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ARTICLE

A large-sample assessment of possible association between ischaemic stroke and rs12188950 in the *PDE4D* gene

Håkan Lövkvist[*,1,6], Sandra Olsson[2], Peter Höglund[3,6], Olle Melander[4,6], Christina Jern[2], Marketa Sjögren[4,6], Gunnar Engström[4,6], J Gustav Smith[4,6], Bo Hedblad[4,6], Gunnar Andsberg[1,6], Hossein Delavaran[1,6], Katarina Jood[2], Ulf Kristoffersson[5,6], Holger Luthman[4,6], Bo Norrving[1,6] and Arne Lindgren[1,6]

Previous reports have shown ambiguous findings regarding the possible associations between ischaemic stroke (IS) and single nucleotide polymorphisms (SNPs) in the phosphodiesterase 4D (*PDE4D*) gene region. The SNP rs12188950 (or SNP45) has often been studied in this context. We performed a multi-centre study involving a large sample of 2599 IS patients and 2093 control subjects from the south and west regions of Sweden to replicate previous studies regarding IS risk and rs12188950. Subjects from Lund Stroke Register (LSR), Malmö Diet and Cancer Study (MDC) and Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS) were enroled. Subgroups of participants with hypertension and participants <55 years of age, as well as the TOAST subgroups large vessel disease, small vessel disease and cardioembolism, were also assessed. Univariate odds ratios (ORs) and ORs controlling for hypertension, diabetes and current smoking were calculated. We additionally performed a meta-analysis including 10 500 patients and 10 102 control subjects from 17 publications (including the present study). When assessing pooled data from LSR, MDC and SAHLSIS we obtained no association between IS and rs12188950 for all participants (OR=0.93; 95% confidence interval (CI): 0.83–1.05). Significant associations were not found for hypertensive participants or participants with age <55, or when separately evaluating patients from the three different TOAST subgroups. The meta-analysis showed no significant overall estimate (OR=0.96; 95% CI: 0.89–1.04) with significant heterogeneity for random effect (*P*=0.042). No effect from rs12188950 on IS was found from either our pooled multi-centre data or the performed meta-analysis. We did not find any association between the examined subgroups and rs12188950 either.

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INTRODUCTION

Stroke is a major cause of death and functional disability in industrialised countries. The clinical presentation of stroke includes intracranial bleedings as well as cerebral diseases with a cardiovascular aetiology. A genetic contribution to stroke risk, also for common ischaemic stroke (IS) subtypes, is considered likely, but results have been equivocal.

Two previous reports, one from an Icelandic study in 2003 and one reported by us in 2008, suggested that the minor allele in the single nucleotide polymorphism (SNP) in rs12188950 (also denoted SNP45), located at the 5′ end of the phosphodiesterase 4D (*PDE4D*) gene in chromosome 5, might have an inhibiting effect on IS risk.[1,2] Our previous study also showed a significant inhibiting effect of rs12188950 minor (T) allele on IS among subjects with hypertension.[2] However, a meta-analysis in our previous publication in 2008 showed ambiguous results concerning the possible association between IS risk and variants in rs12188950.[3–12]

We were aware of three replicating studies, that showed significant allelic association with IS by detecting and examining haplotype blocks located at the 5′ end region of the *PDE4D* gene.[4,13,14] Moreover, a meta-analysis including four studies indicated significantly increased IS risk when considering an at-risk haplotype, including rs12188950.[15] These findings suggest that rs12188950, as a polymorphism located in the centre of the 5′ end region of *PDE4D*, might be particularly interesting for further study to confirm or refute a relation to IS.

In the present multi-centre study involving three case–control groups from the west and south of Sweden, we intended to test for replication of previous findings regarding rs12188950 by assessing a large number of individuals. We also updated our previous meta-analysis by including new publications and our new results regarding the subject of the possible association between rs12188950 and IS. We focused on possible discrepancies between included populations and subpopulations because of sex, nationality or ethnicity, rather than discrepancies between the publications themselves in this updated meta-analysis.

[1]Department of Clinical Sciences, Neurology, Lund University, Lund, Sweden; [2]Institute of Neuroscience and Physiology, Department of Clinical Neuroscience and Rehabilitation, The Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden; [3]Department of Laboratory Medicine, Clinical Pharmacology, Lund University, Lund, Sweden; [4]Department of Clinical Sciences, Lund University, Malmö, Sweden; [5]Department of Clinical Genetics, Lund University, University and Regional Laboratories, Lund, Sweden; [6]Skåne University Hospital, Lund, Sweden
*Correspondence: H Lövkvist, Competence Centre for Clinical Research, Skåne University Hospital, Lund SE-221 85, Sweden. Tel: +46 46 177932; Fax: +46 46 176085; E-mail: hakan.lovkvist@skane.se

MATERIAL AND METHODS

Study population and sample characteristics

Phenotypic and genotypic data for subjects from Lund Stroke Register (LSR), the Malmö Diet and Cancer study (MDC) and the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS) were included in our analyses. Originally, the total number of individuals consisted of 2682 IS patients and 2139 control subjects. After exclusion of 129 subjects owing to genotyping failure for rs12188950, the remaining 2599 patients and 2093 control subjects were included in our multi-centre study.

Complete characteristics of the data used are described elsewhere.[2,16–21] Briefly, LSR is a prospective, consecutive epidemiological study from which we have included 920 patients with first-ever stroke from the local catchment area of Skåne University Hospital in Lund, and 566 control subjects from the same geographical area, selected randomly after stratification by age and sex to match the patient group (when including intracranial bleedings as well as cerebral infarctions).[2,17] The LSR data was collected between March 2001 and August 2007. In a previous study by us from 2008 we assessed 932 IS patients and 396 control subjects from LSR.[2] In order to perform a replication being independent of assessments made previously, we did not include any of these participants in the current study. However, the results from 2008 were included as a separate study/population in our meta-analysis.

MDC is a prospective population-based study that was initiated in the 1990s as a cohort study at Skåne University Hospital in Malmö, Sweden.[16,21] MDC initially included a total of 28 499 randomly selected men, born between 1923 and 1945, and women, born between 1923 and 1950. Cases with a first stroke after the baseline examination ($n=874$) were matched for age, sex and month of the baseline examination with control subjects ($n=881$) free from stroke at the time of the stroke event of the corresponding patient.

SAHLSIS is a consecutive study comprising patients from four stroke units situated in the west of Sweden. SAHLSIS involves patients presenting with first-ever or recurrent acute IS before reaching the age of 70 years.[18–20] A total of 805 IS patients and 646 controls, recruited between 1998 and 2008 within the SAHLSIS project, were included in this study.

All three above mentioned studies are approved by the ethics committee of the University of Gothenburg (SAHLSIS data) and by the ethics committee of Lund University (LSR and MDC data). All participants or, when necessary, their next of kin provided informed consent before enrolment.

Definition of IS

The diagnosis of IS was determined in accordance with World Health Organization criteria.[22] All patients in the study had computer tomography or magnetic resonance scan, or autopsy findings, compatible with IS. In addition, a classification of IS according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) nomenclature was made for the LSR and SAHLSIS populations. Three main TOAST subgroups of IS were defined: (i) Large vessel disease/large artery atherosclerosis (LVD); (ii) small vessel disease/small artery occlusion (SVD) and iii) cardioembolism or significant aortic plaques (CE).[23] Three patients diagnosed with aortic plaques were included in the LVD group in SAHLSIS.

Definition of intermediate phenotypes

Three risk factors were ascertained for all participants in the study. (1) Hypertension was defined as 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 hospitalisation. (2) Diabetes mellitus was defined as dietary or medical treatment for diabetes mellitus or measurements at discharge or at least 1 week of hospitalisation 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 alternatively more than 11 mmol/l at one occasion with concurrent symptoms suggestive of diabetes mellitus (measurements of hypertension and diabetes mellitus among SAHLSIS cases were performed at 3 months follow-up).[18] (3) Current smoking was defined through self-report (current smokers *versus* previous smokers and never smokers).

For LSR and SAHLSIS, age was defined at first-time stroke onset for patients and at inclusion for control subjects. A nested case–control design was used in MDC. Age at the time of the stroke event was used both for MDC stroke cases

and their corresponding control subjects (age was omitted for 63 control subjects of the MDC owing to the absence of matching patients).

Genotyping

Genotyping of data from the three included populations was performed by matrix-assisted, laser desorption ionisation time-of-flight mass spectrometry on a MASSarray platform with iPLEX genotyping technology (Sequenom, San Diego, CA, USA).[20] All laboratory analyses were performed by the SWEGENE Resource Center for Profiling Polygenic Disease (Skåne University Hospital, Malmö, Sweden), with no access to case/control status or personal identities of the samples. Data were stored at the Bioinformatics Unit at the same facility.

Data analysis and statistical methods

Allelic association tests were used to compare patients and control subjects regarding possible allelic association between variants in rs12188950 and IS. Odds ratio (OR) estimates of the minor (alternative) T allele over the major (reference) C allele against cases and controls are presented with 95% confidence intervals (CI). OR estimates for each subpopulation (LSR, MDC and SAHLSIS) were calculated as well as an overall pooled OR estimate for the total of these subpopulations were joined together. We correspondingly assessed a specific subgroup of 1727 patients and 1056 control subjects with hypertension. Similarly, we defined a subgroup of 382 patients and 321 control subjects below 55 years of age for a separate assessment. We also evaluated a subsample of patients with either SVD, CE or LVD according to TOAST classification criteria compared with control subjects for LSR and SAHLSIS data joined together.[23]

Furthermore, we carried out unweighted and weighted simple logistic regression (LR) as well as weighted multiple LR for individuals genotyped using an additive model counting 0, 1 or 2 minor alleles per individual. Regression weights were created by sex and age (categorized into three age groups: participants below 55 years; participants between 55 and 65 years; and participants 65 years or more). Patients and control subjects of LSR, MDC and SAHLSIS (ie six strata referring to cases/controls in three geographical domains of study) were thus adjusted within each stratum to a weight variable referring to sex and age distribution in uniformity with the age and sex distribution of the entire sample of data.

We performed a goodness-of-fit test (a χ^2 test using one degree of freedom) for possible departure from Hardy–Weinberg equilibrium in control subject data.

Statistical computations were performed by using PASW (SPSS) Statistics (version 18) statistical software (PASW/SPSS software, IBM Corporation, Armonk, NY, USA). Power tests were carried out by using the PS Power and Sample Size Calculations software (version 3.0.14; Department of Biostatistics, Vanderbilt University, Nashville, TN, USA).

Meta-analysis

We searched the electronic PubMed database (using http://www.ncbi.nlm.nih.gov/pubmed/) for studies including the search keywords 'STRK1', 'PDE4D' or 'Phosphodiesterase 4D' in combination with keywords 'Stroke' or 'Cerebrovascular'. All keywords were searched in titles or abstracts of publications issued between January 2003 and August 2011. A total of 58 publications were found and thereafter manually reviewed. We excluded 42 publications owing to the following reasons: 17 publications were studies comprising reviews or meta-analyses; 3 publications were editorials or correspondence articles; 5 publications were association studies excluding rs12188950 because the SNP was found to be monomorphic after being genotyped; 8 were association studies that did not consider rs12188950 at all; whereas 9 publications were not allelic association studies assessing genotypes *versus* IS risk.

After this process, 16 publications, comprising 19 distinguishable populations suitable for the meta-analysis, remained.[1–13,24–26] The meta-analysis thus involved a total of 20 populations from 17 publications when our current study was included.

The 42 publications that remained after the above selection for the meta-analyses were either studies comprising reviews or meta-analyses (11 publications); or editorials or correspondence articles (3 publications); or association studies where rs12188950 was excluded because of non-polymorphic traits

(5 publications), or studies that were not including rs12188950 or in other respects were irrelevant for our meta-analysis (23 publications).

We performed a random effects DerSimonian–Laird meta-analysis, and our intention was to examine the effect of rs12188950 on IS in a large population providing genetic information, as well as comparing different population subgroups regarding the possible association between rs12188950 allelic variation and IS risk. R version 2.12.0 (The R Foundation for Statistical Computing c/o Institute for Statistics and Mathematics, Vienna, Austria) was used for the meta-analysis calculations.

RESULTS

The 2599 IS patients were older than the 2030 control subjects with non-missing values of age (see Table 1). The LSR subpopulation showed a higher rate of men in control subjects than in patients, whereas the SAHLSIS subpopulation conversely showed a higher rate of women. As expected, there were higher prevalences of the vascular risk factors hypertension, diabetes mellitus and current smokers among patients compared with control subjects.

The genotyping success rate for rs12188950 was 98% when considering all data. The genotype distribution in LSR, MDC and SAHLSIS control subject data joined together conformed to Hardy–Weinberg equilibrium ($P=0.072$). According to a power test, using the parameters alpha=0.05; 1-beta=0.8, we found the entire sample size of our data to be sufficient to detect significant OR estimates when true OR values were below 0.84 or above 1.18. For a subgroup of 382 patients and 321 control subjects (covering the number of participants below 55 years of age), a true OR <0.61 or >1.52 would be required for a significant finding.

Univariate association tests

For the entire study data, we did not detect any statistically significant association between IS risk and allelic variants in rs12188950. Moreover, we did not obtain statistically significant ORs when assessing LSR, MDC and SAHLSIS data separately. Separate assessments of hypertensive participants or participants younger than 55 years of age did not show any results with such significance either. When examin-

Table 1 Phenotype characteristics of patients and control subjects included in Lund Stroke Register (LSR), Malmö Diet and Cancer study (MDC) and the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS)

	Patients	Control subjects	P-value[a]
LSR (N=1486)	*N=920*	*N=566*	
Age: mean (±SD)	74.8 (±12.3)	73.9 (±12.4)	0.163[b]
Age: median, min, max	76.7, 18.2, 101.7	76.3, 18.0, 96.4	0.207[c]
Age: non-missing *N*	920	566	
Men: frequency/non-missing *N* (%)	463/920 (50.3)	324/566 (57.2)	0.010
Hypertension: frequency/non-missing *N* (%)	618/896 (69.0)	319/566 (56.4)	<0.001
Diabetes mellitus: frequency/non-missing *N* (%)	256/875 (29.3)	45/562 (8.0)	<0.001
Current smokers: frequency/non-missing *N* (%)	171/907 (18.9)	60/565 (10.6)	<0.001
MDC (N=1755)	*N=874*	*N=881*	
Age: mean (±SD)	69.3 (±7.0)	69.4 (±7.0)	0.680[b]
Age: median, min, max	69.9, 48.4, 82.7	70.1, 48.4, 82.7	0.630[c]
Age: non-missing *N*	874	818[d]	
Men: frequency/non-missing *N* (%)	481/874 (55.0)	476/881 (54.0)	0.701
Hypertension: frequency/non-missing *N* (%)	645/873 (73.9)	513/880 (58.3)	<0.001
Diabetes mellitus: frequency/non-missing *N* (%)	86/874 (9.8)	25/881 (2.8)	<0.001
Current smokers: frequency/non-missing *N* (%)	283/854 (33.1)	185/869 (21.3)	<0.001
SAHLSIS (N=1451)	*N=805*	*N=646*	
Age: mean (±SD)	56.2 (±10.6)	56.0 (±10.4)	0.698[b]
Age: median, min, max	58.8, 18.1, 69.8	58.4, 18.6, 70.4	0.471[c]
Age: non-missing *N*	805	646	
Men: frequency/non-missing *N* (%)	527/805 (65.5)	376/646 (58.2)	0.005
Hypertension: frequency/non-missing *N* (%)	464/794 (58.4)	224/645 (34.7)	<0.001
Diabetes mellitus: frequency/non-missing *N* (%)	150/805 (18.6)	31/644 (4.8)	<0.001
Current smokers: frequency/non-missing *N* (%)	307/801 (38.3)	127/646 (19.7)	<0.001
All subjects in study (N=4692)	*N=2599*	*N=2093*	
Age: mean (±SD)	67.2 (±12.8)	66.4 (±12.7)	0.033[b]
Age: median, min, max	67.6, 18.1, 101.7	67.4, 18.0, 96.4	0.045[c]
Age: non-missing *N*	2599	2030[d]	
Men: frequency/non-missing *N* (%)	1471/2599 (56.6)	1176/2093 (56.2)	0.790
Hypertension: frequency/non-missing *N* (%)	1727/2563 (67.4)	1056/2091 (50.5)	<0.001
Diabetes mellitus: frequency/non-missing *N* (%)	492/2554 (19.3)	101/2087 (4.8)	<0.001
Current smokers: frequency/non-missing *N* (%)	761/2562 (29.7)	372/2080 (17.9)	<0.001

[a]P-values are based on Fisher's exact test if not footnoted otherwise.
[b]Student's *t*-test (with equal variances assumed).
[c]Mann–Whitney non-parametric test.
[d]Age of ischaemic stroke patient-matched control subjects of whom 63 such subjects from MDC data did not match.

Table 2 Association between rs12188950 (SNP45) and prevalence of ischaemic stroke for Lund Stroke Register (LSR), Malmö Diet and Cancer study (MDC) and the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS)

Population/ Subpopulation	Number of non-missing patients/controls	Number of minor alleles in patients/controls[a]	Allelic association test: OR; 95% CI; P-value[b]
All patients and controls			
LSR	920/566	257/177	0.88; 0.71–1.08; 0.219
MDC	874/881	244/248	0.99; 0.82–1.20; 0.923
SAHLSIS	805/646	192/168	0.91; 0.73–1.13; 0.396
Total (LSR, MDC, SAHLSIS)	2599/2093	693/593	0.93; 0.83–1.05; 0.251
Patients and controls with hypertension			
LSR	618/319	174/94	0.95; 0.72–1.24; 0.728
MDC	645/513	176/145	0.96; 0.76–1.22; 0.762
SAHLSIS	464/224	116/59	0.94; 0.67–1.32; 0.730
Total (LSR, MDC, SAHLSIS)	1727/1056	466/298	0.95; 0.81–1.11; 0.521
Age below 55 years			
LSR	58/44	16/13	0.92; 0.42–2.04; 1.000
MDC	31/29	10/12	0.74; 0.29–1.87; 0.638
SAHLSIS	293/248	53/63	0.68; 0.46–1.01; 0.061
Total (LSR, MDC, SAHLSIS)	382/321	79/88	0.73; 0.53–1.00; 0.057
IS sub-groups due to TOAST classification[c]			
LVD	161/1212	40/345	0.85; 0.60–1.22; 0.395
SVD	339/1212	85/345	0.86; 0.67–1.11; 0.285
CE	410/1212	123/345	1.06; 0.85–1.33; 0.605

Abbreviations: CE, cardiembolism; CI, confidence interval; LVD, large vessel disease; OR, odds ratio; SVD,small vessel disease.
[a]Minor alleles T, major alleles C.
[b]CIs computed using Wald approximation of variances, *P*-values computed using Fisher's exact test. A small divergence may cause conflicting null-hypothesis conclusions.
[c]TOAST classification of 1678 cases and1786 control subjects from LSR and SAHLSIS was performed, MDC is not included.

ing cases defined as IS patients with either LVD, SVD or CE subgroup, we did not find any significant association with rs12188950 (Table 2).[23]

LR analyses of additive rs12188950 data
From the weighted simple LR analyses we obtained results for LSR, MDC and SAHLSIS after adjustments for heterogeneity caused by dissimilar distributions on sex and age. No significant associations were found (Table 3). These results were approximately similar to the corresponding univariate OR estimates (Table 2).

The weighted multiple LRs, controlling for the dichotomous phenotype risk factors hypertension, diabetes mellitus and current smoking, did not change the results. All of these risk factor covariates were significant with $P < 0.001$, but no significant contribution from rs12188950 could be seen.

Meta-analysis
The outcome of the meta-analysis of 10 500 IS patients and 10 102 subjects from 17 published studies (including our previous study) and this study, containing 20 distinguishable populations is visualised in Figure 1. The overall OR of 0.96 (95% CI: 0.89–1.04) indicates no significant overall effect in these 20 populations. The heterogeneity test ($P = 0.042$) shows a slightly significant difference between the ORs of the 20 populations, indicating that the OR estimates from these populations may be caused by other reasons than random variations.

Only two populations showed ORs that significantly suggest allelic association between variants in rs12188950 and IS risk.[1,2] When carrying out a meta-analysis that only comprised the Icelandic and the Swedish populations (five studies including the present study), the OR remained insignificant (OR=0.88; 95% CI: 0.76–1.01; *P*-value from heterogeneity test=0.047).[1,2,5,9]

DISCUSSION
In this large multi-centre study we could not detect an association between rs12188950 and IS. This might be in conflict with the results of our previous study and the Icelandic study, although the upper CI limit of 0.91 and 0.92 from these studies, respectively, are close to the estimated OR (0.93) for rs12188950 in this present study.[1,2]

Considerations about IS subgroups
None of the TOAST-classified subgroups of IS met the significance level in our analyses. A previous Swedish study by Kostulas *et al*[5] in 2007 showed a relative risk (RR) of 1.43 (to be compared with 1.07 when including all IS causes and transient ischaemic attack) when testing the major (G) allele of rs12188950 against LVD risk. Another study by Kuhlenbäumer *et al*[6] in 2006 found an OR estimate of 1.06 when testing major alleles of rs12188950 against LVD risk among a German population (OR=1.05 when assessing all IS causes). The Icelandic study by Gretarsdottir *et al*[1] showed RR=1.77 when assessing patients with combined carotid and cardiogenic causes of stroke (all IS causes provided an RR estimate of 1.31). From these findings, only the Icelandic RR estimates were significant. However, the tendency

Table 3 Simple and multiple logistic regression (LR) performing an additive approach for assessing possible association between rs12188950 and ischaemic stroke risk for Lund Stroke Register (LSR), Malmö Diet and Cancer study (MDC) and the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS)

Population	Covariate	Simple LR		Weighted Simple LR[a]		Weighted Multiple LR[a]	
		OR; 95% CI; P-value	Number of non-missing cases/controls	OR; 95% CI; P-value	Number of non-missing cases/controls	Controlling for IS risk factors[b] OR; 95% CI; P-value	Number of non-missing cases/controls
LSR	rs12188950	0.88; 0.72–1.08; 0.214	920/566	0.86; 0.70–1.06; 0.154	920/566	0.88; 0.70–1.10; 0.253	856/562
	Hypertension					1.73; 1.37–2.18; <0.001	
	Diabetes					4.18; 2.94–5.96; <0.001	
	Current smoker					2.61; 1.92–3.55; <0.001	
MDC	rs12188950	0.99; 0.82–1.20; 0.920	874/881	0.93; 0.77–1.13; 0.478	874/818	0.98; 0.80–1.21; 0.873	849/795
	Hypertension					2.02; 1.63–2.50; <0.001	
	Diabetes					4.73; 2.86–7.82; <0.001	
	Current smoker					2.45; 1.95–3.07; <0.001	
SAHLSIS	rs12188950	0.90; 0.72–1.13; 0.372	805/646	0.91; 0.73–1.14; 0.419	805/646	0.86; 0.67–1.09; 0.215	793/645
	Hypertension					2.52; 2.00–3.16; <0.001	
	Diabetes					3.86; 2.64–5.66; <0.001	
	Current smoker					3.37; 2.56–4.42; <0.001	
Pooled data	rs12188950	0.93; 0.83–1.05; 0.240	2599/2093	0.90; 0.80–1.02; 0.095	2599/2030	0.91; 0.80–1.04; 0.159	2498/2002
	Hypertension					2.02; 1.77–2.29; <0.001	
	Diabetes					4.40; 3.50–5.53; <0.001	
	Current smoker					2.64; 2.28–3.07; <0.001	

Abbreviations: CI, confidence interval; OR, odds ratio.
[a]Weight obtained by age and sex standardization of included populations.
[b]Dichotomous risk factors for ischaemic stroke: hypertension, diabetes mellitus and current smoking.
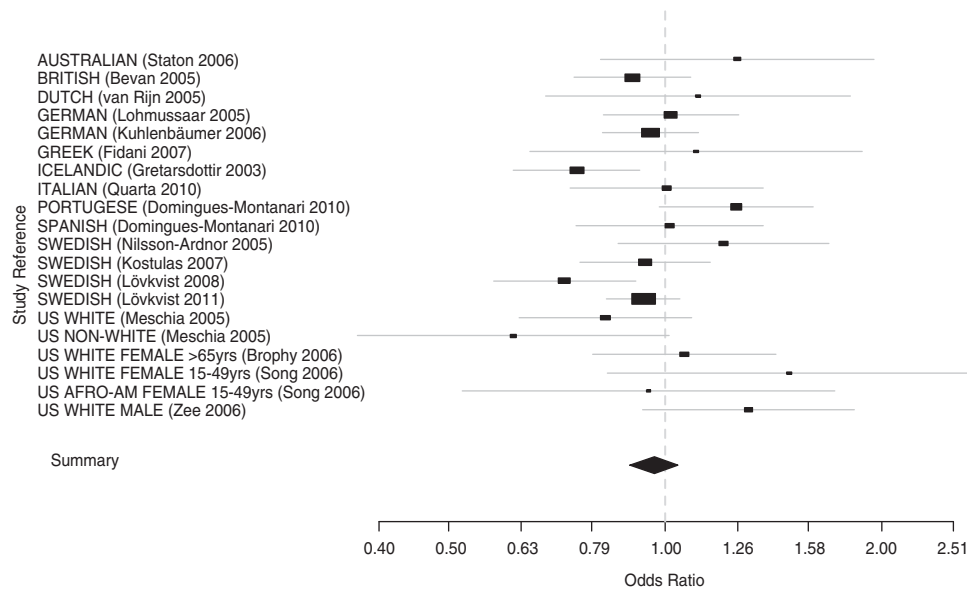
Figure 1 Random effects (DerSimonian–Laird) meta-analysis of 16 previously published studies and the present study, comprising 20 distinguishable populations assessing the association between the minor (T) allele of rs12188950 and IS in 10 500 IS patients and 10 102 controls. Overall OR: 0.96 (95% CI: 0.89–1.04), test for heterogeneity $\chi^2=30.9$ ($P=0.0416$).

towards a protecting effect of minor alleles against IS in the three studies as a whole might be an interesting subject of further studies of LVD subgroups.

Discrepancy between present and previously reported LSR data
Our previously reported study showed a protecting effect against IS that could not be found in our current study, despite similar LSR data collection routines.[2] The dataset in the previous study published in 2008 (LSR-I) did not differ considerably from the present LSR data (LSR-II) regarding mean age and proportion of current smokers. Nevertheless, LSR-I comprises a lower frequency of hypertensive controls (33.7%) than LSR-II (56.4%). Patients and controls with diabetes mellitus were also more frequent in LSR-II (29.1% and 8.1%, respectively) compared with that in LSR-I (21.0% and 6.3%, respectively). LSR-I was collected between 2001 and 2004, and the major part of LSR-II was collected between 2004 and 2008.

Meta-analysis findings
Our meta-analysis that included populations from Europe, North America and Australia did not find any overall tendency of IS being affected by rs12188950 minor alleles in neither protective nor augmenting direction. A previously published meta-analysis, with similarities to ours regarding selection of publication, presented a significant overall OR=1.29 ($P=0.05$) after removal of publications causing heterogeneity.[27]

The slightly significant heterogeneity test ($P=0.042$) suggests a non-random effect explaining the difference between the ORs among the 20 compared populations in our meta-analysis. Figure 1 visualises this ambiguity regarding the OR estimates. Dissimilar ethnicities in the studied populations might be one explaining factor. However, other demographic attributes (such as age, sex or occupational status of participants) may also contribute to such an effect. Besides, conclusions regarding possible ethnical differences might be connected with various problems regarding adequate definitions of a particular group of study.[28]

We found five publications in our PubMed search that presented monomorphic allele representation regarding rs12188950.[14,29–32] Moreover, we found a Chinese meta-analysis on Asian populations that had excluded rs12188950 from five studies regarding *PDE4D* gene data because of total absence of individuals with heterozygote allele pairs within that SNP.[33] A number of published studies examined SNPs in the *PDE4D* gene without including rs12188950. Uncommented exclusion of a SNP might cause selection bias in meta-analyses.

Multi-centre data considerations
One main issue when assessing multi-centre data is how to cope with a non-homogenous group. When performing a heterogeneity test of the three subpopulations (LSR, MDC and SAHLSIS) using the algorithm applied in the meta-analysis, we obtained a non-significant P-value of 0.6735. However, although the subpopulations were heterogeneous because of age distribution, we fitted weighted LR models using age- and sex-standardizing stratum weights. We thereby obtained OR estimates that were more similar than those obtained when not using such stratum weights (Table 3). Another source of heterogeneity between LSR, MDC and SAHLSIS is the registration of vascular risk factors (hypertension, diabetes and current smoking). Data for these phenotypes was collected at stroke onset for LSR and SAHLSIS, but at the baseline time of screening for MDC. The MDC data collection regarding these risk factors was therefore performed at an estimated mean time of about 5.5 years before stroke onset.

CONCLUSION
Our assessment in a large study including 2599 patients and 2093 control subjects did not show any association between rs12188950 and IS. A meta-analysis including 10 500 patients and 10 102 control subjects provided the same result. No association was detected in any of the subgroups. However, a hypothetical protecting effect (although it is non-significant with a P-value of 0.057 in this study)

of the rs12188950 minor allele on IS among younger individuals might be subject for further studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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