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ARTICLE

# Ischaemic stroke in hypertensive patients is associated with variations in the *PDE4D* genome region

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**Previous Icelandic studies reported that single nucleotide polymorphisms (SNPs) in the phosphodiesterase 4D (*PDE4D*) region and the 5-lipoxygenase activating protein *ALOX5AP* were associated with ischaemic stroke, whereas other studies reported ambiguous findings. We examined 932 ischaemic stroke patients from a Swedish population-based stroke register, and 396 control subjects. We assessed possible associations between ischaemic stroke and nine preselected SNPs in the chromosome regions of the *PDE4D* gene, including rs12188950 (SNP45) and rs3887175 (SNP39); the *ALOX5AP* gene, including rs17222814 (SG13S25) and the promoter region of the MHC class II transactivator, *MHC2TA*. The T allele of SNP45 showed negative association with ischaemic stroke (odds ratio, OR = 0.72; 95% confidence interval (CI): 0.58–0.91; *P* = 0.0055). Among hypertensive subjects, this influence of the T allele of SNP45, and the T allele of SNP39, were more pronounced (with OR = 0.52; 95% CI: 0.37–0.73; *P* = 0.0001 and OR = 0.57; 95% CI: 0.41–0.79; *P* = 0.0007, respectively). These SNPs also interacted with hypertension with a relative excess risk due to interaction of −1.66 (*P* = 0.0002) for SNP45 and −1.65 (*P* = 0.0005) for SNP39. The *P*-values remained significant after correction for multiple testing. Among nonhypertensives, the A allele of SG13S25 indicated increased stroke risk (OR = 1.82; 95% CI: 1.21–2.74; *P* = 0.0039; not significant after Bonferroni correction). SNP45 was associated with ischaemic stroke even when controlling for hypertension, diabetes, heart disease and smoking. Our meta-analysis of 13 studies (including ours) showed no overall influence of SNP45 on ischaemic stroke. However, the 13 studies may differ because of nonrandom causes, as suggested by the heterogeneity test (*P* = 0.042). This might support previously undetected mechanisms causing fluctuating ischaemic stroke risk.**
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## Introduction

Two previous studies of an Icelandic population used a genomewide approach to search for stroke susceptibility loci and reported an association between single nucleotide polymorphisms (SNPs) in the chromosome regions of phosphodiesterase 4D (*PDE4D*) and 5-lipoxygenase activating protein (*ALOX5AP*) and stroke.[1,2] The study which

showed the most convincing association, suggested that the *PDE4D* region of chromosome 5q12 may be associated with stroke related to atherosclerosis. However, other studies attempting to replicate this finding in different populations have shown conflicting results.[3–15] We therefore aimed to further evaluate possible associations between ischaemic stroke and five SNPs in the 5′ region of *PDE4D* on chromosome 5, including rs12188950 (SNP45) that showed the strongest association in the original report.

We also examined three SNPs in the region encoding *ALOX5AP* on chromosome 13. *ALOX5AP* encodes the enzyme FLAP which regulates production of leukotriene inflammatory mediators, shown to be of importance in atherogenesis and plaque rupture.[2,11,12]

Additionally we assesssed one SNP located in one of the promoters of the gene encoding the major histocompatibility complex class II transactivator (*MHC2TA*) on chromosome 16. *MHC2TA* encodes a protein involved in regulation of expression of the antigen presenting molecule MHC class II, that is, associated with increased susceptibility to myocardial infarction. *MHC2TA* has previously been reported as a genetic marker for diseases with inflammatory aetiology, including myocardial infarction and subsequently an increased risk of cardiovascular mortality.[16,17] *MHC2TA* has not to our knowledge previously been examined for association with ischaemic stroke, although cardiovascular risk and ischaemic stroke are both vascular diseases.

In two previous genetic association studies, hypertension was considered as a confounder when assessing a possible influence of the *PDE4D* region on ischaemic stroke.[3,15] *PDE4D* is an enzyme which selectively degrades cAMP and is expressed in multiple tissues including vascular smooth muscle cells (VSMC).[18] Pharmacologic studies have shown that selected *PDE* inhibitors reduce proliferation of VSMC[19] providing a potential mechanism behind the influence of hypertension on the effect of *PDE4D*. However, one study did not reveal any clearly significant interaction pattern between hypertension and *PDE4D* SNPs on ischaemic stroke risk.[15] Nevertheless, we considered the sample size of our data to be large enough to in more detail assess the possible role of hypertension.

We therefore wanted to perform a large study regarding *PDE4D*, *ALOX5AP* and *MHC2TA* on ischaemic stroke, including interaction with hypertension.

## Materials and methods

### Population, sample and data collection

A total of 932 first-ever ischaemic stroke patients, included between 1 March 2001 and 30 September 2004 in the Lund Stroke Register (LSR), participated in this study. LSR is a prospective, population-based epidemiologic register that consecutively includes all patients with first-ever stroke from the catchment area of Lund University Hospital ($N = 234505$ inhabitants on 31 December 2001). We also included 396 control subjects without stroke in the same geographic area, randomly selected from the official Swedish Population Register with similar age and gender distribution as the patients in LSR. Patients were detected at both hospital and primary care facilities. Vascular risk factors including hypertension, heart disease, diabetes mellitus and smoking (definitions – see below) were collected for patients and control subjects in a similar way. Blood samples for DNA extraction were collected from patients with ischaemic stroke who had agreed (personally or through their next of kin) to participate in the study and from control subjects. The median age was 76 years (range: 17–102 years), 75% of the subjects were above 66 years and 44% were women. The local Ethics Committee approved the study.

### Definition of ischaemic stroke

The diagnosis of stroke was made using WHO criteria.[20] All patients in this study had CT or MR scan or autopsy findings compatible with ischaemic stroke.

### Definitions of intermediate phenotypes

The following phenotype data regarding risk factors were registered for ischaemic stroke patients as well as for control subjects: hypertension – medical treatment for hypertension, or blood pressure 160/90 mm Hg or higher at the time of discharge or after at least 1 week of hospitalization; heart disease – diagnosis of angina pectoris, myocardial infarction, congestive heart failure, atrial fibrillation/flutter, medical treatment for heart disease or history of cardiac surgery; diabetes mellitus – dietary or medical treatment for diabetes mellitus or measurements at discharge or at least 1 week of hospitalization as follows: blood glucose 6.1 mmol/l or higher at two occasions, plasma glucose 7.0 mmol/l or higher at two occasions (or more than 11 mmol/l at one occasion with concurrent symptoms suggestive of diabetes mellitus). Smoking was defined through self-report as: current smoking (current smokers *versus* previous smokers and nonsmokers) and never started smoking (nonsmokers *versus* previous smokers and current smokers).[21]

### Genotyping

DNA was extracted from EDTA-treated venous blood samples. Genotyping was performed using the fluorogenic 5′ nuclease assay on an ABI 7900HT real-time PCR System. Probe and primer sequences are available on request. All laboratory analyses were performed blind to phenotype and identity at the Genotyping Lab, SWEGENE Resource Center for Profiling Polygenic Disease, Lund University, Malmö University Hospital, Malmö, Sweden. Data were stored at the Bioinformatics Unit at the same facility.

We directly typed the SNPs in the *PDE4D* gene region with the most significant stroke association described in the previous Icelandic study,[1] rs2910829 (SNP87) and rs12188950 (SNP45) as well as the additional SNPs rs3887175 (SNP39), rs26956 (SNP37) and rs27653 (SNP34). The SNPs in the *ALOX5AP* region were selected to optimise the genetic information for the haplotype blocks covering the gene: rs17222814 (SG13S25), rs17222772 and rs17222828 were selected. Finally, we selected rs3087456, located in one of the promoters of the *MHC2TA* gene, reported to be associated with diseases with inflammatory components.[16,17] We examined our results regarding haplotype structures at the *PDE4D* locus using the Solid Spine of LD definition in Haploview[22] (Treshold of LD: $D' > 0.8$). We also compared our data with all SNPs showing significant association in the initial deCODE report: rs12153798 (SNP41), rs12188950 (SNP45), rs966221 (SNP83), rs2910829 (SNP87) and rs1396476 (SNP89)[1] using HapMap data[23] (CEPH sample, release 21, NCBI B35 assembly, dBSNP b125). SNP56 also showed significant association in the deCODE report[1] but was excluded as it is not genotyped in HapMap.

### Data analysis and statistical methods

We used allelic association tests to compare patients and control subjects regarding allelic variation in each of the nine SNPs in our study. Odds ratios (OR) of the minor (alternative) allele over the major (reference) allele in cases and controls were thus estimated and are presented with 95% confidence intervals (CIs). In the same way we assessed the SNPs' association with ischaemic stroke considering hypertensive and nonhypertensive subjects separately. We also assessed possible interaction of each SNP with hypertension on ischaemic stroke by calculating the relative excess risk due to interaction (RERI).[24] The RERI estimates can be interpreted as follows: a positive value indicates an increased ischaemic stroke risk caused by the minor allele of a SNP among hypertensives compared to nonhypertensives, whereas a negative value suggests an increased ischaemic stroke risk caused by the minor allele of a SNP among nonhypertensives compared to hypertensives. Furthermore, we performed multiple logistic regression analyses of patients and control subjects on SNP45 T-allele variation and intermediate phenotypes (hypertension, diabetes mellitus, heart disease and smoking) to assess the effects of these covariates simultaneously. An additional multiple logistic regression model also took interaction between SNP45 and hypertension into consideration.

When performing the allelic association tests and the RERI estimate calculations, we considered each allele as a sample unit, while the multiple logistic regression of the vascular risk suggestible phenotypes was performed regarding each patient or control subject as a sample unit. In the logistic regression analyses, SNP45 was defined as a categorical variable (as explained in the Table 4 footnote).

All significance tests were two-tailed, and the $\alpha$-level was set to 0.05. *P*-values above 0.10 are omitted in the tables. All CIs and *P*-values of estimated ORs were calculated using the Wald's approximation. We used SPSS statistical software (version 15.0) for our analyses.

Our analyses of SNP45 were preceded by the formulation of well-founded hypotheses in accordance with the Icelandic report,[1] while the remaining eight SNPs were assessed in a more explorative manner. Therefore, we adjusted the resulting *P*-values for mass significance using the Bonferroni method. We adjusted the critical $\alpha$-value, $\alpha = 0.05$, by dividing it by the number of compared SNPs. Hence, we calculated a Bonferroni-corrected $\alpha$-value of $0.05/9 = 0.0056$ to be used for rejecting the null-hypotheses of the SNP analyses presented in Table 2, and the RERI analyses in Table 3. To correct the 18 compared ORs in Table 3 (presenting two subgroups with nine SNPs each), we calculated a Bonferroni-corrected $\alpha$-value of $0.05/18 = 0.0028$.

We also performed a random effects (DerSimonian–Laird) meta-analysis with the intention to examine the effect of SNP45 on ischaemic stroke risk in a large population providing comparable genetic information. Considering our and the Icelandic *PDE4D* study, we examined reviews regarding Caucasian people. All studies published after the initial deCODE report in October 2003 on *PDE4D* and ischaemic stroke were considered, detected by searching of the terms '*PDE4D*', 'Phosphodiesterase 4D' and 'STRK1'.[1,3,5–7,9,11–15,25] We subsequently reviewed all abstracts as well as all reference lists of included articles. We then performed the meta-analysis comprising 13 studies (including ours) with a total of 6221 ischaemic stroke patients and 6750 control subjects from Caucasian populations.

## Results

Data regarding personal and phenotype characteristics about patients and control subjects are described in Table 1, while results from the allelic association analyses are presented in Tables 2 and 3. All nine SNPs were in Hardy–Weinberg equilibrium (with *P*-values of 0.16 and above).

We formed a haplotype block with four of our SNPs: rs12188950 (SNP45), rs3887175 (SNP39), rs26956 (SNP37) and rs27653 (SNP34), when we examined the haplotype structure of our data. This is shown in Figure 2. One SNP from the Icelandic study, rs12153798 (SNP41), was linked to that haplotype block according to HapMap data (data not shown). SNPs rs966221 (SNP83) and rs1396476 (SNP89) from the Icelandic study (data not shown), and SNP rs2910829 (SNP87) from our study, were not part of the haplotype block due to shortage in linkage disequilibrium with the other SNPs.

**Table 1** Characteristics of 932 patients and 396 control subjects concerning data regarding personal and phenotype characteristics

| | *Patients (mean age = 73.6 years, SD = 11.5)* | | *Control subjects (mean age = 73.6 years, SD = 11.9)* | | |
|---|---|---|---|---|---|
| *Background and phenotype data* | *%* | *Frequency* | *%* | *Frequency* | P-*value** |
| Gender (proportion of women) | 44.0 | 410 | 43.2 | 171 | NS |
| Age above 75 years | 52.0 | 485 | 52.0 | 206 | NS |
| Age above 65 years | 76.2 | 710 | 78.8 | 312 | NS |
| Age above 55 years | 93.1 | 868 | 93.2 | 369 | NS |
| Hypertension | 61.9 | 576 | 33.6 | 133 | $<0.0001$ |
| Diabetes mellitus | 20.9 | 195 | 6.3 | 25 | $<0.0001$ |
| Heart disease | 41.2 | 384 | 26.0 | 103 | $<0.0001$ |
| Current smokers | 19.3 | 178 | 9.1 | 36 | $<0.0001$ |
| Never started smoking | 49.7 | 457 | 51.6 | 204 | NS |

Missing values: hypertension: one patient; current smoking and never started smoking: 12 patients and one control subject.*$P$-values obtained by $\chi^2$-tests to examine possible differences between patients and control subjects. $P$-values are denoted NS if $P>0.10$.

**Table 2** Prevalence of SNPs among patients with ischaemic stroke and control subjects

| *SNP* | *Major/ minor allele* | *Number of non-missing individuals: patients, controls* | *Number of minor alleles in patients, controls* | *Allelic Association test patients/control subjects OR (95% CI)* | P-*value** |
|---|---|---|---|---|---|
| *PDE4D* | | | | | |
| rs2910829 (SNP87) | T/C | 929, 394 | 847, 352 | 1.04 (0.88–1.23) | NS |
| rs12188950 (SNP45) | C/T | 929, 395 | 245, 137 | 0.72 (0.58–0.91) | 0.0055** |
| rs3887175 (SNP39) | A/T | 912, 390 | 314, 160 | 0.81 (0.65–1.00) | 0.0460 |
| rs26956 (SNP37) | G/T | 925, 292 | 543, 231 | 1.00 (0.83–1.20) | NS |
| rs27653 (SNP34) | G/T | 925, 393 | 795, 331 | 1.04 (0.87–1.23) | NS |
| *ALOX5AP* | | | | | |
| rs17222814 (SG13S25) | G/A | 928, 393 | 182, 66 | 1.19 (0.88–1.59) | NS |
| rs17222772 | T/A | 926, 394 | 610, 235 | 1.16 (0.96–1.38) | NS |
| rs17222828 | C/A | 923, 394 | 891, 369 | 1.06 (0.90–1.25) | NS |
| *MHC2TA* | | | | | |
| rs3087456 | A/G | 927, 391 | 487, 223 | 0.89 (0.74–1.08) | NS |

Abbreviation: SNP, single nucleotide polymorphism.
SNPs are ordered after position (in base pair units) within each chromosome region. SNP denotations within parentheses indicate SNP identity according to references.[1,2]
*$P$-values are denoted NS if $P>0.10$.
**$P$-value significant even after Bonferroni correction of 9 comparisons (ie 9 compared SNPs) providing a critical value of $\alpha=0.0056$.

The T allele of SNP45 had a risk-reducing effect regarding ischaemic stroke (OR: 0.72; 95% CI: 0.58–0.91; $P=0.0055$), as shown in Table 2. This $P$-value remained significant after correction for multiple testing. A weak similar association was also found for the T allele of SNP39 (OR: 0.81; 95% CI: 0.65–1.00; $P=0.046$), which became not significant after correction for multiple testing. No significant associations were observed between overall ischaemic stroke and SNPs in *ALOX5AP* or *MHC2TA*.

When assessing a subset of subjects below the median age of 76 years we only found modest changes in results. The T allele of SNP45 still had a significant risk-reducing effect (OR: 0.64; 95% CI: 0.47–0.89; $P=0.0074$) while SNP39 was not significantly associated with ischaemic stroke (OR: 0.84; 95% CI: 0.62–1.13; $P=0.0765$). Neither of these two SNPs showed significant association with ischaemic stroke after correction for multiple testing. All the other SNPs were not associated with ischaemic stroke for subjects under 76 years of age (data not shown).

When analysing hypertensive and nonhypertensive subjects separately, there were tangible differences in the allelic associations between these two groups (Table 3). The T allele of SNP45 was significantly related to decreased ischaemic stroke risk in the hypertensive group (OR: 0.52; 95% CI: 0.37–0.73; $P=0.0001$) but not among nonhypertensive subjects (OR: 0.95; 95% CI: 0.69–1.30; $P>0.10$). Similarly, the T allele of SNP39 showed a reduced stroke risk among hypertensive subjects (OR: 0.57; 95% CI: 0.41–0.79; $P=0.0007$) but not among nonhypertensive subjects (OR: 1.08; 95% CI: 0.81–1.43; $P>0.10$). Correction for multiple testing did not change any conclusion regarding SNP45 and SNP39. Additionally, the A allele of SG13S25 in

**Table 3** Prevalence of SNPs among ischaemic stroke patients and control subjects. Hypertensive and non-hypertensive subjects considered separately

| | | *Hypertensive patients/control subjects* | | | | *Non-hypertensive patients/control subjects* | | | | *RERI estimate* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SNP | *Major/ minor allele* | *Number of non-missing individuals: patients, controls* | *Number of minor alleles in patients, controls* | *OR (95% CI)* | P-*value** | *Number of non-missing individuals: patients, controls* | *Number of minor alleles in patients, controls* | *OR (95% CI)* | P-*value** | Est. RERI** | P-*value** |
| *PDE4D* | | | | | | | | | | | |
| rs2910829 (SNP87) | T/C | 575, 133 | 511, 130 | 0.84 (0.64–1.09) | NS | 353, 261 | 336, 222 | 1.23 (0.98–1.54) | 0.0783 | −0.85 | 0.0952 |
| rs12188950 (SNP45) | C/T | 575, 133 | 142, 57 | 0.52 (0.37–0.73) | 0.0001*** | 353, 262 | 103, 80 | 0.95 (0.69–1.30) | NS | −1.66 | 0.0002**** |
| rs3887175 (SNP39) | A/T | 565, 131 | 179, 65 | 0.57 (0.41–0.79) | 0.0007*** | 346, 259 | 135, 95 | 1.08 (0.81–1.43) | NS | −1.65 | 0.0005**** |
| rs26956 (SNP37) | G/T | 571, 132 | 347, 74 | 1.12 (0.83–1.51) | NS | 353, 261 | 195, 157 | 0.89 (0.69–1.14) | NS | 0.47 | NS |
| rs27653 (SNP34) | G/T | 570, 133 | 496, 99 | 1.30 (0.99–1.71) | 0.0619 | 354, 260 | 298, 232 | 0.90 (0.72–1.14) | NS | 0.91 | 0.0484 |
| *ALOX5AP* | | | | | | | | | | | |
| rs17222814 (SG13S25) | G/A | 574, 132 | 98, 30 | 0.73 (0.47–1.12) | NS | 353, 261 | 84, 36 | 1.82 (1.21–2.74) | 0.0039 | −1.78 | 0.0127 |
| rs17222772 | T/A | 572, 132 | 391, 75 | 1.31 (0.98–1.76) | 0.0731 | 353, 262 | 218, 160 | 1.02 (0.80–1.30) | NS | 0.90 | NS |
| rs17222828 | C/A | 570, 133 | 541, 132 | 0.92 (0.70–1.20) | NS | 352, 261 | 349, 237 | 1.18 (0.94–1.48) | NS | −0.48 | NS |
| *MHC2TA* | | | | | | | | | | | |
| rs3087456 | A/G | 573, 131 | 304, 71 | 0.97 (0.72–1.31) | NS | 353, 260 | 182, 152 | 0.84 (0.65–1.08) | NS | 0.07 | NS |

Abbreviations: RERI, relative excess risk due to interaction; SNP, single nucleotide polymorphism.
**P*-values are denoted NS if $P > 0.10$.
**A negative RERI indicates a stronger risk-reducing effect of the minor allele on ischaemic stroke among hypertensives compared to non-hypertensives.
****P*-value significant even after Bonferroni correction of 18 comparisons (ie, 18 compared SNPs in two compared subsets) providing a critical value of $\alpha = 0.0028$.
*****P*-value significant even after Bonferroni correction of 9 comparisons (ie, 9 compared SNPs) providing a critical value of $\alpha = 0.0056$.

**Table 4** Multiple logistic regression analysis of SNP45 (rs12188950) with intermediate risk factors affecting prevalence of ischaemic stroke

| | *No interaction term included* | | | *Interaction term (SNP45 × Hypertension) included* | | |
|---|---|---|---|---|---|---|
| | *Estimated β-Coefficient* | *Estimated OR* | P-*value** | *Estimated β-Coefficient* | *Estimated OR* | P-*value** |
| SNP45** | — | — | 0.0329 | — | — | NS |
| Heterozygote CT or TC allele indicator | −0.334 | 0.716 | 0.0247 | −0.080 | 0.923 | NS |
| Homozygote TT allele indicator | −0.614 | 0.541 | NS | −0.069 | 0.933 | NS |
| Hypertension | 1.069 | 2.912 | <0.0001 | 1.259 | 3.523 | <0.0001 |
| Interaction term: SNP45 × hypertension | | | | — | — | 0.0575 |
| Heterozygote CT or TC allele indicator × hypertension | | | | −0.563 | 0.569 | 0.0562 |
| Homozygote TT allele indicator × hypertension | | | | −1.261 | 0.283 | NS |
| Diabetes mellitus | 1.135 | 3.111 | <0.0001 | 1.128 | 3.090 | <0.0001 |
| Heart disease | 0.624 | 1.865 | <0.0001 | 0.625 | 1.869 | <0.0001 |
| Current smokers | 1.236 | 3.443 | <0.0001 | 1.227 | 3.411 | <0.0001 |
| Never started smoking | 0.262 | 1.300 | 0.0615 | 0.259 | 1.295 | 0.0662 |

Abbreviation: SNP, single nucleotide polymorphism.
*$P$-values are denoted NS if $P > 0.10$.
**SNP45 is treated as a trichotomeous categorical variable (denoting CC homozygote alleles, heterozygote alleles and TT homozygote alleles) in the multiple logistic regression analysis, and is therefore represented by two dichotomeous design variables, the former encoded 1 if the alleles are heterozygotes and 0 otherwise, the latter encoded 1 if the alleles are TT homozygotes and 0 otherwise. The interaction term SNP45 × hypertension is designed in a similar way.

the *ALOX5AP* gene showed an increased risk for ischaemic stroke among nonhypertensives. (OR: 1.82; 95% CI: 1.21–2.74; $P = 0.0039$). This result was not significant when correcting for multiple testing.

The negative estimated RERI values in Table 3 indicated a decreased ischaemic stroke risk among hypertensives in relation to nonhypertensives for the minor alleles of SNP45 (RERI = −1.66; $P = 0.0002$), SNP39 (RERI = −1.65; $P = 0.0005$) and SG13S25 (RERI = −1.78; $P = 0.013$). An enhanced ischaemic stroke risk among hypertensives in relation to nonhypertensives (indicated by a positive RERI estimate) was noticed for SNP34 (RERI = 0.91; $P = 0.048$). The RERIs of SNP45 and SNP39 remained significant when corrected for multiple testing.

The multiple logistic regression analysis of SNP45 is presented in Table 4 (two different models are shown). There was a significant difference between CC homozygotes, heterozygotes and TT homozygotes regarding the effect on ischaemic stroke risk, even when controlling for the effect of hypertension, diabetes mellitus, heart disease, current smoking and never started smoking simultaneously. When considering the SNP45 × hypertension interaction term, the SNP45 main effect factor was nonsignificant ($P > 0.10$) while the interaction term was nonsignificant with $P = 0.0575$. However, interaction terms expressed as a product of two categorical variables are difficult to interpret due to computational problems.

The outcome of the meta-analysis is presented in Figure 1. There was a significant risk-reducing effect of SNP45 only in our and the Icelandic study,[1] a similar nonsignificant tendency in four other studies,[11,13,14,25] and a nonsignificant tendency to increased risk in the remaining seven studies.[3,5–7,9,12,15] The overall OR: 0.95 (95% CI: 0.86–1.05) indicates no significant overall effect in the 13 studies. However, the heterogeneity test ($P = 0.042$) suggests that the ORs of the 13 studies differ due to other reasons than random variations.

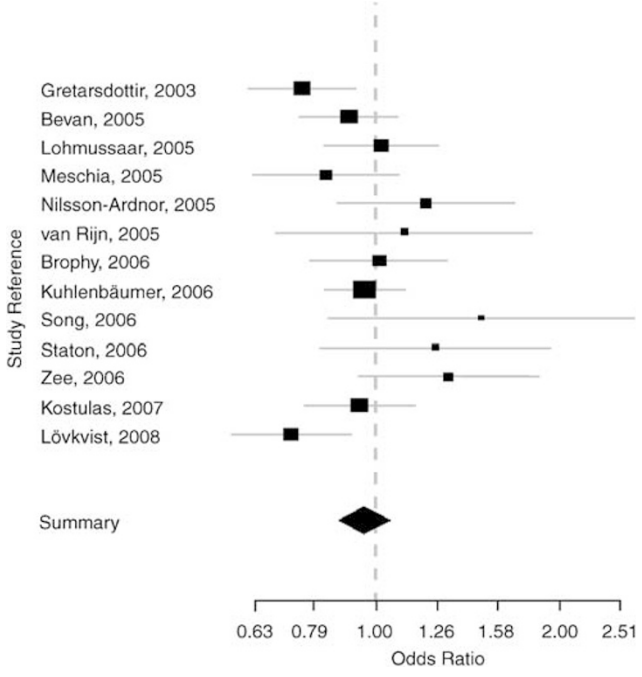


**Figure 1** Random effects (DerSimonian–Laird) meta-analysis of published studies examining the association between the T allele of SNP45 and ischaemic stroke in 6221 Caucasian patients and 6750 controls. Overall OR: 0.95 (95% CI: 0.86–1.05), test for heterogeneity $\chi^2 = 21.6$ ($P = 0.0419$).

## Discussion

Our findings revealed that the T allele of SNP45 in the *PDE4D* region was associated with a decreased ischaemic stroke risk among individuals in southern Sweden. This effect was more substantial for individuals with hypertension. The T allele of SNP39, being in LD with SNP45 ($R^2 = 0.63$ as shown in Figure 2), showed similar results. These findings put a new perspective on the association between ischaemic stroke and the *PDE4D* region and propose the involvement of SNP45 regarding the pathogenesis of ischaemic stroke, especially among hypertensive individuals. However, conclusions regarding genetic markers closely linked to stroke-related SNPs should be handled cautiously, as stated in a comment by Rosand *et al*.[26] We also found that the A allele of SG13S25 in the *ALOX5AP* gene may confer an increased risk of ischaemic stroke among nonhypertensives.

We selected the SNPs in our study to correspond to those selected by Gretarsdottir.[1] However, because of limited resources, we performed a selection of a limited number of SNPs that we considered to provide the best information regarding genetic variation in the part of interest of the examined genes. We subsequently performed a haplotype analysis in which we included the five SNPs in the *PDE4D* gene analysed in our study. Two other SNPs, rs966221 (SNP83) and rs12153798 (SNP41) were analysed in the study by Gretarsdottir *et al*,[1] but not in ours. Nevertheless, according to HapMap data (CEPH sample, release 21, NCBI B35 assembly, dBSNP b125), both rs966221 (SNP83) and rs12153798 (SNP41) studied by Gretarsdottir *et al*, are in LD with our typed variants rs2910829 (SNP87) and rs12188950 (SNP45), $R^2 = 0.59$ and 0.94, respectively. Three SNPs, rs3887175 (SNP39), rs26956 (SNP37) and rs27653 (SNP34) were not reported in Gretarsdottir's study but were analysed by us. We selected these SNPs due to their 'strategic' position at the 5′ end of *PDE4D*, which also is shown by the haplotype block structure formed (Figure 2), when using a cut-off point of $D' = 0.80$.

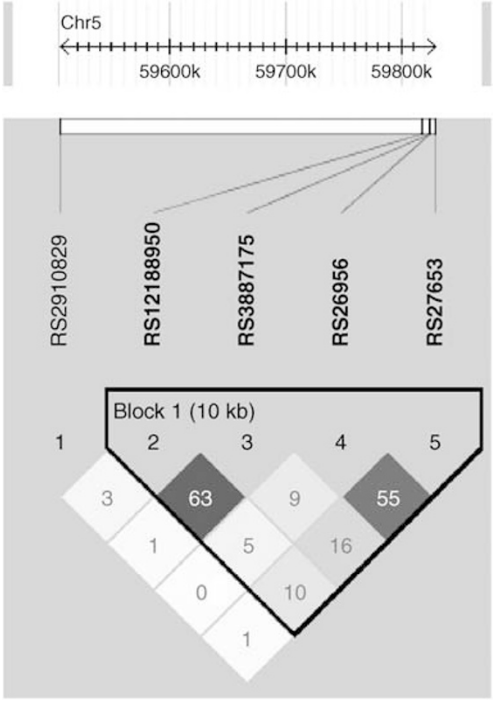


**Figure 2** Linkage disequilibrium plot of the five SNPs from the 5′ end of the *PDE4D* gene in chromosome 5, included in our analysis. The analysis is made by using our study data, and the block formation is based upon $D'$ values above 80% ($D'$ values are not shown). $R^2$ values of linkage disequilibrium are displayed within the squares.

Our examination of a hypertensive factor (by stratification and by analysing the interaction between selected SNPs and hypertension) raises an important issue: will some selected genetic markers have a 'protective' (or conversely, an 'enhancing') effect on ischaemic stroke, which is stronger among hypertensive than among nonhypertensive subjects? Our analyses confirmed the hypothesis regarding a protective effect of SNP39 and SNP45 in the *PDE4D* gene against ischaemic stroke when considering a Scandinavian population.

The intermediate phenotypes hypertension, diabetes mellitus, heart disease and smoking habits are important indicators for ischaemic stroke (Table 1) and may confound the effect of the T allele of SNP45. All these phenotypes (except the indicator for never smokers) are strongly significant covariates when included with SNP45 in a multiple logistic regression model with ischaemic stroke (patients *versus* control subjects) as the response variable (Table 4). However, the noninteractive alternative of our multiple logistic regression model showed a persistent association between ischaemic stroke and SNP45, even when controlling for these vascular risk-indicating phenotypes.

When assessing our data (both ischaemic stroke patients and control subjects as one group) with cross tabulations between SNP45 and the intermediate phenotypes we did not find any significant associations, except for smoking habits (ie, current smokers, previous smokers and never smokers conferring $P = 0.05$ when performing a $\chi^2$-test).

Subtyping of our ischaemic stroke patients would have been valuable, especially because carotid and cardiogenic stroke are related to atherosclerosis. However, TOAST classifications of our patients were not available. It would also have been of interest with an analysis of the microsatellite AC008818-1, which was presented in the report by Gretarsdottir *et al*. This was not done because of technical difficulties.

### Comparisons with other studies

According to our meta-analysis, previous published reports seem to have shown equivocal results regarding the association between ischaemic stroke and SNP45. Two other studies, not included in the meta-analysis, found SNP45 to be monomorphic in a biracial North American

population[4] and in a Japanese population,[10] of which the latter is consistent with HapMap data. Apart from the possibility of publication bias, the divergence regarding the studies in the meta-analysis might reflect hitherto unknown gene–gene or gene–environment interactions that differ between populations. This may be explained by variations between the study populations, including age, distribution of males and females, appearance of hypertension, other vascular risk factors, and cultural and ethnic factors.

In Brophy's study,[15] hypertensive and nonhypertensive subjects were analysed separately, suggesting a possible tendency towards decreased risk of SNP45 on ischaemic stroke in the former group and an increased risk in the latter. This is in accordance with our study although the results presented by Brophy *et al*, regarding SNP45 were not significant.

It is possible that SNP45 is not itself the causal variant but is in linkage disequilibrium with the actual causal variant, and that the level of linkage disequilibrium varies between populations. Indeed, the studies have shown a tendency towards a protective role of SNP45 against ischaemic stroke among Scandinavians, who might be more similar in patterns of linkage disequilibrium than other populations. Further studies in the chromosome region of *PDE4D* in Scandinavians as well as in other populations may help to further clarify the role of *PDE4D* in ischaemic stroke.

## Concluding remarks

Our study of 1328 individuals indicates that the T alleles of SNP45 and SNP39 in the *PDE4D* genome region are ischaemic stroke risk reducers among Scandinavians. Thus, selected genotypes in the *PDE4D* gene might act as inhibitors regarding ischaemic stroke risk, especially among individuals with hypertension.

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## Disclosure

The authors report no conflict of interest.

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