Association of PAI-1 rs1799889 Polymorphism with Susceptibility to Ischemic Stroke: a Huge Meta-Analysis based on 44 Studies

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ABSTRACT

Background: the PAI-1 rs1799889 polymorphism has been reported to be associated with susceptibility to ischemic stroke. However, the results of previous studies have been inconsistent or controversial. Hence, we performed a systematic review and meta-analysis to evaluate the association of PAI-1 rs1799889 polymorphism with ischemic stroke risk. Methods: A comprehensive literature search was performed on PubMed, Web of Science, Scopus, SciELO, CNKI, and CBD databases up to November 05, 2019. Pooled odds ratio (OR) with 95% confidence interval (CI) were used to access the strength of this association in fixed- or random-effects model. Results: A total of 44 case-control studies with 8,620 cases and 10,260 controls were selected. Pooled data showed a significant association between PAI-1 rs1799889 polymorphism and ischemic stroke risk in the overall populations (GG vs. AA: OR = 0.791, 95% CI 0.633–0.988, p = 0.039; GA vs. AA: OR = 0.807, 95% CI 0.683–0.953, p = 0.012; and GG+GA vs. AA: OR = 0.795, 95% CI 0.637–0.993, p = 0.043). Subgroup analysis by ethnicity revealed a significant association in Asian and Mixed populations, but not in Caucasians. Moreover, stratified analysis by country of origin revealed an increased risk of ischemic stroke in Chinese populations, but not among Dutch (Netherlands) and Swedish. Conclusions: This meta-analysis result suggested that PAI-1 rs1799889 polymorphism was associated with an increased risk of ischemic stroke, especially in Asian and Mixed populations.

KEYWORDS

ischemic stroke; cerebrovascular accident; PAI-1 gene; rs1799889; polymorphism; meta-analysis

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INTRODUCTION

Stroke is the second leading cause of death globally and leading cause of long-term disability worldwide (1). It poses a huge threat to public health and is the leading cause of death in developed and developing countries (2). It is estimated that approximately 70% of new strokes are ischemic in origin, 51% stroke death, and 58% of stroke disability-adjusted life years are because of ischemic stroke (3). The exact etiology of ischemic stroke is multifactorial and a complex interaction between modifiable and non-modifiable conventional risk and genetic factors could be behind the pathogenesis of this disease (4). Several variants at low-penetrance and high-penetrance genes have been identified as potential ischemic stroke susceptibility loci. Numerous studies have found that Plasminogen activator inhibitor-1 (PAI-1) also serpin E1 was involved in the pathogenesis of ischemic stroke (5). Therefore, PAI-1 gene polymorphisms and its circulating levels may be associated with the development of ischemic stroke (5, 6).

Human PAI-1 gene is located at chromosome 7q21.3–q22, contains 9 exons and spans 12.3 kb (7). PAI-1, a secreted single-chain glycoprotein, is one of the early inflammatory response genes, and its expression level changes dramatically in response to many stimuli, including growth factors and endotoxins (8, 9). Several polymorphisms within the PAI-1 gene have clearly been postulated to modulate the expression of PAI-1 (10, 11). Among SNPs of the PAI-1 gene, rs1799889 (4G/5G) polymorphism has been extensively studied in different disease (7, 12). PAI-1 rs1799889 is an inserted or deleted in the 4G sequence polymorphism in the PAI-1 promoter (4G/5G) at 675 bp upstream from the start of transcriptional start site in the promoter region. Studying the association of PAI-1 gene with different disease will help us to understand the mechanism of PAI-1 regulation and the role of PAI-1 in many physiological and pathological processes (12, 13).

Studies have shown that the 4G/4G genotype has been linked to higher PAI-1 level, compared with the 5G/5G genotype, with the heterozygous genotype associated with intermediate levels. In 2003, Chen et al., have reported that PAI-1 rs1799889 polymorphism alone is not associated with an increased risk of ischemic stroke. However, they revealed a significant contribution of PAI-1 4G/4G genotype with an increased triglyceride and decreased HDL cholesterol levels in the healthy group (14). There are several numbers of epidemiological studies have evaluated association between PAI-1 rs1799889 polymorphism and ischemic stroke risk, but their results were inconsistent or even contradictory. For example, Adamski et al., and Esparza-García et al., have reported that PAI-1 rs1799889 polymorphism was not associated with an increased risk of ischemic stroke in Polish and Mexican populations, receptively (15, 16). By contrast, Xu et al. results supported that PAI-1 rs1799889 polymorphism might be associated with an increased risk of ischemic stroke in Han Chinese (17). In recent years, some studies already studied potential associations PAI-1 rs1799889 polymorphism with risk of ischemic stroke. Nevertheless, the results of these studies were not always consistent and the sample size of each study was also statistically insufficient. Thus, we performed a meta-analysis to offer a more comprehensive estimation of the association between PAI-1 rs1799889 and ischemic stroke susceptibility in globally populations.

MATERIALS AND METHODS

SEARCH STRATEGY

We have performed a comprehensive literature search in PubMed, MEDLINE, EMBASE, Cochrane Library, Web of Science, Elsevier, SciELO, SID, WanFang, VIP, Chinese Biomedical Database (CBD) and Chinese National Knowledge Infrastructure (CNKI) to identify all eligible studies on PAI-1 4G/5G (rs1799889) polymorphism and risk of ischemic stroke up to November 05, 2019. The following keywords were adopted in the electronic searches: ("Ischemic Strok" OR "Atherothrombotic Cerebral Infarction") AND ("Plasminogen Activator Inhibitor-1 Gene" OR "PAI-1" OR "SERPINE1") AND ("insertion/deletion polymorphism" OR "4G/5G polymorphism" OR "4G/5G promoter polymorphism" OR "rs1799889" OR "–675 4G/5G") AND ("Gene" OR "Genotype" OR "Polymorphism SNP" OR "Mutation" OR "Variation" OR "Variant"). Publication language was restricted to English, Chinese, and Farsi. Also a manual search of the reference lists performed to retrieved articles for additional potential studies.

INCLUSION AND EXCLUDING CRITERIA

The inclusion criteria for the gene association studies in this meta-analysis were as follows: 1) studies with case-control or cohort design; 2) full-text published studies; 3) studies evaluated the association between PAI-1 rs1799889 polymorphism and ischemic stroke risk; and 4) provided the genotype distribution in both cases and controls for estimating an odds ratio (OR) with 95% confidence interval (CI). Additionally, studies were excluded if one of the following criteria was fulfilled: 1) studies without detailed raw data regarding PAI-1 rs1799889 polymorphism; 2) case only studies; 3) family-based, sibling, twins and linkage studies; 4) abstracts, review, letters, comments, conference editorials, presentations, case reports, case series previous meta-analyses; 5) duplicates or overlapping studies. If the authors published two or more studies using the same data (with overlapping data), the newest publication or the publication with the largest sample size was included. There was no any limitation by ethnicity, race, placed or geography area. Moreover, non-English publications were translated and included in the meta-analysis.

DATA EXTRACTION

Two authors (HN and MJA) systematically extracted data from all eligible studies using a standardized form. Then, they have checked the data extraction results and reached consensus. If different results were generated, the two authors carried out discussions until a consensus was reached or a third author was invited to resolve the disagreement and then a final decision were made by the

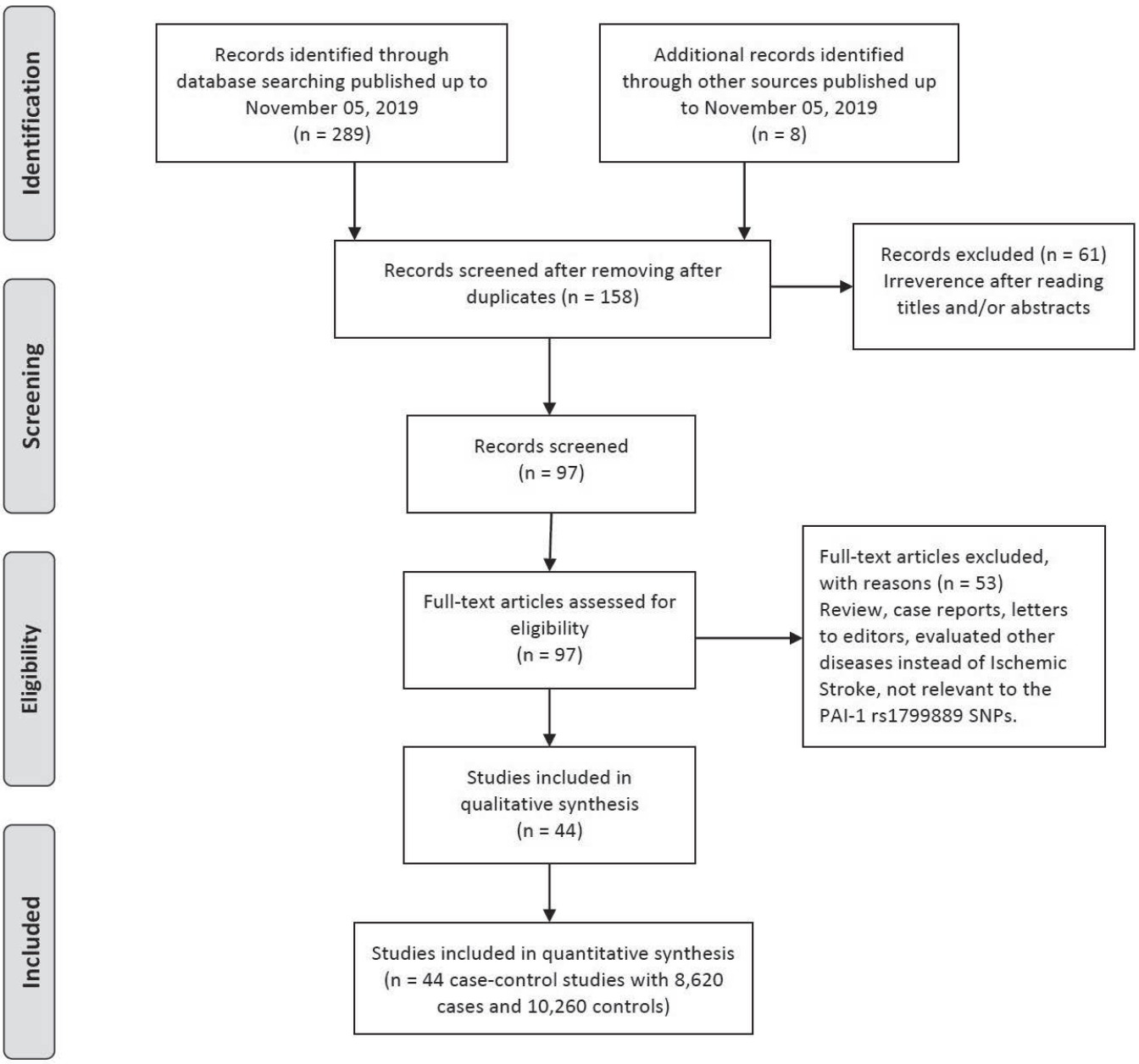

Fig. 1 The study selection and inclusion process.

majority of the votes. The collected data were: first author's name, publication year, country of origin, ethnicity (Caucasian, Asian, African, Mixed populations), total numbers of cases and controls, genotypes frequencies of cases and controls, minor allele frequencies (MAFs) and Hardy-Weinberg equilibrium test in control subjects.

STATISTICAL ANALYSIS

An ethical approval was not necessary as this study was a meta-analysis based on previous studies. The strength of the associations PAI-1 rs1799889 (4G/5G) polymorphism and susceptibility to ischemic stroke was measured by odds ratios (ORs) with 95% confidence intervals (CIs). The statistical significance of the pooled OR was determined using the Z-test. Pooled estimates of the OR were obtained by calculating a weighted average of OR from each study. The pooled ORs was calculated under all five genetic models, i.e., allele (G vs. A), homozygote (GG vs. AA), heterozygote (GA vs. AA), dominant (GG+GA vs. AA) and recessive (GG vs. GA+AA). Between-studies heterogeneity was assessed by a Chi-squared Q-test and I^2 statistics ($P < 0.05$). The heterogeneity between studies was estimated by Cochran's $\chi 2$ based Q-statistic test, in which it was considered to be statistically significant at $P \leq 0.01$. In addition, I^2 test was used to quantify the effect of heterogeneity, with the range of 0 to 100%, and 0–40% meant no risk of heterogeneity, 30–60% meant a low risk of heterogeneity, 50–90% meant substantial heterogeneity and 75–100% meant considerable heterogeneity. Accordingly, when between-study heterogeneity existed a random-effects model weighted (the DerSimonian-Laird method) was applied to give a more conservative result; otherwise, a fixed-effects model weighted (the Mantel-Haenszel method) method was selected. Hardy-Weinberg equilibrium (HWE) of the genotype distribution in controls was conducted by Pearson's $\chi 2$ test, in which it was considered to be statistically significant at $P \leq 0.05$. A subgroup analysis by

Tab. 1 Characteristics of studies included in this meta-analysis.

First Author	Country (Ethnicity)	Case/ Control	Cases					Controls					MAFs	HWE
			Genotypes			Allele		Genotypes			Allele			
			AA	AG	GG	A	G	AA	AG	GG	A	G		
Catto 1997	UK(Caucasian)	558/172	150	274	134	574	542	56	80	36	192	152	0.442	0.454
Liu 1998	China(Asian)	107/95	44	43	20	131	83	25	48	22	98	92	0.484	0.910
Jeppesen 1998	Denmark(Caucasian)	177/93	48	92	37	188	166	26	49	18	101	85	0.457	0.552
Endler 2000	Austria(Caucasian)	136/115	42	63	31	147	125	48	48	19	144	86	0.373	0.287
Elbaz 2001	Netherlands(Caucasian)	461/461	125	223	113	473	449	129	245	87	503	419	0.454	0.123
Gottl 2001	Germany(Caucasian)	198/951	65	91	42	221	175	275	473	203	1023	879	0.462	0.988
Bang 2001	Korea(Asian)	60/100	25	25	10	75	45	21	53	26	95	105	0.525	0.530
Sun 2001	China(Asian)	50/60	21	20	9	62	38	15	30	15	60	60	0.500	1.000
Zhang 2001a	China(Asian)	95/60	50	31	14	131	59	15	30	15	60	60	0.500	1.000
Zhang 2001b	China(Asian)	65/60	28	25	12	81	49	16	35	9	67	53	0.441	0.157
Kain 2002	UK(Caucasian)	101/102	22	58	21	102	100	36	54	12	126	78	0.382	0.075
Hindorff 2002	USA(Caucasian)	41/385	7	24	10	38	44	115	187	83	417	353	0.458	0.668
Crainich 2003	USA(Caucasian)	265/753	81	143	41	305	225	200	387	166	787	719	0.477	0.410
Zhang 2003	China(Asian)	113/121	48	47	18	143	83	23	70	28	116	126	0.521	0.080
Chen 2003	Taiwan(Asian)	100/150	40	46	14	126	74	58	68	24	184	116	0.386	0.588
Zhan 2003	China(Asian)	54/83	11	30	13	52	56	25	30	6	80	42	0.344	0.485
Guan 2004	China(Asian)	222/215	75	105	42	255	189	46	121	48	213	217	0.504	0.065
Yeh 2004	China(Asian)	213/200	79	103	31	261	165	71	102	27	244	156	0.390	0.309
Yi 2004	China(Asian)	52/57	20	22	10	62	42	28	27	2	83	31	0.271	0.138
Tang 2005	China(Asian)	122/50	66	35	21	167	77	13	26	11	52	48	0.48	0.768
Jood 2005	Sweden(Caucasian)	600/600	162	307	131	631	569	186	280	134	652	548	0.456	0.144
Van Goor 2005	Netherlands(Caucasian)	123/123	33	61	29	127	119	36	58	29	130	116	0.472	0.550
Wiklund 2005a	Sweden(Caucasian)	89/218	42	33	14	117	61	67	109	42	243	193	0.442	0.844
Wiklund 2005b	Sweden(Caucasian)	222/542	94	85	43	273	171	174	261	107	609	475	0.438	0.609
Xu 2006	China(Asian)	72/77	15	29	28	59	85	5	35	37	45	109	0.707	0.386
Komitopoulou 2006	Greece(Caucasian)	87/101	23	50	14	96	78	23	55	23	101	101	0.500	0.370
Attia 2007	Australia(Caucasian)	171/182	63	71	37	197	145	62	89	31	213	151	0.415	0.922
Saidi 2007	Tunisia(African)	135/118	23	74	38	120	150	33	58	27	124	112	0.475	0.875
Liu 2008	China(Asian)	220/140	48	114	58	210	230	43	70	27	156	124	0.497	0.876
Tang 2008	China(Asian)	90/30	40	36	16	116	68	6	19	5	31	29	0.483	0.142
Adamski 2009	Poland(Caucasian)	390/291	120	189	81	429	351	89	136	66	314	268	0.377	0.018
Sabino 2011	Brazil(Mixed)	127/201	33	52	42	118	136	93	65	43	251	151	0.376	≤0.001
Balcerzyk 2011	Poland(Caucasian)	70/133	23	35	12	81	59	47	60	26	154	112	0.421	0.389
Pruissen 2011	Netherlands(Caucasian)	841/310	261	111	29	633	169	71	157	82	299	321	0.518	0.802
Maguire 2011	Australia(Caucasian)	612/600	198	279	135	675	549	169	302	129	640	560	0.467	0.784
Assawamakin 2012	Taiwan(Asian)	179/229	51	97	31	199	159	67	110	52	244	214	0.467	0.594
Babu 2012	India(Asian)	516/513	236	238	42	710	322	258	223	32	739	287	0.284	0.028
Huang 2014	China(Asian)	285/919	115	156	14	386	184	310	520	89	1140	698	0.380	≤0.001
Natesirinilkul 2014	Thailand(Asian)	29/40	2	20	7	24	34	1	32	7	34	46	0.575	≤0.001
Supanc 2014	Croatia(Caucasian)	155/150	44	51	60	139	171	28	46	76	102	198	0.660	≤0.001
García 2015	Mexico(Mixed)	204/204	23	94	87	140	268	16	87	101	119	289	0.708	0.646
Ranellou 2015	Greece(Caucasian)	40/65	2	36	2	40	40	4	44	17	52	78	0.600	≤0.001
Akhter 2017	India(Asian)	100/100	34	56	10	124	76	24	54	22	102	98	0.490	0.421
Coen Herak 2017	Croatia(Caucasian)	73/100	19	37	17	75	71	27	53	20	107	93	0.465	0.514

A

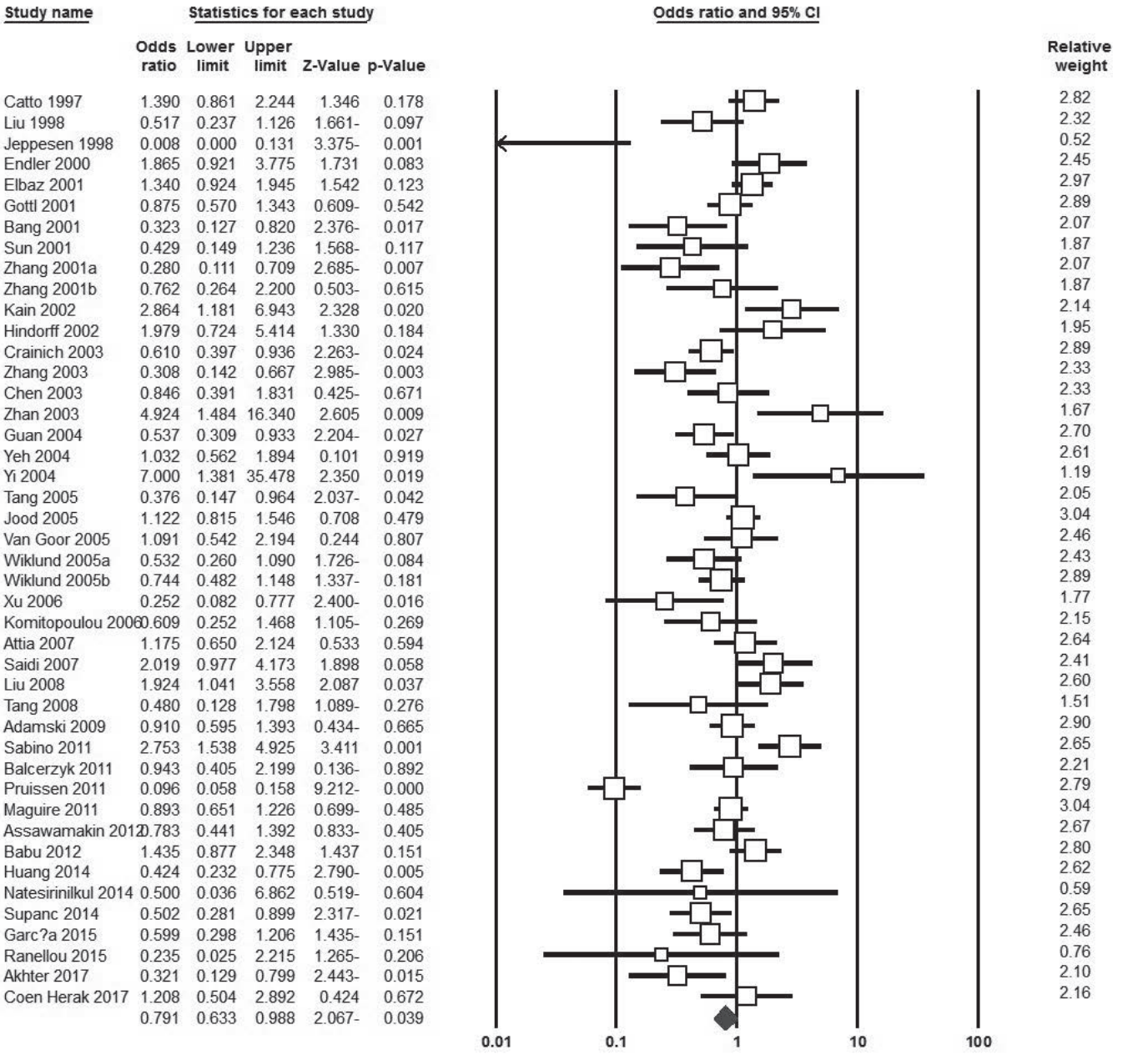

Fig. 2A Forest plot for the association of PAI-1 rs1799889 Polymorphism with Susceptibility to Ischemic Stroke: overall population (homozygote model: GG vs. AA).

ethnicity, country of origin, and source of controls was performed to explore potential sources of between-study heterogeneity (18, 19). To check the stability of the pooled data, a sensitivity analysis was performed by omitting each individual study in turn from the all selected studies and reanalyzing the remainder. Moreover, sensitivity analysis was performed by excluding HWE-violating studies. The potential publication bias was explored visually by Egger's linear regression test and Begg's quantitative test (20). The asymmetric plot of Egger's test and the P-value of Begg's test less than 0.05 were considered a significant publication bias. All statistical analyses were performed using Comprehensive Meta-Analysis (CMA) Software version 2.0 (Biostat, Englewood, NJ). All tests were two-sided, and the P values of < 0.05 were considered statistically significant.

RESULTS

CHARACTERISTICS OF INCLUDED STUDIES

By electronic and manual searches concerning the association of PAI-1 rs1799889 polymorphism and ischemic stroke risk, 297 relevant studies up to November 05, 2019 were identified. After reading titles and abstracts, 139 irrelevant and duplicate articles were excluded. Another 95 articles were subsequently excluded because not reporting useful data for meta-analysis, review, case only study, and not being case-control studies. Finally, a total of 44 case-control studies (5, 14–16, 21–49) with 8,620 ischemic stroke cases and 10,260 controls were included in the meta-analysis. Characteristics of included studies are presented in Table 1. All eligible studies were published in English and Chinese between April 1997 and November 2017. Among

B

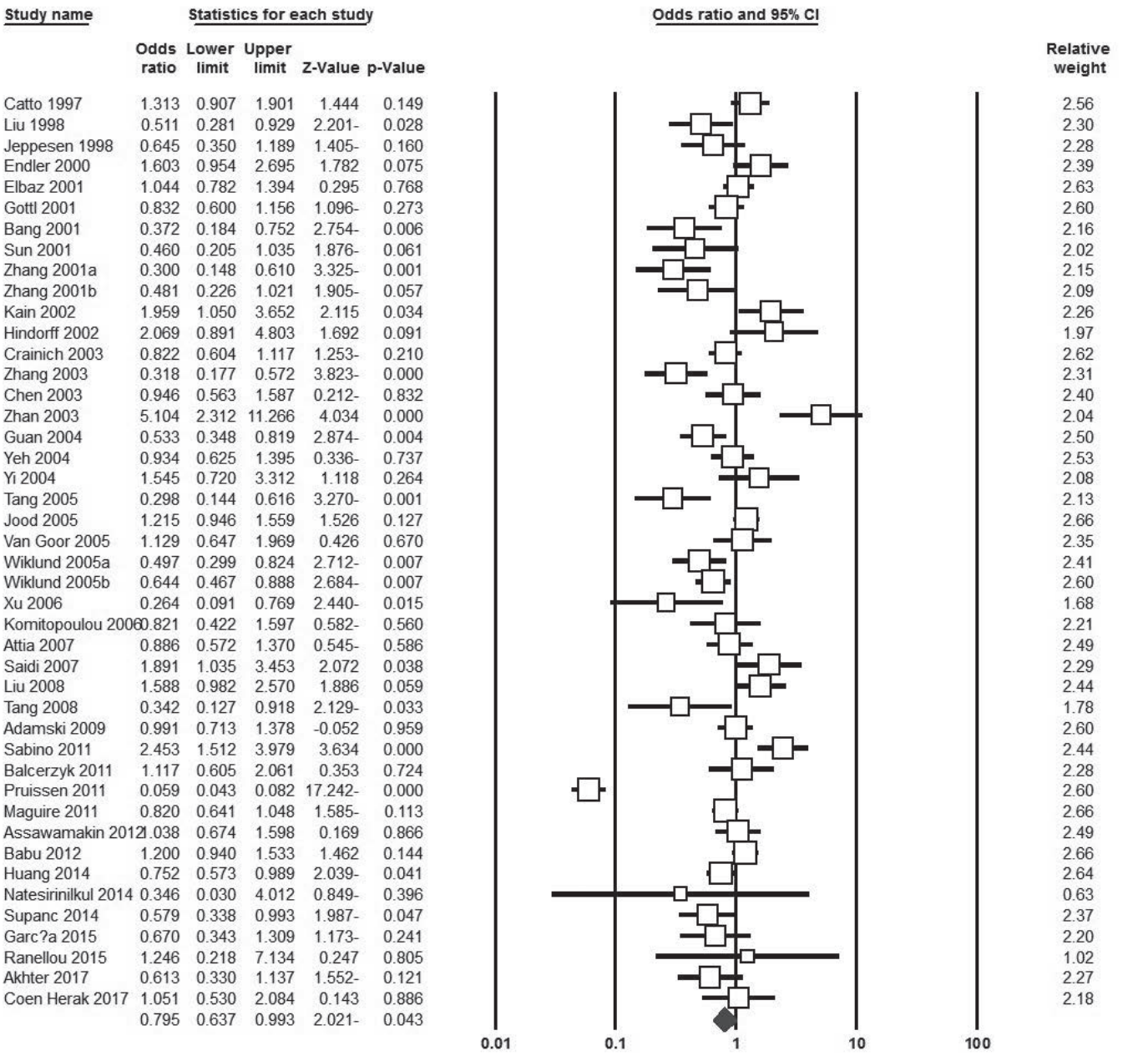

Fig. 2B Forest plot for the association of PAI-1 rs1799889 Polymorphism with Susceptibility to Ischemic Stroke: overall population (dominant model: GG + GA vs. AA).

them, 21 studies were based on Caucasian populations (5,410 cases and 6,438 controls), 20 studies based on Asian populations (3,137 cases and 3,700 controls), two studies based on mixed populations (331 cases and 405 controls), and one study was based on African populations (135 cases and 118 controls). The selected studies were conducted in UK, USA, Sweden, Greece, Australia, Austria, Poland, Denmark, Netherlands, Germany, Croatia, China, Taiwan, Thailand, Korea, India, Brazil, Mexico and Tunisia. The allele, genotype and minor allele frequency (MAF) distributions in the cases and controls are shown in Table 1. Moreover, the distribution of genotypes in the controls was in agreement with Hardy-Weinberg equilibrium (HWE) for all selected studies, except for seven studies (Table 1).

QUANTITATIVE DATA SYNTHESIS

The summary of the meta-analysis of the association of between PAI-1 rs1799889 polymorphism and ischemic stroke are shown in Table 2. Pooled data revealed that there was a significant association between PAI-1 rs1799889 polymorphism and an increased risk of ischemic stroke in the overall population under three genetic models, i.e., homozygote (GG vs. AA: OR = 0.791, 95% CI 0.633–0.988, p = 0.039, Fig 2A), heterozygote (GA vs. AA: OR = 0.807, 95% CI 0.683–0.953, p = 0.012) and dominant (GG+GA vs. AA: OR = 0.795, 95% CI 0.637–0.993, p = 0.043, Fig 2B). Moreover, we have performed subgroup analyses by ethnicity and country of origin. Subgroup analysis by ethnicity showed that there was a significant association between PAI-1 rs1799889 polymorphism and ischemic stroke risk in

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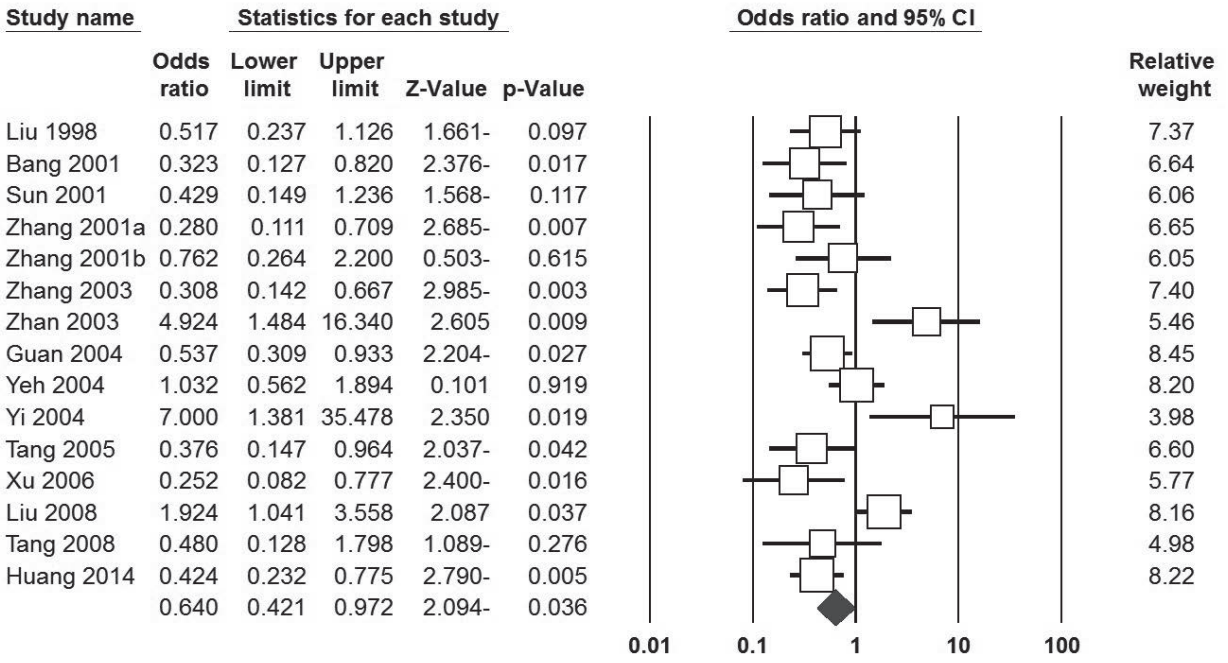

Fig. 2C Forest plot for the association of PAI-1 rs1799889 Polymorphism with Susceptibility to Ischemic Stroke: Chinese population (homozygote model: GG vs. AA).

Asians (G vs. A: OR = 0.829, 95% CI 0.697–0.987, p = 0.035; GA vs. AA: OR = 0.663, 95% CI 0.518–0.848, p = 0.001; and GG+GA vs. AA: OR = 0.683, 95% CI 0.521–0.897, p = 0.006) and Mixed population (G vs. A: OR = 3.255, 95% CI 1.041–10.181, p = 0.043), but not in Caucasians. When stratified analysis by country of origin performed a significant association was found among Chinese population (G vs. A: OR = 0.798, 95% CI 0.637–0.999, p = 0.049; GG vs. AA: OR = 0.640, 95% CI 0.421–0.972, p = 0.036, Fig 2C; GA vs. AA: OR = 0.577, 95% CI 0.427–0.778, p ≤ 0.001; and GG+GA vs. AA: OR = 0.620, 95% CI 0.438–0.876, p = 0.007), but not in Dutch (Netherlands) and Swedish.

BETWEEN-STUDY HETEROGENEITY TEST

As shown in Table 2, there was statistically moderate to high between-study heterogeneity in the overall population under all five genetic models, i.e., allele (I^2 = 91.95, $P_H \leq 0.001$), homozygote (I^2 = 79.67, $P_H \leq 0.001$), heterozygote (I^2 = 77.98, $P_H \leq 0.001$), dominant (I^2 = 89.86, $P_H \leq 0.001$), and recessive (I^2 = 78.03, $P_H \leq 0.001$). To explore the potential sources of heterogeneity, subgroup analyses by ethnicity, country of origin and HWE was performed. The results suggested that the above mentioned factors did not contribute to between-study heterogeneity in the current meta-analysis.

SENSITIVITY ANALYSIS

A sensitivity analysis was used to test the effects of each study on pooled ORs. There were no significant differences observed upon removal of any of the studies, suggesting that our findings were statistically robust and reliable. Moreover, we performed sensitivity analysis by excluding the HWE-violating study (Figure 3). When this study was excluded, the results were not changed in overall population and also by subgroup analyses, indicating that our meta-analysis was statistically robust and reliable.

PUBLICATION BIAS

Begg's funnel plot and Egger's test were inspected to evaluate the possible publication bias in this meta-analysis. Results of publication bias were shown in Table 2 and Figure 4. The shape of the funnel did not show any obvious asymmetry in all of the genetic models. Moreover, Egger's test was statistically revealed that there was no a significant bias under all five genetic models in the overall populations all five genetic models, i.e., allele (P_{Beggs} = 0.112; P_{Eggers} = 0.859), homozygote (P_{Beggs} = 0.198; P_{Eggers} = 0.307), heterozygote (P_{Beggs} = 0.107; P_{Eggers} = 0.267), dominant (P_{Beggs} = 0.172; P_{Eggers} = 0.841), and recessive (P_{Beggs} = 0.723; P_{Eggers} = 0.876).

DISCUSSION

The PAI-1 rs1799889 polymorphism association to ischemic stroke was first described by Catto et al. in 1997 (44). Since several epidemiological studies have been evaluated association between PAI-1 rs1799889 polymorphism and risk of ischemic stroke (17, 45). However, the results of these studies remain contradictory. It is clear that a single study may fail to demonstrate a complicated genetic relationship completely because of small sample size, which has low statistical power. Larger studies could overcome these disadvantages. Therefore, we performed a comprehensive meta-analysis of all eligible studies evaluated the association of PAI-1 rs1799889 polymorphism with risk ischemic stroke.

Tab. 2 Summary risk estimates for association of PAI-1 rs1799889 polymorphism with risk of ischemic stroke.

Subgroup	Genetic Model	Type of Model	Heterogeneity		Odds Ratio				Publication Bias	
			I^2 (%)	P_H	OR	95% CI	Z_{test}	P_{OR}	P_{Beggs}	P_{Eggers}
Overall	G vs. A	Random	91.95	≤0.001	0.854	0.727-1.003	-1.928	0.054	0.112	0.859
	GG vs. AA	Random	79.67	≤0.001	0.791	0.633-0.988	-2.067	0.039	0.198	0.307
	GA vs. AA	Random	77.98	≤0.001	0.807	0.683-0.953	-2.526	0.012	0.107	0.267
	GG+GA vs. AA	Random	89.86	≤0.001	0.795	0.637-0.993	-2.021	0.043	0.172	0.841
	GG vs. GA+AA	Random	78.03	≤0.001	0.868	0.726-1.038	-1.555	0.120	0.723	0.876
Ethnicity										
Caucasian	G vs. A	Random	87.76	≤0.001	1.076	0.884-1.311	0.730	0.465	0.620	0.561
	GG vs. AA	Random	56.91	0.003	1.002	0.807-1.243	0.018	0.986	0.921	0.907
	GA vs. AA	Random	55.09	0.005	0.978	0.822-1.163	-0.255	0.798	0.373	0.588
	GG+GA vs. AA	Random	61.67	0.001	0.983	0.825-1.172	-0.189	0.850	0.428	0.611
	GG vs. GA+AA	Random	48.82	0.017	0.994	0.839-1.178	-0.072	0.942	0.766	0.681
Asian										
	G vs. A	Random	77.84	≤0.001	0.829	0.697-0.987	-2.113	0.035	0.820	0.389
	GG vs. AA	Random	94.75	≤0.001	0.988	0.446-2.189	-0.031	0.975	0.581	0.497
	GA vs. AA	Random	71.16	≤0.001	0.663	0.518-0.848	-3.276	0.001	0.144	0.014
	GG+GA vs. AA	Random	79.02	≤0.001	0.683	0.521-0.897	-2.749	0.006	0.284	0.079
	GG vs. GA+AA	Random	49.29	0.007	0.881	0.704-1.102	-1.111	0.267	0.314	0.410
Mixed										
	G vs. A	Random	96.28	≤0.001	3.255	1.041-10.181	2.029	0.043	NA	NA
	GG vs. AA	Random	90.73	0.001	1.301	0.292-5.795	0.345	0.730	NA	NA
	GA vs. AA	Random	83.11	0.015	1.333	0.455-3.908	0.524	0.600	NA	NA
	GG+GA vs. AA	Random	89.45	0.002	1.310	0.367-4.670	0.416	0.678	NA	NA
	GG vs. GA+AA	Random	86.25	0.007	1.156	0.492-2.719	0.333	0.739	NA	NA
Country										
China	G vs. A	Random	80.33	≤0.001	0.798	0.637-0.999	-1.967	0.049	0.766	0.871
	GG vs. AA	Random	71.92	≤0.001	0.640	0.421-0.972	-2.094	0.036	0.373	0.836
	GA vs. AA	Random	70.45	≤0.001	0.577	0.427-0.778	-3.599	≤0.001	0.373	0.104
	GG+GA vs. AA	Random	80.61	≤0.001	0.620	0.438-0.876	-2.706	0.007	0.766	0.383
	GG vs. GA+AA	Random	52.16	0.010	0.895	0.680-1.178	-0.793	0.428	0.373	0.243
Netherlands	G vs. A	Random	99.29	≤0.001	0.498	0.095-2.260	-0.822	0.411	1.000	0.959
	GG vs. AA	Random	96.06	≤0.001	0.586	0.184-1.862	-0.907	0.364	1.000	0.920
	GA vs. AA	Random	97.24	≤0.001	0.519	0.089-3.014	-0.731	0.465	1.000	0.825
	GG+GA vs. AA	Random	98.94	≤0.001	0.410	0.052-3.208	-0.849	0.396	1.000	0.899
	GG vs. GA+AA	Random	97.82	≤0.001	0.518	0.093-2.885	-0.751	0.453	1.000	0.730
Sweden	G vs. A	Random	75.65	0.016	0.855	0.647-1.129	-1.105	0.269	0.296	0.210
	GG vs. AA	Fixed	56.85	0.099	0.907	0.711-1.155	-0.794	0.427	0.296	0.219
	GA vs. AA	Random	87.54	≤0.001	0.737	0.401-1.352	-0.986	0.324	1.000	0.341
	GG+GA vs. AA	Random	86.60	0.001	0.751	0.437-1.292	-1.034	0.301	0.296	0.328
	GG vs. GA+AA	Fixed	0.00	0.829	0.951	0.769-1.177	-0.459	0.646	0.296	0.321
HWE	G vs. A	Random	92.62	≤0.001	0.843	0.700-1.015	-1.799	0.072	0.161	0.964
	GG vs. AA	Random	80.14	≤0.001	0.778	0.609-0.994	-2.010	0.044	0.277	0.418
	GA vs. AA	Random	79.04	≤0.001	0.765	0.633-0.926	-2.754	0.006	0.107	0.346
	GG+GA vs. AA	Random	90.70	≤0.001	0.763	0.590-0.988	-2.051	0.040	0.266	0.932
	GG vs. GA+AA	Random	79.09	≤0.001	0.871	0.714-1.062	-1.365	0.172	0.743	0.938

NA: Not Applicable.

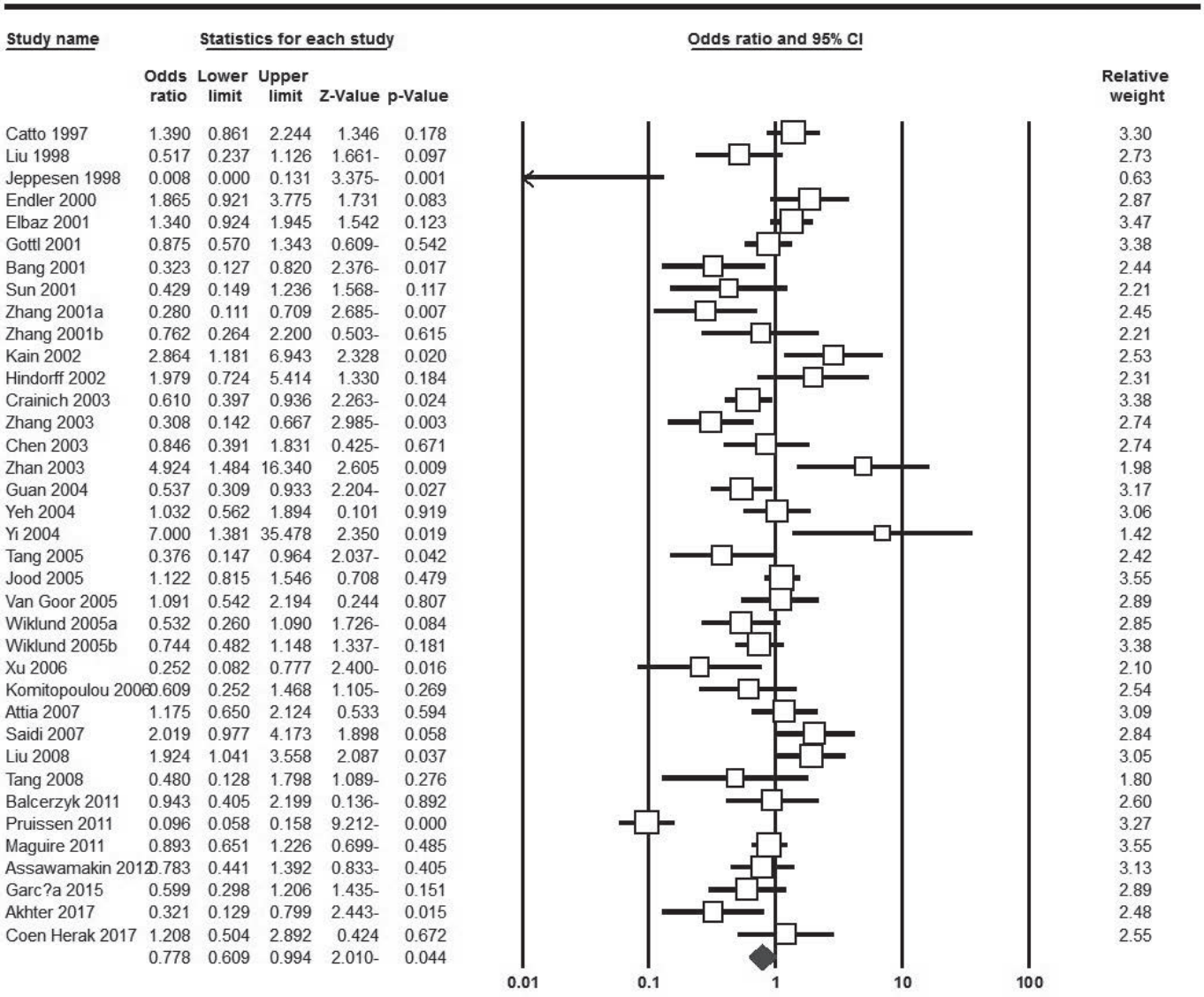

Fig. 3 Forest plot for the association of PAI-1 rs1799889 Polymorphism with Susceptibility to Ischemic Stroke after excluding Hardy-Weinberg equilibrium (HWE) violating studies under the homozygote genetic model (GG vs. AA).

In the current meta-analysis, we have selected a total of 44 eligible case-control studies with 8,620 ischemic stroke cases and 10,260 controls to evaluate the association of PAI-1 rs1799889 polymorphism with ischemic stroke risk. Our pooled data showed that PAI-1 rs1799889 polymorphism was significantly associated with an increased risk of ischemic stroke in the overall population. Moreover, subgroup analyses revealed that PAI-1 rs1799889 polymorphism was associated with significantly increased risk of ischemic stroke in Asian and mixed populations, but not in Caucasians. When stratified analysis by country of origin performed a significant association was found among Chinese population, but not in Dutch (Netherlands) and Swedish. This finding indicated that the carriers with the 4G allele of the PAI-1 rs1799889 polymorphism in Asians and mixed populations might be predisposed to ischemic stroke, but not in Caucasian populations. Moreover, this finding suggested a possible influence among environmental exposures and different genetic backgrounds in development of ischemic stroke in different populations. Therefore, more studies are warranted to further validate genetic background difference in the effect of PAI-1 rs1799889 polymorphism in susceptibility to ischemic stroke, especially in Caucasians. Cao et al., in a meta-analysis of eleven case-control studies with 1,358 cases and 1,134 controls evaluated the association of PAI-1 rs1799889 polymorphism with susceptibility to ischemic stroke in the Chinese population. Their results showed a significant association between PAI-1 rs1799889 polymorphism and ischemic stroke risk. However, their meta-analysis results reliability and the number of studies are considerably smaller than that needed to receive the robust conclusions (45). Here, we have extended the meta-analysis with a more relevant recently published studies and subgroup analysis by ethnicity. Moreover, Hu et al., in meta-analysis of 39 studies with 8,336 cases and 14,403 controls evaluated PAI-1 polymorphisms with risk of stroke. Their results revealed a significant association between PAI-1 rs1799889 polymorphism and an increased risk of ischemic stroke in adult, but not pediatric. Their stratified analysis showed a significant association in Asians, but not Caucasians. Moreover, they found that PAI-1–844 G>A, but not 11,053 T>G polymorphism was associated with an increased risk of ischemic stroke and a tendency

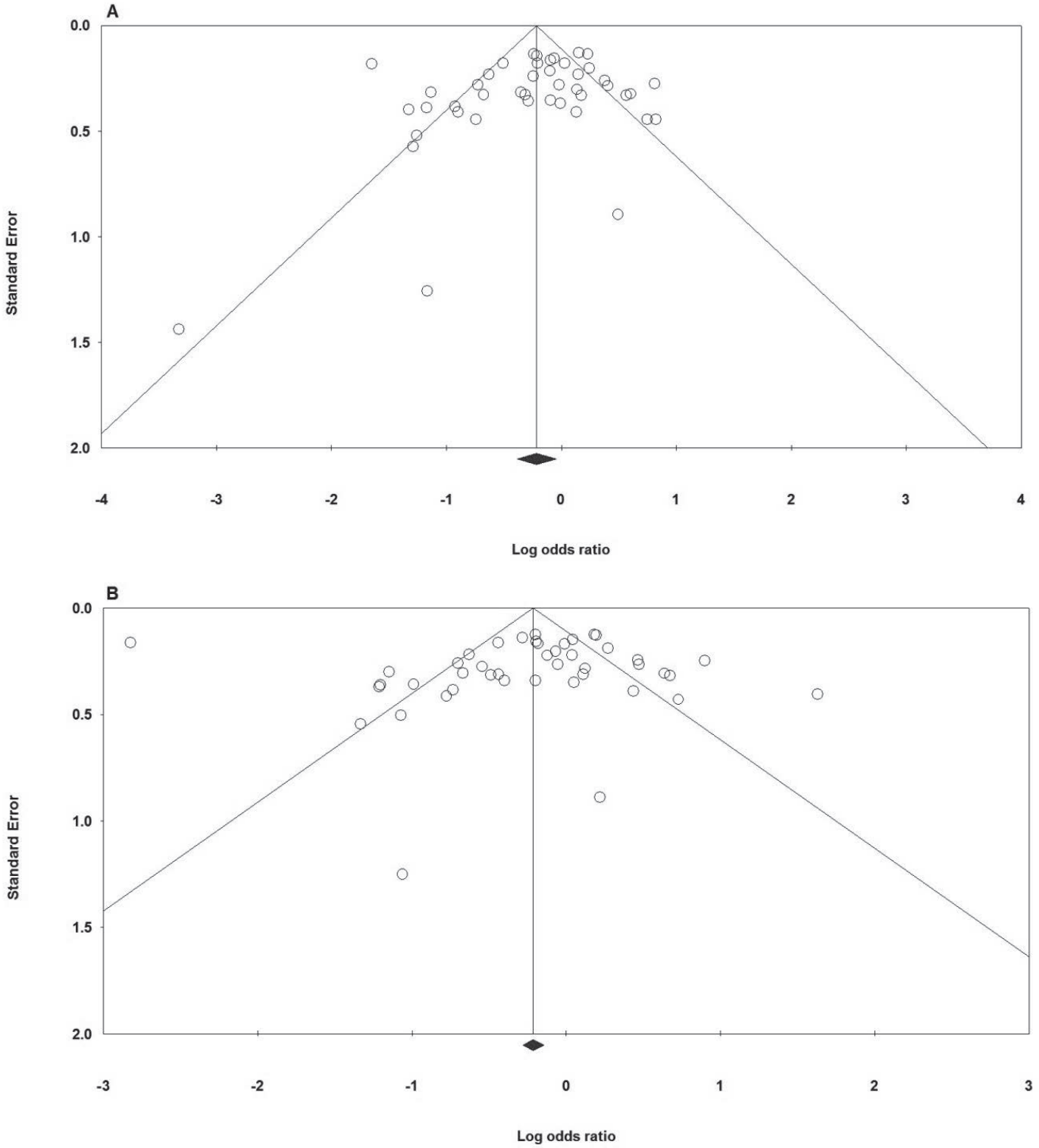

Fig. 4 Begg's funnel plots of between PAI-1 rs1799889 Polymorphism with Susceptibility to Ischemic Stroke. A: heterozygote model (GA vs. AA); B: dominant model (GG+GA vs. AA). Each point represents a separate study for the indicated association.

of PAI-1 rs1799889 polymorphism towards a decreased risk of hemorrhagic stroke (50).

Between-study heterogeneity is a common issue in a meta-analysis on genetic association (51–53). It could be attributable to differences in several factors such as environmental factors, including criteria or methodological factors in design and conduct of the studies (54, 55). Thus, identifying the potential sources of heterogeneity is one of the most important goals of meta-analysis. When all the eligible studies were pooled in this meta-analysis, there was significant between-study heterogeneity under all genetic models. However, after subgroup analyses by ethnicity the heterogeneity not effectively disappeared or decreased, which indicated that ethnicity did not play a crucial role in the existence of between-study heterogeneity in the current meta-analysis.

The current meta-analysis had some advantages. First, this was the most comprehensive and accurate me-

ta-analysis to evaluate association of PAI-1 rs1799889 polymorphism with ischemic stroke, which involved Asian, Caucasian, mixed populations. Second, the current meta-analysis search not restricted to studies published in indexed journals. Third, we have evaluated the association under all five genetic models. Forth, there was no evidence of publication bias by Begg's funnel plot and Egger's test in this meta-analysis. Finally, sensitivity analysis confers the reliability and stability of our pooled data. However, some limitations of this meta-analysis should be mentioned. First, the sample size of the included studies was not large enough by ethnicity among African and Mixed populations. Therefore, there was a lack of statistical power to better calculate association of PAI-1 rs1799889 polymorphism with risk of stroke among African and Mixed populations. Second, all included studies were published in English or Chinese which may be brought some bias. Third, in this meta-analysis between-study heterogeneity was detected under all five genetic models in the overall population and by subgroup analyses, which may be distorting the pooled data. Finally, our results were based on single-factor estimations without adjustment for other risk factors such as age, gender, and environmental factors.

In summary, this meta-analysis result revealed that PAI-1 rs1799889 polymorphism was significantly associated with an increased risk of ischemic stroke, especially in Asian populations. Moreover, there was a significant association between PAI-1 rs1799889 polymorphism and ischemic stroke risk. Future studies with large sample sizes and well designs in the Mixed and African populations and gene-gene and gene-environment interaction studies are warranted to confirm these findings.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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