No Association Between Single-Nucleotide Polymorphism 56 (SNP56) in Phosphodiesterase 4D (PDE4D) Gene and Susceptibility to Ischemic Stroke: A Meta-Analysis of 15 Studies

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Data Collection B
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Background: Recent studies demonstrated that polymorphisms in the *PDE4D* gene were associated with several processes involved in the occurrence of ischemic stroke (IS). The association between specific *PDE4D* single-nucleotide polymorphism 56 (SNP56) and IS risk was initially identified via genome-wide association studies (GWAS), although the GWAS in different populations produced inconclusive results. Thus, we performed a meta-analysis to better explain the association between *PDE4D* SNP56 and IS risk.

Material/Methods: A literature search was conducted using PubMed, Embase, and Web of Science up to June 1, 2015. A fixed-effects or random-effects model was used to calculate the pooled odds ratios (ORs) based on the results from the heterogeneity tests.

Results: Finally, we performed a meta-analysis of 15 studies, involving 8731 IS patients and 10,756 controls. The results showed nonsignificant association between *PDE4D* SNP56 and IS risk (T *vs.* A: OR=1.01, 95%CI=0.88–1.15, *P*=0.90). Similarly, in the subgroup analysis by ethnicity, no significant association was observed in Asian (T *vs.* A: OR=1.08, 95%CI=0.80–1.44, *P*=0.62) or European (T *vs.* A: OR=0.96, 95%CI=0.86–1.08, *P*=0.54) population. Moreover, funnel plots and Egger regression testing showed no evidence of publication bias.

Conclusions: In summary, current evidence suggested that *PDE4D* SNP56 might not be associated with an increased susceptibility to IS. However, this conclusion needs further validation by well-designed studies with large sample sizes.

MeSH Keywords: **Meta-Analysis • Polymorphism, Genetic • Stroke**

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Background

Stroke is the leading cause of disability and third cause of death in developed countries; an estimated 5.7 million people die of stroke annually worldwide [1,2]. Ischemic stroke (IS), the most common type of stroke, accounts for approximately 85% of all strokes. Recently, various epidemiologic studies in families and twins have revealed that genetic factors played an important role in the pathogenesis of IS, including *IL1-β*, *HDAC9*, *TNF-α*, and *ACOT4* [3,4]. These genetic findings permit the early detection of people at risk for IS.

Phosphodiesterase 4D (*PDE4D*) is a large gene spanning >1.5 Mb on chromosomal region 5q1; *PDE4D* has 22 exons, 8 splice variants, and several hundred SNPs [5]. *PDE4D* is associated with several processes involved in the occurrence of stroke, including cell proliferation, migration, and inflammation [6–9]. Recent studies showed that polymorphisms in *PDE4D* gene might be risk factors for IS [1,10–13]. Gretarsdottir et al. first demonstrated that SNP56 in *PDE4D* gene was associated with the IS risk via whole-genome linkage screens in the Icelandic population [14]. To date, the following GWAS in different populations that investigated the association between *PDE4D* SNP56 and IS have produced inconclusive results. For instance, several studies suggested a positive association between SNP56 and IS susceptibility [15–17]; nevertheless, some studies could not replicate it [18–21]. These discrepancies may be due to a variety of explanations, including studies with a small sample sizes, inadequate statistical power, different analytical methods, ethnic differences, and different stroke subtypes. Meta-analysis is an efficient method to provide more credible evidence by systematically summarizing all eligible data from independent studies [22]. To date, meta-analysis-involved SNP56 was not reported. Thus, we aimed to perform a meta-analysis to clarify the effect of *PDE4D* SNP56 on susceptibility to IS.

Material and Methods

Search strategy

A comprehensive electronic search was conducted of PubMed, Embase, and Web of Science, using the keywords stroke, polymorphism OR mutation OR variant, SNP56 OR rs702553, and *PDE4D* OR phosphodiesterase 4D. The search was last performed in April 2015. The reference lists of reviews and retrieved articles were hand-checked for additional potential studies.

Inclusion and exclusion criteria

Studies were considered eligible in our meta-analysis if the following criteria were fulfilled: (1) the study reported the association between *PDE4D* SNP56 and risk of ischemia stroke; (2)

the study provided sufficient information of allele or genotype frequencies; (3) the study was a prospective cohort or case-control study. Comments, letters, and review articles were excluded, as were studies containing overlapping data with other studies and studies having no control population. A study reporting the results for different subpopulations was treated as separate studies.

Data extraction

The following data were extracted independently from each study by 2 authors: first author, year of publication, ethnicity of the individuals (categorized as Europeans or Asians), numbers of cases and controls, sex and mean age in cases and controls, and genotype frequency. In addition, the corresponding author was contacted for detailed data when there was insufficient information of genotype distributions.

Statistical analysis

The odds ratios (ORs) and corresponding 95% CIs were used to measure the strength of the association between *PDE4D* SNP56 and susceptibility to IS. The significance of the pooled OR was determined by the Z test, and $P<0.05$ was considered significant. Moreover, the pooled ORs were estimated for T *vs.* A, TT *vs.* AA (homozygote comparison), TC *vs.* AA (heterozygote comparison), TT *vs.* TA+AA (recessive model), and TT+TA *vs.* AA (dominant model). Subgroup analysis was performed according to ethnicity. Finally, the Hardy-Weinberg equilibrium (HWE) in the control group was also assessed, and a $P<0.05$ was considered significant disequilibrium.

Heterogeneity across studies was evaluated by using the Chi-square – based Q test and I^2 test. For the Q test, a P value of <0.05 was considered significant heterogeneity [22,23]. I^2 values range between 0% and 100%, with higher values denoting a greater degree of heterogeneity (considered when $I^2>50\%$). A random-effects model (DerSimonian-Laird method) was used when $P<0.05$ or $I^2>50\%$; otherwise, a fixed-effects model (Mantel-Haenszel method) was used.[24] A metaregression was used to investigate whether any particular covariates could explain the observed between-study heterogeneity [25].

To evaluate the stability of the results, sensitivity analysis was performed by sequentially excluding 1 study each time, to assess the effect of a single study on the pooled ORs [26]. Moreover, a cumulative analysis was carried out to measure the genetic effects as they accumulated over time [27]. Publication bias was assessed using visual inspection of funnel plots and the Egger regression test; $P<0.05$ was considered significant [28]. All statistical analyses were performed by STATA software, version 12 (StataCorp LP, College Station, Texas, USA).

Results

Characteristics of the included studies

The systematic literature search identified 68 publications in PubMed, Embase, and Web of Science. After a preliminary screening, 21 articles that met the inclusion criteria and full texts were reviewed and analyzed in detail; 7 articles reported other variants in *PDE4D* gene [29–35]. Two studies investigated the association of *PDE4D* variants with other diseases [36,37]. Figure 1 shows a flow chart of the selection process. Finally, 12 articles on *PDE4D* SNP56 and IS risk were included [14–21,38–41]. Among them, Domingues-Montanari et al. and Matsushita et al. reported on 2 and 3 subpopulations, respectively, and were treated independently. Thus, 15 publications were included in the present meta-analysis, and the characteristics of all included studies are summarized in Table 1.

Quantitative synthesis

A total of 15 studies involving 19,487 subjects (including 8731 patients and 10,756 control subjects) were included in the
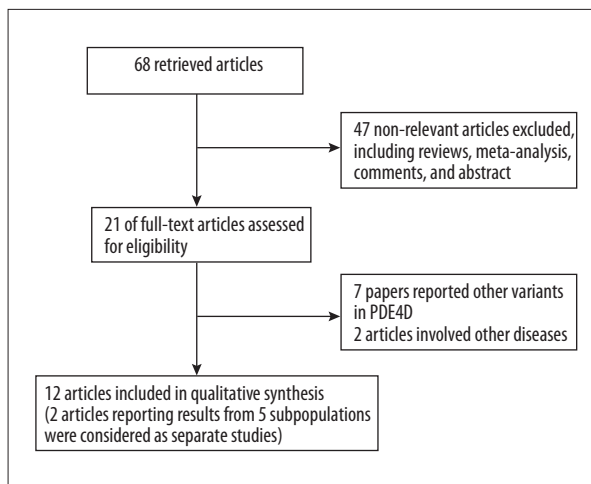

Figure 1. Study selection procedures for a meta-analysis of *PDE4D* SNP56 variant and ischemic stroke risk.

meta-analysis. Here, we observed a wide spectrum of the T allele frequency across different ethnicities. Briefly, European control subjects carried a higher frequency of *PDE4D* SNP56 T allele (65.68±4.13%), compared with that in Asian controls (40.93±5.68%; *P*<0.01; Figure 2).

Table 1. Characteristics of Eligible Studies in a Meta-analysis of the *PDE4D* SNP56 and ischemic stroke risk.

| First author	Year	Ethnicity	Cases				Controls			
			Number	Age	Males (%)	Hypertension (%)	Number	Age	Males (%)	Hypertension (%)
Brophy	2006	Europeans	248	73.9±5.9	NR	56.5	560	70.3±4.5	NR	32.7
Domingues-Montanari	2010	Europeans	527	70.6±11.9	54.5	59.2	263	72.1±6.9	45.7	44.7
Domingues-Montanari	2010	Europeans	565	52.4±9.3	63.9	57.2	518	63.0±6.8	45.9	37.7
Sun	2009	Asians	649	73.2±9.4	56.0	71.3	761	73.3±7.3	55	48.2
Matsushita	2009	Asians	24	NR	NR	NR	1566	NR	NR	NR
Matsushita	2009	Asians	1112	70.2±10.0	60.7	78.1	1112	70.1±10.1	60.7	53.7
Matsushita	2009	Asians	1711	69.0±9.3	64.7	75.4	1786	64.8±15.4	54.3	52.2
Gretarsdottir	2003	Europeans	864	NR	NR	NR	908	NR	NR	NR
Kuhlenbäumer	2006	Europeans	1181	66.9±14.6	54.0	77.0	1569	55.9±13.7	49.0	41.0
Lin	2007	Asians	190	NR	NR	NR	211	NR	NR	NR
Munshi	2012	Asians	516	49.3±17.3	69.8	53.2	513	49.0±16.8	69.8	29.6
Staton	2006	Europeans	151	67.3±11.7	66.2	NR	164	66.1±11.8	62.8	NR
Zee	2006	Europeans	259	62.1±0.5	Nr	19.8	259	61.7±0.5	NR	15.5
Meschia	2005	Europeans	377	64.8±15.0	53.6	68.5	263	60.0±14.7	38.0	38.8
Woo	2006	Europeans	357	69.0	43.7	NR	303	68.0	44.2	NR

NR – not report.
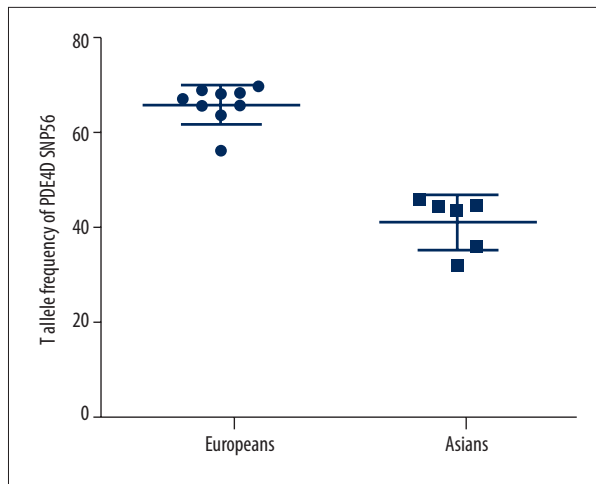
Figure 2. T allele frequencies (%) of *PDE4D* SNP56 in European or Asian control groups. Each data point represents a separate study for the indicated association.

The meta-analysis results of the association between the allele contrast and genetic models of the *PDE4D* SNP56 polymorphism and the susceptibility to IS was shown in Table 2. The results of combined analyses comprising 6448 patients and 9700 controls revealed a nonsignificant association between the SNP56 variant in *PDE4D* gene and risk of IS (T *vs.* A: OR=1.01, 95%CI=0.88–1.15, *P*=0.90; TT *vs.* AA: OR=1.00, 95%CI=0.75–1.34,

P=0.99; TA *vs.* AA: OR=1.00, 95%CI=0.83–1.21, *P*=0.98; TT+TA *vs.* AA: OR=0.99, 95%CI=0.79–1.24, *P*=0.93; TT *vs.* TA+AA: OR=1.03, 95%CI=0.86–1.25, *P*=0.74). In the subgroup analysis stratified by ethnicity, no significant association was observed between *PDE4D* SNP56 and IS susceptibility in Europeans (T *vs.* A: OR=0.96, 95%CI=0.86–1.08, *P*=0.54) or Asians (T *vs.* A: OR=1.08, 95%CI=0.80–1.44, *P*=0.62; Figure 3).

Test of heterogeneity

The results showed that there was significant heterogeneity in most comparisons in the analysis of IS (T *vs.* A: *P*<0.01 for Q test, I^2=84.7%; TT *vs.* AA: *P*<0.01 for Q test, I^2=83.3%; TA *vs.* AA: *P*<0.01 for Q test, I^2=70.0%; TT+TA *vs.* AA: *P*<0.01 for Q test, I^2=81.0%; TT *vs.* TA+AA: *P*<0.01 for Q test, I^2=78.5%; Table 2). We carried out metaregression analysis to assess the source of heterogeneity by ethnicity, sample sizes, and year of publication, which did not indicate any sources that contributed to the substantial heterogeneity.

Sensitivity analyses and cumulative meta-analysis

Sensitivity analysis showed that no single study qualitatively changed the pooled ORs, indicating that the results of this meta-analysis are highly stable (Figure 4). Excluding 2 studies that deviated from HWE, the pooled ORs did not change at all. [Data

Table 2. Summary ORs and heterogeneity of the *PDE4D* SNP56 and risk of ischemic stroke.

Comparison	Variables	No. of studies	Test of association		Model	Test of heterogeneity	
			OR (95% CI)	P-value		I² (%)	P-value
T *vs.* A	Overall	15	1.01 (0.88–1.15)	0.90	R	84.7	<0.01
	Europeans	9	0.96 (0.86–1.08)	0.54	R	65.8	<0.01
	Asians	6	1.08 (0.80–1.44)	0.62	R	92.2	<0.01
TT *vs.* AA	Overall	13	1.00 (0.75–1.34)	0.99	R	83.3	<0.01
	Europeans	7	0.89 (0.67–1.18)	0.42	R	61.8	0.02
	Asians	6	1.16 (0.68–1.99)	0.58	R	90.5	<0.01
TA *vs.* AA	Overall	13	1.00 (0.83–1.21)	0.98	F	70.0	0.12
	Europeans	7	0.92 (0.73–1.15)	0.46	R	41.5	<0.01
	Asians	6	1.09 (0.81–1.47)	0.57	R	81.0	<0.01
TT+TA *vs.* AA	Overall	13	0.99 (0.79–1.24)	0.93	R	81.0	<0.01
	Europeans	7	0.91 (0.72–1.16)	0.45	R	53.9	0.04
	Asians	6	1.09 (0.75–1.57)	0.65	R	89.1	<0.01
TT *vs.* TA+AA	Overall	13	1.03 (0.86–1.25)	0.74	R	78.5	<0.01
	Europeans	7	0.97 (0.79–1.18)	0.76	R	69.4	<0.01
	Asians	6	1.13 (0.76–1.69)	0.54	R	85.9	<0.01

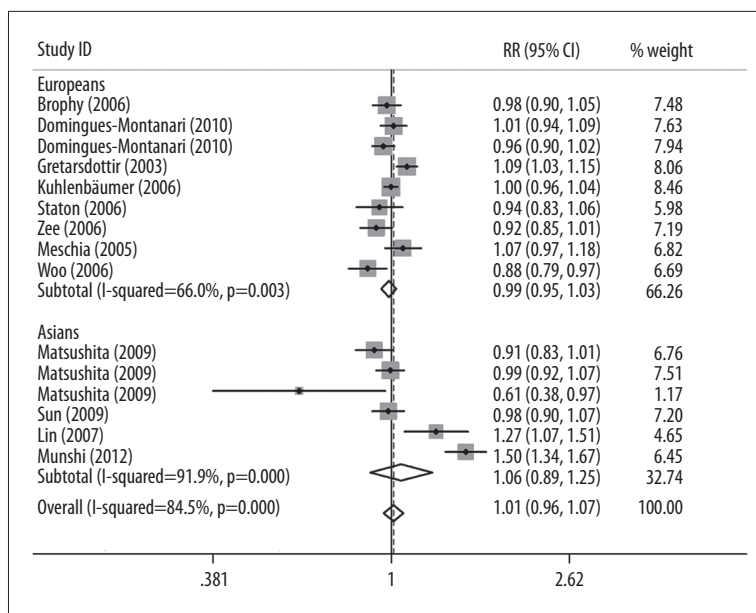OR – odds ratio; CI – confidence interval.

Figure 3. Forest plot of ischemic stroke risk associated with *PDE4D* SNP56 variant. The strength of the association was calculated by the odds ratios and corresponding 95% CIs. The sizes of the squares reflect the weighting of included studies; the center of diamonds reflects summary effect, and the left and right extremes of diamonds reflect 95% CI.
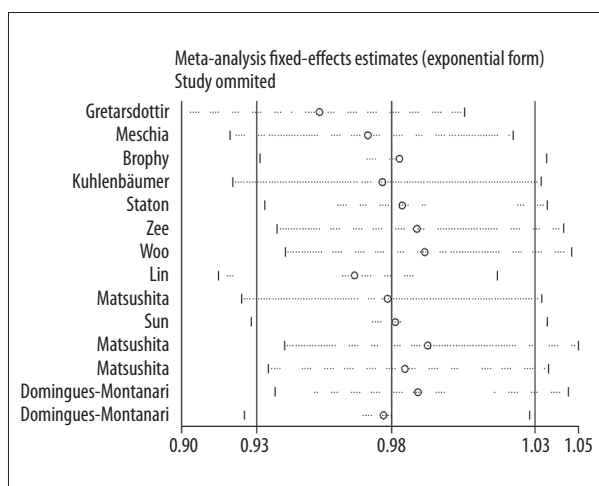

Figure 4. Sensitivity analysis for the association between *PDE4D* SNP56 and ischemic stroke risk. Results were computed by omitting each study (left column) in turn. Bars represent 95% CIs.

not shown,] Cumulative meta-analysis conducted by publication year demonstrated that estimated pooled ORs tended to be stable with the accumulation of more data over time (Figure 5).

Publication bias

Funnel plots and the Egger test were performed to assess publication bias. As shown in Figure 6, the shapes of the funnel plots did not show any evidence of obvious asymmetry for *PDE4D* SNP56 and IS. In addition, the results of the Egger test also did not show any evidence of publication bias: T *vs.* A: *P*=0.53, TT *vs.* AA: *P*=0.52, TA *vs.* AA: *P*=0.22, TT+TA *vs.* AA: *P*=0.24, TT *vs.* TA+AA: *P*=0.76.

Discussion

Despite substantial progress in prevention and treatment, stroke, a multifactorial disease, remains the leading cause of disability and is also responsible for 10% of deaths each year in developed countries [2,42]. Previous studies showed that development of IS can be attributed to environmental and genetic factors [1,43]. In the last few decades, a series of genetic studies have provided evidence supporting an important role for genetics in the pathogenesis of IS, such as variants in interleukin-6, leptin receptor, and angiotensin-converting enzyme [44–46].

One of the widely studied candidate genes was *PDE4D*, which belongs to a large superfamily of phosphodiesterases (PDEs). *PDE* genes encode PDE enzyme, which can regulate cAMP levels and is also the key signal transduction molecule in multiple tissues, including kidney, macrophages, B and T lymphocytes, monocytes, as well as vascular smooth muscle cells [47,48]. Moreover, several studies have demonstrated that *PDE4D* was associated with cell proliferation, migration, and inflammation – processes involved in stroke occurrence [6–9]. The association between specific *PDE4D* SNP56 and IS was initially identified via GWAS by the deCODE group [14]. However, replication of these results in other populations has proven difficult. A meta-analysis is a proper method that can overcome the problem of small sample sizes and inadequate statistical power in different genetic studies by combining results from individual studies. To date, accumulated meta-analysis has been performed to clarify the association of genetic polymorphisms with IS risk. Variants in several genes were identified as risk factors for IS, including *apolipoprotein B*, *beta-2 adrenergic receptor*, methylenetetrahydrofolate reductase (*MTHFR*),

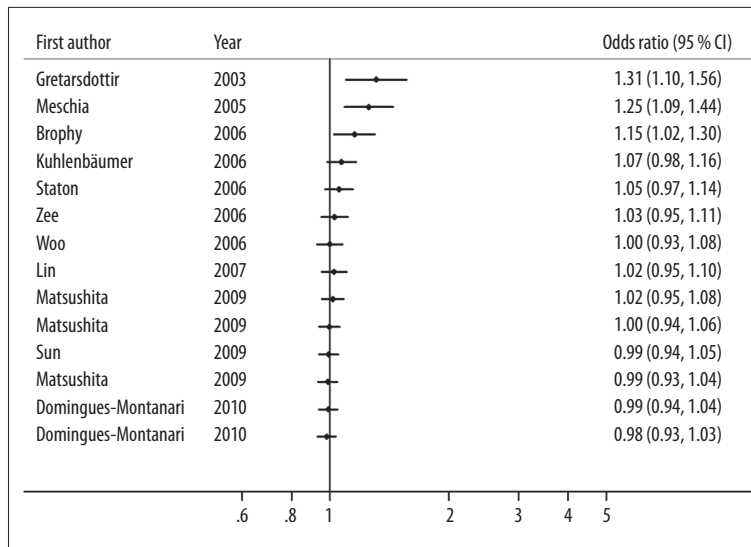| First author	Year		Odds ratio (95 % CI)
Gretarsdottir	2003		1.31 (1.10, 1.56)
Meschia	2005		1.25 (1.09, 1.44)
Brophy	2006		1.15 (1.02, 1.30)
Kuhlenbäumer	2006		1.07 (0.98, 1.16)
Staton	2006		1.05 (0.97, 1.14)
Zee	2006		1.03 (0.95, 1.11)
Woo	2006		1.00 (0.93, 1.08)
Lin	2007		1.02 (0.95, 1.10)
Matsushita	2009		1.02 (0.95, 1.08)
Matsushita	2009		1.00 (0.94, 1.06)
Sun	2009		0.99 (0.94, 1.05)
Matsushita	2009		0.99 (0.93, 1.04)
Domingues-Montanari	2010		0.99 (0.94, 1.04)
Domingues-Montanari	2010		0.98 (0.93, 1.03)

Figure 5. Cumulative meta-analyses of the association between *PDE4D* SNP56 and ischemic stroke risk. Pooled odds ratio estimates with the corresponding 95% CI as information accumulates at the end of each year (left column). Bars represent 95% CIs.
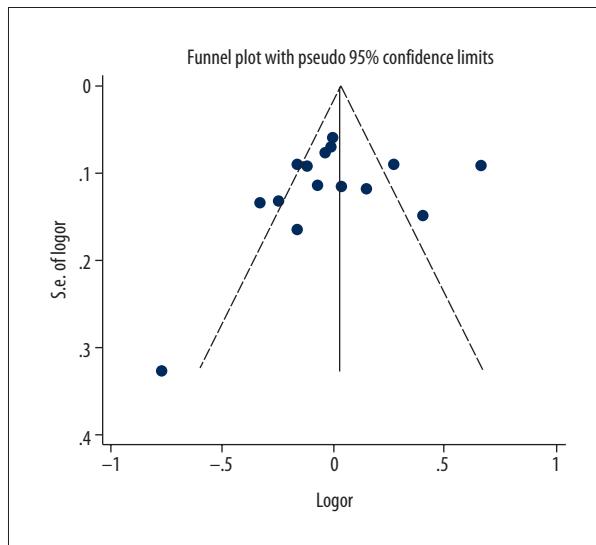
Figure 6. Funnel plots of the association between *PDE4D* SNP56 and ischemic stroke risk. No significant funnel asymmetry that could indicate publication bias was observed. Logor – natural logarithm of the odds ratio; SE – standard error.

and *HDAC9* [49–53]. However, some other variants, such as *IL-6* G572C, *PON1* L55M, and *CYP11B2* C-344T were not associated with susceptibility to IS [44,54,55]. Thus, we saw the need to perform a meta-analysis aimed to investigate the contribution of SNP56 in the *PDE4D* gene to IS.

In this comprehensive meta-analysis involving 8731 patients and 10,756 controls, the results showed a wide spectrum of T allele frequency of *PDE4D* SNP56 across different ethnicities. Compared with Asian controls, European controls carried a higher frequency of T allele (Asians *vs.* Europeans: 40.93±5.68% and 65.68±4.13%, respectively; *P*<0.01). It is widely accepted

that genetic markers in predisposition to IS vary across different ethnic populations. For instance, *MTHFR* A1298C polymorphism was strongly associated with IS risk in Asians but not in Europeans [56–58]. These results suggested that *PDE4D* SNP56 might be an ethnic population – specific genetic marker for IS patients.

In the overall analysis, we found that SNP56 in the *PDE4D* gene might not be significantly associated with IS risk. Subsequently, in the subgroup analysis stratified by ethnicity, no significant association between SNP56 and susceptibility to IS was found in either European or Asian population. However, the results might not be conclusive because IS is a complex multifactorial disease and environmental factors also play an important role in the development of IS [1,2,42]. Thus, the lack of association might also be attributed to variations in climate and in lifestyle, diet, and economic status of different individuals.

Heterogeneity was significant for the most comparisons of *PDE4D* SNP56 and IS risk. We then performed subgroup analysis and metaregression to identify the source of heterogeneity. The results of metaregression did not show any sources that contribute to the heterogeneity, including year of publication, sample size, and ethnicity. However, subtype information of IS might contribute to the significant heterogeneity [59]. For example, the significant association in the first GWAS was strongest for carotid and cardiogenic stroke [14]. In this meta-analysis, it is difficult to carry out a subgroup analysis by stroke subtype because of the lack of sufficient data. Additionally, hypertension status of patients and controls also accounted for the heterogeneity [60]. Compared with those with normal blood pressure, patients with hypertension were at a higher risk for diabetes, another stroke risk factor [61]. Thus, it might be easier to discern the effect of *PDE4D* SNP56 on IS risk in the absence of hypertension. Moreover, a difference in

the proportion of hypertensives might also contribute to the inconsistent results.

This meta-analysis significantly increased statistical power by pooling data from different studies; however, several limitations of this meta-analysis need to be considered for interpretation of our results. First, significant heterogeneity between studies was observed in the current meta-analysis, which might be attributed to different genetic backgrounds, different lifestyles, distinct environments, stroke subtypes, hypertension status, and abnormal physiologic variables. Second, we only included studies published in English, which might introduce a language bias. Distinct IS subtypes had different risk factors and etiologies, thus a subgroup analysis by types of IS was necessary in further meta-analysis. Finally, the development of IS is multifactorial, including genetic and environmental factors. Insufficient data prevented us from performing gene-environment or gene-gene interactions.

Conclusions

In conclusion, despite these limitations, the current meta-analysis included 15 genetic studies suggested that SNP56 in *PDE4D* gene might not be associated with the susceptibility to IS both in Europeans and Asians. Further well-designed studies with large sample size are required to validate our results. Moreover, gene-gene and gene-environment interactions should also be investigated to clarify possible roles of multiple risk factors in the development of IS.

Conflict of interest

The authors declare no conflict of interest.

References:

1. Domingues-Montanari S, Mendioroz M, del Rio-Espinola A et al: Genetics of stroke: A review of recent advances. Expert Rev Mol Diagn, 2008; 8: 495–513

2. Feigin VL: Stroke epidemiology in the developing world. Lancet, 2005; 365: 2160–61

3. Chehaibi K, Hrira MY, Trabelsi I et al: Gene variant and level of IL-1beta in ischemic stroke patients with and without type 2 diabetes mellitus. J Mol Neurosci, 2015; 57(3): 404–9

4. Auer PL, Nalls M, Meschia JF et al: Rare and coding region genetic variants associated with risk of ischemic stroke: The NHLBI Exome Sequence Project. JAMA Neurol, 2015; 72: 781–88

5. Wang D, Deng C, Bugaj-Gaweda B et al: Cloning and characterization of novel *PDE4D* isoforms *PDE4D*6 and *PDE4D*7. Cell Signal, 2003; 15: 883–91

6. Ariga M, Neitzert B, Nakae S et al: Nonredundant function of phosphodiesterases 4D and 4B in neutrophil recruitment to the site of inflammation. J Immunol, 2004; 173: 7531–38

7. Pan X, Arauz E, Krzanowski JJ et al: Synergistic interactions between selective pharmacological inhibitors of phosphodiesterase isozyme families PDE III and PDE IV to attenuate proliferation of rat vascular smooth muscle cells. Biochem Pharmacol, 1994; 48: 827–35

8. Palmer D, Tsoi K, Maurice DH: Synergistic inhibition of vascular smooth muscle cell migration by phosphodiesterase 3 and phosphodiesterase 4 inhibitors. Circ Res, 1998; 82: 852–61

9. Johnson-Mills K, Arauz E, Coffey RG et al: Effect of CI-930 [3-(2H)-pyridazinone-4,5-dihydro-6-[4-(1H-imidazolyl) phenyl]-5-methyl-monohydrochloride] and rolipram on human coronary artery smooth muscle cell proliferation. Biochem Pharmacol, 1998; 56: 1065–73

10. Dhamija RK, Ranjan P, Kumar B et al: Association of *PDE4D* gene with ischaemic stroke. Int J Stroke, 2012; 7: E8

11. Lovkvist H, Olsson S, Hoglund P et al: A large-sample assessment of possible association between ischaemic stroke and rs12188950 in the *PDE4D* gene. Eur J Hum Genet, 2012; 20: 783–89

12. Kalita J, Somarajan BI, Kumar B et al: Phosphodiesterase 4 D gene polymorphism in relation to intracranial and extracranial atherosclerosis in ischemic stroke. Dis Markers, 2011; 31: 191–97

13. Milton AG, Aykanat VM, Hamilton-Bruce MA et al: Association of the phosphodiesterase 4D (*PDE4D*) gene and cardioembolic stroke in an Australian cohort. Int J Stroke, 2011; 6: 480–86

14. Gretarsdottir S, Thorleifsson G, Reynisdottir ST et al: The gene encoding phosphodiesterase 4D confers risk of ischemic stroke. Nat Genet, 2003; 35: 131–38

15. Meschia JF, Brott TG, Brown RD Jr. et al: Phosphodiesterase 4D and 5-lipoxygenase activating protein in ischemic stroke. Ann Neurol, 2005; 58: 351–61

16. Munshi A, Roy S, Thangaraj K et al: Association of SNP41, SNP56 and a novel SNP in *PDE4D* gene with stroke and its subtypes. Gene, 2012; 506: 31–35

17. Lin HF, Liao YC, Liou CW et al: The phosphodiesterase 4D gene for early onset ischemic stroke among normotensive patients. J Thromb Haemost, 2007; 5: 436–38

18. Brophy VH, Ro SK, Rhees BK et al: Association of phosphodiesterase 4D polymorphisms with ischemic stroke in a US population stratified by hypertension status. Stroke, 2006; 37: 1385–90

19. Domingues-Montanari S, Fernandez-Cadenas I, del Rio-Espinola A et al: Association of a genetic variant in the ALOX5AP with higher risk of ischemic stroke: A case-control, meta-analysis and functional study. Cerebrovasc Dis, 2010; 29: 528–37

20. Staton JM, Sayer MS, Hankey GJ et al: Association between phosphodiesterase 4D gene and ischaemic stroke. J Neurol Neurosurg Psychiatry, 2006; 77: 1067–69

21. Sun Y, Huang Y, Chen X et al: Association between the *PDE4D* gene and ischaemic stroke in the Chinese Han population. Clin Sci, 2009; 117: 265–72

22. Trikalinos TA, Salanti G, Zintzaras E, Ioannidis JP: Meta-analysis methods. Adv Genet, 2008; 60: 311–34

23. Zintzaras E, Lau J: Synthesis of genetic association studies for pertinent gene-disease associations requires appropriate methodological and statistical approaches. J Clin Epidemiol, 2008; 61: 634–45

24. DerSimonian R, Laird N: Meta-analysis in clinical trials. Control Clin Trials, 1986; 7: 177–88

25. Kriston L, Harms A, Berner MM: A meta-regression analysis of treatment effect modifiers in trials with flexible-dose oral sildenafil for erectile dysfunction in broad-spectrum populations. Int J Impot Res, 2006; 18: 559–65

26. Copas J, Shi JQ: Meta-analysis, funnel plots and sensitivity analysis. Biostatistics, 2000; 1: 247–62

27. Kung TN, Dennis J, Ma Y et al: RFC1 80G>A is a genetic determinant of methotrexate efficacy in rheumatoid arthritis: A human genome epidemiologic review and meta-analysis of observational studies. Arthritis Rheumatol, 2014; 66: 1111–20

28. Egger M, Davey Smith G, Schneider M, Minder C: Bias in meta-analysis detected by a simple, graphical test. BMJ, 1997; 315: 629–34

29. Bevan S, Porteous L, Sitzer M, Markus HS: Phosphodiesterase 4D gene, ischemic stroke, and asymptomatic carotid atherosclerosis. Stroke, 2005; 36: 949–53

30. Kostulas K, Gretarsdottir S, Kostulas V et al: *PDE4D* and ALOX5AP genetic variants and risk for Ischemic Cerebrovascular Disease in Sweden. J Neurol Sci, 2007; 263: 113–17

31. Kumar A, Sagar R, Kumar P et al: Identification of genetic contribution to ischemic stroke by screening of single nucleotide polymorphisms in stroke patients by using a case control study design. BMC Neurol, 2013; 13: 136

32. Song Q, Cole JW, O'Connell JR et al: Phosphodiesterase 4D polymorphisms and the risk of cerebral infarction in a biracial population: the Stroke Prevention in Young Women Study. Hum Mol Genet, 2006; 15: 2468–78

33. Saleheen D, Bukhari S, Haider SR et al: Association of phosphodiesterase 4D gene with ischemic stroke in a Pakistani population. Stroke, 2005; 36: 2275–77

34. Nilsson-Ardnor S, Wiklund PG, Lindgren P et al: Linkage of ischemic stroke to the *PDE4D* region on 5q in a Swedish population. Stroke, 2005; 36: 1666–71

35. Lohmussaar E, Gschwendtner A, Mueller JC et al: ALOX5AP gene and the *PDE4D* gene in a central European population of stroke patients. Stroke, 2005; 36: 731–36

36. Heyer EJ, Mergeche JL, Ward JT et al: Phosphodiesterase 4D single-nucleotide polymorphism 83 and cognitive dysfunction in carotid endarterectomy patients. Neurosurgery, 2013; 73: 791–96; discussion 796

37. Liao YC, Lin HF, Guo YC et al: Sex-differential genetic effect of phosphodiesterase 4D (*PDE4D*) on carotid atherosclerosis. BMC Med Genet, 2010; 11: 93

38. Kuhlenbaumer G, Berger K, Huge A et al: Evaluation of single nucleotide polymorphisms in the phosphodiesterase 4D gene (*PDE4D*) and their association with ischaemic stroke in a large German cohort. J Neurol Neurosurg Psychiatry, 2006; 77: 521–24

39. Matsushita T, Kubo M, Yonemoto K et al: Lack of association between variations of *PDE4D* and ischemic stroke in the Japanese population. Stroke, 2009; 40: 1245–51

40. Woo D, Kaushal R, Kissela B et al: Association of Phosphodiesterase 4D with ischemic stroke: a population-based case-control study. Stroke, 2006; 37: 371–76

41. Zee RY, Brophy VH, Cheng S et al: Polymorphisms of the phosphodiesterase 4D, cAMP-specific (*PDE4D*) gene and risk of ischemic stroke: A prospective, nested case-control evaluation. Stroke, 2006; 37: 2012–17

42. Feigin VL, Lawes CM, Bennett DA, Anderson CS: Stroke epidemiology: A review of population-based studies of incidence, prevalence, and case-fatality in the late 20th century. Lancet Neurol, 2003; 2: 43–53

43. Flossmann E, Schulz UG, Rothwell PM: Systematic review of methods and results of studies of the genetic epidemiology of ischemic stroke. Stroke, 2004; 35: 212–27

44. Kumar P, Yadav AK, Kumar A et al: Association between Interleukin-6 (G174C and G572C) promoter gene polymorphisms and risk of ischaemic stroke: A meta-analysis. Ann Neurosci, 2015; 22: 61–69

45. Tang H, Zhang Z, Li ZK et al: Association of leptin receptor gene polymorphisms with genetic susceptibility to ischemic stroke. J Stroke Cerebrovasc Dis, 2015; 24(9): 2128–33

46. Yuan H, Wang X, Xia Q et al: Angiotensin converting enzyme (I/D) gene polymorphism contributes to ischemic stroke risk in Caucasian individuals: A meta-analysis based on 22 case-control studies. Int J Neurosci, 2015 [Epub ahead of print]

47. Essayan DM: Cyclic nucleotide phosphodiesterases. J Allergy Clin Immunol, 2001; 108: 671–80

48. Conti M, Richter W, Mehats C et al: Cyclic AMP-specific PDE4 phosphodiesterases as critical components of cyclic AMP signaling. J Biol Chem, 2003; 278: 5493–96

49. Cao Y, Fan X, Zhu W et al: Association of C7673T polymorphism in apolipoprotein B gene with ischemic stroke in the Chinese population: a meta-analysis. Int J Neurosci, 2015 [Epub ahead of print]

50. Kumar A, Prasad M, Kumar P et al: Association between beta adrenergic receptor polymorphism and ischemic stroke: A meta-analysis. J Stroke, 2015; 17: 138–43

51. Carty CL, Keene KL, Cheng YC et al: Meta-analysis of genome-wide association studies identifies genetic risk factors for stroke in African Americans. Stroke, 2015; 46(8): 2063–68

52. Cui T: MTHFR C677T mutation increased the risk of Ischemic Stroke, especially in large-artery atherosclerosis in adults: An updated meta-analysis from 38 researches. Int J Neurosci 2015.

53. Gu L, Wu G, Su L et al: Genetic polymorphism of beta-fibrinogen gene-455G/A can contribute to the risk of ischemic stroke. Neurol Sci, 2014; 35: 151–61

54. Shao P, Qu DJ, Song RY et al: Association between PON1 L55M polymorphism and ischemic stroke: A systematic review and meta-analysis. Int J Clin Exp Med, 2015; 8: 3429–37

55. Pi Y, Zhang LL, Chang K et al: Lack of an association between CYP11B2 C-344T gene polymorphism and ischemic stroke: a meta-analysis of 7,710 subjects. PLoS One, 2013; 8: e68842

56. Kang S, Wu Y, Liu L et al: Association of the A1298C polymorphism in MTHFR gene with ischemic stroke. J Clin Neurosci, 2014; 21: 198–202

57. Balcerzyk A, Niemiec P, Kopyta I et al: Methylenetetrahydrofolate reductase gene A1298C polymorphism in pediatric stroke – case-control and family-based study. J Stroke Cerebrovasc Dis, 2015; 24: 61–65

58. Lv QQ, Lu J, Sun H, Zhang JS: Association of methylenetetrahydrofolate reductase (MTHFR) gene polymorphism with ischemic stroke in the Eastern Chinese Han population. Genet Mol Res, 2015; 14: 4161–68

59. Dichgans M, Malik R, Konig IR et al: Shared genetic susceptibility to ischemic stroke and coronary artery disease: A genome-wide analysis of common variants. Stroke, 2014; 45: 24–36

60. Tsukinoki R, Okamura T, Watanabe M et al: Blood pressure, low-density lipoprotein cholesterol, and incidences of coronary artery disease and ischemic stroke in Japanese: the Suita study. Am J Hypertens, 2014; 27: 1362–69

61. Bruno RM, Taddei S: New-onset diabetes in hypertensive patients and mortality: timing is everything. Eur Heart J, 2015 [Epub ahead of print]