

RESEARCH ARTICLE

Correlation between platelet-derived growth factor-B gene polymorphism and coronary heart disease

Yaru Lu¹  | Hui Liu² | Bo Dong¹ | Jingyu Yang¹ | Lu Kou¹ | Qin Qin¹

¹Department of Cardiology, Tianjin Chest Hospital, Chest Hospital Tianjin University, Tianjin, China

²Department of Hematology, Qilu Hospital (Qingdao), Cheeloo College of Medicine, Shandong University, Qingdao, China

Correspondence

Qin Qin, Department of Cardiology, Tianjin Chest Hospital, Chest Hospital Tianjin University, 261 Taierzhuangnan Road Jinnan District, Tianjin 300022, China.

Email: qinqin6351@163.com

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Abstract

Object: The aim of the present work was to investigate the correlation of plasma platelet-derived growth factor (PDGF)-BB level and single nucleotide polymorphism (SNP, rs1800817 and rs2285094) of PDGF-B gene with the onset and stability condition of coronary heart disease (CHD).

Methods: Totally, 335 subjects were included in and divided into CHD ($n = 247$) and control group ($n = 88$) according to coronary angiography. Besides, the patients in the CHD group were divided into acute coronary syndrome (ACS) group ($n = 165$) and stable angina pectoria (SAP) group ($n = 82$), based on CHD stability condition. The plasma PDGF-BB level was measured by ELISA, and the genotype of PDGF-B was examined through qPCR assay.

Results: The PDGF-BB level was positively correlated with hsCRP level ($r = 0.149$, $p < 0.05$). The genotype frequencies of SNP rs1800817 and rs2285094 match Hardy-Weinberg equilibrium. There was weak linkage disequilibrium between SNP rs1800817 and rs2285094: $D' = 0.419$, $r^2 = 0.04$, which has no correlation with CHD. There was no statistical difference in plasma PDGF-BB level among different genotypes in rs1800817 and rs2285094. There were no differences in the plasma PDGF-BB level among patients with any genotype of SNP rs1800817 and rs2285094, no matter how it was grouped. Logistic regression results indicated that the plasma PDGF-BB level was the independent risk factor of CHD onset (OR = 1.003, 95% CI 1.001–1.006, $p = 0.014$).

Conclusions: High plasma PDGF-BB level is the risk factor of CHD and has correlation with instability of CHD. The plasma PDGF-BB level change may be related to inflammatory response. PDGF-B gene rs1800817 and rs2285094 polymorphisms are not correlated with CHD.

KEYWORDS

coronary heart disease, platelet-derived growth factor, single nucleotide polymorphism

Yaru Lu and Hui Liu contributed equally to this article.

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1 | INTRODUCTION

Coronary atherosclerotic heart disease, abbreviated as coronary heart disease (CHD), is the major cardiovascular disease with high incidence and fatality rate. The disease is caused by various genetic factors and environmental factors.¹⁻³ Migration and hyperplasia of smooth muscle cells (SMC) are part of the main mechanisms for the plaque formation of atherosclerosis (AS). Platelet-derived growth factor (PDGF)-B is a 24-ku cation glycoprotein, mainly exists in the platelet α granules and injured endothelial cells, macrophages, smooth muscle cells, fibroblasts, etc., in active form of homozygous dimer PDGF-BB. PDGF-BB possesses chemotactic effect, promotion of cell division, and vasoconstriction effect.^{4,5} Malabanan et al.⁶ found that PDGF-BB could promote the proliferation and migration of smooth muscle cells. Therefore, PDGF-B plays a very important role in the formation of AS and CHD onset. Human PDGF-B gene is a coding product of proto-oncogene *c-sis*, located in chromosome 22q12.3-q13.1, composed of seven exons. Several polymorphic sites of PDGF-B gene have been reported, but the correlation between PDGF-B gene polymorphism and disease was few reported.⁷ There is still no study on the correlation of either plasma PDGF-BB level or PDGF-B gene polymorphism with CHD onset or plaque stability. Therefore, by detecting plasma PDGF-BB level and PDGF-B single nucleotide polymorphism (SNP) in CHD patients as well as control group, and exploring the correlation of PDGF-BB level and PDGF-B gene polymorphism with CHD onset and disease stability, we hope to provide a new target for early-stage diagnosis of CHD and recognition on high-risk patients. In this study, we focused on two SNPs: rs2285094 and rs1800817. rs2285094 (chr22:39226555) is located in the intron region of PDGFB transcript variant 1,2 mRNA, and rs1800817 (chr22:39243848) is located in the intron region of PDGFB transcript variant 1 mRNA and upstream of transcript variant 2 mRNA (https://www.ncbi.nlm.nih.gov/snp/rs2285094#variant_details).

2 | MATERIALS AND METHODS

2.1 | Subjects inclusion

Three hundred and thirty-five patients were recruited from the Department of Cardiology, Tianjin Chest Hospital between May 2014 and October 2015. There were 214 male and 121 female (total 335), of 40–78 years old, average age at 60.54 ± 7.64 years. After completing relevant examinations, all the enrolled patients received coronary angiography by Judkins method.⁸ Coronary angiography diameter method was used to measure the coronary artery (left main coronary artery, anterior descending branch, circumflex artery, right coronary artery), and its main branches (diagonal branch, obtuse marginal branch, posterior descending coronary artery, posterior branch of left ventricle). Those who have at least one of the branches showing diameter stenosis $\geq 50\%$ were diagnosed as coronary heart disease (CHD group). The patients without stenosis were

considered control group. The patients in CHD group were further divided into stable angina pectoris (SAP) group and acute coronary syndrome (ACS) group according to the stability condition of CHD.⁹ The patients with percutaneous coronary intervention history, other cardiovascular diseases, cardiac insufficiency, hepatic and renal insufficiency, coagulation disorders, other systemic diseases, acute infection, administration of inflammation inhibition drugs (such as nonsteroids, anti-inflammatory analgesic), administration of statin or aspirin within 1 month were excluded.

2.2 | Methods

The patients received routine blood examination such as fasting blood glucose (Glu), total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDLc), low-density lipoprotein (LDLc), white blood cell count (WBC), platelet count (Plt), high-sensitivity C-reactive protein (hsCRP), and ultrasonic cardiography after hospitalization, and their clinical information such as age, gender, body mass index (BMI), hypertension history, diabetes history, CHD family history, and smoking history were all recorded. Venous blood (5 ml) was taken in the morning after fasting for 12 h, anticoagulated with EDTA, centrifuged at 1500g/min for 15 min to separate plasma and hemocytes, and stored at -20°C . Blood genome DNA extraction system (RelaxGene Blood DNA System (TIANGEN BIOTECH CO. LTD)) was used to extract DNA from leukocytes. The plasma PDGF-BB levels were measured by ELISA, according to instruction of the kit (BlueGene Biotech).

The gene sequences near rs1800817 and rs2285094 taken from the Genebank, together with the immunofluorescence probe and primer designed by Primer Premier 5.0 and Gene Runner were synthesized by Thermo Fisher Scientific. The primer and probe sequences of rs1800817 loci are shown in Table 1, and the primer and probe of rs2285094 loci refer to Assay ID C_15962706_10. Taqman Quantitative Real-Time PCR (Prism7500, ABI, USA) was used to detect the genotypes. PCR reaction system (10 μl): 2 \times Taqman PCR MIX (5 μl), 20 \times (Primer-F, Primer-R, MGB probe) (0.5 μl), 100 ng/ μl DNA (1 μl), deionized water (3.5 μl). PCR reaction condition: [95 $^{\circ}\text{C}$, 10 min] \rightarrow [95 $^{\circ}\text{C}$, 15 s \rightarrow 60 $^{\circ}\text{C}$, 60 s], 40 cycles.

Totally 35 samples were randomly selected from different genotypes and verified by Thermo Fisher Scientific. The sequence was

TABLE 1 Immunofluorescence probes and primers

Loci		Sequence (5'-3')
rs1800817	Upstream primer	GGGCTGGTCTTCATTTCATTACCTT
	Downstream primer	TGCAAGGGTCCAAAGTTCCT
	Probe 1	CCCCC <u>AC</u> CTTCTG
	Probe 2	CCCCC <u>CC</u> CTTCTG

Note: rs2285094 probes and primers: Assay ID C_15962706_10, Thermo Fisher Scientific. The mutation sites of rs1800817 are underlined in the sequence of the Prob1 and 2.

compared with the homologous one in the database from <http://blast.ncbi.nlm.nih.gov/Blast.cgi>. The sequence comparison result proved that the PCR amplification fragment was the target gene one.

2.3 | Statistical method

SPSS20.0 software was used to analyze the data. Measurement data were analyzed by normality test. The measurement data meeting with normal distribution were demonstrated with mean \pm SD. The measurement data between the two groups were analyzed by *t* test, and those among groups were analyzed by univariate ANOVA. The pairwise comparison was done by *q* test. The measurement data that did not match normal distribution were expressed as median and quartile M (P_{25} , P_{75}). The comparison between two groups was analyzed by non-parametric Mann-Whitney *U* test, and that among multiple groups was analyzed by nonparametric Kruskal-Wallis univariate ANOVA test. Enumeration data were shown by *n* (%), and the comparison between groups was analyzed by χ^2 test. The correlation of abnormal distribution was analyzed by Spearman correlation analysis. The correlation of variables with coronary heart disease was analyzed by binomial logistic regression analysis. Bilateral $p < 0.05$ was considered statistically significant. PDGF-B gene SNP rs1800817 and rs2285094 allele frequencies and Hardy-Weinberg genetic equilibrium were analyzed by HaploView software and THESIAS 3.1.

3 | RESULTS

3.1 | General conditions of the included subjects

There were no statistical differences of age, gender, BMI, hypertension history, diabetes history, CHD family history, smoking history, and laboratory examination results (Glu, TG, TC, LDLc, HDLc, WBC,

Plt, hsCRP), between CHD group and control group or among control group, SAP group and ACS group ($p > 0.05$), as shown in Table 2.

3.2 | Laboratory examination results and plasma PDGF-BB level

The plasma PDGF-BB level in the CHD group was significantly higher than that in control group ($p < 0.05$). There were no significant differences in WBC, Plt, Glu, TC, TG, LDLc between the two groups ($p > 0.05$). The HDLc level in CHD group was significantly lower than that in control group ($p < 0.05$), and hsCRP level in CHD group was significantly higher than that in control group ($p < 0.05$), as shown in Table 3.

There were no statistical differences in WBC, Plt, Glu, TC, TG, LDLc levels among control group, SAP group, and ACS group ($p > 0.05$). There were statistical differences in PDGF-BB and hsCRP levels among the three groups ($p < 0.01$). Those in the ACS group were significantly higher than that of the other two groups ($p < 0.01$). However, the difference between the latter two groups was not statistically different ($p > 0.05$). There was statistical difference in HDLc level among the three groups ($p < 0.05$), and SAP group was significantly lower than control group ($p < 0.05$). Although HDLc level in ACS group was lower than that of control group, there was no statistical significance between the two groups, as shown in Table 4. PDGF-BB level was positive correlated with hsCRP level ($r = 0.149$, $p = 0.009$).

3.3 | SNP analysis of PDGF-B

There was linkage disequilibrium between PDGF-B gene SNP rs1800817 and rs2285094: $D' = 0.419$, $r^2 = 0.04$. Only one case with CC homozygote was detected in the rs1800817 loci, and others were AA and AC genotypes. The allele frequency A 0.940,

TABLE 2 General condition comparisons between control group and CHD group

	Control group (n = 88)	CHD group (n = 247)	SAP group (n = 82)	ACS group (n = 165)
Age (years)	60.60 \pm 7.95	60.52 \pm 7.55	61.44 \pm 7.24	60.06 \pm 7.67
Male (n (%))	54 (61.4)	160 (64.8)	56 (68.3)	104 (63.0)
Hypertension (n (%))	46 (52.3)	151 (61.1)	49 (59.8)	102 (61.8)
Diabetes (n (%))	16 (18.2)	68 (27.5)	21 (25.6)	47 (28.8)
Smoking history (n (%))	30 (34.1)	109 (44.1)	32 (39.0)	77 (46.7)
Family history (n (%))	22 (25.0)	77 (31.2)	23 (28.0)	54 (32.7)
BMI (kg/m ²)	25.51 \pm 3.28	25.52 \pm 3.06	25.58 \pm 3.38	25.49 \pm 2.90

Note: The measurement data (Age, BMI) were shown by mean \pm SD, and the comparison between control group and CHD group was analyzed by *t* test, and those among control group, SAP group, and ACS group were analyzed by univariate ANOVA. Enumeration data (male, hypertension, diabetes, smoking history, family history) were shown by *n* (%), and the comparison between groups was analyzed by χ^2 test. BMI = Weight (kg)/height² (m²).

Abbreviations: ACS, acute coronary syndrome; BMI, body mass index; CHD, coronary heart disease; SAP, stable angina pectoria.

	Control group (n = 86)	CHD group (n = 247)	t/Z	p
Glu (mmol/L)	5.50 ± 1.09	5.69 ± 1.47	-1.330	0.185
TC (mmol/L)	4.62 ± 1.13	4.59 ± 1.17	0.219	0.827
TG (mmol/L)	1.58 ± 0.85	1.86 ± 1.39	-1.761	0.079
HDLc (mmol/L)	1.16 ± 0.33	1.05 ± 0.41	2.451	0.015*
LDLc (mmol/L)	2.88 ± 0.91	2.88 ± 1.01	-0.010	0.992
WBC (×10 ⁹ /L)	6.39 ± 1.50	6.67 ± 1.53	-1.478	0.140
Plt (×10 ⁹ /L)	234.25 ± 54.07	228.60 ± 55.82	0.823	0.411
hsCRP (mg/L)	0.83 (0.44, 2.32)	1.41 (0.63, 3.28)	2.573	0.010**
PDGF-BB (pg/ml)	224.10 ± 111.83	258.37 ± 132.40	-2.168	0.031*

TABLE 3 Comparisons of laboratory examination results and PDGF-BB levels between control group and CHD group

Note: The measurement data (Glu, TC, TG, HDLc, LDLc, WBC, Plt, PDGF-BB) were shown by mean ± SD, and the comparison between control group and CHD group was analyzed by t test. The data of hsCRP level that did not match normal distribution were expressed as median and quartile M (P25, P75), the comparison between groups was analyzed by nonparametric Mann-Whitney U test. Other measurement data between control group and CHD group were analyzed by t test (* $p < 0.05$, ** $p < 0.01$).

Abbreviations: CHD, coronary heart disease; Glu, Glucose; HDLc, high-density lipoprotein cholesterol; hsCRP, high sensitivity C-reactive protein; LDLc, low-density lipoprotein cholesterol; PDGF-BB, platelet-derived growth factor-BB; Plt, blood platelet; TC, total cholesterol; TG, triglyceride; WBC, white blood cells.

TABLE 4 Comparison of laboratory examination results and PDGF-BB levels among control group, SAP group, and ACS group

	Control group (n = 86)	SAP group (n = 79)	ACS group (n = 134)	F/Z	p
Glu (mmol/L)	5.50 ± 1.09	5.70 ± 1.53	5.69 ± 1.45	0.667	0.514
TC (mmol/L)	4.62 ± 1.13	4.50 ± 0.89	4.63 ± 1.29	0.360	0.698
TG (mmol/L)	1.58 ± 0.85	1.88 ± 1.34	1.85 ± 1.50	1.559	0.212
HDLc (mmol/L)	1.16 ± 0.33	1.01 ± 0.25 ^A	1.06 ± 0.46	3.554	0.030*
LDLc (mmol/L)	2.88 ± 0.91	2.80 ± 0.81	2.92 ± 1.10	0.349	0.706
WBC (×10 ⁹ /L)	6.39 ± 1.49	6.49 ± 1.57	6.76 ± 1.50	1.938	0.146
Plt (×10 ⁹ /L)	234.25 ± 54.07	222.78 ± 55.04	231.48 ± 56.14	1.016	0.363
hsCRP (mg/L)	0.83 (0.44, 2.32)	1.02 (0.49, 2.42)	1.44 (0.75, 3.91) ^{ab}	10.653	0.005**
PDGF-BB (pg/ml)	224.10 ± 111.83	231.00 ± 134.50	271.98 ± 129.62 ^{ab}	5.262	0.006**

Note: The measurement data (Glu, TC, TG, HDLc, LDLc, WBC, Plt) were shown by mean ± SD, and those among control group, SAP group, and ACS group were analyzed by univariate ANOVA (* $p < 0.05$, ** $p < 0.01$). The data of hsCRP level that did not match normal distribution were expressed as median and quartile M (P25, P75), that among three groups was analyzed by nonparametric Kruskal-Wallis univariate ANOVA test (* $p < 0.05$, ** $p < 0.01$). The pairwise comparison was analyzed by q test (Δ compared with control group $p < 0.05$; Superscript letter a compared with control group, $p < 0.01$; Superscript letter b compared with SAP group, $p < 0.01$).

Abbreviations: ACS, acute coronary syndrome; CHD, coronary heart disease; Glu, Glucose; HDLc, high-density lipoprotein cholesterol; hsCRP, high sensitivity C-reactive protein; LDLc, low-density lipoprotein cholesterol; PDGF-BB, platelet-derived growth factor-BB; Plt, blood platelet; SAP, stable angina pectoria; TC, total cholesterol; TG, triglyceride; WBC, white blood cells.

C 0.060, genotype frequency AA 0.884, AC 0.113, CC 0.003, $\chi^2 = 0.036$, $p = 0.850$ match the Hardy-Weinberg equilibrium. There was no statistical difference in rs1800817 loci genotype or allele frequency between CHD group and control group, or among control group, SAP group, and ACS group ($p > 0.05$). rs2285095 loci allele frequency T 0.782, C 0.218, genotype frequency TT 0.603, TC 0.358, CC 0.039, $\chi^2 = 0.869$, $p = 0.351$ match the Hardy-Weinberg equilibrium. There was no statistical differences in the genotype and allele frequency between CHD group and control group, or among control group, SAP group, and ACS group ($p > 0.05$) (Tables 5 and 6).

3.4 | Correlation between PDGF-B genotype and plasma PDGF-BB level

There was no statistical difference in the plasma PDGF-BB level in three different genotypes on both SNP rs1800817 and rs2285094 among all the subjects ($p > 0.05$), as shown in Table 7.

The plasma PDGF-BB level of the patients with AA genotype at rs1800817 loci in CHD group was significantly higher than that of control group ($p < 0.05$). There was no statistical difference in AC+CC genotype between CHD group and control group ($p > 0.05$). The plasma PDGF-BB level in CHD group had no statistical difference

TABLE 5 Genotype and allele frequency comparison between control group and CHD group

SNPs	Genotype/allele	Control group (n = 88)	CHD group (n = 247)	χ^2	p
rs1800817	AA	81 (92.0)	215 (87.0)	0.249	0.143
	AC+CC	7 (8.0)	32 (13.0)		
	A	169 (0.960)	461 (0.933)	1.702	0.266
	C	7 (0.040)	33 (0.067)		
rs2285094	TT	54 (61.4)	148 (59.9)	1.294	0.524
	TC	29 (33.0)	91 (36.8)		
	CC	5 (5.7)	8 (3.2)		
	T	137 (0.778)	387 (0.783)	0.016	0.916
	C	39 (0.222)	107 (0.217)		

Note: Genotype/allele data were shown by n (%), and the comparison between groups was analyzed by χ^2 test.

Abbreviations: CHD, coronary heart disease; SNP, single nucleotide polymorphism.

TABLE 6 Genotype and allele frequency comparison among control group, SAP group, and ACS group

SNPs	Genotype/allele	Control group (n = 86)	SAP group (n = 82)	ACS group (n = 165)	χ^2	p
rs1800817	AA	81 (92.0)	71 (86.6)	144 (87.3)	1.603	0.449
	AC+CC	7 (8.0)	11 (13.4)	21 (12.7)		
	A	169 (0.960)	153 (0.933)	308 (0.933)	1.689	0.430
	C	7 (0.040)	11 (0.067)	22 (0.067)		
rs2285094	TT	54 (61.4)	53 (64.6)	95 (57.6)	2.474	0.649
	TC	29 (33.0)	27 (32.9)	64 (38.8)		
	CC	5 (5.7)	2 (2.4)	6 (3.6)		
	T	137 (0.778)	133 (0.811)	254 (0.770)	1.114	0.573
	C	39 (0.222)	31 (0.189)	59 (0.230)		

Note: Genotype/allele data were shown by n (%), and the comparison between groups was analyzed by χ^2 test.

Abbreviations: ACS, acute coronary syndrome; CHD, coronary heart disease; SAP, stable angina pectoria; SNP, single nucleotide polymorphism.

TABLE 7 Comparison of PDGF-BB levels of different genotypes

SNPs	Genotype	n	PDGF-BB (pg/ml)	t/F	p
rs1800817	AA	296	248.98 ± 126.89	-0.151	0.880
	AC+CC	39	252.28 ± 138.24		
rs2285094	TT	202	249.10 ± 125.35	0.008	0.992
	TC	120	250.19 ± 136.58		
	CC	13	245.81 ± 90.55		

Note: PDGF-BB level was shown by mean ± SD, and the comparison between genotype AA group and AC+CC genotype was analyzed by t test, and that among genotype TT, TC, CC groups was analyzed by univariate ANOVA.

Abbreviations: PDGF-BB, platelet-derived growth factor-BB; SNP, single nucleotide polymorphism.

between AA and AC+CC genotypes ($p > 0.05$). There is also no such statistical difference in control group ($p > 0.05$). Because the sample size of AC and CC genotypes in rs1800817 loci was small, the PDGF-BB levels in control group, SAP group, and ACS group were not compared, as shown in Table 8.

There is no statistical difference of plasma PDGF-BB level of different genotypes at rs2285094 between control group and CHD group, or within either CHD group or control group ($p > 0.05$).

Among control group, SAP group, and ACS group, the plasma PDGF-BB level of TT genotype at rs2285094 showed the trend of ACS group > SAP group > control group, which did not achieve statistical difference ($p > 0.05$). However, the CC genotype sample size was small, we combined TC genotype with CC genotype. There was statistical difference of plasma PDGF-BB level of TC+CC genotypes at rs2285094 among control group, SAP group, and ACS group. The plasma PDGF-BB level of ACS group is higher than SAP group and

control group. There was no statistical significance between SAP group and control group ($p > 0.05$). The plasma level of different genotypes within each group was not statistically different ($p > 0.05$) as shown in Tables 8 and 9.

3.5 | Logistic regression analysis

Coronary heart disease onset was taken as a dependent variable, and age, gender, BMI, hypertension history, diabetes history, CHD family history, smoking history, Glu, blood fat levels (TG, LDLc, HDLc), WBC, Plt, hsCRP, PDGF-BB, genotypes of rs1800817 loci and rs2285094 loci were taken as independent variables. The result of binary logistic regression analysis indicated that plasma PDGF-BB level is the risk factor of CHD onset (OR = 1.003, 95% CI 1.001 ~ 1.006, $p = 0.014$).

4 | DISCUSSION

Platelet-derived growth factor-BB plays very important roles in various physiological and pathological processes, such as trauma tissue

repair, onset and treatment of malignant tumors, formation of pulmonary arterial hypertension, etc.¹⁰⁻¹² Leitzel¹³ reported that the normal plasma PDGF-BB level was 0.32 ± 0.14 ng/ml (0.10–0.69 ng/ml), and the level of 15% of 131 cancer patients was significantly increased > 0.69 ng/ml. The level was not related to age or gender in both cancer patients and normal people. The plasma PDGF-BB level was proven to have positive correlation with diabetic foot syndromes, pulmonary fibrosis, etc.; it has negative correlation with hepatic fibrosis caused by hepatitis B.¹⁴⁻¹⁶ The study of relationship between the plasma PDGF-BB level and AS or CHD was few reported.

There was no statistical difference in hypertension, diabetes, blood fat level between CHD group and control group in our study. Our results showed that PDGF-BB level and hsCRP level in the CHD group were significantly higher than the control group. AS plaque fibrous cap thinning, rupture, and thrombosis are considered as the main pathological basis for instability of coronary artery disease, as well as the major reasons for ACS occurrence. According to the stability of AS plaque, the CHD group was further divided into ACS and SAP groups. The PDGF-BB level and hsCRP level among the control group, SAP group, and ACS group

TABLE 8 Comparison of plasma PDGF-BB levels of different genotypes between control group and CHD group

SNPs	Genotype	Control group		CHD group		t	p
		n	PDGF-BB (pg/ml)	n	PDGF-BB (pg/ml)		
rs1800817	AA	81	220.02 ± 105.72	215	259.89 ± 132.60	-2.430	0.016*
	AC+CC	7	271.27 ± 171.76	32	248.13 ± 132.73	0.397	0.694
	T		-1.166		0.468		
	p		0.247		0.640		
rs2285094	TT	54	231.92 ± 129.79	148	255.37 ± 123.55	-1.178	0.240
	TC	29	215.09 ± 79.30	91	261.37 ± 148.94	-1.600	0.112
	CC	5	191.81 ± 45.43	8	279.56 ± 97.42	-1.868	0.089
	F		0.429		0.163		
	p		0.653		0.850		

Note: PDGF-BB level was shown by mean ± SD, and that between control group and CHD group of different genotypes was analyzed by t test ($*p < 0.05$). In control group or CHD group, the comparison between genotype AA group and genotype AC+CC group was analyzed by t test, and that among genotype TT, TC, CC groups was analyzed by univariate ANOVA ($*p < 0.05$).

Abbreviations: CHD, coronary heart disease; PDGF-BB, platelet-derived growth factor-BB; SNP, single nucleotide polymorphism.

TABLE 9 Comparison of PDGF-BB levels of different genotypes in rs2285094 among control group, SAP group and ACS group

Genotype	Control group		SAP group		ACS group		F	p
	n	PDGF-BB (pg/ml)	n	PDGF-BB (pg/ml)	n	PDGF-BB (pg/ml)		
TT	54	231.92 ± 129.79	53	235.99 ± 132.29	95	266.18 ± 117.73	1.690	0.187
TC+CC	34	211.67 ± 75.20	29	221.81 ± 140.33	70	279.84 ± 144.71 ^{Δ§}	4.030	0.020*
t		0.826		0.454		-0.668		
p		0.411		0.651		0.505		

Note: PDGF-BB level was shown by mean ± SD, and that among control group, SAP group, and ACS group of different genotypes were analyzed by univariate ANOVA ($*p < 0.05$). The pairwise comparison was analyzed by q test (^Δcompared with control group $p < 0.05$; [§]compared with SAP group, $p < 0.05$). The comparison of PDGF-BB level between TT and TC+CC genotypes was analyzed by t test ($*p < 0.05$).

Abbreviations: ACS, acute coronary syndrome; CHD, coronary heart disease; PDGF-BB, platelet-derived growth factor-BB; SAP, stable angina pectoria.

had statistical differences. The levels in ACS group were significantly higher than those in SAP group, but there was no statistical difference between SAP group and control group. PDGF-BB level was positive correlated with hsCRP level. The results indicated that high plasma PDGF-BB level was related to CHD onset and instability condition, and the change of plasma PDGF-BB level was related to inflammatory response.

Ho et al.¹⁷ found that SMC treated by CRP was more sensitive to PDGF-BB, suggesting inflammatory response could improve the effects of PDGF-BB. After inflammation and mechanical injury caused arterial intimal injury, the coagulation mechanism will be activated. The thrombin stimulates large amount of PDGF-BB release from platelet at the same time of activation of clotting cascade.^{18,19} PDGF-BB changes the SMC phenotype, turns contraction type into synthetic type that possesses synthesis, secretion, migration, and proliferation functions. It migrates from media to intima, and secretes lots of extracellular matrix, protease, various cytokines, which is beneficial to vessel remodeling and AS plaque formation and stability.²⁰⁻²² Cui et al.²³ carried out exogenous PDGF-BB transfected with plasmid to rat acute myocardial infarction (AMI) model. After infarction, at Day 28, neonatal vessels with mature tissue structure were formed in the exogenous PDGF-BB group, and the infarction area shrunk. Compared with the control group, LVd and LVDs significantly reduced, and LVEF significantly increased, suggesting PDGF-BB played a very important role in post-infarction myocardium repairing and cardiac function improvement. Therefore, increase in plasma PDGF-BB level in the ACS patients might be the manifestation of body repair after AS plaque instability and fracture.

Results of many clinical investigations supported above opinions. Koizumi et al.²⁴ reported that the plasma PDGF-BB level in coronary circulation increased in the AMI patients receiving emergent PCI, suggesting PDGF-BB participated in the instability process of AS plaque, and the increase of the level was the manifestation during AMI acute phase. Nakagawa et al.²⁵ found that in the carcass after ACS stenting, during Day 24 and 55, the PDGF-B was highly expressed in both the macrophages accumulated under neonatal intima and highly differentiated SMC. At Month 6, the expression significantly decreased and could not be detected in the neonatal intima after 1-5 years, suggesting that PDGF-B was related to the intima repair after PCI surgery within time limit. However, Rattik et al.²⁶ found that the high PDGF-BB level was related to stable plaque during the investigation on 202 patients receiving carotid endarterectomy, 384 patients with coronary artery events, and 409 control patients. Yeboah et al.²⁷ believed that low plasma PDGF-BB level was the risk factor of cardiovascular diseases. The investigation on patients with Type II diabetes and CHD indicated that the plasma PDGF-BB level is higher in diabetes patients without cardiovascular disease history than those with cardiovascular disease.

Up to now, the reported polymorphic sites of PDGF-B gene SNP mainly included rs1800817, rs1800818, rs2285094, rs2040399, rs2267406, rs2285097, rs130650. SNP rs1800817 (+1135C/A) is located in the first intron, and rs2285094 is located in the intron near the mRNA splice site.⁷ Ben-Ari et al.²⁸ found in post-orthotopic

liver transplantation patients with PDGF-B gene SNP rs1800817 AA genotype, the hepatic C recurrence rate was high; liver fibrosis tends to be more serious. While all these were not related to SNP rs1800818. Bicanski et al.²⁹ investigated IgA nephropathy patients in Germany and Italy and found that four SNP polymorphism sites of PDGF-B gene (rs2285094, rs2040399, rs2267406, rs2285097), allele distributions matched the Hardy-Weinberg linkage equilibrium, and each SNP genotype distribution was not related to disease onset and severity. Anat et al.³⁰ studied 70 patients with heart transplantation and found that patients with GG genotype at PDGF-B gene SNP rs1800818 had lower cardiac allograft vasculopathy (CAV) rate, longer survival time, and lower fatality rate. The occurrence risk of CAV in AA patients was higher, suggesting GG genotype might be the protective factor. The occurrence risk of CAV in patients with rs1800817 CC genotype was relatively low, and CC genotype was the protective factor. Osadnik et al.²⁷ followed up 265 patients with stable CHD implanted with bare metal stent and found that late lumen loss of patients with PDGF-B gene rs2285094 CC genotype was larger than those with CT and TT genotypes. The results of Gensini score indicated that the genotype of rs2285094 was not related to coronary artery disease severity.

The PDGF-B gene SNP rs1800817 and rs2285094 genotypes and allele frequency distribution match the Hardy-Weinberg linkage equilibrium. The detection rate of CC homozygote in rs1800817 was low, and only one patient of CC genotype was detected in CHD group (CC and AC genotypes were combined to perform the statistical analysis). This was similar with our results of investigation on the patients with restenosis after stenting. No CC homozygous genotype was detected, and there was no statistical difference in AA and AC genotype distribution between the instant restenosis group and non-instant restenosis group.³¹ The genotypes of rs1800817 and rs2285094 had no statistical significance among the control group, SAP group, and ACS group. The haplotype analysis indicated that SNP rs1800818 and rs2285094 presented weak linkage disequilibrium, but it was not related to coronary heart disease onset.

Up to now, there is no relevant report found on the correlation between PDGF-B gene polymorphism and plasma PDGF-BB level, as well as the correlation between CHD onset and instability condition. To compare different genotype plasma levels in PDGF-B gene SNP rs1800817 and rs2285094, we found that there was no difference in PDGF-BB level among different genotypes in rs2285094. The level of TC+CC of CHD group was significantly higher than control group (because the number of cases with CC genotype was small, the cases with TC and CC types were combined for analysis). There was no statistical significance in the plasma PDGF-BB level of TT genotype between the two groups. The PDGF-BB level of TC+CC genotype had statistical difference among the control group, SAP group, and ACS group. The level in the ACS group was significantly higher than those in the SAP group and control group, but there was no statistical difference between the latter two groups. For the TT genotype, the level in the ACS group was higher than the other two groups but without

statistical difference. Moreover, there was no statistical difference in the level between the different genotypes in each group. The above results suggested that plasma PDGF-BB level had no correlation with rs2285094 genotype, but correlated with different types of coronary heart disease. High-plasma PDGF-BB level might be related to the instability of coronary lesions.

In rs1800817 loci, the plasma PDGF-BB level between AA and AC+CC genotype had no statistical difference. The plasma PDGF-BB level in the AA genotype of CHD group was significantly higher than that of the control group. The level of AC+CC genotype between CHD and control group had no statistical difference. The level of different genotypes in both the CHD group and control group had no statistical difference. Because there were only seven patients with AC+CC genotype in the control group, and 32 in the CHD group, we did not compare the plasma levels of different genotypes among the three groups. The above results indicated that plasma PDGF-BB level was not related to rs1800817 genotype.

The CHD onset was taken as a dependent variable, and gender, age, BMI, hypertension history, diabetes history, CHD family history, smoking history, Glu, blood fat levels (TG, LDLc, HDLc), WBC, Plt, hsCRP, PDGF-BB levels, PDGF-B genes rs1800817 and rs2285094 were taken as the independent variables. The result of binary logistic regression analysis indicated that plasma PDGF-BB level was the risk factor of CHD onset.

5 | CONCLUSION

The study indicated that high plasma PDGF-BB level was the risk factor for CHD onset, and the manifestation of coronary artery lesion instability. PDGF-B gene rs1800817 and rs2285094 polymorphisms were not correlated with the plasma PDGF-BB level, CHD onset, and coronary artery lesion instability. Up to now, the correlation between plasma PDGF-BB level and CHD is still controversial. The causality between the PDGF-BB and occurrence of CHD and ACS is still unclear, nor is the mechanism, which need further exploration.

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CONFLICT OF INTEREST

Not reported.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in https://pan.baidu.com/s/1_v9NINi5NLKQ3C_wLwILNg, password: en1h.

ORCID

Yaru Lu  <https://orcid.org/0000-0003-3836-8516>

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