SHORT REPORT

Association between *phosphodiesterase 4D* gene and ischaemic stroke

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Background: An association between the *phosphodiesterase 4D* (*PDE4D*) gene and risk of ischaemic stroke in an Icelandic population has been suggested by the deCODE group.

Methods: A case–control study of 151 hospitalised patients with first-ever ischaemic stroke and 164 randomly selected age-matched and sex-matched community controls was conducted. PDE4D genotypes for the six single-nucleotide polymorphisms (SNPs) previously reported to be independently associated with stroke were determined, common haplotypes were inferred using the expectation-maximisation algorithm, and SNP and haplotype associations with stroke were examined. A meta-analysis of published studies examining the association between PDE4D and stroke was also carried out.

Results: Our study of Australian patients with stroke showed an independent association between ischaemic stroke and PDE4D SNP 89 (CC: odds ratio (OR) 5.55, 95% confidence interval (CI) 1.02 to 30.19; CA: OR 1.68, 95% CI 0.96 to 2.96; AA: OR 1 (reference)), SNP 87 (CC: OR 2.13, 95% CI 1.08 to 4.20; TC: OR 1.64, 95% CI 0.89 to 3.00; TT: OR 1 (reference)) and SNP 83 (TT: OR 2.16, 95% CI 1.08 to 4.32; TC: OR 1.37, 95% CI 0.77 to 2.43; CC: OR 1 (reference)), and between ischaemic stroke and PDE4D haplotypes at SNP 89–87–83 (A–C–C: OR 2.13, 95% CI 1.15 to 3.96; C–C–T: OR 2.25, 95% CI 1.29 to 3.92), but no association between ischaemic stroke and PDE4D SNP 56, SNP 45 or SNP 41, or with PDE4D haplotypes at SNP 56–45–41. A meta-analysis of nine case–control studies (including our current results) of 3808 stroke cases and 4377 controls confirmed a significant association between stroke and PDE SNP 87 (pooled $p=0.002$), SNP 83 (0.003) and SNP 41 (0.003). However, there was statistical heterogeneity ($p<0.1$) among the studies in the direction of association for each of the individual SNPs tested.

Conclusions: Our results and the pooled analyses from all the studies indicate a strong association between PDE4D and ischaemic stroke. This strengthens the evidence that PDE4D plays a key part in the pathogenesis of ischaemic stroke. Heterogeneity among the studies in the direction of association between individual SNPs and stroke suggests that the SNPs tested are in linkage disequilibrium with the causal allele(s).

Stroke is one of the leading causes of death and long-term disability worldwide. About 80% of strokes are ischaemic in origin, most commonly caused by atherosclerosis.[1] By recognising and treating individuals and populations at risk, the burden of stroke can be reduced. As only about two thirds of ischaemic strokes seem to be attributable to known environmental risk factors,[2] there are likely to be other as yet unknown causal risk factors for ischaemic stroke.

In recent years, there have been several reports of genetic polymorphisms that are associated with an increased (or decreased) risk of stroke.[3–6] In 2002, the deCODE group published the results of a genomewide screen for stroke susceptibility genes in Iceland.[7] Among 260 phosphodiesterase 4D (PDE4D) single-nucleotide polymorphisms (SNPs) examined, six were significantly associated with stroke after adjustment for multiple comparisons.

To establish a causal association between the *PDE4D* gene and stroke, the results of the study by the deCODE group need to be validated externally in independent datasets. Recently published studies from different populations[8–15] provide apparently conflicting evidence on the association between the *PDE4D* gene and stroke. We explored the relationship between PDE4D genotype and ischaemic stroke in a case–control study of consecutive Australian patients admitted to hospital with ischaemic stroke and a similar number of randomly selected community controls. We determined PDE4D genotypes among patients and controls for the six SNPs found by the deCODE group to be markedly associated with stroke (SNP 89, SNP 87, SNP 83, SNP 56, SNP 45 and SNP 41) and examined the association between these SNPs and PDE4D haplotypes and ischaemic stroke. We also carried out a meta-analysis of published studies to explore the consistency of the association between PDE4D and stroke.

PATIENTS AND METHODS

The study was approved by the Institutional Review Board of the Royal Perth Hospital (Perth, Australia) and informed consent was provided by all study patients.

Patient selection

The methods of patient selection have been previously described.[16] Consecutive patients presenting to the Royal Perth Hospital between March 1996 and June 1998 with first-ever ischaemic stroke were approached for consent to participate in the study. Patients with cerebral haemorrhage, cerebral venous thrombosis or liver disease were not included.

Baseline demographic data (age, sex), history of conventional vascular risk factors (hypertension, diabetes, hypercholesterolaemia, current smoker) and history of previous vascular events (myocardial infarction, angina, claudication, amputation for peripheral vascular disease) were obtained.

All patients underwent computed tomography of the brain. Echocardiography and an extracranial duplex ultrasound were carried out at the discretion of the clinician.

Abbreviations: PDE4D, phosphodiesterase 4D; SNP, single-nucleotide polymorphism

Table 1 Prevalence of phosphodiesterase 4D genotypes among patients and controls and their association with ischaemic stroke

PDE4D SNPs	Patients (n = 151)	Controls (n = 164)	HWE test p value	OR (95% CI; adjusted*)	p Value
89, n (%)					
AA	99 (66.0)	127 (77.4)	1.00	1 (reference)	
CA	45 (30.0)	35 (21.3)		1.68 (0.96 to 2.96)	0.07
CC	6 (4.0)	2 (1.2)		5.55 (1.02 to 30.19)	0.048
87, n (%)					
TT	34 (22.5)	56 (34.2)	0.159	1 (reference)	
TC	72 (47.7)	72 (43.9)		1.64 (0.89 to 3.00)	0.11
CC	45 (29.8)	36 (22.0)		2.13 (1.08 to 4.20)	0.03
83, n (%)					
CC	43 (28.7)	61 (37.2)	0.631	1 (reference)	
TC	68 (45.3)	75 (45.7)		1.37 (0.77 to 2.43)	0.28
TT	39 (26.0)	28 (17.1)		2.16 (1.08 to 4.32)	0.03
56, n (%)					
TT	55 (37.2)	65 (39.6)	0.861	1 (reference)	
AT	66 (44.6)	78 (47.6)		1.14 (0.65 to 1.98)	0.66
AA	27 (18.2)	21 (12.8)		1.76 (0.83 to 2.76)	0.14
45, n (%)					
GG	106 (70.7)	121 (73.8)	0.742	1 (reference)	
AG	38 (25.3)	41 (25.0)		1.00 (0.56 to 1.78)	0.99
AA	6 (4.0)	2 (1.2)		3.52 (0.63 to 19.63)	0.15
41, n (%)					
AA	101 (68.2)	112 (68.3)	0.421	1 (reference)	
AG	38 (25.7)	45 (27.4)		0.85 (0.48 to 1.52)	0.59
GG	9 (6.1)	7 (4.3)		1.50 (0.50 to 4.47)	0.47

HWE, Hardy–Weinberg equilibrium; PDE4D SNP, phosphodiesterase 4D single-nucleotide polymorphism.
*Adjusted for baseline differences between cases and controls. All PDE4D genotypes tested were in HWE.

Controls were randomly selected from the Western Australian electoral roll, stratified by 5-year age groups, sex and postal code.

A blood sample was obtained from patients and controls for genetic analysis.

Choice of polymorphisms

We selected the six SNPs that had shown marked association with stroke after adjustment for multiple testing in the original study.

Statistical analyses

The distribution of PDE4D SNPs among controls was tested for the Hardy–Weinberg equilibrium using an exact test. Pairwise linkage disequilibrium was estimated using Lewontin's method, and haplotypes with ambiguous phase were inferred using the expectation-maximisation algorithm.[17 18] Logistic regression was applied to determine haplotype effects after assigning the most common haplotype as the reference and with adjustment for baseline differences between patients and controls.

All analyses were carried out using SAS V.9.1. Results are expressed as odds ratios (ORs) and their 95% confidence intervals (CIs). An OR of <1 corresponds with a reduced risk of disease; an OR of >1 with an increased risk. A two-sided p value <0.05 was considered significant.

Meta-analysis

We searched the electronic Medline database from 1966 to January 2006 for studies reporting the association between PDE4D and stroke, using the following terms singly and in combination: phosphodiesterase 4D, PDE, PDE4D, stroke, cerebrovascular, gene. Studies were included if they reported the prevalence of PDE4D genotypes in patients with stroke compared with a control population without a history of stroke.

Data from studies that did not include family controls were pooled in two stages. Firstly, we examined the association between the *PDE4D* gene and stroke using the Fisher method for pooling p values,[19] thereby providing an overall estimate of association that is independent of the direction of the association.[20] Secondly, we pooled results for individual genotypes using the DerSimonian Laird method under a random effects model,[21 22] thereby attempting to provide a best estimate of the strength and direction of the association between individual PDE4D SNPs and stroke. Heterogeneity was explored using the Q test.[22] We adjusted for multiple comparisons using the Benjamini–Liu method.[23 24]

RESULTS

The study group consisted of 151 consecutive patients admitted with ischaemic stroke (100 men, 51 women; mean age 67.3 years (standard deviation (SD) 11.7)) and 164 controls (103 men, 61 women; mean age 66.1 years (SD 11.8)).

Baseline characteristics

No significant differences were observed in the age and sex distribution of patients with ischaemic stroke and controls. Baseline conventional vascular risk factors—that is, smoking, hypertension, previous vascular event and diabetes—were significantly more common among patients ($p<0.001$), but the prevalence of hypercholesterolaemia was not significantly different among patients compared with controls ($p = 0.59$).

Association between PDE4D and ischaemic stroke

A significant association was found between ischaemic stroke and PDE4D haplotypes at SNP 89–87–83 (A–C–C: OR 2.13, 95% CI 1.15 to 3.96; C–C–T: OR 2.25, 95% CI 1.29 to 3.92) but not at SNP 56, SNP 45 or SNP 41, or with PDE4D haplotypes at SNP 56–45–41.

Table 1 presents the results of analyses.

Meta-analysis data

We identified eight additional studies, which along with our current results made a total of 3808 patients with stroke and 4377 controls that met our criteria for inclusion. One study that used family controls was not included in our meta-analysis.[10]

Meta-analysis of the case–control studies showed a significant association between stroke and PDE SNP 87 (pooled $p = 0.002$), SNP 83 (0.003) and SNP 41 (0.003). These associations remained significant after adjustment for multiple comparisons by the Benjamini–Liu method and are also consistent with the findings of van Rijn *et al*[10] using family controls. However, there was statistical heterogeneity ($p<0.1$) among the studies for each of the individual SNPs tested.

DISCUSSION

Our study adds to a growing number of publications that have independently confirmed a relationship between PDE4D and sporadic ischaemic stroke risk in populations outside the Icelandic community. Furthermore, the consistency of our results showing an independent association between PDE4D SNP 89, SNP 87, SNP 83 and PDE4D haplotypes at SNP 89–87–83 with the Icelandic group and the pooled analyses from all the studies are consistent with the conclusion that PDE4D is a marker of risk for ischaemic stroke.

Differences exist between the studies in the direction of the association between individual PDE4D SNPs and stroke, as reflected by the statistical heterogeneity for the pooled ORs. For example, in our study of Australian patients with stroke, the at-risk alleles are the converse of those reported by the deCODE group, and we did not show a significant association between stroke and PDE4D SNP 45 or SNP 41 or PDE4D haplotypes involving these markers, which were the most strongly associated with ischaemic stroke in the Icelandic population. Other studies also reported results that differed in the magnitude or direction of the association between PDE4D alleles and stroke compared with the deCODE group.[9 11–15] The most likely explanation of these apparently conflicting findings is that the SNPs tested are not causally linked to stroke but instead are in linkage disequilibrium with the causative alleles, which may differ across populations.

The roughly twofold increase in risk conferred by at-risk PDE4D haplotypes and the 10–20% prevalence of at-risk PDE4D haplotypes among patients with stroke is similar to the strength and prevalence of established cardiovascular risk factors for stroke, including hypertension, diabetes and smoking, and seems to be independent of, and in addition to, that conferred by traditional risk factors.

Our data strengthen the evidence that PDE4D plays a part in the pathogenesis of ischaemic stroke. If a causal association is ultimately confirmed, an investigation to determine the mechanisms of the association could lead to the development of pharmacological interventions that lower the risk of stroke in those predisposed by PDE4D genotype.

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REFERENCES

1 **Warlow CP**. Epidemiology of stroke. *Lancet* 1998;**352**(Suppl 3):SIII1–4.
2 **Whisnant JP**. Modeling of risk factors for ischemic stroke. The Willis Lecture. *Stroke* 1997;**28**:1840–4.
3 **Endler G**, Funk M, Haering D, *et al*. Is the factor XIII 34Val/Leu polymorphism a protective factor for cerebrovascular disease? *Br J Haematol* 2003;**120**:310–4.
4 **Sun L**, Li Z, Zhang H, *et al*. Pentanucleotide TTTTA repeat polymorphism of apolipoprotein(a) gene and plasma lipoprotein(a) are associated with ischemic and hemorrhagic stroke in Chinese: a multicenter case-control study in China. *Stroke* 2003;**34**:1617–22.
5 **Gretarsdottir S**, Thorleifsson G, Reynisdottir ST, *et al*. The gene encoding phosphodiesterase 4D confers risk of ischemic stroke. *Nat Genet* 2003;**35**:131–8.
6 **Goto S**. Cilostazol: potential mechanism of action for antithrombotic effects accompanied by a low rate of bleeding. *Atheroscler Suppl* 2005;**6**:3–11.
7 **Gretarsdottir S**, Sveinbjornsdottir S, Jonsson HH, *et al*. Localization of a susceptibility gene for common forms of stroke to 5q12. *Am J Hum Genet* 2002;**70**:593–603.
8 **Lohmussaar E**, Gschwendtner A, Mueller JC, *et al*. ALOX5AP gene and the PDE4D gene in a central European population of stroke patients. *Stroke* 2005;**36**:731–6.
9 **Nakayama T**, Asai S, Sato N, *et al*. Genotype and haplotype association study of the STRK1 region on 5q12 among Japanese: a case-control study. *Stroke* 2006;**37**:69–76.
10 **van Rijn MJ**, Slooter AJ, Schut AF, *et al*. Familial aggregation, the PDE4D gene, and ischemic stroke in a genetically isolated population. *Neurology* 2005;**65**:1203–9.
11 **Saleheen D**, Bukhari S, Haider SR, *et al*. Association of phosphodiesterase 4D gene with ischemic stroke in a Pakistani population. *Stroke* 2005;**36**:2275–7.
12 **Nilsson-Ardnor S**, Wiklund PG, Lindgren P, *et al*. Linkage of ischemic stroke to the PDE4D region on 5q in a Swedish population. *Stroke* 2005;**36**:1666–71.
13 **Bevan S**, Porteous L, Sitzer M, *et al*. Phosphodiesterase 4D gene, ischemic stroke, and asymptomatic carotid atherosclerosis. *Stroke* 2005;**36**:949–53.
14 **Meschia JF**, Brott TG, Brown RD Jr, *et al*. Phosphodiesterase 4D and 5-lipoxygenase activating protein in ischemic stroke. *Ann Neurol* 2005;**58**:351–61.
15 **Woo D**, Kaushal R, Kissela B, *et al*. Association of phosphodiesterase 4D with ischemic stroke: a population-based case-control study. *Stroke* 2006;**37**:371–6.
16 **McQuillan AM**, Eikelboom JW, Hankey GJ, *et al*. Protein Z in ischemic stroke and its etiologic subtypes. *Stroke* 2003;**34**:2415–9.
17 **Thakkinstian A**, D'Este C, Attia J. Haplotype analysis of VDR gene polymorphisms: a meta-analysis. *Osteoporos Int* 2004;**15**:729–34.
18 **Fallin D**, Cohen A, Essioux L, *et al*. Genetic analysis of case/control data using estimated haplotype frequencies: application to APOE locus variation and Alzheimer's disease. *Genome Res* 2001;**11**:143–51.
19 **Fisher RA**. *Statistical methods for research workers*. Edinburgh: Oliver and Boyd, 1932.
20 **Guerra R**, Etzel CJ, Goldstein DR, *et al*. Meta-analysis by combining p-values: simulated linkage studies. *Genet Epidemiol* 1999;**17**(Suppl 1):S605–9.
21 **DerSimonian R**, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986;**7**:177–88.
22 **Egger M**, Davey Smith G, Altman DG, eds. *Systematic reviews in health care: meta-analysis in context*. London: BMJ Books, 2001.
23 **Benjamini Y**, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B* 1995;**57**:289–300.
24 **Benjamini Y**, Drai D, Elmer G, *et al*. Controlling the false discovery rate in behavior genetics research. *Behav Brain Res* 2001;**125**:279–84.