The 981C>T polymorphism in protein tyrosine phosphatase 1B is associated with decreased risk of coronary artery disease in Chinese Han population

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ABSTRACT

Objective: The present study was designed to investigate the potential association between the 981C>T polymorphism in protein tyrosine phosphatase 1B (PTP-1B) and coronary artery disease (CAD).

Methods: We conducted a hospital-based case–control study with 864 CAD patients and 1008 controls to explore the association between the PTP-1B 981C>T polymorphism and risk of CAD in Chinese Han population.

Results: Subjects with the variant genotypes (CT + TT) had a 52% decreased risk of CAD relative to CC carriers (adjusted odds ratio, 0.48; 95% confidence interval, 0.39–0.60). The 981C>T polymorphism was associated with a higher body mass index and serum triglyceride levels in both CAD patients and controls. Moreover, this polymorphism was found to be associated with a lower serum glucose levels in cases, but not in controls.

Conclusion: Our study demonstrates that the PTP-1B 981C>T polymorphism is associated with decreased risk of CAD in Chinese Han population.

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1. Introduction

Coronary artery disease (CAD) is one of the major causes of death in most countries, including China [1]. Many risk factors for CAD have been identified in the past few decades, including smoking, hypertension, diabetes, dyslipidemia and obesity. Most recently, genetic studies have revealed a series of candidate genes susceptibility variants that may also contribute to the pathogenesis of CAD [2–4].

Protein phosphorylation at tyrosine is a key regulatory event that modulates intracellular signalling pathways involved in signal transduction. The ubiquitously expressed protein tyrosine phosphatase 1B (PTP-1B), encoded by the protein tyrosine phosphatase non-receptor type 1 (PTPN1) gene, catalyzes the dephosphorylation of tyrosine residues from the insulin receptor kinase activation segment [5] and insulin receptor substrate 1 [6] resulting in the down-regulation of insulin signalling. PTP-1B also inhibits leptin signalling through the dephosphorylation of JAK2 and STAT3 [7,8]. The disruption of the PTP-1B gene in mice results in increased insulin sensitivity, resistance to diet-induced obesity [9] and enables normalization of blood glucose levels [10]. Moreover, it has been shown that the inactivation of PTPN1 with antisense oligonucleotides regulates the expression of genes involved in lipogenesis, suggesting that PTP-1B may play a role in the enlargement of adipocyte energy storage [11].

The PTP-1B gene is located on human chromosome 20q13. The 10 exons of the gene span more than 74 kb of sequence. The open reading frame includes 1305 bp and codes for a protein of 435 amino acids [12]. Several single nucleotide polymorphisms (SNPs) in the PTP-1B gene have been shown to be associated with insulin resistance, diabetes, obesity and dyslipidemia in different populations. The PTP-1B IVS6 + G82A polymorphism was associated with body mass index (BMI), albuminuria, and hypertension in type 2 diabetic patients. The 981T/T genotype of the Pro303Pro might have some protective role against the development of type 2 diabetes [13]. A rare Pro387Leu variant was associated with type 2 diabetes in a Danish population [14], but not in a German population [15]. The frequent IVS5 + 3666delT SNP was associated with obesity in a French Caucasian subjects and in the obese group, P303P was associated with lower apolipoprotein A1 levels, whereas P387L was associated with higher triglyceride and apolipoprotein B [16]. The PTP-1B SNPs have also been demonstrated to be associated with essential hypertension in Chinese population [17,18]. One of the most frequently studied polymorphisms, 981C>T, was reported to be associated with protection from type 2 diabetes and impaired glucose tolerance in a Canadian population [19]. Until now, there have not been any studies that have evaluated the association of the 981C>T polymorphism with CAD risk. Therefore, the present...
case–control study was designed to explore the potential association between the PTP-1B 981C>T polymorphism and risk of CAD in Chinese Han population.

2. Methods

2.1. Study subjects

The study enrolled 864 consecutive patients with CAD admitted to the First Affiliated Hospital of Nanjing Medical University. The diagnosis of CAD was confirmed by coronary angiography performed with the Judkins technique using a quantitative coronary angiographic system [20]. CAD was defined as angiographic evidence of at least one segment of a major coronary artery including the left anterior descending, left circumflex or right coronary artery with more than 50% organic stenosis. Patients with CAD were divided into 1-, 2-, and 3-vessel disease subgroups according to the number of significantly stenosed vessels with reference to the Coronary Artery Surgery Study classification. Two cardiologists who were responsible for the assessment of angiograms both underwent strict training and complied with the same diagnostic criteria. This study also included 1008 unrelated control subjects randomly selected from outpatients (staff of local companies and administration agencies) who underwent regular physical examinations during the same time in the same hospital. Those control subjects with a history of angina, symptoms or signs of other atherosclerotic vascular diseases and an abnormal electrocardiogram were excluded. All subjects enrolled in this study were of Han Chinese origin and residing in or near Jiangsu Province. They had no history of significant concomitant diseases including cardiomyopathy, bleeding disorders, renal failure, previous thoracic irradiation therapy and malignant diseases. Blood pressure was measured in the right arm with the participant seated and the arm bare using an automatic sphygmomanometer (Omron HEM–7200). Two readings were recorded for each subject and the average was recorded. Fasting blood samples were collected and blood glucose and lipid profile were measured on a Roche Hitachi 902 auto-analysers (Roche Diagnostics Co., Indianapolis, IN) using standard Roche enzymatic kits (Roche Diagnostics, Basel, Switzerland). Hypertension was defined as a systolic pressure ≥140 mmHg or a diastolic pressure ≥90 mmHg or receiving antihypertensive treatment. Diabetes was defined as fasting blood glucose ≥7.0 mmol/L or use of antidiabetic drug therapy. Dyslipidemia was defined as serum total cholesterol (TC) concentration ≥5.72 mmol/L or triglyceride (TG) concentration ≥1.70 mmol/L or use of lipid-lowering therapy. Individuals who formerly or currently smoked ≥10 cigarettes per day for at least 2 years were defined as smokers. BMI was calculated as weight (kilograms) divided by height (meters) squared. This study was approved by the institutional ethics committees of the First Affiliated Hospital of Nanjing Medical University and informed consent was obtained from each participant.

2.2. DNA extraction and genotyping

Peripheral venous blood was drawn from each participant. Genomic DNA was extracted using the AxyPrep DNA Blood Kit (Axygen Scientific Inc., Union City, CA, USA). The 981C>T polymorphism was genotyped by the PCR-LDR sequencing method, as reported previously [21,22]. A 315 bp DNA fragment containing the polymorphic site was amplified by PCR in the ABI 9600 (Applied Biosystems, Foster City, CA) using the forward primer 5’-GACACACCGCAATGTTAAC-3’ and the reverse primer 5’-CTTCAACCCCAACAAGGT-3’. The PCR was carried out in a total volume of 15 μL containing 1.5 μL 10× PCR buffer, 0.2 μL 10 pmol each primer, 0.3 μL dNTP, 0.25 μL Taq polymerase (MBI Fermentas), 2 μL of genomic DNA and 10.75 μL H₂O. The PCR cycling parameters were 35 cycles of 94 °C at 30 s and 72 °C for 45 s. Ligase detection reaction (LDR) was performed in a total volume of 10 μL containing 2 μL PCR product, 1 μL 10× Taq DNA ligase buffer, 0.125 μL 40 U/μL Taq DNA ligase (NEB), 1 μL 10 pmol probes (0.33 μL each of probe), and 5.875 μL H₂O. LDR probes were composed of 1 common probe and 2 discriminant probes (designed by the Shanghai Generay Biotech Co., Ltd.). Subsequently, LDR products were analyzed by DNA sequencing (Model 377, Applied Biosystems). All assays were conducted blindly without the knowledge of case or control status. Additionally, about 10% of the samples were randomly selected and retested by direct DNA sequencing on a 3730xl DNA analyzer (Applied Biosystems) and the results were 100% concordant.

2.3. Statistical analysis

Statistical analyses were conducted with the SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Normality was tested using the Kolmogorov–Smirnov test. Differences of continuous variables without skewness between two groups were calculated by the Student’s t-test. Differences of continuous variables departing from the normal distribution even after transformation between two groups were analyzed by Mann–Whitney U-test. Pearson χ²-test was used to compare allele distribution and qualitative variables represented as frequencies. Odds ratio (OR) and 95% confidence interval (CI) were calculated to estimate the correlation between the 981C>T polymorphism and CAD risk. Two-tailed P<0.05 were considered as statistical significance.

3. Results

The characteristics of our study subjects are presented in Table 1. Patients with CAD were much older, smoked more cigarettes, had significantly higher BMI, TC, TG and LDL-C, and were more likely to be diabetic, hypertensive and dyslipidemic than the control subjects. In terms of coronary angiographic findings, 330 (38.2%) CAD cases had single- vessel disease, 281 (32.5%) had double- vessel disease and 253 (29.3%) had triple-vessel disease.

The genotype and allele distribution of 981C>T polymorphism are presented in Table 2. We used a dominant genetic model for analysis in the present study. The allele frequencies of SNP 981C>T in CAD group and control group were 80.4%/19.6% and 70.4%/29.6%, respectively. Table 2 also shows the risk estimates for the variant
Table 2
Genotype and allele distribution of the 981C>T polymorphism in cases and controls.

<table>
<thead>
<tr>
<th>Genotype, n (%)</th>
<th>Cases (n = 864)</th>
<th>Controls (n = 1008)</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted* OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>592 (68.5)</td>
<td>539 (53.5)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>CT</td>
<td>205 (23.7)</td>
<td>341 (33.8)</td>
<td>0.55 (0.44–0.67)</td>
<td>0.52 (0.41–0.65)</td>
</tr>
<tr>
<td>TT</td>
<td>67 (7.8)</td>
<td>128 (12.7)</td>
<td>0.48 (0.35–0.66)</td>
<td>0.43 (0.30–0.62)</td>
</tr>
<tr>
<td>CT + TT</td>
<td>272 (31.5)</td>
<td>469 (46.5)</td>
<td>0.53 (0.44–0.64)</td>
<td>0.48 (0.39–0.60)</td>
</tr>
</tbody>
</table>

Distributions of the 981C>T genotype in control group was in Hardy–Weinberg equilibrium (P = 0.648). CI, confidence interval; OR, odds ratio.

* Adjusted for age, sex, body mass index, hypertension, diabetes, dyslipidemia and smoking.

Table 3
BML, lipid profiles and glucose of cases and controls in the PTP-1B 981C>T genotypes.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases</th>
<th>Controls</th>
<th>P</th>
<th>Cases</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m2)</td>
<td>24.42 ± 2.26</td>
<td>25.98 ± 2.68</td>
<td>0.023</td>
<td>23.08 ± 2.05</td>
<td>24.25 ± 2.62</td>
<td>0.038</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.21 ± 1.20</td>
<td>4.03 ± 1.14</td>
<td>NS</td>
<td>3.89 ± 0.93</td>
<td>4.05 ± 1.26</td>
<td>NS</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.58 ± 0.85</td>
<td>1.92 ± 0.95</td>
<td>0.035</td>
<td>1.20 ± 0.65</td>
<td>1.78 ± 0.92</td>
<td>0.026</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.13 ± 0.45</td>
<td>1.06 ± 0.39</td>
<td>NS</td>
<td>1.28 ± 0.31</td>
<td>1.40 ± 0.45</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.63 ± 0.90</td>
<td>2.50 ± 0.82</td>
<td>NS</td>
<td>2.34 ± 0.82</td>
<td>2.21 ± 0.73</td>
<td>NS</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>6.34 ± 1.84</td>
<td>5.37 ± 1.65</td>
<td>0.016</td>
<td>5.15 ± 1.24</td>
<td>4.96 ± 0.96</td>
<td>NS</td>
</tr>
</tbody>
</table>

TC, TG, HDL-C, LDL-C and glucose (expressed as mean ± SD) were abnormal distributed and analyzed by Mann–Whitney U-test. BMI (expressed as mean ± SD) was normal distributed and analyzed by Student’s t-test. BMI, body mass index; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.

981C>T genotypes among CAD patients compared with controls. Overall, after being adjusted for the risk factors including age, sex, BMI, hypertension, diabetes, dyslipidemia and smoking, the OR for subjects with the variant genotypes (CT and TT) was 0.48 (95% CI = 0.39–0.60). We also found that TT genotype carriers had a 57% reduction in risk of CAD compared with the CC carriers (adjusted OR = 0.43, 95% CI = 0.30–0.62).

Table 3 shows BMI, lipid profiles and glucose levels according to the 981C>T genotypes in the CAD patients and controls. The 981C>T polymorphism was found to be associated with a higher BMI and serum triglyceride levels in both CAD patients and controls. Moreover, the association between the 981C>T polymorphism and serum glucose levels was significant in the CAD patients, but not in controls.

4. Discussion

The PTP-1B gene is responsible for negatively regulated insulin signalling by dephosphorylating the phosphotyrosine residues of the insulin receptor kinase activation segment [23]. Disruption of the murine homolog of PTP-1B was associated with increased insulin sensitivity, resistance to diet-induced obesity [9] and enables normalization of blood glucose levels [10], which suggested that PTP-1B plays a crucial role in the modulation of insulin sensitivity and energy metabolism. In previous studies, several polymorphisms in the PTP-1B gene have been identified to be associated with insulin resistance, diabetes, obesity, dyslipidemia and essential hypertension [13–18]. However, there have not been any studies that have evaluated the association between the 981C>T polymorphism and risk of CAD. Therefore, we conducted a hospital-based case–control study with 864 cases and 1008 controls to explore the association between the PTP-1B 981C>T polymorphism and CAD risk in Chinese Han population.

The study by Mok et al. [19] reported that subjects with the PTP-1B 981T/981C genotype were approximately 40% less likely to have impaired glucose tolerance or type 2 diabetes as subjects with the 981C/981C genotype. In our study, a 52% decreased risk of CAD was observed in subjects with the PTP-1B 981C/T/T genotypes compared with the CC carriers, indicating that the T allele might confer a protective effect against CAD.

In the present study, we also evaluated the association of the 981C>T polymorphism with BMI, lipid profiles and glucose levels in CAD patients and controls. The 981C>T polymorphism was found to be associated with a higher BMI and serum triglyceride levels in both CAD patients and controls. Moreover, this polymorphism was associated with a lower serum glucose levels in CAD patients, which might be one of the potential mechanisms for the reduced risk of CAD.

Our study had several limitations. Firstly, although we selected controls from individuals with no history of angina, no symptoms or signs of other atherosclerotic vascular diseases and normal electrocardiogram, without performing coronary angiography, we could not rule out CAD completely. Nevertheless, the prevalence of CAD in an asymptomatic population appeared to be low [24]. Secondly, our data were obtained at the time of diagnosis, thus prospectively followed-up clinical outcome including severe cardiac events may be required to analyze the association between the 981C>T polymorphism and the CAD prognosis. Thirdly, our study was performed in Chinese Han population, and the data should be extrapolated to other regions and ethnic groups cautiously. Lastly, the positive findings in this study could not exclude the possibility of linkage between the 981C>T polymorphism and other unmeasured variants in the PTP-1B gene.

In conclusion, our study demonstrates for the first time that the PTP-1B 981C>T polymorphism is associated with decreased risk of CAD in Chinese Han population. Further investigations are required to explore the underlying mechanisms of our findings.

Acknowledgment

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2011.05.021.

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