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# Functional polymorphism rs189037 in the promoter region of *ATM* gene is associated with angiographically characterized coronary stenosis

Sheyu Li[a,b,1], Li Zhang[a,1], Tie Chen[c,1], Baochun Tian[a], Xiangbing Deng[a], Zhuoxian Zhao[a,b], Ping Yuan[d], Birong Dong[c], Yonggang Zhang[a,b,c], Xianming Mo[a,b,c,*]

[a] *Department of Cardiology, Laboratory of Stem Cell Biology, State Key Laboratory of Biotherapy, West China Hospital, West China Medical School, Sichuan University, Chengdu 610041, China*

[b] *Department of Endocrinology, Laboratory of Stem Cell Biology, State Key Laboratory of Biotherapy, West China Hospital, West China Medical School, Sichuan University, Chengdu 610041, China*

[c] *Department of Geriatrics, Laboratory of Stem Cell Biology, State Key Laboratory of Biotherapy, West China Hospital, West China Medical School, Sichuan University, Chengdu 610041, China*

[d] *Department of Epidemiology, West China College of Public Health, Sichuan University, Chengdu 610041, China*

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ABSTRACT

*Objectives:* To evaluate the association between the single nucleotide polymorphism rs189037 of the ataxia-telangiectasia mutated (*ATM*) gene and angiographically characterized coronary stenosis as well as the molecular basis of this association.

*Results:* In 562 patients treated at the Department of Cardiology, West China Hospital, a significant association was found between polymorphism rs189037 and angiographically characterized coronary stenosis. For the T versus C allele, the adjusted OR was 0.79 (95%CI 0.67–0.92, $P = 0.003$), using the allele frequency model; for TT versus CT/CC, the adjusted OR was 0.36 (95%CI 0.21–0.59, $P = 0.00006$), using the recessive model; and for TT/CT versus CC, the adjusted OR was 0.54 (95%CI 0.29–1.02, $P = 0.06$), using the dominant model. An antagonism was found between polymorphism rs189037 and diabetes mellitus ($P = 0.003$). In coronary artery disease (CAD) patients, the TT genotype of rs189037 was associated with higher ATM mRNA expression ($F = 4.23$, $P = 0.02$) in peripheral mononuclear cells than the CC or CT genotypes.

*Conclusion:* Polymorphism rs189037 may influence the expression of ATM mRNA in CAD patients. It is also associated with the degree of coronary stenosis. Moderately low expression of the *ATM* gene may be associated with the development of coronary atherosclerosis.



## 1. Introduction

Coronary artery disease (CAD) is a prominent illness and among the leading causes of death in the world [1]. It is now clear that CAD mainly results from atherosclerosis, a chronic fibroproliferative-inflammatory response of the arterial intima, which leads to the formation of atherosclerotic plaques [2]. Recent evidence has shown that oxidative stress-induced DNA damage and telomere dysfunction, which may activate the p53 pathway, play an important role in the pathogenesis of atherosclerosis [3,4].

The product of the ataxia-telangiectasia mutated (*ATM*) gene, which is a critical protein in the p53 pathway, has been reported to be a nuclear protein involved in several signaling pathways, including DNA damage recognition, cell cycle control, and meiotic recombination [5]. Homozygous loss of the *ATM* gene causes ataxia telangiectasia (AT), a neurological autosomal-recessive disorder. Various epidemiological studies have suggested that the rate of ischemic heart disease-related mortality is significantly higher among heterozygous ATM carriers than among the general population [6,7]. In animal experiments, coronary and aortic atherosclerosis and its associated diseases have been observed in ATM-deficient mice [8], suggesting that the decreased expression level of ATM is a risk factor for vascular diseases. However, whether common genetic polymorphisms of ATM contribute to the pathogenesis of atherosclerosis remain unknown.

Previously, we identified rs189037, a single nucleotide polymorphism (SNP) in the promoter of the *ATM* gene, is associated with human longevity, and we demonstrated that the polymorphism may affect the expression of ATM mRNA by differentially binding to activator protein 2α (AP-2α) [9]. In this study, we aimed to investigate the association between polymorphism

* Corresponding author at: Laboratory of Stem Cell Biology, West China Hospital, West China Medical School, Sichuan University, No. 1, Keyuan 4 Lu, High-Tech District, Chengdu 610041, China. Tel.: +86 28 85164017; fax: +86 28 85164047.
*E-mail address:* xmingmo@yahoo.cn (X. Mo).

[1] The first three authors contributed equally to the current study.

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**Table 1**
Clinical characteristics of patients in the study and the association between the rs189037 polymorphism and coronary stenosis.

| Variable | Patient group | | *P* value |
|---|---|---|---|
| | SCS+ *N* = 366 | SCS− *N* = 196 | |
| Age, year | 65.4 ± 9.8 | 62.8 ± 9.8 | 0.003 |
| Male, *n* (%) | 303 (82.8) | 125 (63.8) | <0.001 |
| Diabetes mellitus, *n* (%) | 86 (23.5) | 29 (14.8) | 0.015 |
| Hypertension, *n* (%) | 206 (56.3) | 77 (39.3) | <0.001 |
| Dyslipidemia, *n* (%) | 140 (38.3) | 50 (25.5) | 0.002 |
| Smoking, *n* (%) | 200 (54.6) | 73 (37.2) | <0.001 |
| Family history, *n* (%) | 29 (7.9) | 14 (7.1) | 0.74 |
| WBC, $\times 10^9$/L | 6.7 ± 2.2 | 6.0 ± 2.3 | 0.001 |
| Platelets, $\times 10^9$/L | 146.3 ± 60.1 | 144.3 ± 51.7 | 0.71 |
| Total cholesterol, mmol/L | 4.7 ± 1.0 | 4.0 ± 1.2 | <0.001 |
| LDL-C, mmol/L | 2.9 ± 0.8 | 2.4 ± 0.9 | <0.001 |
| HDL-C, mmol/L | 1.1 ± 0.4 | 1.3 ± 0.4 | <0.001 |
| Triglyceride, mmol/L | 1.8 ± 1.3 | 1.8 ± 1.2 | 0.55 |
| Genotypes, % | | | |
| TT | 58 (15.8) | 62 (31.6) | |
| CT | 181 (49.5) | 78 (39.8) | |
| CC | 127 (34.7) | 56 (28.6) | <0.001 |
| T allele frequency, % | 40.6 | 51.5 | <0.001 |

| | OR (95%CI) | *P* value |
|---|---|---|
| Recessive model | | |
| Unadjusted | 0.41 (0.27–0.61) | 0.00001 |
| Adjusted | 0.38 (0.23–0.62) | 0.0001 |
| Dominant model | | |
| Unadjusted | 0.75 (0.52–1.10) | 0.14 |
| Adjusted | 0.74 (0.47–1.17) | 0.20 |
| Allele model | | |
| Unadjusted | 0.64 (0.50–0.82) | 0.0004 |
| Adjusted | 0.79 (0.68–0.93) | 0.003 |

Normally collected clinical characteristics of age, WBC, and platelets are given as the average values ± standard deviation, and other values are given as the number of individuals with percentages in parentheses. Adjusted ORs and *P* values were corrected for age, gender, WBC, HDL-C, smoking, family history of CAD, and histories of hypertension, dyslipidemia, and diabetes mellitus. SCS+: with significant coronary stenosis; SCS−: without significant coronary stenosis; WBC: white blood cell count, LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; CI: confidence Interval; OR: odds ratio.

rs189037 and angiographically characterized coronary stenosis in the Han Chinese population and suggest a molecular basis for this association.

## 2. Materials and methods

This study was approved by the Ethics Committee of the West China Hospital, Sichuan University (Chengdu, China). Written, informed consent was obtained from all participants.

In order to examine the association between the rs189037 locus and stenosis, we recruited 562 Han Chinese patients consecutively from the Department of Cardiology, West China Hospital between September 2007 and January 2010. The inclusion criteria were as follows: (1) established or suspected CAD with a Class I recommendation of AHA/ACC for angiography [10]; (2) selective coronary angiography with or without stents; and (3) a willingness to participate in the research. Patients with unrelated chronic diseases were excluded from the study. The patients were divided into 2 groups, those with and without significant coronary stenosis (SCS+ and SCS−, respectively). Patients with at least one lesion showing >50% diameter stenosis were considered as SCS+, while all others were considered as SCS− [11,12].

DNA was purified using resin DNA purification kits (Beijing SBS Genetech Co., Ltd., Beijing, China) within 1 month after the blood samples were collected. The forward and reverse primers were 5′-GCTGCTTGGCGTTGCTTC-3′ and 5′-CATGAGATTGGCGGTCTGG-3′, respectively. The PCR cycling conditions used were as follows: 94 °C for 4 min; 32 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s; and a final extension of 72 °C for 5 min. The PCR products were digested with SacII at 37 °C overnight. Finally, the products were resolved by electrophoresis on a 10% polyacrylamide gel and then stained with silver nitrate. The genotypes were determined as follows: three fragments of 46 bp, 116 bp, and 125 bp for the CC genotype; two fragments of 125 bp and 162 bp for the TT genotype; and four fragments of 46 bp, 116 bp, 125 bp, and 162 bp for the CT genotype. The results were entirely consistent with the direct sequence obtained from an ABI PRISM 3730 automatic sequencer (data not shown).

In order to examine the mRNA expression of the *ATM* gene in CAD patients, we also recruited 36 Han Chinese patients with acute coronary syndrome (ACS) admitted to the West China Hospital of Sichuan University from September 1st to October 31st 2010. Total RNA was extracted using the QIAamp RNA Blood Mini Kit (Qiagen) from peripheral mononuclear cells isolated from blood samples with lymphocyte-separating medium (Institute of Hematology, Chinese Academy of Medical Sciences, Tianjin, China), and reverse-transcribed into cDNA (PrimeScript RT-PCR Kit, TaKaRa, Japan). The forward and reverse primers were 5′-GCTCCTAATCCACCTCAT-3′ and 5′-CTCCTCCTAAGCCACTTT-3′, respectively. The PCR cycling conditions were as follows: 94 °C for 3 min; 30 cycles of 94 °C for 30 s, 51 °C for 30 s, 72 °C for 30 s; and a final extension of 72 °C for 5 min. The PCR product (256 bp) was visualized on a 2% agarose gel by staining with ethidium bromide. Semi-quantitative PCR was performed as a limited dilution of templates, and analysis of the amplified products was performed using an image analyzer (ChemiDoc™ XRS, BIO-RAD, USA).

The overall power of the test in our study (562 cases) was 0.988, when the odds ratio (OR) is 0.407 in the recessive model of the univariate chi-square test. The allele frequencies in both groups were found to conform to Hardy–Weinberg equilibrium proportions based on the chi-square test. Genotypes and allele frequencies