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ORIGINAL ARTICLE

Correlation between the GP78 Gene Polymorphism and Coronary Atherosclerotic Heart Disease

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Abstract *Objective:* To study the correlation between the GP78 gene polymorphism and blood fat, blood glucose, blood pressure and coronary atherosclerotic heart disease.
Methods: A total of 72 patients with coronary atherosclerotic heart disease were selected as the observation group, and 68 healthy participants were selected as the control group. The gp78 gene polymorphism of both groups was studied via polymerase chain reaction-restriction fragment length polymorphism (RFLP). At the same time, the multiple expression quantities of the GP78 gene in the tissues of both groups were tested via fluorogenic quantitative PCR, enzyme-linked immunosorbent assay (ELISA) and Western-blotting assay. Furthermore, the blood fat, blood glucose and blood pressure of subjects in both groups were tested.
Results: The percentages of the gp78 gene polymorphisms of Arg/Arg, Arg/Gly and Gly/Gly at the 145 locus of the study subjects in the observation group were 12.3%, 43.2% and 44.5%, respectively, while those in the control group were 74.3%, 11.2% and 14.5%, respectively, and there were significant differences between both groups. Based on the test results of the blood fat, blood glucose and blood pressure of the objects in the observation group and control group, significant differences were found between the two groups ($P<0.05$).
Conclusion: There was a significant correlation between the 145 locus of the gp89 gene and coronary atherosclerotic heart disease, indexes of blood fat, blood glucose and blood pressure.

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1. Introduction

Coronary atherosclerotic heart disease, CAD, is mainly caused by the abnormal metabolism of lipid in the body, which leads to lipid sedimentation in blood vessels and forms a porridge-like white plaque.[1] As the quantity of lipid sedimentation in blood vessels grows, blood volume decreases[2] and blood circulation becomes blocked. This leads to heart ischemia and manifests mainly as angina pectoris in the clinic.[3] Studies in recent years have suggested that[4–6] the sterol regulatory element binding proteins (SREBPs) signal pathway plays an important role in degrading excessive lipids, such as cholesterol. For those lipids, especially the lipid sedimentation in blood vessels, degradation is conducted mainly through the 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMGGR) signal pathway, which is induced by related lipids, and through the ubiquitin-proteasome.[7] Research[8] shows that the GP78 protein plays a key role in that process. For example, as research has shown, the contents of lipids, such as cholesterol, increased in an abnormal way. The Insig protein would integrate with the HMGGR protein and the GP78 protein at the same time, and the GP78 ubiquitinated HMGGR protein would be transported to the proteasomes for degradation.[9,10] The GP78 protein can catalyze the ubiquitination of lysine on HMMGR at position 89, and the ubiquitinated HMGGR protein will be transported to the proteasome for degradation [11]. Previous studies have shown that the GP78 gene and CAD are correlated. The expression level of the GP78 protein in some patients with CAD was significantly different from that in healthy people, while there was no significant difference in the expression level of the GP78 protein between other patients with CAD and healthy people, but the mechanism is not clear. Gene polymorphism has been shown to correlate with the occurrence of diseases. A previous study showed that the polymorphism of the KM1 gene is closely related to the occurrence of thyroid disease [12], the expression level of the KM1 gene with A at position 178 was significantly higher than that of the KM1 gene with T at the same position, leading to a higher incidence of thyroid disease in those people. However, no study on the correlation between the polymorphism of the GP78 gene and CAD has been reported. Our study aimed to investigate the correlation between the polymorphism of the GP78 gene and CAD and to examine the polymorphism of GP78 in healthy participants and patients with different types of CAD to provide a theoretical and experimental basis for further studies on the pathogenesis of CAD.

2. Patients and Methods

2.1. Patients' information

A total of 72 patients, diagnosed according to the criterion of *Clinical Diagnosis of CAD*, were admitted to our hospital from September 2013 to September 2015 and were selected as study subjects or placed into the observation group. The latter contained 38 males and 34 females with a mean age of 46.3±18.4 years. Meanwhile, 68 healthy persons, including 38 males and 34 females with a mean age of 43.6±15.8 years were selected as the control group. People in the control group were physically healthy, particularly without heart disease and other correlated diseases.

2.2. Reagents and equipment

Reagents: Molecular reagents, such as ExTaq DNA polymerase, dNTP, 10x Buffer and so on, were purchased from Dalian TAKARA Corporation (Dalian, Liaoning, China), restriction endonucleases Rsa1 and PmlI were purchased from THERMO Corporation (Waltham, MA, USA), and the animal cellular genome extraction kit was purchased from AMYGEN Corporation (Waltham, MA, USA). Equipment: LongoGene A300 PCR equipment, a mini electrophoresis tank (Beijing Liuyi, Beijing, China), a Gel Doc 2000+ gel imager (Bio-Rad, Hercules, CA, USA) and a thermostatic metal bath were purchased from TIANGEN Biotech Co., Ltd (Tianjin, China).

2.3. Samples obtained

The observation and control groups were set as study samples. After the guardians and families provided consent to participate in the study and the study was approved by the ethics committee of this hospital, 5 ml of venous blood were drawn from every study sample and were centrifuged at 3000 rpm/min. The supernatant was collected and preserved at −70°C, and the leucocytes were preserved in liquid nitrogen after the addition of cryoprotectant for genome extraction.

2.4. Genome extraction

The genome in the cells of the study samples was extracted with the animal genome extraction kit from AXYGEN Corporation according to the manufacturer's instructions. Detailed protocols have been described in a previous report.[11]

2.5. RFLP and quantitative PCR

The primers used in this study were all synthesized by Shanghai Biological Engineering Co., Ltd. (Shanghai, China). The fluorogenic quantitative inverse transcription kit used in this study was purchased from TAKARA, and the instructions from TAKARA were followed. The fluorogenic quantitative reaction system included: YBR Premix Ex Taq II (2×) 5 μl, PCR forward primer (10 μM) 0.5 μl, PCR reverse primer (10 μM) 0.5 μl, cDNA 1 μl, dH_2O 3 μl, (the primer sequences were as follows in Table 1).

2.6. Enzyme-linked immunosorbent assay (ELISA)

Total protein was extracted from the blood samples of subjects with atherosclerosis. ELISA was applied to test the level of GP78 protein expression in different genotypes according to the specific instructions of ELISA kit (German QIAGEN).

2.7. Western-blotting

Samples previously kept under −80°C were selected for total protein extraction. Approximately 150 mg of tissue were taken out of liquid nitrogen and pulverized in a

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