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Relationship Between Polymorphism of Adiponectin Gene SNPS+276 and Coronary Heart Disease

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ABSTRACT

Background: This study aims to investigate the relationship between polymorphism of adiponectin (ADIPOQ) gene SNPS+276 and the severity of coronary heart disease (CHD) and coronary artery disease (CAD).

Methods: A total of 582 inpatients were enrolled and divided into Group CHD (342 cases) and the control group (CON, 240 cases), according to their angiographic results from June 2014 to April 2016 for the genotype (G/T) analysis of ADIPOQ SNPs+276 by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results: Group CHD: GG 110 (32%), GT 205 (59%), and TT27 (8%); Group CON: GG 36 (15%), GT 161 (67%), and TT 43 (18%) ($P < .05$). The frequency of allele G in group CHD was 62.1% and 48.5% in group CON ($P < .05$). The frequencies of genotype GG, GT, and TT were 67 (33.3%), 107 (53.2%), and 27 (13.5%), respectively, in the group with single vascular lesion, and 64 (45.4%), 53 (37.6%), and 24 (17%), respectively, in the group with multiple vascular lesions. There was statistical significance between the two groups ($P < .05$).

Conclusions: The 276G gene of adiponectin may be a susceptibility gene of CHD, and the genotype GG may be related to the severity of this disease.

INTRODUCTION

Adiponectin is a small molecule protein synthesized by the adipocytes, which has a wide range of biological effects [Yamauchi 2007]. In recent years, studies have found that hypoadiponectinemia is closely related to insulin resistance, inflammatory reaction, or atherosclerosis [Wang 2016; Achari 2017], and is related to the pathogenesis of coronary heart disease and cerebral infarction [Kanhai 2013]. Studies also have shown that hypoadiponectinemia is an independent risk factor for coronary heart disease [Hao 2013]. Scholars have found that the adiponectin single nucleotide polymorphism (SNP)+276 is associated with the risk of coronary heart disease (CHD) [Qi 2006; Gable 2007]. Clinical control studies have shown that the frequency of adiponectin rs3774261G gene in group CHD is higher than the control group, while the frequency of rs2082940T gene is significantly lower than the control group [Kanu 2016]. In populations with non-alcoholic fatty liver disease, the frequency of genotype G in patients with CHD is significantly higher than those without CHD [Du 2016]. Another study has shown that the populations with the genotype GG of adiponectin SNP have 3.06-time risk of possible metabolic syndrome than those with the genotype TT and 3.24-time risk than those with the genotype TT+TG [Li 2015]. This shows that the genotype G is a causative gene of adiponectin. In this paper, we observed the distribution of adiponectin SPNS+276 phenotype, aiming to explore the relationship between its polymorphism and the severity of CHD and coronary artery disease (CAD).

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MATERIALS AND METHODS

Subjects: A total of 582 inpatients in our department were enrolled from June 2014 to April 2016. All patients met the diagnostic criteria for CHD and were confirmed, by coronary angiography, stenosis ≥50% in at least one vessel of the left anterior descending branch, left circumflex artery branch, right coronary artery branch, or their main branches [Eagle 2004]. The patients were divided into group CHD (342 patients) and group CON (240 patients, without obvious stenosis in the above main arteries or stenosis <50%). Exclusion criteria included cardiomyopathy, valvular heart disease, severe liver and renal insufficiency; various acute and chronic infectious diseases, cancer; recent major trauma or major surgery; a history of stroke within six months; or autoimmune disease. There were 326 males and 256 females, with an average age as (66.9 ± 9.58) years. The research subjects all came from non-blood-related Han populations in Luoyang. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Zhengzhou University. Written informed consent was obtained from all participants.

Collection of general information: Specific personnel was assigned to measure the height and weight of the patients and calculated the body mass index (BMI) = weight/height^2 (kg/m^2). Specialists in the department of cardiology were assigned to collect each patient's past medical history on the day of admission.

Detection of adiponectin gene polymorphism: Specimen collection: The fasting blood glucose and lipid were tested on the next morning of admission after emptying the bladder.

Extraction of genomic DNA: 2 mL of peripheral venous blood was extracted from each patient and placed into

Table 1. Comparison of Clinical Data Between the Two Groups

	CHD (N = 342)	CON (N = 240)
M/F (N)	218/124	108/132
Age (years)	67.1 ± 9.42	65.6 ± 9.83
Hypertension	245 (72.06)	166 (69.17)
FBG (mmol/L)	5.92 ± 1.26*	4.14 ± 1.18
Smoking	138 (40.59)	67 (27.92)
BMI (kg/m^2)	25 ± 3.02	24.9 ± 2.66
TC (mmol/L)	4.58 ± 0.76	4.52 ± 0.48
LDL-C (mmol/L)	2.94 ± 0.83	2.80 ± 0.92
HDL-C (mmol/L)	0.95 ± 0.26	0.97 ± 0.32
TG (mmol/L)	1.86 ± 1.23*	1.27 ± 0.67

FBG: fast blood glucose; BMI: body mass index; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglyceride; *Table 4. Relationship of coronary lesions with polymorphism of SPNS+276.

Table 2. Comparison of Genotype SNPs+276G/T Between Group CHD and Group CON

SNPs	N	GG	GT	TT
CHD	342	110 (32%)*	205 (59%)*	27 (8%)*
CON	240	36 (15%)	161 (67%)	43 (18%)

*Table 4. Relationship of coronary lesions with polymorphism of SPNS+276.

one EDTA anticoagulant tube. The Leukocyte DNA was extracted with one blood genomic DNA extraction kit (Beijing Tianwei Time BioTech Co., Ltd.) and measured the absorbance by one UV spectrophotometer for determining the purity and quantitation. The sample was then stored at -20 °C for future use.

Polymerase chain reaction (PCR) primers and synthesis: The Primer 5 biological primer design was used to design the primers, and all the primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. The upstream primer 5'-GTcTcTccTTG-GcTGAcAGTG-3'; the downstream primer: 5'-AAT-CATTCAGGTTGCTTATGGTTA-3'. The PCR reaction system was 20ul, and the procedure was: pre-denaturation at 95°C for 15min; denaturation at 94°C for 30s, annealing at 60°C for 90s, and extension at 72°C for 30s, for 35 cycles; the sample was finally extended at 72°C for 10min and stored at 4°C, the theoretic length of the PCR product is 456bp. The PCR products were analyzed by 3% agarose gel electrophoresis.

Selection of polymorphic specific endonuclease: the endonuclease Bsm I (BioLabs Inc., New Zealand) was selected. The restriction site of BsmI is at 5'-GAAGGCN-3'. When GAAGTC is changed to GAAGGC, the PCR product can be digested into two fragments (82bp and 374bp, respectively). The results of digestion of SNP+276 (G/T mutation): TT type: 82bp and 374bp; G/T heterozygote: 456bp, 82bp, and 374bp: GG homozygote: 456bp.

Table 3. Comparison of Allele Distribution of SNPs+276G/T Between Group CHD and Group CON

SNPs+276	N	Allele G	Allele T
CHD	342	425 (62.1%)*	259 (37.9%)*
CON	240	233 (48.5%)	247 (51.5%)

*P < .05 compared with group CON

Table 4. Relationship of Coronary Lesions with Polymorphism of SPNS+276

Genotype	N	Single vascular lesion	Multi-vessel lesions (including 2 lesions and 3 lesions)
GG	131	67 (33.3%)	64 (45.4%)
GT	160	107 (53.2%)	53 (37.6%)
TT	51	27 (13.5%)	24 (17.0%)

Evaluation of severity of CAD lesions: Coronary angiography was performed using the Judkins method [Plank 2016]. The coronary artery lesions were observed from the conventional projection view. The angiographic images were analyzed by two experienced cardiovascular physicians for evaluating stenosis in the left main branch, left anterior descending branch, left circumflex branch, right coronary artery, and their primary branches. Angiographic stenosis ≥50% in one, two, or three major coronary arteries were defined as single, two, or three lesions.

Statistical analysis: The data were analyzed using SPSS17.0 statistical software; the calculation of the Hardy-Weinberg equilibrium test used the χ^2 test; the measurement data were expressed as ±s and compared using the t test between group; the frequencies of SNPs alleles and SNPs genotypes, as well as the distribution of SNPs genotype combination between group CHD and group CON, were compared using the χ^2 test, with P < .05 considered as statistical significance.

RESULTS

Comparison of general, clinical, and biochemical indicators: There was no significant difference in the gender, age, hypertension, smoking history, BMI, total cholesterol, low density lipoprotein (LDL) cholesterol, or high density lipoprotein (HDL) cholesterol between the two groups, but the fasting blood glucose and triglyceride in group CHD were significantly higher than group CON, and the differences were statistical significance (P < .05) (Table 1).

Comparison of genotype SNPs+276G/T: The frequencies of genotype distribution in group CHD were GG 110 (32%), GT 205 (59%), and TT27 (8%), and GG36 (15%), GT 161 (67%), and TT 43 (18%) in Group CON. The differences between the two groups were statistically significant (P < .05)

(Table 2). The alleles in group CHD were G 425 (62.1%) and T 259 (37.9%). There were statistical significance between the two groups ($P < .05$) (Table 3).

Comparison of allele distribution of SNPs+276G/T: The allele distribution was G 425 (62.1%) and T 259 (37.9%) in group CHD and G 233 (48.5%) and T 247 (51.5%) in group CON, and the differences between the two groups were statistically significant ($P < .05$) (Table 3).

Relationship of coronary lesions with polymorphism of SPNS+276: The frequencies of genotype GG, GT, and TT in the group with single vascular lesion were 67 (33.3%), 107 (53.2%), and 27 (13.5%), and those in the groups with multi-vessel lesions (including two lesions and three lesions) were 64 (45.4%), 53 (37.6%), and 24 (17%), and the comparison showed statistical significance ($P < .05$). The distribution frequency of combined genotype GT+TT was higher in the patients with single vascular lesion than those with multi-vessel lesions (66.7% versus 54.6%, $P < .05$) (Table 4).

DISCUSSION

The Adiponectin gene is located in 3q27, including three exons and two introns, so it has rich SNP polymorphism. This region contains the susceptibility sites of Type 2 diabetes, coronary heart disease, or metabolic syndrome [Melistas 2009; Mohammadzadeh 2009], so the relationship between adiponectin+276 SNPs polymorphism and CHD has always been a hot research topic. Bacci et al [Bacci 2004] has confirmed that the patients carrying SNP+276 T homozygotes have significantly lower risk of CHD ($P < .05$). Qi et al [Qi 2005] also found that the incidence of CHD and cerebrovascular accident in the patients with SNP+276T/T homozygous is significantly lower than those carrying other genotypes.

The present study finds that there exists statistical significance in the genotype frequencies of SNP+276G between group CHD and Group CON: GG 110 (32%), GT 205 (59%), and TT27 (8%) in group CHD and GG36 (15%), GT 161 (67%), and TT 43 (18%) in group CON ($P < .05$). The alleles were G 425 (62.1%) and T 259 (37.9%) in group CHD and G 233 (48.5%) and T 247 (51.5%) in group CON ($P < .05$), suggesting that the 276G gene of adiponectin may be the susceptibility gene of CHS, and is closely related to the incidence of CHD, consistent with Kanu et al [Kanu 2016], in whose study the adiponectin SNPs+276G>T genotype GG+G/T shows significantly increased risk of CHD than TT. However, Jung et al [Jung 2006] did not find the association between SNP 276G/T polymorphism and CHD in 156 Korean patients, and the SNP+276G/T polymorphism of adiponectin shows no statistical significance between group CHD and group CON. The differences existing in the above studies may be related to the differences in their study population and ethnics, and even the population in the same race show different susceptibility to diseases. Furthermore, it can't rule out that the research about the correlation of adiponectin gene polymorphism with CHD is more concentrated toward the populations with common CHD or CHD combined with diabetes, which also mixes diabetes, an important risk factor for CHD [Skrabal 2011].

In addition, this study shows that the genotype frequencies of GG, GT, and TT of SPNS+276 show statistical significance between the patients with single coronary lesion and those with multi-coronary lesions. The frequency of genotype GT+TT is significantly higher in the patients with single coronary lesion than those with multi-coronary lesions (66.7% versus 54.6%, $P < .05$), suggesting that the allele T (GT+TT) carriers have less likelihood of having multiple coronary lesions. The reason may be associated with the fact that the adiponectin gene SNP+276T is related to high plasma adiponectin levels [Menzaghi 2007], and adiponectin can play its protective effect against cardiovascular diseases through anti-inflammation, anti-atherosclerosis, anti-platelet aggregation, and improving insulin resistance [Behre 2008]. In short, CHD is a disease caused by multiple factors and genes, and the cause is too complex to be decided by one gene. The sample number in this study is relatively small, so it still needs further studies.

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