Association of plasminogen activator inhibitor-1 4G5G Polymorphism with risk of diabetic nephropathy and retinopathy: a systematic review and meta-analysis

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Abstract

Background The 4G5G polymorphism of Plasminogen activator inhibitor-1 (PAI-1) gene is reported to be associated with diabetes nephropathy and retinopathy (DNR) risk. However, the findings are conflicting. Herein, we conducted a case-control and meta-analysis study to explore the association of PAI-1 4G5G polymorphism with risk of DNR.

Methods We retrieved PubMed, EMBASE, Web of Knowledge, and CNKI databases and screened eligible studies up to August 15, 2020. The strength of associations was assessed by odd ratio (OR) and the corresponding 95% confidence interval (95% CI).

Results A total of 27 case-control studies including 16 studies with 1,825 cases and 1,731 controls on DN and eleven studies with 1,397 cases and 1,545 controls on DR were selected. Pooled data showed that the PAI-1 4G5G polymorphism was significantly associated with DN (allele model: OR = 0.674, 95% CI 0.524–0.865, $p = 0.002$; homozygote model: OR = 0.536, 95% CI 0.351–0.817, $p = 0.004$; heterozygote model: OR = 0.621, 95% CI 0.427–0.903, $p = 0.013$; dominant model: OR = 0.575, 95% CI 0.399–0.831, $p = 0.003$; and recessive model: OR = 0.711, 95% CI 0.515–0.981, $p = 0.038$) and DR (homozygote model: OR = 0.770, 95% CI 0.621–0.955, $p = 0.017$) risk. Stratified analyses by ethnicity indicated that PAI-1 4G5G polymorphism was associated with DN and DR risk in Asians and Caucasians, respectively.

Conclusions The present meta-analysis revealed that the PAI-1 4G5G polymorphism was associated with increased risk of DN and DR risk. However, well-designed large-scale clinical studies are required to further validate our results.

Keywords Diabetic Mellitus · Nephropathy · Retinopathy · PAI-1 Gene · Meta-Analysis

Introduction

The vascular complications of diabetes mellitus (DM) are the most serious manifestations which affecting several important organ systems of human body [1–3]. Diabetic nephropathy

and retinopathy (DNR) are most common complications in DM patients with long-standing hyperglycemia [4–7]. Diabetic nephropathy (DN) is a common microvascular complication of Type 1 Diabetes (T1D) which traditionally defined as urine albumin excretion (UAE) > 300 mg/24hrs and

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declining renal function in the absence of urinary tract infection or any other renal disease, referred to as overt nephropathy or clinical proteinuria [8, 9]. DN remains the leading cause of end-stage renal disease (ESRD) necessitating transplantation or dialysis among people in the working age. DN accounts for approximately 20% of diabetes-related deaths directly [10, 11]. Moreover, diabetic retinopathy (DR) is diabetic complication, which histopathology is based on capillary damage with typical loss of endothelial cells and pericytes, deposition of glycoproteins in capillary basement membrane and increased vascular permeability [12–14]. DR is frequently symptomless until evolving into a severe vision-threatening form among the working aged population [15, 16]. DR increases progressively with diabetes duration and reaches 100% prevalence after 15–20 years. An epidemiological study reported that the prevalence of DR in patients with Type 2 Diabetes (T2D) was 8.2% in the United States [17, 18]. The common pathological mechanism, leading to DR and DN, may present microangiopathic damage of capillaries of affected organs, kidneys and eyes [19].

There is very close relationship between DR and DN in both epidemiology and pathogenesis [20]. Epidemiological studies have demonstrated that genetic susceptibility together with environmental factors contributes to the development of DR and DN [21]. Genetic model analyses revealed that a single or two plausibly causal genetic variant with a dominant or recessive effect interacting with environmental factors, or a few genes with a moderate effect in a similar interaction in development of DR and DN [2, 22]. Plasminogen activator inhibitor-1 (PAI-1) is a multifunctional protein which plays a crucial role in wide range of pathophysiological conditions, including wound healing, obesity, metabolic syndrome, cardiovascular disease, and cancer.[23–25] the elevated PAI-1 concentrations associated with insulin resistance, impaired glucose tolerance (IGT), and T2D.[26] Moreover, mouse model studies revealed that PAI-1 has emerged as a powerful fibrogenic mediator in DN and anti-Thy-1-antibody-mediated glomerulonephritis [27, 28].

The human PAI-1, a serine protease inhibitor (also known as Serpin E1) is located on chromosome 7q21.3-q22, consists of 9 exons and with a length of 12.2 kb [29]. The PAI-1 promoter contains a common –675 4G5G polymorphism that may affect both basal and inducible PAI-1 expression [30, 31]. The progress in the knowledge about the role of PAI-1 gene in up regulation of hyperinsulinemia and hyperglycemia have led to several studies have tested a potential association of the PAI-1 4G5G polymorphism with development of DR and DN in diabetic patients [32], but the results were conflict. Thus, we performed a case-control and meta-analysis to evaluate the association of the PAI-1 4G5G polymorphism with DNR risk.

Materials and methods

Case-control study

Subjects, genomic DNA extraction and genotyping

The case-control study was performed on 360 subjects (pediatrics and adults), including 120 diabetic patients with nephropathy, 120 diabetic patients with retinopathy, and 120 diabetic patients without DN and DR. The subjects were matched for age, sex and BMI. From each subject 4 ml of blood was drawn into vacutainer tubes containing EDTA and stored at 4° C. then, the genomic DNA was extracted from peripheral blood using the QiAamp DNA Mini Kit (Qiagen, Hilden, Germany). The polymerase chain reaction (PCR) followed by restriction enzyme digestion was used to genotyping PAI-1 4G5G polymorphism. Oligonucleotide primers used were forward 5'-CACAGAGAGAGTCTGGACACGTGA-3' and reverse 5'-TGCAGCCAGCCACGTGATTGTCTAG-3'. Each sample contained 80 ng of genomic DNA, 6–8 pmoles of each primer, 0.25 mM of dNTPs, 10 mmol/l Tris HCl pH 8.3, 50 mmol/l KCl, 1.5 mmol/l MgCl₂, and 1 unit of Taq DNA polymerase (Sinagen, Iran). PCR conditions were: 95 °C for 5 min, following by 95 °C for 50 s, 69 °C for 50 s, 72 °C for 50 s for 30 cycles and finally 72 °C for 10 min. Then, the amplification products were digested with 5 units of BseRI restriction enzyme (New England Biolabs) at 55 °C for 3 hr, according to the manufacturer's protocol. Digestion was visualized following electrophoresis on a 3% agarose gel containing ethidium bromide. The PAI-1 wild type allele (5G) showed 148 bp fragment (not digested), and mutated allele (4G) with a BseRI endonuclease site was digested into two 110 bp and 38 bp fragments.

Statistical analysis

All the statistical analysis was performed using the Excel 2013 and version 20.0 software (SPSS Company, Chicago, Illinois, USA), in which $P < 0.05$ was considered to indicate a statistically significant difference. Genotype distribution analysis between DNR and diabetic patients without nephropathy and retinopathy (DNR) was carried out by χ^2 test. The strength of the association of the PAI-1 4G5G polymorphism with DNR risk was measured by odds ratios (ORs) with 95% confidence intervals (CIs).

Meta-analysis

Search strategy

A comprehensive literature search on electronic databases including PubMed, EMBASE, Web of Science, Elsevier, Google Scholar, Cochrane Library, SciELO, SID, WanFang,

VIP, Chinese Biomedical Database (CBD) and Chinese National Knowledge Infrastructure (CNKI) to identify all relevant studies on the association of PAI-1 4G5G Polymorphism with diabetic nephropathy and retinopathy up to August 15, 2020. We retrieved the relevant studies using the following terms: (“Diabetes” OR “Diabetes Mellitus” OR “Diabetic Nephropathy” OR “Diabetic Retinopathy”) AND (“Plasminogen Activator Inhibitor-1” OR “PAI-1” OR “Serpin E1” OR “insertion (4G)/deletion (5G)” OR “-675 4G5G” OR “PAI-1 4G5G” OR “rs1799889”) AND (“Polymorphism” OR “Mutation” OR “Genotype” OR “Allele” OR “Variation” OR “Variant”). There were no limitations on the language of publication. The search was focused on studies that had been conducted in humans. Only full-text articles and the most recent studies were included in this meta-analysis.

Inclusion and exclusion criteria

The following major criteria should be met by the included studies: (a) published case-control study or cohort study; (b) evaluated the association of PAI-1 4G5G polymorphism with DN and DR; (c) adequate information provided to calculate the odds ratio (OR) and the corresponding 95% confidence interval (95% CI). Accordingly, studies with the following characteristics were excluded: (a) not case-control study or cohort; (b) animal studies or invitro studies; (c) studies without sufficient available data or lacking of genotypes distribution data; (d) linkage studies and family based studies (sibling, twins and trios-parents studies); (e) abstracts, posters, case reports, reviews, previous meta-analyses, commentaries, editorials, conference articles, and proceedings; (f) duplicates of previous published studies or studies with overlapping data. If more than one study was published by the same author(s) using repeated or overlapped data, the most complete one or more recently published study was selected.

Data extraction

Two investigators independently extracted the data from each study according to the above criteria. Any disagreement was subsequently resolved by discussion with a third author. The following data were extracted from each study: the name of first author, year of publication, country, ethnicity, genotyping methods, sample size, allele and genotype frequency in cases and controls, Minor Allele Frequency (MAFs) and Hardy-Weinberg equilibrium (HWE) in healthy controls. The ethnicity was divided into Asian and Caucasian or others. In addition, studies was performed on different populations were considered as independent studies.

Statistical analysis

The overall association of PAI-1 4G5G polymorphism with diabetes mellitus type 2 related nephropathy and retinopathy risk was measured by odds ratio (OR) and corresponding 95 percent confidence interval (95% CI). The Z-test was employed to determine the significance of the pooled ORs, and a P value less than 0.05 was considered statistically significant. The pooled ORs for PAI-1 4G5G polymorphism were performed under five genetic models, i.e., allele model (5G vs. 4G), homozygote (5G5G vs. 4G4G); heterozygote (5G4G vs. 4G4G); dominant (5G5G + 5G4G vs. 4G4G); and recessive (5G5G vs. 5G4G + 4G4G). The heterogeneity among the studies was calculated by Chi-square-based Q-tests with significance set at $P < 0.05$ level. In addition, the I^2 test was used to assess the proportion of statistical heterogeneity. If heterogeneity existed, the random-effect model (DerSimonian and Laird method) would be used²⁸. Or else, the fixed-effect model was adopted (the Mantel–Haenszel method). Z-test was used to estimate the pooled OR with significance set at $P < 0.05$ level. The HWE was assessed by using Fisher’s exact test with significance set at $P < 0.05$ level. The potential publication bias was estimated by adopting the funnel plot. The funnel plot symmetry was evaluated by using Egger’s linear regression test on the natural logarithm scale of the OR and significance was set at $P < 0.05$ level. To evaluate whether our results were influenced by the presence of any individual study, we conducted a sensitivity analysis by systematically removing each study and reassessing the significance of the result. When a significant heterogeneity existed across the included studies ($I^2 > 50\%$), the random effect model was used⁵⁸; When there was no significant heterogeneity across the included studies ($I^2 < 50\%$), the fixed effect model was used. To assess whether our results were substantially influenced by the presence of any individual study, we conducted a sensitivity analysis by systematically removing each study and recalculating the significance of the result. Begg’s funnel plot was performed to examine the publication bias, in which a P -value of < 0.05 was considered to indicate existence of a publication bias existence. Then the Duval and Tweedie non-parametric “trim and fill” method was used to assess the effect of publication bias on pooled results. Analyses were carried out using the Comprehensive Meta-Analysis (CMA) 2.0 software (Biostat, USA). P values < 0.05 were considered statistically significant.

Results

Case-control study

The distribution of genotype and allele frequencies of PAI-1 4G5G polymorphism in DN ($n = 118$), DR ($n = 117$), and

DWNR ($n = 120$) are shown in Table 1. The frequencies of the three PAI-1 4G5G genotypes in DN were 32.2% (4G4G), 44.9% (4G5G) and 22.9% (5G5G), in DR patients were 36.7% (4G4G), 41.9% (4G5G) and 21.4% (5G5G), while in DWNR patients were 46.7% (4G4G), 25.0% (4G5G) and 28.3% (5G5G). Our results showed that the 4G4G or 4G5G genotypes of the PAI-1 4G5G polymorphism were associated with an increased risk of DN in the Iranian diabetic patients (4G4G: OR = 0.543, 95% CI = 0.320–0.920, $P = 0.023$; and 4G5G: OR = 2.446, 95% CI = 1.412–4.239, $P = 0.001$). In addition, there is a significant association between 4G5G genotype of the PAI-1 4G5G polymorphism and increased risk of DR in the Iranian diabetic patients (4G5G: OR = 2.162, 95% CI = 1.244–3.758, $P = 0.006$; Table 1).

Meta-analysis

Baseline characteristics

As shown in Fig. 1 and 194 articles were identified through the initial search in the database and hand searching. Based on the selected criteria, we screened the titles and abstracts of the initial obtained articles, and excluded 126 irrelevant articles. Full texts were reviewed for the remaining 68 articles, and 41 of them were further excluded because there were reviews, case reports, case only, healthy controls subjects, evaluated other polymorphisms of PAI-1 gene, insufficient data or overlapping data, or the subjects in the control group was not DWNR. Finally, a total of 27 case-control studies with

3,222 DNR and 3,276 DWNR subjects addressing the association between the PAI-1 4G5G polymorphism and DNR risk were included. The characteristics of the included studies are presented in Table 2. Among the 27 studies, there were 16 studies comprising 1,825 cases DN and 1,731 DWNR on DN risk [33–47], and eleven studies comprising 1,397 DR cases and 1,545 DWNR on DR risk [40, 43, 48–55]. Of them, eight studies were published in Chinese and the rest in English. Seventeen studies focused on Asians (Japan, China, India, and Iran), seven on Caucasians (Italy, France, Ireland, USA, Spain, Slovenia, and Germany), two on Africans (Tunisia and Egypt), and one on a mixed population (Brazil). Four genotyping methods were used, including direct sequencing, PCR, RFLP-PCR, and AS-PCR. Table 2 also shows the genotypes and alleles frequency of the PAI-1 4G5G polymorphism in DNR and DWNR subjects.

Quantitative synthesis

Diabetic nephropathy

The association of PAI-1 4G5G polymorphism with DN risk are summarized in Table 2. We pooled all the 15 case-control studies to assess the overall association of PAI-1 4G5G polymorphism with DN risk. Overall pooled analysis suggest a significant association between PAI-1 4G5G polymorphism and DN risk under all five genetic models, i.e., allele model (OR = 0.674, 95% CI 0.524–0.865, $p = 0.002$, Fig. 2a), homozygote (OR = 0.536, 95% CI 0.351–0.817, $p = 0.004$);

Table 1 The genotype and the allele frequencies of PAI-1 4G5G in DNR and DWNR patients

	DNR (%)	DWNR (%)	OR (95% CI)	P-value
Nephropathy ($n = 118$)				
Genotypes				
4G4G	38(32.2)	56(46.7)	Ref.	
4G5G	53(44.9)	30(25.0)	2.446(1.412–4.239)	0.001
5G5G	27(22.9)	34(28.3)	0.750(0.418–1.347)	0.336
Allele				
4G	129(54.6)	142(59.2)	Ref.	
5G	107(45.4)	98(40.8)	1.202(0.836–1.728)	0.321
Retinopathy ($n = 117$)				
Genotypes				
4G4G	43(36.7)	56(46.7)	Ref.	
4G5G	49(41.9)	30(25.0)	2.162(1.244–3.758)	0.006
5G5G	25(21.4)	34(28.3)	0.687(0.379–1.245)	0.216
Allele				
4G	136(58.2)	142(59.2)	Ref.	
5G	98(41.8)	98(40.8)	1.044(0.724–1.505)	0.817

DNR: diabetic nephropathy and retinopathy; DWNR: diabetic without nephropathy and retinopathy

OR: Odds Ratio; CI: Confidence Interval

Fig. 1 Flow diagram of included studies for this meta-analysis

heterozygote (OR = 0.621, 95% CI 0.427–0.903, $p = 0.013$); dominant (OR = 0.575, 95% CI 0.399–0.831, $p = 0.003$); and recessive (OR = 0.711, 95% CI 0.515–0.981, $p = 0.038$, Fig. 2b). A stratified analysis by ethnicity indicated a significantly increased risk of DN in Asians under four genetic models, i.e., allele model (OR = 0.702, 95% CI 0.548–0.899, $p = 0.005$), homozygote (OR = 0.453, 95% CI 0.256–0.801, $p = 0.006$); heterozygote (OR = 0.529, 95% CI 0.328–0.853, $p = 0.009$); and dominant (OR = 0.487, 95% CI 0.302–0.783, $p = 0.003$). No significant increase was found in Caucasians (Table 3).

Diabetic retinopathy

We pooled all the eleven case-control studies to assess the overall association of PAI-1 4G5G polymorphism with DR risk. Overall pooled analysis suggest a significant association between PAI-1 4G5G polymorphism and DR risk under homozygote model (OR = 0.770, 95% CI 0.621–0.955, $p = 0.017$, Fig. 3). A stratified analysis by ethnicity indicated a

significantly increased risk of DR in Caucasians under two genetic models, i.e., allele model (OR = 0.813, 95% CI 0.673–0.982, $p = 0.032$) and recessive (OR = 0.655, 95% CI 0.478–0.898, $p = 0.009$). No significant increase was found in Asians (Table 3).

Test of heterogeneity and sensitivity analysis

Q -test and I^2 statistics were employed to test heterogeneity between the selected studies on PAI-1 4G5G polymorphism and DNR susceptibility. Significant between-study heterogeneity was detected in any genetic model for DN and DR studies. Thus, random effects model was used to synthesize the data. Hence, we performed subgroup analysis by ethnicity. The sensitivity analysis indicated the results of our meta-analysis were relatively consistent even when some studies were excluded. After excluding studies that deviated from HWE in controls, no other studies were found to significantly influence the pooled effects in each genetic model, which

Table 2 Main characteristics of studies included in this meta-analysis

First Author	Country (Ethnicity)	Genotyping Method	Case/ Control	DNR					DWNr					MAFs	HWE
				Genotypes			Allele		Genotypes			Allele			
				4G4G	5G4G	5G5G	4G	5G	4G4G	5G4G	5G5G	4G	5G		
Nephropathy															
Kimura 1998	Japan(Asian)	Sequencing	98/110	28	58	12	114	82	36	58	16	130	107	0.409	0.342
De Cosmo 1999	Italy(Caucasian)	RFLP-PCR	175/136	52	81	42	185	165	30	75	31	135	137	0.503	0.229
Wong 2000	China(Asian)	AS-PCR	95/46	39	36	20	114	76	7	30	9	47	48	0.521	0.037
Tarnow 2000	France(Caucasian)	RFLP-PCR	197/191	54	104	39	212	182	63	80	48	206	176	0.460	0.029
Li 2002	China(Asian)	AS-PCR	79/64	31	34	14	126	58	21	30	13	72	56	0.437	0.703
Liu 2004	China(Asian)	RFLP-PCR	77/70	30	28	19	88	66	12	47	11	71	69	0.492	0.004
Tang 2004	China(Asian)	PCR	59/49	33	15	11	96	37	17	17	15	51	47	0.479	0.032
Martin 2007	Ireland(Caucasian)	Sequencing	222/361	70	114	38	254	190	111	179	71	401	321	0.444	0.939
Wang 2007	China(Asian)	PCR	76/38	43	23	10	109	43	13	14	11	40	36	0.473	0.107
Zheng 2007	China(Asian)	AS-PCR	167/80	65	68	34	198	136	17	43	20	77	83	0.518	0.493
Yang 2008	China(Asian)	PCR	125/92	44	56	25	144	106	10	36	46	56	128	0.695	0.466
Prasad 2010	India(Asian)	RFLP-PCR	196/225	57	90	49	204	188	52	117	56	221	229	0.508	0.545
Xue 2010	China(Asian)	PCR	70/50	45	21	4	111	29	4	21	25	29	71	0.710	0.888
Liu 2011	China(Asian)	AS-PCR	29/34	10	12	7	32	26	13	18	3	44	24	0.352	0.353
Xu 2016	China(Asian)	AS-PCR	42/65	16	20	6	52	32	24	33	8	81	49	0.377	0.514
Present 2020	Iran(Asian)	RFLP-PCR	118/120	38	53	27	129	107	56	30	34	142	98	0.405	≤0.001
Retinopathy															
Nagi 1997	USA(Caucasian)	AS-PCR	70/102	14	44	12	72	68	18	45	39	81	125	0.602	0.427
Broch 1998	Spain(Caucasian)	AS-PCR	82/95	17	46	19	80	84	19	48	28	86	104	0.989	0.847
Wong 2000	China(Asian)	AS-PCR	84/57	31	38	15	100	68	16	38	13	70	64	0.565	0.263
Santos 2003	Brazil(Mixed)	PCR	99/111	24	41	34	89	109	22	41	30	85	71	0.543	0.281
Petrovic 2003	Slovenia(Caucasian)	RFLP-PCR	124/80	39	58	27	136	112	25	58	15	108	88	0.449	0.052
Liu 2004	China(Asian)	RFLP-PCR	56/91	15	26	15	56	56	27	26	15	80	56	0.411	0.082
Zietz 2004	Germany(Caucasian)	AS-PCR	131/240	48	55	28	151	111	112	55	73	279	201	0.418	≤0.001
Murata 2004	Japan(Asian)	RFLP-PCR	188/143	78	86	24	242	103	43	86	14	172	114	0.398	0.002
Ezzidi 2009	Tunisia(African)	RFLP-PCR	383/473	77	167	139	321	445	54	167	177	275	521	0.654	0.149
Abdel Rasol 2012	Egypt(African)	AS-PCR	63/33	42	14	7	98	28	7	22	4	36	30	0.454	0.047
Present 2020	Iran(Asian)	RFLP-PCR	117/120	43	50	24	136	98	56	30	34	142	98	0.405	≤0.001

PCR: Polymerase Chain Reaction Restriction; PCR–RFLP: Polymerase Chain Reaction Restriction Fragment Length Polymorphism; DNR: diabetes with nephropathy or retinopathy; DWNr: diabetes without nephropathy or retinopathy; MAFs: Minor Allele Frequencies; HWE: Hardy-Weinberg Equilibrium

indicating that the robustness of the summary estimate derived from this meta-analysis.

Publication bias

The Begg's funnel plot and Egger's linear regression test were performed to appraise the publication bias among the studies

included in this meta-analysis. Begg's funnel plot and Egger's test did not reveal any publication bias for of PAI-1 4G5G polymorphism and DR under all five genetic models. However, possible publication bias was suggested between PAI-1 4G5G polymorphism and DN under two genetic models i.e., heterozygote ($P_{\text{Begg's}} = 0.022$ and $P_{\text{Egger's}} = 0.015$) and dominant ($P_{\text{Begg's}} = 0.022$ and $P_{\text{Egger's}} = 0.006$, Fig. 4).

Fig. 2 Forest plots for the association of the PAI-1 4G5G polymorphisms and DN risk. **a** allele model (5G vs. 4G); **b** recessive model (5G5G vs. 5G4G + 4G4G)

Moreover, subgroup analysis by ethnicity in DN studies showed that there was a significant publication bias in Asians under dominant model (P_{Begg} = 0.086 and P_{Egger} = 0.027, Table 3). The above results indicated that publication bias was evident in this meta-analysis on DN. Thus, we have employed the Duval and Tweedie non-parametric “trim and fill” method to adjust the publication bias under those genetic models. However, the pooled result did not change with and without “trim and fill” test, indicating that the current meta-analysis results were statistically robust.

Discussion

The search for genetic factors that predict risk for DNR is important for several reasons, although the genetic risk factors and inheritance pattern is not known yet. An insertion (4G)/deletion (5G) polymorphism in the promoter region of the PAI-1 gene (-675) was identified in 675 base pairs before the onset of the transcript. A single guanine base pair deletion (4G4G) results in increased PAI-1 levels, while carriers of 5G5G have reduced levels, and 4G5G individuals have

Table 3 Summary of the association between PAI-1 4G5G polymorphism and DNR risk

Subgroup	Genetic Model	Type of Model	Heterogeneity		Odds Ratio				Publication Bias	
			I ² (%)	P _H	OR	95% CI	Z _{test}	P _{OR}	P _{Begg}	P _{Eggers}
Nephropathy										
Overall	Allele	Random	83.59	≤ 0.001	0.674	0.524–0.865	-3.094	0.002	0.013	0.057
	Homozygote	Random	75.71	≤ 0.001	0.536	0.351–0.817	-2.899	0.004	0.092	0.073
	Heterozygote	Random	78.73	≤ 0.001	0.621	0.427–0.903	-2.497	0.013	0.022	0.015
	Dominant	Random	80.91	≤ 0.001	0.575	0.399–0.831	-2.951	0.003	0.022	0.006
	Recessive	Random	69.88	≤ 0.001	0.711	0.515–0.981	-2.077	0.038	0.552	0.511
Ethnicity										
Asian	Allele	Random	71.81	≤ 0.001	0.702	0.548–0.899	-2.801	0.005	0.275	0.551
	Homozygote	Random	78.91	≤ 0.001	0.453	0.256–0.801	-2.724	0.006	0.192	0.211
	Heterozygote	Random	78.88	≤ 0.001	0.529	0.328–0.853	-2.611	0.009	0.086	0.060
	Dominant	Random	81.84	≤ 0.001	0.487	0.302–0.783	-2.965	0.003	0.086	0.027
	Recessive	Random	74.93	≤ 0.001	0.662	0.426–1.029	-1.832	0.067	0.837	0.706
Caucasian	Allele	Fixed	0.00	0.823	0.942	0.804–1.103	-0.748	0.455	1.000	0.820
	Homozygote	Fixed	0.00	0.903	0.863	0.626–1.186	-0.902	0.367	1.000	0.744
	Heterozygote	Fixed	66.03	0.053	1.028	0.793–1.333	0.210	0.834	1.000	0.740
	Dominant	Fixed	46.40	0.155	0.978	0.766–1.249	-0.177	0.860	1.000	0.709
	Recessive	Fixed	0.00	0.586	0.860	0.653–1.132	-1.078	0.281	1.000	0.590
Retinopathy										
Overall	Allele	Random	87.10	≤ 0.001	0.908	0.666–1.239	-0.608	0.543	0.350	0.472
	Homozygote	Fixed	18.24	0.270	0.770	0.621–0.955	-2.378	0.017	0.876	0.711
	Heterozygote	Random	80.06	≤ 0.001	0.874	0.568–1.345	-0.612	0.541	1.000	0.666
	Dominant	Random	85.52	≤ 0.001	0.832	0.520–1.333	-0.764	0.445	0.019	0.067
	Recessive	Fixed	44.84	0.053	0.880	0.681–1.137	-0.979	0.328	0.755	0.909
Ethnicity										
Asian	Allele	Random	88.47	≤ 0.001	0.910	0.500-1.657	-0.309	0.757	0.308	0.541
	Homozygote	Fixed	0.00	0.450	0.966	0.648–1.441	-0.168	0.867	1.000	0.847
	Heterozygote	Random	82.33	0.001	1.013	0.469–2.189	0.032	0.975	0.734	0.612
	Dominant	Random	89.44	≤ 0.001	0.916	0.351–2.395	-0.178	0.859	1.000	0.898
	Recessive	Fixed	44.41	0.145	0.990	0.692–1.417	-0.054	0.957	0.734	0.482
Caucasian	Allele	Fixed	40.19	0.171	0.813	0.673–0.982	-2.144	0.032	0.308	0.161
	Homozygote	Fixed	12.15	0.332	0.822	0.567–1.194	-1.029	0.304	0.308	0.540
	Heterozygote	Random	71.67	0.014	1.197	0.643–2.228	0.568	0.570	1.000	0.461
	Dominant	Random	81.51	0.001	0.751	0.340–1.657	-0.710	0.478	0.089	0.168
	Recessive	Fixed	51.51	0.103	0.655	0.478–0.898	-2.626	0.009	1.000	0.987

DNR: Diabetes Nephropathy and Retinopathy

intermediate levels [56]. The 4G5G sequence polymorphism in the promoter region of PAI-1 gene, has been postulated to be associated with the risk of DNR. However, the results are conflicting and inconclusive. Here, we analyzed pooled data from 27 case-control studies with 3,222 DNR and 3,276 DNR subjects to determine the role of PAI-1 4G5G polymorphism with susceptibility to DNR. The pooled results showed that PAI-1 4G5G polymorphism was associated with a significantly increased risk of DNR. Moreover, in the subgroup analyses by ethnicity, increased risk of DN and DR was also found in Asians and Caucasian, respectively.

Our meta-analysis results are in agreement with previous meta-analyses on DNR; however, in this meta-analysis more studies were included. Therefore, the current meta-analysis has higher statistical power than previous meta-analyses. In the present study, we found that PAI-1 4G4G and 4G5G genotypes were significantly associated with increased risk of DN in Iranian diabetic patients. Similarly, Xu et al., have found that PAI-1 4G4G genotype was associated with the increased risk of diabetic macroalbuminuria in T2D [39]. Moreover, in current case-control study we found that PAI-1 4G5G polymorphism may be associated with susceptibility to DR in an Iranian

Fig. 3 Forest plots for the association of the PAI-1 4G5G polymorphisms and DR risk under the homozygote model (5G5G vs. 4G4G)

patients with T2D. Our results are in agreement only with Nagi et al.,[48] and Ezzidi et al.,[54] studies among the Pima Indians and Tunisian, respectively. Recently, Saleem et al. not found a significant association between PAI-1 4G5G polymorphism and development and progression of DR in a Pakistani population.[57] In addition, Siokas et al. have reported that PAI-1 gene polymorphisms including rs2227667, rs2070682, rs2227690, rs2227692, and rs1050813 might not be associated with risk of DR in a Greek population.[58] In addition, our results inconsistent with the most previous studies performed among Asians, Caucasians, African and mixed populations.

The heterogeneity plays an important role when interpreting a meta-analysis and finding the source of heterogeneity is very important for the final result of meta-analysis. The ethnicity,

genotyping method, sample size, population selection, lifestyle, environmental background and other factors might be a source of between-study heterogeneity. In this meta-analysis, heterogeneity was found under all five genetic models for DN and three genetic models for DR. We hypothesized the ethnicity may be a potential origin of between-study heterogeneity. When stratified by ethnicity the heterogeneity was disappeared in Caucasians for DN. However, heterogeneity still existed among Asians and for DR. Publication bias is another significant problem which has negative effect on the results. In this meta-analysis, we found a significant publication bias in two genetic models for DN. However, the pooled results with and without “trim and fill” did not draw different results, suggesting the reliability of our results.

Fig. 4 Begg's funnel plots of the PAI-1 4G5G polymorphisms and DN risk for publication bias test under the dominant model (5G5G + 5G4G vs. 4G4G). Without “blue” and with “red” Trim and Fill test

This meta-analysis had some advantages as follows: First, compared to previous meta-analysis the sample size was larger, thus the association of PAI-1 4G5G polymorphism on DNR risk is statistically more powerful than any other single study and also previous meta-analyses. Second, strict searching strategy was conducted which combination computer assisted with reviewing potential articles and tracing the reference document especially for studies published in Chinese, make the eligible studies included as much as possible. Third, in this meta-analysis the potential source of heterogeneity was evaluated by subgroup analysis, however; the previous meta-analysis did not do.

There were several limitations of this meta-analysis that should be addressed. First, because most of the included studies were conducted on Asians and a few on Caucasians, results must be interpreted with caution. Further studies concerning populations in other areas such as Africa and Latinos populations are required to diminish the ethnic variation-produced biases. Second, the sample size on DNR was relatively small for stratified analyses and might not have provided sufficient power to estimate the associations. Third, although the present meta-analysis of all studies and a set of subgroup analyses have been undertaken, significant heterogeneity still persisted, limiting the interpretation of pooled risk estimates. Fourth, the present analyses were based on unadjusted estimates because most studies did not provide adjusted data. More precise analysis including individual data, lifestyle factors, and environmental factors, should be conducted if possible. Fifth, in this meta-analysis funnel plots and Egger's tests showed publication bias among DN studies because only published studies in English or Chinese were retrieved. Finally, it has been well known that DNR as diabetes mellitus is a polygenic and multifactorial in nature, however, the effects of gene-gene and gene-environment interactions were not addressed in the present meta-analysis, and thus the potential roles of the PAI-1 4G5G polymorphism may be masked or magnified by other polymorphisms of the PAI-1 gene, gene-gene, and gene-environment interactions.

In summary, this meta-analysis systematically analyzed the association between PAI-1 4G5G polymorphism and the risk of DNR. The pooled results suggest that the PAI-1 4G5G polymorphism is significantly associated with risk of DNR. Moreover, this meta-analysis demonstrates that PAI-1 4G5G polymorphism probably increase the susceptibility to DN and DR in Asians and Caucasians, respectively. Considering the limited sample size and ethnicities included in the meta-analysis, our results need to be proved in further large scaled and well-designed studies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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