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Original Article

Correlation between single nucleotide polymorphisms of *ATM* and coronary artery disease susceptibility

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Abstract: Aims: The planning of this study was to explore the correlation between ataxia-telangiectasia mutated (*ATM*) gene rs373759, rs664143 polymorphisms and the occurrence risk of coronary artery disease (CAD). Methods: 98 patients with CAD and 109 healthy volunteers who were matched with the former by age and gender, were enrolled in this study. The single nucleotide polymorphisms (SNPs) of *ATM* were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The distribution difference of SNP genotypes and alleles between case and control groups was checked by the chi-square test and genotype frequencies difference in control group was assessed by Hardy-Weinberg equilibrium (HWE). The susceptibility of CAD was expressed by odds ratio (OR) and 95% confidence interval (CI). The linkage disequilibrium (LD) and haplotype analyses were conducted based on haploview software. Results: The AA genotypes of *ATM* rs373759 between case and control groups had significant difference (*P*=0.040), and AA genotype carriers was easily subject to CAD, compared with GG genotype (OR=2.428, 95% CI=1.031-5.718). In addition, two SNPs were strong LD and haplotype $G_{rs373759}$-$C_{rs664143}$ were more frequent in control group (*P*=0.001) and might be a protective factor for CAD (OR=0.366, 95% CI=0.196-0.682). Conclusion: This study discovered that *ATM* rs373759 polymorphism is likely to be associated with the increased risk of CAD, but not rs664143. Besides, $G_{rs373759}$-$C_{rs664143}$ haplotype plays a protective role in CAD.

Keywords: *ATM*, polymorphism, CAD, haplotype

Introduction

Coronary artery disease (CAD), as a common disease, results from the abnormal blood and oxygen supply to the hear due to atherosclerotic coronary artery [1-3]. Its morbidity is in the first place in cardiovascular disease (CVD) around the world with the younger-age trend [4]. The clinical symptoms of CAD include subclinical myocardial ischemia (latent CAD), stenocardia, myocardial infarction, ischemic heart failure (ischemic heart disease), and sudden death [5]. The pathogenesis of CAD is still unclear and plenty of studies have proved that this disease is caused by multi-factors, such as genetic factors and related environmental factors like stature, weight (obesity), regional diversity, dyslipidemia, hypertension, diabetes, smoking, alcohol intake [6-8]. It has been verified in population from different regions and hierarchies in the world [9]. CAD is predicted that cardiovascular disease is still the leading cause of death throughout the country in the next few years [10].

Ataxia-telangiectasia mutated (ATM) gene is a type of gene which is found in the research of ataxia-telangiectasia (AT) by Shiloh and Rotman in 1995. It is located in human chromosome 11q22-11q23 and encodes a kind of protein composed of 3056 amino acid residues, namely ATM protein belonging to phosphatidylinositol 3-kinase (PI3K) family [11-13]. The carboxyl terminal of ATM protein possesses serine/threonine protein kinase activity and involves in various signaling pathways, including DNA damage/repair, the regulation of cell cycle, restructuring of meiosis, reducing the oxidative stress reaction, and protecting telomeres [14]. All normal cells have two main cell cycle checkpoints activated by the signal transduction system, called G1/S phase and G2/M phase; meanwhile, *ATM*

Table 1. Primer sequences of *ATM* rs373759, rs664143 polymorphisms

SNP	Primer sequence		Annealing temperature
rs373759	For.	5'-GCTGAGAGAGAAGGAAAAGTGATGC-3'	60°C
	Rev.	5'-AATAGGAAAGTCTTAAGCTGGAGAG-3'	
rs664143	For.	5'-ATCCTGGCTGACTCCTTCTT-3'	58°C
	Rev.	5'-TCCTCTAGTAATGGATGCTGACAAT-3'	

gene plays an important part in this process. Furthermore, DNA damage can cause diverse diseases including cancer, senility, neurodegenerative disease, cardiovascular disease, and other tissue toxicity. The study indicates that high expression of *ATM* gene regulated by polymorphism can prevent relative oxidative stress response of coronary artery endothelial cells and diabetes in animal experiment, and reduce the risk of coronary stenosis caused by diabetes [15].

However, the report about the relationship of *ATM* polymorphism with CAD is few. In this research, we chose and analyzed the correlation between rs373759, rs664143 SNPs of *ATM* and CAD risk and provided basic prediction for clinical CAD risk groups and individual-based treatment.

Materials and methods

Research objects

98 CAD patients diagnosed by coronary angiography in X-ray departments of The First Affiliated Hospital, Harbin Medical University from June, 2012 to June, 2013 were enrolled as the case group. The inclusion criteria of CAD patients were at least one main coronary artery branch's stenosis degree was more than 50%, or had the history of myocardial infarction. 109 healthy people were from physical examination center of The First Affiliated Hospital, Harbin Medical University, who were matched with the cases in terms of age and gender. They all performed the comprehensive physical examination and excluded the individuals who might have got hypertension, diabetes, ischemic cardiomyopathy, chronic heart failure and tumors.

Case report form was designed in our study and the gender, age, blood pressure, smoking, drinking, body mass index (BMI), along with disease history of diabetes, hyperlipidaemia, liver and renal diseases from every subject were recorded in detail by questionnaire. At the same time, biochemical index including blood glucose, blood fat were recorded as well. All people from the case and control groups were Chinese Han population. Plans of this project were reviewed by Medical Ethics Committee of The First Affiliated Hospital, Harbin Medical University. All selected individuals were informed the research process and signed informed consents.

Sample collection

2 ml peripheral venous blood of fasting participants was extracted and collected in centrifuge tube with EDTA anticoagulant. And then genome DNA of all samples was extracted using blood genome DNA extraction kit bought from Beijing TIANGEN biochemical Co. Ltd., according to the manufacturer's instructions. Finally, the DNA was stored at -20°C for standby application.

Genotyping based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

According to *ATM* gene sequences and primer design optimization principles provided by Genebank database from NCBI website, we deigned PCR primers of rs373759 and rs664143 using Primer Premier 5.0 software. Primer was synthesized by Shanghai Sangon Biotech Co., Ltd. and the detailed information was shown in **Table 1**. A volume of 25 μl PCR amplification system was prepared on the ice, including each 1 μl of the former and reverse primers, 0.5 μl TaqDNA polymerase, 1 μl DNA template, 5 μl 10× Buffer buffer solution, 1 μl dNTPs, and 15.5 μl ddH_2O. The whole PCR program was as follows: 95°C predenaturation for 3 min; and then 35 cycles of 94°C denaturation for 30 s, 60°C (rs373759) and 58°C (rs664143) annealing for 35 s, and 72°C extension for 90 s; 72°C final extension for 8 min and preserved at 4°C.

The restriction enzymes *Xho* I (rs373759) and *Xba* I (rs664143) were used to digest the PCR products. The enzyme digestion system was a total of 20 μl solution, including the above-mentioned 0.2 μl restriction enzyme, 10 μl PCR products, 2 μl 10× Buffer buffer solution, and 7.8 μl sterilization double distilled water. The

Table 2. Genotype and allele distribution comparison of *ATM* gene rs373759, rs664143 polymorphisms

Genotype/allele		Case n=98 (%)	Control n=109 (%)	χ^2	*P*	OR (95% CI)
rs373759	GG	30 (30.61)	46 (42.20)	-	-	1.000
	GA	49 (50.00)	51 (46.79)	1.584	0.208	1.473 (0.805-2.696)
	AA	19 (19.39)	12 (11.01)	4.222	0.040	2.428 (1.031-5.718)
	G	109 (55.61)	143 (65.60)	-	-	1.000
	A	87 (44.39)	75 (34.40)	4.319	0.038	1.522 (1.023-2.263)
rs664143	CC	28 (28.57)	32 (29.36)	-	-	1.000
	TC	48 (48.98)	59 (54.13)	0.051	0.822	0.930 (0.493-1.753)
	TT	22 (22.45)	18 (16.51)	0.667	0.414	1.397 (0.626-3.119)
	C	104 (53.06)	123 (56.42)	-	-	1.000
	T	92 (46.94)	95 (43.58)	0.471	0.493	1.145 (0.777-1.688)

Table 3. Analyses of LD and haplotypes in alleles of *ATM* gene rs373759, rs664143 polymorphisms

Haplotype SNP1-SNP2	G-T	A-C	G-C
Case 2n=196 (%)	92 (46.94)	87 (44.39)	17 (8.67)
Control 2n=218 (%)	95 (43.58)	75 (34.40)	48 (22.02)
χ^2	-	0.705	10.435
P	-	0.401	0.001
OR (95% CI)	1.000	1.198 (0.786-1.826)	0.366 (0.196-0.682)

Note: SNP1: rs373759; SNP2: rs664143.

enzyme digestion products were separated by 2% agarose gel electrophoresis (AGE) and stained with ethidium bromide.

Statistical analysis

Direct counting method and chi-square test were used to compare frequency differences of rs373759, rs664143 SNPs in this study and check genotype distribution in the control group whether conformed to Hardy-Weinberg equilibrium (HWE). All data in this article were expressed by $\bar{x}\pm s$ or n (%). The correlation between genotype, allele and CAD susceptibility were analyzed and represented by odds ratio (OR) with corresponding 95% confidence interval (CI). All statistical analyses applied PASW Statistics 18.0 software, and $P<0.05$ had statistically significance meaning. Besides, the linkage disequilibrium (LD) and haplotype were also analyzed according to haploview software and the article of Song et al. [16].

Results

Clinically detailed information of all subjects

This study totally covered 98 cases diagnosed by coronary angiography, including 62 males and 36 females, their age range was 32~78 with the mean age of 56.08±11.23. At the same time, 67.89% of 109 controls recorded in this study were males and only 32.11% were females, their mean age was 55.16± 10.16 with the age range of 32~74. According to χ^2 test, there was no obvious distribution difference between the cases and controls in terms of age and gender ($P>0.05$). Besides, BMI, blood lipid level, hypertension history, and alcohol, cigarette intake history of two groups had no obvious difference, either. Additionally, the genotypes distribution of *ATM* rs373759, rs664143 in the control group conformed to HWE ($P>0.05$) which indicated our study population was a Mendelian population and had a representativeness.

Correlation between ATM gene polymorphisms and susceptibility of CAD

The genotype and allele distribution s of *ATM* rs373759, rs664143 polymorphisms were displayed in **Table 2**. The genotype frequencies of rs373759 were 30.61%, 50.00%, 19.39% for the cases and 42.20%, 46.79%, 11.01% for the controls, the order of genotypes from left to right was GG, GA, AA respectively. AA genotype had a higher frequency in cases than controls and its carriers were high risk to suffer from CAD, compared with GG genotype carriers (OR=2.428, 95% CI=1.031-5.718), so was A allele (OR=1.522, 95% CI=1.023-2.263). Referring to rs664143 polymorphism, we found that

there was no significant difference between the cases and controls based on genotypes and alleles (*P*=0.822, 0.414, 0.493, respectively).

Haplotype analysis of ATM gene

Between *ATM* rs373759 and rs664143 polymorphisms existed the strong linkage disequilibrium (LD) (D'=1.0, r^2=0.677), a total of three haplotypes for rs373759-rs664143 were identified, namely G-C, A-C, and G-T haplotypes. The frequency of haplotype G-C in cases was obviously lower than that of in controls (8.67% & 22.02%, *P*=0.001) and it significantly decreased the susceptibility to CAD, compared with haplotype G-T (OR=0.366, 95% CI=0.196-0.682), which indicated that haplotype G-C might play a protective in the development of CAD independently.

Discussion

In recent years, the study emphasis of cardiovascular disease has been converted from clinic to more basic researches, trying to know more about ethology and pathology of this disease so as to better prevent the occurrence of disease and look for new and effective treatment methods. CAD is a kind of polygenic inheritable disease with complicated pathogenesis. Its basic physiological changes are derived from atherosclerosis and thrombosis caused by lipid accumulation, inflammatory cells invasion, and NO accumulation. Oxidative stress reaction has been proved to be an important part in the pathological process of CAD [17, 18]. What's more, with the improvement of people's living standard and life style change in our country, obesity, hypertension, and diabetes as the risk factors of CAD are increasingly prevalent. The data from randomized trials and clinical experience demonstrate that active and multiform treatment aiming at ameliorable risk factors of CAD can reduce or even eliminate the occurrence of major adverse cardiac event [19].

ATM is the main disease-causing gene of ataxia-telangiectasia. Its C-terminal contains FRAP, ATM, and TRAPP domains (also called FATC domain) with 35 highly conserved residues, which play a key role in regulating *ATM* kinase activity and combining other regulatory proteins [20]; N-terminal contains HEAT domain which lacks of distinctive helical structures and are apart from kinase functional area. The latter was identified as binding domains of enzymes and that's why it may affect the interaction of ATM with other proteins. In addition, ATM also covers some functional areas of helical and superhelical structures with uncertain functions. ATM protein mainly expresses in cell nucleus, however, studies also point out that it has different location in different tissues and cells: referring to proliferation cells, ATM mainly expresses in nucleus, but when it's in differentiated cells like oocytes and cerebral neuronsin, it is expressed in cytoplasm [21].

When the DNA damage occurs in cells, ATM protein is activated and phosphorylates multiple proteins which participating in cell cycle control and repair of DNA damage, consisting of tumor suppressor proteins p95, Brca1, p53 and cell cycle regulation kinase chk2, SMC1, BLM and FANCD2 [22]. Therefore, as a recognition factor, it can detect the damaged DNA and activate a series of repairing reaction [23]. Furthermore, *ATM* also a kind of tumor suppressor gene and several studies show that its germline mutation and polymorphisms may increase the susceptible to various tumors [24]. For the past few years, many studies also indicated that single nucleotide polymorphisms (SNPs) of *ATM* gene might influence its function and then affect the sensitivity of cells to radiation, which is closely related to the occurrence of tumor [25-27]. *ATM* mutation has high heterogeneity with complicated mutational pattern and detection. More than 400 mutant types of *ATM* gene have been reported abroad until now and they are divided into two kinds: one is AT homozygote (*ATM* double chain mutation) and another is AT heterozygote (*ATM* single strand mutation). Most AT patients who don't show the typical features of AT are *ATM* heterozygotes with the incidence of 0.35%~1.00% in population, compared with normal people, the incidences of its tumor, insulin resistance and ischaemic heart disease significantly increase [28, 29]. Feng et al. demonstrate that the activity of ATM protein kinase and *p53* which *ATM* relies on decrease with the increasing age of mice and the latter can accelerate the formation of atherosclerosis [30].

SNP is the most common genetic variation in humans. As a genetic marker, SNP plays an important part in research of medical genetics with the rapid development of genetics and

genomics [31], especially attracting extensive attention in the study of CAD. Since the 21st century, genome-wide association study (GWAS) of CAD has achieved dramatic development. The association of SNPs discovered in whole-genome with various phenotypes and clinical events of CAD has become the hot spot of current research [32]. In this study, two SNPs rs373759, rs664143 in *ATM* were identified the association with CAD. In rs373759, AA genotype had a higher frequency in cases than controls and its carriers were high risk for suffering from CAD, compared with GG genotype. Furthermore, A allele also showed an association with CAD occurrence. But rs664143 didn't reveal an independently significant association with CAD risk. In addition, these two SNPs presented the strong LD and haplotype $G_{rs373759}$-$C_{rs664143}$ significantly decreased the susceptibility to CAD, compared with $G_{rs373759}$-$T_{rs664143}$ haplotype.

In conclusion, as a CAD risk predictor, *ATM* polymorphism may be used to discover early CAD by the way of testing non-diagnostic group. However, the genotype and allele distributions of *ATM* polymorphism are associated with region, race, and environment. That is why we need to conduct studies on larger-scale population with different races to prove this result. The occurrence of CAD is complicated and includes multiple genes which may only play small role, even combines with individual behaviors (like smoking, high fat diet) and environmental factors. Gene-gene, gene-environment, and even polymorphisms of the same gene all can regulate CAD development. That also needs further research.

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Disclosure of conflict of interest

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