Review

Neuro
epidemiology

Neuroepidemiology 2011;36:213–222
DOI: 10.1159/000327915

Received: December 1, 2010
Accepted: March 30, 2011
Published online: June 16, 2011

Meta-Analysis of Homogeneous Subgroups Reveals Association between *PDE4D* Gene Variants and Ischemic Stroke

Dankyu Yoon[a] Sue K. Park[c–e] Daehee Kang[c–e] Taesung Park[b] Ji Wan Park[f]

[a]Interdisciplinary Program in Bioinformatics, and [b]Department of Statistics, College of Natural Science, Seoul National University, [c]Department of Preventive Medicine, Seoul National University College of Medicine, [d]Cancer Research Institute, Seoul National University, and [e]Department of Biomedical Science, Seoul National University Graduate School, Seoul, and [f]Department of Medical Genetics, Hallym University College of Medicine, Chuncheon, Republic of Korea

Key Words
Phosphodiesterase 4D · Ischemic stroke · Meta-analysis · Single nucleotide polymorphism · Genetic association study

Abstract
Background: An Icelandic study showed a significant positive association between phosphodiesterase 4D *(PDE4D)* gene variants and stroke. However, subsequent studies reported conflicting results, possibly due to small sample sizes and the heterogeneity of the studies. ***Method:*** We performed a meta-analysis on 6 SNPs of the *PDE4D* gene to investigate the association between this gene and ischemic stroke by integrating the results of previous studies, comprising 11,834 cases and 15,233 controls. A pooled genotypic odds ratio (OR) for each SNP was determined under 3 genetic models (i.e. dominant, recessive, and codominant) using both fixed- and random-effects models with consideration for heterogeneity and publication bias across studies. ***Results:*** Among the SNPs included in this study, SNP56 (rs702553) showed the most significant association with ischemic stroke in a meta-analysis comprised of 7 homogenous studies. The overall OR of the TT genotype compared to the AA genotype was 1.29 (95% CI 1.03–1.61; p = 0.022). For SNP83 (rs966221), a protective effect of the ancestral allele T was observed only in Asian populations (ORTT 0.79, 95% CI 0.69–0.90; p = 0.0005). This meta-analysis revealed a significant association of *PDE4D* gene variants with the risk of ischemic stroke, and further investigations are warranted to evaluate possible ethnic-specific effects.

Introduction

According to the World Health Organization (WHO), stroke is a leading cause of death and disability and was responsible for 5.7 million deaths worldwide in 2005 [1]. Ischemic stroke, the most common subtype of stroke, accounts for 85% of all strokes [2]. Previous genetic epidemiological studies provided substantial evidence that susceptibility to ischemic stroke is determined by a complex interplay of genetic and environmental factors. Conventional risk factors include nonmodifiable factors (i.e. age, gender, and ethnicity), modifiable environmental factors (i.e. cigarette smoking and a high-calorie diet), and disease-related phenotypes (i.e. homocysteine con-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0251–5350/11/0364–0213$38.00/0

Accessible online at:
www.karger.com/ned

Dr. Ji Wan Park
Department of Medical Genetics
Hallym University College of Medicine, 39 Hallymdaehak-gil
Chuncheon, Gangwon-do 200-702 (Republic of Korea)
Tel. +82 33 248 2691, E-Mail jwpark@hallym.ac.kr

centration, hypertension, diabetes, etc.) [3]. Genetic predisposition may differ depending on the age, gender, ethnicity, and stroke subtype (i.e. small-vessel stroke, large-vessel stroke, and cardio-embolic stroke) of the study population [4–6]. There are no published population-based data on heritability for ischemic stroke. Alternatively, heritability estimates for such intermediate phenotypes of stroke as cerebral white matter lesions and intima-media thickness were in the ranges of 55–70 and 30–60%, respectively [7, 8].

Phosphodiesterase 4D *(PDE4D)* (MIM 600129) is a very large gene spanning >1.5 Mb on chromosomal region 5q12 with 22 exons, 8 splice variants, and several hundred SNPs [9, 10]. Gretarsdottier et al. [10] of the Iceland DeCODE study reported evidence for the linkage of a susceptibility gene for ischemic stroke to the 5q13 chromosomal region in 2002 and subsequently found a significant association between the *PDE4D* gene and ischemic stroke in 2003. Thus far, more than 20 studies have attempted to replicate the findings of the Icelandic study, but some of these studies have reported conflicting results [11–13]. Even 3 publications that conducted analyses identical to that of the initial study using the same markers in the same stroke subtype did not replicate the original findings of Gretarsdottir et al. [2, 4, 10–12]. There could be a variety of explanations for these discrepancies, including insufficient statistical power due to a small sample size, nonstandardized adjustments for modifiable and nonmodifiable risk factors for stroke, and different analytical methods, ethnic populations, diagnostic criteria, and stroke subtypes applied [14]. Some of these problems can be resolved through meta-analysis, an efficient method to increase statistical power by pooling samples obtained from independent studies [15].

However, meta-analysis on the association between the *PDE4D* gene and stroke has proven difficult to perform because of the lack of accumulated information on each SNP, different SNPs selected as target markers across studies, and the heterogeneity of the phenotypes examined among studies [2]. To date, 5 meta-analyses have been reported with respect to the relationship between the *PDE4D* gene and stroke. Staton et al. [13] (2006) included 9 case-control studies composed of 3,808 patients with stroke and 4,377 controls without consideration of subtype. Three SNPs, i.e. 41 ($p = 0.003$), 83 ($p = 0.003$), and 87 (pooled $p = 0.002$), showed a significant association with overall stroke even after adjustment for multiple comparisons, although heterogeneity was detected using the Q test ($p < 0.1$). Lövkvist et al. [16] (2008) analyzed only SNP45 in their meta-analyses of the random-effects model which was comprised of 13 studies (6,221 Caucasian cases and 6,750 controls) and showed no significant overall effect of SNP45 on ischemic stroke. The heterogeneity test suggests that the odds ratios (ORs) of the 13 studies are more heterogeneous than expected by random variation alone ($p = 0.042$). Bevan et al. [17] (2008) performed a series of meta-analyses of 16 studies only under the fixed-effects model. The associations of 6 individual SNPs (i.e. SNP 26, 45, 56, 83, 87, and 89) were tested for both carriers (i.e. under the dominant model) and homozygotes (i.e. under the recessive model). None of the meta-analyses for ischemic stroke cases could replicate the statistical significance of an association reported in previous studies. Recently, 2 ethnic specific meta-analyses were published. Domingues-Monatanari et al. [18] (2010) reported that none of the SNPs in the *PDE4D* gene was associated with ischemic stroke in the Iberian population. However, Xu et al. [19] (2010) found an association between SNP83 and ischemic cerebral infarction in Asian populations.

Nonetheless, the *PDE4D* gene encoding an enzyme that selectively degrades cAMP appears to be a potential candidate gene for stroke through its enzyme activity in regulating the response of human tissues to injury or infections [20, 21]. The significant heterogeneity of the studies being combined might be responsible for the failure of these meta-analyses to replicate previous findings. Therefore, we performed a meta-analysis on 6 SNPs (i.e. SNPs 26, 45, 56, 83, 87, and 89) with consideration of heterogeneity and bias being introduced in the studies to evaluate the effect of the *PDE4D* gene variants in the occurrence of ischemic stroke.

Materials and Methods

Systematic Review of Studies

Using a systematic search of the MEDLINE electronic database (PubMed, http://www.ncbi.nlm.nih.gov/sites/entrez/) and SCOPUS (http://www.scopus.com/scopus/home.url) from January 2003 to August 2010, we identified all studies on the association of *PDE4D* gene variants with ischemic stroke. The terms used for the search were 'ischemic (ischaemic) stroke genotype cohort', 'phosphodiesterase 4D ischemic (ischaemic) stroke genotype', 'cAMP-specific *(PDE4D)* ischemic (ischaemic) stroke genotype', and 'ischemic (ischaemic) stroke *PDE4D*'. Studies were included in our meta-analysis if the following criteria were fulfilled: firstly, an association between ischemic stroke and SNPs near or in the *PDE4D* gene was examined; secondly, the study was a prospective cohort, nested case-control, or case-control study, and thirdly, studies that provided genotype or allele frequencies on common SNPs among independent studies were included. In case of lack of genotype frequency information from papers, we contacted

Table 1. *PDE4D* gene variants evaluated for an association with ischemic stroke in previous studies

Study	Ethnicity	Number of cases/controls	SNP[a]
Gretarsdottir et al. [10]	Icelandic	864/908	SNPs 26, 45, 56, 83, 87, 89
Bevan et al. [11]	English	737/933	SNPs 26, 45, 87
Lohmussaar et al. [12]	German	639/736	SNPs 26, 45, 87
Meschia et al. [39]	European-American, others	377/263	SNPs 45, 56, 83
Nakayama et al. [28]	Japanese	208/270	SNP83
Nilsson-Ardnor et al. [43]	Swedish	275/550	SNP45
Saleheen et al. [30]	Pakistani	200/250	SNPs 83, 87
van Rijn et al. [42]	Dutch	91/200	SNPs 45, 83
Woo et al. [40]	European-American, African-American	357/303	SNPs 56, 83, 87, 89
Brophy et al. [37]	European-American	248/560	SNPs 26, 45, 56
Kuhlenbaumer et al. [41]	German	1,181/1,569	SNPs 45, 56, 83, 87, 89
Song et al. [36]	European-American, African-American	224/211	SNPs 45, 83, 89
Staton et al. [13]	Australian	151/164	SNPs 45, 56, 83, 87, 89
Zee et al. [38]	European-American	259/259	SNPs 26, 45, 56
Kostulas et al. [44]	Swedish	524/751	SNP45
Fidani et al. [45]	Greek	97/102	SNP45
Lin et al. [31]	Taiwanese	190/211	SNPs 56, 83, 87
Banerjee et al. [32]	Indian	176/212	SNP83
Lövkvist et al. [16]	Swedish	932/396	SNPs 45, 87
Xue et al. [34]	Chinese	639/887	SNPs 83, 87
Matsushita et al. [29][b]	Japanese	2,890/4,412	SNPs 56, 83, 87, 89
Sun et al. [35]	Chinese	649/761	SNPs 56, 83, 87
Munshi et al. [33]	Indian	250/250	SNP83

[a] SNPs included in each study among the 6 SNPs that were analyzed in the Icelandic study by Gretarsdottir et al. [10].
[b] Included 2 case-control studies and 1 cohort study.

each author to obtain the genotype data. Studies were excluded if they were cross-sectional studies or clinical trials, and studies were limited to human studies published in the English language. A total of 23 studies were included in the subsequent meta-analysis from a total of 168 independent references identified through the systematic searches (fig. 1).

Statistical Analysis

Data were analyzed using the meta-analysis package, 'meta', implemented in the statistical software environment R. (http://www.r-project.org/). Meta-analyses were carried out for the 6 genetic markers, i.e. SNP26 (rs40512), SNP45 (rs12188950), SNP56 (rs702553), SNP83 (rs966221), SNP87 (rs2910829), and SNP89 (rs1396476), for which data were available in at least 2 different studies. Our analyses were based on the genotypic effect under 3 genetic models (i.e. dominant, recessive, and codominant). We calculated pooled ORs with 95% confidence intervals (CI) for each of the 6 SNPs using both fixed- and random-effects models. We applied the Mantel-Haenszel method to estimate the fixed-effects summary ORs and the DerSimonian and Laird method for the summary ORs under the random-effects model [22, 23].

The degree of heterogeneity among the study results was assessed using the I^2 statistic, and the significance of heterogeneity for each meta-analysis was evaluated using the χ^2 test at $p < 0.01$ [23–25]. In the presence of significant heterogeneity, we performed the sensitivity analysis using sequential algorithms to explore the source of heterogeneity and to achieve homogeneity by removing one heterogeneous study at a time [26]. Since among-study variability may influence the conclusions of a meta-analysis (for instance, the effects in opposite directions across studies due

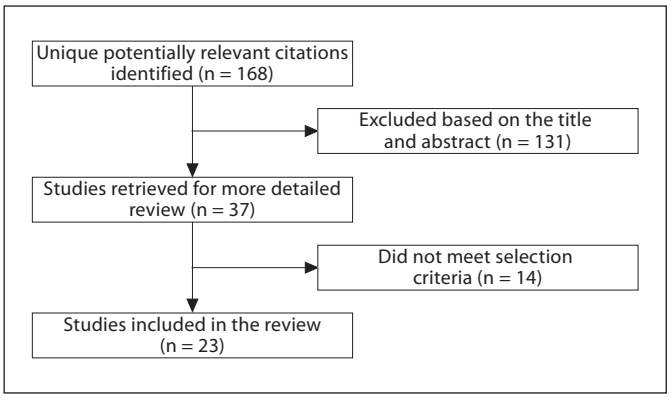

Fig. 1. Schematic flow chart depicting the selection of studies for meta-analysis.

Table 2. Characteristics of 23 studies reporting an association between *PDE4D* gene variants and ischemic stroke

Study	Study design	Cases/controls			Adjustment variables
		selection	mean age, years	female, %	
Gretarsdottir et al. [10]	Cohort	Hospital/population	NA	NA	NA
Bevan et al. [11]	Case-control	Hospital/population	65 ± 12.5/65 ± 8.9	41.0/43.0	Age, sex, smoking, DM, HT, HCL
Lohmussaar et al. [12]	Case-control	Hospital/population	65 ± 18.2/62 ± 11.7	37.7/39.3	NA
Meschia et al. [39]	Case-control, cohort	Hospital/spouses, friends	64.8 ± 15.0/60 ± 14.7	46.4/62.0	Age, sex, race, BMI, family history, smoking, etc.
Nakayama et al. [28]	Case-control	Hospital/hospital	66 ± 12.4/66.1 ± 5.8	39.4/47.8	NA
Nilsson-Ardnor et al. [43]	Nested case-control	Population/population	55 ± 8.3	42.9	Matching for sex, age, cohort, etc.
Saleheen et al. [30]	Case-control	Hospital/population	62.4 ± 12.4/54.1 ± 8.9	70.5/74.0	Age, sex, DM, HT
van Rijn et al. [42]	Case-control	Population/population	64.1 ± 12.2/56.8 ± 11.6	40.7/60.0	Age, sex
Woo et al. [40]	Case-control	Hospital/population	69/68	56.3/55.8	BMI, HT, DM, smoking, family history, etc.
Brophy et al. [37]	Nested case-control	Hospital/hospital	73.9 ± 5.9/70.3 ± 4.5	Female only	Age, weight, smoking, DM
Kuhlenbaumer et al. [41]	Cohort	Hospital/hospital	66.9 ± 14.6/55.9 ± 13.7	46.4/51.2	Age, sex, DM, HT, HCL
Song et al. [36]	Case-control	Hospital/population	41.7/39.6	Female only	Age
Staton et al. [13]	Case-control	Hospital/population	67.3 ± 11.7/66.1 ± 11.8	33.8/37.2	NA
Zee et al. [38]	Nested case-control	Prospective cohort	62.1 ± 0.5/61.7 ± 0.5	Male only	Matching for age, smoking, length of follow-up
Kostulas et al. [44]	Case-control	Hospital/population	69.7 ± 11.4/46.8 ± 14.9	43.9/41.0	NA
Fidani et al. [45]	Cohort	Hospital/population	75.8 ± 6.9/72.7 ± 6.1	48.5/49.0	NA
Lin et al. [31]	Case-control	Hospital/population	NA	NA	Smoking, HT, DM
Banerjee et al. [32]	Case-control	Hospital/population	58.6 ± 14.2/57.4 ± 8.8	35.8/32.5	Age, sex, smoking, HT, DM
Lövkvist et al. [16]		Prospective cohort	73.6 ± 11.5/73.6 ± 11.9	44/43.2	Matching for age, sex, HT, DM, HD, smoking
Xue et al. [34]	Case-control	Hospital/population	60.8 ± 9.2/60.7 ± 8.2	37.4/42.4	Age, sex, BMI, SBP, DBP, glucose, HDL, etc.
Matsushita et al. [29][a]	Case-control	Hospital/hospital	70.2 ± 10.0/70.1 ± 10.1	39.3/39.3	Matching for age and sex
	Case-control	Hospital/hospital	69.0 ± 9.3/64.8 ± 15.4	35.3/45.7	Stratification by HT
	Cohort	Prospective cohort	≥40	NA	NA
Sun et al. [35]	Case-control	Hospital/hospital	73.2 ± 9.4/73.3 ± 7.3	44/45	SNP83, age, sex, BMI, DM, HT, HD
Munshi et al. [33]	Case-control	Hospital/population	48.5 ± 16.3/47.0 ± 17.8	24.8/26.0	Matching for age and sex

BMI = Body mass index; DM = diabetes mellitus; HT = hypertension; HD = heart disease; HCL = hypercholesterolemia; SBP = systolic blood pressure; DBP = diastolic blood pressure; HDL = high-density lipoprotein cholesterol; NA = not available.

[a] Included 2 case-control studies and 1 cohort study.

to differences in population characteristics may dilute the true effect of a variant to the susceptibility of a disease), we calculated pooled ORs for homogenous substudies in the event that significant heterogeneity was observed [25]. We drew forest plots to summarize the ORs and 95% CI for each meta-analysis showing a statistically significant association [23]. In addition, we examine funnel plots to find evidence of publication bias and used the rank correlation method for testing asymmetry [23, 27].

Results

We initially performed meta-analyses between each of the 6 SNPs reported in the Icelandic study and ischemic stroke. The subjects included in our meta-analysis consisted of 11,834 cases and 15,233 controls. Each of the original studies analyzed different numbers of study subjects and SNPs (table 1). While the study populations of most studies (n = 15) were of European descent, as in the original Icelandic study, 8 studies were from Japan, Pakistan, Taiwan, India, and China [28–35]. Table 2 summarizes the population characteristics and study designs of 23 studies included in subsequent analysis. We observed significant heterogeneity across studies with respect to ethnicity, study design, nonstandardized adjustments for modifiable and nonmodifiable risk factors for stroke, etc. Most studies recruited their case subjects from hospitals and their control subjects from the general population; however, 5 studies recruited case and control subjects from the general population of prospective cohort studies, and 3 studies obtained both groups of study subjects from hospitals. The mean age of subjects in most studies was within the range of 50–75 years, except in the studies by Munshi et al. [33] (i.e. cases, 48.5; controls, 47 years old) and Song et al. [36] (cases, 41.7;

Table 3. Genotype frequencies for each of the 6 SNPs observed in the original publications

Study		Cases	Controls
SNP26	(rs40512)	TT/CT/CC	
	Gretarsdottir et al. [10]	162/270/95	118/203/72
	Bevan et al. [11]	258/338/138	300/441/170
	Brophy et al. [37]	80/122/46	192/272/96
	Lohmussaar et al. [12]	176/339/107	244/374/108
	Zee et al. [38]	91/123/45	78/134/47
SNP45	(rs12188950)	CC/CT/TT	
	Gretarsdottir et al. [10]	488/216/19	300/167/25
	Bevan et al. [11]	529/184/21	659/240/29
	Brophy et al. [37]	176/66/6	406/142/12
	Fidani et al. [45]	68/25/4	72/28/2
	Kuhlenbaumer et al. [41]	825/301/31	1,140/394/29
	Kostulas et al. [44]	276/89/8	545/188/18
	Lohmussaar et al. [12]	441/140/14	550/167/17
	Meschia et al. [39]	186/165/25	113/120/29
	Nilsson-Ardnor et al. [43]	164/50/6	342/94/6
	Song et al. [36]	143/48/4	143/41/3
	Staton et al. [13]	106/38/6	121/41/2
	van Rijn et al. [42]	65/19/5	139/47/5
	Zee et al. [38]	178/73/8	192/64/3
	Lövkvist et al. [16]	702/209/18	271/111/13
SNP56	(rs702553)	AA/AT/TT	
	Gretarsdottir et al. [10]	52/211/287	66/292/257
	Brophy et al. [37]	30/113/105	62/249/249
	Kuhlenbaumer et al. [41]	123/463/545	157/653/738
	Meschia et al. [39]	68/151/136	67/90/97
	Staton et al. [13]	27/66/58	21/78/65
	Woo et al. [40]	64/170/111	39/131/114
	Zee et al. [38]	32/122/105	20/118/121
	Lin et al. [31]	60/80/44	90/87/33
	Matsushita et al. [29]	346/549/186	1,185/1,872/771
	Sun et al. [35]	212/309/117	241/379/139
SNP83	(rs966221)	CC/CT/TT	
	Gretarsdottir et al. [10]	167/249/94	98/167/84
	Banerjee et al. [32]	32/49/31	38/110/64
	Kuhlenbaumer et al. [41]	434/546/179	595/700/254
	Meschia et al. [39]	164/139/55	90/120/46
	Nakayama et al. [28]	3/42/163	4/56/210
	Saleheen et al. [30]	55/96/47	49/139/69
	Song et al. [36]	40/93/59	45/93/48
	Staton et al. [13]	44/68/39	61/75/28
	van Rijn et al. [42]	34/37/17	70/86/32
	Woo et al. [40]	108/170/68	97/134/46
	Lin et al. [31]	6/54/117	13/51/147
	Xue et al. [34]	27/144/253	29/255/603
	Matsushita et al. [29]	11/167/561	85/910/2,734
	Sun et al. [35]	40/223/385	35/230/496
	Munshi et al. [33]	26/124/100	5/100/145
SNP87	(rs2910829)	CC/CT/TT	
	Gretarsdottir et al. [10]	148/315/179	156/290/137
	Bevan et al. [11]	154/360/212	214/464/245
	Kuhlenbaumer et al. [41]	216/505/293	353/759/452
	Lohmussaar et al. [12]	128/296/174	146/366/216
	Saleheen et al. [30]	76/57/37	86/78/39
	Staton et al. [13]	45/72/34	36/72/56
	Woo et al. [40]	80/175/97	58/134/76
	Lövkvist et al. [16]	187/473/269	72/208/114
	Lin et al. [31]	120/52/8	149/54/7
	Xue et al. [34]	12/119/293	26/257/604
	Matsushita et al. [29]	826/248/18	2,840/950/57
	Sun et al. [35]	439/182/25	539/202/20
SNP89	(rs1396476)	TT/GT/GG	
	Gretarsdottir et al. [10]	411/159/5	295/140/15
	Kuhlenbaumer et al. [41]	813/301/32	1,093/432/39
	Song et al. [36]	140/49/4	146/37/3
	Staton et al. [13]	100/45/6	127/16/21
	Woo et al. [40]	237/88/9	204/65/5
	Matsushita et al. [29]	989/103/2	3,519/329/11

Genotypes were determined according to the allelic designation and allele frequencies of the Icelandic study by Gretarsdottir et al. [10].

controls, 39.6 years old). In terms of gender composition, most studies were composed of about 25–70% women; however, Brophy et al. [37] and Song et al. [36] studied only women, while Zee et al. [38] included only men. Controlled variables differed across studies; Meschia et al. [39] and Woo et al. [40] adjusted for various variables, including smoking, hypertension, diabetes mellitus, and hypercholesterolemia; however, some studies did not provide any information on whether any variables were controlled for statistical analyses.

We summarize the genotype frequencies for each of the 6 SNPs by case-control status obtained from the 23 original publications that were included in our meta-analysis (table 3). The reference genotypes for all studies were determined according to those of the Icelandic study. Genotype distributions of SNP83 in the case and control groups of 8 studies which are composed mostly of Asian populations seemed different from those of 6 other studies in Caucasians [28–35]. The ORs and 95% CI of previous studies were not directly comparable because

Table 4. Results of the meta-analysis under 3 genetic models and the test for heterogeneity of published studies that examined the association between each of 6 SNPs and ischemic stroke

SNPs	Studies	Cases, n	Dominant[b] (carrier)		Recessive (homozygote)[b]		Codominant (heterozygote/homozygote)[b]	
(M/m)[a]	n	Controls, n	OR (95% CI)[c]	I^2 (p)[c]	OR (95% CI)	I^2 (p)	OR (95% CI)	I^2 (p)
SNP26	5	TT/CT/CC						
rs40512		767/1,192/431	1.01	43.60	1.05	0.00	0.99/1.05	34.80/8.40
(T/C)		932/1,424/493	(0.85–1.18)	(0.131)	(0.90–1.21)	(0.877)	(0.85–1.17)/(0.88–1.24)	(0.189)/(0.358)
SNP45	14	CC/CT/TT						
rs12188950		4,347/1,623/175	0.96	31.50	1.01	38.60	0.96/1.01	0.00/45.90
(C/T)		4,993/1,844/193	(0.87–1.06)	(0.123)	(0.75–1.36)	(0.069)	(0.88–1.04)/(0.74–1.39)	(0.530)/(0.031)[c]
	11	2,971/1,033/113	1.05	0.00	1.28	0.00	1.03/1.29	0.00/0.00
		4,309/1,446/126	(0.96–1.15)	(0.958)	(0.98–1.67)	(0.719)	(0.94–1.13)/(0.99–1.68)	(0.989)/(0.691)
SNP56	9	AA/AT/TT						
rs702553		1,678/2,023/691	1.05	29.60	1.08	61.80	1.03/1.13	0.00/63.10
(A/T)		2,900/3,657/1,309	(0.95–1.17)	(0.181)	(0.89–1.32)	(0.007)	(0.94–1.12)/(0.91–1.41)	(0.560)/(0.005)[c]
	7	1,332/1,474/505	1.11	37.70	1.19	8.60	1.03/1.29	0.70/41.70
		1,715/1,785/538	(0.96–1.27)	(0.141)	(1.02–1.39)	(0.362)	(0.93–1.14)/(1.03–1.61)	(0.418)/(0.112)
					p = 0.022		p = 0.022	
SNP83	14	CC/CT/TT						
rs966221		1,024/1,952/2,074	0.85	69.40	0.92	63.30	0.87/0.86	58.10/70.30
(C/T)		1,216/3,059/4,922	(0.68–1.07)	(1×10^{-4})	(0.78–1.06)	(0.0007)	(0.71–1.06)/(0.65–1.13)	(0.003)/(1×10^{-4})[c]
	10	872/1,520/1,635	0.95	51.50	0.97	19.70	0.95/0.98	45.00/37.50
		1,072/2,490/4,077	(0.77–1.16)	(0.029)	(0.87–1.09)	(0.261)	(0.77–1.16)/(0.78–1.21)	(0.059)/(0.109)
Asian studies	6	163/608/996	0.66	22.40	0.79	0.00	0.72/0.64	31.00/16.50
		168/841/1,589	(0.50–0.87)	(0.265)	(0.69–0.90)	(0.711)	(0.53–0.99)/(0.48–0.85)	(0.203)/(0.307)
			p = 0.0034		p = 0.0005		p = 0.046/0.0025	
SNP87	11	CC/CT/TT						
rs2910829		2,283/2,539/1,460	0.992	0.00	1.02	0.00	0.98/1.02	0.00/3.90
(C/T)		4,519/3,544/1,886	(0.91–1.07)	(0.539)	(0.93–1.11)	(0.518)	(0.90–1.06)/(0.90–1.15)	(0.795)/(0.405)
Asian studies	5	1,473/658/381	0.98	0.00	1.12	0.00	0.96/1.18	0.00/0.00
		3,640/1,541/727	(0.88–1.11)	(0.427)	(0.92–1.35)	(0.860)	(0.85–1.08)/(0.89–1.57)	(0.495)/(0.883)
SNP89	5	TT/GT/GG						
rs1396476		2,279/586/53	1.14	44.50	0.82	50.10	1.29/0.89	77.50/29.00
(T/G)		5,089/879/79	(0.95–1.37)	(0.125)	(0.43–1.55)	(0.091)	(0.95–1.75)/(0.53–1.50)	(0.001)[c]/(0.228)
	4	2,179/541/47	1.05	8.70	1.13	0.00	1.04/1.12	12.00/0.00
		4,962/863/58	(0.92–1.19)	(0.349)	(0.75–1.69)	(0.845)	(0.91–1.20)/(0.75–1.69)	(0.332)/(0.820)

ORs and 95% CIs were computed using random effects models. The p value was listed below OR if p was <0.05. I^2 = Heterogeneity (%); (p) = p value for heterogeneity (Q statistics).

[a] Major/minor alleles were determined by the allele frequency reported by Gretardottir et al. [10].

[b] Genetic model: dominant, MM vs. Mm + mm; recessive, MM + Mm vs. mm, and codominant, heterozygote MM vs. Mm, homozygote MM vs. mm.

[c] Subgroup analysis was performed in the genetic model being marked.

the statistical models and/or genetic models used in each study were different; furthermore, adjusted covariates varied across studies as shown in table 2.

We performed a meta-analysis using fixed- and random-effect models comprised of 23 independent studies. The analysis proceeded excluding original studies due to the variability introduced by a stronger genetic effect of the first study compared to those subsequent studies showing less significance [36, 37]. Table 4 lists the pooled genotypic ORs and the 95% CI for each SNP, as determined using 3 genetic models (estimates with the fixed effects models are not shown). After sensitivity analyses excluding the studies responsible for the significant heterogeneity (I^2), we performed a meta-analysis on the group of homogenous studies [26]. Heterogeneity among studies was determined using the $I^2 > 50\%$ or Q statistics $p < 0.1$. We present the pooled ORs of homogeneous substudies.

In the meta-analysis, we failed to find a significant association of SNP26 with ischemic stroke. For SNP45, we performed the initial meta-analysis using 14 studies and subsequently identified significant heterogeneity in the

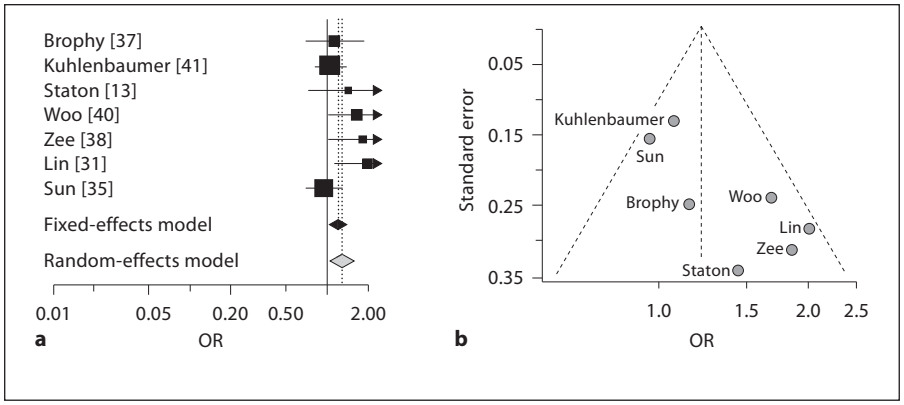

Fig. 2. Forest (**a**) and funnel (**b**) plots for SNP56 after removing the studies responsible for significant heterogeneity in the meta-analysis. The first author of each study is presented in **a** and **b**.

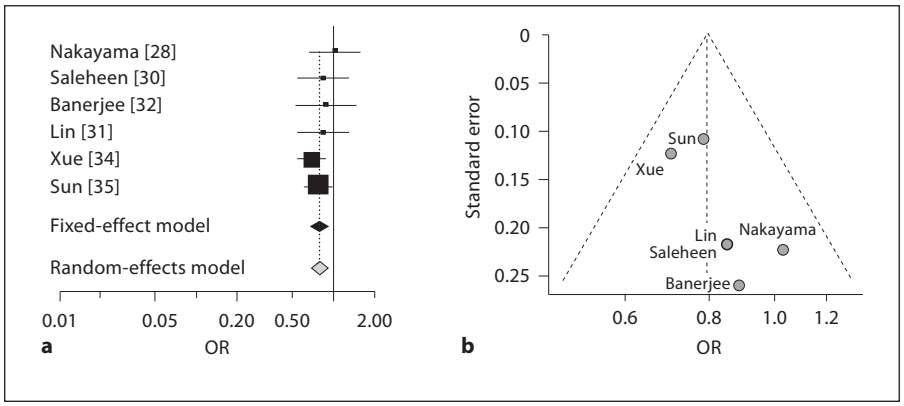

Fig. 3. Forest (**a**) and funnel (**b**) plots for SNP83 using Asian studies after removing the studies responsible for significant heterogeneity in the meta-analysis. The first author of each study is presented in **a** and **b**.

codominant model. Sensitivity analysis identified that 3 studies (i.e. Gretarsdottir et al. [10], Lövkvist et al. [16], and Meschia et al. [39]) introduced considerable heterogeneity. After removing these 3 studies from the subsequent meta-analysis of 11 studies, we observed a marginally significant overall OR in the TT homozygote group under the codominant model (OR = 1.29, 95% CI = 0.99–1.68; p = 0.05).

The major allele of SNP56 in the Icelandic population was allele A, and the genotype frequencies included in the meta-analysis were 1,678/2,023/691 and 2,900/3,657/1,309 for case and control groups, respectively. The interstudy heterogeneity was introduced by the studies of Matsushita et al. [29] and Meschia et al. [39] in the meta-analysis of 9 studies (I^2 = 63.10%; p = 0.005). After removing these studies from the subsequent meta-analysis, we observed the most significant overall OR in a codominant model by comparison of individuals with the TT genotype to the AA reference group (OR = 1.29, 95% CI = 1.03–1.61; p = 0.022). However, a recessive mode of inheritance might be a better fit to SNP56 because the OR for the AT heterozygous group was close to the baseline value of 1 in the codominant model. Figure 2a illustrates the ORs and 95% CIs of individual studies, as well as the overall ORs of the SNP56 in each of the fixed- and random-effects models. As shown in figure 2b, neither interstudy heterogeneity nor publication bias for the SNP56 seemed to exist anymore (I^2 = 41.70%; p = 0.112).

In the case of SNP83, four different studies were excluded from the analysis due to heterogeneity and possible publication bias (i.e. Staton et al. [13], Saleheen et al. [30], Munshi et al. [33], and Xue et al. [34]). However, the ORs estimated in 10 homogenous studies were close to 1. Since the genotype frequencies of SNP83 were different by ethnicity, we performed ethnic-specific meta-analyses in Asian populations [28, 30–32, 34, 35] and non-Asian populations [4, 13, 36, 40–42], respectively. The studies of Matsushita et al. [29] and Munshi et al. [33] were excluded from the Asian subgroup analysis because of its large heterogeneity estimated in the sensitivity analysis. A protective effect of the rare T allele was observed in Asian populations and the effect seems to best fit the recessive genetic model (OR = 0.79, 95% CI = 0.69–0.90; p = 0.0005) (fig. 3a). No significant association was observed in non-Asian populations (data not shown).

For SNP87, eleven studies did not show any significant association. We performed a meta-analysis in Asian groups (i.e. Matsushita et al. [29], Saleheen et al. [30], Lin et al. [31], Xue et al. [34], and Sun et al. [35]) and found no evidence of a genetic association of SNP87 with ischemic stroke. After removing Staton et al. [13] from the initial analysis, the heterogeneity of SNP89 significantly decreased. However, the effect size was close to null.

Discussion

The current meta-analysis consisted of 11,834 cases and 15,233 controls. In this meta-analysis, we included 7 more studies (i.e. Lövkvist et al. [16], Matsushita et al. [29], Lin et al. [31], Banerjee et al. [32], Munshi et al. [33], Xue et al. [34], and Sun et al. [35]) published after the study by Bevan et al. [17]. Among the 6 SNPs analyzed, our meta-analysis of 7 homogenous studies showed a significant association between SNP56 and ischemic stroke ($p = 0.022$). The result showed that individuals with the SNP56 TT genotype were 29% more likely to develop ischemic stroke compared with the common AA genotype under the random-effects model. In terms of ethnic difference, a protective effect of the ancestral T allele at SNP83 was observed only in Asian populations (ORTT = 0.79; $p = 0.0005$). This result is in agreement with those of Xu et al. [19].

The reversed direction of the effects of *PDE4D* gene variants observed in different studies suggested the possibility of a spurious association detected in Icelanders or in other study populations. It is important to note that some studies designated alleles based on the 3′-to-5′ orientation of the gene, while other studies followed the 5′-to-3′ orientation on the chromosome. For instance, the allele of SNP45 was reported as C/T in 4 (i.e. Bevan et al. [11], Lövkvist et al. [16], Song et al. [36], and van Rijin et al. [42]) of 14 studies but was reported as A/G in the rest of the studies, including the Icelandic study. Thus, the allelic designation for an SNP must be taken into consideration when the result of the SNP is compared across studies. In addition, a major genotype that is conventionally used as a reference genotype in genetic association studies may not always be the same among different ethnic populations as in the case of SNP83. In the current study, the summarized OR was computed from genotypic frequencies, and the reference genotypes were consistently determined for all studies according to the allelic designation and allele frequencies of the Icelandic study.

Heterogeneity across studies may have confounded or diluted the overall estimate of the meta-analysis if conducted with all of these studies without any consideration for incomparability. In the current study, the OR for SNP83 for all of the studies combined was close to 1, while it was 0.79 in Asian studies. The frequency of the C allele in Asians (i.e. 0.156 for JPT and 0.211 for HCB) is significantly different from that in Europeans (i.e. 0.592). The difference in allele frequency between Asian and European populations and the meta-analysis of Asian studies indicates that SNP83 may affect the risk of ischemic stroke in an Asian-specific manner. Bevan et al. [17] performed their meta-analysis only in the basic fixed-effects model without consideration of significant heterogeneity. It might be responsible for the failure of this study to replicate previous findings. The analysis strategy considering the design of each study would be another critical factor for removing spurious findings [41]. For instance, Matsushita et al. [29] reported that significant association of SNP56 in the prospective cohort, while they failed to detect the association in 2 case-control studies. Thus, careful consideration of multiple confounding factors and heterogeneity among studies may be necessary to perform a meta-analysis.

Some important concerns are discussed below. Firstly, different allele frequencies across study populations, particularly in different ethnic groups, might cause inconsistency among research findings and may result in a loss of statistical power. Secondly, stratification analysis by factors causing heterogeneity across studies may be necessary to reveal more reliable information. Due to the limited number of studies available for this meta-analysis, we could not perform separate meta-analyses for case-control studies and prospective cohort studies, which may differ in various aspects. Finally, confounding variables (e.g. age and sex) were not adjusted in the meta-analysis because covariate data were not given in most references. Nonetheless, this meta-analysis included the most studies published so far on the genetic association of *PDE4D* gene variants and ischemic stroke. In the presence of significant heterogeneity, influence analysis objectively explored all combinations of studies to achieve homogeneity and to prevent heterogeneity from having influence across studies in the overall effect estimate at a given SNP. The genotypic frequencies of previous studies were obtained from the authors of each study if only allelic frequencies were shown in the publication, and the accuracies of the genotypic frequencies and configurations were thoroughly checked before combining the data from all of the studies. Owing to the complexity of finding suit-

able genetic models for SNPs, the association of each SNP was tested under 3 different genetic models, allowing for heterogeneity across studies.

Insignificant results observed in SNPs other than SNP56 and SNP83 do not rule out a true association of any of the reported variants with ischemic stroke, particularly in the case of SNP45. In consideration of the issues discussed above, applications of valid meta-analytic methods along with continuous efforts to combine the results of the growing number of related studies may allow investigators to confirm the true effects of the *PDE4D* gene with ischemic stroke in different ethnic groups.

Acknowledgements

This study was supported by the Center for Genome Science, Republic of Korea, (National Institute of Health research contract 2007-E00004-00). We thank Dr. Lövkvist, who provided genotype data, and we thank Dr. Daniel Woo, Dr. James F. Meschia, Dr. Robert Y.L. Zee, Dr. J. M. Staton, Dr. John W. Eikelboom, and Dr. Steve Bevan for helpful discussions.

Disclosure Statement

The authors declare no conflict of interest.

References

1 Strong K, Mathers C, Bonita R: Preventing stroke: saving lives around the world. Lancet Neurol 2007;6:182–187.
2 Rosand J, Bayley N, Rost N, de Bakker PI: Many hypotheses but no replication for the association between PDE4D and stroke. Nat Genet 2006;38:1091–1092; author reply 1092–1093.
3 Goldstein LB, Adams R, Alberts MJ, Appel LJ, Brass LM, Bushnell CD, Culebras A, DeGraba TJ, Gorelick PB, Guyton JR, Hart RG, Howard G, Kelly-Hayes M, Nixon JV, Sacco RL: Primary prevention of ischemic stroke: a guideline from the American Heart Association/American Stroke Association Stroke Council – cosponsored by the Atherosclerotic Peripheral Vascular Disease Interdisciplinary Working Group; Cardiovascular Nursing Council; Clinical Cardiology Council; Nutrition, Physical Activity, and Metabolism Council; and the Quality of Care and Outcomes Research Interdisciplinary Working Group: The American Academy of Neurology affirms the value of this guideline. Stroke 2006;37:1583–1633.
4 Meschia JF: Subtyping in ischemic stroke genetic research. J Stroke Cerebrovasc Dis 2002;11:208–219.
5 Dichgans M, Markus HS: Genetic association studies in stroke: methodological issues and proposed standard criteria. Stroke 2005;36:2027–2031.
6 Touze E, Rothwell PM: Sex differences in heritability of ischemic stroke: a systematic review and meta-analysis. Stroke 2008;39:16–23.
7 Schulz UG, Flossmann E, Rothwell PM: Heritability of ischemic stroke in relation to age, vascular risk factors, and subtypes of incident stroke in population-based studies. Stroke 2004;35:819–824.
8 Moskau S, Golla A, Grothe C, Boes M, Pohl C, Klockgether T: Heritability of carotid artery atherosclerotic lesions: an ultrasound study in 154 families. Stroke 2005;36:5–8.
9 Bolger GB, Erdogan S, Jones RE, Loughney K, Scotland G, Hoffmann R, Wilkinson I, Farrell C, Houslay MD: Characterization of five different proteins produced by alternatively spliced mRNAs from the human camp-specific phosphodiesterase PDE4D gene. Biochem J 1997;328:539–548.
10 Gretarsdottir S, Thorleifsson G, Reynisdottir ST, Manolescu A, Jonsdottir S, Jonsdottir T, Gudmundsdottir T, Bjarnadottir SM, Einarsson OB, Gudjonsdottir HM: The gene encoding phosphodiesterase 4D confers risk of ischemic stroke. Nat Genet 2003;35:131–138.
11 Bevan S, Porteous L, Sitzer M, Markus HS: Phosphodiesterase 4D gene, ischemic stroke, and asymptomatic carotid atherosclerosis. Stroke 2005;36:949–953.
12 Lohmussaar E, Gschwendtner A, Mueller JC, Org T, Wichmann E, Hamann G, Meitinger T, Dichgans M: ALOX5AP gene and the PDE4D gene in a central European population of stroke patients. Stroke 2005;36:731–736.
13 Staton JM, Sayer MS, Hankey GJ, Attia J, Thakkinstian A, Yi Q, Cole VJ, Baker R, Eikelboom JW: Association between phosphodiesterase 4D gene and ischaemic stroke. J Neurol Neurosurg Psychiatry 2006;77:1067–1069.
14 Worrall BB, Mychaleckyj JC: PDE4D and stroke: a real advance or a case of the emperor's new clothes? Stroke 2006;37:1955–1957.
15 Berlin JA, Kim CJ: The use of meta-analysis in pharmacoepidemiology; in Strom BL (ed): Pharmacoepidemiology, ed 4. New York, Wiley, 2007, pp 681–707.
16 Lövkvist H, Smith JG, Luthman H, Hoglund P, Norrving B, Kristoffersson U, Jonsson A-C, Lindgren AG: Ischaemic stroke in hypertensive patients is associated with variations in the PDE4D genome region. Eur J Hum Genet 2008;16:1117–1125.
17 Bevan S, Dichgans M, Gschwendtner A, Kuhlenbaumer G, Ringelstein EB, Markus HS: Variation in the PDE4D gene and ischemic stroke risk: a systematic review and meta-analysis on 5,200 cases and 6,600 controls. Stroke 2008;39:1966–1971.
18 Domingues-Montanari S, Fernández-Cadenas I, del Rio-Espinola A, Corbeto N, Krug T, Manso H, Gouveia L, Sobral J, Mendioroz M, Fernández-Morales J, Alvarez-Sabin J, Ribó M, Rubiera M, Obach V, Martí-Fàbregas J, Freijo M, Serena J, Ferro JM, Vicente AM, Oliveira SA, Montaner J: 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;29:528–537.
19 Xu X, Li X, Li J, Ou R, Sheng W: Meta-analysis of association between variation in the PDE4D gene and ischemic cerebral infarction risk in Asian populations. Neurogenetics 2010;11:327–333.
20 Conti M, Richter W, Mehats C, Livera G, Park JY, Jin C: Cyclic AMP-specific PDE4 phosphodiesterases as critical components of cyclic AMP signaling. J Biol Chem 2003;278:5493–5496.
21 Hakonarson H: Role of FLAP and PDE4D in myocardial infarction and stroke: target discovery and future treatment options. Curr Treat Options Cardiovasc Med 2006;8:183–192.
22 DerSimonian R, Laird N: Meta-analysis in clinical trials. Control Clin Trials 1986;7:177–188.
23 Sutton AJ, Abrams KR, Jones DR, Sheldon TA, Song F: Methods for meta-analysis in medical research. New York, Wiley, 2000.
24 Higgins JP, Thompson SG, Deeks JJ, Altman DG: Measuring inconsistency in meta-analysis. BMJ 2003;327:557–560.
25 Higgins JP, Thompson SG: Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21:1539–1558.

26 Patsopoulos NA, Evangelou E, Ioannidis JP: Sensitivity of between-study heterogeneity in meta-analysis: proposed metrics and empirical evaluation. Int J Epidemiol 2008;37:1148–1157.
27 Egger M, Smith GD, Schneider M, Minder C: Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629–634.
28 Nakayama T, Asai S, Sato N, Soma M: Genotype and haplotype association study of the STRK1 region on 5q12 among Japanese: a case-control study. Stroke 2006;37:69–76.
29 Matsushita T, Kubo M, Yonemoto K, Ninomiya T, Ashikawa K, Liang B, Hata J, Doi Y, Kitazono T, Ibayashi S, Iida M, Kiyohara Y, Nakamura Y: Lack of association between variations of PDE4D and ischemic stroke in the Japanese population. Stroke 2009;40:1245–1251.
30 Saleheen D, Bukhari S, Haider SR, Nazir A, Khanum S, Shafqat S, Anis MK, Frossard P: Association of phosphodiesterase 4D gene with ischemic stroke in a Pakistani population. Stroke 2005;36:2275–2277.
31 Lin HF, Liao YC, Liou CW, Liu CK, Juo SH: The phosphodiesterase 4D gene for early onset ischemic stroke among normotensive patients. J Thromb Haemost 2007;5:436–438.
32 Banerjee I, Gupta V, Ahmed T, Faizaan M, Agarwal P, Ganesh S: Inflammatory system gene polymorphism and the risk of stroke: a case-control study in an Indian population. Brain Res Bull 2008;75:158–165.
33 Munshi A, Babu MS, Kaul S, Shafi G, Anila AN, Alladi S, Jyothy A: Phosphodiesterase 4D (PDE4D) gene variants and the risk of ischemic stroke in a South Indian population. J Neurol Sci 2009;285:142–145.
34 Xue H, Wang H, Song X, Li W, Sun K, Zhang W, Wang X, Wang Y, Hui R: Phosphodiesterase 4D gene polymorphism is associated with ischaemic and haemorrhagic stroke. Clin Sci (Lond) 2009;116:335–340.
35 Sun Y, Huang Y, Chen X, Liu Y, Lu X, Shi Y, Tang W, Yang J, Chen W, Zhao X, Gao L, Li S, Feng G, He L: Association between the PDE4D gene and ischaemic stroke in the Chinese Han population. Clin Sci (Lond) 2009;117:265–272.
36 Song Q, Cole JW, O'Connell JR, Stine OC, Gallagher M, Giles WH, Mitchell BD, Wozniak MA, Stern BJ, Sorkin JD, McArdle PF, Naj AC, Xu Q, Gibbons GH, Kittner SJ: Phosphodiesterase 4D polymorphisms and the risk of cerebral infarction in a biracial population: the Stroke Prevention in Young Women Study. Hum Mol Genet 2006;15:2468–2478.
37 Brophy VH, Ro SK, Rhees BK, Lui LY, Lee JM, Umblas N, Bentley LG, Li J, Cheng S, Browner WS, Erlich HA: Association of phosphodiesterase 4D polymorphisms with ischemic stroke in a US population stratified by hypertension status. Stroke 2006;37:1385–1390.
38 Zee RY, Brophy VH, Cheng S, Hegener HH, Erlich HA, Ridker PM: Polymorphisms of the phosphodiesterase 4D, cAMP-specific (PDE4D) gene and risk of ischemic stroke: a prospective, nested case-control evaluation. Stroke 2006;37:2012–2017.
39 Meschia JF, Brott TG, Brown RD Jr, Crook R, Worrall BB, Kissela B, Brown WM, Rich SS, Case LD, Evans EW, Hague S, Singleton A, Hardy J: Phosphodiesterase 4D and 5-lipoxygenase activating protein in ischemic stroke. Ann Neurol 2005;58:351–361.
40 Woo D, Kaushal R, Kissela B, Sekar P, Wolujewicz M, Pal P, Alwell K, Haverbusch M, Ewing I, Miller R, Kleindorfer D, Flaherty M, Chakraborty R, Deka R, Broderick J: Association of phosphodiesterase 4D with ischemic stroke: a population-based case-control study. Stroke 2006;37:371–376.
41 Kuhlenbaumer G, Berger K, Huge A, Lange E, Kessler C, John U, Funke H, Nabavi DG, Stogbauer F, Ringelstein EB, Stoll M: 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;77:521–524.
42 van Rijn MJ, Slooter AJ, Schut AF, Isaacs A, Aulchenko YS, Snijders PJ, Kappelle LJ, van Swieten JC, Oostra BA, van Duijn CM: Familial aggregation, the PDE4D gene, and ischemic stroke in a genetically isolated population. Neurology 2005;65:1203–1209.
43 Nilsson-Ardnor S, Wiklund PG, Lindgren P, Nilsson AK, Janunger T, Escher SA, Hallbeck B, Stegmayr B, Asplund K, Holmberg D: Linkage of ischemic stroke to the pde4d region on 5q in a Swedish population. Stroke 2005;36:1666–1671.
44 Kostulas K, Gretarsdottir S, Kostulas V, Manolescu A, Helgadottir A, Thorleifsson G, Gudmundsson LJ, Thorsteinsdottir U, Gulcher JR, Stefansson K, Hillert J: PDE4D and ALOX5AP genetic variants and risk for ischemic cerebrovascular disease in Sweden. J Neurol Sci 2007;263:113–117.
45 Fidani L, Clarimon J, Goulas A, Hatzitolios AI, Evans W, Tsirogianni E, Hardy J, Kotsis A: Association of phosphodiesterase 4D gene G0 haplotype and ischaemic stroke in a Greek population. Eur J Neurol 2007;14:745–749.