A Novel Missense Mutation (L\textsuperscript{296}Q) in Cholesteryl Ester Transfer Protein Gene Related to Coronary Heart Disease

Ke-Qin ZHENG, Si-Zhong ZHANG*, Li ZHANG\textsuperscript{1}, De-Jia HUANG\textsuperscript{1}, Lin-Chuan LIAO\textsuperscript{2}, and Yi-Ping HOU\textsuperscript{2}

(\textsuperscript{1}Department of Medical Genetics, West China Hospital; Division of Human Morbid Genomics, Key Laboratory of Biotherapy of Human Diseases of Ministry of Education, Sichuan University, Chengdu 610041, China; \textsuperscript{2}School of Basic and Forensic Medicine, Sichuan University, Chengdu 610041, China)

Abstract  Cholesteryl ester transfer protein (CETP) is a key participant in the reverse transport of cholesterol from the peripheral tissues to the liver. To understand the role that CETP gene plays in the pathogenesis of coronary heart disease (CHD), the promoter region, all 16 exons and adjacent intronic regions of CETP gene were screened for single nucleotide polymorphisms (SNPs) in 203 CHD patients and 209 controls by a combination of PCR, denaturing high performance liquid chromatography (DHPLC), molecular cloning, and DNA sequencing. A novel missense mutation in the CETP gene was identified. This mutation (L\textsuperscript{296}Q) was a T-to-A conversion at codon 296 of exon 10 which replaced the codon for leucine (CTG) with the codon for glutamine (CAG). Association study revealed that L\textsuperscript{296}Q mutation was associated with CHD with a significantly higher mutant allele frequency in the CHD patients than that in the controls (0.160 vs. 0.091, \(\chi^2 = 9.014, P = 0.003\), and that the odds ratio for the development of CHD was 1.83 for the \(296Q\) allele carriers relative to \(296LL\) homozygotes. Statistical analyses demonstrated that the mutant \(296Q\) allele carrier patients displayed significantly higher total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) concentrations than non-carrier patients. The results of the present study suggest that the \(L296Q\) mutation is related to CHD, and the identification of new mutations in the CETP gene will afford the opportunity to investigate the relationship between CETP gene and CHD.

Key words  cholesteryl ester transfer protein gene (CETP gene); coronary heart disease (CHD); missense mutation; plasma lipids; single nucleotide polymorphism (SNP)

Cholesteryl ester transfer protein (CETP) is responsible for the net transfer and heteroexchange of triglycerides (TG) and cholesteryl ester (CE) among the high density lipoprotein (HDL), the low (LDL), and very low density lipoproteins (VLDL) fractions [1]. Plasma CETP is a highly hydrophobic glycoprotein containing 476 amino acids and 4 N-linked glycosylation sites [2]. The human CETP gene has 16 exons encompassing 25 kb genomic DNA and is located on chromosome 16q21[3]. In human, CETP mRNA is highly expressed in the liver as well as in spleen and adipose tissue, with lower levels expressed in the small intestine, adrenal, kidney and heart [2,4].

Several mutations in the CETP gene have been identified in the Japanese and Caucasian populations [5–8]. The CETP D\textsuperscript{442}G mutation that replaces an aspartic acid (D) with a glycine (G) in the exon 15 was first reported in 1993 and was observed to be associated with elevated high density lipoprotein cholesterol (HDL-C) concentration [7]. Japanese subjects with homozygous G\textsuperscript{442} genotypes showed a corneal opacity and coronary heart disease (CHD) despite the increased HDL-C level. Other mutations of CETP gene were also detected in different ethnic groups and were noticed to be related to the variation of the plasma lipid and lipoprotein values as well as CHD status [5,6,8,9].
In present study, we reported a novel missense mutation (T→A conversion) at the nucleotide 13,161 (relative to the transcription start site) in exon 10 of the CETP gene, as the result of screening for single nucleotide polymorphisms (SNPs) in 203 CHD patients and 209 controls, and the association of this mutation with biochemical and clinical manifestations of CHD.

Materials and Methods

Study subjects

203 CHD patients were selected from the West China Hospital, Sichuan University. The patients had at least one coronary artery with a stenosis of more than 60 percent as documented by angiography. In addition, 209 unrelated age- and gender-matched subjects, with no clinical or biochemical signs of CHD, recruited at the same hospital via routine health examination, were used as controls in the study.

Plasma lipid and lipoprotein assay

Venous blood was collected from all subjects after an overnight fast. Plasma was separated from the blood cell by centrifugation and used immediately for lipid and lipoprotein analysis. The levels of plasma total cholesterol (TC), HDL-C, LDL-C, VLDL-C and triglyceride (TG) were determined by enzymatic kits (Boehringer-Mannheim) according to the manufacturer’s instruction.

DNA isolation and PCR amplification

Genomic DNA was extracted from leucocytes by the ‘salting-out’ method [10]. Fragments containing the 5' flanking region and individual exon of the CETP gene, including all intron-exon boundaries were amplified by PCR. Oligonucleotide primers for amplification were synthesized according to published sequence data (GenBank accession No. AC010550). A sequence of 5501 bp was amplified.

Denaturing high performance liquid chromatography (DHPLC) screening

DHPLC screening for single nucleotide polymorphisms was performed on an automated HPLC instrument (HP1100, Hewlett Packard). The heteroduplex molecules are generally eluted ahead of the homoduplex molecules, therefore the additional following peaks or shoulders during DHPLC was interpreted as an indication of a single base mismatch in heteroduplex DNA fragments.

DNA sequencing

Both the location and chemical nature of the mismatch were determined by sequencing the reamplified product. Then the heterozygous and homozygous DNA samples were cloned into the pMD18-T vector (TaKaRa), then sequenced in both directions on the ABI prism 377 DNA sequencer using the BigDye terminators cycle sequencing kit. The sequencing was done commercially in Sangon Company, Shanghai.

Statistical analysis

All the statistical analyses were carried out by using SPSS10.0 software. The data were presented as $\bar{x} \pm s$ or as percentages. Differences in lipid and lipoprotein values of the various genotypes were evaluated with student t test, and differences in frequencies of alleles and genotypes of the mutation between the two groups were detected by $\chi^2$ test. The odds ratios (OR) for CHD were derived from the logistic regression analysis.

Results

Subject characteristics

The biochemical features of the subjects are provided in Table 1. Compared with the matched control group, the CHD group had significantly larger body mass index (BMI), significantly higher plasma TG and LDL-C levels. On the contrary, the level of HDL-C was significantly lower in patients than in controls.

Table 1  Characteristics of CHD patients and controls

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>CHD patients</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>203</td>
<td>209</td>
<td>–</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>137/66</td>
<td>140/69</td>
<td>–</td>
</tr>
<tr>
<td>Age (y)</td>
<td>55.4± 6.5</td>
<td>54.8± 8.7</td>
<td>0.441</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.9± 23.24</td>
<td>23.89± 2.73</td>
<td>0.000**</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.51± 0.85</td>
<td>1.26± 0.79</td>
<td>0.001**</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>5.01± 1.01</td>
<td>4.90± 0.99</td>
<td>0.601</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.17± 0.42</td>
<td>1.31± 0.49</td>
<td>0.017*</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.36± 0.76</td>
<td>2.19± 0.75</td>
<td>0.041*</td>
</tr>
<tr>
<td>VLDL-C (mmol/L)</td>
<td>0.89± 0.48</td>
<td>0.94± 0.42</td>
<td>0.534</td>
</tr>
<tr>
<td>TC / HDL-C</td>
<td>4.12± 1.25</td>
<td>4.01± 1.12</td>
<td>0.651</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.1.

Identification of DNA sequence change

Screening of the fragments containing promoter region and all 16 exons of the CETP gene with DHPLC detected a putative sequence variation showing double peaks in the chromatogram. Sequencing analysis revealed a missense mutation (T→A conversion) at nucleotide 13,161 in exon 10 of the CETP gene that results in amino acid substitution of a polar glutamine (codon CAG) for a nonpolar leucine (codon CTG) at residue 296 (Fig. 1).

Effect of the mutation on risk of CHD and plasma lipid levels

Table 2 presents the frequencies of alleles and genotypes in CHD patient group and control group. The patients displayed significantly higher mutated allele frequency and mutation genotype frequency than controls. The odds
ratio of the development of CHD was 1.83 for the 296Q allele carriers versus 296LL homozygotes with the 95% confidence interval 1.04–2.87. Significant differences with regard to lipid or lipoprotein values were also observed between genotypes within CHD group (Table 3). The 296Q allele carriers showed significantly higher TC and LDL-C levels than 296LL genotype.

**Discussion**

In the present study, we have described a new mutation in the CETP gene, L296Q in exon 10. To our knowledge, this is the first report on identifying novel CETP missense mutation in Chinese, and the tenth mutation in the coding region of the CETP gene with published allele frequency data [11,12].

Studies have shown that genetic variability in the CETP gene is associated with plasma lipid concentration and is a significant independent risk factor for CHD [7,9]. The first reported CETP missense mutation, D442G within exon 15, was found to lead to the reduction in CETP synthesis and increase of HDL-C. The 442G heterozygote had an increase of 3-fold in HDL concentration and markedly decreased plasma CETP mass and activity. Cellular expression of mutant cDNA resulted in secretion of only 30% of wild type CETP activity [7]. The results of study on Japanese-American men demonstrated that the overall prevalence of definite CHD was higher in men with CETP mutations than those without mutations, and the adjusted relative risk

**Table 2** Frequencies of alleles and genotypes of L296Q mutation in CHD patients and controls

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Genotype (%)</th>
<th>χ²</th>
<th>P</th>
<th>Allele (%)</th>
<th>χ²</th>
<th>P</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>203</td>
<td>145(71.4)</td>
<td>58(28.6)</td>
<td>6.856</td>
<td>0.008**</td>
<td>0.840</td>
<td>0.160</td>
<td>9.014</td>
</tr>
<tr>
<td>Control</td>
<td>209</td>
<td>172(82.3)</td>
<td>37(17.7)</td>
<td></td>
<td></td>
<td>0.909</td>
<td>0.091</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3** Comparisons of lipid and lipoprotein levels between genotypes in CHD patients

<table>
<thead>
<tr>
<th>Lipids(mmol/L)</th>
<th>LL (n=145)</th>
<th>LQ (n=51)</th>
<th>QQ (n=7)</th>
<th>LL vs. LQ</th>
<th>LL vs. QQ</th>
<th>LQ vs. QQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>1.49±0.79</td>
<td>1.51±0.81</td>
<td>1.57±0.59</td>
<td>0.854</td>
<td>0.687</td>
<td>0.637</td>
</tr>
<tr>
<td>TC</td>
<td>4.88±1.03</td>
<td>5.20±0.92</td>
<td>5.81±1.09</td>
<td>0.044*</td>
<td>0.031*</td>
<td>0.151</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.16±0.41</td>
<td>1.13±0.30</td>
<td>1.21±0.57</td>
<td>0.744</td>
<td>0.645</td>
<td>0.315</td>
</tr>
<tr>
<td>LDL-C</td>
<td>2.26±0.88</td>
<td>2.47±0.67</td>
<td>2.89±0.80</td>
<td>0.026*</td>
<td>0.039*</td>
<td>0.083</td>
</tr>
<tr>
<td>VLDL-C</td>
<td>0.91±0.37</td>
<td>0.88±0.41</td>
<td>0.93±0.51</td>
<td>0.884</td>
<td>0.891</td>
<td>0.583</td>
</tr>
</tbody>
</table>

*P < 0.05.

**Fig. 1** Detection of the L296Q mutation in the CETP gene by DNA sequencing

The arrows indicate the T to A transversion. (A) 296L allele; (B) 296Q allele.
of CHD was 1.61 ($P=0.024$) in men with the D442G mutation [9]. This result is consistent with those studies in which CETP transgenic mice with hypertriglyceridemia expressing both human CETP and apo C-III genes had reduced atherosclerosis [13]. Another study has shown a significant relation between sequence variation at the CETP gene locus and the progression of coronary atherosclerosis that is independent of plasma HDL-C levels [14]. Clearly, the CETP gene may exert effects on cardiovascular risk that are independent of HDL-C level. Another 2 missense mutations, A373P and R451Q, found only in Caucasians, were also shown to be related to plasma lipoprotein levels [8,15].

Unlike the D442G and R351Q that are close to the active site of CETP, L296Q mutation is far from the C-terminus and leads to the increase in TC and LDL-C levels as well as CHD risks of the 296Q allele carriers. The mechanism through which this mutation exerts its effect on the population is not clear. With the aid of specific monoclonal antibodies [16] and site-directed mutagenesis [17], the lipid transfer domain of CETP has been localized to the C-terminal 12 amino acids. A putative hinge sequence [1], lipid-protein binding [17] and HDL binding sites [18] have also been identified towards the C-terminus of the protein. It seems that L296Q mutation can not change the structure of CETP dramatically. On the other hand, leucine is a nonpolar amino acid while glutamine is a polar amino acid, and they have different side chains. Such amino acid substitution can change the hydrophobic nature of the protein and hence may have different effects on the secondary structure of CETP. Therefore, L296Q mutation may modify the structure of CETP and somehow elevated TC and LDL-C levels which are the well-known risk factors to CHD. Further studies of structure and function of CETP in carriers with this genetic variant may help to elucidate the correlation.

In conclusion, a novel missense mutation L296Q in exon 10 of the CETP gene in Chinese was identified in this paper. Association study revealed that this mutation was related to CHD since a significantly higher 296Q allele frequency was found in CHD patients than in controls. The mutation affected the levels of plasma lipids with the 296Q allele carriers displaying considerably higher TC and LDL-C concentrations than noncarriers. The identification of mutations in the CETP gene will afford the opportunity to investigate the role that CETP gene plays in the pathogenesis of CHD in Chinese.

References


