European Review for Medical and Pharmacological Sciences 2019; 23 (3 Suppl): 287-293

Gene polymorphism of adiponectin in restenosis after coronary stenting

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Abstract. – OBJECTIVE: The objective of the present study was to investigate the relationship between adiponectin (APN)+45T/G and +276G/T polymorphisms and in-stent restenosis (ISR).

PATIENTS AND METHODS: A total of 150 patients treated with percutaneous coronary intervention (PCI) were divided into the ISR group and non-ISR group. The levels of blood biochemical indicators were measured, and APN+45T/G and +276G/T polymorphisms were detected by TaqMan probes.

RESULTS: Cholesterol levels in the IRS group were significantly higher than those in the non-ISR group ($p<0.05$). The frequency of the GG genotype and G allele of the APN+45T/G locus in the ISR group were significantly higher than those in the non-ISR group ($p<0.05$). The frequency of the GG genotype and G allele of the APN+276G/T locus in the ISR group were significantly higher than those in the non-ISR group ($p<0.05$).

CONCLUSIONS: APN+45T/G and +276G/T polymorphisms were associated with susceptibility to ISR, and carrying the G allele of the APN+45T/G and +276G/T loci can significantly increase the risk of ISR.

Key Words:
Percutaneous coronary intervention, Restenosis, Adiponectin, Single nucleotide polymorphism.

Introduction

The incidence of coronary atherosclerotic heart disease (CAD) increases annually. CAD seriously threatens human health and has become a disease with one of the highest rates of disability and mortality[1]. One of the primary methods of clinical treatment of CAD is percutaneous coronary intervention (PCI), which can quickly relieve coronary artery stenosis, restore myocardial oxygen supply, and relieve chest pain and other symptoms. However, PCI treatment can damage the vascular endothelium, which can cause vascular smooth muscle cell migration and proliferation, eventually leading to coronary remodeling and postoperative in-stent restenosis (ISR)[2]. Some studies have shown that ISR occurs in 20-30% of patients within 6 months after PCI, and 15% of patients require retreatment[3]. Drug-coated stents have been applied in recent years, and can significantly reduce the incidence of restenosis, although the incidence remains as high as 10%[4]. The occurrence of ISR seriously affects the treatment efficacy and prognosis of patients with CAD. Adiponectin (APN), as a specific fat factor, is mainly secreted by lipocytes. After entering vascular tissue, APN can regulate the proliferation and apoptosis of smooth muscle and endothelial cells through various mechanisms to achieve its role in regulating vascular function[5]. It was found that polymorphisms of the +45T/G and +276G/T loci on the APN gene are related to the occurrence of cardiovascular disease[6-10]. APN plays an important anti-inflammatory role in arteriosclerosis and can reduce the incidence of ISR[11]. However, studies on the correlation between APN gene polymorphisms and the occurrence of ISR are lacking. In this work, we observed patients who underwent PCI in our department. The occurrence of ISR was detected by imaging techniques. The single-nucleotide polymorphism (SNP) typing technique was used to detect polymorphisms of the +45T/G and +276G/T loci on the APN gene. The relationship between APN polymorphisms and intraventricular restenosis after PCI was analyzed.

Patients and Methods

Patients

A total of 150 patients who underwent PCI in our hospital from June 2014 to June 2016 were selected. All patients belonged to the Han nationality. There were 98 males and 52 females, with a mean age of 57.32 ± 7.48 years. Inclusion

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criteria: 1. Age between 18 and 70-years-old; 2. Clinical diagnosis of myocardial ischemia; 3. Stenosis detected by coronary angiography, with stenosis rate ≥ 70%, and lesion length ≤ 40 mm; 4. Total number of lesions ≤ 2; 5. Lesion diameter between 2.5 and 5.0 mm, and lesion length ≤ 40 mm. Exclusion criteria: 1. Patients with lesions of the left main coronary artery, left circumflex artery, anterior descending branch, and right coronary artery opening; 2. Patients with lesions causing total occlusion of the bifurcation region and blood vessels; 3. Acute myocardial infarction that occurred within 1 week prior to the study; 4. Lesion length > 40 mm; 5. Patients combined with severe heart failure; 6. Patients with recent cerebrovascular accidents, active peptic ulcers, other bleeding diseases, and who could not be treated with anticoagulant therapy; 7. Allergy to anticoagulants, antiplatelet agents, and contrast agents; 8. Pregnant women. All patients signed the informed consent. This study was approved by the Ethics Committee of QILU Hospital of Shandong University.

All subjects underwent coronary angiography and PCI, and patients were informed after surgery. Patients were subjected to coronary angiography immediately in our hospital if any chest pain occurred. Otherwise, coronary angiography was performed for patients 6 months after surgery. Simultaneously, the content of cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and fasting blood glucose (FPG) were measured. Anticoagulation vacuum (EDTA) blood collection tubes were used to collect 3 ml of fasting venous blood. ISR diagnosis: if coronary angiography showed a restenosis rate of the vasculature within the stent, or within the area 5 mm around the stent ≥ 50%, myocardial ischemia recurred. Patients were divided into the ISR group and non-ISR group according to the results of coronary angiography. There were 28 cases in the ISR group, and 122 cases in the non-ISR group. There were no significant differences in age, sex ratio, preoperative vascular diseases, number of implanted stents, or stent length between the two groups (p>0.05).

DNA Extraction

DNA was extracted using a whole blood genomic DNA extraction midi kit (Beijing Bio Teke Biotechnology Co., Ltd., Beijing, China) according to the manufacturer's instructions. The purity and concentration of all DNA samples were determined using a Nanodrop-2000 Ultraviolet Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The purity and concentration of all DNA samples met the experimental requirements.

Analysis of APN Gene Polymorphisms

The SNP genotypes of the +45T/G and +276G/T loci of the APN gene were analyzed using a TaqMan® SNP Genotyping Assays kit (Applied Biosystems, Foster City, CA, USA).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 19.0 (IBM, Armonk, NY, USA) software was used for statistical analyses. Numerical data are presented as `x±s. Comparisons between two groups were by independent sample t-test. The likelihood ratio χ^2-test was used to test whether the distribution of genotype was consistent with Hardy-Weinberg equilibrium. The genotype calculation was counted directly. The genotype and allele frequencies between groups were compared by R×C chi-square test. p<0.05 was considered statistically significant.

Results

Comparison of the Levels of Blood Biochemical Indicators

The TC level in the IRS group was significantly higher than that of the non-ISR group (p<0.05). There were no significant differences in TG, FPG, HDL-c, or LDL-c between the two groups (p>0.05) (Figure 1).

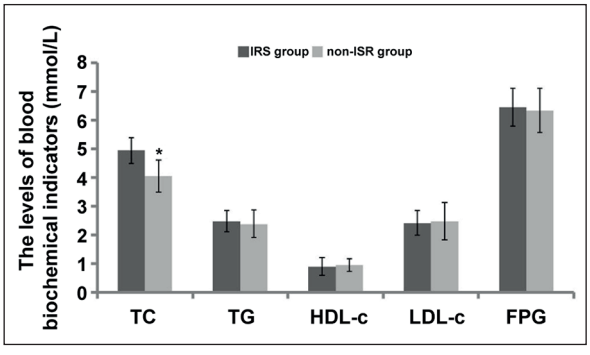

Figure 1. Comparison of the levels of blood biochemical indicators between the two groups. The TC level in the IRS group was significantly higher than that of the non-ISR group (p<0.05), while no significant differences in TG, FPG, HDL-c, or LDL-c were found between the two groups (p>0.05). Note: *compared with the IRS group, p<0.05.

Table I. The examination of distribution frequency of APN+45T/G.

Groups	Cases	GG		GT		TT		χ^2-value	*p*-value
		Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency		
ISR	28	7	4.72	9	13.55	12	9.72	3.16	0.206
Non- ISR	122	10	8.39	44	47.21	68	66.39	0.56	0.754

Table II. The examination of distribution frequency of APN+276G/T.

Groups	Cases	GG		GT		TT		χ^2-value	*p*-value
		Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency		
IISR	28	18	17.29	8	9.43	2	1.28	0.73	0.643
Non- ISR	122	50	43.68	46	58.64	26	19.68	5.67	0.059

Hardy-Weinberg Equilibrium Test by Likelihood Ratio χ^2-test

The distribution frequency of the APN +45T/G and +276G/T genotype was tested. The *p*-value of distribution frequency was higher than 0.05, indicating that the sample was representative of the population (Tables I and II).

Distribution of APN +45T/G Variance in the ISR Group and Non-ISR Group

The frequencies of distribution of the GG, GT, and TT genotypes in the ISR group were 25%, 32.14%, and 42.86%, respectively. The GG, GT, and TT genotypes in the non-ISR group were 8.20%, 36.06%, and 55.74%, respectively. The distribution of the APN +276G/T genotype in the two groups was significantly different from each other (p=0.039), indicating that the occurrence of ISR may be related to the APN + 45T/G polymorphism.

The different genotypes were further compared. The odds ratio (OR) of the GG genotype compared with the TT genotype was 2.874, and the 95% CI was 1.259-5.561. The OR of the GT genotype compared with the TT genotype was 1.091, and the 95% CI was 0.633-1.881, indicating that the occurrence of ISR is related to the homozygous mutation of APN + 45T/G, and the risk of ISR is increased by the GG homozygous mutation of APN + 45T/G. The occurrence of ISR is not related to the heterozygous mutation of APN + 45T/G (Table III).

Comparison of G and T Allele Distribution of APN + 45T/G

The frequencies of the G and T alleles in the ISR group were 41.07% and 58.93%, respectively. The frequencies of the G and T alleles in the non-ISR group were 26.23% and 73.77%, respectively. The frequency of risk allele G in the ISR group was significantly higher than that of the non-ISR group (p=0.027), the OR was 1.566, and the 95% CI was 1.073-2.285 (Table IV).

Distribution of APN + 276G/T Variants in the ISR and Non-ISR Groups

The distribution of the GG, GT, and TT genotypes in the ISR group were 64.29%, 28.57%, and 7.14%, respectively. The distribution of the GG, GT, and TT genotypes in the non-ISR group

Table III. The comparison of the distribution of APN+45T/G genotypes between the two groups.

Groups	Cases	Genotype [case (%)]			χ^2	*p*
		GG	GT	TT		
ISR	28	7 (25)	9 (32.14)	12 (42.86)	6.481	0.039
Non-ISR	122	10 (8.20)	44 (36.06)	68 (55.74)		

Table IV. Comparison of G and T allele distribution of APN + 45T/G between the two groups.

Groups	Cases	Genotype [case (%)]		χ^2	*p*
		G	T		
ISR 28	23 (41.07)	33 (58.93)	4.872	0.027	
Non-ISR	122	64 (26.23)	180 (73.77)		

were 40.98%, 37.71%, and 21.31%, respectively. The distribution of the APN + 276G/T genotype in the two groups was significantly different from each other (p=0.047), indicating that the occurrence of ISR may be related to the APN + 276G/T polymorphism.

The different genotypes were further compared. The OR of the GG genotype compared with the TT genotype was 1.368, and the 95% CI was 1.100-1.702. The OR of the GT genotype compared with the TT genotype was 1.252, and the 95% CI was 0.878-1.786, indicating that the occurrence of ISR is related to the homozygous mutation of APN+276G/T, and the risk of ISR was increased by the GG homozygous mutation of APN+276G/T. The occurrence of ISR is not related to the heterozygous mutation of APN+276G/T (Table V).

Comparison of G and T Allele Distribution of APN + 276G/T

The frequencies of G and T alleles in the ISR group were 78.57% and 21.43%, respectively. The frequencies of G and T alleles in the non-ISR group were 59.84% and 40.16%, respectively. The frequency of risk allele G in the ISR group was significantly higher than that of the non-ISR group (p=0.007), the OR was 1.313, and the 95% CI was 1.107-1.558 (Table VI).

Discussion

The occurrence of ISR is a complex pathophysiological process involving multiple factors. Some researchers found that the process of PCI in patients with CAD can directly damage the vascular endothelium, resulting in inflammation. This, in turn, leads to the formation of thrombosis within blood vessels, and eventually to restenosis[13]. The development of ISR is mediated by a variety of complex mechanisms, including various cytokines, vasoactive substances, immune mechanisms, gene expression, protein translation, and other processes[13,14]. Endometrial hyperplasia is one of the main mechanisms of ISR formation[2,15]. Some studies have shown that injury of vascular endothelial cells during PCI can lead to the loss of vascular intimal integrity, exposure to subcutaneous matrix, platelet aggregation and adhesion, local induced inflammatory response, vascular smooth muscle cell migration and pro-

Table V. The comparison of the distribution of the APN+276G/T genotypes between the two groups.

Groups	Cases	Genotype [case (%)]			χ^2	*p*
		GG	GT	TT		
ISR	28	18 (64.29)	8 (28.57)	2 (7.14)	6.095	0.047
Non-ISR	122	50 (40.98)	46 (37.71)	26 (21.31)		

Table VI. Comparison of G and T allele distribution of APN+276G/T between the two groups.

Groups	Cases	Genotype [case (%)]		χ^2	*p*
		G	T		
ISR	28	44 (78.57)	12 (21.43)	7.35	0.007
Non-ISR	122	146 (59.84)	98 (40.16)		

liferation, and formation of vascular neointima, eventually leading to the occurrence of ISR[16]. If the newly generated endothelial cells can quickly cover the intima from PCI injury, hyperplasia of the intima will stop, and the incidence of ISR can be reduced. Therefore, vascular endometrial lesions are the basis of the occurrence of ISR, and endothelial dysfunction is an important factor in the development of ISR.

APN is secreted by adipocytes. In humans, APN has anti-atherosclerotic, anti-inflammatory, and vasoprotective functions. The human APN gene is located on chromosome 3q27 and consists of two introns and three exons. It is 17 kb in size and encodes 244 amino acids[17]. Previous studies[17,18] have shown that APN can inhibit extracellular signal-regulated kinase signaling pathways, thereby reducing the proliferation and migration of vascular smooth muscle cells. It has been shown[19] that APN can promote nitric oxide (NO) synthase expression in endothelial cells to reduce the oxidative stress response in endothelial cells, and to increase the activity of NO for the protection of vascular endothelium. Another report[20] of patients followed-up after PCI showed that the incidence of ISR and the expression of serum APN were positively correlated. The increased serum APN levels were shown to reduce the incidence of ISR. APN reduced the incidence of ISR by protecting the damaged vascular endothelium after PCI, inhibiting the vascular endometrial inflammatory response, and inhibiting the proliferation of vascular smooth muscle cells.

With the development of genome-wide association analysis, the sites of polymorphism of the APN gene were proven to be susceptible sites for coronary heart disease and metabolic syndrome, and gene polymorphisms were widely investigated in recent years[6-10]. Two SNP loci (+ 45T/G and + 276G/T) have been widely studied in recent years. Lacquemant et al[6] found that APN + 45T/G was significantly associated with CAD in Swedish and French Caucasian patients with type 2 diabetes mellitus, and was an independent risk factor for the formation of CAD. AI-Daghri et al[7] found that carrying the APN + 45T/G allele G can significantly increase the risk of CAD. In 2004, Jang et al[9] found that APN + 276G/T was closely related to the pathogenesis of CAD in patients with type 2 diabetes mellitus, and the incidence of CAD in patients with the TT genotype was significantly lower than that in patients with the GG and GT genotypes. In Korean CAD patients without diabetes mellitus, it was found that patients with the GG genotype of the APN +276G/T locus had a higher oxidative state and higher serum TG and LDL-c levels than those of patients with the TT genotype. Moreover, the LDL-c particles were smaller in patients with the GG genotype than those of patients with the TT genotype, indicating that APN + 276G/G carriers had higher risk of cardiovascular disease[9,10]. Esteghamati et al[21] explored the association between APN polymorphisms and type 2 diabetes mellitus combined with CAD, and found that the risk of CAD in patients with type 2 diabetes mellitus was significantly reduced by the existence of the T allele on the APN + 276G/T locus. Further association analysis revealed that carrying two haplotypes of 45T-276T and 45G-276T could reduce the risk of CAD. Thus, APN gene + 45T/G and +276G/T polymorphisms were closely correlated with the occurrence and development of coronary atherosclerosis.

In this work, the relationship between the APN + 45T/G and +276G/T polymorphisms and the occurrence of ISR after PCI was investigated. We found that the APN + 45T/G gene polymorphism was significantly associated with the risk of ISR. The risk of ISR was significantly increased by the GG homozygous mutations of the + 45T/G locus, and carrying of G allele can significantly increase the risk of ISR. The APN + 276G/T gene polymorphism was significantly associated with the risk of ISR. The risk of ISR was increased because of GG homozygous mutation of APN + 276G/T, and carrying the G allele could significantly increase the risk of ISR. However, the mechanism of how SNPs at the + 45T/G and + 276G/T loci affect the formation of ISRs remains unclear. The present investigation suggests that the + 276G/T site may be linked to other SNPs located in the promoter region, or linked to SNPs that affect the cleavage of an mRNA precursor, thereby affecting the transcription of APN mRNA. It is also possible that the + 276G/T site can directly affect the cleavage of an mRNA precursor, which in turn affects mRNA expression and protein translation[22-24]. Further studies are still required to determine the specific mechanism. The occurrence of ISR is the combined result of genetic and environmental factors. Genetic backgrounds are highly varied among different racial groups, and the intensity of SNPs are also different. Similarly, multiple SNP loci can affect each other. Eating habits, living environment, and experimental sample-size can also affect relevant research results.

Conclusions

We found that APN+45T/G and +276G/T polymorphisms were associated with ISR. However, multi-center and multi-site studies between different populations are required for the correlation analysis between APN polymorphisms and ISR.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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