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Common SNPs of APM1 Gene Are Not Associated With Hypertension or Obesity in Chinese Population

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Objective To investigate whether the common variants 45T/G and 276G/T in *APM1* gene were associated with hypertension combined with obesity (HO) and related clinical features in Chinese Han population. **Methods** A case-control study design was applied. Common polymorphisms of 45T/G and 276G/T were genotyped by PCR product sequencing in 484 cases with HO and 502 controls with normal blood presure and BMI < 25. **Results** The genotype and allele frequencies of 45T/G, 276G/T, and haplotype defined by the two variants in cases did not differ from those in controls. The means of blood pressure, BMI and waist-hip ratio did not differ among genotypes of the two polymorphisms and haplotypes. Among lipid profiles, only serum high-density lipoprotein cholesterol (HDL-C) levels were significantly lower in T allele carriers than that in non-T carriers after adjusting possible confounding factors (1.21 vs 1.32 mmol/L, *P*=0.0001). **Conclusion** Polymorphisms of 45T/G and 276G/T in APM1 gene are not associated with hypertension or obesity, or their clinical features in Chinese Han population. Common polymorphism of 45T/G might be associated with serum HDL-C levels in Chinese.

Key words: Adiponectin; HDL-C; Hypertension; Obesity

INTRODUCTION

Adiponectin, an adipocyte-derived peptide, is decreased in subjects with obesity[1-2], type 2 diabetes[3], essential hypertension[4] and coronary heart disease[3,5]. All these diseases are closely associated with insulin resistance. Adiponectin encoded by *APM1*, which is located at chromosome 3q27 where a diabetes and metabolic syndrome susceptible locus has recently been mapped[6-7]. Studies conducted in different ethnic populations provide evidence for the association between genetic variants in *APM1* gene and type 2 diabetes as well as insulin resistance[8-10]. Insulin resistance is widely accepted as a common etiology of obesity, type 2 diabetes and essential hypertension. The current study aimed to investigate whether the two common variants in *APM1* gene were associated with HO and related features in Chinese Han population.

MATERIALS AND METHODS

Subjects

Four hundred and eighty-four unrelated subjects with HO and 502 unrelated age-matched controls were recruited from Beijing, Shanxi Province and Hebei Province. Recruited cases met the following inclusion criteria: 20-69 years of age, Chinese Han nationality, systolic blood pressure (SBP) ⩾160 mmHg and/or diastolic blood pressure (DBP) ⩾90 mmHg or subjects receiving anti-hypertensive therapies, body mass index (BMI) ⩾28. To be eligible for our study, controls had to meet the following criteria: no history of any anti-hypertensive therapies with their SBP<130 mmHg and DBP<85 mmHg, BMI<25. Subjects with secondary hypertension, secondary obesity, coronary heart disease (CHD) or typical clinical symptoms, such as stroke and myocardial infarction (MI)，cancer or type 2 diabetes were excluded from this study. A set of

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questionnaires including demographic information, detailed medical history, family history of hypertension, obesity, type 2 diabetes and stroke, medication use as well as alcohol consumption and cigarette smoking were completed. Venous blood was drawn from all subjects after an overnight fast. Serum and plasma were separated immediately and stored at –70℃. Blood pressure (BP) was measured three times by trained and certified observers according to a common protocol recommended by the American Heart Association[11]. A standardized mercury sphygmomanometer and an appropriately sized cuff were used. Blood pressure was measured at sitting position on the right arm after resting for 5 minutes. Three measurements were taken at an interval of 30 seconds. The mean value of three consecutive measurements was used for statistical analyses. Anthropometric height, weight, waist, and hip circumference were measured by standard protocols and then BMI and waist-hip-ratio were calculated.

All investigators and staff members successfully completed a training program. All observers and interviewers participated in a special training session on the use of standardized protocols for BP, anthropometric measurements, and administration for the study questionnaires.

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

Biological Measurements

Serum lipids including total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) were measured by enzymatic methods with an autoanalyzer (HITACHI 7060 Automatic Analyzer) and reagent kits. HDL-C was estimated as cholesterol after precipitation of apoB-containing lipoproteins with phosphotungstic Mg^{2+}. Low-density lipoprotein cholesterol (LDL-C) was calculated by Friedewald's equation for samples with TG values <4.5 mmol/L. Lipids measurements were conducted in a clinical biochemistry laboratory standardized for lipid measurements according to the criteria of the CDC-National Heart, Lung and Blood Institute Lipid Standardization Programs[12].

Genotyping of Polymorphisms

Genomic DNA was prepared by standard method. PCR product direct sequencing was used to determine the genotypes 45 T/G and 276 G/T. PCR was performed on 50 ng DNA in 25 μL volume mixture containing 1.0U Taq ploymerase (TaKara), 7 mmol/L dNTPs, 0.5 pmol forward and reverse primer respectively, 50 mmol/L $MgCl_2$ and 2.5 μL buffer (TaKara) for 32 cycles of denaturation at 94℃ for 60 s, annealing at 60℃ for 40 s, extension at 70℃ for 60 s in 9700 thermal cycler. Primers for amplifying the 1.3 kb fragment containing both polymorphisms were as follows: forward 5′---gag aac agc aag gga taa g ---3′ and reverse 5′---atg cag caa agc caa agt c ---3′, from 110 257 to 111 608 in contig NT-005962.11 (Genbank). Primer for sequencing reaction was 5′---tgg gct gca ata ttc aga a ---3′. Sequencing of 576 bp fragment (from 111 033 to 111 608 in contig NT-005962.11) was carried out by using Big Dye Terminator Cycle sequencing FS Ready Reaction Kit on ABI3700 (Applied Biosystems). Oligo 6.0 was used to design primers.

Statistical Analyses

Genotype and allele frequencies of polymorphisms between case and control group were compared by χ^2 analyses. EH software (http://linkage.rockefeller.edu/software/eh) was used to estimate and compare the haplotype frequencies between case and control group. Phase 1.0 (http://www.stat.washington.edu/stephens/) was used to estimate haplotype pair of each individual. The differences in means of continuous variables between genotypes and haplotypes were analyzed by Student's *t*-test or ANOVA. Multivariate analyses of covariance were performed to adjust possible confounding factors and assess interactions among them.

Hardy-Weinberg Equilibrium (HWE) test was assessed by the χ^2 test. Linkage disequilibrium strength between 2 polymorphisms was evaluated by using 2LD software (University of London, http://www.jop.kcl.ac.uk/IoP/Departments/PsychMed /GEpiBSt/software.stm). Data analyses except for the test of linkage equilibrium were performed by SAS statistical software. $P<0.05$ was considered statistically significant.

GenBank accession number: Human APM-1 mRNA, NM004797.

Blast result: APM-1 gene 111 197 to 116 609 in contig NT_005962.11 (5412 bp) on chromosome 3.

RESULTS

General Clinical Description of Subjects

Table 1 presented the general clinical description of subjects in case and control groups. The mean age of case subjects did not significantly differ from that of control group. The proportion of gender, smokers and drinkers, the mean levels of lipid profiles (except for HDL-C), SBP, DBP, and fasting glucose in case group were significantly higher than those in control group.

TABLE 1

Comparison of Clinical Characteristics between Case and Control Groups ($\bar{x} \pm s$)

	Cases (n=494)	Controls (n=502)
Male/Female[a*]	273/221	209/276
Average Age, y	49 ± 9	48 ±10
TC, mmol/L[a*]	5.14 ± 0.98	4.76 ± 0.94
HDL-C, mmol/L[a,b]	1.13 ± 0.26	1.33 ± 0.33
TG, mmol/L[a, b]	1.83 ± 1.07	1.21 ± 0.69
LDL-C, mmol/L[a]	2.94 ± 1.18	2.88 ± 0.83
FBS, mmol/L[a, b]	5.43 ± 1.08	4.91 ± 0.48
SBP, mmHg[a]	144.5 ± 16.5	116.7 ± 10.1
DBP, mmHg[a]	94.9 ± 10.3	74.6 ± 5.6
BMI[a], kg/m^2	30.8 ± 2.5	22.4 ± 1.8
Smokers[a]	217	167
Drinkers[a]	184	125

Note. [a]P<0.05. [b]Significance was tested on log-transformed values.

Association Analyses Between Genetic Variants in APM1 and HO

HWE test showed no significant change in 45T/G and 276G/T. Table 2 gave the distribution of genotypes and alleles of the 2 polymorphisms in cases and controls, with T being the major allele at position 45 (T=0.7351) and *G* being the major allele at position 276 (G=0.7395). χ^2 test showed significant differences in genotype or allele frequencies at the 2 positions between case and control groups, which suggested no association between the 2 polymorphisms and the HO in Chinese Han population. After adjusting gender, age, cigarette smoking, alcohol consumption and lipid profiles, stepwise logistic regression analyses suggested that the polymorphisms 45 and 276 might not be a significant determinant of HO in the present study population.

TABLE 2

Comparison of Genotypic and Allelic Distribution of 45T/G and 276G/T Between Case and Control Groups

	Genotypes			P	Alleles		P
	n (%)				n (%)		
45T/G	T/T	T/G	G/G		T	G	
Case	201(41.70)	186(38.59)	95(19.71)		588(60.99)	376(39.00)	
Control	222(44.67)	203(40.85)	72(14.49)	NS	647(65.12)	347(34.91)	NS
276G/T	G/G	G/T	T/T		G	T	
Case	259(53.73)	184(38.17)	39(8.09)		702(72.82)	262(27.18)	
Control	274(55.13)	187(37.63)	36(7.24)	NS	735(73.94)	259(26.06)	NS

Note. NS shows no significance.

The genotype and allele frequencies of polymorphisms 45 and 276 in the controls did not significantly differ from those in Japanese non-diabetic subjects[9].

Estimations and comparison of haplotype and haplotype pairs' frequencies defined by polymorphisms 45 and 276 between case and control group are listed in Table 3. The haplotype frequency in case group was significantly different from that in control group by using EH program (χ^2=11.57, P<0.025) while the χ^2 test by using of SAS software showed a reverse result (χ^2=7.93, P=0.34). The estimated G/T haplotype being zero in control group might be the explanation. Though the χ^2 test suggested a significant difference in haplotype pairs' frequencies between case and control group (χ^2=11.57, P<0.025), we failed to identify the risk haplotype.

TABLE 3

Distribution of Haplotype and Haplotype Pairs Defined by 45T/G and 276G/T in Case and Control Groups

Haplotype	Haplotype Frequency		Haplotype Pair	Haplotype Pair Frequency	
	Case	Control		Case	Control
TG	0.4840	0.4740	TG/TG	0.2227	0.2628
TT	0.2556	0.2605	TG/TT	0.2227	0.2218
GG	0.2441	0.2655	TG/GG	0.2479	0.2382
GT	0.0163	0.0000	TT/TT	0.0693	0.0760
–	–	–	TT/GG	0.1702	0.1273
–	–	–	TT/GT	0.0084	0.0021
–	–	–	GG/GT+GG/GG	0.0588	0.0719

Note. Comparison of the haplotype frequencies between case and control groups by EH software: χ^2=11.57, P<0.025. Comparison of the haplotype frequencies between case and control groups by SAS software: χ^2=7.93, P=0.34.

Effect of Polymorphisms and Haplotypes on Lipid Profiles, FBS, and Other Related Features

Univariate analysis showed that HDL-C levels were significantly different among genotypes at position 45 (P=0.0003) in all subjects (Table 4). Multivariate analyses were performed for adjusting possible confounding factors such as age, gender, cigarette smoking and alcohol consumption (Table 5). The adjusted mean HDL-C levels of T/T carriers did not significantly differ from that of T/G carriers either in all subjects or in controls. The difference in the HDL-C levels only existed between G/G carriers and T allele carrier (T/T+T/G) by at least 0.1 mmol/L. We also found that TT haplotype carriers (TT/TT+TT/XX, n=432) had significantly lower HDL-C levels than non-TT haplotype carriers (XX/XX, n=531) (1.21 vs 1.25 mmol/L, P=0.02). After an adjustment for age, gender, BMI, and other traditional risk factors the difference remained significant (P<0.01).

TABLE 4

Lipid Profiles Among Genotypes of Polymorphism 45 in Controls ($\bar{x} \pm s$)

Lipid Profiles	Genotypes			F	P
(mmol/L)	T/T(222)	T/G(203)	G/G(72)		
TC	4.77 ± 1.0	4.71 ± 0.9	4.89 ± 0.9	0.94	NS
TG[a]	1.20 ± 0.6	1.26 ± 0.9	1.10 ± 0.6	1.79	NS
HDL-C[a]	1.31 ± 0.3	1.29 ± 0.3	1.46 ± 0.3	8.29	0.0003
LDL-C	2.90 ± 0.8	2.84 ± 0.8	2.93 ± 0.8	0.42	NS

Note. NS shows no significance. [a]Significance was tested on log-transformed values.

TABLE 5

Association Between Polymorphism 45 and HDL-C Levels by Multivariate Analysis

Subjects	Adjusted HDL-C Levels (mmol/L)			P	Adjusted Power	P Value of Adjusted Variables					
	T/T	T/G	G/G			Age	Gender	BMI	Drink	Smoke	45T/G
All Subjects	1.21	1.22	1.32	0.0001	0.897	NS	0.0001	0.0001	NS	0.007	0.0001
Controls	1.31	1.29	1.45	0.0001	0.963	NS	0.0001	0.0001	NS	–	0.0007

Note. T/T, T/G, and G/G, refer to genotypes of polymorphism 45; P refers to the P value of the model; Adjusted power was calculated by using Power calculation for GLMs ("%Power: A Simple Macro for Power and Sample Size Calculations" available as Technical Support document TS-272 at the web address: http://www.sas.com/service/techsup/tnote/tnote_stat.html). NS indicates not significant. Drink refers to alcohol consumption: 1-current or ever drinking, 0-never drinking; Smoke refers to cigarette smoking: 1-current or ever smoking, 0-never smoking.

No difference in BP, body weight, BMI, or waistline was found among genotypes at position 45 in control subjects. For polymorphism 276, G allele carriers in the case group had significantly greater body weight than T/T homozygotes (82.6 vs 85.0 kg, P=0.02). But the difference disappeared after an adjustment for age, gender, BMI, cigarette smoking, and alcohol consumption. On additional univariate and multivariate analyses no any association between polymorphism 276 and lipid profiles or other hypertension- or obesity-related phenotypes has been found.

DISCUSSION

Adiponectin (also known as Acrp30) is an adipocyte-derived hormone with multiple important biological functions. Matsuzawa and co-workers reported that adiponectin may have putative anti-atherogenic properties *in vitro*[13-14]. Scherer and co-workers[15-16] reported that an acute increase in circulating Acrp30 levels lowers hepatic glucose production. A recent study provided a strong evidence that globular adiponectin protects ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis[17]. Circulating adiponectin level is commonly decreased in obesity[1-2] and essential hypertension patients[4]. Polymorphisms 45 and 276 can affect adiponectin levels and insulin resistance in different populations[8-10]. We then hypothesized that the variation in adiponectin gene might lead to obesity, hypertension or both by affecting the circulating adiponectin levels. However, we failed to detect any significant association between polymorphisms 45, 276, or haplotypes and related features in Chinese population in the present study. We assumed that insulin resistance was not likely the main pathway of APM1 or adiponectin contributing to hypertension or obesity.

The particular interesting finding in our study is the association between T allele of polymorphism 45 and the haplotype TT and HDL-C levels. Though the difference in HDL-C levels was moderate (0.1 mmol/L), the association between APM1 polymorphisms and HDL-C levels is worthy to pay attention because of the important role of HDL-C in cardiovascular diseases. Several studies have reported good correlations between plasma adiponectin and HDL-C levels in overweight obese and diabetic subjects[18-21]. A recent study conducted in Chinese population revealed that a T/G polymorphism at exon 2 in *APM1* gene has an effect on the mRNA level in adipose tissue[22]. The mechanism of how the variants in *APM1* gene influence serum HDL-C levels remains unclear and further study is expected.

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