Association of CYP4F2 and CTRP9 polymorphisms and serum selenium levels with coronary artery disease

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Abstract
Aims to explore the interaction between serum selenium level and CYP4F2 and CTRP9 gene polymorphisms in the development of coronary artery disease (CAD).

A total of 200 cases of CAD were selected from the Central Hospital of Enshi Tujia and Miao Autonomous Prefecture, Hubei, China, and 200 healthy subjects cases were served as controls. The polymorphism of CYP4F2 and CTRP9 gene was detected by Sanger sequencing, and the serum selenium level was measured by hydride generation atomic fluorescence spectrometry.

The serum selenium level in the CAD group was significantly lower than that in the control group. The risk of CAD was decreased in the patients carrying the AA genotype in CYP4F2 rs3093135, while the frequency of the CC genotype of CTRP9 rs9553238 in CAD patients was higher than that in control subjects. Low serum selenium level and CTRP9 rs9553238 CC genotype play a positive role in the occurrence of CAD.

The serum selenium level is negatively correlated with CAD. The polymorphism of the CYP4F2 rs3093135 and CTRP9 rs9553238 was significantly related to the susceptibility of CAD, and there is a synergistic effect between the serum selenium level and the CTRP9 rs9553238 CC genotype, which significantly increases the risk of CAD.

Abbreviations: CAD = coronary artery disease, CI = confidence interval, CTRP = C1qTNF related protein, DBP = diastolic blood pressure, GHAFC = generation hydride atomic fluorescence spectrometry, GWAS = genome wide association studies, PCR = polymerase chain reaction, SNPs = single nucleotide polymorphisms

Keywords: coronary artery disease, CTRP9, CYP4F2, polymorphism, selenium

1. Introduction
Coronary artery disease (CAD) is a common cardiovascular disease, with a high mortality rate worldwide.[1] At present, it is generally considered that CAD is a disease involving many factors, including the complex interaction between many genetic and environmental factors.[2–4] In recent years, genome wide association studies (GWAS) provide a powerful tool for finding new potential genes that may increase the risk of CAD. The largest GWAS performed on CAD has identified multiple genes associated with disease susceptibility in different populations and provided molecular-based insights into the disease.[5,6] For example, GWAS data show that certain single nucleotide polymorphisms (SNPs), such as CYP17A1 rs1004467 and AT2B1 rs2681492, are associated with susceptibility to essential hypertension.

CYP4F2 gene is located on human chromosome 19, containing 12 introns and 13 exons.[7,8] There are several common SNPs, such as rs3093100, rs3093105, rs3093166, rs1558139, and rs2108622.[7,8] CYP4F2 protein belongs to human cytochrome P450 superfamily, which is an essential enzyme for the production of 20-hydroxy eicosane arachidonic acid (20-HETE),[9,10] and increases the level of 20-HETE and vascular oxidative stress, endothelial dysfunction and high peripheral vascular resistance.[11] As a biologically active eicosapentaenoic acid and therapeutic intervention target, 20-HETE is involved in a variety of vascular events, such as regulation of blood pressure, renal function, cerebral blood flow, and pulmonary circulation.

The C1qTNF related protein (CTRP) family is a newly discovered homologue of adiponectin.[11] The CTRP family has 15 members, with different structures and functions.[12] Among them, CTRP9 has the highest amino acid homology with adiponectin and is secreted as glycoprotein in adipose tissue. At present, it has been reported that CTRP9 has a protective effect on the cardiovascular system, with a higher vasoactive effect than adiponectin. It has a protective effect on remodeling after acute myocardial infarction, reducing inflammation and inhibiting the proliferation of vascular smooth muscle cells.[13–15]

Selenium is an important part of many enzymes. It has been widely studied for its potential role in the prevention of many chronic diseases.[16] Selenium deficiency may have a negative
impact on the immune system, leading to susceptibility to bacterial and viral infections, and increasing the risk of fatal cardiomyopathy.\cite{17} Since, Salonen et al\cite{18} found that there is a negative correlation between selenium and CAD risk, many researches began to explore the effect of selenium on CAD. Previous animal studies\cite{19,20} have confirmed that selenium is beneficial to the prevention of myocardial and Keshan disease. A cross-sectional study\cite{21} shows that selenium supplementation can be used to prevent cardiovascular disease, and the increase of serum selenium level may be related to metabolic syndrome and increase of fasting blood glucose.

The above evidences show that there is an association between CYP4F2 and CTRP9 and CAD. However, there are few studies on the interaction of serum selenium level with CYP4F2 and CTRP9 gene polymorphisms in the development of CAD. Therefore, a case-control study was carried out to explore the interaction between serum selenium level and CYP4F2 and CTRP9 gene polymorphisms in the development of CAD.

2. Methods

2.1. Study population

Our study included 200 unrelated individuals of Tuja Chinese with CAD, and all the patients are from the Central Hospital of Enshi Tuja and Miao Autonomous Prefecture, Hubei, China. All the recruits received coronary angiography. The definition of CAD was significant coronary stenosis (≥50%) in no less than one third of main coronary arteries or their major branches (branch diameter ≥2 mm). The 200 healthy controls were determined to be free of CAD according to medical history, questionnaires, electrocardiography, and clinical examination.

Diagnosis of hypertension was established if patients were taking antihypertensive medication, if the mean of three resting measurements of systolic blood pressure was above 140 mm Hg, or diastolic blood pressure (DBP) was >90 mm Hg. Diabetes mellitus was diagnosed by a fasting blood glucose above 7.0 mmol/L or by use of antidiabetic drug therapies. Hyperlipidemia was diagnosed by a total cholesterol ≥5.72 mmol/L and/or triglyceride ≥1.7 mmol/L. The research was approved by the Ethical Committees of Central Hospital of Enshi Tuja and Miao Autonomous Prefecture. Written informed consent conforming to the tenets of the Declaration of Helsinki (1983 Revision).

2.2. Blood specimen collection

All subjects were fasting for more than 8 hours before venous blood sampling. Laboratory investigations included current data hematological and bioclinical (cholesterol, triglycerides, glycemia, etc.). The level of serum selenium was detected by the Medical Laboratory of our hospital. The collected venous blood was coagulated at room temperature for 30 minutes, centrifuged at 3000 r/min for 15 minutes, and the supernatant was retained. The serum selenium level was determined by generation hydride atomic fluorescence spectrometry (GHAFS).

2.3. Genotyping

Plasma genomic DNA was extracted using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions and stored at −80°C. The DNA fragment containing the rs3093135 loci of the CYP4F2 and the rs9553238 loci of the CTRP9 gene 3′-UTR was amplified by polymerase chain reaction (PCR) using the extracted genomic DNA as a template. The PCR mixture contained 12.5 μL PCR mix (Elpis-Biotech), 1 μL (10 pmol) each of the primers, 1 μL genomic DNA, and 1.5 μL double distilled water. The PCR conditions were as follows: pre-denaturation at 94°C for 2 minutes, denaturation at 94°C for 1 minute, annealing at 60°C for 40 seconds, and extension at 72°C for 4 minutes, in a total of 30 cycles. After PCR, Sanger sequencing was performed, and the genotypes were determined by comparing the sequencing results with the sequences in the NCBI database.

2.4. Statistical analysis

SPSS 16.0 software was used for data analysis, measurement data indicators were tested for normality, and comparisons between the two groups were analyzed by t test or analysis of variance. The genotypes and allele frequencies of genes polymorphisms were calculated by frequency counting method, and whether the frequency distribution of genotypes in CAD group and control group was in accordance with Hardy–Weinberg equilibrium (HWE). The difference in genotype was calculated using logistic regression model, and the hazard ratio (OR) and its 95% confidence interval (CI) were calculated after adjusting for confounding factors. The serum selenium content was converted to count data according to the upper limit (0.109 mg/L) of the normal content reference value [0.079 ± 0.03 mg/L], and the relationship between serum selenium level and CAD was analyzed. \( P < .05 \) indicates that the difference is statistically significant.

3. Results

3.1. Baseline characteristics

The clinical data of all included subjects are shown in Table 1. The proportion of male in CAD group was higher than that in control group (71% vs 54%, \( P < .01 \)). There was no significant difference in age between CAD group and control group (\( P > .05 \)). However, the incidence rate of CAD, hypertension and diabetes was significantly higher in group CAD than control group (\( P < .05 \)). Besides, some important clinical biochemical indexes (such as triacylglycerols and fasting blood glucose) were statistically significant in the two groups (\( P < .05 \)).

3.2. CYP4F2 rs3093135 and CTRP9 rs9553238 associated with CAD

Two SNPs (rs3093135 and rs9553238) were successfully genotyped in all CAD patients and healthy controls (Table 2). The results of Sanger sequencing were shown in Figure 1. All the genotype frequencies were in HWE (\( P > .05 \)). We found significant differences of genotypic distribution in rs3093135 and rs9553238. The frequency of the AA genotype (rs3093135) in CAD patients was lower than that in control subjects (OR = 0.540, 95% CI: 0.333–0.875, \( P = .012 \)), while CC (rs9553238) was higher than that of control subjects (OR = 1.308, 95% CI: 1.245–1.749, \( P = .03 \)).

3.3. Relationship between serum selenium level and CAD

The serum selenium level of CAD group was (0.079 ± 0.028) mg/L, which was significantly lower than that of control group (0.114 ± 0.027) mg/L. The upper limit (0.109 mg/L) of the
Table 1
Comparison of baseline data between the CAD group and the control group.

|                | Control group | CAD group | $\chi^2$ | P    |
|----------------|---------------|-----------|----------|------|
| Gender         |               |           |          |      |
| Male (n, %)    | 108 (54.00%)  | 142 (71.00%) |          | < .01|
| Age (Mean ± SD, years) | 57.00 ± 6.22 | 63.46 ± 12.38 | -1.74 | .57  |
| Arterial pressure (Mean ± SD, mm Hg) |               |           |          |      |
| Systolic pressure | 129.97 ± 20.17 | 159.74 ± 27.16 | -6.16 | .02  |
| Diastolic pressure | 81.09 ± 17.2  | 97.56 ± 19.01 | -3.41 | .04  |
| Total cholesterol (Mean ± SD, mmol/L) | 3.77 ± 0.78 | 4.99 ± 1.49 | -1.84 | .28  |
| Triacylglycerols (Mean ± SD, mmol/L) | 1.38 ± 0.80 | 1.65 ± 0.86 | -2.97 | .03  |
| Fasting blood glucose (Mean ± SD, mmol/L) | 5.64 ± 1.69 | 6.49 ± 1.73 | -1.57 | .04  |
| Diabetes (n, %) | 15            | 49        | 24.74    | < .01|

CAD = coronary artery disease.

Table 2
Association of CYP4F2 and CTRP9 gene polymorphism with CAD susceptibility.

| CYP4F2 rs3093135 | Control | CAD | $\chi^2$ | P    |
|------------------|---------|-----|----------|------|
| AA               | 55      | 34  |          |      |
| AT               | 98      | 112 |          |      |
| TT               | 47      | 54  |          |      |
| OR (95%CI)       | 0.540 (0.333–0.875) | 1.325 (0.894–1.963) | 1.204 (0.706–1.892) | 0.862 (0.504–1.472) | 0.980 (0.659–1.457) | 1.308 (1.245–1.749) |
| P                | .012    | .161| .421     | .586 | .919 | .03 |

| CTRP9 rs9553238 | TT      | CT  | CC       |
|-----------------|---------|-----|----------|
| Control         | 34      | 85  | 81       |
| CAD             | 20      | 74  | 106      |
| OR (95%CI)      | 0.862 (0.504–1.472) | 0.980 (0.659–1.457) | 1.308 (1.245–1.749) |
| P               | .586    | .919| .03      |

CAD = coronary artery disease.

Figure 1. Display of Sanger sequencing results. (A) Sequencing of CYP4F2 gene locus in a sample; (B) sequencing of CTRP9 gene locus in a sample.
The susceptibility of CTRP9 gene polymorphism and CAD with the level of serum selenium.

### Table 3
**Relationship between serum selenium levels and CAD.**

| The level of serum selenium | Control | CAD | OR (95%CI) | P   |
|-----------------------------|---------|-----|------------|-----|
| <0.109 mg/L                 | 79      | 132 |            |     |
| ≥0.109 mg/L                 | 121     | 68  | 28.175     | 0.336 (0.224–0.505) | <.01 |

**Table 4**
**The susceptibility of CTRP9 gene polymorphism and CAD with the level of serum selenium <0.109 mg/L.**

| Genotype | Control (n = 79) | CAD (n = 132) | OR (95%CI) | P   |
|----------|-----------------|---------------|------------|-----|
| TT       | 12              | 10            | 0.451 (0.185–1.099) | .080 |
| CT       | 30              | 41            | 0.721 (0.401–1.296) | .274 |
| CC       | 28              | 71            | 2.078 (1.169–3.695) | .013 |

CAD = coronary artery disease.

### 3.4. Interaction between serum selenium level and CTRP9 rs9553238 gene polymorphism in the development of CAD

The exposure factors were serum selenium level <0.109 mg/L and CC with CTRP9 rs9553238 genotype. We performed subgroup analysis on subjects with serum selenium levels below 0.109 mg/L, and the results are presented in Table 4. The OR with its 95% CI was TT: 0.451 (0.185–1.099) and CT: 0.721 (0.401–1.296). However, the OR was 2.078 (1.169–3.695) only when exposed to serum selenium <0.109 mg/L and carrying CTRP9 rs9553238 CC genotype. The risk of CAD increased by 1.94 times only when exposed to serum selenium level <0.109 mg/L, while the risk increased by 2.51 times when exposed to serum selenium level ≥0.109 mg/L, the difference was statistically significant (P < .01, Table 3).

### 4. Discussion

The incidence of CAD involves many factors such as heredity and environment. Age, gender, smoking, obesity, hypertension, diabetes and family genetic history are considered as common risk factors. But with the further research, more and more new genetic factors have been found, such as inflammation, apolipoproteins, and so on.

CYP4F2 gene is highly polymorphic and has become an attractive candidate gene for cardiovascular disease genetic association research. Previous studies in the Asian population have significant local distribution characteristics. The reason may be that CAD is affected by heredity and environment. Mutations in the primary structure of CYP4F2 affect enzyme activity, leading to changes in drug metabolism, physiology and pathophysiology. Previous study determined that CYP4F2 can influence the genetic factors of Japanese response to warfarin through GWAS. CAD is caused by the interaction between environment and genetic factors. In this study, CYP4F2 gene, an important member of P450 gene family, was selected as a candidate gene for case-control study. It was found that rs3093135 in CYP4F2 gene was significantly related to the risk of CAD. Singh et al. found that androgen induced CYP4A8 expression decreased CYP2C23 expression and increased the production of 20-HETE, and then cyclooxygen acid decreased, thus affecting vasoconstriction, which was also confirmed in another study. However, the exact mechanism of CAD susceptibility is not clear. Moreover, CYP4F2 polymorphism is also associated with other cardio cerebral diseases, such as ischemic stroke, stent thrombosis, etc.

The frequency of the CC genotype of CTRP9 rs9553238 in CAD patients was higher than that in control subjects. The association between CTRP family members and cardiac metabolic abnormalities has been documented in previous studies. CYP4F2 can be considered critical players in the pathogenesis of CAD, since its expression is dysregulated in metabolic diseases. Specifically, the circulating CTRP9 levels were independently associated with increased risk of CAD. A significant association of serum CTRP9 levels with adhesion molecules in CAD as well as serum TNF-α levels in CAD individuals was noted. In addition, CTRP9 is predominantly secreted by adipose tissue and the CTRP9 gene is up-regulated in the adipose tissue of obese mice. Serum CTRP9 levels were found to be inversely correlated with BMI in individuals with T2DM and reduced CTRP9 levels were observed in obese patients following bariatric surgery. It has been suggested that CTRP9 activates Akt, AMPK and p42/44 MAPK and increases glucose uptake. Some studies also suggest that CTRP9 mainly regulates the inflammatory response of CAD. Positive correlations between CTRP9 and inflammatory cytokines (IL-6 and TNF-α) in CAD individuals, indicating a compensatory response to inflammatory milieu in patients with CAD. CTRP9 inhibits inflammatory responses in macrophages and was shown to improve plaque stability by reducing inflammatory cytokine secretions in mice.

A meta-analysis involving 16 studies found that selenium supplementation reduced serum CRP and glutathione peroxidase (GSH-Px) levels. CRP is a major inflammatory biomarker and has been considered as an important risk factor for CAD because CRP level is directly related to the presence and severity of coronary, cerebral and peripheral arteriosclerosis. Previous epidemiological study described the association between elevated CRP levels and increased CAD risk. The effects of selenium on the pathogenesis of CAD mainly include:

1. improving inflammatory response and vascular endothelial damage;
2. anti-oxidative stress and anti-lipid peroxidation;
3. alleviating vascular calcification and platelet aggregation.

At present, there is not enough evidence to elucidate the mechanism of interaction between serum selenium level and CYP4F2 and CTRP9 polymorphism, which may be due to the fact that both selenium and CYP4F2 and CTRP9 genes are involved in inflammation, vasoconstriction, and other processes.

In addition, our research has some limitations:

1. This study is a case-control study in single center, which leads to the limited number of subjects we recruited.
We selected two gene loci for testing, which may not fully reflect the role of these genes in the development of CAD.

In conclusion, the frequency of the AA genotype (rs3093135) in CAD patients was lower than that in control subjects, while CC (rs9553238) was higher than that of control subjects. Furthermore, we found that there was a positive interaction between serum selenium and CTRP9 rs9553238 gene polymorphism after analyzing the relationship between serum selenium level and CTRP9 gene polymorphism. It is suggested that low serum selenium level and CTRP9 rs9553238 may have synergistic effect on the occurrence of CAD, but the specific mechanism needs further study.

**Author contributions**

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