The 161TT genotype in the exon 6 of the peroxisome-proliferator-activated receptor γ gene is associated with premature acute myocardial infarction and increased lipid peroxidation in habitual heavy smokers

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ABSTRACT

PPAR (peroxisome-proliferator-activated receptor) is a nuclear receptor. Activation of PPARγ by its ligands could modulate gene transcription, thereby leading to multiple anti-atherogenic and fibrinolytic effects. However, the association between the 161T allele in exon 6 of the PPARγ gene and premature AMI (acute myocardial infarction) is not clear. We recruited 146 patients with premature AMI (onset age ≤ 50 years) and 146 controls. The C161T polymorphism was examined using PCR and restriction-fragment-length polymorphism. Plasma levels of Ab-ox-LDL (antibody against oxidized low-density lipoprotein) were measured in 27 male smokers, whose genotypes have been identified. The frequency of the PPARγ TT genotype among patients with AMI was significantly higher than that in controls [13 % compared with 5.5 %; OR (95 % CI) 2.7, (1.1–6.5), where OR and CI are odds ratio and confidence interval respectively]. This association was not observed in CC or CT genotypes. Using multivariate logistic regression analyses, we found that the homozygous TT genotype [OR (95 % CI), 3.1 (1.2–7.9)], smoking [OR (95 % CI), 3.5, (2.1–6.0)], hypertension [OR (95 % CI), 3.6, (1.9–6.9)] and diabetes mellitus [OR (95 % CI), 3.5 (1.5–8.4)] were independent risk factors for premature AMI. Plasma levels of Ab-ox-LDL were significantly higher in healthy volunteers with the TT genotype compared with those with the CC genotype (49.3 ± 18.1 compared with 24.2 ± 15.2 units/l respectively; P = 0.02). Therefore in our study we observed an association between the PPARγ 161 TT genotype and premature AMI. Lipid peroxidation was significantly influenced by the 161T allele.

INTRODUCTION

Atherosclerosis is a process of responses to endothelial damage with the involvement of lipids, inflammation and smooth muscle cell proliferation and migration [1–3]. The most important pathogenic mechanism in AMI (acute myocardial infarction) is occlusive thrombus formation at the site of a ruptured atherosclerotic plaque

Key words: acute myocardial infarction, atherosclerosis, oxidized low-density lipoprotein, peroxisome-proliferator-activated receptor (PPAR), polymorphism, smoking.

Abbreviations: AMI, acute myocardial infarction; BMI, body mass index; CAD, coronary artery disease; CI, confidence interval; LDL, low-density lipoprotein; Ab-ox-LDL, antibody against oxidized LDL; PAI-1, plasminogen activator inhibitor-1; OR, odds ratio; PPAR, peroxisome-proliferator-activated receptor.

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in the coronary artery [4–6]. In addition to conventional coronary risk factors, there is an increasing awareness of the contribution of genetic risk factors for coronary atherothrombosis [7–10].

PPARγ (peroxisome-proliferator-activated receptor γ) is a nuclear receptor expressed at high levels in mammalian adipose tissue and leads to the regulation of transcription of several genes involved in preadipocyte differentiation and insulin-mediated glucose uptake in peripheral tissues [11,12]. Insulin resistance could be improved markedly by activation of this receptor [13] and thiazolidinedione, which has significant anti-diabetic effects, is one of the activators of PPARγ [14,15]. In addition, several non-hypoglycaemic effects of this ligand, which are potentially beneficial for prevention of atherothrombosis, have been reported [16]. Thiazolidinedione can modify LDL (low-density lipoprotein)-cholesterol oxidation [17], reduce PAI-1 (plasminogen activator inhibitor-1) and fibrinogen levels [18–20] and platelet aggregation [21], decrease smooth muscle cell migration [22] and intima–media thickness [23], and inhibit inflammatory response [17,24–26]. In addition, activation of PPARγ could reduce reactive oxygen species [27]. Therefore PPARγ may have anti-atherogenic, anti-oxidative, anti-inflammatory and anti-fibrinolytic effects, and a deficiency of this protein could cause acute vascular events.

Recently, a genetic variant (C161T polymorphism) in exon 6 of the PPARγ gene was identified [28,29]. The 161T allele was associated with an increased plasma leptin level [29], a marker of inflammation [30–32], which might be theoretically involved in the genesis of AMI [33,34]. However, the association of AMI and the influence on oxidative modification of LDL with this genetic variant has not been investigated extensively. Therefore we conducted a case-controlled study to evaluate whether the 161T allele was associated with atherothrombosis and thus associated with AMI at relatively young ages. In addition, we evaluated the relationship of Ab-ox-LDL (antibody against oxidized LDL) with the 161T allele among habitual heavy smokers.

METHODS

Study subjects

Consecutively eligible patients with premature AMI (onset age ≤ 50 years) were enrolled from January 1997 to June 2002 at a teaching hospital in southern Taiwan. Diagnosis of AMI was based on ischaemic chest symptoms, typical electrocardiographic changes and elevation in the serum of conventional cardiac enzymes (creatine kinase and its MB isoenzyme to more than twice the upper level of normal) or cardiac-specific troponin T (more than 2 ng/dl). Controls, who were admitted to the same hospital for routine health examinations, were recruited consecutively and did not show any clinical or electrocardiographic evidence of AMI or CAD (coronary artery disease) and had no history of cerebrovascular or peripheral artery diseases on physical examination and review of medical charts. For patients, demographic data were taken from the medical records at the time of admission for AMI; for controls, data were collected at the time of hospital admission for the routine health examination. Hypertension was defined as elevated blood pressure (> 140/90 mmHg) being measured on three occasions or being treated with antihypertensive agents. Subjects with diabetes mellitus were identified if they had a fasting blood glucose level ≥ 126 mg/dl on at least two separate occasions or were being controlled with either lifestyle modification or hypoglycaemic agents. BMI (body mass index) was calculated as the ratio of weight (kg) to height (m²). Plasma levels of Ab-ox-LDL were measured in 27 healthy male volunteers (age ≥ 20 < 45 years), who smoked at least 20 cigarettes daily for more than 5 years.

Informed consent was obtained from all participants, and this study was approved by our Institutional Research Committee.

PCR and restriction-fragment-length polymorphism

Genomic DNA was extracted from 3 ml of peripheral blood using a Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN, U.S.A.), according to the manufacturer’s instructions. PCR was used to detect the C161T at exon 6 of the PPARγ gene [29]. The sequences of the forward and reverse primers were 5’-CAAGACAACTGCTACAAGC-3’ and 5’-TTCTTTGATCTCCTGAGC-3’ respectively. The amplification was performed in a 25 μl volume containing 100 ng of DNA, 20 pmol of each primer, 2.0 mmol/l MgCl2, 50 mmol/l KCl, 25 μmol/l dNTP, 5 mmol/l Tris/HCl (pH 8.3) and 1 unit of Taq polymerase. PCR was run for 1 min of initial denaturation at 94 °C, followed by 34 cycles, with each cycle consisting of 30 s of denaturation at 94 °C, 30 s of annealing at 56 °C, 1 min of extension at 72 °C and a final extension at 72 °C for 5 min. The reaction yielded a 200 bp DNA fragment. The PPARγ C161T polymorphism was detected by the restriction-fragment-length polymorphism method. PCR products were digested with a PmlI restriction enzyme (recognition site, CACGTG) and separated on a 8 % (w/v) polyacrylamide gel (GeneGel Excel Kit; Amersham Biosciences, San Francisco, CA, U.S.A.) at 15 °C with 400V for 1.5 h. The gels were then visualized by a silver-staining protocol using Hoefer Automated Gel Stainer (Amersham Biosciences). This resulted in two fragments (120 and 80 bp) for the CC genotype and one fragment (200 bp) for the TT genotype, when the restriction site was eliminated by the C161T substitution.
Measurement of the plasma levels of Ab-ox-LDL
Plasma assay for Ab-ox-LDL was performed using an ELISA (oLAB kit; Biomedica, Vienna, Austria), according to the manufacturer’s instructions, which have been described previously [35]. The coefficient of variation of these measurements within and between each test was < 10%.

Statistical analysis
PPARγ genotypes and conventional coronary risk factors were presented as a percentage of patients with the condition, and differences in frequencies were evaluated by the χ² test. Continuous variables were expressed as means ± S.D., and differences between the groups were evaluated by Student’s t test. Multiple logistic regression analyses were performed to adjust for possible confounding effects of the conventional coronary risk factors. Risk factors that appeared to be potential significant predictors (P < 0.1) in single-variate analyses were included in the multiple logistic regression analyses. For comparison of plasma levels of Ab-ox-LDL between different genotypes, unpaired Student’s t test or one-way ANOVA was used as indicated. Statistical significance was defined as P < 0.05. All statistical analyses were performed using SPSS 10.0 for Windows.

RESULTS

Distribution of the PPARγ C161T polymorphism
A total of 146 patients were recruited. Among them, 67 (45.9 %) had anterior wall myocardial infarction. Accordingly, 146 unmatched controls were recruited. All patients and controls were Han Chinese and came from the same geographic area, and the two groups had similar mean ages (P = 0.74) and proportions of men (P = 0.14; Table 1). We have previously tested the frequency of the TT genotype in 250 healthy volunteers from the same geographic area and ethnic backgrounds (results not shown), and the frequency (4.8 %) was very similar to that in our controls. This observation suggests that the genetic background of our control group is representative of the normal subjects of our general population. Table 2 shows the distribution and frequency of the PPARγ C161T polymorphism in the study population. The frequency of TT genotype was higher in the patient group (Table 2).

Table 1  Clinical characteristics of study subjects

<table>
<thead>
<tr>
<th>Value</th>
<th>Patients with AMI (n = 146)</th>
<th>Patients with AMI (n = 146)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45.3 ± 8.2</td>
<td>45.0 ± 3.6</td>
</tr>
<tr>
<td>Men (%)</td>
<td>82.2</td>
<td>88.4</td>
</tr>
<tr>
<td>Systemic hypertension (%)</td>
<td>12.3</td>
<td>35.6 *</td>
</tr>
<tr>
<td>Diabetes mellitus (%)</td>
<td>5.5</td>
<td>17.8 *</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>48.6</td>
<td>75.3 *</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.6 ± 1.0</td>
<td>24.3 ± 1.2</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.10 ± 0.83</td>
<td>5.29 ± 1.10</td>
</tr>
<tr>
<td>Anterior wall AMI (%)</td>
<td>—</td>
<td>45.9</td>
</tr>
</tbody>
</table>

This mutation was not associated with the presence of diabetes mellitus in the patients with AMI or the control group (results not shown).

Table 2  Frequency of the genotypes of the PPARγ gene in control subjects and patients with premature AMI

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls (n = 146)</th>
<th>Patients with AMI (n = 146)</th>
<th>OR (95 % CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>71 (48.6)</td>
<td>63 (43.2)</td>
<td>1.0</td>
</tr>
<tr>
<td>CT</td>
<td>67 (45.9)</td>
<td>64 (43.8)</td>
<td>1.1 (0.7–1.7)</td>
</tr>
<tr>
<td>TT</td>
<td>8 (5.5)</td>
<td>19 (13)</td>
<td>2.7 (1.1–6.5)</td>
</tr>
<tr>
<td>CT + TT</td>
<td>75 (51.4)</td>
<td>83 (56.8)</td>
<td>1.3 (0.8–2.0)</td>
</tr>
</tbody>
</table>

Comparison of conventional coronary risk factors between AMI patients and controls
Conventional coronary risk factors, including hypertension, diabetes mellitus, smoking and serum total cholesterol level, were compared between patient and control groups (Table 1). Significant differences were found in the frequencies of hypertension, diabetes mellitus and smoking between the two groups. However, the differences in BMI and serum total cholesterol level between the two groups were not statistically significant.

Independent predictors for premature AMI
In multivariate analyses, potential risk factors in the single-variate analyses, including hypertension, diabetes mellitus, smoking and the PPARγ TT genotype, were included in the multiple logistic regression model. We observed that all these variables were independent risk factors for premature AMI and that the PPARγ TT genotype had an OR (odds ratio) of 3.1 [95 % CI (confidence interval), 1.2–7.9] after adjusting for the effects of hypertension [OR (95 % CI), 3.6 (1.9–6.9)], diabetes mellitus [OR (95 % CI), 3.5 (1.5–8.4)] and smoking [OR (95 % CI), 3.5 (2.1–6.0)].
The C161T polymorphism of the human PPARγ 161T allele might be a genetic risk factor for premature AMI after adjustment of conventional coronary risk factors. Oxidative stress plays a crucial role in the pathogenesis of endothelial dysfunction in patients with conventional coronary risk factors, including tobacco smoking [40]. Ox-LDL is believed to play a major role in the development of atherosclerosis [41]. Oxidative modification of LDL induces immunogenic epitopes, and the presence of Ox-LDL has been found in human sera [42]. Increased titres of Ox-LDL have been found in patients with AMI [35,43,44], indicating the association with the inflammatory response and the role in the development of plaque instability [35,43]. In addition, tobacco smoking is the major risk factor for AMI at a younger age [38,45]. Therefore our present study evaluated plasma levels of Ox-LDL in smokers and demonstrated that these were significantly higher in subjects with the CT genotype compared with those with the CC genotype, despite no statistical significance (P = 0.06).

**DISCUSSION**

The present study evaluated the association between the PPARγ 161T allele and premature AMI. Our data indicated that the homozygous TT genotype was a risk factor of premature AMI, independent of the conventional coronary risk factors, including hypertension, diabetes mellitus and smoking. Furthermore, plasma levels of Ab-ox-LDL were significantly higher in habitual heavy smokers with the PPARγ 161T allele. Furthermore, according to previous reports, activation of PPARγ by ligands could reduce PAI-1 and fibrinogen levels [18–20] and platelet aggregation [21]. In addition, PPARγ ligands have been shown to reduce expression of MMP-9 (matrix metalloproteinase-9), which is related to plaque destabilization [46,47]. These observations support an effect of PPARγ gene mutations on premature AMI. In addition, a large body of evidence supports the definite role of inflammation on the genesis of AMI [48,49]. PPARγ activators have a substantial anti-inflammatory effect [17,24–26], including inhibition of leptin, a known inflammatory marker [30–32]. Leptin itself could up-regulate both phagocytosis and the production of proinflammatory cytokines [29,31] as well being induced by IL-1β (interleukin-1β) [31]. Furthermore, leptin was associated with an increased risk of AMI [33], and elevation of this serum marker was observed during AMI [34]. Our present study showed that homozygous carriers of the human PPARγ 161T allele might be a genetic risk factor for premature AMI after adjustment of conventional coronary risk factors. Oxidative stress plays a crucial role in the pathogenesis of endothelial dysfunction in patients with conventional coronary risk factors, including tobacco smoking [40]. Ox-LDL is believed to play a major role in the development of atherosclerosis [41]. Oxidative modification of LDL induces immunogenic epitopes, and the presence of Ab-ox-LDL has been found in human sera [42]. Increased titres of Ab-ox-LDL have been found in patients with AMI [35,43,44], indicating the association with the inflammatory response and the role in the development of plaque instability [35,43]. In addition, tobacco smoking is the major risk factor for AMI at a younger age [38,45]. Therefore our present study evaluated plasma levels of Ab-ox-LDL in smokers and demonstrated that these were significantly higher in subjects with the PPARγ 161T allele. Furthermore, according to previous reports, activation of PPARγ by ligands could reduce PAI-1 and fibrinogen levels [18–20] and platelet aggregation [21]. In addition, PPARγ ligands have been shown to reduce expression of MMP-9 (matrix metalloproteinase-9), which is related to plaque destabilization [46,47]. These observations support an effect of PPARγ gene mutations on premature AMI. In addition, a large body of evidence supports the definite role of inflammation on the genesis of AMI [48,49]. PPARγ activators have a substantial anti-inflammatory effect [17,24–26], including inhibition of leptin, a known inflammatory marker [30–32]. Leptin itself could up-regulate both phagocytosis and the production of proinflammatory cytokines [29,31] as well being induced by IL-1β (interleukin-1β) [31]. Furthermore, leptin was associated with an increased risk of AMI [33], and elevation of this serum marker was observed during AMI [34]. The C161T substitution in exon 6 of the PPARγ gene was reported to be associated with increased plasma leptin level [29]. Taken together,
this mutation theoretically and functionally operates on the genesis of AMI.

A number of PPARγ gene variants, including Pro12Ala and Pro115Gln, have been studied, but no significant association with the occurrence of CAD was observed [50]. Furthermore, there are some controversies regarding the association of the 161T allele of the PPARγ gene with CAD [28,50]. In comparison with the previous studies [28,29,50], frequencies of the CT and TT genotypes were higher in our population and, therefore, the present study can generate more reliable risk estimates.

Although there was no clinical or electrocardiographic evidence of CAD in the control group, we cannot exclude the presence of patients with previous unrecognized AMI in the control group. The aim of our present study was to examine this genetic variation in AMI patients of relatively young age, among whom the genetic coronary risk factor may be more prominent. Therefore our present study was limited by small sample size.

In summary, we observed a higher frequency of the PPARγ 161T allele in Chinese population in comparison with Caucasians. Lipid peroxidation was significantly influenced with the 161T allele. We also found a significant association between the homozygous carriers of the PPARγ 161T allele, but not the heterozygous carriers, and the occurrence of premature AMI after adjustment for conventional coronary risk factors. Further longitudinal studies investigating the possible association between the TT genotype of the PPARγ gene and recurrent ischaemia are needed to confirm the possible role of TT genotype as a risk factor of premature AMI observed in our present study.

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