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REVIEW ARTICLE

Phosphodiesterase 4 D (PDE4D) gene polymorphisms and risk of ischemic stroke: A systematic review and meta-analysis

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Abstract

Background and purpose Studies on the relationship between Phosphodiesterase 4 D (PDE4D) gene polymorphism with the risk of ischemic stroke (IS) have shown discordant results. The present meta-analysis was aimed to clarify the relationship between PDE4D gene polymorphism with the risk of IS by estimating pooled analysis of published epidemiological studies.

Methods A comprehensive literature search for all the published articles was performed in various electronic databases, including PubMed, EMbase, Cochrane Library, Trip Database, Worldwide Science, CINAHL, and Google Scholar up to 22nd December 2021. Pooled Odds ratios (ORs) with 95% Confidence Intervals (CIs) under dominant, recessive, and allelic models were calculated. Subgroup analysis based on ethnicity (Caucasian vs. Asian) was performed to examine the reliability of these findings. Sensitivity analysis was also performed to detect the heterogeneity between studies. Finally, Begg's funnel plot was used to assess the potential for publication bias.

Results In our meta-analysis, we identified a total of 47 case–control studies with 20,644 ischemic stroke (IS) cases and 23,201 control subjects, including 17 studies of Caucasian descent and 30 studies of Asian descent. Our findings suggest that there was a significant relationship between SNP45 gene polymorphism and risk of IS (Recessive model: OR = 2.06, 95% CI 1.31–3.23), SNP83 overall (allelic model: OR = 1.22, 95% CI 1.04–1.42), Asian (allelic model: OR = 1.20, 95% CI 1.05–1.37), and SNP89 Asian (Dominant model: OR = 1.43, 95% CI 1.29–1.59, recessive model: OR = 1.42, 95% CI 1.28–1.58) respectively. However, no significant relationship was found between SNP32, SNP41, SNP26, SNP56, and SNP87 gene polymorphisms and risk of IS.

Conclusion Findings of this meta-analysis conclude that SNP45, SNP83, and SNP89 polymorphism could be capable of increasing stroke susceptibility in Asians but not in the Caucasian population. Genotyping of SNP 45, 83, 89 polymorphisms may be used as a predictor for the occurrence of IS.

Keywords Phosphodiesterase 4 D · Gene polymorphism · Stroke · Ischemic stroke · Meta-analysis

Introduction

Stroke is a heterogeneous complex disorder and the second most common cause of death and long-term disability with higher incidence among adults and older people worldwide and is a significant public health problem [1]. Stroke can be divided into two subtypes: 20% of cases are hemorrhagic (intracerebral hemorrhage), and 80% are ischemic stroke (IS) [2, 3]. Characterization of a sudden decrease in blood flow to one or more areas within the central nervous system is considered 'Ischemic stroke'. In contrast, hemorrhagic stroke occurs when a weakened blood vessel rupture.

Both genetic and lifestyle-related factors may increase the risk of stroke. Genetic factors involving single nucleotide polymorphisms (SNPs) can indicate susceptibility to

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specific stroke subtypes. Numerous mutations in microsatellite markers and SNPs of the STRK1 locus, chromosome 5q12, encoding phosphodiesterase (PDE) type 4D (PDE4D), were identified as independent risk factors for IS [3]. PDEs are intracellular enzymes that degrade the cyclic nucleotides adenosine (cAMP) and/or guanosine monophosphate (cGMP), thereby modulating cellular signaling via the cAMP/cGMP pathways [4]. In IS, suppression of PDE4D protects the blood–brain barrier and reduces both inflammation and thrombosis. Inflammation plays a significant role in the development of atherosclerosis, and PDE4 has been significantly expressed in inflammatory cells during cerebral ischemia [5]. Variants and polymorphisms in the PDE4D gene have been investigated in different IS population to ascertain any associated risks [6]. In the present study, we retrospectively reviewed studies focusing on the relationship of PDE4D gene polymorphism with increasing or decreasing risk of IS in patients published to date.

Methods

Literature search

The current systematic review was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement guidelines, and a meta-analysis was conducted with the extracted data [7]. Relevant publications (until 31st October 2021) were identified by searching various electronic databases, including PubMed, EMbase, Cochrane Library, Trip Database, Worldwide Science, CINAHL, and Google Scholar. The following key terms were used: 'Phosphodiesterase 4 D' OR 'PDE4D' AND 'polymorphism' OR 'variant' OR 'mutation' OR 'genotype' AND 'stroke' OR 'ischemic stroke' OR 'Cerebral Infarction' OR 'Brain Infarction' OR 'Cerebrovascular disease' OR 'Cerebrovascular disorder' in combination with 'SNP 45' OR 'SNP 56' OR 'SNP 87' OR 'SNP 89' OR 'SNP 32' OR 'SNP 41' OR 'SNP 83' OR SNP '26' OR 'rs966221' OR 'rs40512' OR 'rs12188959' OR 'rs702553' OR 'rs2910829' OR 'rs1396476'. Additionally, the reference list of the retrieved studies, review articles, and previous meta-analyses, were manually searched for collecting more relevant studies often missed while performing the electronic search. Two authors (MN and PS) independently conducted the search for various studies, and any discrepancy regarding the inclusion or exclusion of individual studies was resolved by consulting the corresponding author (PK).

Eligibility criteria

Studies were included if they met the following inclusion criteria: (1) case–control studies investigating the relationship of PDE4D gene polymorphism with the risk of IS; (2) clinically confirmed diagnosis of IS using CT or MRI scan; (3) patients aged < 18 years for pediatric and > 18 years for adult population; (4) numbers available for patient and control groups for PDE4D SNP 45, 32, 41, 26, 56, 83, 87, 89 genotype or data provided from which the numbers could be calculated. No restriction of language was applied.

Data extraction

Two investigators (PK and PS) independently extracted the data. Following data were extracted from each study: first author's name, published year, ethnicity, country, number of cases and controls, matching criteria, sample source, duration of inclusion, mean age, genotyping method and frequency distribution of PDE4D SNP 45, 32, 41, 26, 56, 83, 87, 89 genotypes. Hardy–Weinberg Equilibrium (HWE) was calculated for allelic frequency distribution. Ethnicities were categorized as either Asian or Caucasian, and the population was classified into adult and paediatric groups.

Quality assessment

Newcastle–Ottawa Scale (NOS) [8] was used for assessing the quality of the included studies based on three components: selection, comparability and ascertainment of the outcome. Scores ranged from 01 to 09. Two authors (PS and MN) independently assessed the quality of the included studies. Discrepancies over quality scores were resolved by discussion among all the authors and subsequent consensus was reached.

Statistical analysis

Odds ratios (ORs) with 95% Confidence Intervals (CIs) were calculated to investigate the relationship between PDE4D (SNP 45, 32, 41, 26, 56, 83, 87, 89) gene polymorphism and risk of IS using fixed (Mantel–Haenszel method) or random effects (Dersimonian and Laird method) models [9, 10]. Heterogeneity between studies was compared using Cochran's Q statistic and I^2 metric [11, 12]. I^2 metric was used to describe the degree of heterogeneity between included studies, where 0–25% indicated no observed heterogeneity and larger values showed increasing heterogeneity, with 25–50% regarded as low, 50–75% as moderate and 75–100% as high. If $I^2 < 50\%$, a fixed-effects model was used, and if $I^2 > 50\%$, a random-effects model was used. In addition, heterogeneity between studies was adjusted by subgroup analysis, HWE status, and meta-regression by quality score of the included studies. Meta-regression was conducted using the Restriction maximum likelihood (REML) estimate with Knapp-Hartung modification for determining the between-study variance [13].

One-way sensitivity analyses were performed to assess the stability of the results. A single study in the meta-analysis was deleted each time to reflect the influence of the individual dataset on the pooled OR. Funnel plots and Egger's linear regression test were used to diagnose the potential publication bias [14, 15]. Presence of selection bias in control participants was evaluated by calculating HWE, and genotypic frequencies of the control subjects were compared using the chi-square test. Stratified analyses based on ethnicity (Asian vs. Caucasian) were performed. The reliability and accuracy of the results were validated by two investigators (PK and PS), who independently analyzed the data using the software. All statistical analyses were performed using STATA 13.0 and Review Manager 5.3 software. The p values were two-sided, and a p value < 0.05 was statistically significant.

Results

Literature search

The initial search yielded 72 records from PubMed, EMbase, Scopus, Web of Science, CENTRAL (Cochrane Central Register of Controlled Trials), and Google scholar databases. Of them, 22 were excluded after the review of title/abstract, leaving 50 potential studies for full-text information review. Finally, 47 studies met the inclusion criteria and were included in this study (Fig. 1).

Characteristics of eligible studies

The main characteristics of included studies are presented in Table 1. The publication years of the studies included in our analysis ranged from 2003 to 2019. The sample size in each study ranged from 91 to 2890 in IS cases and 44 to 4412 in controls subjects for all the included studies in our meta-analysis. Forty-seven case–control studies (18 studies for SNP45, 12 for SNP56, 29 for SNP83, six for SNP89, five for SNP26, seven for SNP41, four studies for SNP32, and 27 studies for SNP87) were included in our meta-analysis. Studies were carried out in two major ethnic populations; 30 studies were in the Asian while 17 studies were in the Caucasian population. All studies in this meta-analysis had controls in HWE. The quality scores of all included studies were moderately high. Out of 47 studies, 27 studies had hospital-based and 20 studies had a population-based source of controls. Table 1 summarizes the characteristics

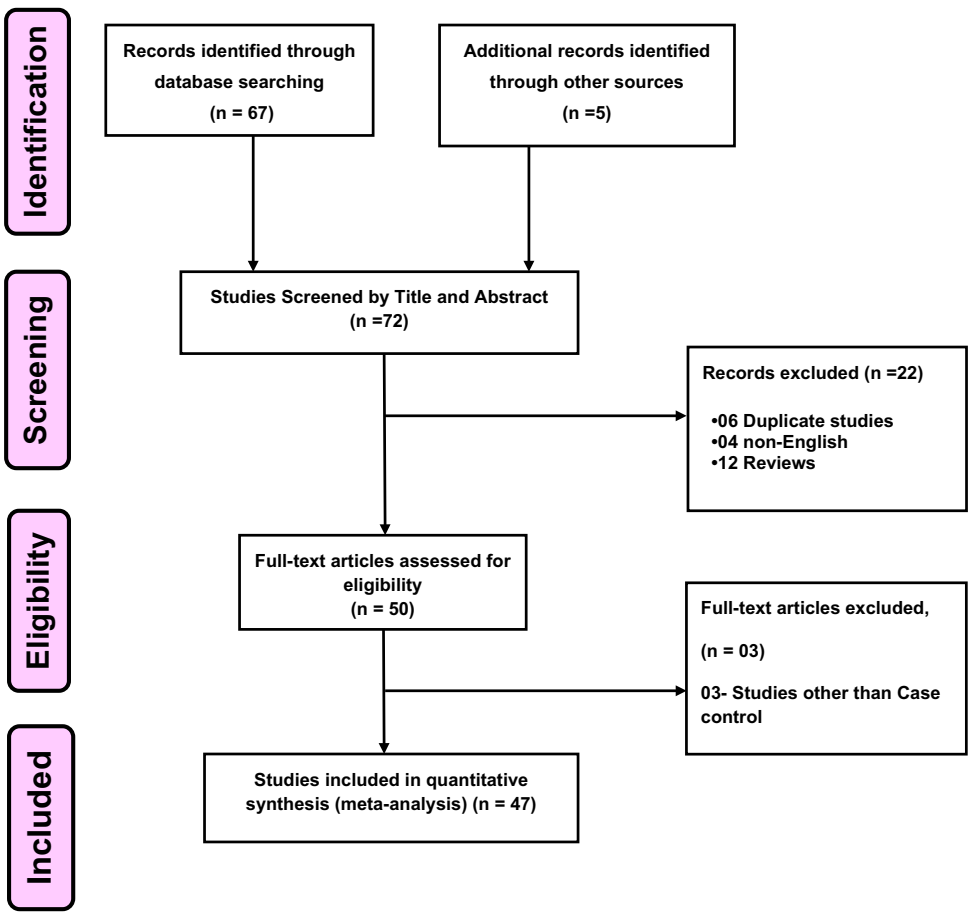


Fig. 1 Flow diagram for the selection of studies and specific reasons for exclusion from the present meta-analysis

Table 1 Characteristic of the included studies in the meta-analysis for the relationship between PDE4D gene polymorphism with the risk of stroke

S.No	References	Country	Ethnicity	Total IS cases	Total control	Variant studied	Genotype method	HWE	Source of control	NOS Quality score
1	Lin et al. [16]	China	Asian	190	211	SNP 87, 56, 83	Taqman	Yes	HB	5
2	Banerjee et al. [17]	India	Asian	176	212	SNP 83	PCR	Yes	PB	4
3	Munshi et al. [18]	India	Asian	250	250	SNP 83	PCR	Yes	HB	4
4	Fidani et al. [19]	Greece	Caucasian	97	102	SNP 45	PCR- RFLP	Yes	HB	6
5	Kostulas et al. [20]	Iceland	Caucasian	524	715	SNP, 45	PCR- RFLP	Yes	PB	6
6	Van Rijn et al. [21]	Netherland	Caucasian	91	200	SNP 83, 45,	PCR	Yes	PB	6
7	Staton et al. [22]	Australia	Caucasian	151	164	SNP 89, 87, 83, 56, 45, 41	Not known	Yes	PB	6
8	Zee et al. [23]	Boston	Caucasian	259	259	SNP 56, 32, 45, 26	PCR–RFLP	Yes	PB	7
9	He et al. [24]	China	Asian	186	232	SNP 87	PCR–RFLP	Yes	PB	6
10	Ying et al. [25]	China	Asian	400	400	SNP 87	PCR–RFLP	Yes	PB	5
11	Kalita et al. [26]	India	Asian	145	188	SNP 87, 83, 32	PCR	Yes	PB	6
12	Li et al. [27]	China	Asian	371	371	SNP 83, 87	PCR–RFLP	Yes	HB	5
13	Sun et al. [28]	China	Asian	649	761	SNp 56, 83, 87, 41	PCR	Yes	HB	5
14	Xue et al. [29]	China	Asian	639	887	SNP 83, 87, 32	PCR–RFLP	Yes	HB	7
15	Munshi et al. [30]	India	Asian	516	513	SNP 41, 56	PCR–RFLP	Yes	HB	6
16	Saleheen et al. [31]	Pakistan	Asian	200	250	SNP 87, 83, 32	PCR	Yes	PB	5
17	Ma et al. [32]	China	Asian	373	377	SNP 87, 83	PCR	Yes	HB	3
18	Hseih et al. [33]	Taiwan	Asian	108	280	SNP 87, 41	DS	Yes	PB	6
19	Shao et al. [34]	China	Asian	459	462	SNP 83, 45, 87	SpectroCHIP	Yes	HB	4
20	Skvortsova et al. [35]	Russia	Caucasian	577	270	SNP 41, 87	Taqman	Yes	PB	6
21	Gretarsdottir et al. [3]	America	Caucasian	864	908	SNP 83, 45, 56,89, 26, 87	PCR	Yes	HB	8
22	Meschia et al. [36]	America	Caucasian	377	263	SNP 83, 45, 56	PCR	Yes	HB	5
23	Woo et al. [37]	America	Caucasian	357	303	SNP 83, 56, 89, 87	TaqMan	Yes	PB	5
24	Song et al. [38]	America	Caucasian	224	211	SNP 83, 45, 89	DHPLC	Yes	HB	6
25	Kuhlenbaumer et al. [39]	Germany	Caucasian	1181	1569	SNP 83, 45, 56, 89, 87	TaqMan	Yes	PB	4
26	Matsushita et al. [40]	Japan	Asian	2890	4412	SNP 83, 56, 89, 87	TaqMan	Yes	PB	5
27	Wang et al. [41]	China	Asian	235	105	SNP 83	PCR	Yes	HB	5
28	Cheng et al. [42]	China	Asian	280	258	SNP 83	PCR-LDR	Yes	HB	4
29	Zhang et al. [43]	China	Asian	122	44	SNP 83	PCR-LDR	Yes	HB	4
30	Xu et al. [44]	China	Asian	116	110	SNP 83	PCR–RFLP	Yes	HB	5
31	Zhao et al. [45]	China	Asian	396	300	SNP 83	PCR	Yes	HB	5
32	Luo et al. [46]	China	Asian	712	744	SNP 83	PCR	Yes	HB	6
33	Zhao et al. [45]	China	Asian	682	598	SNP 83, 87	PCR	Yes	HB	6
34	Yue et al. [47]	China	Asian	200	193	SNP 83, 87	Illumina Miseq	Yes	HB	7

Table 1 (continued)

S.No	References	Country	Ethnicity	Total IS cases	Total control	Variant studied	Genotype method	HWE	Source of control	NOS Quality score
35	Zhang et al. [48]	China	Asian	881	892	SNP 87	PCR–RFLP	Yes	HB	6
36	Shi et al. [49]	China	Asian	256	250	SNP 87	PCR–RFLP	Yes	HB	6
37	Bevan et al. [50]	Germany	Caucasian	737	933	SNP 45, 26, 87	PCR	Yes	PB	7
38	Brophy et al. [51]	California	Caucasian	248	560	SNP 45, 56, 26	RT-PCR	Yes	PB	5
39	Lohmussaar et al. [52]	Germany	Caucasian	639	736	SNP 45, 87, 26	MALDI-TOF	Yes	PB	6
40	Ardnor et al. [53]	Sweden	Caucasian	275	550	SNP 45	TaqMan	Yes	PB	7
41	Lovkvist et al. [54]	Sweden	Caucasian	932	396	SNP 45, 87,	RT-PCR	Yes	PB	7
42	Nakayama et al. [55]	Japan	Asian	208	270	SNP 83	TaqMan PCR	Yes	HB	7
43	Kumar et al. [56]	India	Asian	250	250	83, 45, 87	PCR–RFLP	Yes	HB	7
44	Wang [57]	China	Asian	610	618	SNP 45, 83, 87, 56	PCR	Yes	HB	5
45	Kim et al. [58]	Korea	Asian	205	103	41	PCR	Yes	HB	6
46	Milton et al. [59]	Australia	Caucasian	180	301	41, 45	PCR	Yes	PB	7
47	Zhang et al. [60]	China	Asian	226	220	87	PCR	Yes	HB	6

IS Ischemic Stroke, *HWE* Hardy Weinberg Equilibrium, *PB* Population-Based, *HB* Hospital-based, *PCR–RFLP* Polymerase Chain Reaction-Restriction Fragment Length Polymorphism, *SNP* Single Nucleotide Polymorphism, *NOS* New-Castle Ottawa Scale

and methodological quality of all the included studies. In our meta-analysis, a total of 47 case–control studies involving 20,644 IS cases and 23,201 controls were included.

Relationship between PDE4D SNP 45 gene polymorphism and ischemic stroke risk

No significant relationship was observed between PDE4D SNP45 gene polymorphism and risk of IS, under overall 18 studies, dominant model (OR = 1.00, 95% CI 0.95–1.04), recessive model (OR = 1.11, 95% CI 0.83–1.47), and allelic model (OR = 1.02, 95% CI 0.90–1.16). Upon conducting subgroup analysis on the basis of ethnicity of study population, significant association was observed in Asian population in case of recessive model (OR = 2.06, 95% CI 1.31–3.23); but not in dominant model (OR = 0.98, 95% CI 0.87–1.09) and allelic model (OR = 1.32, 95% CI 0.81–2.17) respectively [Figs. 2A, 3A, 4A)] [Table 2]. In Caucasian population, no significant association was observed under dominant model (OR = 1.00, 95% CI 0.95–1.05), recessive model (OR = 0.98, 95% CI 0.75–1.29) and allelic model (OR = 0.97, 95% CI 0.88–1.07) respectively [Figs. 2A, 3A, 4A] [Table 2].

Relationship between PDE4D SNP 56 gene polymorphism and ischemic stroke risk

Our results revealed that there were no significant relationship between PDE4D SNP56 gene polymorphism and risk of IS, under dominant model (OR = 1.01, 95% CI 0.96–1.07), recessive model (OR = 1.04, 95% CI 0.91–1.18), and allelic model (OR = 1.04, 95% CI 0.90–1.20). Upon subgroup analysis of the data on ethnicity of the study population, no significant association was observed in the Asian population under dominant model (OR = 1.06, 95% CI 0.94–1.20), recessive model (OR = 1.17, 95% CI 0.87–1.57) and allelic model (OR-1.16, 95% CI 0.87–1.55) respectively [Figs. 2B, 3B, 4B)] [Table 2]. Additionally, we did not find any correlation of SNP56 polymorphism with an increased or decreased risk of IS in all the three genetic models in Caucasian population, as can be observed under dominant model (OR = 0.99, 95% CI 0.93–1.07), recessive model (OR = 1.00, 95% CI 0.90–1.11) and allelic model (OR = 0.97, 95% CI 0.84–1.12) respectively [Figs. 2B, 3B, 4B)] [Table 2].

Relationship between PDE4D SNP 83 gene polymorphism and ischemic stroke risk

For SNP83, upon analysing 29 studies, results showed no significant relationship between PDE4D SNP83 gene polymorphism and risk of IS, under dominant model (OR = 0.98, 95% CI 0.94–1.03), and recessive model (OR = 1.11, 95% CI 0.97–1.27); but a significant association was observed under allelic model (OR = 1.22, 95% CI 1.04–1.42). Upon performing subtype analysis on the basis of ethnicity of the study population, no significant association was observed in the Asian population in dominant model (OR = 0.97, 95% CI 0.91–1.03), and recessive model (OR = 1.19, 95% CI 0.97–1.45); but a significant association in allelic model (OR-1.20, 95% CI 1.05–1.37) was observed [Figs. 2D, 3D, 4D] [Table 2]. In the Caucasian population, no significant association was observed under dominant model (OR = 1.01, 95% CI 0.94–1.08), recessive model (OR = 0.98, 95% CI 0.89–1.09) and allelic model (OR = 1.26, 95% CI 0.80–1.98) respectively [Figs. 2D, 3D, 4D] [Table 2].

Relationship between PDE4D SNP 26 gene polymorphism and ischemic stroke risk

For SNP26, we failed to find a significant genetic association between PDE4D SNP26 gene and risk of IS, under overall dominant model (OR = 0.99, 95% CI 0.91–1.08), recessive model (OR = 0.99, 95% CI 0.89–1.11), and allelic model (OR = 0.98, 95% CI 0.89–1.08) [Figs. 2F, 3F, 4F] [Table 2].

Relationship between PDE4D SNP 89 gene polymorphism and ischemic stroke risk

We did not find any association of the SNP89 gene polymorphism with increased or decreased risk of stroke under dominant model (OR = 1.06, 95% CI 0.88–1.28), recessive model (OR = 1.02, 95% CI 0.85–1.24), and allelic model (OR = 0.99, 95% CI 085–1.15) respectively. After the data were stratified according to ethnicity of the study population, our results showed significant association in the Asian population under dominant model (OR = 1.43, 95% CI 1.29–1.59), and recessive model (OR = 1.42, 95% CI 1.28–1.58); but no significant association was observed in allelic model (OR–0.93, 95% CI 0.74–1.16) [Figs. 2E, 3E, 4E] [Table 2]. In the Caucasian population, no significant association was observed under dominant model (OR = 0.97, 95% CI 0.90–1.05), recessive model (OR = 0.97, 95% CI 0.88–1.05) and allelic model (OR = 1.00, 95% CI 0.83–1.21) respectively [Figs. 2E, 3E, 4E] [Table 2].

Relationship between PDE4D SNP 32 gene polymorphism and ischemic stroke risk

No significant relationship was observed between PDE4D SNP32 gene polymorphism and risk of IS, under overall dominant model (OR = 0.89, 95% CI 0.71–1.12), recessive model (OR = 1.08, 95% CI 0.82–1.44), and allelic model (OR = 1.25, 95% CI 0.96–1.61). Upon conducting subgroup analysis on the basis of ethnicity of study population, no significant association was observed in Asian population under dominant model (OR = 0.87,

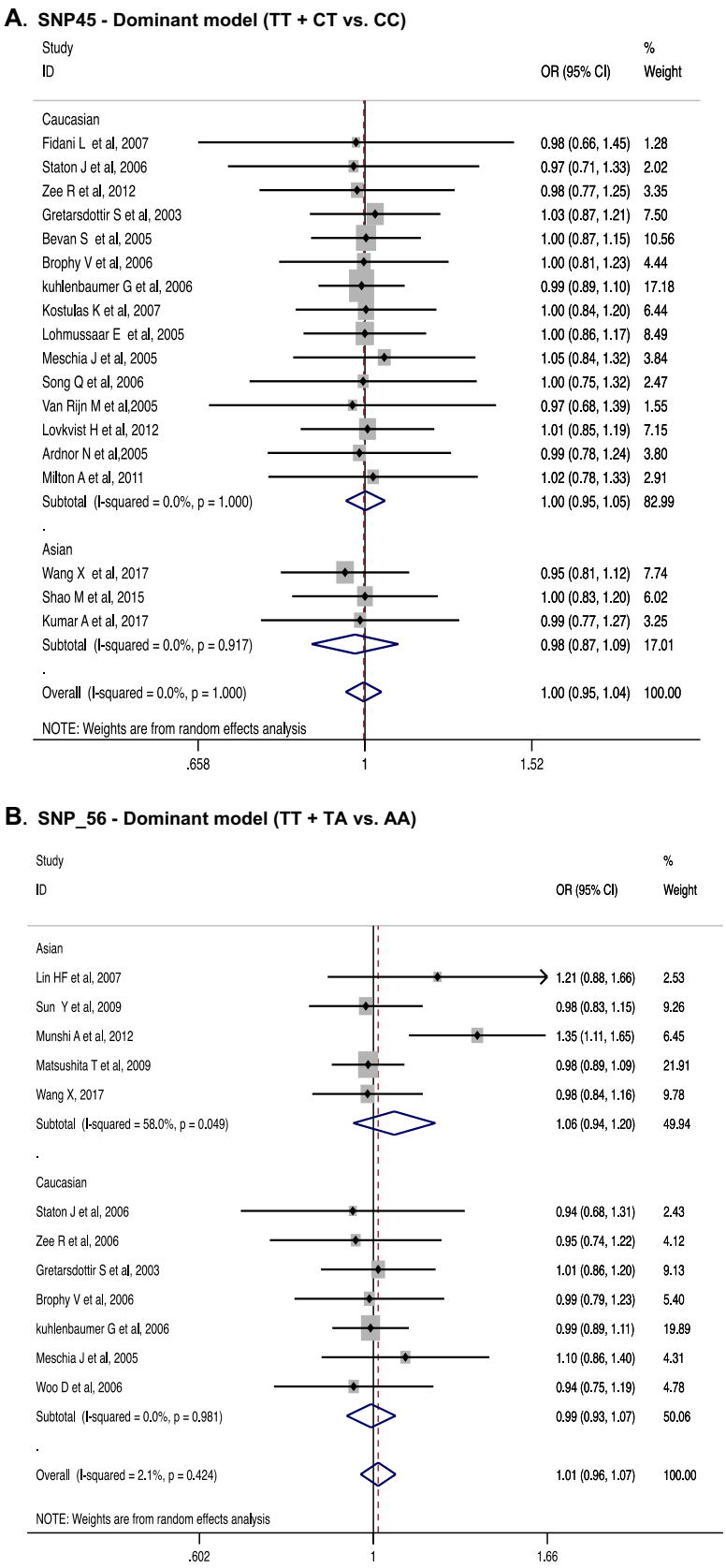
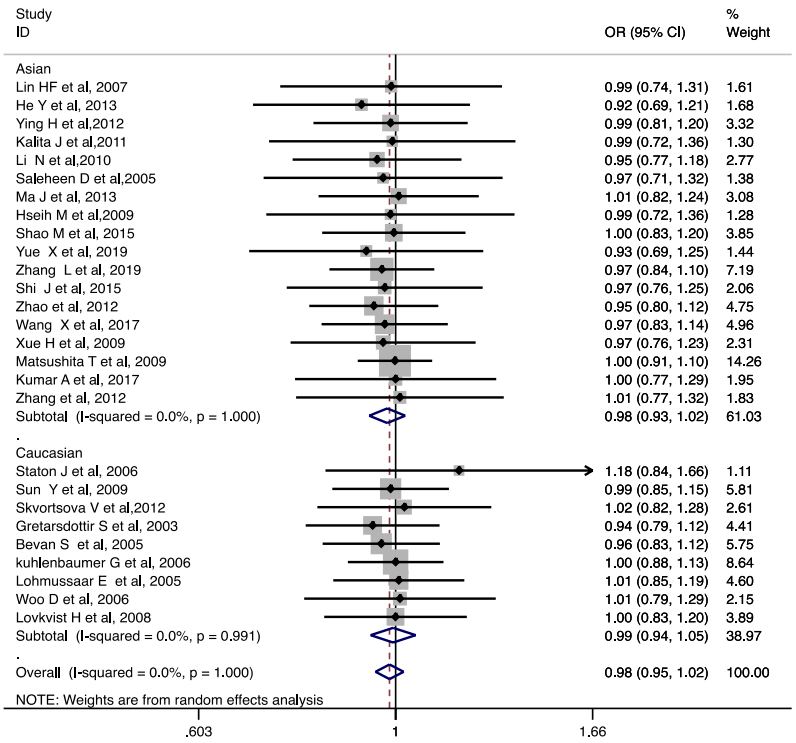


Fig. 2 **A**–**H**: Forest plot for the relationship between PDE4D SNP45, SNP56, SNP87, SNP83, SNP89, SNP26, SNP41, and SNP32 Polymorphism with the Risk of Ischemic Stroke using the Dominant Model

Fig. 2 (continued)

C. SNP 87 - Dominant model (CC + CT vs. TT)



D. SNP 83 - Dominant model (CC + TC vs. TT)

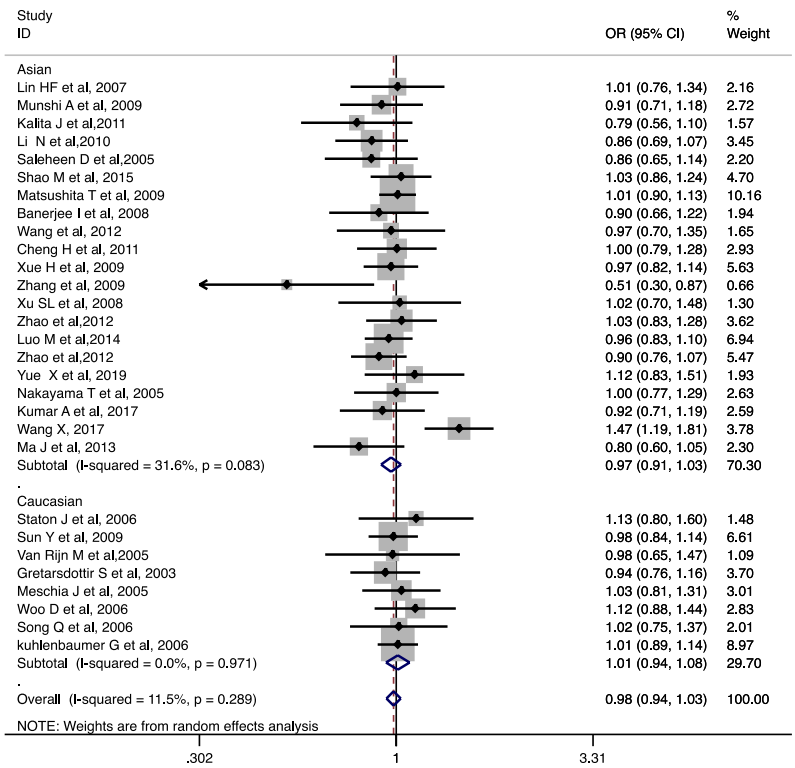
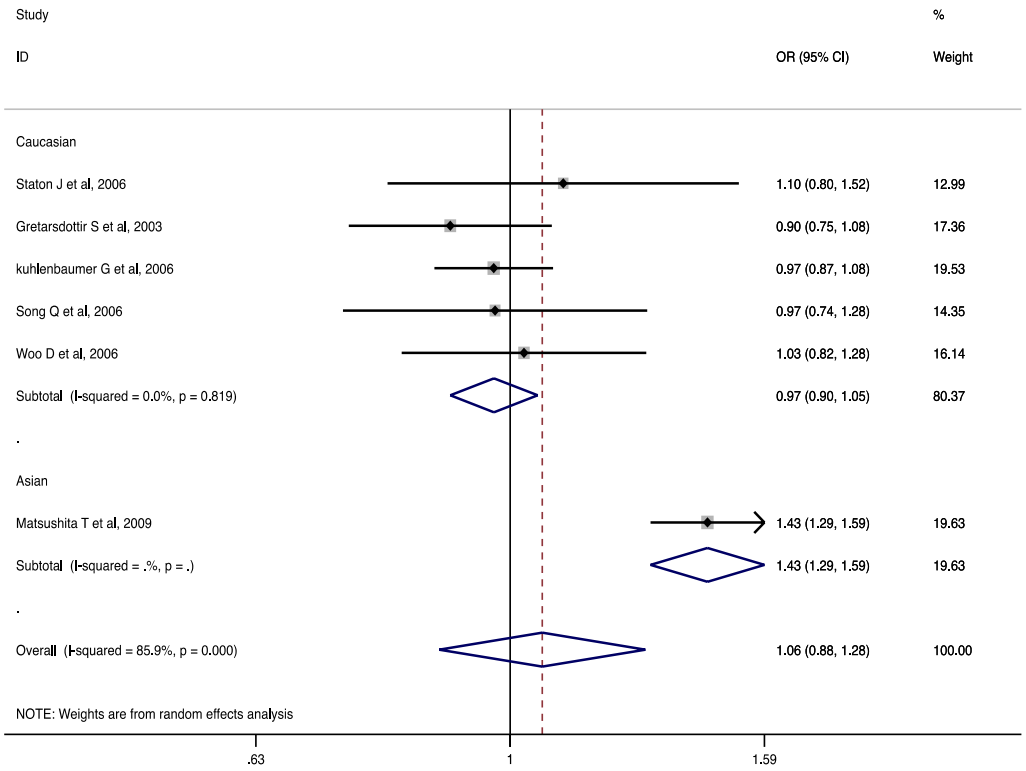


Fig. 2 (continued)

E. SNP 89 - Dominant model (CC + CT vs. TT)



F. SNP 26 - Dominant model (CC + CT vs. TT)

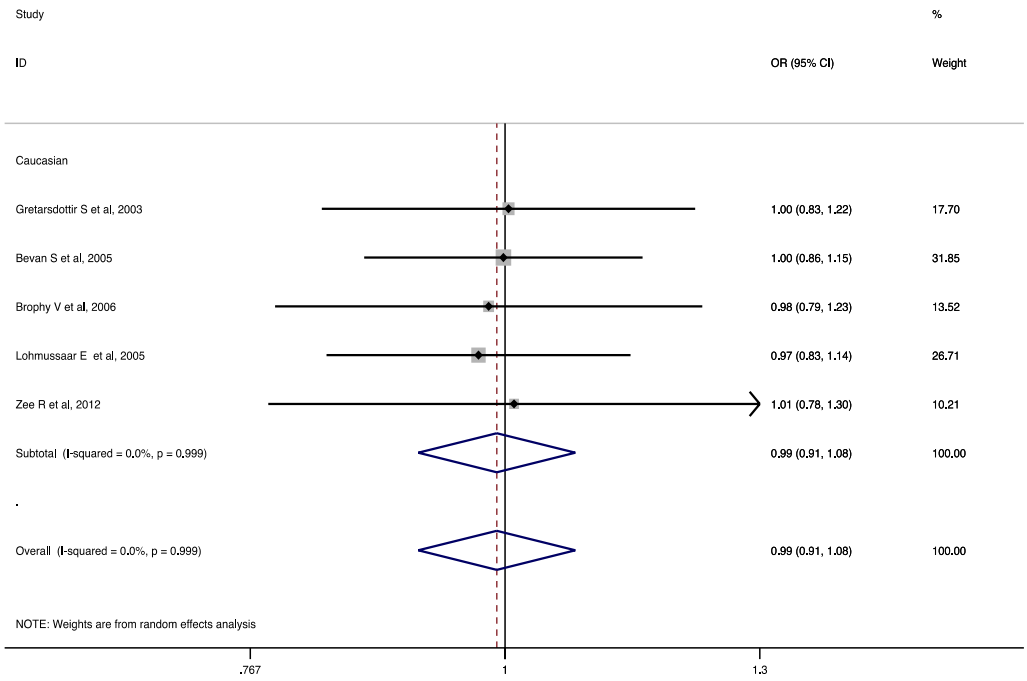
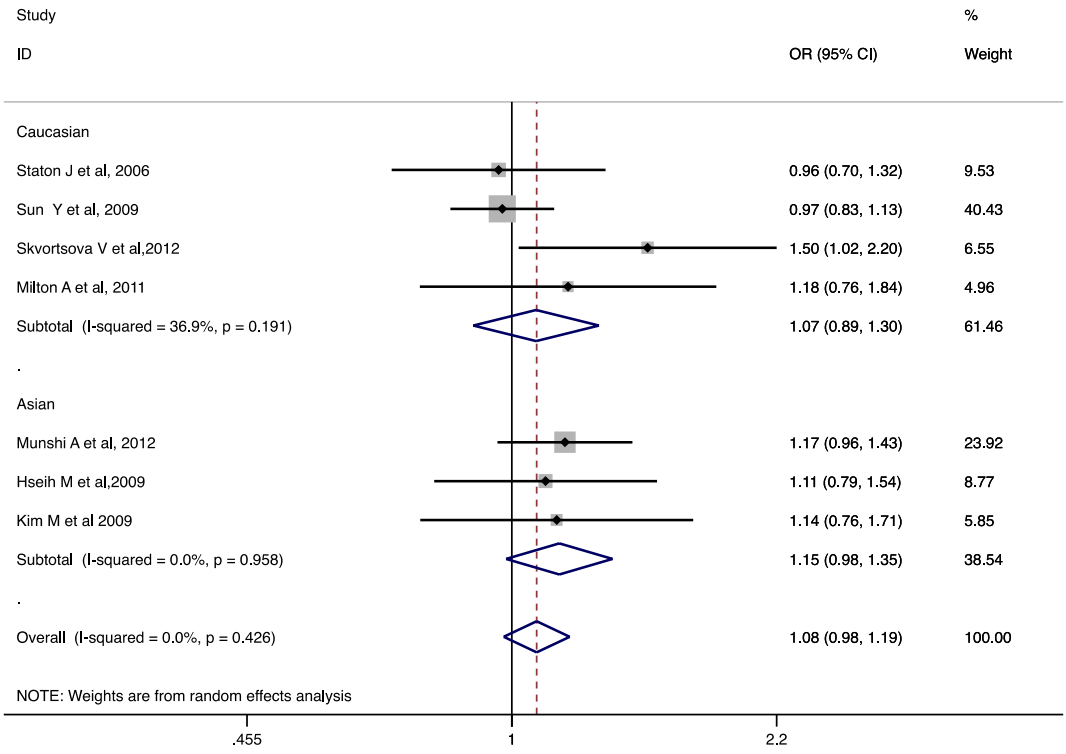


Fig. 2 (continued) **G. SNP 41 - Dominant model (CC + CT vs. TT)**



H. SNP 32 - Dominant model (CC + CT vs. TT)

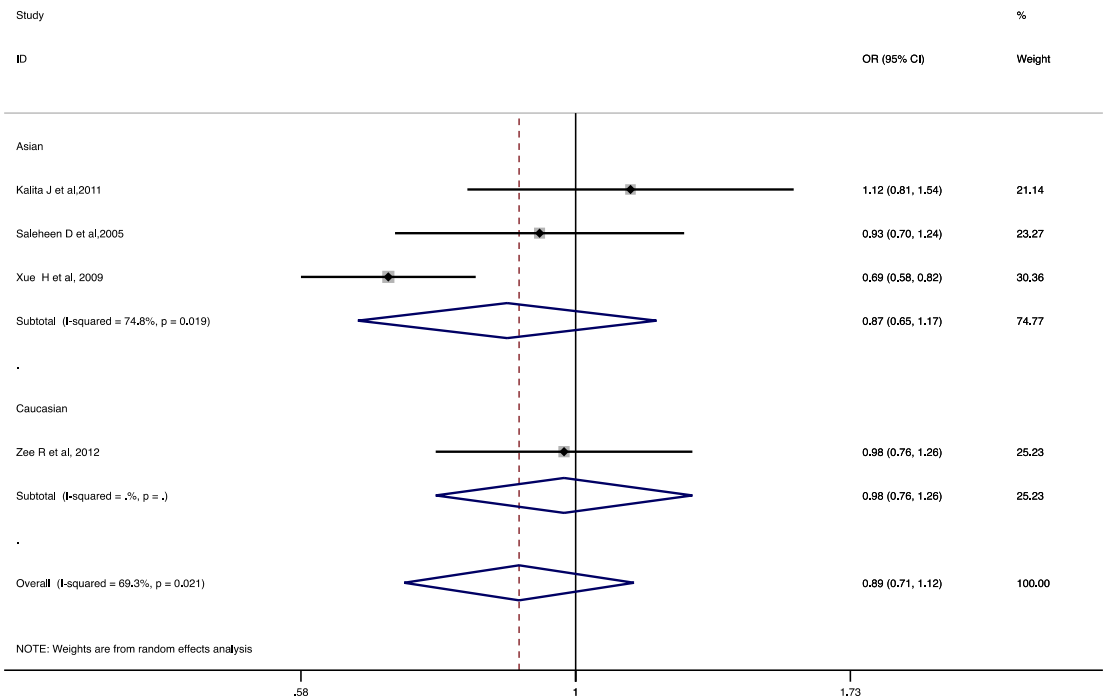
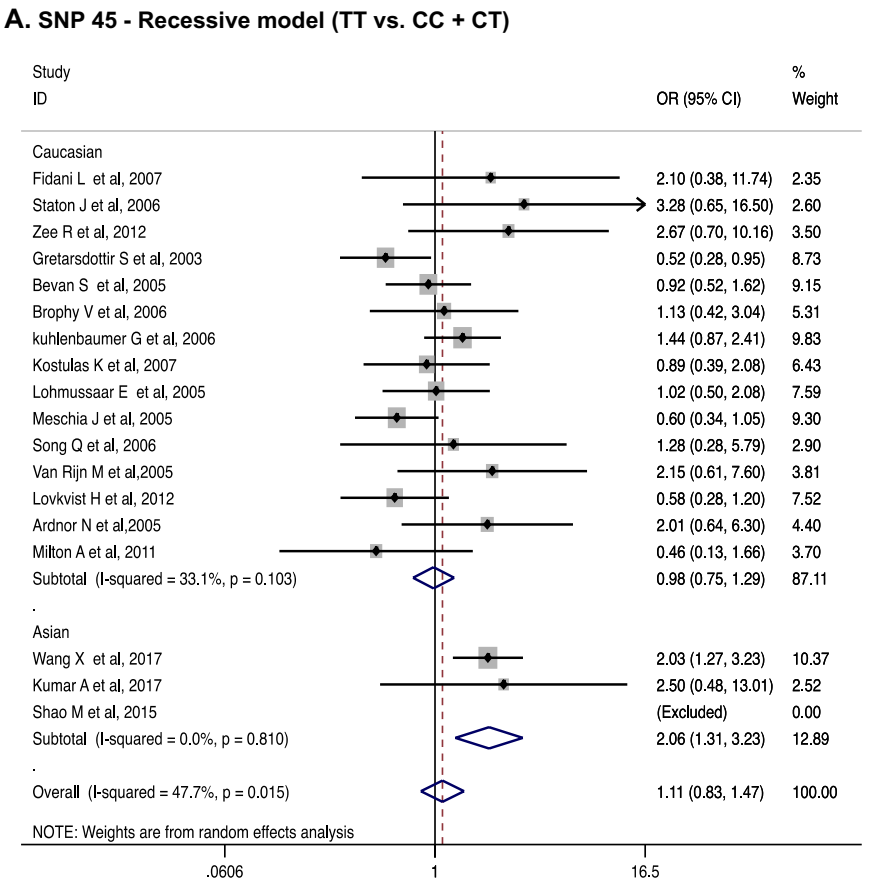


Fig. 3 **A**–**H**: Forest plot for the relationship between PDE4D SNP45, SNP56, SNP87, SNP83, SNP89, SNP26, SNP41, and SNP32 Polymorphism with the Risk of Ischemic Stroke using the Recessive Model



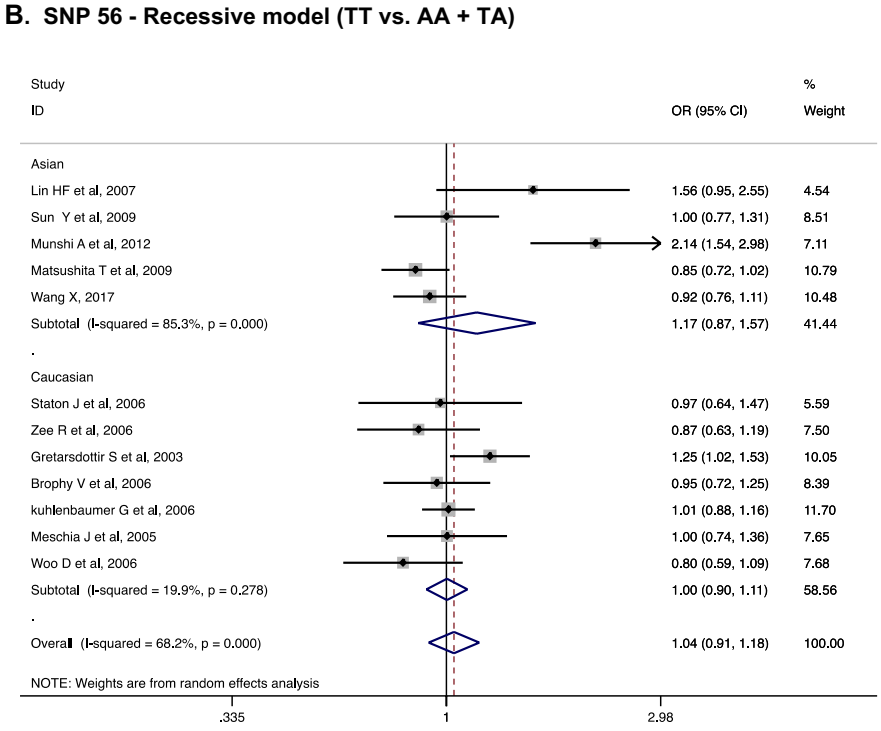
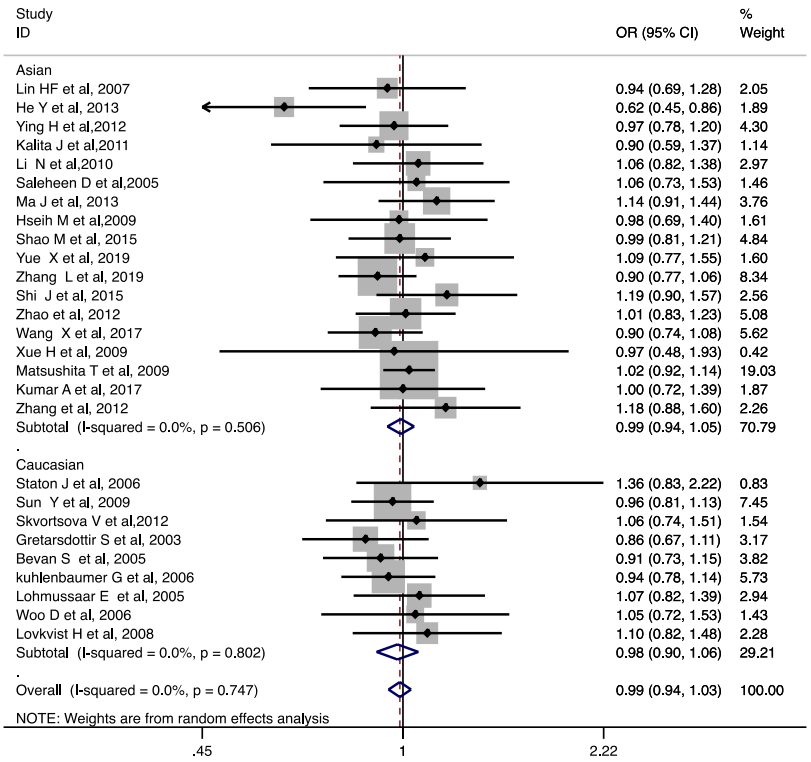


Fig. 3 (continued)

C. SNP 87 - Recessive model (CC vs. CT+ TT)



D. SNP 83 - Recessive model (CC vs. TT+ CT)

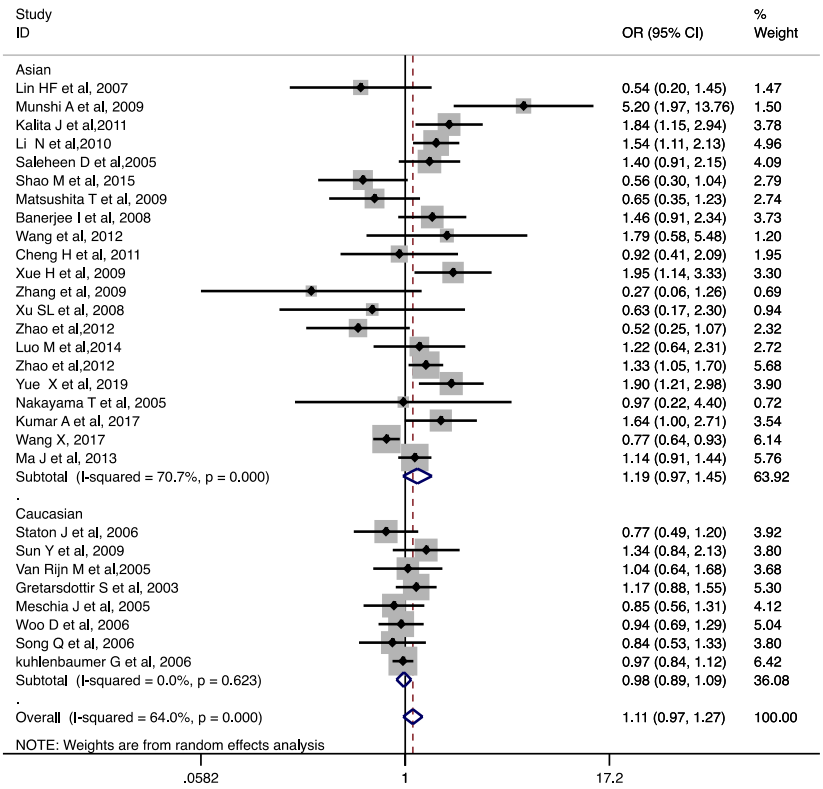
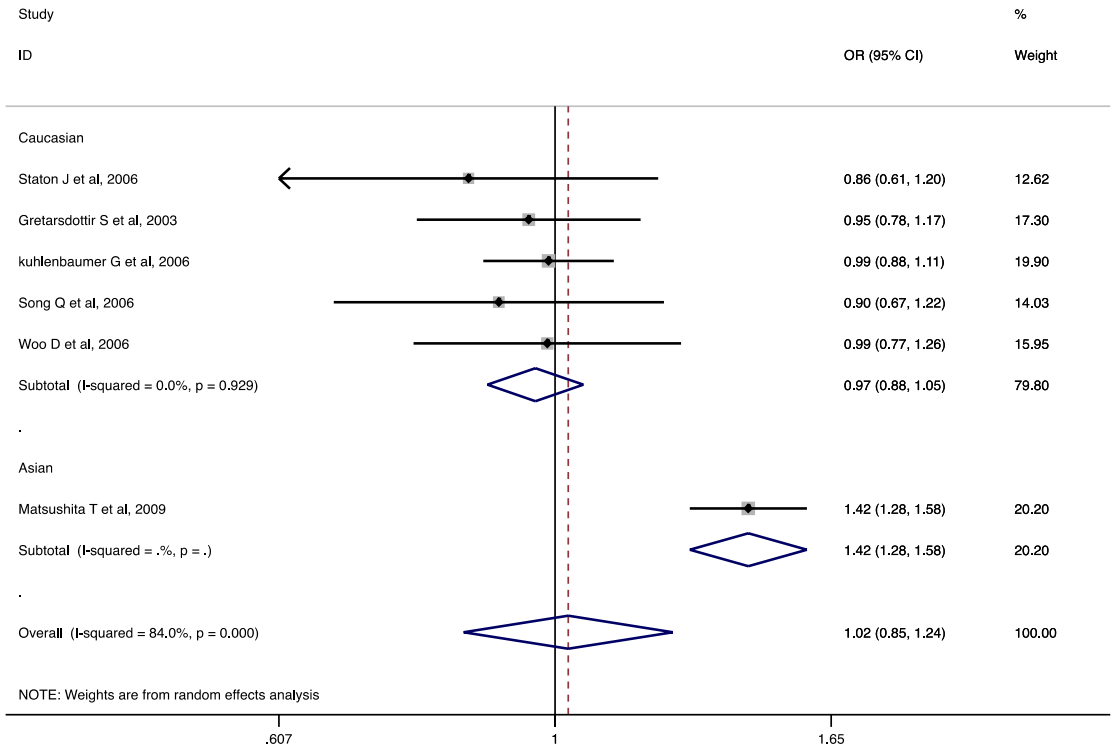


Fig. 3 (continued)

E. SNP 89 - Recessive model (CC vs. CT+ TT)



F. SNP 26 - Recessive model (CC vs. CT+ TT)

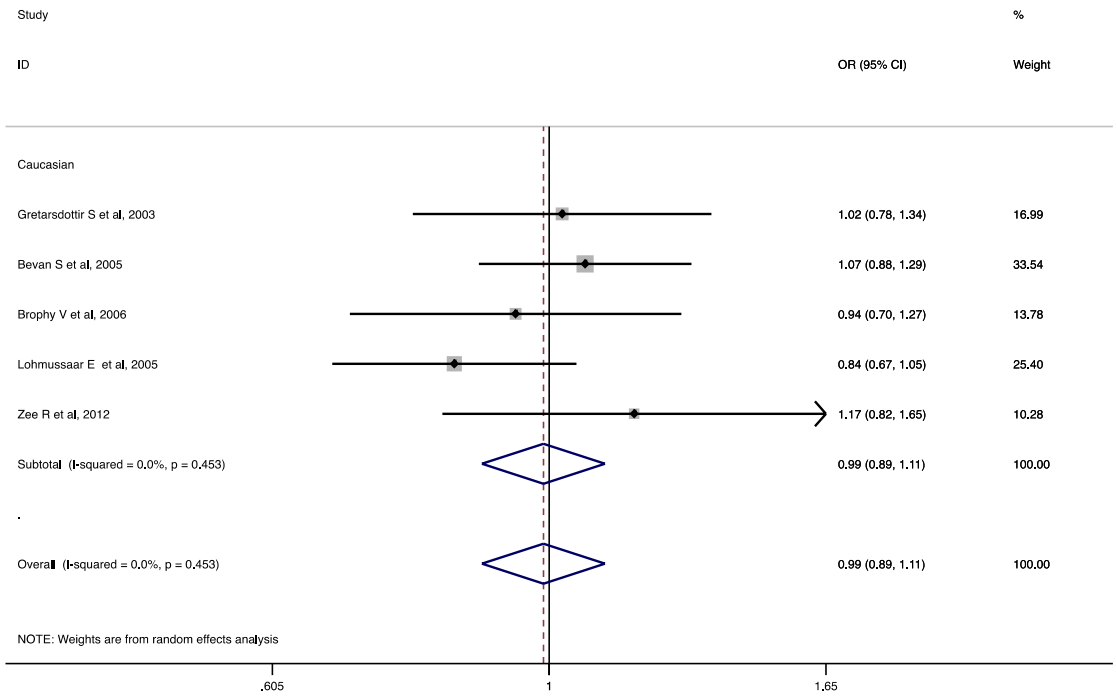
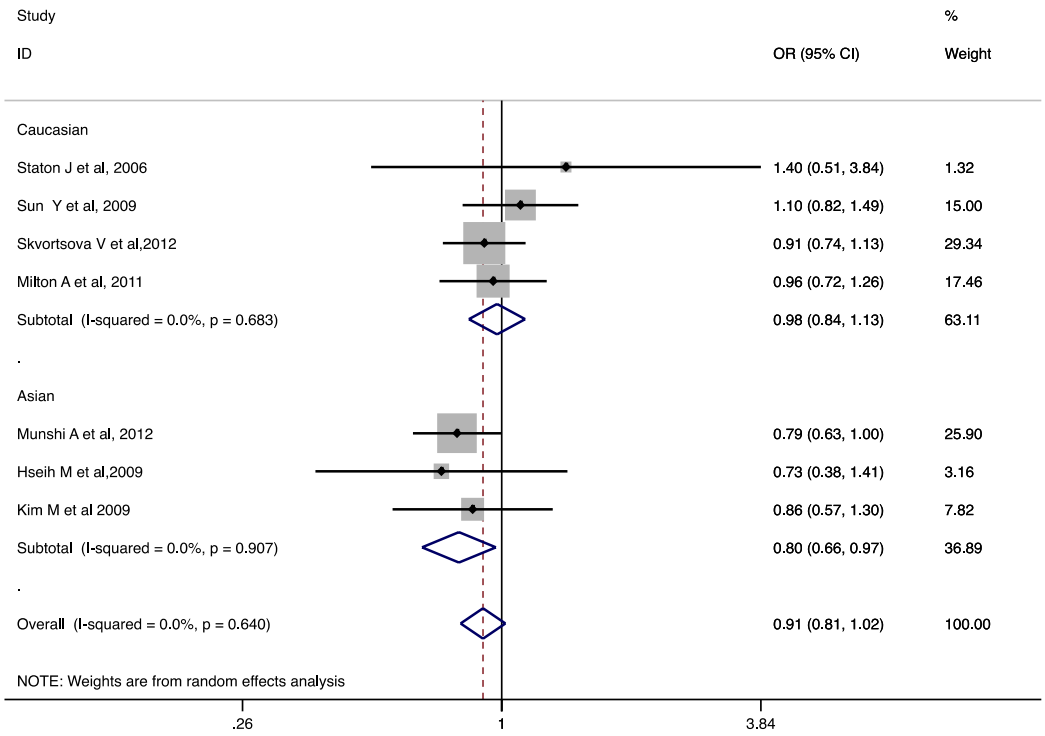
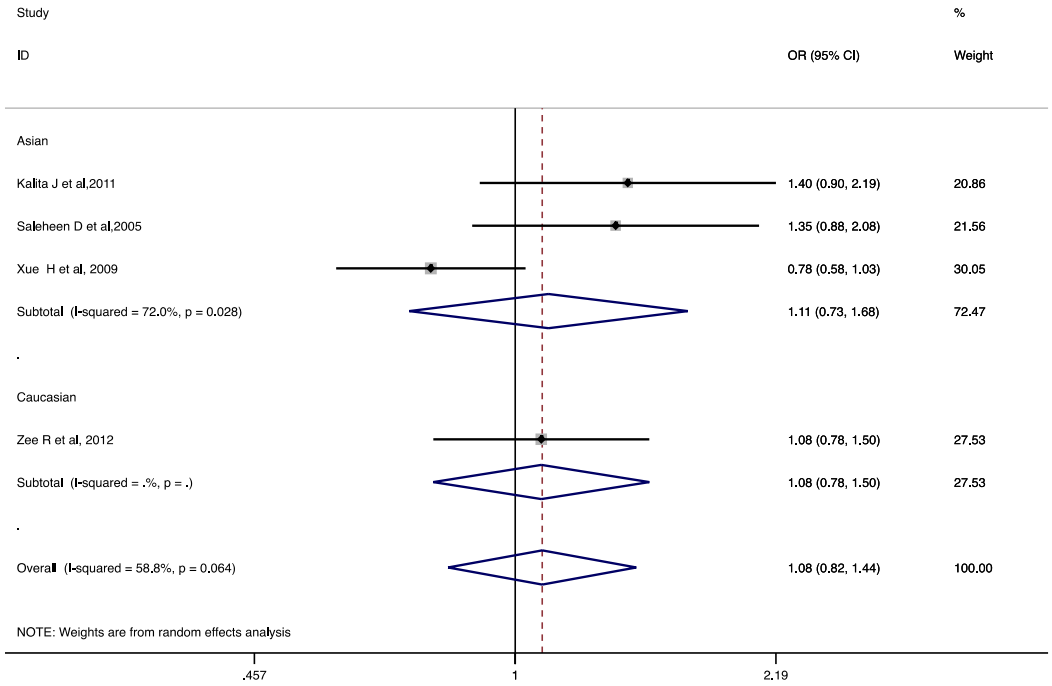


Fig. 3 (continued)

G. SNP 41 - Recessive model (CC vs. CT+ TT)



H. SNP 32 - Recessive model (CC vs. CT+ TT)



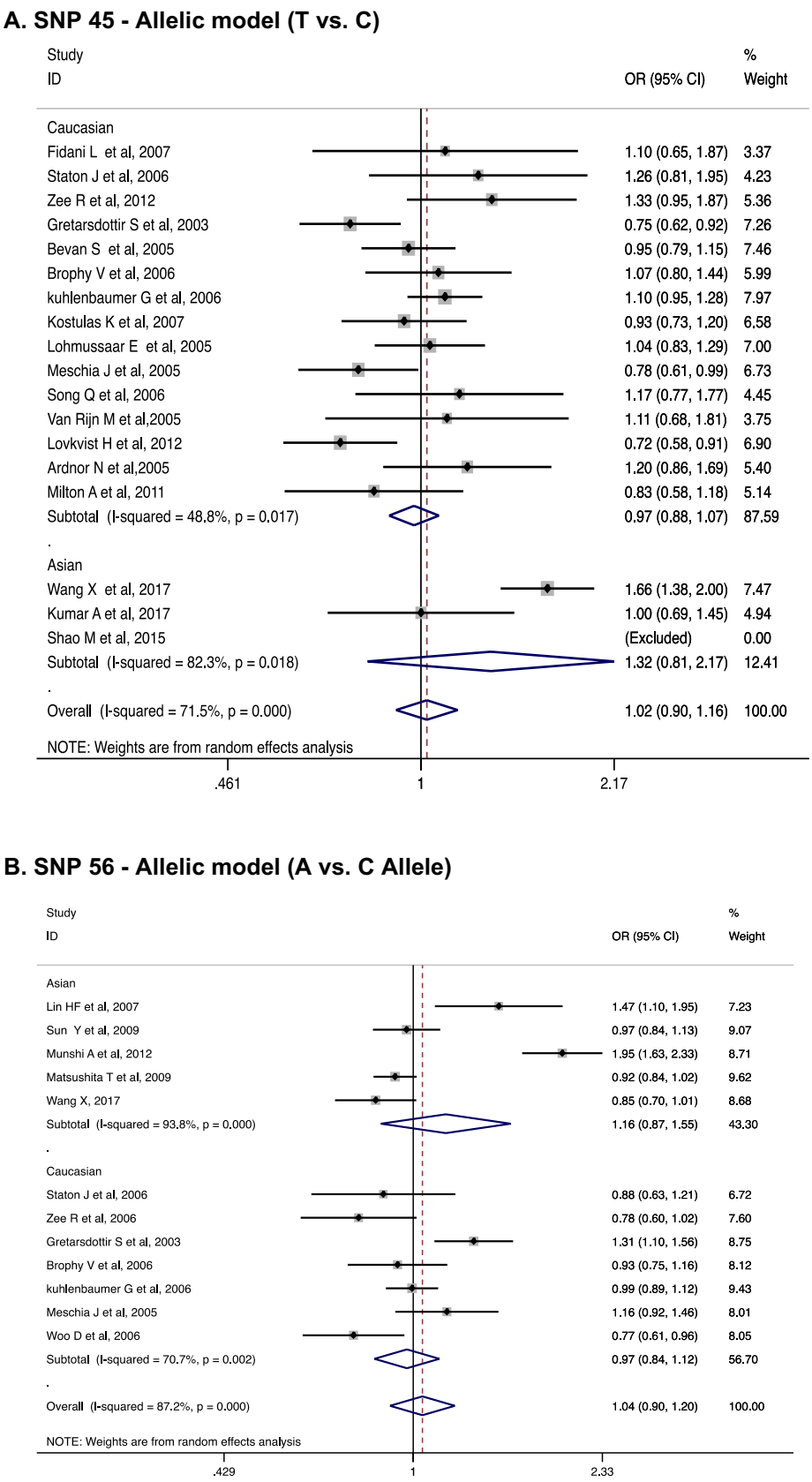
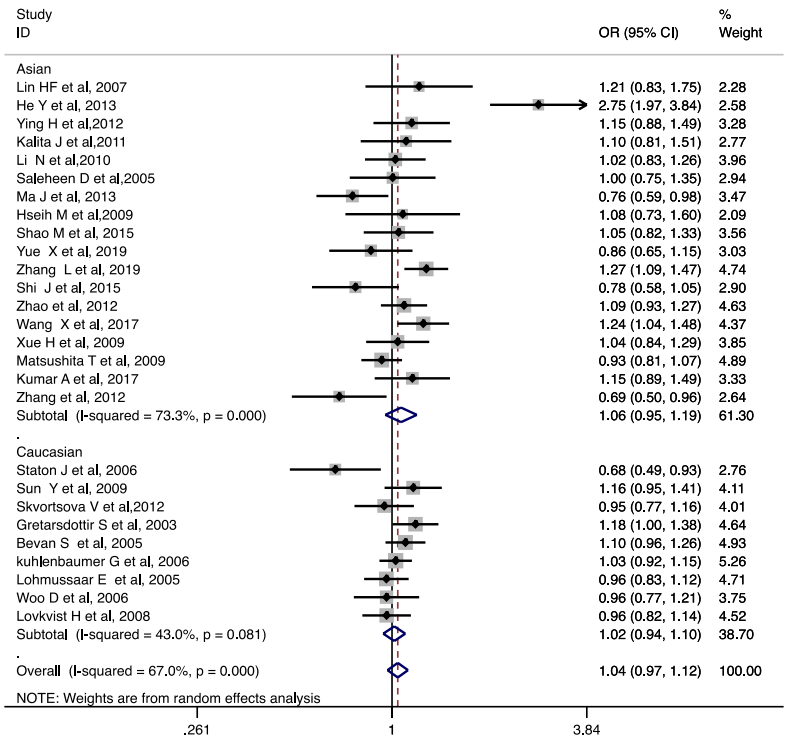


Fig. 4 **A**–**H**: Forest plot for the relationship between PDE4D SNP45, SNP56, SNP87, SNP83, SNP89, SNP26, SNP41, and SNP32 Polymorphism with the Risk of Ischemic Stroke using the Allelic Model

Fig. 4 (continued)

C. SNP 87 - Allelic model (T vs. C Allele)



D. SNP 83 - Allelic model (C vs. T Allele)

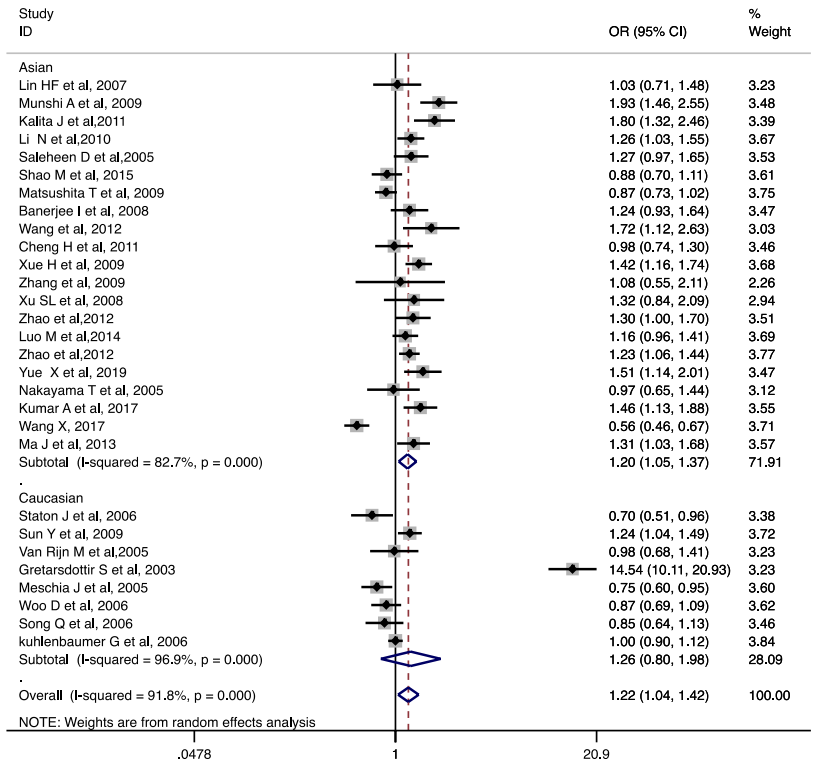
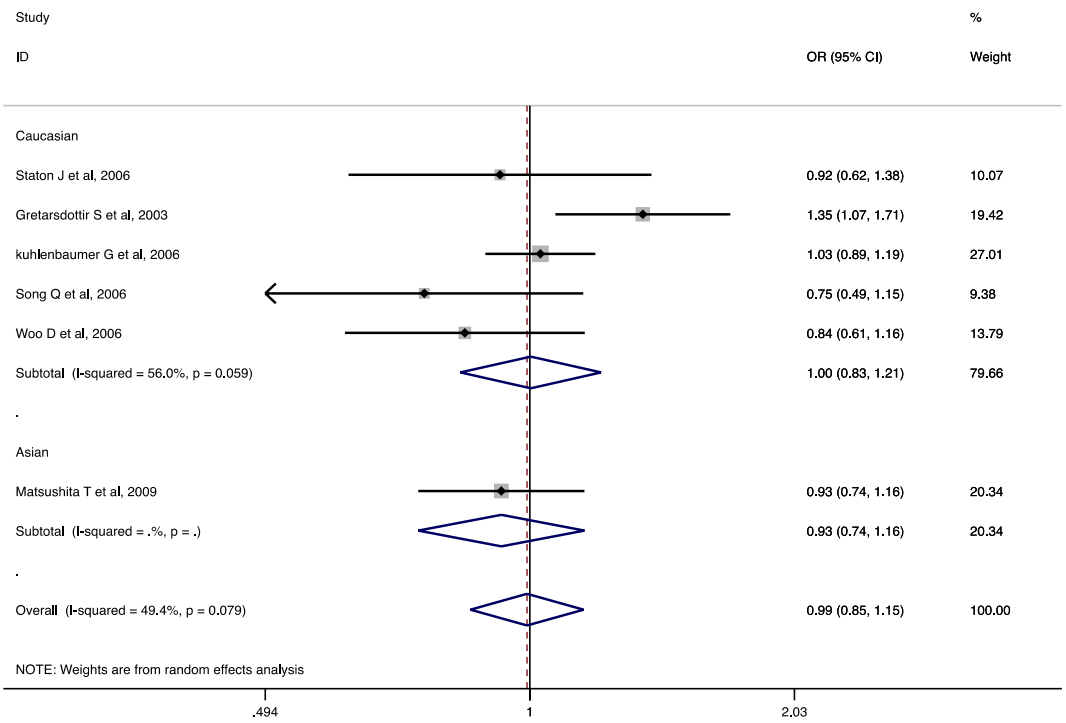


Fig. 4 (continued)

E. SNP 89 - Allelic model (T vs. C Allele)



F. SNP 26 - Allelic model (T vs. C Allele)

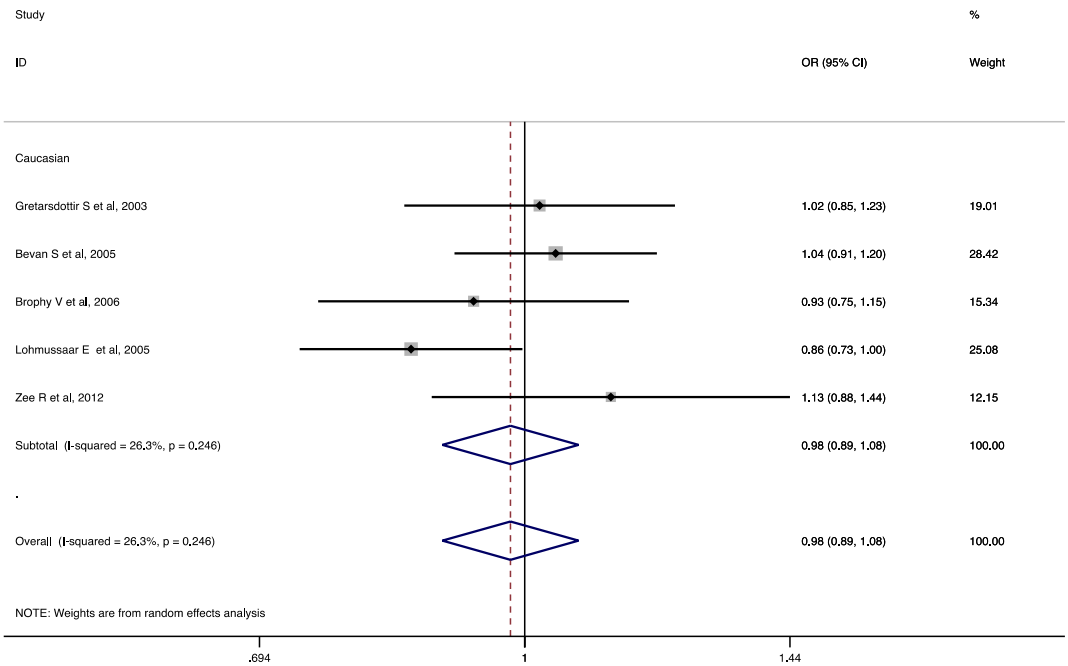
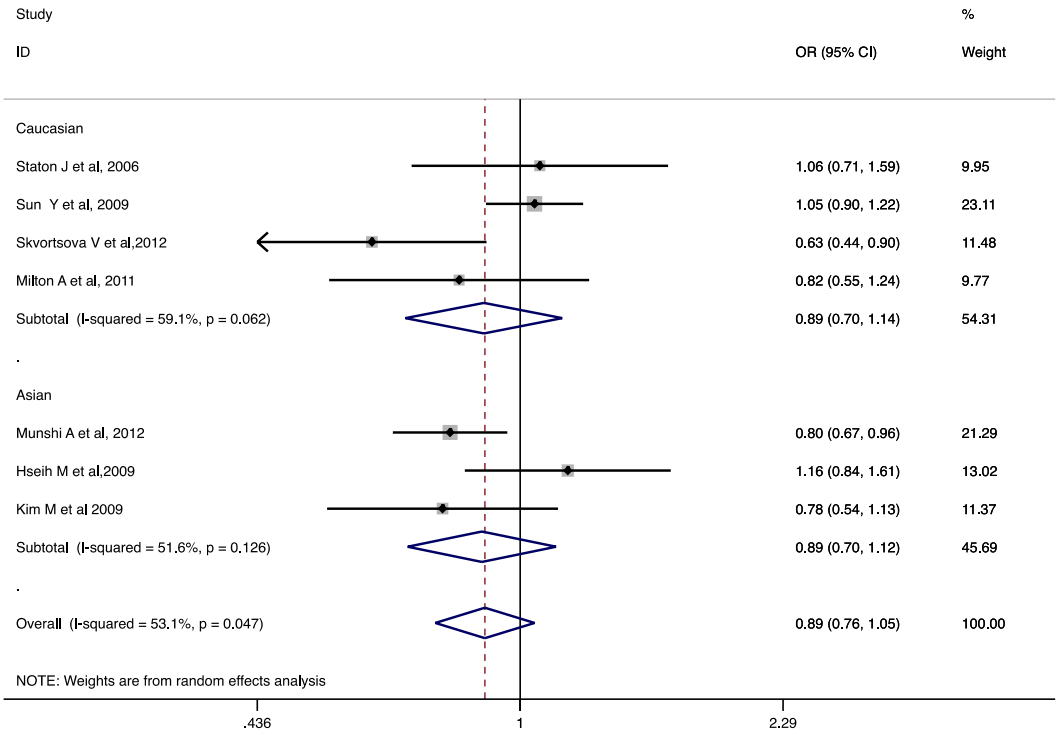


Fig. 4 (continued)

G. SNP 41 - Allelic model (T vs. C Allele)



H. SNP 32 - Allelic model (T vs. C Allele)

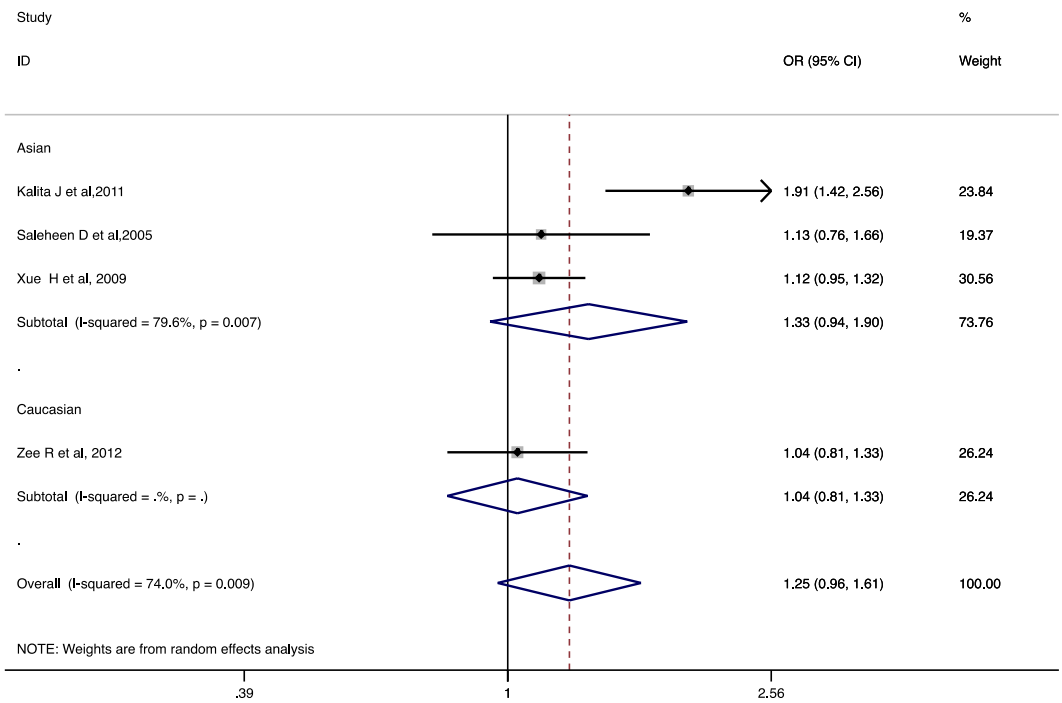


Table 2 Meta-analyses of overall population and subgroup analyses using different genetic models

PDE4D genotype	Sample size (Cases/ Control)	Dominant model			Recessive model			Allelic model		
SNP45		OR (95% CI)	I^2	p value	OR (95% CI)	I^2	p value	OR (95% CI)	I^2	p value
Overall	7651/8661	1.00 (0.95–1.04)	0.0%	1.000	1.11 (0.83–1.47)	47.7%	0.015	1.02 (0.90–1.16)	71.5%	0.000
Asian	1319/1330	0.98 (0.87–1.09)	0.0%	0.917	**2.06 (1.31–3.23)**	0.0%	0.810	1.32 (0.81–2.17)	82.3%	0.018
Caucasian	6332/7331	1.00 (0.95–1.05)	0.0%	1.000	0.98 (0.75–1.29)	33.1%	0.103	0.97 (0.88–1.07)	48.8%	0.017
SNP 56										
Overall	6064/9612	1.01 (0.96–1.07)	2.1%	0.424	1.04 (0.91–1.18)	68.2%	0.0	1.04 (0.90–1.20)	87.2%	0.0
Asian	2415/5928	1.06 (0.94–1.20)	58.0%	0.049	1.17 (0.87–1.57)	85.3%	0.0	1.16 (0.87–1.55)	93.8%	0.0
Caucasian	3649/3684	0.99 (0.93–1.07)	0.0%	0.981	1.00 (0.90–1.11)	19.9%	0.278	0.97 (0.84–1.12)	70.7%	0.002
SNP 83		OR (95% CI)	I^2	p value	OR (95% CI)	I^2	p value	OR (95% CI)	I^2	p value
Overall	10,639/14234	0.98 (0.94–1.03)	11.5%	0.289	1.11 (0.97–1.27)	64.0%	0.000	**1.22 (1.04–1.42)**	91.8%	0.000
Asian	7121/ 10,433	0.97 (0.91–1.03)	31.6%	0.083	1.19 (0.97–1.45)	70.7%	0.000	**1.20 (1.05–1.37)**	82.7%	0.000
Caucasian	3518/3801	1.01 (0.94–1.08)	0.0%	0.971	0.98 (0.89–1.09)	0.0%	0.623	1.26 (0.80–1.98)	96.9%	0.000
SNP 26		OR (95% CI)	I^2	p value	OR (95% CI)	I^2	p value	OR (95% CI)	I^2	p value
Overall	2390/2849	0.99 (0.91–1.08)	0.0%	0.999	0.99 (0.89–1.11)	0.0%	0.453	0.98 (0.89–1.08)	26.3	0.246
Asian	0/0	0	0	0	0	0	0	0	0	0
Caucasian	2390/2849	0.99 (0.91–1.08)	0.0%	0.999	0.99 (0.89–1.11)	0.0%	0.453	0.98 (0.89–1.08)	26.3	0.246
SNP 89		OR (95% CI)	I^2	p value	OR (95% CI)	I^2	p value	OR (95% CI)	I^2	p value
Overall	3162/6325	1.06 (0.88–1.28)	85.9%	0	1.02 (0.85–1.24)	84.0%	0.0	0.99 (0.85–1.15)	49.4%	0.079
Asian	739/3729	**1.43 (1.29–1.59)**	0	0	**1.42 (1.28–1.58)**	0	0	0.93 (0.74–1.16)	0	0
Caucasian	2423/2596	0.97 (0.90–1.05)	0.0%	0.819	0.97 (0.88–1.05)	0.0%	0.929	1.00 (0.83–1.21)	56.0%	0.059
SNP 32		OR (95% CI)	I^2	p value	OR (95% CI)	I^2	*p* value	OR (95% CI)	I^2	p value
Overall	1243/1584	0.89 (0.71–1.12)	69.3%	0.021	1.08 (0.82–1.44)	58.8%	0.064	1.25 (0.96–1.61)	74.0	0.009
Asian	984/1325	0.87 (0.65–1.17)	74.8%	0.019	1.11 (0.73–1.68)	72.0%	0.028	1.33 (0.94–1.90)	79.6%	0.007
Caucasian	259/259	0.98 (0.76–1.26)	0	0	1.08 (0.78–1.50)	0	0	1.04 (0.81–1.33)	0	0
SNP 41		OR (95% CI)	I^2	p value	OR (95% CI)	I^2	p value	OR (95% CI)	I^2	p value
Overall	2378/2382	1.08 (0.98–1.19)	0.0%	0.426	0.91 (0.81–1.02)	0.0%	0.640	0.89 (0.76–1.05)	53.1%	0.047
Asian	821/886	1.15 (0.98–1.35)	0.0%	0.958	**0.80 (0.66–0.97)**	0.0%	0.907	0.89 (0.70–1.12)	51.6%	0.126
Caucasian	1557/1496	1.07 (0.89–1.30)	36.9%	0.191	0.98 (0.84–1.13)	0.0%	0.683	0.89 (0.70–1.14)	59.1%	0.062
SNP 87		OR (95% CI)	I^2	p value	OR (95% CI)	I^2	p value	OR (95% CI)	I^2	p value
Overall	12,651/ 16,133	0.98 (0.95–1.02)	0.0%	1.000	0.99(0.94–1.03)	0.0%	0.747	1.04(0.97–1.12)	67.0%	0.000
Asian	7016/10,478	0.98 (0.93–1.02)	0.0%	1.000	0.99 (0.94–1.05)	0.0%	0.506	1.06 (0.95–1.19)	73.3%	0.000
Caucasian	5635/5655	0.99 (0.94–1.05)	0.0%	0.991	0.98 (0.90–1.06)	0.0%	0.802	1.02 (0.94–1.10)	43%	0.081

OR Odds ratio, *CI* Confidence Interval

Bold values of OR represent statistically significant results (p value < 0.05)

95% CI 0.65–1.17), recessive model (OR = 1.11, 95% CI 0.73–1.68) and allelic model (OR-1.33, 95% CI 0.94–1.90) respectively [Figs. 2H, 3H, 4H] [Table 2]. Also in the Caucasian population, no significant association was observed under dominant model (OR = 0.98, 95% CI 0.76–1.26), recessive model (OR = 1.08, 95% CI 0.78–1.50) and allelic model (OR = 1.04, 95% CI 0.81–1.33) respectively [Figs. 2H, 3H, 4H] [Table 2].

Relationship between PDE4D SNP 41 gene polymorphism and ischemic stroke risk

The pooled effect among all studies estimates that there were no significant association between PDE4D SNP41 gene polymorphism and risk of IS, under overall dominant model (OR = 1.08, 95% CI 0.98–1.19), recessive model (OR = 0.91, 95% CI = 0.81–1.02), and allelic model (OR = 0.89, 95% CI 0.76–1.05) respectively. Upon conducting subgroup analysis on the basis of ethnicity of the study population, no significant association was observed in the Asian population under dominant (OR = 1.15, 95% CI 0.98–1.35) and allelic models (OR = 0.89, 95% CI 0.70–1.12) respectively. However, a protective association was observed under the recessive model (OR = 0.80, 95% CI 0.66–0.97) [Figs. 2G, 3G, 4G] [Table 2]. Also in Caucasian population no significant association was observed under dominant model (OR = 1.07, 95% CI 0.89–1.30), recessive model (OR = 0.98, 95% CI 0.84–1.13) and allelic model (OR = 0.89, 95% CI 0.70–1.14) respectively [Figs. 2G, 3G, 4G] [Table 2].

Relationship between PDE4D SNP 87 gene polymorphism and ischemic stroke risk

We conducted a meta-analysis for 27 eligible studies. In the overall population, no significant association was observed between SNP87 and IS under dominant model (OR = 0.98, 95% CI 0.95–1.02), recessive model (OR = 0.99, 95% CI 0.94–1.03), and allelic model (OR = 1.04, 95% CI 0.97–1.12) respectively. Eighteen independent analyses of Asian and nine independent analyses of Caucasian populations were conducted. Subgroup analysis on the basis of ethnicity of study population observed no significant association in the Asian population under dominant model (OR = 0.98, 95% CI 0.93–1.02), recessive model (OR = 0.99, 95% CI 0.94–1.05) and allelic model (OR—1.06, 95% CI 0.95–1.19) respectively [Figs. 2C, 3C, 4C] [Table 2]. Also in the Caucasian population, no significant association was observed under dominant model (OR = 0.99, 95% CI 0.94–1.05), recessive model (OR = 0.98, 95% CI 0.90–1.06) and allelic model (OR = 1.02, 95% CI 0.94–1.10) respectively [Figs. 2C, 3C, 4C] [Table 2].

Publication bias

For SNPs 45, 32, 41, 26, 56, 83, 87, 89, publication bias arising from the literature was qualitatively estimated by funnel plots and quantitatively examined by Begg's and Egger's test. It was observed that all the plots were roughly symmetrical, indicating no publication bias was present as shown in the supplementary file as figures: S1-A, S2-A, S3-A, S4-A, S5-A, S6-A, S7-A and S8-A. In addition, Begg's and Egger's test did not have any significant p values for the different effect sizes (p value > 0.05).

Sensitivity analyses

Moreover, sensitivity analyses were carried out to assess the influence of each of the SNPs (SNP 45, 32, 41, 26, 56, 83, 87, 89) on the overall study by sequential omission of each eligible study. By removing individual studies, no statistical variation of pooled OR was seen. Our result indicated that no study influenced the quality of the pooled ORs and the current meta-analysis was reliable and robust [Figures: S1-B, S2-B, S3-B, S4-B, S5-B, S6-B, and S7-B].

Meta-regression analysis

Meta-regression analysis based on the quality score for the relationship between PDE4D SNP45 gene polymorphism and the risk of IS did not confirm any deviation from the findings of the meta-analysis (p = 0.86) [Figures: S1-C, S2-C, S3-C, S4-C, S5-C, S6-C, S7-C, and S8-B].

Discussion

The current systematic review and meta-analysis involving 47 studies consisted of 20,644 IS cases and 23,201 controls. The association between eight PDE4D SNPs (45, 32, 41, 26, 56, 83, 87, 89) and the risk of IS was analyzed. For SNP83, we included 29 eligible studies, comprising 10,639 cases and 14,234 controls. SNP83 was associated with a statistically increased risk of IS in overall analysis (OR = 1.22, 95% CI 1.04–1.42) under the allelic model. Moreover, we found a significant association in the subgroup analysis of the Asian population in the allelic model of the polymorphism (OR—1.20, 95% CI 1.05–1.37). In a previously published meta-analysis by Wang et al., 2017 [61], a total of 26 studies were included and a significant association was observed between the SNP83 genetic polymorphism and risk of IS in the Asian population under dominant (OR = 1.19, 95% CI 1.02–1.38) and allelic (OR = 1.25, 95% CI 1.06–1.48) models. Moreover, Yan et al. [62] observed a significant association in the overall population under dominant (OR = 1.15, 95% CI 1.02–1.30), recessive (OR = 1.21, 95% CI 1.02–1.42),

and allelic (OR = 1.19, 95% CI 1.06–1.33) models. On subgroup analysis, a significant association was found in the Asian population under dominant (OR = 1.20, 95% CI 1.13–1.44), recessive (OR = 1.48, 95% CI 1.22–1.79), and allelic (OR = 1.35, 95% CI 1.16–1.57) models, respectively. There was no significant association of SNP83 of PDE4D in the Asian subgroup analysis in our study with the risk of IS under the dominant and recessive genetic models. In the studies by Wu et al. [63] and Xue et al. [64], a significant association was seen in the overall analysis (OR = 1.45, 95% CI 1.19–1.76 and OR = 1.42, 95% CI 1.44–1.77, respectively). However, Yoon et al. [65] only reported the significance of SNP83 association with the risk of IS in the Asian subgroup populations under all three genetic models. Our study did not find any overall significance of SNP83 with the risk of IS. Moreover, the Caucasian populations did not have any significant association of SNP83 in PDE4D and IS risk.

For SNP45, we observed that the PDE4D gene was not significantly associated with IS in the overall analysis, which is in accordance with the findings of Yoon et al. [65]. However, on conducting subgroup analysis stratified by ethnicity, a significant association between SNP45 and risk of IS was found in the Asian population, only under the recessive model (OR = 2.06, 95% CI 1.31–3.23). A meta-analysis by Zhang et al. [66] involving 8731 cases and 10,756 controls showed no significant association of SNP56 in PDE4D with the risk of IS, which is consistent with the findings of our study. Yoon et al. [65] also observed no significant association in their meta-analysis. The analysis of SNP56 consisting of 13 published studies with 6064 cases and 9612 controls, failed to find an association between SNP56 gene polymorphism and risk of IS. The inclusion of SNP87 with 27 studies comprised of 12,651 cases and 16,133 controls also demonstrated that there was no relation between SNP87 and IS. Liang et al. [67] did not find any significance for SNP87 polymorphism in PDE4D and the risk of IS after incorporating data from 18 studies with 8363 cases and 12,223 controls. Moreover, Xu et al. [64] also reported no observable associations between SNP87 PDE4D polymorphism and risk of IS. The present meta-analysis on SNP87 also did not show any association with increased risk of IS in the overall population and the ethnicity subgroups. For SNPs 26 and 89, no correlation was observed with increased or decreased risk of IS among Asian or European populations [65]. We also did not observe any significant association of SNP26 under any of the genetic models of PDE4D with IS risk for Asian and Caucasian ethnicities. However, SNP89 of PDE4D has a significant association with the risk of IS in the Asian population under dominant and recessive models in our meta-analysis, providing a correlation of SNP89 in patients of Asian descent. We also reported the analysis of two additional SNPs, 32 and 41, which were not included in previous meta-analyses. Although SNP 32 was not associated with any significant risk of IS, SNP41 of PDE4D showed significant protective association with IS in the Asian subgroup under the recessive model genetic correlation. Although, the sample sizes for these two SNPs were relatively lower, further studies are warranted to establish their association with IS pathophysiology. The detailed effect sizes of the different meta-analyses previously published on different SNPs of PDE4D have been depicted in Table 3.

We included eight SNPs in our meta-analysis which accounts for the largest meta-analysis on PDE4D done to date for analyzing the association of the risk of IS occurrence. Unlike previous studies, we did not find any significant association between the eight SNPs of PDE4D with the risk of IS in the overall population and the Caucasian subgroup. The significance of risk association of PDE4D polymorphism with IS was observed in the Asian population under specific genetic models in SNPs 45, 83, and 89. Moreover, SNP45 showed a protective association against IS in the Asian population. Polymorphism in PDE4D is diverse, and its relation to the associated risk of cerebral ischemic stroke has been quite intriguing. Despite the heterogeneity in sample size, the overall effect size of the studies showed a significant association in the results without any deviation. The risk conferred by the SNPs of the gene is non-significant in the overall and the Caucasian population. Although the Asian population is predisposed towards IS risk associated with specific polymorphisms of PDE4D, several elements need to be conferred upon, which could have added possible confounding effects to the resultant effect size. Interest in PDE4D stems from its significant involvement in the pathophysiology of IS governing inflammation and structural damage to the neural architecture. SNPs 39 and 45 are found to be involved in small vessel infarction in an isolated Caucasian population group, suggesting the allelic significance of PDE4D polymorphisms in IS physiology [21]. Moreover, targeted PDE4D inhibitors have also depicted favorable outcomes through neuroplastic improvements and anti-inflammatory responses in stroke recovering patients [68].

Despite the significance of a large-scale meta-analysis, our study had certain limitations to be considered for interpreting the results. First, there was significant heterogeneity in the effect size of the outcomes. The studies had variable sample sizes, which affected the individual strength of the studies on overall results. Since most of the studies were of case–control design, the sample sizes were usually < 1000. However, sensitivity analyses and meta-regression did not depict any significant deviation from the observed effect size, validating our results. Therefore, large-scale prospective cohort-based and case–control studies should be conducted to validate these polymorphisms. Second, most of the included studies were from the Asian population, and the observed overall effect size could have been affected by

Table 3 Summary and results of the meta-analysis under three genetic models of published studies that examined the association between each SNP and ischemic stroke

S. No	References	Country	Total no. of studies (Asian/Caucasian)	Sample size Cases/ Control	Model	Over All	Asian	Caucasian
SNP87								
1	Yoon et al. [65]	Korea	11 (5/6)	8614/15857	Dominant	0.992 (0.91–1.07)	0.98 (0.88–1.11)	NA
					Recessive	1.02 (0.93–1.11)	1.12 (0.92–1.35)	NA
					Codominant	0.98/1.02 (0.90–1.06)/ (0.90–1.15)	0.96/1.18 (0.85–1.08)/ (0.89–1.57)	NA
2	Liang et al. [67]	China	18 (11/7)	8363/12223	Dominant	1.01 (0.96–1.06)	NA	NA
					Recessive	1.05 (0.98–1.13)	NA	NA
					Allelic	1.03 (0.97–1.09)	NA	NA
3	Xu et al. [64]	China	7 (7/0)	4856/ 9300		1.22 (1.03–1.43)	NA	NA
SNP83								
4	Yoon et al. [65]	Korea	14 (8/6)	5050/9197	Dominant	0.85 (0.68–1.07)	0.66 (0.50–0.87)	0.95 (0.77–1.16)
5	Wang et al. [61]	China	26 (19/7)	9832/12916	Recessive	0.92 (0.78–1.06)	0.79 (0.69–0.90)	0.97 (0.87–1.09)
					Codominant	0.87/0.86 (0.71–1.06)/ (0.65–1.13)	0.72/0.64 (0.53–0.99)/ (0.48–0.85)	0.95/0.98 (0.77–1.16)/ (0.78–1.21)
					Dominant	1.09 (0.96–1.24)	1.19 (1.02–1.38)	0.87 (0.09–1.11)
6	Xu et al. [64]	China	6 (6/0)	2220/4380	Recessive	1.10 (0.91–1.34)	1.20(0.90–1.61)	0.95 (0.84–1.07)
					Allelic	1.12 (0.99–1.27)	1.25 (1.06–1.48)	0.92 (0.80–1.05)
						1.42 (1.14–1.77)	NA	NA
7	Wu et al. [63]	China	9 (0/9)	2751/3233		1.45 (1.19–1.76)	NA	NA
8	Yan et al. [62]	China	25 (16/9)	8878/12306	Dominant	1.15 (1.02–1.30)	1.20 (1.13–1.44)	0.87 (0.69–1.11)
					Recessive	1.21 (1.02–1.42)	1.48 (1.22–1.79)	0.95 (0.84–1.07)
					Allelic	1.19 (1.06–1.33)	1.35 (1.16–1.57)	0.95 (0.84–1.08)
SNP56								
9	Yoon et al. [65]	Korea	9(7/2)	7703/ 11,904	Dominant	1.05 (0.95–1.17)	1.11 (0.96–1.27)	NA
10	Zhang et al. [66]	China	15 (6/9)	8731/10,576	Recessive	1.08 (0.89–1.32)	1.19 (1.02–1.39)	NA
					Codominant	1.03/1.13 (0.94–1.12)/ (0.91–1.41)	1.03/1.29 (0.93–1.14)/ (1.03–1.61)	NA
					Dominant	0.99 (0.79–1.24)	1.09 (0.75–1.57)	0.91 (0.72–1.16)
SNP26								
11	Yoon et al. [65]	China	5 (5/0)	2390/2849	Dominant	NA	1.01 (0.85–1.18)	NA
					Recessive	NA	1.05 (0.90–1.21)	NA
					Codominant	NA	0.99/1.05 (0.85–1.17)/ (0.88–1.24)	NA
SNP45								

Table 3 (continued)

S. No	References	Country	Total no. of studies (Asian/Caucasian)	Sample size Cases/Control	Model	Over All	Asian	Caucasian
12	Yoon et al. [65]	China	14 (11/3)	6145/7030	Dominant	0.96 (0.87–1.06)	1.05 (0.96–1.15)	NA
					Recessive	1.01 (0.75–1.36)	1.28 (0.98–1.67)	NA
					Codominant	0.96/1.01 (0.88–1.04)/(0.74–1.39)	1.03/1.29 (0.94–1.13)/(0.99–1.68)	NA
SNP89								
13	Yoon et al. [65]	China	5 (1/4)	2918/6047	Dominant	1.14 (0.95–1.37)	1.05 (0.92–1.19)	NA
					Recessive	0.82 (0.43–1.55)	1.13 (0.75–1.69)	NA
					Codominant	1.29/0.89 (0.95–1.75)/(0.53–1.50)	1.04/1.12 (0.91–1.20)/(0.75–1.69)	NA

their association. However, the number of Caucasian studies included in the meta-analysis was the highest to date, and the effect size observed provides significant credibility. Third, the effect of confounders such as age, sex, and IS subtypes was not analyzed in the study, which could have impacted the desired outcome and overall heterogeneity. Lastly, unpublished or ongoing studies were not incorporated in the analysis, possibly introducing selection bias in the observed effect size.

Conclusion

The association between PDE4D gene polymorphism and the risk of IS is debatable. The present meta-analysis showed some possible associations of PDE4D with the risk of IS in Asian populations while other studies did not. Further large-scale genome-wide association studies are warranted to ascertain the association of PDE4D gene polymorphism and IS risk.

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Data availability All data used in this study are included in the article and its supplementary materials.

Declarations

Conflict of interest No potential conflict of interest.

Ethical approval Ethical approval was not required for this manuscript as it was a systematic review and meta-analysis done by using existing published data and tusing existing published data. In addition, the research was not directly conducteddid not involve in any human subjects.

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