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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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

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/896904
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-[3], 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 size, 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 I² test. For the Q test, a P value of <0.05 was considered significant heterogeneity [22,23]. I² values range between 0% and 100%, with higher values denoting a greater degree of heterogeneity (considered when I²>50%). A random-effects model (DerSimonian-Laird method) was used when P<0.05 or I²>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 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 Number | Age       | Males (%) | Hypertension (%) | Controls 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        | 1566             | NR             | NR        | NR        | NR                |
| Matsushita         | 2009 | Asians    | 1112         | 70.2±10.0  | 60.7      | 75.4              | 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        | 908              | NR             | 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        | 211              | NR             | 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      | 164              | 66.1±11.8     | 62.8      | NR        | 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.
The meta-analysis results of the association between the allelic contrast and genetic models of the \textit{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 \textit{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=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 \textit{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).

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, \(I^2=81.0\%\); TT+TA vs. 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.

### 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, \(I^2=81.0\%\); TT+TA vs. AA: \(P<0.01\) for Q test, \(I^2=78.5\%\); Table 2).

### 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 \textit{PDE4D} SNP56 and risk of ischemic stroke.

| Comparison | Variables | No. of studies | Test of association | Model | Test of heterogeneity |
|------------|-----------|----------------|---------------------|-------|----------------------|
| 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.
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),
Compared with Asian controls, European controls carried a high allele frequency of T allele (Asians: 40.93±5.68% and 65.68±4.13%, respectively; Europeans: 40.93±5.68% and 10.756 controls, the results showed a wide spectrum of T allele frequency of SNP56 across different ethnicities. 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 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

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.
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.

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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.
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