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Original Article

Association between phosphodiesterase 4D (*PDE4D*) SNP 87 and ischemic stroke: a meta-analysis

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Abstract: Background and purpose: Data on the association between *PDE4D* SNP 87 and the risk of ischemic stroke are contentious and debatable. The present meta-analysis was undertaken to systematically summarize the possible association. Methods: Based on comprehensive search of PubMed, Embase, and CNKI databases, we identified 18 eligible articles examining the relationship between *PDE4D* SNP 87 and ischemic stroke risk. We evaluated the strength of relationship using odds ratios (ORs) with 95% confidence intervals (CIs). Results: In the overall analysis, *PDE4D* SNP 87 was not found to have effects on the risk of ischemic stroke. The null association persisted in the subgroup analyses according to ethnicity and sample size. Conclusions: Our meta-analysis suggests that *PDE4D* SNP 87 may not represent an independent risk factor for ischemic stroke development.

Keywords: *PDE4D*, SNP, ischemic stroke, risk

Introduction

Stroke is a leading cause of death and disability with a high prevalence among old and very old people worldwide [1, 2]. There are two subtypes of stroke: hemorrhagic stroke and ischemic stroke [3]. Ischemic stroke characterized by a sudden decrease in blood flow to one or more central nervous system areas constitutes 80% of all strokes. Ischemic stroke is a highly complex disease composed of various heterogeneous disorders attributable to both genetic and environmental factors [4, 5]. Experimental evidence demonstrates that genetics should be responsible for a large part of stroke risk [6]. However, the investigations fail to identify candidate genetic factors for the aggressive disease.

Several previous studies of an Icelandic population used a genome-wide approach highlighted the implication of single nucleotide polymorphisms (SNPs) and haplotypes in the 5-lipoxygenase-activating protein (ALOX5AP or FLAP) and phosphodiesterase 4D (*PDE4D*) genes in ischemic stroke [7, 8]. *PDE4D* located on chromosomal region 5q12 is a very large gene that spans 1.5 Mb and consists of 8 splice variants, 22 exons, and several hundred SNPs [9]. *PDE4D* belongs to the large superfamily of cyclic nucleotide phosphodiesterases and has been reported to be involved in inflammation [10], cell proliferation [11], and migration [12] that could consequently result in ischemic stroke. A growing body of replicated reports has sought to challenge the preconceived findings of the Icelandic study in diverse populations, showing conflicting results [13-17]. The relatively small studies using participants of different ethnic backgrounds and different analytical methods may be the major sources of the controversy.

Among the numerous SNPs (SNP 26, 45, 56, 83, 87, and 89) of *PDE4D* gene, SNP 87 (rs2910829) has been extensively investigated in ischemic stroke community [18-21]. However, the efforts did not confirm the susceptibility role of SNP 87 in ischemic stroke. In this study, we hypothetized that SNP 87 was associated with the onset of ischemic stroke. To test this

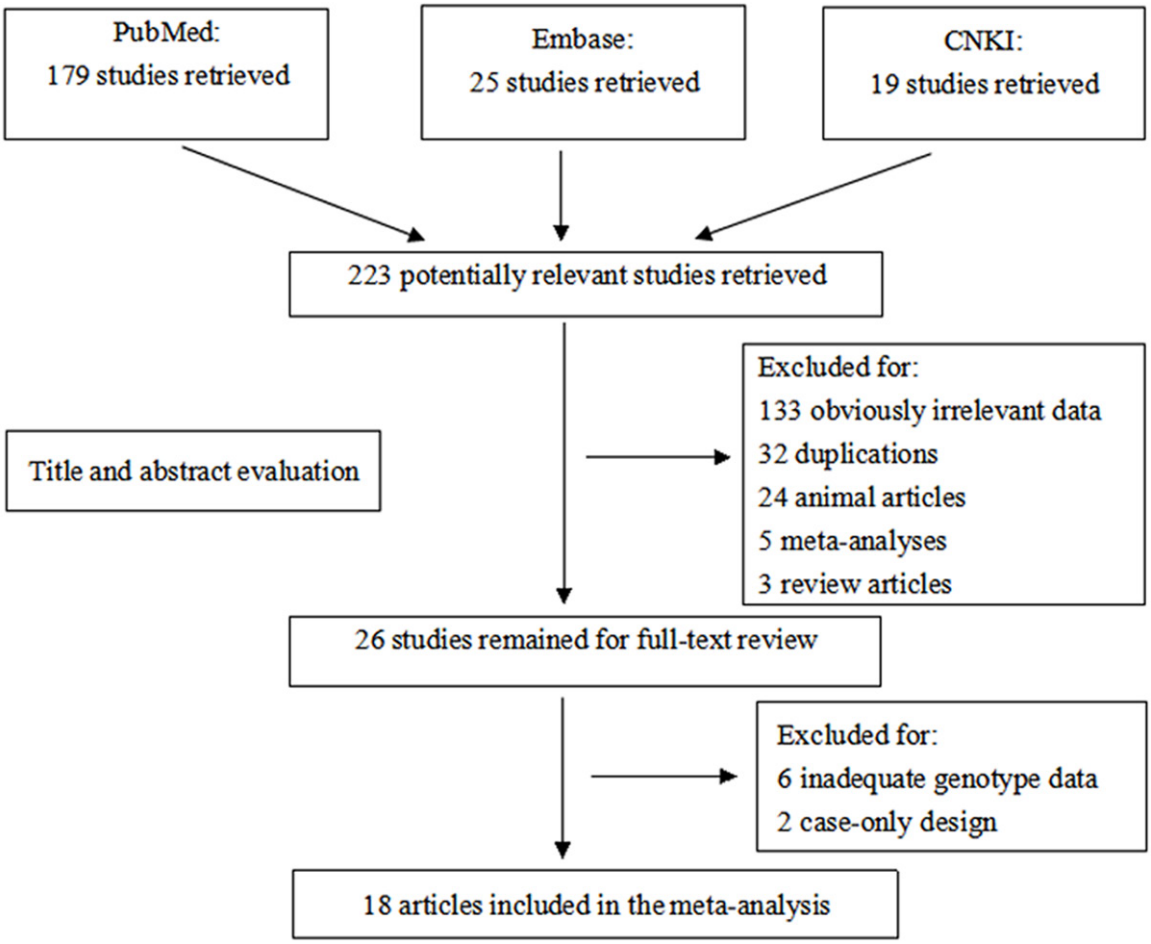

Figure 1. Flow chart for primary selection in this meta-analysis.

hypothesis, we performed a meta-analysis to re-evaluate the association between SNP 87 and ischemic stroke.

Materials and methods

Identification and eligibility of relevant studies

The genetic association studies concerning the association of SNP 87 and ischemic stroke risk published before March 2014 were identified by comprehensively searching PubMed, Embase and CNKI (China National Knowledge Infrastructure) databases. The following search terms were used: (polymorphism) OR (polymorphisms) AND (phosphodiesterase 4D) OR (*PDE4D*) OR (SNP 87) OR (rs2910829) AND (ischemic stroke). We reviewed the abstracts of the retrieved studies to examine their appropriateness for inclusion in the meta-analysis. Then, the full texts of the articles were screened in order to check their eligibility for the present study. Finally, all the reference lists of the eligible articles and the journals known to publish articles relevant to the current topic were systematically reviewed to identify additional published articles. The case-control studies provided the genotype distribution of SNP 87 in ischemic stroke risk were eligible for inclusion in the meta-analysis. For studies used the same series of cases, the latest or the largest study was considered. Review articles, comment letters, case reports were excluded from this meta-analysis.

Data extraction

Two reviewers independently gathered the data from each eligible study and reached a consensus on all items. The information required to be collected was: first author, journal, year of publication, study country, ethnicity, gender and mean age of the cases, sample sizes of the

Table 1. Main characteristics of all studies included in the meta-analysis

First author	Year	Population	Gender	Mean age	Genotyping method	Sample size		Cases			Controls			HWE
						Cases	Controls	CC	CT	TT	CC	CT	TT	
Gretarsdottir	2003	Caucasian	NR	NR	RT-PCR	642	583	148	315	179	156	290	137	0.921
Bevan	2005	Caucasian	F/M	65 ± 12.5	PCR	726	923	154	360	212	214	464	245	0.842
Lohmussaar	2005	Caucasian	F/M	65 ± 18.2	MALDI-TOF	598	728	128	296	174	146	366	216	0.688
Saleheen	2005	Asian	F/M	62.4 ± 12.4	PCR	170	203	76	57	37	86	78	39	0.007
Woo	2006	Caucasian	F/M	69	TaqMan	352	268	80	175	97	58	134	76	0.941
Kuhlenbaumer	2006	Caucasian	F/M	66.9 ± 14.6	RT-PCR	1014	1564	216	505	293	353	759	452	0.313
Staton	2006	Caucasian	F/M	67.3 ± 11.7	NR	151	164	45	72	34	36	72	56	0.164
Lin	2007	Asian	NR	NR	NR	180	210	120	52	8	149	54	7	0.447
Lovkvist	2008	Caucasian	F/M	73.6	RT-PCR	929	394	187	473	269	72	208	114	0.177
Xue	2009	Asian	F/M	60.8 ± 9.2	PCR-RFLP	424	887	12	119	293	26	257	604	0.832
Matsushita	2009	Asian	F/M	69.6	NR	1092	3847	826	248	18	2840	950	57	0.025
Sun	2009	Asian	F/M	73.2 ± 9.4	RT-PCR	646	761	439	182	25	539	202	20	0.837
Hsieh	2009	Asian	F/M	70 ± 11	DS	108	280	71	31	6	187	81	12	0.398
Li	2010	Asian	F/M	63.88 ± 7.36	PCR-RFLP	371	371	170	117	84	160	141	70	< 0.10
Kalita	2011	Asian	F/M	61	PCR	148	188	51	77	20	72	92	24	0.520
Zhang	2012	Asian	F/M	59.9	PCR	226	220	157	58	11	129	78	13	0.791
He	2012	Asian	F/M	61 ± 10	PCR-RFLP	400	400	276	108	16	286	103	11	0.640
He	2013	Asian	F/M	36.5 ± 6.4	PCR-RFLP	186	232	84	82	20	168	58	6	0.712

F: female; M: male; PCR: polymerase chain reaction; PCR-RFLP: PCR-restriction fragment length polymorphism; RT-PCR: real-time PCR; MALDI-TOF: matrix-assisted laser desorption/ionization time-of-flight; DS: direct sequencing; HWE: Hardy-Weinberg equilibrium.

Table 2. Meta-analysis results for *PDE4D* SNP 87 and ischemic stroke risk

Variables (studies)	TT vs. CC			TT + CT vs. CC			TT vs. CT + CC			T vs. C			CT vs. CC		
	OR (95% CI)	P_h	I^2	OR (95% CI)	P_h	I^2	OR (95% CI)	P_h	I^2	OR (95% CI)	P_h	I^2	OR (95% CI)	P_h	I^2
Total (18)	1.05 (0.97, 1.14)	0.292	13.5%	1.01 (0.96, 1.06)	0.293	13.5%	1.05 (0.98, 1.13)	0.417	3.2%	1.03 (0.97, 1.09)	0.015	47.1%	1.00 (0.95, 1.06)	0.425	2.5%
Ethnicity															
Caucasian (7)	1.02 (0.92, 1.12)	0.732	0.0%	1.01 (0.94, 1.07)	0.987	0.0%	1.02 (0.93, 1.12)	0.498	0.0%	1.01 (0.96, 1.06)	0.695	0.0%	1.01 (0.93, 1.09)	0.995	0.0%
Asian (11)	1.11 (0.97, 1.28)	0.125	34.2%	1.02 (0.94, 1.10)	0.045	46.3%	1.13 (0.99, 1.28)	0.366	8.2%	1.07 (0.95, 1.21)	0.002	64.0%	1.00 (0.91, 1.09)	0.080	40.3%
Sample size															
500-1000 (5)	1.06 (0.94, 1.20)	0.582	0.0%	1.02 (0.95, 1.10)	0.911	0.0%	1.08 (0.96, 1.21)	0.616	0.0%	1.03 (0.97, 1.10)	0.669	0.0%	1.02 (0.93, 1.11)	0.952	0.0%
< 500 (11)	1.05 (0.92, 1.20)	0.081	40.1%	1.02 (0.94, 1.11)	0.067	42.4%	1.05 (0.93, 1.19)	0.158	30.3%	1.05 (0.93, 1.19)	0.002	64.5%	1.01 (0.91, 1.13)	0.118	35.1%
> 1000 (2)	1.03 (0.87, 1.23)	0.847	0.0%	0.98 (0.89, 1.08)	0.369	0.0%	1.01 (0.86, 1.18)	0.708	0.0%	0.99 (0.91, 1.07)	0.393	0.0%	0.97 (0.88, 1.08)	0.322	0.0%

P_h: *p* value of heterogeneity test; I^2: heterogeneity (%); CI: confidence interval; OR, odds ratio.

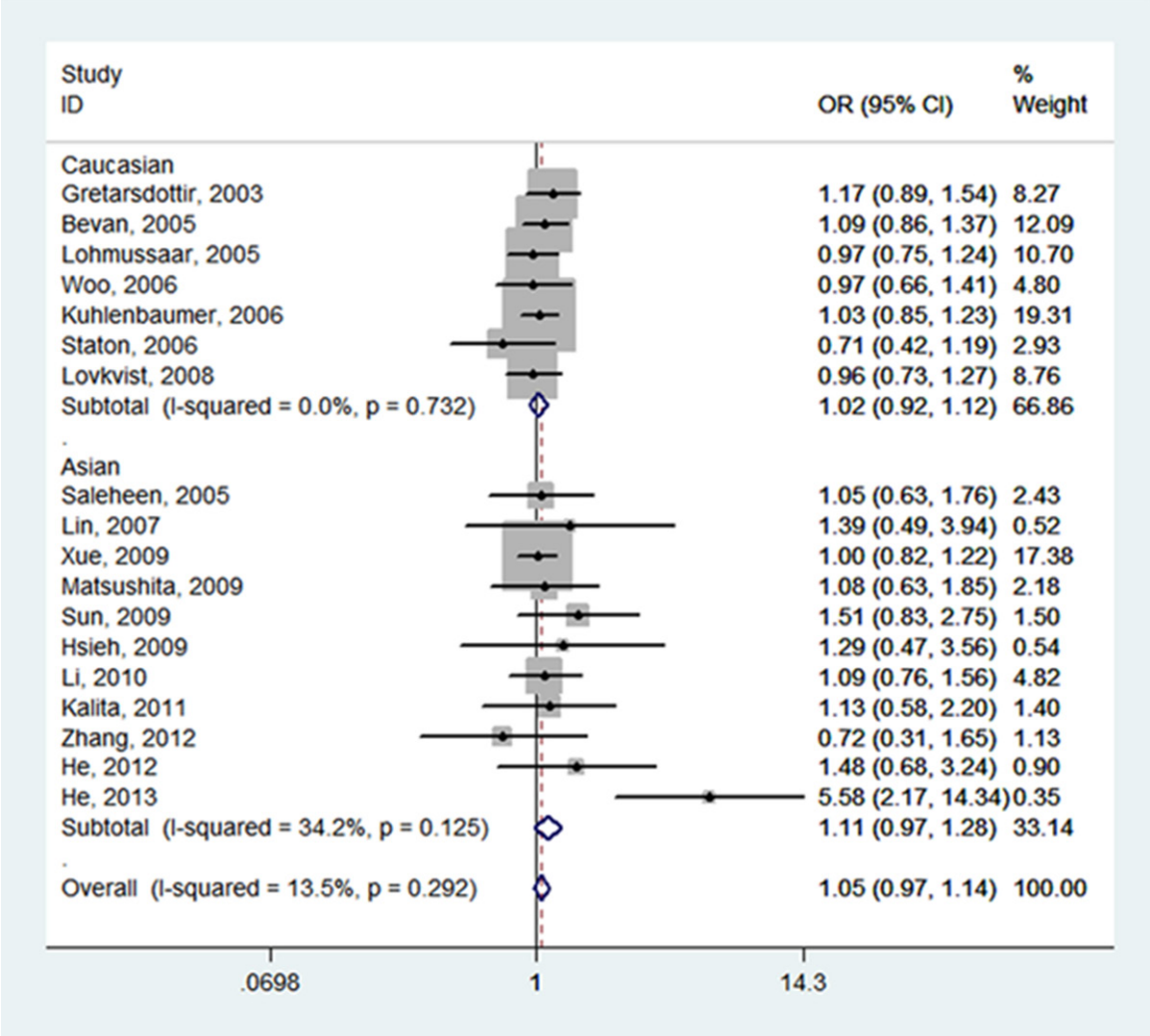

Figure 2. Forest plot of ischemic stroke risk associated with *PDE4D* SNP 87 stratified by ethnicity under TT vs. CC model. The boxes and horizontal lines represent the OR and the corresponding 95% CI. The area of the boxes indicates the weight (inverse of the variance). The diamond corresponds to the summary OR and 95% CI.

cases and controls, genotyping methods and genotype frequencies of SNP87.

Statistical analysis

STATA software (version 12.0) was used for all statistical analyses. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to evaluate the association of SNP 87 and ischemic stroke risk.

A chi-square-based Q test was used to measure between-study heterogeneity. Statistical significance was defined at P < 0.10. In addition, I^2 statistic was calculated to quantify the proportion of the total variation across studies due to heterogeneity (I^2 = 0%-25%: no heterogeneity; I^2 = 25-50%: moderate heterogeneity; I^2 = 50-75%: large heterogeneity; I^2 = 75-100%: extreme heterogeneity) [22]. The ORs were pooled using the fixed effects model (the Mantel-Haenszel method) [23] when the *P* value is above 0.10, and the random effects model (the DerSimonian and Laird method) [24] if P < 0.10. Subgroup analyses by ethnicity (Asian or Caucasian) and sample size (500-1000, < 500, > 1000) were performed to further identify heterogeneity.

Hardy-Weinberg equilibrium (HWE) of the control groups were checked by a Chi-square test. A *P*-value < 0.10 was considered significant. Sensitivity analysis was carried out to identify the study modifying the summary ORs. Begg's

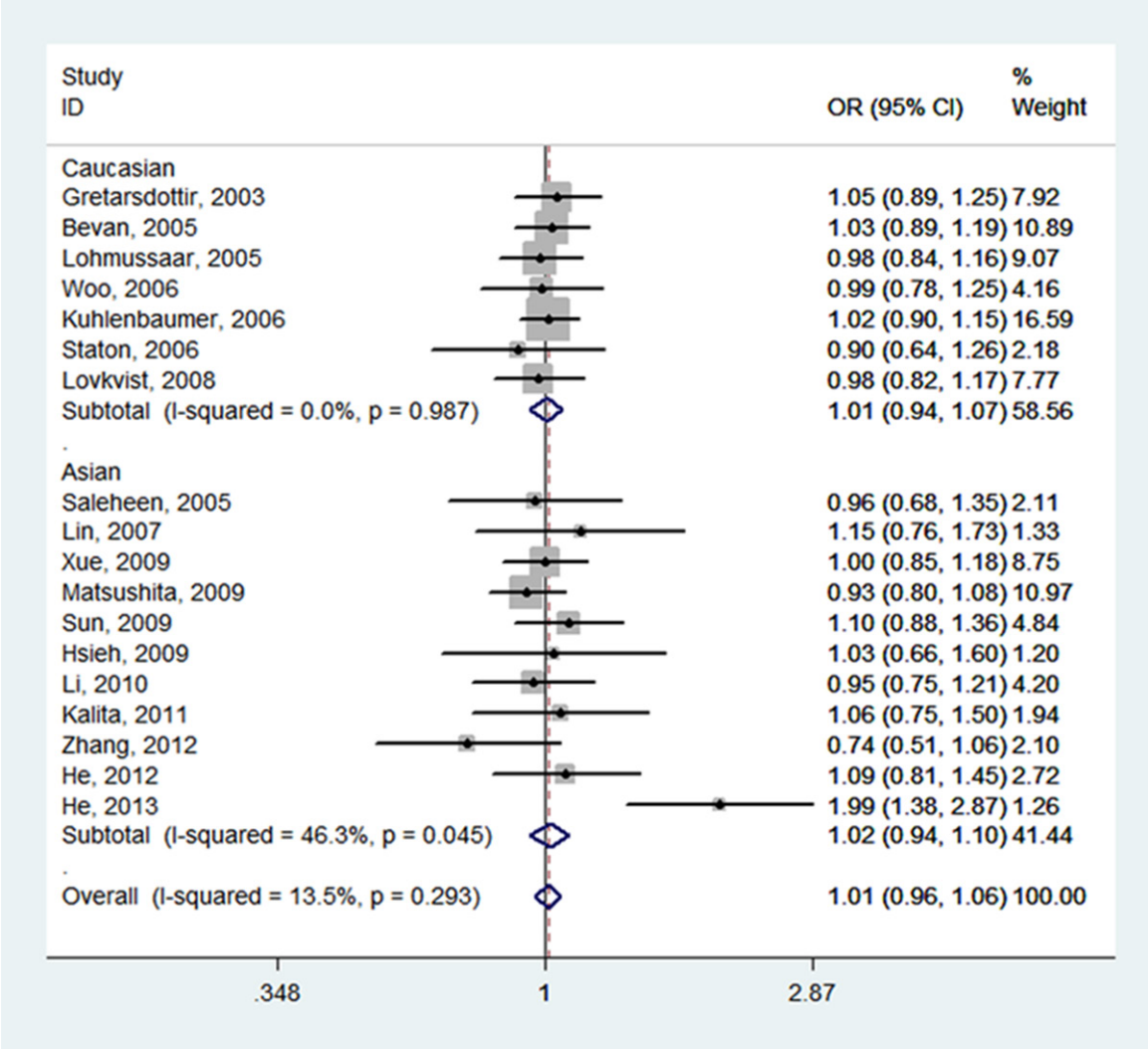

Figure 3. Forest plot of ischemic stroke risk associated with *PDE4D* SNP 87 stratified by ethnicity under TT + CT vs. CC model. The boxes and horizontal lines represent the OR and the corresponding 95% CI. The area of the boxes indicates the weight (inverse of the variance). The diamond corresponds to the summary OR and 95% CI.

funnel plots and Egger's test [25] were used to determine publication bias among the studies included in this meta-analysis. A two-sided *P* value < 0.10 was considered significant.

Results

Characteristics of studies

A flow diagram of the study selection process for the meta-analysis of SNP 87 and ischemic stroke is described in **Figure 1**. The original search provided 223 records. After eliminating duplications, 187 records remained. Of these, 161 were discarded after reviewing the abstracts. The full texts of the remaining 26 studies were examined in detail and 8 articles were further excluded according to the criteria for inclusion. Therefore, we identified 18 records [13-21, 26-34] with 8,363 cases and 12,223 control subjects for the final meta-analysis, including 7 records for Caucasian descent, and 11 records for Asian descent. All 18 articles were based on a case-control design. The main information and genotype frequencies for SNP 87 for ischemic stroke cases and controls in each of the studies included are summarized in **Table 1**.

Quantitative synthesis

Table 2 summarizes for each of the studies the *P* value for heterogeneity and ORs with 95% CIs for the association between SNP 87 and isch-

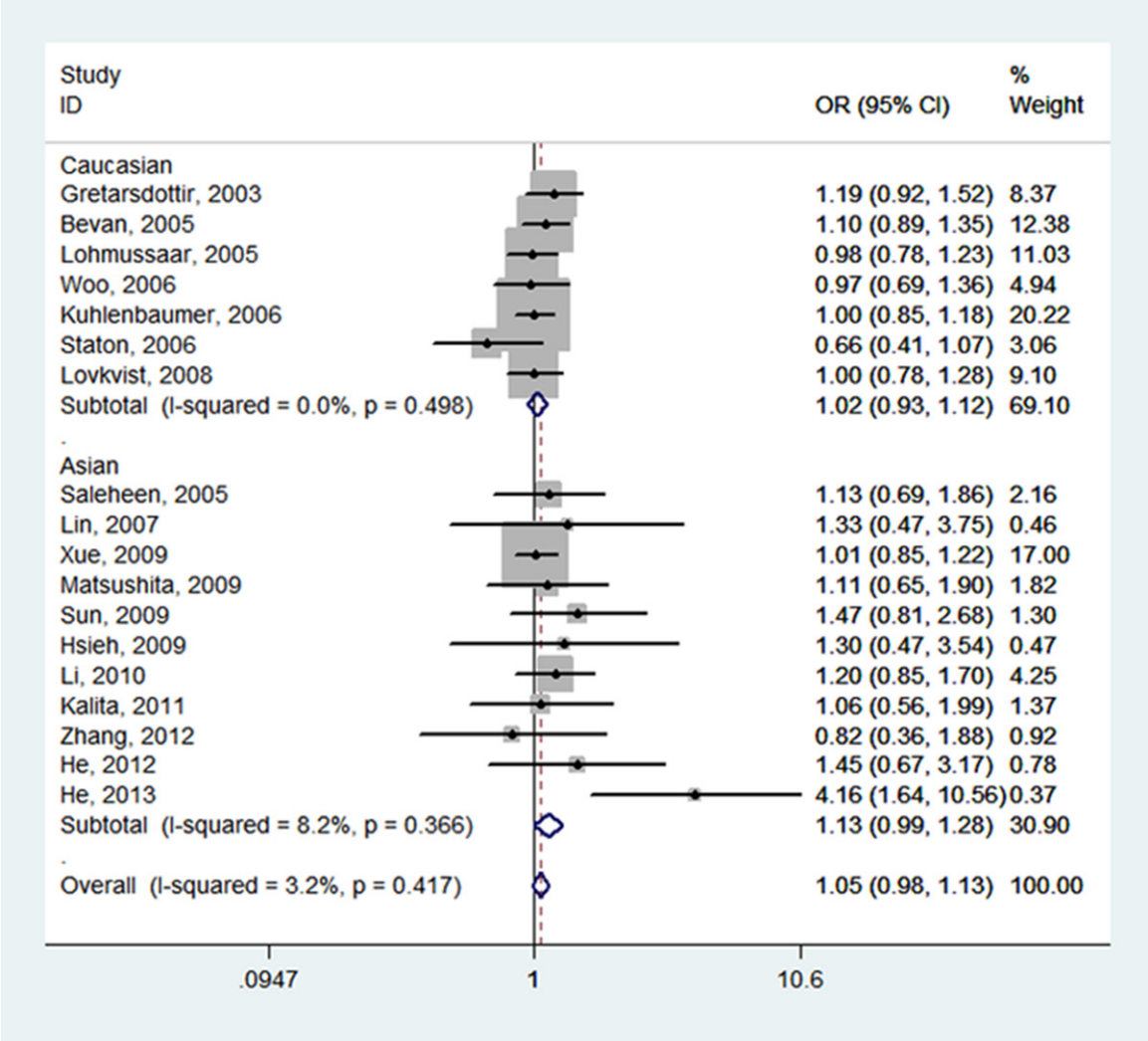

Figure 4. Forest plot of ischemic stroke risk associated with *PDE4D* SNP 87 stratified by ethnicity under TT vs. CT + CC model. The boxes and horizontal lines represent the OR and the corresponding 95% CI. The area of the boxes indicates the weight (inverse of the variance). The diamond corresponds to the summary OR and 95% CI.

emic stroke risk assuming the homozygote and heterozygote genotypes, the dominant, recessive and allele genetic models. The pooled effect estimates among all studies showed that none of the genetic models were significantly associated with an increased or decreased risk of ischemic stroke. However, a tend to increase ischemic stroke risk was indicated under all of the contrast models, and the tendency was more pronounced under the homozygote genotypes (TT vs.CC, OR, 1.05, 95% CI, 0.97-1.14, fixed-effects), the recessive model (TT vs. CT + CC, OR, 1.05, 95% CI, 0.98-1.13, fixed-effects), and the allele model (T vs. C, OR, 1.03, 95% CI, 0.97-1.09, random-effects). Meanwhile, neither the stratified analyses according to ethnicity nor according to sample size did we find the association of SNP 87 and ischemic stroke was significant (**Table 2**; **Figures 2**-**6**).

Test of heterogeneity and sensitivity analysis

No significant heterogeneity was detected under all genetic models except for the allele model (P = 0.015, I^2 = 47.1) (**Table 2**). Subgroup analyses and sensitivity analyses together identified the study by He et al. [34] was the source of the moderate heterogeneity. Excluding this study obviously diminished the heterogeneity and increased the homogeneity among the remaining studies (P = 0.816, I^2 = 0.0%). Nonetheless, the corresponding pooled ORs were not quantitatively altered by removing any single study.

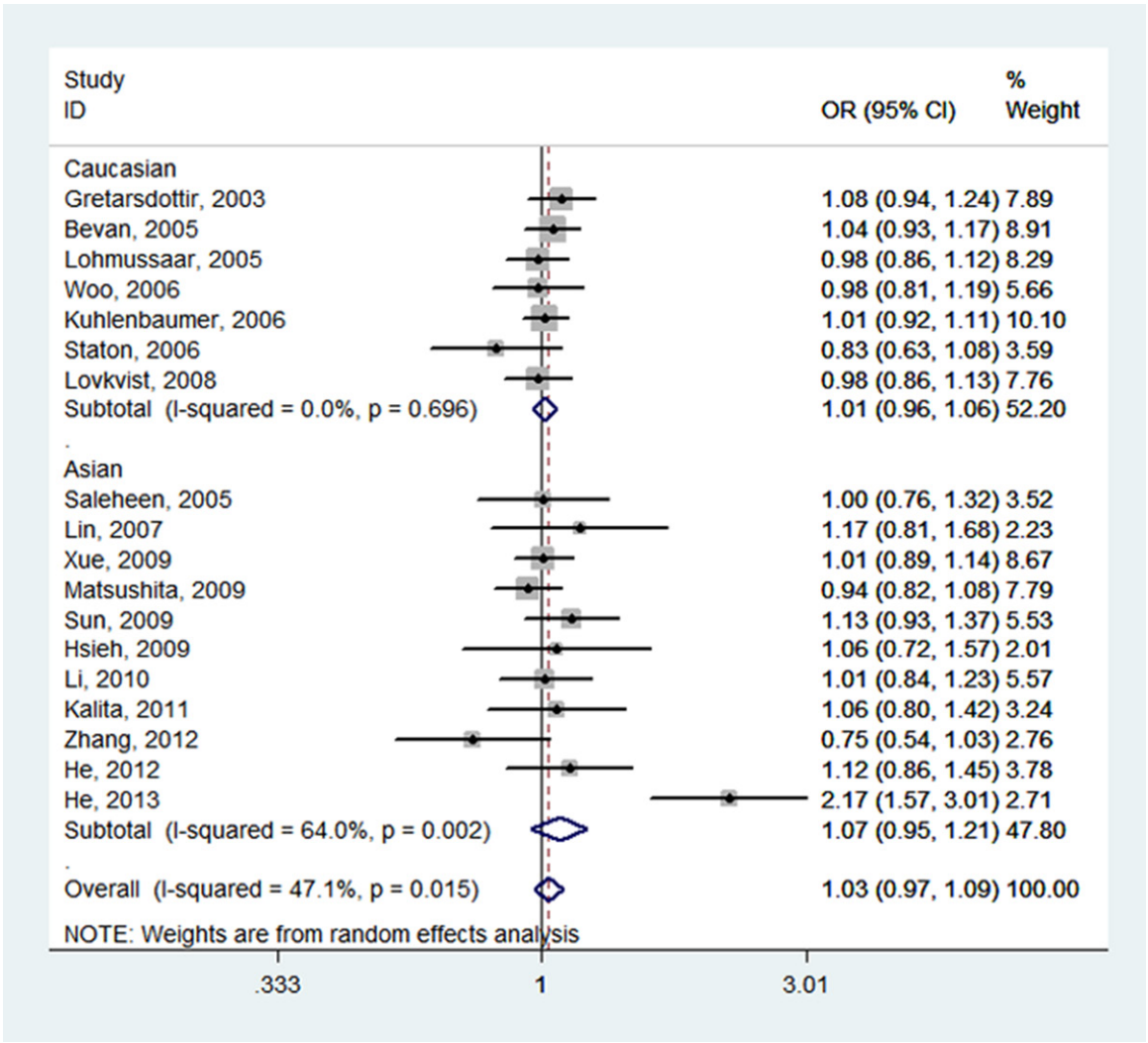

Figure 5. Forest plot of ischemic stroke risk associated with *PDE4D* SNP 87 stratified by ethnicity under T vs. C model. The boxes and horizontal lines represent the OR and the corresponding 95% CI. The area of the boxes indicates the weight (inverse of the variance). The diamond corresponds to the summary OR and 95% CI.

Publication bias

Both Begg's funnel plots and Egger's test were performed to determine the publication bias of the studies included in this meta-analysis. The shapes of the funnel plot did not indicate obvious asymmetry under any genetic model (**Figure 7**), and the statistical evidence of Egger's test suggested no significant publication bias in the meta-analysis (TT vs. CC: P = 0.905).

Discussion

PDE4D selectively degrading cyclic AMP that has effects on the vasculature and nervous system has been implicated to play a pivotal role in the etiology of stroke [18, 35]. Since the first study addressing the associations of *PDE4D* SNPs and ischemic stroke risk was reported [18], an increasing body of research has been subsequently published to assess how the *PDE4D* gene SNPs, especially SNP 87, act in the progress of ischemic stroke [19-21, 31-34]. The investigations failed to reach a consensus on the association between *PDE4D* SNP 87 and the risk of ischemic stroke due to the small sample sizes. This promoted us to conduct the current meta-analysis, in an attempt to assess the controversial association through pooling the data supplied by the eligible studies.

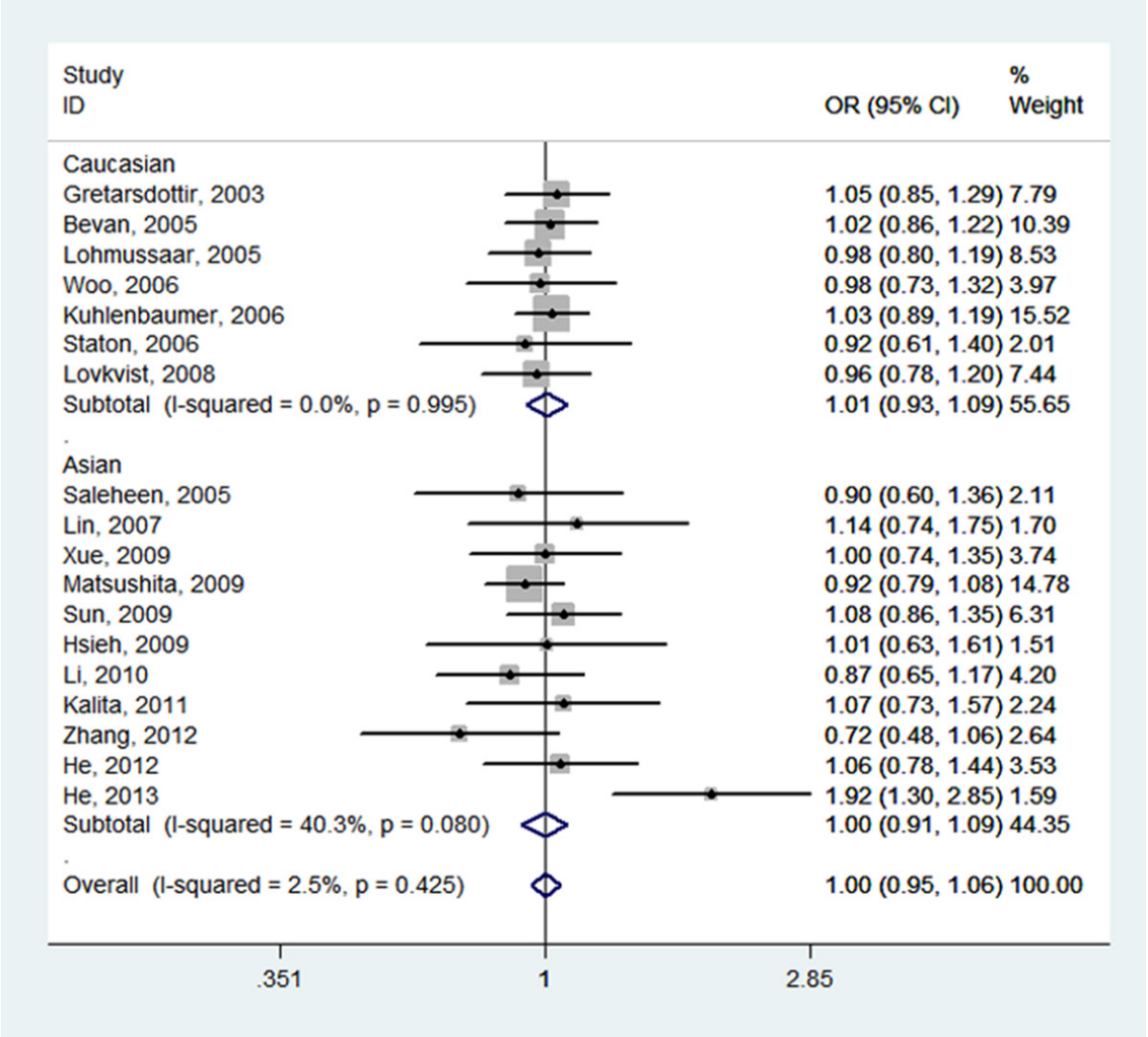

Figure 6. Forest plot of ischemic stroke risk associated with *PDE4D* SNP 87 stratified by ethnicity under CT vs. CC model. The boxes and horizontal lines represent the OR and the corresponding 95% CI. The area of the boxes indicates the weight (inverse of the variance). The diamond corresponds to the summary OR and 95% CI.

This meta-analysis examined all the available data on the association between *PDE4D* SNP 87 and ischemic stroke risk, including a total of 8,363 cases and 12,223 control subjects. Although the pooled results showed that SNP 87 was not associated with the risk of ischemic stroke, the trend to an increased risk of developing ischemic stroke was observed. The stratified analyses based on ethnicity and sample size did not suggest any statistical evidence for a significant association.

By comparing the results of the meta-analyses published before and the present study, we found an implication of great interest. The initial meta-analysis published in 2008 investigating six SNPs (SNP 26, 45, 56, 83, 87, and 89) of *PDE4D* gene suggested that no SNPs examined in *PDE4D* showed a robust and reproducible association to ischemic stroke [36]. Similar results were observed in a following meta-analysis by Domingues-Montanari and the co-authors [37]. Later, two studies based on 7 datasets and 13 datasets respectively revealed a significant association between *PDE4D* SNP 83 and ischemic stroke, but not SNP 87 [38, 39]. Taken together, a uniformly non-significant association was indicated in the four meta-analyses. However, we could draw an interesting conclusion from the five meta-analyses that the more studies were included, the higher risk of developing ischemic stroke was indicated in the results. This implies that the modification

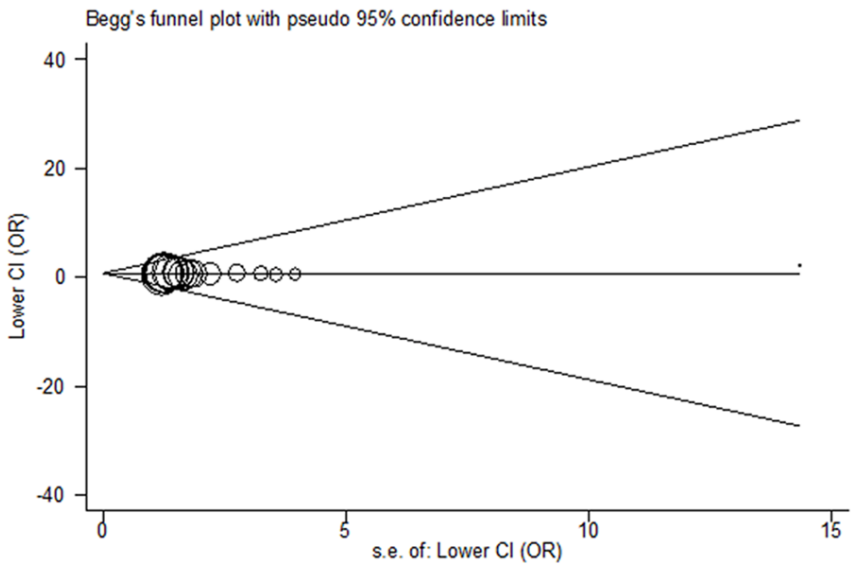

Figure 7. Begg's funnel plot for *PDE4D* SNP 87. Log OR is plotted versus standard error of Log OR for each included study. Each circle dot represents a separate study for the indicated association between *PDE4D* SNP 87 and ischemic stroke risk under TT vs. CC model.

effects of SNP 87 are necessary to be further validated in future larger studies.

Heterogeneity is an important index to evaluate the quality of a meta-analysis. Despite some differences in certain aspects such as study designs, inclusion criteria for participants, sample sizes, and ethnic backgrounds, we only observed moderate between-study heterogeneity for the allele model, but not for the rest of four genetic models. In addition, when we deleted the study resulting in the heterogeneity, no significant alternation occurred in the corresponding ORs with 95% CIs, suggesting our results were statistically reliable.

Some limitations need to be considered when interpreting the results. To start with, although we have included all available data on the association of SNP 87 and ischemic stroke, the sample size does not appear to be sufficiently large enough to detect the potential relationship. Moreover, ischemic stroke is a multifactorial disease that is caused by the interplay of genetic and environmental factors, but we are unable to assess the effects of gene-environment interaction on ischemic stroke on account of lacking related data. Finally, only the published data were considered in this meta-analysis, and the unpublished or the ongoing studies were not included, which may have introduced selection bias.

In conclusion, we found from this meta-analysis that SNP 87 of *PDE4D* gene was not an independent risk factor for ischemic stroke risk. Further larger rigorous genetic association studies that take gene-gene and gene-environment interaction into consideration are needed to provide conclusive evidence for the association between *PDE4D* SNP 87 and ischemic stroke.

Disclosure of conflict of interest

None.

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