fluorescence situ hybridization (7). The entire sequence of the human *PLIN* gene was first released to the public database, the National Center of Biotechnology Information, in May 2002. Four single-nucleotide polymorphisms (SNPs) in the human *PLIN* gene have been reported in Japanese (<http://snp.ims.u-tokyo.ac.jp>). The hypothesis of this study was that the poly-

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<sup>1</sup> Nonstandard abbreviations: HSL, hormone sensitive lipase; SNP, single-nucleotide polymorphism; HO, the combination of hypertension and obesity; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein-cholesterol; FBS, fasting blood sugar; HDL-C, high-density lipoprotein-cholesterol; HWE, Hardy-Weinberg Equilibrium; OR, odds ratio; CI, confidence interval; PCR, polymerase chain reaction; bp, base pair(s).

**Table 1.** Comparison of clinical characteristics between cases and controls

	Cases (N = 503)	Controls (N = 511)
Men/women	284/219*	230/281
Average age (years)	49 ± 9	48 ± 10
TC (mM)	5.16 ± 0.92*	4.74 ± 0.88
HDL-C (mM)	1.13 ± 0.24*	1.33 ± 0.32†
TG (mM)	1.82 ± 1.03*	1.22 ± 0.69†
LDL-C (mM)	3.08 ± 1.00*	2.85 ± 0.77
FBS (mM)	5.29 ± 0.86*	4.92 ± 0.48†
SBP (mm Hg)	144.8 ± 17.0*	116.7 ± 10.2
DBP (mm Hg)	95.0 ± 10.4*	74.8 ± 5.8
BMI (kg/m <sup>2</sup> )	30.8 ± 2.5*	22.4 ± 1.8
Smokers	225*	181
Drinkers	188*	134

\*  $p < 0.05$  level between control and case subjects.

† Significance was tested on log-transformed values.

morphisms in the *PLIN* gene might be associated with the combination of hypertension and obesity (HO) or its related clinical features. Five hundred three cases with HO and 511 controls were recruited from hospitals and communities in the Beijing metropolitan area and Shanxi province according to strict criteria. Polymorphisms 1237 (*T/C*), 1243 (*C/T*), and 1323 (*C/G*) (IMS-JST061886, IMS-JST061887, and IMS-JST061888 in the database of Japanese Single Nucleotide Polymorphisms, respectively) were selected for genotyping in all subjects.

Compared with the control group, the case group had a greater proportion of smokers and drinkers, and much higher BMI, systolic blood pressure (BP) (SBP), and diastolic BP (DBP) levels. The case group also had significantly higher total cholesterol (TC), triglyceride (TG), low-density lipoprotein-cholesterol (LDL-C), and fasting blood sugar (FBS) levels but lower high-density lipoprotein-cholesterol (HDL-C) levels than the control group (Table 1).

The existence of polymorphisms was confirmed by sequencing of 96 samples, which showed T being the major allele at 1237 ( $T = 0.9521$ ) and C being the major allele at 1243 ( $C = 0.7172$ ). Polymorphism 1323 (*C/G*) reported in Japanese was not detected in our population. This finding was confirmed by an additional sequencing in an extra 96 samples. No significant deviation from the Hardy-Weinberg Equilibrium (HWE) was observed for polymorphism 1243. However, polymorphism 1237 showed significant deviation from HWE among cases and controls. The extent of linkage disequilibrium between polymorphisms 1237 and 1243 did not reach significant level ( $p = 0.69$ ).

Univariate analysis indicated that the genotype and allele frequencies of polymorphisms 1237 (*T/C*) and 1243 (*C/T*) were not significantly different between the case and control groups (Table 2). After controlling for age, gender, cigarette smoking, alcohol consumption, and blood lipid level, polymorphisms 1237 and 1243 were not found to contribute to HO (both  $p > 0.05$ ) by multiple logistic regression analyses.

TC levels were significantly different among genotypes for the polymorphism 1243 in all subjects ( $p = 0.023$ , power = 0.55), with mean levels of TC levels being 5.01, 4.91, and 4.84 mM for *CT*, *TT*, and *CC* genotypes, respectively. Genotypes *CT* and *TT* were pooled as a *T* allele carrier group in subsequent analyses because no significant difference of TC levels was identified between the two genotype groups. Among the cases, levels of TC, HDL-C, and LDL-C tended to be significantly higher among 1243T allele carriers (*CT* + *TT*) than among *CC* homozygotes ( $p = 0.02$ , 0.03, and 0.03, respectively) in men (Table 3). The power of the significance was 0.52, 0.43, and 0.45, respectively. However, the lipids difference was absent between the female cases and controls. Covariance analyses showed that after controlling for confounding effects from age, gender, cigarette smoking, alcohol consumption, BMI, BP, and medication use, polymorphism 1243 was not significantly associated with TC levels ( $p = 0.0514$ ) in cases with HO.

To explore further the association between polymorphism 1243 and TC level, all subjects were tentatively subdivided into two groups by TC levels ( $\geq 5.2$  vs.  $\leq 5.2$  mM). The distribution of the genotype of polymorphism 1243 in the

**Table 2.** Genotype and allele frequencies of *PLIN* gene polymorphisms between case and control groups

	Genotypes and alleles	Cases (N = 503)	Controls (N = 511)	$\chi^2$	$p$
SNP1237 ( <i>T/C</i> )	<i>TT/TC/CC</i>	451/28/24	458/38/15	3.583	0.167
	<i>T/C</i>	0.9245/0.075	0.934/0.067	0.624	0.430
SNP1243( <i>C/T</i> )	<i>CC/CT/TT</i>	238/227/38	262/209/40	1.883	0.390
	<i>C/T</i>	0.699/0.301	0.717/0.283	0.832	0.362

**Table 3.** Lipid profiles of case subjects by genotypes and gender at position 1243

	All cases (n = 503)			Male cases (n = 267)			Female cases (n = 236)		
	CC	CT + TT	p	CC	CT + TT	p	CC	CT + TT	p
TC (mM)	5.01 ± 0.87	5.21 ± 0.90	0.01	4.98 ± 0.90	5.23 ± 0.88	0.02	5.03 ± 0.84	5.22 ± 0.96	0.21
HDL-C (mM)*	1.12 ± 0.23	1.16 ± 0.24	0.09	1.07 ± 0.22	1.13 ± 0.23	0.03	1.18 ± 0.23	1.20 ± 0.26	0.61
LDL-C (mM)	3.11 ± 0.78	3.26 ± 0.80	0.03	3.11 ± 0.80	3.31 ± 0.78	0.03	3.09 ± 0.81	3.13 ± 0.99	0.61
TG (mM)*	1.84 ± 1.11	1.81 ± 0.96	0.49	2.02 ± 1.32	1.83 ± 1.03	0.97	1.61 ± 0.71	1.78 ± 0.87	0.28

\* Significance was tested on log-transformed values.

elevated TC group was significantly different from that in the optimal TC group ( $\chi^2 = 13.40$ ,  $p < 0.001$ ). Among subjects with HO, the proportion of T allele carriers (CT + TT) was significantly higher in the elevated TC group than in the optimal TC group [ $\chi^2 = 8.53$ ;  $p < 0.003$ ; odds ratio (OR), 1.69; 95% confidence interval (CI), 1.19~2.42], whereas the significance was absent in controls. Multiple logistic regression analyses performed among all subjects by defining  $y = 1$  for TC level  $\geq 5.2$  mM and  $y = 0$  for TC levels  $< 5.2$  mM showed that after controlling for traditional risk factors such as age, gender, BMI, cigarette smoking, and alcohol consumption, T allele at position 1243 significantly contributed to TC levels (OR, 1.48; 95% CI, 1.14~1.91;  $p = 0.003$ ). Age, BMI, and alcohol consumption were also significant determinants (Table 4). No such associations were identified between polymorphism 1243 and BP, FBS, and BMI. Neither univariate nor multivariate analyses showed any significant association between polymorphism 1237 and continuous variables such as BMI, BP, FBS, or lipid levels.

In this study, no direct associations between the PLIN gene and HO were observed, although previous studies in vivo or vitro have revealed an important role of perilipins in hydrolysis of triacylglycerol in adipocytes (3–6).

However, our study found that the frequent polymorphism 1243 (C/T) in the PLIN gene was associated with TC levels. Although the difference of TC levels among genotypes of polymorphism 1243 were minor, the association between polymorphism 1243 and TC levels among cases with HO indicates a clue to future study.

It is unclear how the perilipins affect TC levels. In adipocytes, perilipin A increases the triacylglycerol content of cells by forming a barrier that reduces the access of soluble lipases to stored lipids, thus inhibiting triacylglycerol hydrolysis (4). HSL hydrolyzes triacylglycerol and cholesterol esters with equal efficiency and appears to be identical to the neutral cholesterol esterase that hydrolyzes cholesterol esters stored in intracellular lipid droplets of steroidogenic cells (8). Translocation of HSL to the lipid droplet has been associated with the greater lipolytic rates in the animal model rat (9). Additionally, tumor necrosis factor- $\alpha$  has been reported to be associated with the function of the perilipins in adipocytes (3,10). Perilipins have been found coating lipid droplets (cholesterol esters) in steroidogenic cells (perilipin A and C) besides adipocytes (11). Thus, it seems justified to assume that perilipin A or C, which are expressed mainly in steroidogenic cells, act on lipolysis of cholesterol esters in a similar way to that in

**Table 4.** Multiple unconditional logistic regression analysis of risk factors contributing to higher or abnormal TC levels (TC  $\geq 5.2$  mM) in all subjects

Variable	Parameter estimate	SE	Standardized estimate	OR	95% CI for OR	P
SNP1243	0.2919	0.1376	0.0805	1.34	1.02 to 1.75	0.03
Age	0.0401	0.0072	0.2167	1.04	1.03 to 1.06	0.0001
Alcohol consumption	0.3816	0.1454	0.0980	1.47	1.10 to 1.95	0.0087
BMI	0.0709	0.0146	0.1862	1.07	1.04 to 1.11	0.0001

Dependent variable was defined as status of abnormal TC level according to TC level ( $\geq 5.2$  or  $< 5.2$  mM), and independent variables included age, gender, BMI, cigarette smoking, alcohol consumption, and polymorphism 1243.

adipocytes. A study on comparison of proteins expressed in ruptured and stable human atherosclerotic plaques has suggested that the perilipins are likely involved in development and process of atherosclerotic plaque (12).

It is unclear how the silent (i.e., without causing an amino acid exchange) polymorphism 1243 can affect metabolism. Generally, it is possible that a silent polymorphism can be in complete or near-complete linkage disequilibrium with a yet-unidentified second polymorphism that has functional relevance, for example, in the promoter region; alternatively, the polymorphism might be in linkage disequilibrium with a functional polymorphism that is in analogy to the situation in *calpain-10* (13). This scenario remains a possibility because intronic regions of the human *PLIN* gene have not yet been systematically screened for SNPs.

More extensive screening of variations in the *PLIN* gene and subsequent association studies with larger sample sizes (at least 1213 to get a power > 0.54 recommended by the power program of SAS) will be necessary to confirm the association between the *PLIN* gene and TC levels and to identify the functional variants that might result in the association between the *PLIN* gene and lipid levels.

## Research Methods and Procedures

### Study Population

A case control study design was applied to identify whether *PLIN* gene polymorphisms were associated with obesity and hypertension. All subjects were recruited from a population in north China where both hypertension and obesity were highly prevalent.

Five hundred three subjects with HO and 511 unrelated age-matched controls who met the following criteria were recruited. Inclusion criteria for cases were as follows: 20 to 69 years of age, Chinese Han nationality, SBP  $\geq$  160 mm Hg and/or DBP  $\geq$  90 mm Hg or subjects on antihypertensive therapies, and BMI  $\geq$  28. To be eligible for our study, controls had to meet the following criteria: no history of any antihypertensive medications and SBP < 130 mm Hg and DBP < 85 mm Hg and BMI < 25. Subjects with secondary hypertension, secondary obesity, coronary heart disease, stroke, cancer, or type 2 diabetes were excluded from this study.

### Phenotypic, Demographic, and Lifestyle Data

A set of questionnaires was completed that included demographic information, details of medical history, and family history including hypertension, obesity, type 2 diabetes, stroke, medication use, alcohol consumption, and cigarette smoking. Three BP measurements were obtained from each participant by trained and certified observers according to a common protocol adapted from procedures recommended by the American Heart Association (14). Anthropometric measurements, including height, weight,

waistline, and hip circumference, were obtained by standard protocols, and then BMI and waist-to-hip ratio were calculated.

Venous blood was drawn from all subjects after an overnight fast. Serum and plasma were separated immediately and stored at  $-70^{\circ}\text{C}$ .

The study protocol was approved by the local research ethics committee of the Cardiovascular Institute and Fu Wai Hospital, Chinese Academy of Medical Sciences. Written informed consents were obtained from all study subjects before data collection.

### Biological Measurements

Serum lipids including TC, TG, and HDL-C as well as FBS were measured by enzymatic methods with a Hitachi 7060 Automatic Analyzer (Hitachi, Tokyo, Japan) and the manufacturer's reagent kits. The clinical biochemistry laboratory was standardized for lipid measurements according to the criteria of the Centers for Disease Control and Prevention-National Heart, Lung, and Blood Institute Lipid Standardization Programs (15).

### Genotyping of Polymorphisms

Ninety-six randomly selected genomic DNA samples were amplified and sequenced from 522,916 to 523,358 in contig NT-010356.11 to confirm the reported polymorphisms 1237, 1243, and 1323 (exon 7) in *PLIN*. Fluorescent dye terminator cycle sequencing was performed, and products were analyzed with an Applied Biosystems 3700 capillary sequencer (Applied Biosystems, Foster City, CA). Polymorphisms 1237 (T/C) and 1243 (C/T) were genotyped by polymerase chain reaction (PCR)-restriction fragment-length polymorphism. The PCR reactions for sequencing to confirm three reported polymorphisms were performed by using primers 5'-aag ggt cag ggg agt tac ca-3' and 5'-aat gtt gcc agg gca ctg ag-3'. PCR was performed on 50 ng of DNA in a 25- $\mu\text{l}$  mixture containing 1.0 U of Taq polymerase (Takara, Kyoto, Japan); 7 mM dNTPs; 0.5 pmol forward and reverse primers, respectively; 50 mM  $\text{Mg}^{2+}$ ; and 2.5  $\mu\text{L}$  of buffer (Takara) for 32 cycles of denaturation at  $94^{\circ}\text{C}$  for 60 seconds, annealing at  $60^{\circ}\text{C}$  for 40 seconds, and extension at  $70^{\circ}\text{C}$  for 60 seconds. The length of the amplification product was 448 base pairs (bp).

For polymorphism 1243, the presence of the 1243C allele created an *Alw26I* site in the 448-bp PCR product for sequencing, which resulted in digested bands of 334 and 114 bp (instead of an undigested band of 448 bp).

Primers 5'-tta cca cag gag gca ctg ac-3' (forward) and 5'-ccc ctt ggt tga gga gag cg-3' (reverse) were applied to amplify fragments that contain polymorphism 1237. An MbiI site (MBI Biotechnology Co. Ltd., Burlington, Ontario, Canada) was introduced in reverse primer when polymorphism 1237 was C. After digestion, presence of a 1237C allele resulted in digested bands of 190 and 18 bp (instead

of an undigested band of 208 bp). The products were analyzed by 3% agarose gel. PCR reactions were performed in 10  $\mu$ L of volume system that contained 25 ng of genomic DNA; 0.2 U of Taq polymerase (Takara); 1.0 mM dNTPs for polymorphism 1237 (2.0 mM for polymorphism 1243); 1.0 pmol forward and reverse primers, respectively; 20 mM  $Mg^{2+}$ ; and 1.0  $\mu$ L of buffer (Takara). PCR conditions for polymorphism 1237 were: 32 cycles of denaturation at 94 °C for 60 seconds, annealing at 62 °C for 30 seconds, and extension at 72 °C for 40 seconds.

All PCR reactions were performed in 9700 thermal cyclers. Oligo 6.0 was applied to design primers.

### Statistical Analysis

The frequencies of genotypes or alleles between case and control groups were compared by the  $\chi^2$  test. Differences in continuous parameters among genotype groups were evaluated by Student's *t* test (two groups) and one-way ANOVA (three groups). FBS, TG, and HDL-C were log-transformed before analysis, and their untransformed data are presented in Tables 1 and 3 by mean  $\pm$  SD. Multivariate ANOVA was performed to evaluate the association between polymorphisms and lipid profiles by adjusting possible confounding factors and interactions among them.

HWE was tested using  $\chi^2$  testing. Linkage disequilibrium strength was evaluated by using the 2LD program (University of London; <http://www.iop.kcl.ac.uk/IoP/Departments/NeuroSci/research.shtml>).

Data analyses, except the test of linkage equilibrium, were performed by SAS version 6.12 (SAS Institute Inc., Cary, NC). Power calculations for general linear models and ANOVAs were computed by the use of SAS macro programs ("Power: A Simple Macro for Power and Sample Size Calculations" available as technical support document TS-272 at [http://www.sas.com/service/techsup/tnote/tnote\\_stat.html](http://www.sas.com/service/techsup/tnote/tnote_stat.html)).  $p < 0.05$  was considered to be statistically significant. The GenBank accession number is NT\_010356.11.

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