

Original Article

Plasminogen activator inhibitor-1 4G/5G polymorphism is associated with coronary artery disease risk: a meta-analysis

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Abstract: Background: The aim of the current study was to evaluate the association of *PAI-1* 4G/5G polymorphism with coronary artery disease (CAD) risk using a meta-analysis. Methods: All eligible studies were identified through a search of PubMed, EMBASE, China National Knowledge Infrastructure (CNKI), Database of Chinese Scientific and Technical Periodicals, and China Biology Medical literature database (CBM) before June 2014. The association between the *PAI-1* 4G/5G polymorphism and CAD risk was estimated by odds ratio (OR) and 95% confidence interval (CI). Results: A total of 72 studies including 23557 cases and 21526 controls were eventually collected. The *PAI-1* 4G/5G polymorphism was significant associated with CAD risk in overall population (OR=1.19, 95% CI 1.10-1.28, $P < 0.00001$). The combination of adjusted ORs for CAD was 1.20 (95% CI 1.03-1.40, $P=0.02$). This polymorphism was associated with CAD risk in Caucasians (OR=1.10, 95% CI 1.02-1.19, $P=0.01$) and Asians (OR=1.46, 95% CI 1.21-1.75, $P < 0.0001$). This polymorphism significantly increased MI risk (OR=1.15, 95% CI 1.06-1.25, $P=0.001$). In the subgroup analysis by age, this polymorphism was significantly associated with early-onset CAD risk (OR=1.21, 95% CI 1.02-1.43, $P=0.03$). In the gender subgroup analyses, a statistically significant association was found in male CAD patients (OR=1.10, 95% CI 1.01-1.20, $P=0.04$). Both T2DM patients and non-T2DM patients carrying 4G allele showed increased CAD risks (OR=2.23, 95% CI 1.27-3.92, $P=0.005$ and OR=1.64, 95% CI 1.19-2.25, $P=0.002$, respectively). Conclusions: This meta-analysis suggested that *PAI-1* 4G/5G polymorphism was a risk factor for CAD.

Keywords: Coronary artery disease, plasminogen activator inhibitor-1, meta-analysis, genetic

Introduction

Cardiovascular diseases, the first cause of death in the Western countries are a real common health problem. Despite the high responsibility of factors such as high level of total cholesterol, systemic hypertension, smoking, type 2 diabetes (T2DM) in coronary artery disease (CAD), evidence from family studies show that genetic factors contribute to the predisposition to CAD.

The plasminogen activator inhibitor-1 (*PAI-1*), a 52 kDa glycoprotein belong to the serine proteinase inhibitor super family, is a multifaceted proteolytic factor. It is the principal inhibitor of tissue and urinary plasminogen activators, and therefore constitutes an important regulatory

protein in fibrinolysis [1]. Impaired fibrinolysis due to high *PAI-1* activity has been shown to be associated with an increased risk of thrombotic events [2]. *PAI-1* overexpression may also promote development of weak plaques with thin fibrous caps by inhibiting both u-PA receptor- and integrin-mediated cell adhesion and migration [3]. In addition, increased plasma *PAI-1* levels have been reported in survivors of myocardial infarction (MI) compared with the general population [4]. Therefore, *PAI-1* might play an important role in the pathogenesis of CAD.

The *PAI-1* gene, located in 7q21.3-22, spans 12.3 kb and contains 9 exons and 8 introns. The polymorphism of the 4G/5G gene is located in the *PAI-1* gene promoter region. The most commonly studied functional variant in the

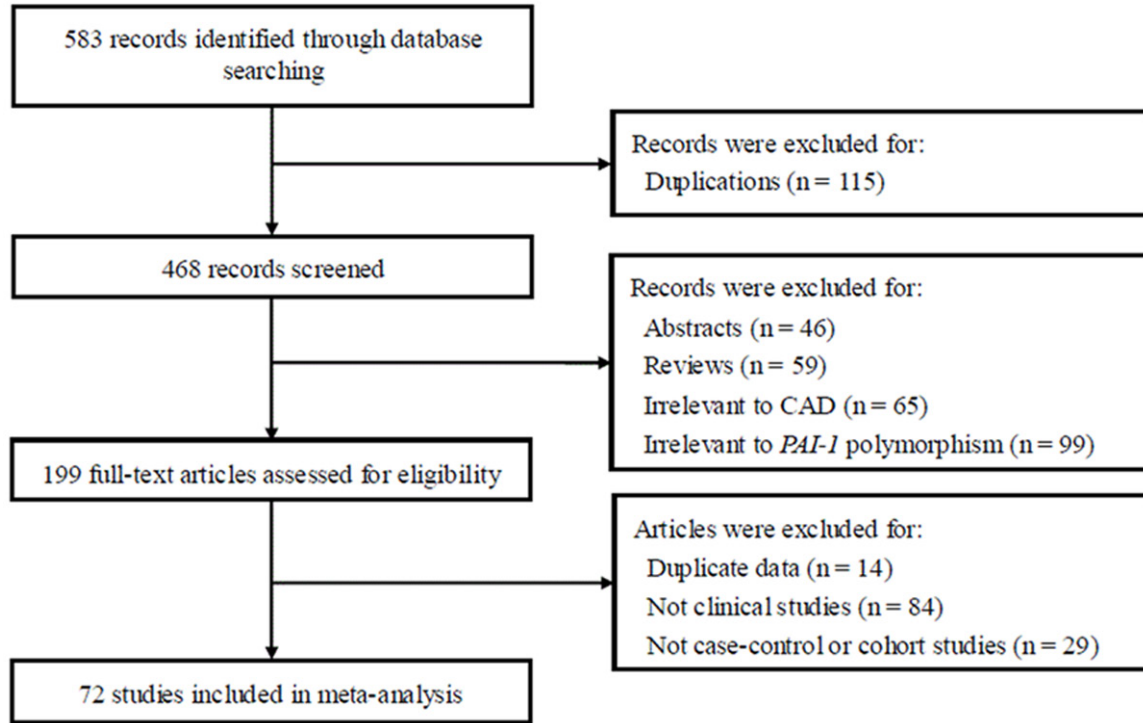


Figure 1. Flow of study identification, inclusion, and exclusion.

Table 1. Characteristics of the included studies

First author	Year	Country	Ethnicity	Endpoint	Age		Case Control		Adjustment for covariates
					of patients	Female (%)	(n)	(n)	
Dawson	1993	Sweden	Caucasian	MI	< 45 (39.9±0.4)	14	107	73	NA
Eriksson	1995	Sweden	Caucasian	MI	< 45	0	93	100	NA
Ye	1995	France/UK	Caucasian	MI	25-64	0	476	601	NA
Mansfield	1995	UK	Caucasian	CAD	61-70	0	38	122	NA
Burzotta	1997	Italy	Caucasian	MI	> 45 (59±7)	25	108	175	NA
Ridker	1997	USA	Caucasian	MI	62.9±8.8	0	374	495	NA
Ossei-Gerning	1997	UK	Caucasian	MI	59.8	NA	158	150	NA
Iwai	1998	Japan	Asian	MI	59.3±10.3	22.5	204	148	NA
Kohler	1998	Finland	Caucasian	MI	57-59	27.7	181	188	NA
Margaglione	1998	Italy	Caucasian	MI	22-65	23	198	981	NA
Pastinen	1998	Finland	Caucasian	MI	58.1±4.9	19.2	151	150	NA
Junker	1998	Germany	Caucasian	MI	38.6±4.4	0	241	179	NA
Sugano	1998	Japan	Asian	MI	63.1±9.2	12.1	66	62	NA
Ardissino	1999	Italy	Caucasian	MI	40.7±4.1	7.5	200	200	NA
Anderson 1	1999	USA	Caucasian	MI	63.7±11.6	23	375	978	NA
Anderson 2	1999	USA	Caucasian	CAD	62.5±10.9	20	898	329	NA
Doggen	1999	Netherlands	Caucasian	MI	56.1±9.0	0	331	302	NA
Gardemann 1	1999	Germany	Caucasian	CAD	62.7	0	1791	594	NA
Gardemann 2	1999	Germany	Caucasian	MI	62.2	0	1214	1351	NA
Grancha	1999	Spain	Caucasian	CAD	56±5	100	41	62	NA
Beneš	2000	Czech	Caucasian	CAD	49.5±4.5	0	175	222	NA
Canavy	2000	France	Caucasian	CAD/MI	55	22	244	244	NA
Hooper	2000	USA	African	MI	60.7±9.2	53	110	185	NA
Mikkelsen	2000	Finland	Caucasian	MI	47.9±9	0	68	164	NA
Song	2000	Korea	Asian	CAD	60.7±9.2	37.3	158	139	NA

PAI-1 polymorphism and CAD risk

Fu	2000	China	Asian	MI	51.3±6.7	42.5	87	92	NA
Viitanen	2001	Finland	Caucasian	CAD	56±1	40.7	118	110	NA
Dai	2001	China	Asian	CAD/MI	57±9	NA	250	95	NA
Fu	2001	China	Asian	CAD	66±10	50	123	172	NA
Shang	2001	China	Asian	CAD	NA	NA	38	80	NA
Ortlepp	2002	Germany	Caucasian	CAD	58±12.8	68	100	100	NA
Yamada	2002	Japan	Asian	MI	62.5±10.8	100	589	704	NA
Guan	2002	China	Asian	CAD	34-90	38.1	126	121	NA
Li	2002	China	Asian	CAD	60 ± 8	33.3	36	16	NA
ATVBISG	2003	Italy	Caucasian	MI	< 45	12.3	1210	1210	Smoking, diabetes, hypertension, family history, body mass index, hypercholesterolemia, alcohol, cocaine, physical exercise
Crainich	2003	USA	Caucasian	MI	73.5±5.5	40.2	264	753	NA
Juhan-Vague	2003	Europe	Caucasian	MI	< 60	0	483	507	NA
Leander 1	2003	Sweden	Caucasian	MI	58.3±7.1	0	851	1051	Age, residential area
Leander 2	2003	Sweden	Caucasian	MI	61.5±6.8	100	361	505	Age, residential area
Petrovič	2003	Slovenia	Caucasian	MI	58.3±11.3	33.8	154	194	NA
Zhan	2003	China	Asian	MI	67.1±10.4	21.4	56	83	NA
Ding 1	2003	China	Asian	CAD	NA	NA	60	109	Age, body mass index, family history
Ding 2	2003	China	Asian	CAD	NA	NA	49	63	Age, body mass index, family history
Wang	2003	China	Asian	CAD	59±12	24	67	30	NA
Zhai	2003	China	Asian	CAD	62.8±9	32	122	172	NA
Tobin	2004	UK	Caucasian	MI	61.9±9.2	32	547	505	NA
Pegoraro	2005	Indian	Asian	MI	< 45	NA	195	300	NA
Whiting	2005	USA	Caucasian	CAD	NA	NA	881	261	Diabetes, family history
Zak	2005	Poland	Caucasian	CAD	45.9±6	34.9	146	121	NA
Agirbasli	2006	Turkey	Caucasian	CAD	< 55	20	100	100	NA
Su	2006	China	Asian	CAD	54.5±8.9	21.6	812	931	Age, sex, BMI, HDL-C, LDL-C, hypertension, diabetes, and smoking
Xia	2006	China	Asian	CAD	57.7±8.1	28.6	166	63	NA
Morange	2007	France	Caucasian	MI	51.91±5.44	0	510	543	NA
Sampaio	2007	Brazil	Caucasian	MI	34.4±4.9	38.1	115	104	Age, gender, ethnic background, hypertension, diabetes, hypercholesterolemia, obesity, smoking, stress, and sedentary lifestyle
Taymaz	2007	Turkey	Caucasian	CAD	NA	NA	115	41	NA
Onalan	2008	Turkey	Caucasian	MI	59±11	19.9	156	281	NA
Saely	2008	Austria	Caucasian	CAD	NA	NA	406	266	Age, gender, BMI, smoking, hypertension, LDL cholesterol, HDL cholesterol, triglycerides, and use of aspirin, statins, angiotensin converting enzyme inhibitors and beta adrenoreceptor blocking agents
Sarecka	2008	Poland	Caucasian	CAD	43.8±6.1	32.6	178	202	Smoking, elevated level of total cholesterol, LDL-cholesterol, triacylglycerols, overweight or obesity
Zhang	2008	China	Asian	CAD	63.6±4.9	27	155	190	NA
Isordia-Salas	2009	Mexico	Caucasian	MI	40±4.6	16.5	127	127	NA
Tàssies	2009	Spain	Caucasian	CAD	60±13	23	248	200	NA
Var	2009	Turkey	Caucasian	CAD	55.3±11.3	35	86	90	Age, sex, smoking and hypertension
Chen	2009	China	Asian	CAD	60±11	51	293	178	NA

PAI-1 polymorphism and CAD risk

Abboud	2010	Tunisia	African	MI	59.0±12.0	19	305	328	NA
Cao	2010	China	Asian	MI	64.62	33.6	116	60	NA
Koch	2010	Germany	Caucasian	MI	64±12	24.2	3657	1211	Age, gender, history of arterial hypertension, history of hypercholesterolaemia, current cigarette smoking, and diabetes mellitus
Agirbasli	2011	Turkey	Caucasian	CAD	45.4±7	43.3	90	90	NA
Ahmed	2011	Pakistan	Caucasian	MI	52.1±11.3	19.7	229	217	NA
Ashavaid	2011	India	Asian	CAD	58.6±10.4	19.7	446	473	NA
Lima	2011	Brazil	Caucasian	CAD	60	50	123	38	NA
Zhao	2012	China	Asian	CAD	40-82	32.9	146	113	NA
Lin	2012	China	Asian	CAD	43±14	38	65	132	NA

MI, myocardium infarction; HDL, high-density lipoprotein; LDL, low-density lipoprotein; BMI, body mass index; NA, not available.

Table 2. Distribution of *PAI-1* -675 4G/5G polymorphism among patients and controls

Study	Case			Control			HWE
	4G/4G	4G/5G	5G/5G	4G/4G	4G/5G	5G/5G	
Dawson	29	51	27	23	24	26	No
Eriksson	40	38	15	26	54	20	Yes
Ye	148	230	98	189	271	141	No
Mansfield	20	15	3	37	67	18	Yes
Burzotta	32	46	30	52	86	37	Yes
Ridker	101	191	82	133	247	115	Yes
Ossei-Gerning	59	73	26	36	65	49	Yes
Iwai	83	99	22	53	76	19	Yes
Kohler	66	91	27	54	86	48	Yes
Margaglione	68	85	45	239	493	249	Yes
Pastinen	46	74	31	30	80	40	Yes
Junker	86	112	43	52	93	34	Yes
Sugano	5	28	33	6	27	29	Yes
Ardissino	38	93	69	32	102	66	Yes
Anderson 1	105	193	77	303	457	218	Yes
Anderson 2	267	433	198	97	155	77	Yes
Doggen	88	170	73	84	150	68	Yes
Gardemann 1	624	985	362	167	305	122	Yes
Gardemann 2	382	606	226	409	684	258	Yes
Grancha	6	23	12	11	30	21	Yes
Beneš	53	91	31	77	103	42	Yes
Canavy	48	97	56	64	121	59	Yes
Hooper	7	42	59	11	79	104	Yes
Mikkelsen	18	38	12	29	78	57	Yes
Song	62	64	32	54	60	25	Yes
Fu	39	29	19	25	45	22	Yes
Viitanen	29	65	24	28	51	31	Yes
Dai	85	110	55	12	48	35	Yes
Fu	58	49	16	38	85	49	Yes
Shang	13	18	7	20	37	23	Yes
Ortlepp	36	48	16	24	54	22	Yes
Yamada	215	300	75	315	316	73	Yes

PAI-1 gene is the guanine deletion polymorphism at position -675 nucleotides relative to the transcription start site (rs179-9889). The *PAI-1* -675 4G allele has higher transcriptional activity than the *PAI-1* -675 5G allele and homozygous possession of -675 4G is associated with higher plasma *PAI-1* levels [5]. A number of papers investigated the association between this polymorphism and CAD risk. However, the results remained inconclusive [6-73]. Metaanalysis is a useful method for investigating associations between genetic factors and diseases, because a quantitative approach is used to combine the results from different studies on the same topic, thereby providing more reliable conclusions. Thus, we performed a meta-analysis to clarify the association of *PAI-1* 4G/5G polymorphism with CAD.

Methods

Publication search

A computerized literature search was performed to identify the relevant studies from five electronic databases including PubMed, EMBASE, China National Knowledge Infrastructure (CNKI), Database of Chinese Scientific and Technical Periodicals, and China

PAI-1 polymorphism and CAD risk

Guan	50	52	24	23	70	28	Yes	<i>Inclusion and exclusion criteria</i> The following criteria were used for the literature selection: first, studies should concern the association of PAI-1 4G/5G polymorphism with CAD risk; second, studies must be observational studies (case-control or cohort); third, papers must offer the size of the sample, odds ratios (ORs) and their 95% confidence intervals (CIs), the genetic distribution or the information that can help infer the results. Studies were excluded if one of the following existed: first, studies were not relevant to PAI-1 or CAD; second, the design based on family or sibling pairs; third, sample size or OR and 95% CI were not reported; fourth, reviews and abstracts. As for the studies from the same institution, only the one with the largest sample size was included. No language restrictions were imposed.
Li	13	18	5	5	11	0	Yes	
ATVBISG	335	589	286	342	588	280	Yes	
Crainich	70	136	58	200	387	166	Yes	
Juhan-Vague	125	249	109	133	269	105	Yes	
Leander 1	256	415	153	283	542	203	Yes	
Leander 2	103	180	61	153	226	110	Yes	
Petrović	45	74	35	68	89	37	Yes	
Zhan	40	14	2	25	52	6	No	
Ding 1	8	26	26	15	39	55	Yes	
Ding 2	15	23	11	10	25	28	Yes	
Wang	8	35	24	2	7	21	Yes	
Zhai	58	49	16	38	85	49	Yes	
Tobin	159	280	108	162	237	106	Yes	
Pegoraro	42	99	54	65	132	103	Yes	
Whiting	263	427	191	78	121	62	Yes	
Zak	34	74	38	44	58	19	Yes	
Agirbasli	28	46	26	23	60	17	Yes	
Su	272	390	150	275	446	210	Yes	
Xia	79	67	20	18	28	17	Yes	
Morange	105	236	120	96	254	124	Yes	
Sampaio	23	47	45	16	45	43	Yes	
Taymaz	31	58	26	15	20	6	Yes	
Onalan	51	75	30	73	112	96	Yes	
Saely	NA	NA	NA	NA	NA	NA	Yes	
Sarecka	38	94	46	69	103	30	Yes	
Zhang	58	62	35	52	87	51	Yes	
Isordia-Salas	9	64	54	17	38	72	Yes	
Tässies	56	121	71	48	92	60	Yes	
Var	43	24	19	24	36	30	Yes	
Chen	100	140	53	47	99	32	Yes	
Abboud	88	156	61	42	180	106	Yes	
Cao	61	41	14	15	27	18	Yes	
Koch	1091	1787	779	360	590	261	Yes	
Agirbasli	36	35	19	24	43	23	Yes	
Ahmed	64	86	79	52	89	76	Yes	
Ashavaid	112	218	116	113	247	113	Yes	
Lima	46	34	43	12	12	14	Yes	
Zhao	46	68	32	23	57	33	Yes	
Lin	29	28	8	34	63	35	Yes	

HWE, Hardy-Weinberg equilibrium.

Biology Medical literature database (CBM). The search terms were used as follows: (coronary artery disease or coronary heart disease or atherosclerosis) and (polymorphism or variant or mutation) and (plasminogen activator inhibitor-1 or PAI-1). All searched studies were retrieved and the bibliographies were checked for other relevant publications.

Data extraction

Data were extracted by two authors independently. If encountered the conflicting evaluations, an agreement was reached following a discussion; if could not reached agreement, another author was consulted to resolve the debate. The following information was extracted from each study: first author, year of publication, original country, ethnicity, endpoint, age, gender, sample size, covariates.

Statistical analysis

OR and 95% CI were employed to evaluate the strength of the association between 4G/5G polymorphism and the risk of CAD in dominant model. Departure from Hardy-Weinberg equilibrium (HWE) in controls was tested by the chi-square test. The Q statistic and the I² statistic were used to assess the degree of heterogeneity among the studies included in the meta-analysis. The random-effects model was used to estimate the pooled OR (the DerSimonian and Laird method).

Table 3. The effect of PAI-1 -675 4G/5G polymorphism on CAD risk

Comparison	Study	No. of studies	Test of association			Heterogeneity		
			OR (95% CI)	Z	P Value	χ^2	P Value	I ² (%)
4G/4G + 4G/5G vs. 5G/5G	Overall	72	1.19 (1.10-1.28)	4.54	< 0.00001	144.13	< 0.00001	51
4G/4G + 4G/5G vs. 5G/5G	Adjusted	10	1.20 (1.03-1.40)	2.29	0.02	13.95	0.12	36
4G/4G + 4G/5G vs. 5G/5G	Caucasian	45	1.10 (1.02-1.19)	2.54	0.01	70.61	0.007	38
4G/4G + 4G/5G vs. 5G/5G	Asian	24	1.46 (1.21-1.75)	4.03	< 0.0001	55.19	0.0002	58
4G/4G + 4G/5G vs. 5G/5G	African	2	1.38 (0.70-2.70)	0.93	0.35	5.12	0.02	80
4G/4G + 4G/5G vs. 5G/5G	MI	39	1.15 (1.06-1.25)	3.27	0.001	68.35	0.002	44
4G/4G + 4G/5G vs. 5G/5G	Early-onset	12	1.21 (1.02-1.43)	2.20	0.03	6.31	0.71	0
4G/4G + 4G/5G vs. 5G/5G	Late-onset	4	0.90 (0.72-1.13)	0.89	0.37	1.83	0.61	0
4G/4G + 4G/5G vs. 5G/5G	Male	13	1.10 (1.01-1.20)	2.10	0.04	7.23	0.84	0
4G/4G + 4G/5G vs. 5G/5G	Female	4	1.03 (0.89-1.19)	0.34	0.73	4.54	0.21	34
4G/4G + 4G/5G vs. 5G/5G	T2DM	4	2.23 (1.27-3.92)	2.80	0.005	0.48	0.79	0
4G/4G + 4G/5G vs. 5G/5G	Non-T2DM	3	1.64 (1.19-2.25)	3.03	0.002	0.57	0.75	0

MI, myocardium infarction; T2DM, type 2 diabetes.

Subgroup analyses were carried out by ethnicity, endpoint, age, gender and T2DM. We defined the early-onset CAD was the first event before 50 years old. Sensitivity analysis was performed through sequentially excluded individual studies to assess the stability of the results. The potential publication bias was examined visually in a funnel plot of log [OR] against its standard error (SE), and the degree of asymmetry was tested using Egger's test [74].

All statistical tests were performed using Revman 5.1 software (Nordic Cochrane Center, Copenhagen, Denmark) and STATA 11.0 software (Stata Corporation, College Station, TX, USA). A *P* value < 0.05 was considered statistically significant, except for tests of heterogeneity where a level of 0.10 was used.

Results

Study characteristics

The flow chart in **Figure 1** summarizes this literature review process. In this current study, a total of 68 eligible studies met the inclusion criteria [6-73]. Four articles reported two cohorts, and each cohort was considered as a case-control study. Finally, a total of 72 studies involving 23557 cases and 21526 controls were included in this meta-analysis. There were 24 studies performed using Asians, 45 studies using Caucasians, and 2 studies using Africans. Thirteen studies included only male CAD patients, and four studies included female CAD patients. Ten studies reported adjusted ORs and CIs and four studies reported the information of T2DM. Three studies were not in HWE. The characteristics of each study included in

this meta-analysis are presented in **Table 1**. Genotype frequencies and HWE examination results are listed in **Table 2**.

Quantitative data synthesis

The results of this meta-analysis are shown in **Table 3**. We found that PAI-1 4G/5G polymorphism was significant associated with CAD risk in overall population (OR=1.19, 95% CI 1.10-1.28, *P* < 0.00001, **Figure 2**). The combination of adjusted ORs for CAD was 1.20 (95% CI 1.03-1.40, *P*=0.02). In the subgroup analysis according to ethnicity, the results suggested that PAI-1 4G/5G polymorphism was associated with CAD risk in Caucasians (OR=1.10, 95% CI 1.02-1.19, *P*=0.01) and Asians (OR=1.46, 95% CI 1.21-1.75, *P* < 0.0001). However, no significant association was observed in Africans (OR=1.38, 95% CI 0.70-2.70, *P*=0.35). In terms of subgroup analyses by endpoint, the PAI-1 4G/5G polymorphism significantly increased MI risk (OR=1.15, 95% CI 1.06-1.25, *P*=0.001). In the subgroup analysis by age, the PAI-1 4G/5G polymorphism was significantly associated with early-onset CAD risk (OR=1.21, 95% CI 1.02-1.43, *P*=0.03) but not with late-onset CAD risk (OR=0.90, 95% CI 0.72-1.13, *P*=0.37). In the gender subgroup analyses, a statistically significant association was found in male CAD patients (OR=1.10, 95% CI 1.01-1.20, *P*=0.04) but not with female CAD patients (OR=1.03, 95% CI 0.89-1.19, *P*=0.73). Stratification by T2DM status showed that both T2DM patients and non-T2DM patients carrying 4G allele were associated with increased CAD risks (OR=2.23, 95% CI 1.27-3.92, *P*=0.005 and OR=1.64, 95% CI 1.19-2.25, *P*=0.002, respectively).

PAI-1 polymorphism and CAD risk

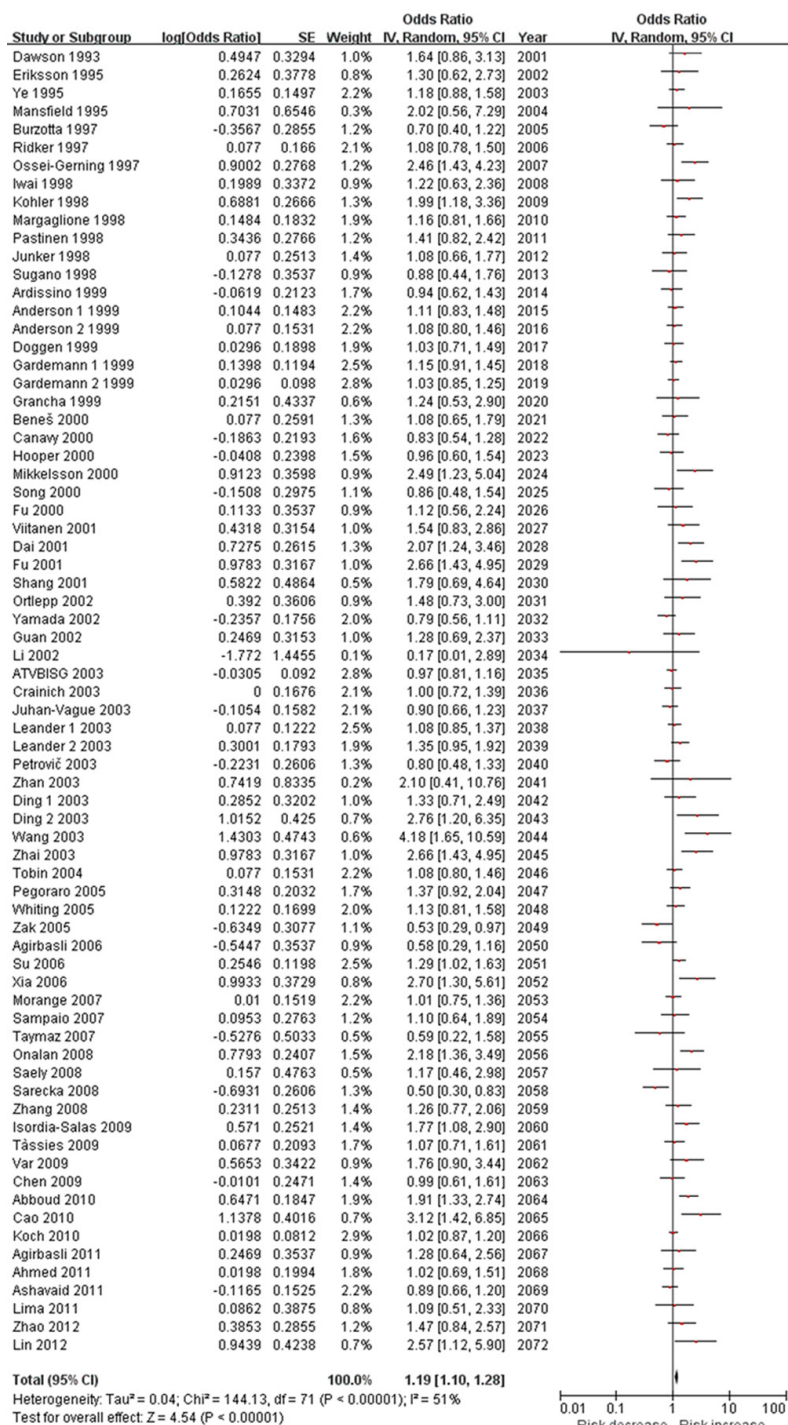


Figure 2. Meta-analysis of the association between the *PAI-1* 4G/5G polymorphism and CAD risk.

Sensitivity analysis was used to evaluate the stability of the overall results by sequential omission of individual studies. In this meta-analysis, the results of sensitive analysis showed that any single study did not influence

the overall results qualitatively (data not shown).

Funnel plots and the Egger's test were used to assess publication bias. In the funnel plot analysis, the shape of the funnel plot seemed symmetrical (**Figure 3**). Furthermore, Egger's test did not detect any publication bias ($P=0.239$). Therefore, there was no significant publication bias in the studies included in current analyses.

Discussion

This present meta-analysis investigating the relationship between *PAI-1* 4G/5G polymorphism and risk of CAD. Seventy-two studies with a total of 45083 subjects were eligible. At the overall analysis, the *PAI-1* 4G/5G polymorphism was significantly associated with CAD risk. Even the studies reporting adjusted ORs were included, the result was still significant. We also found that this polymorphism increased MI risk significantly. In the subgroup analysis by ethnicity, we noted that Asians and Caucasians carrying the 4G allele had an increased CAD risk. Only two studies investigated the association between *PAI-1* 4G/5G polymorphism and risk of CAD in Africans. Therefore, more studies are still needed. In the stratified analysis by age, we found *PAI-1* 4G/5G polymorphism showed increased early-onset CAD risk but not late-onset CAD risk. There were

only four studies about late-onset CAD risk, the positive association between *PAI-1* 4G/5G polymorphism and late-onset CAD risk could not be ruled out, because studies with small sample size may have insufficient statistical

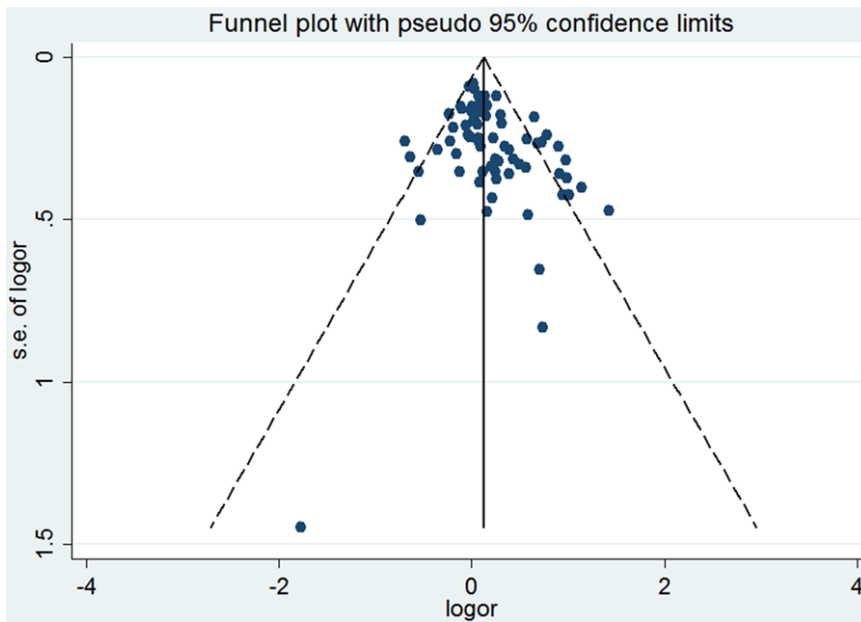


Figure 3. Funnel plot of the association between the *PAI-1* 4G/5G polymorphism and CAD risk.

power to detect a slight effect. The subgroup analysis based on gender found that this polymorphism showed increased CAD risk in male patients but not in female patients. Since the number of studies included in female subgroup analysis was small, the results lacked sufficient reliability to confirm or refute an association in a definitive manner. In the future, more studies should be designed to analyze these associations. When subgroup analysis was performed according to T2DM status, significant associations were showed in T2DM patients and non-T2DM patients. This result suggested that T2DM did not change the effect of *PAI-1* 4G/5G polymorphism on CAD. Previous meta-analysis has assessed the association between *PAI-1* 4G/5G polymorphism and risk of CAD. For example, Koch and coworkers found that the risk of MI in 4G allele carriers was found to be significantly elevated [67]. Li suggested that *PAI-1* 4G/5G polymorphism was associated with increased CAD risk in Chinese Han population [75]. Nikolopoulos et al. also indicated that *PAI-1* 4G allele slightly increased the risk for MI [76]. These results were all in line with our results. However, our study had some advantages. First, it was the first time studying T2DM and *PAI-1* 4G/5G polymorphism interactions. Second, we sought to find as many publications as we could by means of various searching approaches. Third, the main result remained

statistically significant when the adjusted ORs were combined.

PAI-1 is a glycoprotein that belongs to the serine protease inhibitor superfamily. It is equimolecularly combined with the tissue plasminogen activator (tPA) single chain, double chains, and double chain urokinase plasminogen activator (uPA). Consequently, tPA and uPA activities are rapidly inhibited by *PAI-1*. Mice in which *PAI-1* gene was invalidated were protected from thrombotic risk after vascular injury [77]. Case-

control studies in humans have shown that high *PAI-1* plasma levels were associated with an increased risk of CAD and that plasma levels of *PAI-1* were higher in patients with MI than in control individuals [78]. Therefore, *PAI-1* might be involved in the development of CAD. *PAI-1* 4G/5G polymorphism is one of the DNA sequence variations that plays a key role in regulating *PAI-1* gene expression. Studies have shown that the *PAI-1* activity of the 4G allele promoter is higher than that of 5G in a cytokine-stimulated state. Unlike the 5G allele that binds a transcription repressor protein, resulting in low *PAI-1* expression, the 4G allele does not bind a transcription repressor, thus conferring a high *PAI-1* expressor nature to the allele [5].

Some limitations should be addressed. First, there was only two case-control study investigated the association of *PAI-1* 4G/5G polymorphism and risk of CAD in Africans. Therefore, more studies with large sample sizes are needed to further identify the association among Africans. Second, because small negative studies are less likely to be published, the possibility of publication bias cannot be ruled out completely, even though the Egger's test and funnel plots did not provide the evidence of publication bias in this meta-analysis. Third, a lack of original data from the eligible studies limited evaluation of the effects of the gene-gene and

gene-environment interactions during CAD development.

In conclusion, this meta-analysis suggested that *PAI-1* 4G/5G polymorphism was associated with increased CAD risk. Further studies with large sample size were needed to confirm our findings.

Disclosure of conflict of interest

None.

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References

- [1] Pepper MS. Role of the matrix metalloproteinase and plasminogen activator-plasmin systems in angiogenesis. *Arterioscler Thromb Vasc Biol* 2001; 21: 1104-17.
- [2] Cortellaro M, Cofrancesco E, Boschetti C, Mussoni L, Donati MB, Cardillo M, Catalano M, Gabrielli L, Lombardi B, Specchia G. Increased fibrin turnover and high *PAI-1* activity as predictors of ischemic events in atherosclerotic patients. A case-control study. The PLAT Group. *Arterioscler Thromb* 1993; 13: 1412-7.
- [3] Sobel BE. Increased plasminogen activator inhibitor-1 and vasculopathy. A reconcilable paradox. *Circulation* 1999; 99: 2496-8.
- [4] Zorio E, Gilabert-Estellés J, España F, Ramón LA, Cosín R, Estellés A. Fibrinolysis: the key to new pathogenetic mechanisms. *Curr Med Chem* 2008; 15: 923-9.
- [5] Dawson S, Hamsten A, Wiman B, Henney A, Humphries S. Genetic variation at the plasminogen activator inhibitor-1 locus is associated with altered levels of plasma plasminogen activator inhibitor-1 activity. *Arterioscler Thromb* 1991; 11: 183-90.
- [6] Dawson SJ, Wiman B, Hamsten A, Green F, Humphries S, Henney AM. The two allele sequences of a common polymorphism in the promoter of the plasminogen activator inhibitor-1 (*PAI-1*) gene respond differently to interleukin-1 in HepG2 cells. *J Biol Chem* 1993; 268: 10739-45.
- [7] Eriksson P, Kallin B, van't Hooft FM, Bavenholm P, Hamsten A. Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. *Proc Natl Acad Sci U S A* 1995; 92: 1851-5.
- [8] Ye S, Green FR, Scarabin PY, Nicaud V, Bara L, Dawson SJ, Humphries SE, Evans A, Luc G, Cambou JP, et al. The 4G/5G genetic polymorphism in the promoter of the plasminogen activator inhibitor-1 (*PAI-1*) gene is associated with differences in plasma *PAI-1* activity but not with risk of myocardial infarction in the ECTIM study. *Etude CasTemoins de l'infarctus du Myocarde. Thromb Haemost* 1995; 74: 837-41.
- [9] Mansfield MW, Stickland MH, Grant PJ. Plasminogen activator inhibitor-1 (*PAI-1*) promoter polymorphism and coronary artery disease in non-insulin-dependent diabetes. *Thromb Haemost* 1995; 74: 1032-1034.
- [10] Burzotta F, Di Castelnuovo A, Amore C, D'Orazio A, Donati MB, Iacoviello L. 4G/5G polymorphism in the promoter region of the *PAI-1* gene is not a risk factor for familial myocardial infarction in subjects over 45 years. *Thromb Haemost* 1997; 78: 1294-5.
- [11] Ridker PM, Hennekens CH, Lindpaintner K, Stampfer MJ, Miletich JP. Arterial and venous thrombosis is not associated with the 4G/5G polymorphism in the promoter of the plasminogen activator inhibitor gene in a large cohort of US men. *Circulation* 1997; 95: 59-62.
- [12] Ossei-Gerning N, Mansfield MW, Stickland MH, Wilson IJ, Grant PJ. Plasminogen activator inhibitor-1 promoter 4G/5G genotype and plasma levels in relation to a history of myocardial infarction in patients characterized by coronary angiography. *Arterioscler Thromb Vasc Biol* 1997; 17: 33-7.
- [13] Iwai N, Shimoike H, Nakamura Y, Tamaki S, Kinoshita M. The 4G/5G polymorphism of the plasminogen activator inhibitor gene is associated with the time course of progression to acute coronary syndromes. *Atherosclerosis* 1998; 136: 109-14.
- [14] Kohler HP, Stickland MH, Ossei-Gerning N, Carter A, Mikkola H, Grant PJ. Association of a common polymorphism in the factor XIII gene with myocardial infarction. *Thromb Haemost* 1998; 79: 8-13.
- [15] Margaglione M, Cappucci G, Colaizzo D, Giuliani N, Vecchione G, Grandone E, Pennelli O, Di Minno G. The *PAI-1* gene locus 4G/5G polymorphism is associated with a family history of coronary artery disease. *Arterioscler Thromb Vasc Biol* 1998; 18: 152-6.
- [16] Pastinen T, Perola M, Niini P, Terwilliger J, Salomaa V, Vartiainen E, Peltonen L, Syvänen A. Array-based multiplex analysis of candidate genes reveals two independent and additive genetic risk factors for myocardial infarction in the Finnish population. *Hum Mol Genet* 1998; 7: 1453-62.
- [17] Junker R, Heinrich J, Schulte H, Tataru M, Köhler E, Schönfeld R, Nowak-Göttl U, Assmann

PAI-1 polymorphism and CAD risk

- G. Plasminogen activator inhibitor-1 4G/5G-polymorphism and factor V Q506 mutation are not associated with myocardial infarction in young men. *Blood Coagul Fibrinolysis* 1998; 9: 597-602.
- [18] Sugano T, Tsuji H, Masuda H, Nakagawa K, Nishimura H, Kasahara T, Yoshizumi M, Nakahara Y, Kitamura H, Yamada K, Yoneda M, Maki K, Tatsumi T, Azuma A, Nakagawa M. Plasminogen activator inhibitor-1 promoter 4G/5G genotype is not a risk factor for myocardial infarction in a Japanese population. *Blood Coagul Fibrinolysis* 1998; 9: 201-4.
- [19] Ardissino D, Mannucci PM, Merlini PA, Duca F, Fetiveau R, Tagliabue L, Tubaro M, Galvani M, Ottani F, Ferrario M, Corral J, Margaglione M. Prothrombotic genetic risk factors in young survivors of myocardial infarction. *Blood* 1999; 94: 46-51.
- [20] Anderson JL, Muhlestein JB, Habashi J, Carlquist JF, Bair TL, Elmer SP, Davis BP. Lack of association of a common polymorphism of the plasminogen activator inhibitor-1 gene with coronary artery disease and myocardial infarction. *J Am Coll Cardiol* 1999; 34: 1778-83.
- [21] Doggen CJ, Bertina RM, Cats VM, Reitsma PH, Rosendaal FR. The 4G/5G polymorphism in the plasminogen activator inhibitor-1 gene is not associated with myocardial infarction. *Thromb Haemost* 1999; 82: 115-20.
- [22] Gardemann A, Lohre J, Katz N, Tillmanns H, Hehrlein FW, Haberbosch W. The 4G/5G genotype of the plasminogen activator inhibitor 4G/5G gene polymorphism is associated with coronary atherosclerosis in patients at high risk for this disease. *Thromb Haemost* 1999; 82: 1121-6.
- [23] Grancha S, Estellés A, Tormo G, Falco C, Gilbert J, España F, Cano A, Seguí R, Aznar J. Plasminogen activator inhibitor-1 (*PAI-1*) promoter 4G/5G genotype and increased *PAI-1* circulating levels in postmenopausal women with coronary artery disease. *Thromb Haemost* 1999; 81: 516-21.
- [24] Benes P, Muzík J, Benedík J, Frélich M, Elbl L, Vasku A, Znojil V, Vácha J. Single effects of apolipoprotein B, (a), and E polymorphisms and interaction between plasminogen activator inhibitor-1 and apolipoprotein (a) genotypes and the risk of coronary artery disease in Czech male caucasians. *Mol Genet Metab* 2000; 69: 137-43.
- [25] Canavy I, Henry M, Morange PE, Tiret L, Poirier O, Ebagosti A, Bory M, Juhan-Vague I. Genetic polymorphisms and coronary artery disease in the south of France. *Thromb Haemost* 2000; 83: 212-6.
- [26] Hooper WC, Lally C, Austin H, Renshaw M, Dilley A, Wenger NK, Phillips DJ, Whitsett C, Rawlins P, Evatt BL. The role of the t-PA I/D and *PAI-1* 4G/5G polymorphisms in African-American adults with a diagnosis of myocardial infarction or venous thromboembolism. *Thromb Res* 2000; 99: 223-30.
- [27] Mikkelsen J, Perola M, Wartiovaara U, Peltonen L, Palotie A, Penttilä A, Penttilä A, Karhunen PJ. Plasminogen activator inhibitor-1 (*PAI-1*) 4G/5G polymorphism, coronary thrombosis, and myocardial infarction in middle-aged Finnish men who died suddenly. *Thromb Haemost* 2000; 84: 78-82.
- [28] Song J, Yoon YM, Jung HJ, Hong SH, Park H, Kim JQ. Plasminogen activator inhibitor-1 4G/5G promoter polymorphism and coagulation factor VII Arg353->Gln polymorphism in Korean patients with coronary artery disease. *J Korean Med Sci* 2000; 15: 146-52.
- [29] Fu L, Jin H, Song K, Zhang C, Shen J, Huang Y. Relationship between gene polymorphism of the *PAI-1* promoter and myocardial infarction. *Chin Med J (Engl)* 2001; 114 : 266-9.
- [30] Viitanen L, Pihlajamäki J, Halonen P, Lehtonen M, Kareinen A, Lehto S, Laakso M. Association of angiotensin converting enzyme and plasminogen activator inhibitor-1 promoter gene polymorphisms with features of the insulin resistance syndrome in patients with premature coronary heart disease. *Atherosclerosis* 2001; 157: 57-64.
- [31] Dai Y, Gao RL, Ye Y, Wu YJ, Chen JL, et al. The 4G/5G genetic polymorphism of the plasminogen activator inhibitor-1 (*PAI-1*) gene is not associated with plasma *PAI-1* antigen level and the risk of coronary artery disease in Chinese. *Chin Circ J* 2001; 16: 275-8.
- [32] Fu Y, Wang XD, Zhai YL, Fan Z, Yang L, et al. The 4G/5G polymorphism of the plasminogen activator inhibitor-1 gene in patients with coronary heart disease. *J Capital Univers Med Sci* 2001; 22: 119-22.
- [33] Shang SH, Wang C, Yu YC, Gu Y, Wu GT, Wu ZC. Plasminogen activator inhibitor-1 (*PAI-1*) promoter polymorphism and coronary artery disease in type 2 diabetes. *J Tongji Univers (Med Sci)* 2001; 4: 16-8.
- [34] Ortlepp JR, Lauscher J, Janssens U, Minkenberg R, Hanrath P, Hoffmann R. Analysis of several hundred genetic polymorphisms may improve assessment of the individual genetic burden for coronary artery disease. *Eur J Intern Med* 2002; 13: 485-92.
- [35] Yamada Y, Izawa H, Ichihara S, Takatsu F, Ishihara H, Hirayama H, Sone T, Tanaka M, Yokota M. Prediction of the risk of myocardial infarction from polymorphisms in candidate genes. *N Engl J Med* 2002; 347: 1916-23.
- [36] Guan L, Ji X, Wang J, Zhang A, Zhang Y, Zhao I. Association of plasminogen activator inhibitor-1 gene 4G/5G polymorphism and coronary heart disease in Chinese patients. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2002; 19: 393-6.

PAI-1 polymorphism and CAD risk

- [37] Li XS, Xian SX, Huang HQ. Relationship between plasminogen activator inhibitor-1 gene and coronary heart disease with blood-stagnation syndrome. *J Guangzhou Univers Tradition Chin Med* 2002; 19: 261-4.
- [38] Atherosclerosis, Thrombosis, and Vascular Biology Italian Study Group. No evidence of association between prothrombotic gene polymorphisms and the development of acute myocardial infarction at a young age. *Circulation* 2003; 107: 1117-22.
- [39] Crainich P, Jenny NS, Tang Z, Arnold AM, Kuller LH, Manolio T, Sharrett AR, Tracy RP. Lack of association of the plasminogen activator inhibitor-1 4G/5G promoter polymorphism with cardiovascular disease in the elderly. *J Thromb Haemost* 2003; 1: 1799-804.
- [40] Juhan-Vague I, Morange PE, Frere C, Aillaud MF, Alessi MC, Hawe E, Boquist S, Tornvall P, Yudkin JS, Tremoli E, Margaglione M, Di Minno G, Hamsten A, Humphries SE. The plasminogen activator inhibitor-1-675 4G/5G genotype influences the risk of myocardial infarction associated with elevated plasma proinsulin and insulin concentrations in men from Europe: the HIFMECH study. *J Thromb Haemost* 2003; 1: 2322-9.
- [41] Leander K, Wiman B, Hallqvist J, Sten-Linder M, de Faire U. *PAI-1* level and the *PAI-1* 4G/5G polymorphism in relation to risk of non-fatal myocardial infarction: results from the Stockholm Heart Epidemiology Program (SHEEP). *Thromb Haemost* 2003; 89: 1064-71.
- [42] Petrovic D, Globocnik-Petrovic M, Peterlin B. 4G4G genotype of *PAI-1* gene promoter polymorphism is not associated with myocardial infarction in Caucasians with type-2 diabetes. *Cardiology* 2003; 100: 157-8.
- [43] Zhan M, Zhou Y, Han Z. Plasminogen activator inhibitor-1 4G/5G gene polymorphism in patients with myocardial or cerebrovascular infarction in Tianjin, China. *Chin Med J (Engl)* 2003; 116: 1707-10.
- [44] Ding GX, Shen J, Chen JW. The relationship among polymorphism of *PAI-1* gene, type 2 diabetes mellitus, hypertension and coronary heart disease. *Acta Univers Med Nanjing (Nat Sci)* 2003; 1: 4-7.
- [45] Wang YS, Wang SY, Zhang M, Bai CX, Li SQ. Polymorphisms of *PAI-1* 4G/5G in patients with coronary heart disease complicated with or without OSAHS. *New Med* 2003; 34: 674-6.
- [46] Zhai YL, Fu Y, Wang XD, Liu FQ, Qian J, Fan Z, et al. Relation between plasminogen activator inhibitor-1 and the *PAI-1* gene locus 4G/5G polymorphism and their effect on the incidence of coronary heart disease. 2003; 5: 246-8.
- [47] Tobin MD, Braund PS, Burton PR, Thompson JR, Steeds R, Channer K, Cheng S, Lindpaintner K, Samani NJ. Genotypes and haplotypes predisposing to myocardial infarction: a multilocus case-control study. *Eur Heart J* 2004; 25: 459-67.
- [48] Pegoraro RJ, Ranjith N. Plasminogen activator inhibitor type 1 (*PAI-1*) and platelet glycoprotein IIIa (PGIIIa) polymorphisms in young Asian Indians with acute myocardial infarction. *Cardiovasc J S Afr* 2005; 16: 266-70.
- [49] Whiting BM, Anderson JL, Muhlestein JB, Horne BD, Bair TL, Pearson RR, Carlquist JF; Intermountain Heart Collaborative Study Group. Candidate gene susceptibility variants predict intermediate end points but not angiographic coronary artery disease. *Am Heart J* 2005; 150: 243-50.
- [50] Zak I, Balcerzyk A, Sarecka B, Niemiec P, Cierniewski Z, Dylag S. Contemporaneous carrier-state of two or three proatherosclerotic variants of APOE, ICAM1, PPARA and *PAI-1* genes differentiate CAD patients from healthy individuals. *Clin Chim Acta* 2005; 362: 110-8.
- [51] Agirbasli D, Agirbasli M, Williams SM, Phillips JA 3rd. Interaction among 5, 10 methylenetetrahydrofolate reductase, plasminogen activator inhibitor and endothelial nitric oxide synthase gene polymorphisms predicts the severity of coronary artery disease in Turkish patients. *Coron Artery Dis* 2006; 17: 413-7.
- [52] Su S, Chen S, Zhao J, Huang J, Wang X, Chen R, Gu D. Plasminogen activator inhibitor-1 gene: selection of tagging single nucleotide polymorphisms and association with coronary heart disease. *Arterioscler Thromb Vasc Biol* 2006; 26: 948-54.
- [53] Xia DS, Cao J, Song YQ, Hu SY, Guo QY, et al. Association between serotonin transporter and plasminogen activator inhibitor-1 gene polymorphisms and depressive disorder in patients with coronary heart disease. *Chin J of Behavioral Med Sci* 2006; 15: 481-3.
- [54] Morange PE, Saut N, Alessi MC, Yudkin JS, Margaglione M, Di Minno G, Hamsten A, Humphries SE, Tregouet DA, Juhan-Vague I. Association of plasminogen activator inhibitor (*PAI*)-1 (SERPINE1) SNPs with myocardial infarction, plasma *PAI-1*, and metabolic parameters: the HIFMECH study. *Arterioscler Thromb Vasc Biol* 2007; 27: 2250-7.
- [55] Sampaio MF, Hirata MH, Hirata RD, Santos FC, Picciotti R, Luchessi AD, de Quateli Doi S, Armaganijan D, Batlouni M. AMI is associated with polymorphisms in the NOS3 and FGB but not in *PAI-1* genes in young adults. *Clin Chim Acta* 2007; 377: 154-62.
- [56] Taymaz H, Erarslan S, Oner ET, Alkan T, Ağirbaşlı M, Kirdar B. Sequence variations within the genes related to hemostatic imbalance and their impact on coronary artery disease in Turkish population. *Thromb Res* 2007; 119: 55-62.

PAI-1 polymorphism and CAD risk

- [57] Onalan O, Balta G, Oto A, Kabakci G, Tokgozoglu L, Aytemir K, Altay C, Gurgey A, Nazli N. Plasminogen activator inhibitor-1 4G4G genotype is associated with myocardial infarction but not with stable coronary artery disease. *J Thromb Thrombolysis* 2008; 26: 211-7.
- [58] Saely CH, Muendlein A, Vonbank A, Sonderegger G, Aczel S, Rein P, Risch L, Drexel H. Type 2 diabetes significantly modulates the cardiovascular risk conferred by the *PAI-1* -675 4G/5G polymorphism in angiographed coronary patients. *Clin Chim Acta* 2008; 396: 18-22.
- [59] Sarecka B, Zak I, Krauze J. Synergistic effects of the polymorphisms in the *PAI-1* and *IL-6* genes with smoking in determining their associated risk with coronary artery disease. *Clin Biochem* 2008; 41: 467-73.
- [60] Zhang AY, Ji XW, Guan LX, Zhang AJ, Wang JX. Association of *PAI-1* gene polymorphism with prognosis of coronary artery disease. *Chin J Med Genet* 2008; 2: 233-5.
- [61] Isordia-Salas I, Leanos-Miranda A, Sainz IM, Reyes-Maldonado E, Borrayo-Sanchez G. Association of the plasminogen activator inhibitor-1 gene 4G/5G polymorphism with ST elevation acute myocardial infarction in young patients. *Rev Esp Cardiol* 2009; 62: 365-72.
- [62] Tassies D, Roque M, Monteagudo J, Martorell T, Sionis A, Arzamendi D, Heras M, Reverter JC. Thrombin-activatable fibrinolysis inhibitor genetic polymorphisms as markers of the type of acute coronary syndrome. *Thromb Res* 2009; 124: 614-8.
- [63] Var A, Utük O, Akçali S, Sanlıdağ T, Uyanik BS, Dinç G. Impact of hemostatic gene single point mutations in patients with non-diabetic coronary artery disease. *Mol Biol Rep* 2009; 36: 2235-43.
- [64] Chen Y, Zhao LS, Zheng H, Yang F, Kong RN, Zhou S, et al. The association between *PAI-1* and *PAI-1* -675 4G/5G polymorphism and coronary artery disease. *Shangdong Med J* 2009; 5: 61-3.
- [65] Abboud N, Ghazouani L, Saidi S, Ben-Hadj-Khalifa S, Addad F, Almawi WY, Mahjoub T. Association of *PAI-1* 4G/5G and -844G/A gene polymorphisms and changes in *PAI-1*/tissue plasminogen activator levels in myocardial infarction: a case-control study. *Genet Test Mol Biomarkers* 2010; 14: 23-7.
- [66] Cao XL, ZCY, Yin L, Wang SC, Jia XL, Huang H, et al. Reactive protein, plasminogen activator inhibitor type-1 (*PAI-1*) levels, *PAI-1* promoter 4G/5G polymorphism and acute myocardial infarction. *J Geriatr Cardiol* 2010; 7: 147-51.
- [67] Koch W, Schrempf M, Erl A, Mueller JC, Hopmann P, Schömig A, Kastrati A. 4G/5G polymorphism and haplotypes of *SERPINE1* in atherosclerotic diseases of coronary arteries. *Thromb Haemost* 2010; 103: 1170-80.
- [68] Agirbasli M, Guney AI, Ozturhan HS, Agirbasli D, Ulucan K, Sevinc D, Kirac D, Ryckman KK, Williams SM. Multifactor dimensionality reduction analysis of *MTHFR*, *PAI-1*, *ACE*, *PON1*, and *eNOS* gene polymorphisms in patients with early onset coronary artery disease. *Eur J Cardiovasc Prev Rehabil* 2011; 18: 803-9.
- [69] Ahmed W, Malik M, Saeed I, Khan AA, Sadeque A, Kaleem U, Ahmed N, Ajmal M, Azam M, Qamar R. Role of tissue plasminogen activator and plasminogen activator inhibitor polymorphism in myocardial infarction. *Mol Biol Rep* 2011; 38: 2541-8.
- [70] Ashavaid TF, Todur SP, Kondkar AA, Nair KG, Shalia KK, Dalal JJ, Rajani R, Ponde CK. Platelet polymorphisms: frequency distribution and association with coronary artery disease in an Indian population. *Platelets* 2011; 22: 85-91.
- [71] Lima LM, Carvalho Md, Fonseca Neto CP, Garcia JC, Sousa MO. *PAI-1* 4G/5G polymorphism and plasma levels association in patients with coronary artery disease. *Arq Bras Cardiol* 2011; 97: 462-389.
- [72] Zhao XH, Liu P, Han ZQ. The association of plasminogen activator inhibitor type-1 gene 4G/5G polymorphism with coronary heart disease. *Chin Modern Doctor* 2012; 50: 1-5.
- [73] Lin ZX, Yan HF, Lin JD, Zou XB. The association between plasminogen activator inhibitor type-1 gene polymorphism and coronary heart disease. *Guangdong Med J* 2013; 34: 3588-90.
- [74] Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; 315: 629-34.
- [75] Li YY. Plasminogen activator inhibitor-1 4G/5G gene polymorphism and coronary artery disease in the Chinese Han population: a meta-analysis. *PLoS One* 2012; 7: e33511.
- [76] Nikolopoulos GK, Bagos PG, Tsangaris I, Tsiara CG, Kopterides P, Vaiopoulos A, Kapsimali V, Bonovas S, Tsantes AE. The association between plasminogen activator inhibitor type 1 (*PAI-1*) levels, *PAI-1* 4G/5G polymorphism, and myocardial infarction: a Mendelian randomization meta-analysis. *Clin Chem Lab Med* 2014; 52: 937-50.
- [77] Carmeliet P, Stassen JM, Schoonjans L, Ream B, van den Oord JJ, De Mol M, Mulligan RC, Collen D. Plasminogen activator inhibitor-1 gene-deficient mice. II. Effects on hemostasis, thrombosis, and thrombolysis. *J Clin Invest* 1993; 92: 2756-60.
- [78] Hamsten A, de Faire U, Walldius G, Dahlén G, Szamosi A, Landou C, Landou C, Blombäck M, Wiman B. Plasminogen activator inhibitor in plasma: risk factor for recurrent myocardial infarction. *Lancet* 1987; 2: 3-9.