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Peroxisome proliferator-activated receptor γ C161→T polymorphism and coronary artery disease

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Abstract

Background: Peroxisome proliferator-activated receptor γ (PPARγ) as a transcription factor plays an important role in lipid metabolism, glucose homeostasis, insulin sensitivity, obesity, diabetes, foam cell formation and atherogenesis. **Methods and Results:** We have studied distribution of the PPARγ C161→T substitution at exon 6 in 647 Australian Caucasian patients aged ≤65 years (484 men and 163 women) recruited consecutively, with or without angiographically documented coronary artery disease (CAD). The frequencies of the CC, CT and TT genotypes were 69.8%, 27.7% and 2.5% and the 'T' allele frequency 0.163. They were in Hardy–Weinberg equilibrium and not different between men and women. The BMI and waist to hip ratio (WHR) among patients with CC, CT+TT genotypes were not different ($P=0.878$, $P=0.677$). However there was a significant association between the polymorphism and CAD. The 'T' allele carriers (CT+TT) had significantly reduced CAD risk compared to the CC homozygotes (odds ratio: 0.457, 95% CI: 0.273–0.763, $P=0.0045$) in a logistic regression model after controlling other known risk factors. This reduced risk was particularly evident among CT heterozygotes (odds ratio: 0.466, 95% CI: 0.291–0.746, $P=0.0015$), who also had lower apo B and total cholesterol to HDL-C ratios ($P<0.05$). **Conclusion:** We report that the PPARγ C161→T substitution is associated with a reduced CAD risk, particularly among CT heterozygous patients, but not with obesity in Australian Caucasian patients. It implicates that the PPARγ may have a significant role in atherogenesis, independent of obesity and of lipid abnormalities, possibly via a direct local vascular wall effect.

Keywords: Atherosclerosis; Coronary disease; Epidemiology; Lipoproteins; Sequence (DNA)

1. Introduction

Peroxisome proliferator-activated receptor (PPAR) is a family of ligand-activated transcription factors [1]. Three types of PPAR including α, γ and the ubiquitously expressed β/δ have been described in mammalian [2–5]. PPARγ is a group of fine-tuned transcription factors involved in many aspects of metabolic control. It is found predominantly in adipose tissue, where it plays a crucial role in adipocyte differentiation, fat storage and glucose homeostasis [1,2]. Other cell types such as macrophages, skeletal muscles and vascular smooth muscle cells have recently also been shown to express the PPARγ gene [1,6–9].

The PPARγ gene, located at 3p25-24 [10], gives rise to three distinct mRNAs, i.e. PPARγ1, PPARγ2 and PPARγ3 [2,11–14]. While all three PPARγ subtypes contain the common exons 1–6, each differs in their 5′ ends and each is under control of their own promoter [2,11–14]. Although the tissue distributions vary for different PPARγ

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isoforms, they are all expressed in adipose tissue and regulate adipocytes differentiation–a process essential for obesity development [1,2,14,15]. This makes the PPARγ a potential candidate gene for coronary artery disease (CAD) since obesity is a significant contributor to increased CAD risk.

While the link between the PPARγ and CAD could be mediated through its function on adipocyte differentiation, lipid metabolism and glucose homeostasis, PPARγ may also act directly on local vascular wall in promoting foam cell formation. It is known that PPARγ activation shifts free fatty acids into fat cells and induces adipocyte hypertrophy and hyperplasia [1,8]; forced PPARγ2 expression in fibroblasts and myoblasts can differentiate these cells into adipocytes [11,16]. When the PPARγ activation is forced in macrophages, such as by oxLDL, there is also an associated cellular differentiation and lipid accumulation, which could turn them into foam cells [1,17,18]. PPARγ is indeed abundantly expressed in foam cells of atherosclerotic lesions [19]. On the other hand, PPARγ also appears to possess an immune suppressive function [6,7] which could favour an anti-atherogenic effect [20]. Thus PPARγ could potentially exert a double-edged effect on atherogenesis.

In view of the particular relevance of the PPARγ to lipid metabolism, immune suppression and foam cell formation, it is important to assess the role of PPARγ in atherogenesis. However, since PPARγ is a nuclear protein, it is difficult to measure its phenotypic expression in vivo, particularly within local vascular wall environment. Hence, functional DNA variants or variants in linkage with functional change(s) in PPARγ gene could provide an indirect assessment of the local effects. Several mutations have been reported in the PPARγ gene. A Pro12Ala mutation in PPARγ2 specific exon B was found to have a decreased binding affinity to the cognate promoter element and reduced ability to transactivate responsive promoters [21]. While the mutation was associated with a lower BMI (26.2 ± 0.2 vs. 25.0 ± 0.4, $P=0.027$) and improved insulin sensitivity, it was also associated with an increased risk for diabetes ($P=0.028$). The findings are not confirmed by others [22,23]. Meirhaeghe et al. also reported a C161→T substitution at exon 6 of the PPARγ gene associated with circulating leptin levels in obese subjects but not in those of normal BMI [24]. This base change is more common than the Pro115Gln mutation also at exon 6 [25] and occurs in an exon shared by all three forms of PPARγ. Since it is not clear how each of the isoforms is related to atherogenesis, the DNA variants at the common exons of the PPARγ gene could be more informative than isoform specific mutations.

In the present study we explored the PPARγ C161→T substitution in our well-characterised hospital-based patients whose coronary arterial status were documented angiographically. We also investigated the association between the PPARγ polymorphism and commonly used obesity measure–body mass index (BMI) and waist to hip ratio (WHR).

2. Patients and methods

2.1. The patient population

We studied 647 Caucasians aged 65 years or less, both men ($n=484$) and women ($n=163$), consecutively referred to the Eastern Heart Clinic at Prince of Wales Hospital for coronary angiography. Each angiogram was classified as revealing either normal coronary arteries or having coronary lesion with less than 50% luminal stenosis or as having one, two, or three major epicardial coronary arteries with more than 50% luminal obstructions. We obtained each patient's medical history using a questionnaire with standardised choices of answers to be ticked during the interview and DNA samples were collected for each patient as described previously [26]. A written consent was obtained from every patient which was approved by the Ethics Committee of the University of New South Wales.

2.2. Biometric measurements

The height and weight were routinely measured for each patient before the coronary procedures by either a registered nurse or an attending doctor who also interviewed the patients to record the medical history. The waist and hip circumferences were measured and the WHR calculated to assess body fat distribution. The BMI was obtained from the ratio of weight (kg) to height squared (m^2).

2.3. Measurements of lipoproteins and apolipoproteins

The hospital's Clinical Chemistry Department measured levels of total cholesterol (TC), HDL-cholesterol (HDL-C) and triglyceride using standard enzymatic methods; the LDL-cholesterol levels were calculated from the Friedewald formula. Levels of apo AI, apo B and Lp(a) were measured in our laboratory using in-house ELISA methodology as described previously [26].

2.3.1. Determination of the PPARγ exon 6 (C161→T) substitution

The polymerase chain reaction (PCR) was used to detect the C161→T at exon 6 of the PPARγ gene as described by Meirhaeghe et al. [24]. The forward and reverse primers were 5′–CAA GAC AAC CTG CTA CAA GC-3′ and 5′–TCC TTG TAG ATC TCC TGC AG-3′. The amplification was performed in a 25 μl volume containing 100 ng DNA, 20 pmol of each primer, 2.0 mmol/l $MgCl_2$, 50 mmol/l KCl, 25 μmol/l dNTP, 5 mmol/l Tris–HCl (pH

8.3) and 1 Unit Taq polymerase. Samples were subjected to denaturing at 94°C for 1 min followed by 34 cycles of 94°C for 30 s, 56°C for 30 s and 72°C 1 min. The thermal cycles finish with 72°C for 5 min. The 200 bp PCR products were digested with a *Pml*I restriction enzyme (recognition site: 5′ . . . CA**C**$^{\downarrow}$GTG . . . 3′) and run in 8% polyacrylamide gel for 30 min and silver-stained. This resulted in two fragments (120bp and 80bp) for the wild-type and one fragment (200bp) when the restriction site was eliminated by the C161→T transition (5′-CA**T**GTG-3′) (Fig. 1). The genotypes were identified as CC, CT and TT.

2.4. Statistical analysis

A computer package of SPSS Advanced Statistics 9.0 for PC Windows 95 was used to analyse the data. All the continuous variables are presented as mean±SEM. Hardy–Weinberg equilibrium for the PPARγ C161→T genotype distribution was assessed as before [26]. The effects of genotypes on quantitative and categorical variables were analysed and presented for all three genotype groups (CC, CT and TT). However since the number of the rare TT homozygous patients ($n=16$) was small, we also combined the TT and CT into a single 'T' allele carrier group (CT+TT) to improve statistical power as reported by Meirhaeghe et al. [24]. We used an ANOVA F test and a Student t test to estimate relationships between the PPARγ genotypes and quantitative variables. A contingency table chi-square analysis was employed to estimate the contribution of the polymorphism to the presence and severity of CAD, and to evaluate relationships between the genotypes and other medical conditions including myocardial infarction, angina pectoris, diabetes mellitus and hypertension. To assess the independent effect of the PPARγ C161→T polymorphism on BMI, WHR and lipid variables, a general linear model of factorial design of analysis of variance was applied in which other independent factors were controlled as covariates. We measured the independent contribution of the polymorphism to the presence and severity of CAD using a stepwise logistic regression model with other known risk factors entered as covariates and odds ratios (OR) were calculated in the model. The statistical significance was defined as $P<0.05$ and two-tailed P values were reported.

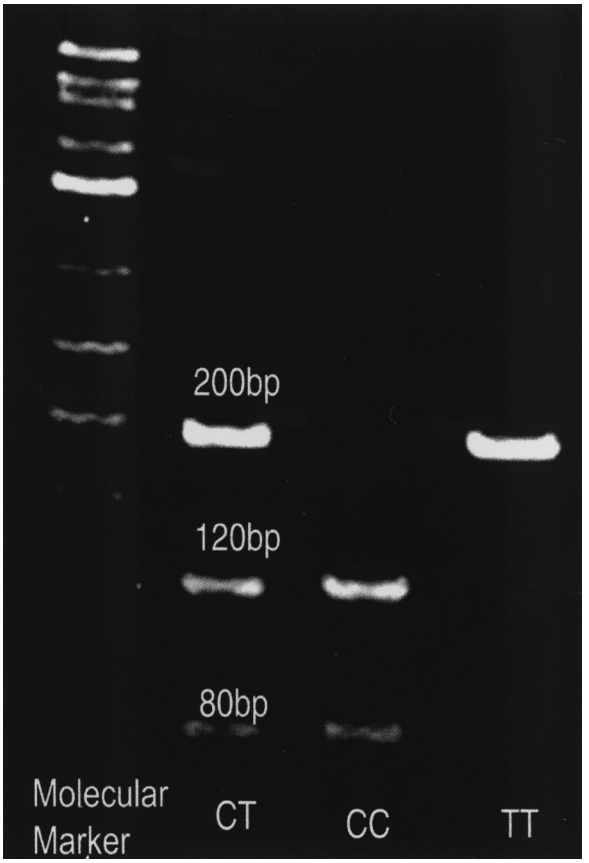

Fig. 1. The PAGE gel shows the PCR products from the amplification of the mutation region of the PPARγ gene. The mutation eliminates a *Pml*I recognition site which digests the 200 bp PCR fragment into 120 and 80 bp fragments. Lane 1 is the molecular marker (Promega, ϕx174 DNA/*Hinf* I), lane 2 is a CT heterozygote, lane 3 shows a common CC homozygote, lane 4 is a rare TT homozygote.

3. Results

We have studied 647 patients (age: 55.7±0.6 years for men and 57.6±0.9 years for women) consecutively enrolled in our program. The frequencies of the PPARγ C161→T genotypes were 69.8% ($n=451$), 27.7% ($n=180$) and 2.5% ($n=16$) for CC, CT and TT respectively and the rare 'T' allele frequency was 0.163. The genotype distribution was in Hardy–Weinberg equilibrium ($\chi^2=0.120$, $P=0.942$) and was not different between men and women ($\chi^2=3.275$, $P=0.194$).

3.1. The C161→T polymorphism and obesity

We measured the obesity by two conventionally used methods – BMI and WHR. The BMI in this hospital-based patient group was much higher than that in general population. While only 22.8% of the patients were of normal weight (BMI: 2–25 kg/m^2), 48.9% were overweight (BMI: 25–30 kg/m^2) and 28.3% were obese (BMI: >30 kg/m^2). As shown in Tables 1–3 there was no association between the PPARγ polymorphism and BMI or WHR in both men and women. The lack of significant association was also true ($P=0.688$) when patients were categorised by overweight and obese. This non-significant association was confirmed after controlling sex, age, cigarette smoking and status of coronary disease in a general factorial model of analysis of variance. However, TT homozygotes tended to be smaller as indicated by the biometric parameters (Tables 1–3). In the general factorial model, age was the only significant predictor of BMI

Table 1
Mean±SEM biometric and lipoprotein profile distributions in relation to the PPARγ C161→T benotypes in all patients[a]

	PPARγ C161→T genotypes			
	CC	CT	TT	CT+TT
N (%)	451 (69.7%)	180 (27.8%)	16 (2.5%)	196 (30.3%)
Weight, kg	80.5±0.6	81.5±1.0	77.5±4.3	81.2±1.0
Height, m	1.69±0.01	1.70±0.01	1.69±0.02	1.70±0.01
Waist, cm	98.3±0.6	99.3±0.9	93.6±3.5	98.8±0.9
Hip, cm	103.8±0.5	105.0±0.7**	97.1±3.1	104.3±0.7
BMI, kg/m^2	28.1±0.2	28.1±0.3	26.8±1.2	28.0±0.3
Waist/hip ratio	0.95±0.001	0.94±0.001	0.96±0.02	0.95±0.01
TC, mmol/l	5.5±0.01	5.3±0.07	5.8±0.22	5.3±0.08
Trig, mmol/l	2.0±0.01	1.8±0.04	2.5±0.31	1.9±0.07
HDL, mmol/l	1.06±0.001	1.10±0.02	1.01±0.06	1.09±0.02
LDL, mmol/L	3.51±0.05	3.38±0.06	3.03±0.22	3.38±0.07
TC/HDL	5.56±0.08	5.18±0.11*	5.97±0.50	5.23±0.11*
Apo AI, g/l	0.96±0.01	0.94±0.02	0.78±0.09	0.92±0.02
Apo B, g/l	0.97±0.01	0.88±0.01***	1.05±0.06	0.90±0.02**
Lp(a), mg/l	287±17	307±24	365±61	312±22

[a] *P<0.05, **P<0.01, ***P<0.001. P values were obtained by ANOVA F tests for comparisons among CC, CT and TT genotypes or Student t tests for comparisons between CC genotype and 'T' allele carriers (CT+TT genotypes).

(P=0.004) and the sex was the only predictor of WHR (P=0.0001). There was no interactive effects between measured variables.

3.2. The C161→T polymorphism and lipoprotein profiles

To compare lipoprotein profiles among three genotype groups by ANOVA F tests, there were significant differences in levels of apo B and TC/HDL-C ratios (Table 1), particularly in men (Table 2). However, the significant differences were confined between the CC and CT genotypes (P=0.001 and 0.004 for apo B and TC/HDL-C respectively) by paired comparisons. While the CT heterozygotes had the lowest levels of apo B and TC/HDL-C, both CC and TT homozygotes had higher levels (Tables 1–3). The levels of apo B and TC/HDL-C in CT+TT combined group, i.e. 'T' allele carriers were also significantly lower than those in CC homozygotes (Tables 1 and 2). Since there was only one female TT homozygote, ANOVA F test was no longer valid for the three group comparisons in females (Table 3). But apo B levels and TC/HDL-C ratios also tended to be lower in female CT heterozygotes. The differences in apo B and TC/HDL-C between genotypes remained significant after controlling

Table 2
Mean±SEM biometric and lipoprotein profile distributions in relation to the PPARγ C161→T genotypes in male patients[a]

	PPARγ C161→T genotypes			
	CC	CT	TT	CT+TT
N (%)	339 (70.1%)	130 (26.8%)	15 (3.1%)	145 (29.9%)
Weight, kg	83.2±0.7	85.4±1.1	79.6±4.0	84.8±1.0
Height, m	1.72±0.01	1.74±0.01*	1.71±0.02	1.73±0.01*
Waist, cm	100.3±0.7	102.5±0.9*	95.1±3.4	101.7±1.1
Hip, cm	103.0±0.5	104.6±0.8*	97.7±3.3	103.8±0.8
BMI, kg/m^2	28.0±0.2	28.2±0.3	27.2±1.2	28.1±0.3
Waist/hip ratio	0.97±0.001	0.98±0.01	0.97±0.01	0.98±0.01
TC, mmol/l	5.4±0.05	5.2±0.08*	5.9±0.22	5.3±0.07
Trig, mmol/l	2.1±0.06	1.9±0.07	2.4±0.33	2.0±0.07
HDL, mmol/l	0.99±0.01	1.01±0.02	1.01±0.07	1.01±0.02
LDL, mmol/l	3.48±0.06	3.33±0.07	3.74±0.21	3.37±0.07
TC/HDL	5.84±0.09	5.43±0.13*	6.10±0.52	5.49±0.13
Apo AI, g/l	0.91±0.01	0.89±0.02	0.80±0.10	0.88±0.02
Apo B, g/l	0.97±0.01	0.89±0.01**	1.05±0.07	0.90±0.02
Lp(a), mg/l	275±15	294±28	378±64	303±26

[a] *P<0.05, **P<0.01, ***P<0.001. P values were obtained by ANOVA F tests for comparisons among CC, CT and TT genotypes or Student t tests for comparisons between CC genotype and 'T' allele carriers (CT+TT genotypes).

Table 3
Mean±SEM biometric and lipoprotein profile distributions in relation to the PPARγ C161→T genotypes in female patients[a]

	PPARγ C161→T genotypes			
	CC	CT	TT	CT+TT
N (%)	112 (68.7%)	50 (30.7%)	1 (0.6%)	51 (31.1%)
Weight, kg	72.7±1.4	71.4±1.8	49.0	70.9±1.8
Height, m	1.59±0.01	1.61±0.01	1.50	1.60±0.01
Waist, cm	92.4±1.4	90.4±1.8	71.0	90.0±1.9
Hip, cm	106.5±1.2	106.1±1.5	87.0	105.7±1.5
BMI, kg/m^2	28.5±0.5	27.7±0.6	21.7	27.6±0.6
Waist/hip ratio	0.87±0.001	0.85±0.01	0.82	0.85±0.01
TC, mmol/l	5.7±0.1	5.6±0.1	4.6	5.5±0.2
Trig, mmol/l	1.8±0.1	1.7±0.1	2.8	1.7±0.1
HDL, mmol/l	1.29±0.04	1.32±0.05	1.10	1.31±0.05
LDL, mmol/l	3.59±0.10	3.50±0.13	2.23	3.40±0.16
TC/HDL	4.66±0.14	4.56±0.22	4.18	4.51±0.22
Apo AI, g/l	1.13±0.04	1.04±0.04	0.56	1.03±0.05
Apo B, g/l	0.96±0.03	0.87±0.02	0.99	0.87±0.03
Lp(a), mg/l	323±32	342±47	161	338±47

[a] *P<0.05, **P<0.01, ***P<0.001. P values were obtained by ANOVA F tests for comparisons among CC, CT and TT genotypes or Student t tests for comparisons between CC genotype and 'T' allele carriers (CT+TT genotypes).

Table 4
Association between the PPARγ C161→T genotypes and the number of significantly diseased vessel (≥50% luminal obstruction)[a]

Number of significantly diseased vessels	PPARγ C161→T genotypes			
	CC	CT	TT*	CT+TT#
0	115 (61.2%)	71 (37.8%)	2 (1.1%)	73 (38.8%)
1	133 (78.7%)	34 (20.1%)	2 (1.2%)	36 (21.3%)
2	100 (71.9%)	36 (25.9%)	3 (2.2%)	39 (28.1%)
3	103 (68.2%)	39 (25.8%)	9 (6.0%)	48 (31.8%)
Total	451	180	16	647

[a] Number of patients is presented in each subgroup with a row percentage in the bracket. *χ^2=24.889, *df*=6, *P*=0.0001 for comparisons among CC, CT and TT genotypes. #χ^2=13.447, *df*=3, *P*=0.004 for comparison between CC genotype and 'T' allele carriers (CT+TT genotypes).

for the main effects of sex, age, lipid lowering drug usage, cigarette smoking status, BMI and WHR in a general factorial model of analysis of variance. The adjusted levels for CC, CT and TT genotypes in this model were 0.96±0.01, 0.88±0.02 and 1.02±0.14 g/l for apo B (*P*=0.003), and 5.26±0.07, 4.99±0.08 and 5.14±0.86 for TC/HDL-C (*P*=0.047) respectively. However, the difference was no longer statistically significant when the main effect of the presence of CAD was also included in the model. The adjusted levels for CC, CT and TT genotypes became 0.93±0.02, 0.88±0.02 and 0.94±0.13 g/l for apo B (*P*=0.157), and 5.08±0.11, 5.02±0.15 and 4.69±0.79 for TC/HDL-C (*P*=0.580) respectively.

3.3. The C161→T polymorphism and angiographically documented CAD

The association between the PPARγ polymorphism and CAD were explored in two ways. We firstly classified the patients according to the number of significantly stenosed major coronary arteries (≥50% luminal obstruction) as a measure of CAD severity. We then classified patients into a group with angiographically normal coronary arteries and patients with angiographically mild and/or significant lesions. By a simple χ^2 comparison, there was a significant association between the PPARγ genotypes and the number of significantly diseased vessels (Table 4). This significant association was further confirmed in a logistic model in which age, sex, cigarette smoking, BMI, WHR, lipoprotein variables, diabetes and hypertension were controlled for (*P*=0.002). The 'T' allele carriers were far more frequent among patients without than those with significantly diseased vessels (*P*=0.004, Table 4). However, when comparisons were conducted among all three genotypes, this protective advantage of the rare 'T' allele was mainly among CT heterozygotes (*P*=0.0001, Table 4). In fact, both CC and TT homozygotes tended to have increased CAD risk (Table 4). The same pattern of associations was also observed for patients classified as with or without angiographically demonstrable CAD (Table 5). The 'T' allele carriers were about 50% more frequent in patients without than those with CAD (*P*=0.003). The CT heterozygosity advantage was also evident in this comparison (*P*=0.001, Table 5).

In a logistic regression model in which age, sex, cigarette smoking, BMI, WHR, lipoprotein variables, diabetes and hypertension were controlled for, the PPARγ CC homozygotes had an increased CAD risk than the 'T' allele carriers (*P*=0.0045). The OR for CC homozygotes to have CAD was 2.19 (95%CI: 1.31–3.65) compared to the 'T' allele carriers. The OR was comparable to those for sex (2.17, 95%CI: 1.21–3.90) and diabetes (3.91, 95%CI: 1.13–13.5). Alternatively, the 'T' allele carriers had a reduced CAD risk compared to the CC homozygotes. The OR was 0.457 (95%CI: 0.273–0.763, *P*=0.0045). This 'T' allele associated low CAD risk was caused by the low risk of the CT heterozygotes when all three genotypes were entered into the model. Comparing to CC homozygotes, the OR was 0.460 (95%CI: 0.291–0.746, *P*=0.0015) for CT heterozygotes, and 1.741 (95%CI: 0.219–13.818, *P*=0.5999) for TT homozygotes. As expected sex, age, cigarette smoking, WHR, apo B, TC/HDL-C and diabetes were also significant predictors of the presence of CAD in the same model.

Table 5
Association between the PPARγ C161→T genotypes and the presence of angiographically demonstrable coronary artery disease[a]

Presence of CAD	PPARγ C161→T genotypes			
	CC	CT	TT*	CT+TT#
No	79 (59.4%)	53 (39.8%)	1 (0.8%)	54 (40.6%)
Yes	372 (72.4%)	127 (24.7%)	15 (2.9%)	142 (27.6%)
Total	451	180	16	647

[a] Number of patients is presented in each subgroup with a row percentage in the bracket. *χ^2=13.523, *df*=2, *P*=0.001 for comparisons among CC, CT and TT genotypes. #χ^2=8.622, *df*=1, *P*=0.003 for comparison between CC genotype and 'T' allele carriers (CT+TT genotypes).

Table 6
Association between the PPARγ C161→T genotypes and other medical conditions[a]

Medical conditions		PPARγ C161→T genotypes			
		CC	CT	TT	CT+TT
Diabetes*	No	398 (69.9%)	158 (27.8%)	13 (2.3%)	171 (30.1%)
	Yes	52 (71.2%)	18 (24.7%)	3 (4.1%)	21 (28.8%)
Hypertension	No	252 (70.0%)	103 (28.6%)	5 (1.4%)	108 (30.0%)
	Yes	195 (69.9%)	73 (26.2%)	11 (3.9%)	84 (30.1%)
Past history of myocardial infarction	No	278 (69.5%)	114 (28.5%)	8 (2.0%)	122 (30.5%)
	Yes	173 (71.2%)	62 (25.5%)	8 (3.3%)	70 (28.8%)
Angina	No	122 (71.8%)	46 (27.1%)	2 (1.2%)	48 (28.2%)
	Stable	158 (71.2%)	62 (27.9%)	2 (0.9%)	64 (28.8%)
	Unstable	170 (68.3%)	67 (26.9%)	12 (4.8%)	79 (31.7%)
Obesity	Normal	101 (67.8%)	40 (28.0%)	6 (4.2%)	48 (32.2%)
	Overweight	223 (71.0%)	82 (26.7%)	6 (2.0%)	91 (29.0%)
	Obesity	127 (69.0%)	52 (29.2%)	3 (1.7%)	57 (31.0%)

[a] Number of patients is presented in each subgroup with a row percentage in the bracket. χ^2 test was used for associations between the genotypes and medical conditions and none of the associations were statistically significant. *There were missing information for histories of diabetes, hypertension, myocardial infarction and angina in 5, 8, 4 and 6 patients respectively.

3.4. The C161→T polymorphism and other medical conditions

There was no association between diabetes, or hypertension or past history of myocardial infarction, or unstable angina and the polymorphism (Table 6). The distribution of the PPARγ genotypes was also not different among normal weight, overweight and obese patients (Table 6).

4. Discussion

Since the C161→T substitution occurs in all three PPARγ isoforms, functional significance of the base change could be diverse and multiple. Our study showed a strong association between this polymorphic marker and CAD. Patients of the 'T' allele carriers (CT+TT) had a significantly reduced CAD risk compared to the common CC genotype (OR: 0.457, 95%CI: 0.273–0.763, $P=0.0045$). This reduced risk was only statistically evident for patients of CT heterozygotes (OR: 0.466, 95%CI: 0.291–0.746, $P=0.0015$) and the OR for the TT homozygotes spanned from a reduced risk to an increased risk (OR: 1.741, 95%CI: 0.219–13.818, $P=0.599$). Since the number of TT homozygous patients was small ($n=16$), the observed wide range of the CAD risk in this group could be incidental. With an assumption of an allele dosage effect, the TT genotype should have an OR about 0.23 comparing to CC genotype. With our calculation based on the allele frequency, effect size and population variance, more than 3000 patients will be needed to approve or disapprove this with a sufficient statistical power. On the other hand, the patients of TT homozygotes could indeed have an elevated CAD risk comparing to CT heterozygotes. This could be explained by a hypothesis of heterozygosity advantage, which is particularly plausible for PPARγ since it can be both pro- and anti-atherogenic. The findings of favourable lipid profiles in CT heterozygous patients lend further support on this hypothesis. Nevertheless, the significant association between the PPARγ polymorphism and CAD, independent of diabetes, BMI, WHR, life-time smoking dose and lipid profiles, implicates a direct local vascular wall effect in relation to atherogenesis. It should be noted however that our findings are based on a high-risk hospital patient population. Among this selected population, we assessed the association between the polymorphic marker and the extent of luminal obstruction of coronary arteries measured angiographically. The results should be interpreted with caution and specific to the studied patients. However, given the potential significance of this nuclear protein, the current observational findings need to be confirmed in other populations and to be used as a lead into more functional studies.

We have also found that the PPARγ C161→T substitution is not associated with obesity (as measured by BMI or WHR), which is consistent with the findings of Meirhaeghe et al. [24]. However, inconsistent correlations between different PPARγ polymorphisms and obesity reported by us and others [21–24] raise several questions. It is either that BMI or WHR is not adequate as a measure of obesity; or that PPARγ does not have an effect significant enough to change these phenotypes; or that the polymorphisms were not functional in altering measurable biometric changes.

In summary, we have found that the PPARγ C161→T base change is associated with a reduced risk for CAD. This protective effect is particularly evident among patients of CT heterozygotes, while it is not observed among the small number of TT homozygotes. Since the 'T' allele frequency is low (0.163), a study in a large population is needed to explore whether this is a true heterozygosity advantage or an allele dosage effect is not shown due to a small number of TT homozygotes. We have further demon-

strated that the polymorphism is not associated with the commonly used obesity measures – BMI and WHR.

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