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The relationship between phosphodiesterase 4D gene polymorphism and coronary heart disease

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ABSTRACT

Coronary atherosclerotic heart disease is one of the most common heart diseases that seriously endanger human health. The study found that intracellular second messenger CAMP plays an important role in inhibiting the proliferation and migration of vascular smooth muscle and the local inflammatory response at the damaged vessel. Phosphodiesterase 4D (PDE4D) can specifically degrade cAMP. The purpose of this article is to investigate the relationship between phosphodiesterase 4D gene polymorphism and coronary heart disease and the effect of phosphodiesterase 4D gene polymorphism on cardiovascular, using polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP) was used to detect 50 patients with coronary heart disease (ACS group) and 100 patients who were diagnosed with coronary artery disease by coronary angiography at the same time as the control group (NC group). The results of the study showed that the frequencies of AA, AG, and GG genotypes in 150 samples were 25.67%, 54.66%, and 22.67%, respectively, which was consistent with Hard Weber's law (X = 2.186, P = 0.101). The distribution of GG genotype (18% vs. 27%), AA + AG genotype (85% vs. 74%), G (57% vs. 55%), A (43% vs. 45 %) There was no statistically significant difference in allele frequency (P <0.05). From this, it can be seen that the rs918592 polymorphism of the PDE4D gene is not associated with coronary heart disease.

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Introduction

The transformation of the social form, the developed economy, the people's living conditions are tense and busy, the competition pressure is large, and the eating habits are gradually westernizing. Insufficiency makes the etiology and risk factors of coronary atherosclerotic heart disease (also known as coronary heart disease) more complicated, and there is a trend of younger-onset and increasing incidence year by year, that is, 1 out of every 6 people. There are an adult and vascular diseases. The diseases caused by the rapid changes of the times are also called “modern civilization diseases”. Phosphodiesterase 4D (PDE4D) can specifically degrade cAMP, and this effect may be related to coronary heart disease (1,2).

Coronary atherosclerotic heart disease is a heart disease caused by coronary atherosclerosis that narrows, blocks, or changes the function of a blood vessel, such as coronary artery spasm, causing myocardial ischemia, hypoxia, or necrosis. Wang studied the relationship between the phosphodiesterase 4D (PDE4D) gene single nucleic acid polymorphism and ischemic stroke and proved that the phosphodiesterase 4D (PDE4D) gene polymorphism can affect the cardiovascular and cerebrovascular (1). Bushueva used real-time PCR and TAPMN allelic discriminant analysis to perform phosphodiesterase 4D polymorphism genotyping in 230 coronary heart disease patients and 180 nearly healthy volunteers (2). In the genome-wide association, Shao found that there is a certain correlation between the polymorphism of the phosphodiesterase 4D gene located in PDE9A and

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cell proliferation and cell autophagy (3). Shi obtained clinical data in a case-control study on coronary heart disease and measured the degree of atherosclerosis in patients by ELISA (4). Song investigated the single nucleotide polymorphism at 87 sites of the phosphodiesterase 4D gene in patients with coronary heart disease and conducted relevant experiments on patients with coronary heart disease by polymerase chain reaction-restriction fragment length polymorphism and genetic testing. The genotypes and allele frequencies of all groups were compared (5). In summary, there are many studies on gallbladder cancer, but there are still few studies on gallbladder cancer by transfecting the statin gene with anti-angiogenesis.

Phosphodiesterase 4D (PDE4D) gene is located on the 12th long arm of human chromosome 5 (5q12), spans about 1.6Mb, contains 24 exons, through the expression of different promoters and selective splicing, the gene 9 protein subtypes with different functions can be expressed. There are many studies on phosphodiesterase 4D (PDE4D) gene polymorphism. Ataman explored the relationship between phosphodiesterase 4D gene and interleukin 6 receptor gene polymorphisms and ischemic stroke in hypertensive people and selected the polymorphic loci rs12188950 and rs918592 in PDE4D to analyze the relationship between cases and controls genotype and association (6). Chien believes that the phosphodiesterase 4D gene is an important factor in regulating atherosclerosis in patients with coronary heart disease, so the polymorphism of the phosphodiesterase 4D gene is related to coronary heart disease (7). Yang explored whether the phosphodiesterase 4D gene polymorphism is related to subclinical hypothyroidism in pregnant women. A non-parametric KWAILL detection method was used, and related data analysis was performed using genotyping technology (8). Yako proposed that the phosphodiesterase 4D gene is located on chromosome P31 and is a candidate gene for schizophrenia, which has been confirmed (9). Lee investigated the interaction between the phosphodiesterase 4D gene rs918592 polymorphism, neuromodulated B receptor and progressive epilepsy myoclonus type 2A genes, and introduced their mechanism of action (10). It can be seen that the phosphodiesterase 4D (PDE4D) gene is an important key gene for regulating cardiovascular and cerebrovascular functions in the human body. It is very important to study the polymorphism of the phosphodiesterase 4D gene for the treatment of coronary heart disease.

This article mainly studies the relationship between phosphodiesterase 4D gene polymorphism and coronary heart disease and the role of phosphodiesterase 4D polymorphism in coronary heart disease. In this paper, the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to detect 50 patients with coronary heart disease (ACS group) and 100 patients who were diagnosed with coronary heart disease by coronary angiography during the same period as the control group (NC group). The rs918592 polymorphisms of the PDE4D gene in the blood, comparing the rs918592 polymorphism difference of PDE4D gene between the two groups were done. The results showed that the frequencies of AA, AG and GG genotypes in 150 samples were 25.67%, 54.66%, and 22.67%, which were in accordance with Hard Weber's law ($X = 2.186$, $P = 0.101$). The innovation of this study is the first to take patients with acute atherosclerotic coronary heart disease as the research object according to the hard weber test standard, including patients with the high echo of cardiac artery plaque into the stable plaque group, and patients with mixed and low echo. In the vulnerable plaque group, compare the genetic differences between the different groups, and at the same time, compare the differences in the cardiac artery. IMT between the different positions of the phosphodiesterase 4D gene to explore their atherosclerosis in patients with atherosclerosis-related possible mechanisms.

Materials and methods

Study Object Inclusion and Exclusion Criteria

Inclusion criteria for this study: selected inpatients in Xuzhou Fourth Hospital Affiliated to Xuzhou Medical University in 2018-2019, admitted to hospital with coronary heart disease and confirmed by coronary angiography 50 cases, mean age 55 years, and selected coronary angiography during the same period 100 patients diagnosed with coronary heart disease were excluded as the control group (NC group), with an average age of 58 years. According to the diagnostic criteria of the 2019 Chinese Coronary Heart Disease Association (ACC) / Chinese Heart

Association (AHA), coronary heart disease diagnosis and treatment with guidelines.

Exclusion criteria: patients with severe liver, kidney, brain and lung disease, severe valvular heart disease, cardiomyopathy, rheumatic heart disease, pulmonary heart disease, severe infection, hyperthyroidism, active tuberculosis, hormone or other immunization within the past month patients with inhibitors, tumors, autoimmune diseases, infectious diseases or acute infections, psoriasis, pregnancy, recent skin and tissue damage, etc.

Experimental Materials

The main equipment used in this experiment: high-performance liquid chromatography, AU620 automatic biochemical analyzer, Y1008 electronic balance, pathological image analyzer, arterial cell detector, phosphodiesterase concentration detector, phosphodiesterase status type divider, fluorescent display, PSCX imaging device, automatic blood pressure detector, etc. Other auxiliary equipment and reagents (Table 1) are needed for this experiment.

Table 1. Other auxiliary equipment and reagents

Group	Usage amount	Source
Magnetic stirrer	1	Comay Group
N-butanol	200 ml	Nanjing Biochemical Company
Sulfuric acid	600 mg	Japan Sanwa Kimono
Electrophoresis tank	1	Bosch, Germany
Cholesterol	320 mg	Chemical Materials Company
Cell extraction instrument	1	Sony Group of Japan

All study subjects collected cubital venous blood in the early morning after fasting for 12 hours, and sodium citrate was used for anticoagulation. The DNA was extracted using the EZP column blood genomic DNA extraction kit provided by Shengong Bioengineering (Nanjing) and operated strictly in accordance with the supporting instructions. Store at -50℃ for future use.

Detection of Polymorphism of rs918592 Locus of Phosphodiesterase 4D Gene

Using the allele-specific PCR (ASPCR) method, Primer5.0 primer design software was used to design two upstream primers and a common downstream primer for each SNP, and each DNA sample was subjected to two PCR reactions at the same time. Add two different upstream primers and a common downstream primer (11). PCR and agarose gel electrophoresis detection PCP products PCR primers are designed by software Primer5.0, synthesized by Shanghai Handsome Company, upstream primer F: 5'-CAGAGTGCTGATCAACATTGGT-3 ', downstream primer R: 5'ATGGAGTCCACAGGGCTTTATT-3' (12). The PCR reaction was carried out on the rs918592 locus gene amplification instrument. The 100 μl reaction system included: 1 × PCR buffer, 0.25 μmol / L each of the upstream and downstream primers, about 800 ng of DNA template, and 2 μl of TaqGold enzyme (13). The PCR amplification conditions were: pre-denaturation at 95°C for 5 min, 30°C at 95°C, 45s at 62°C, and 50s at 72°C for a total of 40 cycles. After extension at 72°C for 10 min, PCR products were determined by agarose electrophoresis (14). Assuming that the two bases that constitute the SNP, site are A and B, for each sample, if it is AA homozygous, only reaction 1 has the amplification product; if it is BB homozygous, only reaction 2 has the amplification product. If it is an AB heterozygote, both reactions 1 and 2 should have amplification products (15). Negative control and a blank control are set during operation to verify the reliability of PCR and assist in judging the credibility of the amplification system (16).

SPSS17.0 statistical software was used for analysis, measurement data were expressed as $x \pm s$, and t-test was used. X2 test was used to compare the genotype frequency with Harad-Weinberg balance and categorical variables; the odds ratio (OR) and 95 % CI indicates that the correlation between the rs918592 point polymorphism of the phosphodiesterase 4D gene and the risk of coronary heart disease was analyzed by LOgistic regression analysis. $P < 0.05$ was considered statistically significant (17).

Results and discussion

Comparison of Various indexes between the Patients with Coronary Heart Disease and the Control Group

Comparison of clinical data between the case group and the control group in this study: We conducted a comparative analysis of 50 patients with ischemic stroke and 100 healthy controls (Table 2): the average age of each case group compared with the control group sample. Gender ratio, total cholesterol, triglycerides, and blood glucose were not statistically significant.

Table 2. Comparison of clinical data between case group and control group

Project name	Control group (n=100)	Observation group(n=50)	P value
Gender (men/women)	67/33	32/18	P>0.05
Age	58 ± 0.5	55 ± 0.5	P>0.05
TG	93 ± 1.435	190 ± 2.628	P>0.05
CBOL	128 ± 2.485	193 ± 3.162	P<0.05
BS	106 ± 1.776	214 ± 2.719	P>0.05

Detection Results of rs918592 Polymorphism of Phosphodiesterase 4D Gene

The results of the study showed that the phosphodiesterase 4D gene rs918592 polymorphism PCR product digestion results in 150 samples of AA, AG, GG genotype frequencies were 22.67%, 56.66%, 20.67%, consistent with Hardy Weinberg's law ($\chi 2$ = 2.686, P = 0.101). PDE4D gene rs918592 site PCR amplification product size is 536bp. After restriction enzyme Apal I digestion, homozygous GG genotype is 388, 148bp two fragments, homozygous AA no enzyme cleavage site, is 1 536bp Fragment, after digestion of heterozygous AG genotype, three bands of 148, 388, and 536 bp were seen Figure 1. The heterozygous AG gene has a certain inhibitory effect on the proliferation of vascular cells under the action of phosphodiesterase 4D gene rs918592.

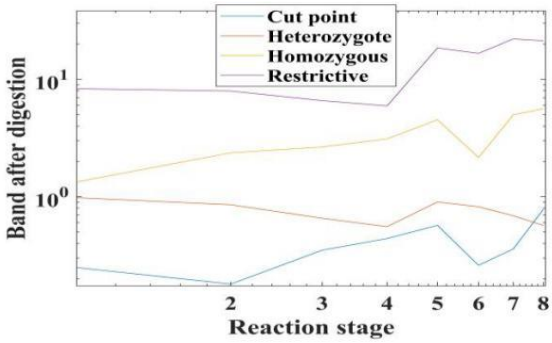

Figure 1. After digestion of heterozygous AG genotype, three bands of 148, 388, and 536bp were seen

Comparison of age, gender composition, hypertension distribution, diabetes distribution, smoking distribution, BMI, TG, TC, LDL-C, HDL-C and other indicators of patients with coronary heart disease and healthy people, we can see that, except for the average age. In addition to the composition of gender and diabetes, the incidence of hypertension, smoking rate, body mass index, TG, TC, LDL-C, HDL-C and other indicators (Figure 2) were statistically significant between the two groups (P <0.05). The incidence of hypertension, smoking rate, body mass index, TG, TC, LDL-C, HDL-C in the coronary heart disease group were significantly higher than that in the control group, proving that diabetes, hypertension, smoking, and obesity are all contributing factors to coronary heart disease.

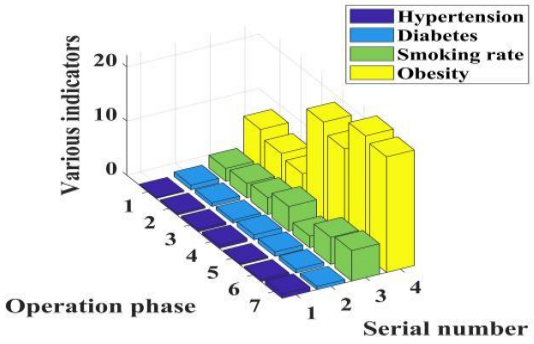

Figure 2. TG, TC, LDL-C, HDL-C and other indicators

Analysis of genotype and allele frequency comparison between coronary heart disease group and control group

In this study, Taqman probe technology was used to detect the rs918592 polymorphism of the phosphodiesterase 4D gene, and it was found that the phenotypes were GG, GA, and AA genotypes. The frequencies of GG, GA, AA genotypes and G and A alleles in patients with non-coronary heart disease were 28.56%, 45.78%, 23.69% and 54.17%, 45.58%, respectively, and the phosphodiesterase 4D of patients with coronary heart disease included in this study. Gene frequency of rs918592 loci polymorphism 34.5%, 43.2%, 22.9% and 58.6%, 47.3% were not significantly different. this paper analyzes the polymorphism of rs918592 locus of PDE4D gene, chi-square test, GG genotype, AA + AG genotype distribution and G, A allele frequency were not significantly different between ACS and NC groups (P> 0.05),as show in Figure 3.

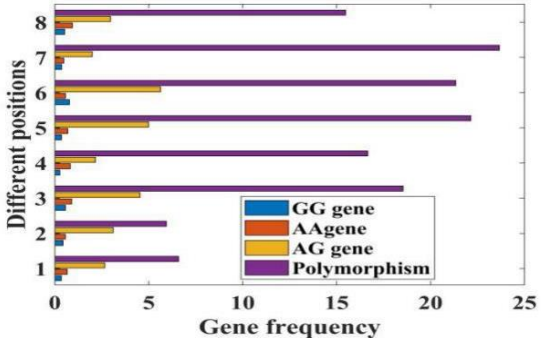

Figure 3. rs918592 locus polymorphism gene frequency

As shown in Figure 4, the results of this study showed that the frequency of PDE4D gene rs918592 polymorphism gene and allele frequency in patients with coronary heart disease was somewhat different from that in the control group, but the difference was not statistically significant (P> 0.05); regression analysis was used to exclude coronary heart disease. The influence of traditional risk factors did not show a correlation (P> 0.05), suggesting that the PDE4D gene rs918592 has nothing to do with the susceptibility to coronary heart disease. This conclusion suggests that the effect of the PDE4D gene on cardiovascular and cerebrovascular diseases is mainly achieved through "PDE4D gene-PDE4D protein expression-cAMP level-atherosclerosis", and the polymorphism gene at rs918592 does not show a crown.

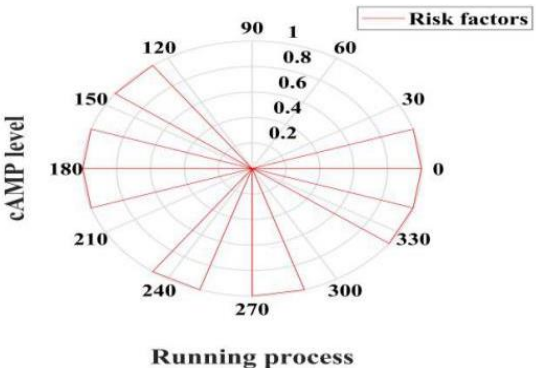

Figure 4. Traditional risk factors for coronary heart disease

Phosphodiesterase (PDE) is a large multi-gene family, consisting of 11 different families, and each family contains different types (4). Each subtype is distributed, expressed, regulated, and sensitive to inhibitors in the cell. Differently, they are involved in the occurrence and development of various pathological processes such as inflammation, asthma, depression, and erectile dysfunction (6). These characteristics have made PDE more and more attention as a new drug target. Phosphodiesterase 4D is a member of the PDE superfamily. The phosphodiesterase 4D gene is located at 5q12 and is about 1.5 Mb in length, with at least 22 exons and encoding at least 9 different isomers (5). The study found that there is no correlation between the activity of the peripheral blood phosphodiesterase 4D gene in coronary heart disease and the severity of cerebral infarction (P> 0.05). Consider the severity of coronary heart disease and the location, scope, and accompanying of cardiovascular infarction disease, treatment and other factors are related, so peripheral blood phosphodiesterase 4D gene activity is not related to the severity of myocardial infarction but may be related to inheritance, such as PDE4D gene polymorphism. In addition, the genomic screening, linkage analysis and haploid analysis of Xuzhou families with a family history of coronary disease showed that the PDE4D gene is associated with coronary heart disease. Especially cardiovascular arterial and cardiogenic myocardial infarction related to atherosclerosis-related, but not related to acute coronary heart disease, so he speculates that the PDE4D gene may cause upregulation of multiple PDE4D isomers, thereby playing an important role in the occurrence and development of atherosclerosis, caused by atherosclerosis myocardial infarction. But there is very little research in this area, and we need further research in this area.

Coronary atherosclerotic heart disease refers to heart disease caused by myocardial ischemia, hypoxia or necrosis due to coronary atherosclerosis that narrows or occludes the lumen, collectively referred to as coronary heart disease or coronary artery disease, referred to as coronary heart disease, attributed to ischemic heart disease, is the most common type of organ disease caused by atherosclerosis (18). The coronary artery structure (Figure 5) is divided into left and right branches, which start from the left and right coronary sinus of the aorta. The main trunk of the left coronary artery is 5 to 10 mm long, and the anterior descending branch and the left circumflex branch are separated from the lower edge of the left main trunk (19). The anterior descending branch and its branches are distributed in the anterior wall of the left ventricle, the anterior papillary muscle, the apex, a small part of the anterior wall of the right ventricle, the anterior 2/3 of the ventricular septum, and the right and left bundle branches of the cardiac conduction system. The branches and their branches are distributed in the left atrium, a small part of the anterior wall of the left ventricle, a part or most of the left ventricular sidewall, and a part or most of the posterior wall of the left ventricle, and even reach the posterior papillary muscle of the left ventricle (20). The right coronary artery is generally distributed in the right atrium, most of the anterior wall of the right ventricle, all of the right and left ventricular sidewalls, and a part of the posterior wall of the left ventricle and the posterior third of the interventricular septum and sinus

node (21). There may be traffic branches between adjacent major coronary arteries (22).

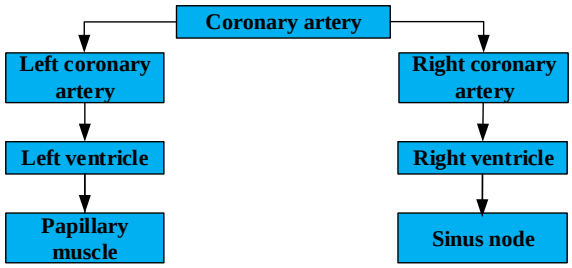

Figure 5. Coronary artery structure

Coronary atherosclerosis can affect the main coronary arteries at the same time or separately. The degree and location of the stenosis determine the ischemic symptoms and prognosis (23). When the luminal stenosis is less than 50%, the myocardial blood supply is generally not affected. When the luminal stenosis is 50% to 75%, the myocardial blood supply is not affected at rest, but during exercise, tachycardia or excitement, the heart oxygen consumption increases can temporarily cause insufficient myocardial blood supply, leading to chronic stable angina (CSA). When atherosclerotic plaque ruptures, erosion, or hemorrhage, the formation of blood clots can cause acute myocardial infarction (AMI) (24). AMI belongs to a special type or advanced stage of coronary heart disease, which refers to myocardial localization or diffuse fibrosis due to long-term myocardial ischemia, resulting in impaired cardiac contraction and/or diastolic function, causing enlarged or stiff heart, congestion heart failure, arrhythmia and other clinical manifestations of the syndrome, its clinical manifestations are similar to idiopathic dilated cardiomyopathy (25-27).

This article believes that the PDE4D gene mainly causes the occurrence of ischemic stroke by promoting the formation of atherosclerosis. Some scholars believe that the increased expression of the phosphodiesterase 4D gene can cause vascular smooth muscle hyperplasia, migration and local inflammation of blood vessels to intensify, thereby promoting the formation of atherosclerosis and leading to increased instability of vascular plaque. This study analyzed the PDE4D gene rs918592 polymorphism. The results showed that there was no statistically significant difference in genotype and allele frequency between the coronary heart disease group and the control group (P> 0.05). In other studies, the research team found that the PDE4D protein levels in the coronary heart disease group and the control group were different, and the PDE4D protein level in the blood of patients with coronary heart disease was higher. The author speculates that this result may be due to multiple polymorphisms in the PDE4D gene. In summary, the rs918592 polymorphism of the PDE4D gene has nothing to do with coronary heart disease.

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None.

Interest conflict

None.

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