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ORIGINAL ARTICLE

Functional Variant of C-689T in the Peroxisome Proliferator-Activated Receptor- γ 2 Promoter is Associated with Coronary Heart Disease in Chinese Nondiabetic Han People

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Objective To investigate the association between the polymorphism of C-689T in the peroxisome proliferator-activated receptor- γ 2 (PPAR γ 2) promoter and coronary heart disease (CHD).

Methods This case-controlled study was conducted in nondiabetic Chinese Han people, which enrolled 455 patients with CHD (cases) and 693 subjects without CHD (controls). Data of clinical indexes were collected, including height, body weight, waist circumference, systolic blood pressure (SBP), diastolic blood pressure (DBP), smoking, drinking, physical activity, as well as body mass index (BMI). Fasting blood glucose (FBG), plasma total cholesterol (TC) and triglyceride (TG) levels were measured. Polymerase chain reaction-restricted fragments length polymorphism (PCR-RFLP) was used to determine the PPAR γ 2 promoter C-689 $\rightarrow$ T substitution. The genotype distribution of PPAR γ 2 promoter C-689T, allelic frequency, clinical indexes, and laboratorial measurements were compared between the two groups. The effect of genotype on the risk of CHD was assessed using univariate and multivariate regression model.

Results The genotype frequencies of CC, CT and TT in PPAR γ 2 promoter C-689T were 89.7%, 9.9% and 0.4% in the case group, and 93.1%, 6.6% and 0.3% in the control group, respectively (CC vs. CT+TT, $\chi^2 = 6.243$, $P = 0.041$). Carriers of -689T allele ($n = 95$) had significantly higher TC level than non-carriers ($n = 1053$) (5.12 ± 1.26 vs. 4.76 ± 1.22 mmol/L, $P = 0.001$). Male carriers of -689T allele ($n = 51$) were

significantly higher in waist circumference, body weight, TC and TG than male non-carriers ($n=656$) (all $P<0.05$). In subjects whose BMI was over 25 kg/m^2 , carriers of -689T allele ($n=82$) had significantly higher levels of waist circumference, BMI, SBP and TC than non-carriers ($n=231$) (all $p<0.05$). The -689T allele was an independent risk factor for CHD ($OR=1.668$, $95\%CI: 1.031-2.705$, $P=0.037$) after adjusting for age, gender, waist circumference, body weight, BMI, smoking, physical activities, SBP, DBP, FBG, TC and TG level.

Conclusion These data support the hypothesis that the -689T allele is associated with an increased risk of CHD, in Chinese Han people and correlates significantly with the profiles of CHD-related risk factors.

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PEROXISOME proliferator-activated receptors (PPARs), which belong to the steroid hormone receptor superfamily, were identified in the past twenty years. To date, three PPAR isotypes have been identified: PPAR α , PPAR β/δ , and PPAR γ .¹ The PPAR γ gene, located on chromosome 3, has three isoforms according to differential promoter usage coupled with alternate splicing: PPAR γ 1 and PPAR γ 3, encode the same protein product; PPAR γ 2 contains an additional 28 amino acids at its amino-terminus. PPAR γ 1 exhibits widespread expression at a low level; PPAR γ 2 and PPAR γ 3 are highly expressed in adipose tissue.² These receptors bind to and are activated by fatty acids, eicosanoids, and numerous structurally dissimilar xenobiotics, known collectively as peroxisome proliferators. Upon activation, PPARs, as heterodimers with retinoid X receptors (RXRs), regulate gene expression by binding to specific PPAR response elements (PPRE), which is defined as a direct repeat of two core recognition motifs AGGTCA spaced by one nucleotide. PPARs binding to the promoter regions of specific target genes results in either activation or suppression of one specific gene.¹ PPAR γ 2 is a nuclear transcript factor with a very wide spectrum of biological activities. It is involved in regulation of many target genes and plays important roles in lipid and glucose metabolism, insulin sensitivity, obesity, cellular proliferation and differentiation, inflammation and atherosclerosis. Activation of PPAR γ 2 improves insulin sensitivity, and exerts antiatherogenic, anti-inflammatory, antioxidative, antifibrinolytic, and a wide range of metabolic effects. Accordingly, it has been generally considered beneficial to coronary heart disease (CHD).

Experimental and clinical studies have reported that thiazolidinediones (TZD), the ligands of PPAR γ , could reduce myocardial infarct size,³ attenuate restenosis after angioplasty,⁴ and significantly reduce circulating platelet activity.⁵ It suggests that PPAR γ 2 is a protective gene for CHD. Moreover, some studies demonstrate that a C-689→T substitution in the promoter of PPAR γ 2 gene reduces the transcription activity of PPAR γ 2, and -689T allele signifi-

cantly affects body weight and LDL-cholesterol.⁶⁻⁷ Therefore, the C-689T polymorphism may be associated with CHD, and affect the profile of CHD-related risk factors. However, data on this polymorphism and CHD still are insufficient. To our best knowledge, no study has focused directly on the relationship between C-689T polymorphism and CHD. The goal of present study is to investigate the association between C-689T polymorphism and CHD.

MATERIALS AND METHODS

Subjects recruiting

This case-control study was conducted in the Affiliated Hospital of Shaoxing University, and the protocol was approved by the local ethical committee. Participants were recruited from in-patients and the annual screening population among Chinese Han people in Shaoxing from 2011 to 2015. A questionnaire survey was administered to collect behavior information such as smoking, drinking and physical activity.

Candidates for the case group were selected from in-patients. Criteria for the diagnosis of CHD included evidences meeting any one of the described below: 1) ischemic symptoms accompanied with either diagnostic changes in electrocardiograms or typical abnormalities of cardiac enzymes; 2) the history of either typical angina pectoris or definite myocardial ischemia on electrocardiograms; 3) the coronary arteriography demonstrating stenosis $\geq 50\%$. Subjects who were concurrently with diabetes, cerebrovascular disease, or life-threatening malignancy were excluded.

Controls were recruited from healthy population who participated annual health check-up at the same hospital. Those with evidence of the followings were excluded: 1) myocardial infarction (MI) on electrocardiograms; 2) the history of angina; 3) MI and heart failure; 4) regular medication on aspirin or anticoagulants; 5) diabetes; 6) cerebrovascular disease; 7) life-threatening malignancy, or 8) renal inadequacy.

Totally, 455 patients with CHD and 693 healthy individuals were enrolled in the case group and the control group respectively. All enrolled subjects had no blood relation to each other.

Clinical measurements and laboratory examinations

All subjects took physical measurements including body weight, body height and waist circumference by trained nurses. Body mass index (BMI) was calculated by the formula: $BMI = \text{body weight (kg)} / \text{body height}^2 (\text{m}^2)$. Blood pressure was measured on the right upper arm using a standard mercury sphygmomanometer, with the subject in sitting position and resting for at least 5 minutes. The mean of two consecutive blood pressure readings was taken for analysis. Current cigarette smokers were defined as who smokes at least one cigarette per day. Alcohol consumption was defined as positive if a subject drinks alcohol such as wine, beer, cider, or spirits over 50 g per day. Being physically active was defined as positive if a subject takes physical activity 3-5 times a week and more than 30 minutes each time.

All blood samples were taken through venipuncture after the subjects had fasted for at least 10 hours, and stored in disodium EDTA tube for laboratory examinations. Fasting blood glucose (FBG) was measured by the glucose oxidase method. Plasma total cholesterol (TC) and triglyceride (TG) levels were enzymatically measured.

Single nucleotide polymorphism identification and genotyping

Genomic DNA was extracted from the buffy coat fraction of 4 ml centrifuged peripheral blood using a Pure gene DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA) according to the manufacturer's instructions, and stored in a -20°C refrigerator. The PPAR γ 2 promoter C-689T polymorphism was detected by the method of polymerase chain reaction-restricted fragments length polymorphism (PCR-RFLP). As no restriction enzyme could recognize the substituted site, we introduced one mispairing base G (shading showed, origin was T) in the third site from 3'-end when designing the forward primer, expecting to give rise to the recognition site 5'-GATC-3' under the condition that the C-689T is C. The sequences of the forward and reverse primers were 5'-TAGAGAACTCCATTTTTTCATTATGACATAGCACTGAT-3' and 5'-ACTGACTGCTATCTAAATTC-TG-3' respectively. They were synthesized by AuGCT Biotechnology (Beijing, China).

The amplification was performed in a 25 μl volume containing 200 ng DNA, 0.3 $\mu\text{mol/L}$ primers, 160 $\mu\text{mol/L}$ dNTP, 1.7 mmol/L MgCl_2 , 1 U of Taq polymerase and 2.5 μl

10 $\times$ Taq Buffer with 100 mmol/L Tris-HCl (pH 8.8 at 25°C), 500 mmol/L KCl, and 0.8% Nonidet P40 (Fermentas). PCR was run for 4 minutes of initial denaturation at 94°C , followed by 35 cycles, with each cycle consisting of 40 seconds of denaturation at 94°C , 45 seconds of annealing at 57.5°C , 40 seconds of extension at 72°C , and a final extension at 72°C for 5 minutes. The reaction yielded a DNA fragment of 214 bp. The PCR products were confirmed by electrophoresis on a 1% agarose gel containing 0.5 $\mu\text{g/ml}$ ethidium bromide, and visualized by UV-induced fluorescence.

The confirmed products were digested with *Mbo* I restriction enzyme [Takara, Bao bio engineering (Dalian) Co. Ltd. China, recognition site 5'-GATC-3'] at 37°C overnight. The digested products were detected by electrophoresis for 30 minutes at 8 V/cm on a 2.5% agarose gel containing 0.5 $\mu\text{g/ml}$ ethidium bromide, and visualized by UV-induced fluorescence. The products of pUC19 DNA digested by *Msp* I enzyme (Fermentas, Canada) were taken as markers. Replication quality control samples were included; and genotypes were detected with 100% concordance.

Statistical analyses

All continuous variables were expressed as means $\pm$ SD, and categorical variables were summarized by counts and percentages. Pearson χ^2 test was used to compare the enumeration data of various genotypes with those expected for the population in Hardy-Weinberg equilibrium, and analyze differences of genotype distributions between cases and controls. Normally distributed clinical indexes (except for FBG, TC and TG) were compared between the two groups by independent samples *t*-test, while log-transformation was applied to FBG, TC and TG. The impacts of various factors on the risk of CHD, including genotypes (CC genotype was used as reference), age, gender, waist circumference, body weight, BMI, smoking, alcohol consumption, physical activities, SBP, DBP, FBG, TC and TG, were evaluated by univariate and multivariate logistic regression analyses. SPSS (version 19.0) was used for statistical analysis. All *P* values presented were two-tailed, and <0.05 were considered statistically significant.

RESULTS

Comparison of clinical characteristics between cases and controls

The age and gender of the case group and the control group were well comparable (both $P>0.05$). However, the case group demonstrated significantly higher body weight ($P=0.021$) and waist circumference ($P<0.001$) than the

control group, but mean BMIs of the two groups were not statistically different ($P=0.621$). Besides, the mean clinical measurements of the case group, including SBP, DBP, FBG, TC and TG, were significantly higher than those of the control group (all $P<0.05$). The details are outlined in Table 1. Additionally, 42% of cases and 20% of controls had a history of hypertension ($\chi^2=4.346$, $P=0.019$), and 34% of cases had a history of percutaneous coronary intervention (PCI) or surgery of coronary artery bypass graft (CABG).

PPAR γ 2 promoter C-689T genotype distribution

PCR-RFLP method was used to detect the expression of PPAR γ 2 promoter C-689T genotype. Theoretically, digestion of the PCR products resulted in two fragments (179 bp and 35 bp) for the CC genotype, three fragments (214 bp, 179 bp and 35 bp) for the CT genotype and one fragment (214 bp) for the TT genotype when the restriction site was eliminated by the C-689T substitution (5'-GATC-3'; the substituted base is underlined). In fact, only 179 bp and

214 bp fragments could be detected because 35 bp fragment was too small. Therefore, the digested products detected were one fragment (179 bp) for the CC genotype, two fragments (214 and 179 bp) for the CT genotype and one fragment (214 bp) for the TT genotype (Fig. 1).

The genotype frequencies of CC, CT and TT in PPAR γ 2 promoter C-689T are listed in Table 2. The distribution of these genotypes between the control group and the case group was significantly different (CC vs. CT+TT, $P=0.041$); the rare frequency of -689T allele showed significantly higher in the case group than that in the control group (5.4% vs. 3.6%, $P=0.040$).

The relative genotype frequencies did not differ significantly between males and females. CC, CT, and TT frequencies were 92.8%, 6.9%, and 0.3% in males, and 90.0%, 9.5%, and 0.5% in females, respectively. The observed genotype frequencies were in accordance with Hardy-Weinberg equilibrium in total population, cases, controls, males, and females with a Chi-square value (P value) of 1.781 (0.182), 0.392 (0.531), 1.439 (0.230), 1.101 (0.294) and 0.595 (0.441), respectively.

Table 1. Comparison of clinical characteristics and laboratory measurements between cases and controls[§]

Variables	Controls ($n=693$)	Cases ($n=455$)	χ^2/t	P value
Gender (male/female, n)	421/272	286/169	0.872	0.367
Age [§] (yrs)	59.67 $\pm$ 7.88	60.07 $\pm$ 10.68	0.625	0.468
Smoking [male/female, n (%)]	196/2	221/5	5.605	0.012
Drinking [male/female, n (%)]	322/189	268/71	0.702	0.431
Waist circumference [§] (cm)	84.97 $\pm$ 10.28	87.31 $\pm$ 11.49	12.364	<0.001
Body weight [§] (kg)	64.64 $\pm$ 10.52	66.16 $\pm$ 11.61	4.289	0.021
BMI [§] (kg/m ²)	24.22 $\pm$ 3.33	24.31 $\pm$ 3.69	0.423	0.621
SBP [§] (mmHg)	129.68 $\pm$ 20.07	139.95 $\pm$ 23.21	11.267	<0.001
DBP [§] (mmHg)	81.06 $\pm$ 10.47	84.74 $\pm$ 14.26	11.891	<0.001
FBG [§] (mmol/L)	5.02 $\pm$ 1.34	5.61 $\pm$ 1.31	12.034	<0.001
TC [§] (mmol/L)	4.74 $\pm$ 1.25	4.87 $\pm$ 1.19	4.023	0.032
TG [§] (mmol/L)	1.37 $\pm$ 1.73	1.53 $\pm$ 1.58	11.017	<0.001

[§]Represents mean and standard deviation.

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBG: fasting blood glucose; TC: total cholesterol; TG: triglyceride.

Figure 1. C-689T polymorphism enzyme restriction spectrum.

1, 6.Marker; 2.PCR product; 3.CC genotype; 4.CT genotype; 5.TT genotype.

Association of genotypes and clinical characteristics

Comparison of clinical indexes between non-carriers and carriers of the -689T allele showed that the mean blood TC level of the carriers was significantly higher than that of the non-carriers (5.12 $\pm$ 1.26 vs. 4.76 $\pm$ 1.22 mmol/L, $P=0.001$) (Table 3). Subjects with C-689-T substitution showed a tendency to a higher TC level ($OR=1.413$, 95%CI: 1.170-1.707, $P<0.001$) after adjusted for age, gender, body weight, waist circumference, BMI, smoking, drinking, physical activities, FBG, SBP, DBP, and TG.

Table 2. Distribution of genotypes and alleles of PPAR γ 2 promoter C-689T [*n*(%)]

Groups	Genotype [*]				Allele [†]		
	<i>n</i>	CC	CT	TT	<i>n</i>	C	T
Controls	693	645(93.1)	46(6.6)	2(0.3)	1386	1336(96.4)	50(3.6)
Cases	455	408(89.7)	45(9.9)	2(0.4)	910	861(94.6)	49(5.4)
Total	1148	1053(91.7)	91(7.9)	4(0.4)	2296	2197(95.7)	99(4.3)

PPAR γ 2: peroxisome proliferator-activated receptor- γ 2.

^{*}CC vs. CT+TT, $\chi^2=6.243$, $P=0.041$; [†]C vs. T, $\chi^2=6.859$, $P=0.040$.

Table 3. Association between genotypes and clinical characteristics in total study population

Clinical Characteristics	Genotypes		χ^2/t	<i>P</i> value
	CC (<i>n</i> =1053)	CT+TT (<i>n</i> =95)		
Gender(male/female, <i>n</i>)	656/397	51/44	6.313	0.013
Age(yrs)	59.79 $\pm$ 9.02	60.19 $\pm$ 9.88	0.412	0.686
Waist circumference(cm)	85.78 $\pm$ 10.66	87.20 $\pm$ 12.56	0.894	0.221
Body weight(kg)	65.18 $\pm$ 10.78	65.96 $\pm$ 13.11	0.551	0.504
BMI(kg/m ²)	24.13 $\pm$ 3.38	24.66 $\pm$ 4.37	1.028	0.154
SBP(mm Hg)	133.60 $\pm$ 21.80	135.46 $\pm$ 23.45	0.637	0.428
DBP(mm Hg)	82.58 $\pm$ 12.30	81.78 $\pm$ 11.66	0.520	0.540
FBG(mmol/L)	5.26 $\pm$ 1.34	5.08 $\pm$ 1.27	0.883	0.247
TC(mmol/L)	4.76 $\pm$ 1.22	5.12 $\pm$ 1.26	10.286	0.001
TG(mmol/L)	1.42 $\pm$ 1.68	1.55 $\pm$ 1.66	1.116	0.137

In male subjects, statistical differences in waist circumference, body weight, TC and TG were detected between carriers and noncarriers of -689T allele (all $P<0.05$). Multivariate logistic regression analysis showed that only waist circumference was significantly different for C-689 $\rightarrow$ T substitution (91.49 $\pm$ 12.89 vs. 87.00 $\pm$ 10.37 cm), with an OR of 1.031 (95%CI: 1.005-1.058, $P=0.020$). In females, only TC was found to be statistically different between carriers and non-carriers of -689T allele (4.98 $\pm$ 1.24 vs. 5.35 $\pm$ 1.27 mmol/L, $P=0.039$), which was consistent with the results from total study population.

In subjects whose BMI was over 25 kg/m², we found that the carriers (*n*=82) and noncarriers of -689T allele (*n*=231) were significantly different in waist circumference (96.19 $\pm$ 12.75 vs. 93.09 $\pm$ 9.05 cm, $P=0.049$), BMI (28.65 $\pm$ 3.46 vs. 27.54 $\pm$ 2.38 kg/m², $P=0.008$), SBP (144.63 $\pm$ 21.08 vs. 137.30 $\pm$ 21.42 mm Hg, $P=0.040$) and TC (5.18 $\pm$ 1.30 vs. 4.81 $\pm$ 1.23 mmol/L, $P=0.036$). By multivariate logistic regression analysis, for C-689 $\rightarrow$ T substitution, significant differences were only found in BMI and TC, with an OR of 1.121 (95%CI: 1.012-1.241, $P=0.029$) and 1.337

(95%CI: 1.017-1.758, $P=0.037$) respectively.

In the case group, carriers of -689T allele had significantly higher waist circumference, body weight, BMI, TC and TG than non-carriers (all $P<0.05$). In the control group, only TC and TG of -689T allele carriers were found to be significantly higher than those of non-carriers (both $P<0.05$) (Table 4). Multivariate logistic regression analysis showed that only the difference in TC between carriers and non-carriers was significant, with an OR of 1.549 (95%CI: 1.008-1.897, $P=0.026$) for C-689 $\rightarrow$ T substitution.

Coronary heart disease risk factors

We analyzed the impact of the -689T allele, as well as the conventional risk factors, on CHD. Univariate logistic regression analysis confirmed that large waist circumference, heavy body weight, smoking, lack of physical activities, high SBP, high DBP, high FBG level, high TC level and high TG level were risk factors of CHD. Importantly, the -689T allele in the PPAR γ 2 promoter was a significant risk factor for CHD, with an OR of 1.548 (95%CI: 1.016-2.358, $P=0.042$) (Table 5).

Results of multivariate logistic regression analysis

showed that the -689T allele was an independent risk factor for CHD, with an *OR* of 1.668 (95%*CI*: 1.031-2.705, *P*=0.037), after adjusted for age, gender, waist circumference, body weight, smoking, physical activities, SBP, DBP, FBG, TC and TG level. Besides, FBG, SBP, smoking and lack

of physical activities were found to have significant impact on the risk of CHD, with an *OR* of 1.196 (95%*CI*: 1.114-1.285, *P*<0.001), 1.024 (95%*CI*: 1.018-1.031, *P*<0.001), 2.340 (95%*CI*: 1.785-3.068, *P*<0.001) and 4.251 (95%*CI*: 3.239-5.578, *P*<0.001), respectively.

Table 4. Association between genotypes and clinical characteristics in controls and cases

Variables	Genotypes in Controls		Genotypes in Cases of CHD	
	CC	CT+TT	CC	CT+TT
<i>n</i>	645	48	408	47
Age (yrs)	59.68±8.23	59.59±9.51	59.97±9.21	60.12±8.98
Waist circumference(cm)	84.78±10.12	85.13±11.14	81.04±10.93	90.46±11.58 [†]
Body weight (kg)	64.25±10.14	64.78±12.52	61.87±10.77	72.17±11.23 [†]
BMI (kg/m ²)	24.12±3.31	24.67±4.07	20.05±3.15	27.32±4.29 [†]
SBP (mm Hg)	129.29±20.03	130.16±21.80	139.62±22.68	140.01±24.13
DBP (mm Hg)	81.01±10.46	81.34±11.02	84.34±13.97	85.24±14.68
FBG (mmol/L)	5.01±1.30	5.09±1.39	5.60±1.28	5.62±1.35
TC (mmol/L)	4.31±1.22	5.12±1.30 [*]	4.46±1.15	5.50±1.28 [†]
TG (mmol/L)	1.09±1.51	1.79±1.62 [*]	1.19±1.53	1.96±1.61 [†]

^{*}*P*<0.05 compared with CC in controls; [†]*P*<0.05 compared with CC in cases.

Table 5. Univariate logistic regression analysis of CHD risk factors

Variables	<i>OR</i>	95% <i>CI</i>	<i>P</i> value
T allele carriers	1.548	1.016-2.358	0.042
Gender (male)	0.915	0.717-1.167	0.473
Age	1.005	0.992-1.018	0.467
Waist circumference	1.020	1.009-1.032	<0.001
Body weight	1.013	1.002-1.024	0.022
BMI	0.991	0.958-1.026	0.621
Smoking	1.992	1.566-2.532	<0.001
Alcohol consumption	1.179	0.897-1.550	0.239
Lack of physical activity	4.087	3.168-5.274	<0.001
SBP	1.022	1.017-1.028	<0.001
DBP	1.025	1.015-1.036	<0.001
FBG	1.165	1.092-1.243	<0.001
TC	1.095	0.974-1.231	0.032
TG	1.075	0.964-1.199	<0.001

CHD: coronary heart disease.

DISCUSSION

Since PPAR γ 2 was identified, studies on the association between PPAR γ 2 gene polymorphisms and diseases have

sprung up. A common variant Pro12Ala in PPAR γ 2 gene has been confirmed to be associated with diseases such as type 2 diabetes mellitus, obesity, metabolic syndrome and cardiovascular disorders. Meirhaeghe *et al*⁶ found that, in French population, carriers of the -689T allele had elevated body weight and LDL-cholesterol concentration compared with the homozygous for the common allele, and a substitution C-689T in the promoter of PPAR γ 2 resulted in lower transcription activity of PPAR γ 2. In another article, the rare alleles of C-689T and Pro12Ala SNPs were reported to be associated with an increased risk of metabolic syndrome compared to the 1431CC genotype.⁷ We assume that the activation of PPAR γ 2 depresses the development of CHD effectively, and C-689→T substitution in the promoter of PPAR γ 2 restrains its activity and therefore may be a significant risk factor for CHD. However, Dallongeville *et al*⁸ reported that there was no major consistent association of PPAR γ C-681G, C-689T, Pro12Ala, and C1431T genotypes or related haplotypes with CHD risks.

In the present study, our results showed that the rare T allele of C-689T, in addition to the traditional risk factors, was a significant risk factor for CHD in nondiabetic Chinese Han people. Because insulin resistance, dyslipidemia, inflammation, atherosclerosis, endothelial dysfunction, and

smooth muscle cells proliferation play important roles in the development of CHD, PPAR γ 2 may have biological effects on improving insulin sensitivity, anti-atherosclerosis, anti-inflammation and protecting endothelial function.

We propose that PPAR γ 2 may take these effects through the following signaling pathways. Firstly, diacylglycerol (DAG) activates protein kinase C (PKC) isoforms and mediates many cellular functions, including cell growth, activation, and differentiation, so that the DAG-PKC signaling pathway mediates the pathogenesis of insulin-resistance. Meanwhile, Diacylglycerol kinase (DGK) can inhibit the DAG-PKC pathway *via* decreasing the intracellular DAG level by phosphorylation of DAG, which yields phosphatidic acid. PPAR γ 2 is able of increasing DGK α production and DGK activity, resulting in suppression of the DAG-PKC signaling pathway, therefore strengthens insulin sensitivity.⁹ Additionally, GLUT2 is a major form of glucose transporter in pancreatic cells and plays an important part in allowing rapid equilibration of glucose across the plasma membrane. It has been described in a previous study that the promoter region of the GLUT2 gene contains PPRE, which indicates that PPAR may maintain glucose homeostasis by regulating the expression of the GLUT2 gene.¹⁰ Therefore, PPAR γ 2 can reduce insulin resistance, and inhibit glucose-stimulated proinsulin biosynthesis and insulin release, and thus decreases fat synthesis and lipid accumulation.

Secondly, as PPAR γ 2 has been revealed to depress the expression of inflammatory response genes, including IFN- γ , IL-1 β , IL-6, TNF- α , VCAM-1, NOS, MCP-1 and C-C chemokine receptor 2, it could promote the recruitment of monocytes and T cells in atherosclerotic lesions and their subsequent differentiation and activation.¹¹ So PPAR γ 2 may control inflammation by inhibiting cytokines and matrix metalloproteinases, and by restraining NF- κ B, AP-1 and STAT-1 signaling pathways.¹²

Thirdly, PPAR γ 2 has been shown to activate ATP-binding cassette transporter A1 (ABCA1) expression indirectly by enhanced transcription of liver X receptor (LXR), therefore promote the efflux of cholesterol from macrophage foam cells.¹³ In vascular smooth muscle cells (VSMCs), PPAR γ 2 has been showed to inhibit MMP-9 expression and down-regulate Ang II type I receptors, which result in blocking the Rho/Rho kinase pathway and inhibiting VSMCs proliferation.¹⁴ The efficacy of PPAR γ 2 in reducing atherosclerosis is hence most likely a consequence of both anti-inflammatory and indirect LXR-mediated effects. Additional anti-atherosclerotic effects might result from reducing macrophage production of inflammatory cytokines, the expression of scavenger receptor type AI/II and the proliferation of VSMCs.¹³

Finally, PPAR γ 2 may take these effects through interacting with environment and other related genes, such as leptin, adiponectin and resistin by gene-environment interactions and gene-gene interactions. In our study, T allele carriers of C-689T were found to have a significant higher mean level of TC than non-carriers in the overall study population. This result may be related to the following two aspects. On one hand, PPAR γ 2 decreases plasma TC synthesis *via* insulin-sensitizing and adipocytes differentiation-inhibitory effects; on the other hand, PPAR γ 2 increases the efflux of TC by controlling the expression of a gene network that mediate cholesterol efflux from cells and its transport in plasma.¹³ Therefore, the T allele carriers have a higher TC level than non-carriers due to the lower activity of PPAR γ 2.

Since PPAR γ 2 is involved in a wide range of metabolic pathways, its activation or inhibition is likely to have complex consequences. Both our study and the study by Meirhaeghe *et al.*⁶ found that the T allele was not associated with TG. Elevated body weight was found to be associated with T allele in the present study, which was different from the study of Meirhaeghe *et al.*⁶ Although T allele was not found to be associated with BMI, FBG, SBP or DBP in the total study population, interestingly, the associations between genotypes and phenotypes were found to be gender related. In males, T allele was associated with elevated waist circumference, body weight, TC and TG, indicating abdominal obesity in males may be associated with T allele. Additionally, in subjects with obesity (BMI ≥ 25 kg/m²), the T allele was associated with increased waist circumference, BMI, SBP and TC.

In the study, we found that in the Han Chinese subjects that were free of diabetes, the total T allele frequency of C-689T was 4.3%, which was significantly lower than 12% as reported in French population.⁶ This finding indicated that genetic mutation is specific to particular ethnic group and environment.

This study excluded subjects with diabetes mellitus, which enhances the power of the results, as diabetes mellitus was reported to be associated with the genetic mutation.¹⁵ The large sample size of this study provided power of data that supported the results of our study. However, specimens of this study came from a single institute and from the population of one city in China, which may weaken the representativeness for the whole Han population.

In conclusion, PPAR γ 2 has a wide range of biological functions. The present study confirmed that the C-689 $\rightarrow$ T substitution in the promoter of PPAR γ 2 is a significant risk factor for CHD, and is associated with an increased risk of CHD in nondiabetic Chinese Han people. Detection of this

genotype may help in diagnosis, prevention and treatment of CHD.¹⁶ Our results indicate the potential of developing PPAR γ 2-targeted pharmaceuticals in the management of CHD. More studies are needed in the future to further verify our results and investigate clinical values.

Conflict of Interest Statement

All authors have no conflict of interests to disclose.

REFERENCES

- Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr Rev* 1999; 20(5):649-88. doi: 10.1210/edrv.20.5.0380.
- Fajas L, Auboeuf D, Raspé E, Schoonjans K, Lefebvre AM, Saladin R, et al. The organization, promoter analysis, and expression of the human PPAR γ gene. *J Biol Chem* 1997; 272(30):18779-89. doi: 10.1074/jbc.272.30.18779.
- Wayman NS, Hattori Y, McDonald MC, Mota-Filipe H, Cuzocrea S, Pisano B, et al. Ligands of the peroxisome proliferator-activated receptors (PPAR- γ and PPAR- α) reduce myocardial infarct size. *FASEB J* 2002; 16(9):1027-40. doi: 10.1096/fj.01-0793com.
- Choi D, Kim SK, Choi SH, Ko YG, Ahn CW, Jang Y, et al. Preventative effects of Rosiglitazone on restenosis after coronary stent implantation in patients with type 2 diabetes. *Diabetes Care* 2004; 27(11):2654-60. doi: 10.2337/11.2654.
- Sidhu JS, Cowan D, Tooze JA, Kaski JC. Peroxisome proliferator-activated receptor-gamma agonist rosiglitazone reduces circulating platelet activity in patients without diabetes mellitus who have coronary artery disease. *Am Heart J* 2004; 147(6):e25. doi: 10.1016/j.ahj.2003.12.035.
- Meirhaeghe A, Tanck MW, Fajas L, Janot C, Helbecque N, Cottel D, et al. Study of a new PPAR γ 2 promoter polymorphism and haplotype analysis in a French population. *Mol Genet Metab* 2005; 85(2):140-8. doi: 10.1016/j.ymgme.2005.02.004.
- Meirhaeghe A, Cottel D, Amouyel P, Dallongeville J. Association between peroxisome proliferator-activated receptor gamma haplotypes and the metabolic syndrome in French men and women. *Diabetes* 2005; 54(10):3043-8. doi: 10.2337/diabetes.54.10.3043.
- Dallongeville J, Iribarren C, Ferrières J, Lyon L, Evana A, Go AS, et al. Peroxisome proliferator-activated receptor gamma polymorphisms and coronary heart disease. *PPAR Res* 2009; 2009:543746. doi: 10.1155/2009/543746.
- Shmueli E, Alberti KG, Record CO. Diacylglycerol/protein kinase C signaling: a mechanism for insulin resistance? *J Intern Med* 1993; 234(4):397-400. doi: 10.1111/j.1365-2796.1993.tb00761.x.
- Kim HI, Kim JW, Kim SH, Cha JY, Kim KS, Ahn YH, et al. Identification and functional characterization of the peroxisomal proliferator response element in rat GLUT2 promoter. *Diabetes* 2000; 49(9):1517-24. doi: 10.2337/diabetes.49.9.1517.
- Jackson SM, Parhami F, Xi XP, Berliner JA, Hsueh WA, Law RE, et al. Peroxisome proliferator-activated receptor activators target human endothelial cells to inhibit leukocyte-endothelial cell interaction. *Arterioscler Thromb Vasc Biol* 1999; 19(9):2094-104. doi: 10.1161/01.ATV.19.9.2094.
- Straus DS, Pascual G, Li M, Welch JS, Ricote M, Hsiang CH, et al. 15-deoxy-delta 12,14-prostaglandin J2 inhibits multiple steps in the NF-kappa B signaling pathway. *Proc Natl Acad Sci USA* 2000; 97(9):4844-9. doi: 10.1073/pnas.97.9.4844.
- Akiyama TE, Sakai S, Lambert G, Nicol CJ, Matsusue K, Pimprale S, et al. Conditional disruption of the peroxisome proliferator-activated receptor gamma gene in mice results in lowered expression of ABCA1, ABCG1, and apoE in macrophages and reduced cholesterol efflux. *Mol Cell Biol* 2002; 22(8):2607-19. doi: 10.1128/MCB.22.8.2607-2619.2002.
- Marx N, Schonbeck U, Lazar M, Libby P, Plutzky J. Peroxisome proliferator-activated receptor gamma activators inhibit gene expression and migration in human vascular smooth muscle cells. *Circ Res* 1998; 83(11):1097-103. doi: 10.1161/01.RES.83.11.1097.
- Khodaeian M, Enayati S, Tabatabaei-Malazy O, Amoli MM. Association between genetic variants and diabetes mellitus in Iranian populations: a systematic review of observational studies. *J Diabetes Res* 2015; 2015:1-21. doi: 10.1155/2015/585917.
- Lavrenko AV, Shlykova OA, Kutsenko LA, Mamontova T, Kaidashev IP. Pharmacogenetic features of the effect of metformin in patients with coronary heart disease in the presence of metabolic syndrome and type 2 diabetes mellitus in terms of PPAR-gamma2 gene polymorphism. *Ter Arkh* 2012; 84(9):35-40. doi: 10.17116/terarkh201284935-40.