Original Article

Relationship between SR-BI genetic polymorphism and coronary heart disease and blood lipid level

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Abstract: To discuss the influence of single nucleotide polymorphism of scavenger receptor class B type I (SR-BI) exon 1 and intron 5 on blood lipid level and susceptibility to coronary heart disease (CHD) of Chinese Han population in Tianjin. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to determine the AluI digestion genotype of SR-BI exon 1 and ApaI digestion genotype of SR-BI intron 5. Their relationship with blood lipid level, CHD and coronary arteriography result was analyzed. Only CC genotype was found in intron 5 of the subjects in two groups. The frequency of alleles G and A of SR-BI exon 1 in the CHD group was 0.988 and 0.012, respectively, and that in the control group was 0.997 and 0.003, respectively. The genotype distribution accorded with the Hardy-Weinberg equilibrium. Regarding to intergroup comparison, there was no significant difference (P>0.05) in genotype frequency and allele frequency of exon 1 AluI digestion polymorphism. In male patients of CHD group, the serum high density lipoprotein cholesterol (HDL-C) and the apolipoprotein AI (ApoAI) level of variation group (subgroup of GA+AA genotype) of exon 1 polymorphism were higher compared to non-variation group (subgroup of GG genotype). The SR-BI exon 1 polymorphism may be related to the susceptibility to CHD and the severity of CHD among Chinese Han population in Tianjin. The alleleA of SR-BI exon 1 of male CHD patients can lead to the increased serum HDL-C and apo AI level.

Keywords: Scavenger receptor class B type I (SR-BI), gene polymorphism, coronary heart disease (CHD), high density lipoprotein cholesterol (HDL-C), apolipoprotein AI (Apo AI)

Introduction

Scavenger receptor class B type I (SR-BI) is a high density lipoprotein receptor that can selectively mediate cholesteryl ester absorption [1, 2]. SR-BI has been verified as the only molecule that mediates the selective absorption of cholesteryl ester by liver and adrenal gland, and the only target that adjusts reverse cholesterol transport [3-5]. Studies both at home and abroad have verified that SR-BI single nucleotide polymorphism is related to blood lipid metabolism [6], postprandial lipid reaction [7], diabetes [8] and insulin resistance [9], etc. After summarizing these studies, we found that the G4A polymorphic site of exon 1 not only influenced the blood lipid of healthy individuals in certain races, but also participated in blood lipid metabolism disorder of diabetics [10]. However, there is no in-depth study yet on the relationship between SR-BI gene polymorphism and coronary heart disease (CHD). Therefore, this study detected the SR-BI exon 1 AluI digestion polymorphism and SR-BI intron 5 ApaI digestion polymorphism of CHD patients and normal population in Tianjin, compared the distributional difference, and analyzed the relationship between SR-BI gene polymorphism and CHD & blood lipid level.

Subjects and methods

Subjects

Five hundred and eighty-eight Chinese Han cases from Tianjin, who were hospitalized in Department of Cardiology of our hospital from October 2010 to October 2011, were selected. They were divided into the following two groups based on coronary angiography (CAG) results: (1) CHD group. There were a total of 370 cases, including 218 males and 152 females, with an average age of 60.55 ± 8.42 years. The blood vessel diameter stenosis ≥50% for at least one
of the left anterior descending artery, left circumflex artery and right coronary artery was confirmed by CAG; (2) Control group. There were a total of 143 cases, including 51 males and 92 females, with an average age of 55.80 ± 7.72 years. Those having CHD were excluded by CAG. All the above patients displayed no severe hepatic or renal insufficiency, non-ischemic cardiomyopathy, valvular heart disease, acute cerebrovascular diseases (cerebral infarction, cerebral hemorrhage), acute infection, trauma or operation in the last 2 weeks, malignant tumor, chronic connective tissue disease, immune disease or blood disease. Clinical data of all the subjects, such as height, weight and past medical history, were collected.

Genotype analysis

5 mL femoral artery blood of the subjects was collected before CAG. Sodium citrate was used for anticoagulation. The genome DNA was extracted by improved saturated phenol-chloroform method. The gene polymorphism of SR-BI exon 1 and intron 5 was detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Specific amplification was conducted for the fragments containing exon 1 and intron 5. The forward primer and the reverse primer of exon 1 were 5'-CCGGCGATGGGGCATAAAACCACT-3' and 5'-CGCCCAGCACAGCGCACAGTAGC-3', respectively. The forward primer and the reverse primer of intron 5 were 5'-GCCCAGACACGCGCACAGTAGC-3', respectively. The sequence was synthesized by Invitrogen Shanghai Trading Co. Ltd. The total volume of PCR reaction was 25 μL, including 12.5 μL 2×Taq PCR Master Mix, 1.5 μL forward primer, 1.5 μL reverse primer, 3.0 μL DNA template and 6.5 μL double distilled water. The PCR reaction condition: 94°C for 5 min, 94°C for 30 S, 65°C for 30 S and 72°C for 1 min, a total of 30 cycles. The elongation at 72°C for 5 min was conducted finally. After the completion of PCR amplified reaction, 5 μL amplification products were taken. The PCR amplification product was identified by 1% agarose gel electrophoresis. The restriction enzyme adopted for exon 1 was AluI (New England Biolabs (Beijing LTD.). The overall reaction system also included 10 μL PCR amplification products, 42 μL of 10×NE Buffer and 0.2 μL of bovine serum albumin (BSA). Then they were incubated in a water-bath overnight at 25°C (4-16 h). The genotype of the enzyme-digested product after reaction was identified by native polyacrylamide gel electrophoresis.

Blood lipid determination

4 mL fasting venous blood of the subjects was drawn in the morning. The enzymatic method was used to detect total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDLC) and high density lipoprotein cholesterol (HDLC). MEGA fully automatic biochemical analyzer from Merck Company, Germany and kit from Beijing Zhongsheng Biotech Company were used to determine apoprotein AI (Apo AI), ApoB and lipoprotein (a) [Lp(a)].

Statistical analysis

Measurement data conformed to normal distribution, which were presented as x̄ ± s. The t-test was adopted for group comparison. The data which did not meet normal distribution were expressed as median (M) and inter-quartile range (QR). Mann-Whitney U test was adopted for comparison of two groups. Enumeration data were presented as percentage (%). x² test or Fisher’s exact test was adopted for group comparison. The representativeness of the sample was detected by Hardy-Weinberg equilibrium. The gene counting method was used to calculate the genotype and the allele frequency of the CHD group and the control group. SPSS 16.0 software was used to carry out statistical analysis. The two-sided critical value was taken, and P<0.05 indicated significant difference.

Results

Comparison of SR-BI exon 1 and intron 5 gene polymorphism of the two groups

The PCR amplification product of intron 5 of the two groups had a fragment length of 291 bp and contained Apal digestion fragment. The amplification product of CC genotype can be cut off completely. Three types of fragments,
The CHD group had significantly more male patients and patients with history of hypertension, diabetes and smoking than the control group (P<0.01). The fasting serum glucose (FSG) and the Lp(a) level of the CHD group were significantly higher than those of the control group (P<0.01, P<0.05; Table 2).

Comparison of blood lipid and coronary artery Gensini score for different SR-BI exon 1 genotypes in CHD group and their gender difference

In the CHD group, the subgroup of GA+AA genotype had significantly higher HDLC and ApoAI levels than the subgroup of GG genotype (P<0.05). There was no significant difference (P>0.05) in the comparison of coronary artery score between the two groups (P>0.05) (Table 3). The difference in the blood lipid level between different genotypes was compared respectively for males and females in the CHD group. The results indicated that the male patients with GA+AA genotype had significantly higher HDLC and ApoAI levels than those with GG genotype (P<0.05; Table 4).

Discussion

Belonging to the receptor superfamily, the scavenger receptors have wide ligand recognition spectrum. They mainly recognize polyan-
ions, including high-density lipoprotein, chemically modified acetylate low-density lipoprotein and oxidized low-density lipoprotein, etc. Human SR-BI is a member of scavenger family. Its gene segment was firstly found by Calvo and Vega in 1993, which was called CLA-1 (CD36 and LIMPII analogous 1) due to its similarity to CD36 receptor and LIMPII sequence [11]. Located at human gene 12q24, including 13 exons and 12 introns, it had a gene length of about 75 kb and can encode a protein containing 509 amino acids [12, 13].

SR-BI participates in the reverse cholesterol transport process [1]. After specific binding of cholesterol in HDL and SR-BI on its surface, they are selectively taken into liver, adrenal gland and ovarian tissue cells. The cholesteryl ester uptake by cells is used to synthesize bile acid and steroid hormone, etc. The HDL granule losing cholesteryl ester separates with SR-BI and returns to the cycle. A study has indicated that integrating the adenovirus which encodes SR-BI gene with the homozygous mouse which has LDL receptor defect, and inducing the overexpression of SR-BI can reduce the area of atherosclerotic plaque and prevent the formation of atherosclerosis [14]. Compared with wild-type control rats, the TC level of SR-BI knockout rats increases by 2.2 times, mainly contributed to the cholesterol in HDL granules. These HDL granules are larger and more strangely-shaped than those in wild-type rats. Although the plasma HDLC level is increased in SR-BI knockout group, there is no difference in the plasma ApoA I level between the two groups, indicating that the increased HDLC is attributed to the damaged selective uptake of cholesterol from HDL [15].

The gene polymorphism of SR-BI exon 1 forms 3 genotypes. Wild-type homozygote GG has the highest frequency, and mutant type heterozygote GA is secondary, followed by mutant type homozygote AA. The allele G is a common allele, followed by the allele A. The SR-BI exon 1 polymorphism has race difference. This study indicated that the A allele frequency of SR-BI exon 1 in Chinese Han population from Tianjin was 0.012 and 0.003, respectively in the CHD group and the control group, and that the intron 5 only had 1 genotype-CC. The mutation rate of SR-BI exon 1 G4A in Chinese Han population from Hubei Province, China [10], Caucasian from southern Europe [4] and non-diabetes controls in Framingham’s study [8] was 0.108, 0.117 and 0.116, respectively. Hong et al. [16] conducted a gene polymorphism study on 137 CHD patients and 124 age-matched controls from Korea, and found that no subjects had an Apal or AluI mutation site.

There are controversies about the relationship between SR-BI exon 1 polymorphism and CHD & diabetes [17-20]. Osgood et al. [8] found that the gene mutation of exon 1 was significantly correlated with type 2 diabetes. The study of Liao Liya et al. [10] indicated that there was no significant difference between diabetes group and control group from Hubei, China in allele A at the G4A polymorphic site of SR-BI exon 1. Yamada et al. [21] screened the myocardial infarction candidate genes of a large population in Japan, and found that the SR-BI G4A polymorphic site was unrelated to myocardial infarction. The results of this study showed that there was no significant difference in genotype distribution frequency of SR-BI exon 1 digested by AluI between the CHD group and the control group. There was no significant difference in the coronary artery score between GA+AA genotype subgroup and GG genotype subgroup of the CHD group. Therefore, we can conjecture that SR-BI exon 1 polymorphism may be unrelated to CHD and coronary artery disease.

However, the relationship between the SR-BI exon 1 polymorphism and the blood lipid level has been verified by lots of studies both at home and abroad [22, 23]. Acton et al. [4] found

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>GG Genotype (n=1080)</th>
<th>GA+AA Genotype (n=30)</th>
</tr>
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<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>25.50 ± 3.28</td>
<td>25.90 ± 3.41</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.98 ± 1.15</td>
<td>4.99 ± 1.16</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.49 ± 0.87</td>
<td>1.69 ± 1.06</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.99 ± 0.93</td>
<td>2.92 ± 0.87</td>
</tr>
<tr>
<td>HDLC (mmol/L)</td>
<td>1.16 ± 0.31</td>
<td>1.48 ± 0.44*</td>
</tr>
<tr>
<td>ApoAl (g/L)</td>
<td>1.12 ± 0.20</td>
<td>1.28 ± 0.27*</td>
</tr>
<tr>
<td>ApoB (g/L)</td>
<td>1.11 ± 0.28</td>
<td>1.25 ± 0.49</td>
</tr>
<tr>
<td>Lp (a) (g/L)</td>
<td>0.27 ± 0.40</td>
<td>0.25 ± 0.41</td>
</tr>
<tr>
<td>Gensini integral</td>
<td>33.50 ± 53.50</td>
<td>47.00 ± 31.00</td>
</tr>
</tbody>
</table>

a: P<0.05 Compared with the GG genotype subgroups.
SR-BI genetic polymorphism and CHD

that the SR-BI G4A mutation of southern European Caucasians was related to increased HDLC and decreased LDLC of males. A later study by Osgood [8] found that the allele A at the SR-BI G4A site was related to low LDLC, low HDLC and decreased diameter of HDL granule. A study which targeted at Hubei, China detected the genotypes of SR-BI exon 1 with G4A variation among 150 cases with type 2 diabetes and 120 cases with normal glucose tolerance. It was found that compared with GG genotype, the cases with allele A in the two groups had low LDLC concentration, and that patients with allele A in the diabetes group had low HDLC concentration [10]. West et al. [24] found that the SR-BI protein level of the population having hyperalphalipoproteinemia was negatively correlated with the HDLC level and the size of HDL granule, positively correlated with the HDLC cholesteryl ester uptake, and unrelated to the lipoprotein level. They also found that individuals with mutant allele A at the 2nd site of exon 1 had a lower HDL granule level compared with those with wild-type allele G. The present study indicated that in the CHD group, patients with GA+AA genotype had a higher HDLC level than those with GG genotype. This also showed that the G4A mutant allele A of SR-BI exon 1 may reduce the SR-BI protein expression, leading to decreased elimination of cholesteryl ester of HDLC and the increased plasma HDLC level. HDLC is considered as a lipoprotein with the property of anti-atherosclerosis [25]. The low HDL level is an independent predictor for the severity of coronary artery disease [26]. A prospective study on a Chinese cohort aged 35-64 years [27] showed that compared with the reference group with HDLC≥1.56 mmol/L, the incidence risk of the ischemic cardiovascular disease increased with the decreased HDLC level. After using lipid-lowering drugs of statins, every 1 mmol/L drop of LDLC can reduce 23% of main coronary artery events, but 77% residual risk of cardiovascular disease remained unsolved [28]. A study [29] showed that the same as LDLC, HDLC can also be oxidized and modified by multiple factors in vitro and vivo and formed oxidized HDLC, resulting in the lost role of anti-atherosclerosis and even reversal. Therefore, the actual protective effect of HDL not only depends on the “quantity” (HDLC level), but also depends on the “quality” (effect of anti-atherosclerosis) [30]. Although there was no significant difference in the frequency of allele A between the CHD group and the control group, we speculate that the SR-BI exon 1 G4A polymorphism may be related to CHD with normal or high HDL level. Moreover, ApoAI is the main apolipoprotein of HDL. The overexpression of ApoAI can increase the level of HDLC and inhibit the progression of atherosclerosis, but its role of anti-atherosclerosis does not depend on the level of HDLC. A study [31] points out that ApoAI significantly decreases in cerebral infarction patients, which is unrelated to the HDLC level.

The study of Acton [4] showed that SR-BI exon 1 gene mutation was related to increase HDLC and decreased LDLC of males, but this phenomenon was not found in the female population. In the present study, there was a difference between males and females in the blood lipid effect after the mutation of SR-BI exon 1. Males in GA+AA genotype subgroup of SR-BI exon 1 had significantly higher HDLC and ApoAI levels than males in GG genotype subgroup, consistent with the research results of Acton et al. The difference in gender can hardly be explained by genetics, which may be explained by the fact that the estrogen has regulatory effect on SR-BI gene expression [32], or the regulating effect of SR-BI on steroidogenesis and hormone, or a accidental phenomenon caused by different gender ratios due to the sample size.
SR-BI genetic polymorphism and CHD

This study indicated that the point mutation at the 4th site of SR-BI exon can reduce the level of SR-BI protein through influencing the expression of SR-BI protein, and weaken the reverse cholesterol transport in HDLC, leading to the increase of plasma HDLC concentration and the decrease of selective HDL cholesterol clearance. However, the SR-BI exon 1 G→A polymorphism had no influence on the susceptibility to CHD among Chinese Han population from Tianjin. This conclusion remains to be validated by a case-control study with a larger sample size.

Disclosure of conflict of interest

None.

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