Original Article

Peroxisome Proliferator-Activated Receptor- γ Coactivator-1 α Polymorphism Is Not Associated with Essential Hypertension and Type 2 Diabetes Mellitus in Chinese Population

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To investigate whether variations in the peroxisome proliferator-activated receptor- coactivator-1 (*PGC-1*) are associated with essential hypertension and type 2 diabetes in a Chinese population. A case-control study design was applied in a Chinese population. Two single nucleotide polymorphisms (SNPs), +1302G > A and G482S, in the *PGC-1* gene were genotyped and compared between 494 unrelated Chinese subjects with essential hypertension and type 2 diabetes and 555 normal control subjects with the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. These two polymorphisms were in highly significant linkage disequilibrium with each other ($p < 0.0001$). The frequency of the 482S allele was 42.9% in the Chinese population, which was similar to the frequency in the Japanese population (43.7%), but much higher than those of Caucasian populations (30.8% to 38.1%). There were no associations of the G482S and +1302G > A polymorphisms and haplotype combinations with essential hypertension and type 2 diabetes. In addition, no associations were found between these two polymorphisms and blood pressure. In conclusion, these results indicated that these two variations in the *PGC-1* gene might not contribute to the risk of hypertension and type 2 diabetes in the Chinese population studied here.

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Key Words: hypertension, type 2 diabetes, peroxisome proliferator-activated receptor- coactivator-1 , association study

Introduction

Hypertension and type 2 diabetes mellitus (T2DM) are components of metabolic syndrome (1). In most patients with hypertension, insulin resistance and hyperinsulinemia are present and may contribute to the pathogenesis of hypertension

(2, 3). The diverse prevalence rates of hypertension and T2DM within and among populations suggest that these two diseases are caused by interaction between environmental and genetic factors (4). However, many of the molecular details have not been fully elucidated (5). Peroxisome proliferator-activated receptor (PPAR)- γ coactivator 1 α (*PGC-1 α* , also named *PPARGC1A*, Gene ID, No. 10891) has been iden-

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tified as a transcriptional coactivator of PPAR α and γ , which are involved in adipogenesis and fatty acid β -oxidation, and other nuclear receptors which participate in blood pressure control (6, 7). *PGC-1 α* plays an important role in several aspects of oxidative metabolism: glucose uptake, the signaling pathway of insulin, and control of the level of endogenous glucose transporter (GLUT4) expression in muscle cells (8). Recent studies showed that *PGC-1 α* was a critical modulator of hepatic gluconeogenesis and a central target of the insulin-cAMP axis in the liver (9). The *PGC-1 α* gene was mapped to chromosome 4p15.1, a region that has been shown to be associated with systolic blood pressure (SBP), fasting insulin level, abdominal subcutaneous fat, and plasma free-fatty acids level (10–13). Several studies have determined that single nucleotide polymorphisms (SNPs) in the *PGC-1 α* gene had effects on the pathogenesis of essential hypertension or T2DM. And yet the replicability of the results was poor (14–17). Due to the intimate relationship between hypertension and diabetes, in the present study we aimed to verify the association of the +1302G>A (a silent transition at amino acid position 394 in exon 8) and G482S (a G-to-A transition that predicted a glycine (G) to serine (S) substitution at amino acid position 482 in exon 8) polymorphisms in the *PGC-1 α* gene with hypertension and T2DM in a Chinese population.

Methods

Subjects

In the present study, 494 subjects with essential hypertension complicated with T2DM and 555 control subjects were enrolled. All subjects (aged 25–75 years) were randomly recruited from the northern area in China. Hypertension was defined as an average SBP ≥ 160 mmHg, an average diastolic blood pressure (DBP) ≥ 90 mmHg, and/or current use of anti-hypertensive medications. Diabetes was diagnosed as a fasting serum glucose concentration ≥ 126 mg/dl (7.0 mmol/l) and/or use of hypoglycemic medications. All control subjects had normal blood pressure (blood pressure $< 135/85$ mmHg) and normoglycemia (fasting glucose < 100 mg/dl (5.6 mmol/l)), and no history of diabetes or hypertension. Subjects were excluded if they had one of the following: secondary hypertension, type 1 diabetes, pregnancy, dysfunction of the liver or kidney, disease of the thyroid or hypothalamus, and history of coronary heart disease, stroke or cancer. The protocol was approved by the local bioethical committee and performed after informed written consent was obtained from all participants.

The present age of all participants and the age at onset of hypertension and diabetes were obtained from direct interviews by trained interviewers. After an initial screen including blood pressure according to the enrollment criteria, three blood pressure measurements were taken from each participant using a standard mercury sphygmomanometer by

trained and certified observers according to a common protocol adapted from procedures recommended by the American Heart Association (18). Anthropometric measurements, such as height, weight, and waist and hip circumferences, were also obtained from all participants. Body mass index (BMI) was calculated by the measurements of weight and height. A BMI of 28 kg/m² may identify the risk factors related to chronic diseases, such as diabetes mellitus and lipoprotein disorders, with a specificity of around 90% (19). In another study, the odds ratio (OR) for clustering of hypertension, myocardial infarction and stroke was remarkably elevated in patients with a BMI ≥ 28 kg/m² (20). And age-adjusted all-cause mortality has been shown to increase in patients with a BMI higher than 28 kg/m² (21). Based on these findings from several recent large-scale epidemiological studies in China, a BMI of 28 kg/m² was recommended as the cut-off point for obesity in the Chinese population by the Working Group on Obesity in China. In this study, we defined individuals as obese when their BMI was ≥ 28 kg/m².

The mean age of patients (246 men, 248 women) was 57.20 ± 9.38 years, and the mean ages of diagnosis of hypertension and diabetes were 48.99 ± 12.02 and 53.47 ± 9.50 years, respectively. Durations of hypertension and diabetes were 8.21 ± 10.06 and 3.73 ± 5.00 years, respectively. In the group of normal control subjects (288 men, 267 women), the average age was 56.49 ± 8.73 years. All subjects enrolled in this study were unrelated, were residents of northern China, and were of Han ethnicity.

Biological Measurements

Blood samples were drawn after an overnight fast, and the serum was segregated for biochemical analysis within 2 h. All samples were stored at -70°C . Serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDLc) and glucose were measured on a Hitachi® 7060 automatic analyzer (Hitachi, Tokyo, Japan) by enzymatic methods. The Lipid Research Laboratory of the Disease Control and Prevention Center (Atlanta, USA) provided serum for quality control.

Detecting Genotypes of SNPs

Genomic DNA for analyses was isolated from blood leukocytes by the standard salt precipitation method. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to detect the genotypes of these two SNPs. The sequences of the primers used to analyze the +1302G>A polymorphisms were 5'-GCCAGTCAATTAATTCCAAACC-3', containing one single-base mismatch (underlined) (14), and 5'-CTTGCTCCAAAGTCTCTC-3'. The sequences of the primers used to assess the G482S polymorphism were 5'-TTGTTCTTCCACAGATT CAGAC-3' and 5'-GAAAAGGACCTTGAACGAGAG-3'. Optimum PCR amplification conditions were achieved with

1 $\times$ PCR buffer, 2 mmol/l MgCl₂, 0.15 μ mol/l of each primer, 0.2 mmol/l dNTP and 1 IU AmpliTaq polymerase. The quantity of genomic DNA was 50 ng for the two polymorphisms. The cycling program for PCR of these two polymorphisms consisted of a denaturation step at 95°C for 5 min, 35 cycles of denaturation at 94°C for 50 s, annealing at 56°C (for the +1302G>A) or 58°C (for the G482S) for 40 s, extension at 72°C for 50 s, and then a final extension step at 72°C for 8 min. PCR products were digested overnight at 37°C with MspI (TaKaRa Biotechnology Co., Ltd., Tokyo, Japan) for these two polymorphisms followed by detection on 3% ethidium bromide-stained agarose gels.

Statistical Analysis

Transformations of continuous variables were made if the equal variance and normal distribution were rejected. Basic continuous characteristics between case and control subjects were analyzed by Student's *t*-test. The χ^2 and Fisher's exact test were used to compare the categorical variables of gender, alleles, genotypes, and haplotypes between groups, and to verify agreement with Hardy-Weinberg expectations. The Estimation Haplotype-frequencies software (EH) (<http://linkage.rockefeller.edu/software/eh>) and PHASE program (<http://www.stat.washington.edu/stephens/>) were used to estimate frequency distributions of haplotypes and haplotype combinations. The standardized linkage disequilibrium statistic (r^2) was estimated using ldmx software (<http://www.sph.umich.edu/csg/abecasis/GOLD/>). The statistical power of this study was estimated using the Power Calculator program (<http://calculators.stat.ucla.edu/powercalc/>).

The general linear model was used to test quantitative variables for differences among genotypic groups adjusting for covariate variables of age, gender, and BMI where appropriate.

To estimate OR with 95% confidence intervals (CI) for each genotype, 2 "dummy" variables with the respective wild-type as the reference were used in multivariate logistic regression analysis adjusted for age, gender, BMI, smoking and drinking status (22).

The OR and 95% CI of each haplotype combination relative to the other haplotype combinations as a group were determined by the method of Horikawa *et al.* (23). In agreement with a previous Japanese study, there was a strong linkage disequilibrium between the two polymorphisms, with the

Table 1. Comparison of Basic Characteristics between Cases and Controls

	Cases (n = 494)	Controls (n = 555)	<i>p</i>
Age (years)	57.20 $\pm$ 9.38	56.49 $\pm$ 8.73	0.2019
Gender (M/F)	246/248 (49.8%)	288/267 (51.89%)	0.4980
SBP (mmHg)	148.31 $\pm$ 18.55	118.45 $\pm$ 9.84	0.0001
DBP (mmHg)	88.02 $\pm$ 11.59	73.98 $\pm$ 6.71	0.0001
Waist circle (cm)	93.27 $\pm$ 10.39	81.84 $\pm$ 9.15	0.0001
BMI (kg/m ²)	27.11 $\pm$ 3.64	23.73 $\pm$ 2.99	0.0001
Glucose (mmol/l)	9.20 $\pm$ 2.95	4.96 $\pm$ 0.38	0.0001
TC (mmol/l)	5.39 $\pm$ 1.07	5.10 $\pm$ 0.97	0.0001
TG (mmol/l)	1.93 $\pm$ 1.05	1.26 $\pm$ 0.62	<0.0001
HDLc (mmol/l)	1.15 $\pm$ 0.27	1.34 $\pm$ 0.35	0.0001
LDLc (mmol/l)	3.33 $\pm$ 0.88	3.19 $\pm$ 0.85	0.0075
Smokers	172 (34.82%)	177 (31.89%)	0.3150
Drinkers	112 (22.67%)	102 (18.38%)	0.0850

SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; TC, total cholesterol; TG, triglyceride; HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol.

Table 2. Genotype and Haplotype Frequencies of *PGC-1 α* Polymorphisms in the Study Subjects

SNP	Genotypes/alleles	Case (n = 494)	Control (n = 555)	<i>p</i>
+1302G>A	GG	305 (61.74%)	340 (61.26%)	0.984
	GA	164 (33.20%)	186 (33.51%)	
	AA	25 (5.06%)	29 (5.23%)	
	G-allele	774 (78.34%)	866 (78.02%)	0.859
	A-allele	214 (21.66%)	244 (21.98%)	
G482S	GG	155 (31.38%)	185 (33.33%)	0.405
	GS	255 (51.62%)	264 (47.57%)	
	SS	84 (17.00%)	106 (19.10%)	
	G-allele	565 (57.19%)	634 (57.12%)	0.975
	S-allele	423 (42.81%)	476 (42.88%)	
Haplotype	+1302G-482S	420 (42.51%)	473 (42.61%)	0.994
	+1302G-482G	354 (35.83%)	393 (35.41%)	
	+1302A-482G	211 (21.36%)	241 (21.71%)	
	+1302A-482S	3 (0.30%)	3 (0.27%)	

PGC-1 α , peroxisome proliferator-activated receptor- γ coactivator-1 α .

six most common haplotype combinations accounting for 99.6% of all haplotype combinations. The frequencies of the rare haplotype combinations (+ 1302AA-482SS, + 1302GA-482SS, + 1302AA-482GS) accounting for less than 0.5% were ignored (24). All analyses were done using Statistical Analysis System software (SAS Institute, Cary, USA). A *p*-value of less than 0.05 (two-tailed) was considered statistically significant.

Results

In the present study, in comparison to controls, cases with hypertension and diabetes had higher BMI and higher levels of TC, low-density lipoprotein cholesterol (LDLc) and TG, and lower levels of HDLc, in addition to higher blood pressure and concentration of serum glucose. The proportions of smokers (34.82% vs. 31.89%, *p* = 0.315) and drinkers (22.67% vs. 18.38%, *p* = 0.085) in patients were similar to those in normal subjects (Table 1).

Fig. 1. PGC-1α +1302G>A genotypes distribution as a function of PGC-1α G482S genotypes (*p* = 0.001).

Genotypic distributions at these two polymorphic sites were both in agreement with Hardy-Weinberg equilibrium in all subjects. Distributions of genotypic and allelic frequencies of these two polymorphisms in the case group were not statistically different from those in the control group (Table 2). In all subjects, these two polymorphisms were in highly significant linkage disequilibrium with each other ($r^2 = 0.19$, *p* < 0.0001). Figure 1 shows that the frequency of the + 1302A allele appeared to increase significantly from non-482G allele carriers to homozygous 482GG carriers; moreover, the homozygous + 1302AA genotype almost coexisted in individuals with the homozygous 482GG genotype. These results suggested that the minor + 1302A allele segregated with the common 482G allele. The frequencies of each haplotype estimated by either the Phase (*p* = 0.994) or EH program (data not shown) did not differ between the case and control groups. The frequencies of haplotype combinations (*p* = 0.892) estimated by the Phase program also did not differ between the two groups. Gender-specific analysis also showed no significant differences in the genotype, allele or haplotype combination frequencies (data not shown). As shown in Table 3, the ORs of haplotype combinations ranged from 0.850 to 1.138, with all *p* values being greater than 0.35; none of the haplotype combinations were classified as increasing the risk of hypertension or diabetes.

Among obese subjects with BMI ≥ 28 kg/m², there were 189 hypertensive patients with diabetes (HD-OB subgroup) (male/female, 95/94; mean age, 54.97 ± 9.23 years; mean BMI, 30.71 ± 2.49 kg/m²) and 44 control subjects (OB subgroup) (male/female, 17/27; mean age, 56.77 ± 8.40 years; mean BMI, 29.96 ± 1.96 kg/m²); while there were 304 hypertensive patients with diabetes (HD-NOB subgroup) (male/female, 151/153; mean age, 58.58 ± 9.24 years; mean BMI, 24.86 ± 2.13 kg/m²) and 508 control subjects (NOB subgroup) (male/female, 268/240; mean age, 56.46 ± 8.78 years; mean BMI, 23.19 ± 2.40 kg/m²) among non-obese subjects. Stratification analysis based on obesity revealed no signifi-

Table 3. Risk of Hypertension and Diabetes by Haplotype Combinations

Haplotype combination	Case (<i>n</i> = 494)	Control (<i>n</i> = 555)	OR (95% CI)	<i>p</i>
+ 1302GG-482GS	159 (32.19%)	168 (30.27%)	1.093 (0.842–1.420)	0.504
+ 1302GG-482SS	83 (16.80%)	104 (18.74%)	0.876 (0.637–1.204)	0.413
+ 1302GA-482GS	95 (19.23%)	96 (17.30%)	1.138 (0.832–1.558)	0.418
+ 1302GA-482GG	69 (13.97%)	89 (16.04%)	0.850 (0.605–1.195)	0.350
+ 1302GG-482GG	63 (12.75%)	68 (12.25%)	1.047 (0.726–1.510)	0.807
+ 1302AA-482GG	23 (4.66%)	28 (5.05%)	0.919 (0.522–1.618)	0.770
Others	2 (0.4%)	2 (0.36%)	NA	NA

NA, not analyzed. The odds ratio (OR) and 95% confidence interval (CI) of each haplotype combination relative to all other haplotype combinations as a group are shown, as in Horikawa *et al.* (23). In agreement with a previous Japanese study, there was strong linkage disequilibrium between the two polymorphisms, with the six most common haplotype combinations accounting for 99.6% of all haplotype combinations. The frequencies of the rare haplotype combinations (+ 1302AA-482SS, + 1302GA-482SS, + 1302AA-482GS) accounting for less than 0.5% were ignored (24).

Table 4. Genotype and Allele Frequencies of *PGC-1α* Polymorphisms in the Study Subjects Stratified Based on Obesity (BMI ≥ 28 kg/m²)

	Obese		<i>p</i>	Non-obese		<i>p</i> [*]
	HD-OB (<i>n</i> = 189)	OB (<i>n</i> = 44)		HD-NOB (<i>n</i> = 304)	NOB (<i>n</i> = 508)	
+ 1302GG	112 (59.26%)	25 (56.82%)	0.6749	192 (63.16%)	313 (61.61%)	0.908
+ 1302GA	68 (35.98%)	18 (40.91%)		96 (31.58%)	167 (32.87%)	
+ 1302AA	9 (4.76%)	1 (2.27%)		16 (5.26%)	28 (5.51%)	
+ 1302G allele	292 (77.25%)	68 (77.27%)	0.996	480 (78.95%)	793 (78.05%)	0.671
+ 1302A allele	86 (22.75%)	20 (22.73%)		128 (21.05%)	223 (21.95%)	
482GG	59 (31.22%)	21 (47.73%)	0.0852	96 (31.58%)	164 (32.28%)	0.5505
482GS	96 (50.79%)	15 (34.09%)		158 (51.97%)	247 (48.62%)	
482SS	34 (17.99%)	8 (18.18%)		50 (16.45%)	97 (19.09%)	
482G allele	214 (56.61%)	57 (64.77%)	0.1623	350 (57.57%)	575 (56.59%)	0.702
482S allele	164 (43.99%)	31 (35.23%)		258 (42.43%)	441 (43.41%)	

HD-OB: subjects with type 2 diabetes, essential hypertension and body mass index (BMI) ≥ 28 kg/m²; OB: simple obese subjects (BMI ≥ 28 kg/m²) without diabetes or hypertension; HD-NOB: type 2 diabetic patients with essential hypertension and BMI < 28 kg/m²; NOB: normal control subjects with BMI < 28 kg/m². *Genotypes are missing for 1 patient and 3 control subjects. *PGC-1α*, peroxisome proliferator-activated receptor- γ coactivator-1 α .

cant differences in genotype, allele, or haplotype combination frequencies (Table 4).

Multivariate logistic regression analysis showed that, in the additive model, genotypes at two polymorphic sites were not associated with hypertension or diabetes (482GS, OR = 1.228, 95% CI = 0.881–1.712; 482SS, OR = 0.932, 95% CI = 0.594–1.462; 1302GA, OR = 0.864, 95% CI = 0.626–1.192; 1302AA, OR = 0.970, 95% CI = 0.492–1.912). The findings of logistic regression analysis indicated that age and BMI had important effects on the risk of hypertension or diabetes, when adjusted for confounding effects from gender, smoking and drinking habits (Table 5).

Discussion

PPAR γ is a transcription factor and molecular target of the thiazolidinedione class of agents, which have been shown to lower fasting blood glucose (25). A meta-analysis including 3,000 individuals from earlier studies reported that the more common proline allele in the PPAR γ gene was associated with a significantly increased risk of diabetes (1.25-fold), which translated into a large population attributable risk of about 25% (26). PPAR γ and its ligands suppress angiotensin II (AII) type 1 receptor (AT1R) gene expression (27, 28). AII activates membrane AT1R and then triggers a variety of signal transduction pathways, including the mitogen-activated protein (MAP) kinase pathway. AII exerts many biological effects and contributes to the progression of hypertension and atherosclerosis (29–31). PPAR γ phosphorylation by the MAP kinase pathway may thus attenuate PPAR γ -mediated AT1R gene transcription suppression through the inhibition of PPAR γ activity (32). Therefore, it is reasonable to assume that PPAR γ plays a role in hypertension by regulating AT1R

Table 5. Multivariate Logistic Regression Results of G482S and G1302A Polymorphisms in the *PGC-1α* Gene in all Subjects

Variable	OR	95% CI		<i>p</i>
		Lower	Upper	
Age	1.023	1.007	1.039	0.0043
Gender, M/F	0.879	0.631	1.225	0.4466
BMI	1.382	1.317	1.450	0.0001
Smoking, Y/N	1.099	0.757	1.596	0.6182
Drinking, Y/N	1.324	0.883	1.987	0.1748
482GS [*]	1.228	0.881	1.712	0.2253
482SS [*]	0.932	0.594	1.462	0.7584
1302GA [#]	0.864	0.626	1.192	0.3726
1302AA [#]	0.970	0.492	1.912	0.9294

Independent variable: hypertension and diabetes status. OR, odds ratio; CI, confidence interval; Y, yes; N, no. * The relative risks associated with the 482GS and 482SS genotypes were estimated in comparison with the 482GG genotype. The relative risks associated with the 1302GA and 1302AA genotypes were estimated in comparison with the 1302GG genotype. *PGC-1α*, peroxisome proliferator-activated receptor- γ coactivator-1 α .

gene expression. As a coactivator of PPAR γ and other nuclear genes, *PGC-1α* regulates PPAR γ and nuclear respiratory factor (NRF)-dependent transcriptions, increases the expression of both nuclear and mitochondrial-encoded genes of oxidative metabolism, induces mitochondrial biogenesis and plays an essential role in energy homeostasis. The region in which the *PGC-1α* gene is situated, chromosome 4p15.1, has been shown to be associated with fasting insulin levels in Pima Indians (12), and to be linked to SBP and plasma free

Table 6. Comparison of Genotype and Allele Frequencies of G482S Polymorphism in Different Populations

		Patients				Controls				<i>p</i>	482S allele frequency		
		482GG	482GS	482SS	Total	482GG	482GS	482SS	Total		Case (%)	Control (%)	<i>p</i>
Danish initial study (14)	T2DM/NGT	186	200	68	454	97	80	21	198	0.1100	37.00	30.80	0.0320
Danish replicated study (14)	T2DM/NGT	76	97	28	201	146	116	31	293	0.0300	38.10	30.40	0.0135
Danish combined study (14)	T2DM/NGT	262	297	96	655	243	196	52	491	0.0035	37.30	30.50	0.0007
French study (16)	T2DM/NDM	280	284	95	659	323	327	98	748	0.7740	36.00	35.00	0.8820
Austrian study (17)*	HT/NT	231	231	47	509	286	315	103	704	0.0140	31.93	37.00	0.0100
Japanese study (15)	T2DM/NDM					178	249	110	537			43.67	
Chinese study	HD/NDM	155	255	84	494	185	264	106	555	0.4050	42.81	42.88	0.9750

T2DM, type 2 diabetes mellitus; NGT, normal glucose tolerance; NDM, non-diabetic subjects; HT, hypertension/hypertensive; NT, normotensive; HD, hypertension and diabetes. * Results for combined male and female subjects from Oberkofler *et al.* (17). Results were from the present study.

fatty acid levels in Dutch dyslipidemic families (13) and to high BMI in Utah pedigrees (33). Amino acids 338–403 of *PGC-1 α* contain a major domain of interaction with PPAR γ , and thereby impact the ability of cells to uptake fatty acids and store them in adipocytes, while residues 403–570 of *PGC-1 α* interact with myocyte enhancer factor 2C (MEF2C), which is responsible for activation of the GLUT4 gene (6, 8). One study showed that *PGC-1 α* expression was significantly reduced in both T2DM subjects and family history-positive nondiabetic subjects (34). These data suggested that the *PGC-1 α* gene might contribute to the pathogenesis of hypertension and diabetes.

It has been reported that the 482S allele was associated with a reduction in the prevalence of hypertension (OR, 0.42; 95% CI, 0.25–0.73) in male Europeans (17). On the other hand, Ek *et al.* found that the G482S polymorphism was significantly associated with T2DM (the 482S allele frequency was 37.3% vs. 30.5%, respectively, $p = 0.0007$) and contributed 1.34-fold (95% CI, 1.13–1.59) relative risk of T2DM in a Danish population (14). However, there were no significant associations between this polymorphism and hypertension or T2DM in Chinese, which finding was similar to those of previous studies in French Caucasians and Japanese (15, 16). Given our sample sizes, we had >65% power to detect an OR of 1.34 at a significance level of 0.05. Thus, if G482S polymorphism had an effect in Chinese similar to that seen in the Danish population, we would have had sufficient power to detect it. Two previous studies suggested that the G482S and other polymorphisms and haplotype combinations in the *PGC-1 α* gene had metabolic consequences on lipid metabolism and played important roles in obesity (35, 36). In order to remove the confounding effect of obesity, we examined the associations of polymorphisms with hypertension and diabetes stratified based on obesity. The stratification analysis showed that there were no significant differences in genotype, allele, or haplotype combination frequencies. Multivariate logistic regression analysis also showed no association of G482S polymorphism with hypertension and

diabetes. So the G482S polymorphism in the *PGC-1 α* gene is unlikely to be a major genetic factor of hypertension and diabetes in the Chinese population.

For another polymorphism, +1302G>A, the minor allele +1302A frequency was lower among diabetic subjects compared to glucose-tolerant Danish control subjects (19.7% vs. 23.9%, $p = 0.02$) in the combined study of Ek *et al.* (14). This relationship was not replicated in our study or in the previous study on the Japanese population (15).

Previous haplotype analysis showed that the +1302A-482S haplotype was attributed to the risk of T2DM in the Japanese population (4.6% vs. 1.3%, $p = 0.00003$) (15), whereas this association was not confirmed in our study. This discrepancy may be explained as follows. Although the frequencies of the two SNPs in control subjects were similar between Chinese and Japanese individuals (for G482S, 185/264/106 vs. 178/249/110, $p = 0.839$; for +1302GA, 340/186/29 vs. 320/184/33, $p = 0.749$), the haplotype +1302A-482S is relatively rare in the Chinese population (6/2,098 [0.29%] vs. 52/1,908 [2.73%], $p = 0.001$). Given the comparable sample sizes, our power to detect a significant effect may be lower than that of the study in Japanese subjects.

As Table 6 shows, the 482S allele frequency distribution of the G482S polymorphism was significantly different among different populations (14–17). For example, the 482S allele frequency was higher in the Chinese population than in Caucasians. Such a difference of allelic frequency among populations may have produced the discrepant results. Other likely causes may have been the poor metabolic characterization (particularly regarding glucose tolerance) and sample biases in ascertainment of the study groups used in the studies listed in Table 6. In our study we determined associations of polymorphisms with two closely related phenotypic traits. However, it should be taken into account that the previously opposite associations of the G482S polymorphism with T2DM (14) and hypertension (17) may overcome the factual association of this polymorphism with diabetes and hypertension when these two phenotypic traits were analyzed to-

gether. Another reference group consisting of hypertensives only or diabetics only will be needed in the future.

In summary, we did not confirm the effects of the G482S and the +1302G>A polymorphisms in the *PGC-1 α* gene on the risk of essential hypertension and T2DM in the Chinese population. Additional studies should be performed to clarify the contribution of variations of the *PGC-1 α* gene to the pathogenesis of hypertension and T2DM in a variety of ethnic groups.

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