

Expression level of lncRNA PVT1 in serum of patients with coronary atherosclerosis disease and its clinical significance

W. QUAN¹, P.-F. HU², X. ZHAO², C.-G. LIANHUA³, B.-R. BATU⁴

¹Department of Cardiovascular, Affiliated Hospital of Inner Mongolia University for Nationalities, Tongliao, China

²Department of Mongolian-Cardiovascular Medicine, Affiliated Hospital of Inner Mongolia University for Nationalities, Tongliao, China

³Chemistry and Chemical Engineering College, Inner Mongolia University for Nationalities, Tongliao, China

⁴Department of Mongolian Medicine Hematology & Oncology, Affiliated Hospital of Inner Mongolia University for Nationalities, Tongliao, China

Abstract. – OBJECTIVE: The purpose of this study was to detect the expression level of PVT1 in the serum of coronary artery disease (CAD) patients, and to explore the clinical significance of PVT1 in CAD.

PATIENTS AND METHODS: A total of 200 CAD patients and 200 healthy controls in the same period were included. The serum level of PVT1 in every subject was detected. Then, the correlation between PVT1 level and Gensini score in CAD patients was analyzed by Spearman correlation test. Finally, multivariable Logistic regression test was conducted to assess risk factors influencing coronary atherosclerosis disease.

RESULTS: It was found that PVT1 was highly expressed in the serum of CAD patients and its level was correlated with Gensini score ($r=0.761$, $p=0.023$). Besides, multivariable Logistic regression test obtained that PVT1 was the risk factor influencing coronary atherosclerosis disease (crude OR = 2.074, 95% CI: 1.642-3.529; adjusted OR = 1.762, 95% CI: 1.382-2.096).

CONCLUSIONS: Serum level of PVT1 helps to distinguish mild and severe CAD. Meanwhile, PVT1 is an independent risk factor influencing the development of coronary atherosclerosis disease.

Key Words:

Coronary artery disease, Atherosclerosis, PVT1, Hallmark.

Introduction

Coronary artery disease (CAD) is the number one fatal disease in the world¹. Acute myocardial

infarction (AMI), which is the major reason for death, occurs mostly due to CAD².

As a chronic inflammatory disease, atherosclerosis is the main pathogenesis of CAD³. Risk factors for cardiovascular diseases, including hypertension, dyslipidemia, smoking, and excessive alcohol consumption, lead to dysfunction in vascular endothelium and oxidative stress. Eventually, formed foam cells and proliferated smooth muscle cells migrate under the endothelium, thus promoting the formation of fibrous plaques. The exacerbated inflammatory reactions trigger plaque rupture and thrombosis, thereafter, resulting in the acute coronary syndrome. Atherosclerosis is a complicated pathological process involving different types of cells and factors⁴.

Long non-coding RNAs (lncRNAs) are non-coding RNAs with over 200 nt transcripts⁵. They display a relatively low conservation and specie-specificity⁶. lncRNAs are extensively involved in cell behaviors, chromosome modification, transcription, and translation regulation^{7,8}. The vital functions of lncRNAs in endothelial dysfunction, phenotypic transformation of smooth muscle cells, reverse cholesterol transport, foam cell formation, and vascular inflammation have been highlighted⁹.

The transcript of PVT1 is a large intergenic non-coding RNA, which is homologous to mouse PVT1 transcript. lncRNA PVT1 was initially identified as a common retroviral integration site in murine leukemia¹⁰. Later, PVT1 has been

found closely linked to human cancer diseases¹¹⁻¹³. Cao et al¹⁴ discovered that PVT1 is capable of influencing atrial fibrosis *via* the miR-128-3p-SP1-TGF- β 1-Smad axis. In this paper, the clinical significance of PVT1 in CAD was mainly explored.

Patients and Methods

Patients

A total of 200 CAD patients undergoing invasive coronary angiography (ICA) in Affiliated Hospital of Inner Mongolia University for Nationalities from June 2017 to May 2018 were included. In the same period, 200 healthy controls undergoing physical examinations were included. Inclusion criteria were: (1) Patients aged over 18 years old, (2) those with 50% stenosis in at least one coronary artery confirmed by ICA, and (3) those whose informed consent was obtained. Exclusion criteria were: (1) Patients with a history of hormone therapy in the last 3 months, (2) those with a history of cancer or infection, or (3) those who were unwilling to participate in this trial. Baseline characteristics and laboratory examination results of each subject were recorded. This study was approved by the Ethics Committee of Affiliated Hospital of Inner Mongolia University for Nationalities. Signed written informed consents were obtained from all participants before the study.

Gensini Score

The degree of artery narrowing was indicated by Gensini score based on the grading criteria of coronary artery image proposed by the American Heart Association (AHA)¹⁵. Gensini score was graded depending on the stenosis and the locations of narrowing (1 grade: stenosis <25%, 2 grades: 25%-49% stenosis, 4 grades: 50-74% stenosis, 8 grades: 75%-90%, 16 grades: 90%-98%, 32 grades: 99-100%). Then, the corresponding coefficients were determined by the location of different narrowing branches of coronary artery (left coronary artery: $\times 5$, proximal LAD: $\times 2.5$, mid-LAD: $\times 1$, distal LAD: $\times 1$, proximal left circumflex: $\times 2.5$, obtuse marginal branch: $\times 1$, distal left circumflex: $\times 1$, posterior descending artery: $\times 1$, posterior lateral branch: $\times 1$, right coronary artery: $\times 1$). According to the calculated Gensini score, included CAD patients were assigned into four groups (I: < 40 grades, II: 40-60 grades, III: 61-80 grades, IV > 80 grades).

Blood Samples

Peripheral blood samples were collected and subjected to EDTA (ethylenediaminetetraacetic acid) anticoagulation and 3000 r/min centrifugation for 5 min. Then, the supernatant was harvested and stored at -80°C.

Real-Time Polymerase Chain Reaction (RT-PCR)

Serum miRNAs were extracted using the miR-Neasy Mini Kit (Qiagen, Hilden, Germany), which were reversely transcribed using the TaqMan microRNA reverse Transcription Kit (Thermo Fisher, Waltham, MA, USA). Next, RT-PCR was performed using the 2 $\times$ SYBR Green PCR Master Mix. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was the internal reference. PVT1: Forward: 5'-GGTTCCACCAGCGTTATTC-3' and Reverse: 5'-CAACTTCCTTTGGGTCTCC-3', and GAPDH: Forward: 5'-GCTCTCTGCTCCTCCTGTTC-3' and Reverse: 5'-ACGACCAAATCCGTTGACTC-3'.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analysis. Data were expressed as mean $\pm$ SD (standard deviation). The *t*-test was performed for analyzing the differences between groups. The correlation between PVT1 level and Gensini score was analyzed by Spearman correlation test. Besides, multivariate Logistic regression test was conducted to assess risk factors influencing coronary atherosclerosis disease. $p < 0.05$ indicated the statistically significant difference.

Results

Baseline Characteristics and Serological Indexes

By analyzing baseline characteristics, no significant differences in age, gender, BMI, history of diabetes, and smoking were found between control and CAD groups ($p > 0.05$). The percentage of hypertension cases was higher in CAD group than controls ($p < 0.05$). In addition, serological indexes were compared as well. No significant differences in WBC, CR and UA were seen between groups ($p > 0.05$). Nevertheless, HDL-C, LDL-C, TG and TC were statistically different ($p < 0.05$) (Table I).

Table I. Baseline characteristics and serological indexes.

Variables	Control (n = 200)	CAD (n = 200)	χ^2/t	<i>p</i>
Male/Female	100/100	100/100	—	—
Age (year)	60 ± 15.02	58.34 ± 12.66	1.195	0.233
BMI	22.92 ± 3.66	23.68 ± 4.15	1.942	0.053
Hypertension (%)	55 (27.5%)	116 (58%)	39.009	< 0.001
Diabetes mellitus (%)	39 (19.5%)	46 (23%)	0.732	0.463
Current smoke (%)	58 (29%)	70 (35%)	1.654	0.238
Biochemistry detection				
WBC count, 10 ⁹ /L	6.26 ± 2.12	6.52 ± 2.98	1.005	0.315
HDL-C, mmol/L	0.92 ± 0.58	1.24 ± 0.63	5.285	< 0.001
LDL-C, mmol/L	2.68 ± 1.05	1.87 ± 0.82	8.598	< 0.001
TG, mmol/L	1.33 ± 0.41	1.95 ± 0.68	11.042	< 0.001
TC, mmol/L	3.61 ± 1.05	4.52 ± 1.88	5.976	< 0.001
CR, mmol/L	97 ± 15.67	95 ± 13.52	1.367	0.173
UA, mmol/L	275 ± 56.92	271 ± 55.83	0.71	0.478

WBC, White blood cell; TG, Triglyceride; TC, Total cholesterol; CR, Creatinine; UA, Uric Acid; BMI, body mass index; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol.

PVT1 Level in Serum of CAD Patients

Compared with that in the controls, PVT1 was highly expressed in serum of CAD patients (Figure 1), suggesting the involvement of PVT1 in the development of CAD.

Correlation Between PVT1 Level and Gensini Score in CAD Patients

CAD patients were classified into four groups depending on their Gensini scores. Interestingly, serum level of PVT1 was increased in order with the highest level in group IV and the lowest in group I (Figure 2). Spearman correlation test also identified a positive correlation between PVT1 level and Gensini score in CAD patients ($r=0.761$, $p=0.023$).

Multivariable Logistic Regression Test on Risk Factors Influencing Coronary Atherosclerosis Disease

Multivariable Logistic regression test obtained that PVT1 (crude OR = 2.074, 95% CI: 1.642-3.529, adjusted OR = 1.762, 95% CI: 1.382-2.096), HDL (crude OR = 1.681, 95% CI: 1.258-2.667, adjusted OR = 1.338, 95% CI: 1.282-2.039), and LDL-C (crude OR = 0.621, 95% CI: 0.506-0.825, adjusted OR = 0.617, 95% CI: 0.529-0.771) were the risk factors influencing CAD (Table II).

Discussion

It is reported that every year, approximately 17.5 million people die of cardiovascular diseases

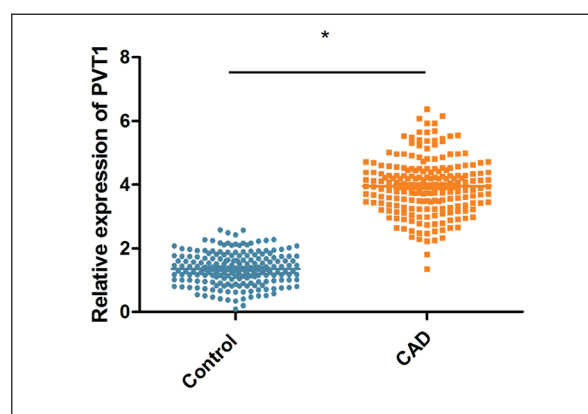
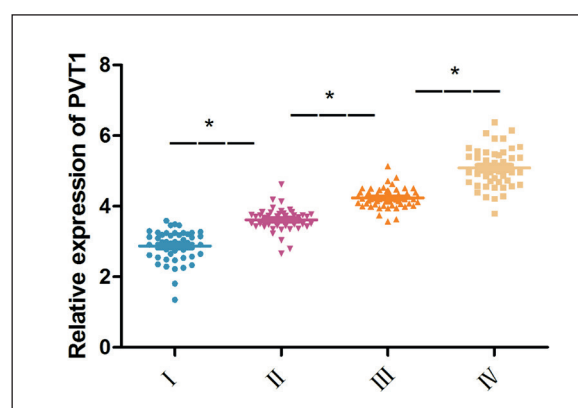
**Figure 1.** Serum level of PVT1 in CAD and control group.**Figure 2.** Serum level of PVT1 in CAD patients of group I, II, III, and IV.

Table II. Multivariable Logistic regression test on risk factors influencing coronary atherosclerosis disease.

Variables	Crude OR (95% CI)	<i>p</i>	OR (95% CI) [#]	<i>p</i> [#]
HDL-C, mmol/L	1.681 (1.258-2.667)	0.026	1.338 (1.282-2.039)	0.033
LDL-C, mmol/L	0.621 (0.506-0.825)	< 0.001	0.617 (0.529-0.771)	< 0.001
TG, mmol/L	1.505 (0.783-1.953)	0.092	1.328 (0.528-1.521)	0.106
TC, mmol/L	0.992 (0.632-2.558)	0.108	1.051 (0.706-1.657)	0.115
PVT1	2.074 (1.642-3.529)	0.002	1.762 (1.382-2.096)	0.009

HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; TG, Triglyceride; TC, Total cholesterol. [#]Values after adjustment of hypertension.

globally, accounting for 31% of global deaths¹⁶, so early prevention is of great significance. Atherosclerosis is one of the major manifestations of cardiovascular diseases, which is also the shared pathological basis of CAD and cerebrovascular diseases¹⁷. Featured by lipid deposition in vessels, atherosclerosis seriously affects health and even threatens lives¹⁸.

Liu et al¹⁹ have shown that lncRNAs may be utilized as hallmarks in cardiovascular diseases. Hence, identifying differentially expressed lncRNAs in CAD patients and exploring their biological functions contribute to improve their clinical outcomes. Chen et al²⁰ demonstrated that the knockdown of TUG1 stimulates endothelial cell apoptosis and thereafter induces atherosclerosis by upregulating miR-26a. Wang et al²¹ pointed out that the interaction between lnc-APF and miR-188-3p enhances autophagy activity and deteriorates myocardial ischemia.

lncRNA PVT1 locates on human chromosome 8q24, where there is the target with the highest DNA copies in cancer cells. Abnormal amplification of this locus indicates a high risk of cancer²². As an oncogene, PVT1 is typically upregulated in many types of cancer samples²³, and it is found to be upregulated in atrial muscles of patients with atrial fibrillation. Overexpression of PVT1 promotes proliferative ability in atrial fibroblasts¹⁴. In this paper, PVT1 was highly expressed in serum of CAD patients. In addition, it was demonstrated that PVT1 level was positively related to Gensini score in CAD patients, suggesting the involvement of PVT1 in the development of atherosclerosis. Furthermore, PVT1 was proven to be an independent risk factor for coronary atherosclerosis disease. It is believed that detection of serum level of PVT1 is of significance, which could assist to monitor the disease progression of CAD. This study reveals, for the first time, that PVT1 is

an important factor affecting coronary atherosclerosis. The detection of PVT1 level is helpful for understanding the prognosis of coronary atherosclerosis and has great significance for understanding the diagnosis and treatment of coronary atherosclerosis. It also lays the foundation for the pathogenesis of coronary atherosclerosis.

Conclusions

Shortly, the serum level of PVT1 helps to distinguish mild and severe CAD. Meanwhile, PVT1 is an independent risk factor influencing the development of coronary atherosclerosis disease.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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