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Phosphodiesterase 4D Gene and Risk of Noncardiogenic Ischemic Stroke in a Korean Population

Recently published studies from different populations provide apparently conflicting evidence on the association between the phosphodiesterase 4D (*PDE4D*) gene and ischemic stroke. The relationship between a representative *PDE4D* genotype and ischemic stroke was explored in a case-control study of 205 consecutive Korean patients with noncardiogenic ischemic stroke and 103 healthy controls who were neurologically and radiologically proven to be stroke-free. We selected and genotyped a *PDE4D* single nucleotide polymorphism (SNP 41, rs152312) as a candidate marker for susceptibility to ischemic stroke because SNP 41 has shown the most significant association with stroke in both a meta-analysis and the original Icelandic study of the *PDE4D* gene. No significant difference was observed between the cases and controls in the distribution of the *PDE4D* SNP 41 genotypes. The results from the adjusted conditional logistic regression analysis (adjusted for age, hypertension, diabetes and smoking status) showed no significant association between *PDE4D* SNP 41 genotypes and an increased risk of noncardiogenic ischemic stroke. The *PDE4D* gene is not a major risk factor for noncardiogenic ischemic stroke in a Korean population, which supports the recent evidence suggesting that the causative genetic variants of ischemic stroke may differ across populations.

Key Words : *Phosphodiesterases 4D; Stroke; Case-Control Study; Genetics*

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INTRODUCTION

Stroke is a major cause of morbidity and mortality worldwide. Although the past 50 yr have seen a decline in the incidence of stroke in Western populations (1), the burden of the disease in the Korean population has increased and is expected to continue to rise with the increasing elderly population. The burden of stroke can be reduced if at-risk individuals are recognized and treated. Since only about two thirds of ischemic strokes seem to be attributable to known environmental risk factors (2), there are likely to be other as yet unknown causal risk factors for ischemic stroke.

Several epidemiologic studies in families and in twins have indicated a distinctive genetic component predisposing to stroke (3). Identification of cerebral infarction susceptibility genes might enhance prediction of disease risk. The completion of the Human Genome Project brought the promise of genomic medicine, which is the use of whole-genome-derived information to prevent, diagnose and treat complex polygenic diseases such as stroke (4). One of the most significant advances in our understanding of the role of genetic factors in the pathogenesis of stroke came from the deCODE Genetics Group in Iceland, who reported the identification and characterization of the phosphodiesterase 4D (*PDE4D*) gene, which appears to confer an increased risk of ischemic stroke (5).

However, recently published studies from different populations provide apparently conflicting evidence on the association between the *PDE4D* gene and ischemic stroke (6-13). Furthermore, none of the previous studies clearly stated whether subjects with silent cerebral infarction or asymptomatic carotid atherosclerosis were appropriately excluded from their control groups. In clinical practice, evidence of silent infarction or asymptomatic carotid stenosis on magnetic resonance imaging (MRI) or MR angiography (MRA) of the head is not uncommon in healthy-looking individuals (14, 15).

In the present study, the relationship between a representative *PDE4D* genotype and ischemic stroke was explored in a case-control study of consecutive Korean patients with ischemic stroke and healthy controls who were neurologically and radiologically proven to be stroke-free. To our knowledge, this is the first report concerning the association of the *PDE4D* gene with ischemic stroke in a Korean population.

MATERIALS AND METHODS

Subjects

Consecutive first-ever ischemic stroke patients admitted

to Chonnam National University Hospital (Gwangju, Korea) between January 2003 and December 2004 were approached for consent to participate in this study. All patients underwent MRI and MRA of the head within 48 hr after stroke onset, and their strokes were classified according to the prespecified Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria (16). In order to ensure homogeneity of samples in terms of the underlying pathomechanism of stroke, only patients with ischemic stroke classified as large artery disease (LAD) or small vessel disease (SVD) were included in the present study.

The control subjects were selected from patients who underwent both MRI and MRA of the head for neurological complaints other than stroke, primarily for headache. Patients having significant increased signal intensity lesions on diffusion-weighted or fluid-attenuated inversion-recovery MR images or vascular abnormalities on MRA of the head were excluded from the study, as were patients with typical vascular headaches.

Baseline demographic data (age, gender), history of conventional vascular risk factors (hypertension, diabetes and current smoker) and history of previous vascular events (myocardial infarction, angina, claudication and peripheral vascular disease) were obtained from each subject.

Individuals with a proven cause of cardioembolism, such as recent myocardial infarction, valvular heart disease, and arrhythmia including atrial fibrillation, and those with a history of previous peripheral vascular events were excluded from the case and control groups.

The study was approved by the Institutional Review Board of the hospital, and informed consent was obtained from all study participants.

Marker selection and genotyping

We selected a *PDE4D* single nucleotide polymorphism (SNP 41, rs152312) as a candidate marker for susceptibility to ischemic stroke in a Korean population because SNP 41 has shown the most significant association with stroke in a meta-analysis of nine case-control studies and in the original Icelandic study (5, 17).

Genomic DNA was extracted from peripheral blood lymphocytes using a standard protocol. Polymerase chain reaction (PCR) primers for *PDE4D* SNP 41 were designed based on GenBank sequences (accession number: NM_006203, AY245866): 5′-ATACATGTGCCATGCTGGTG-3′ (forward) and 5′-CCTCCTAGGCTGGTGTGAAG-3′ (reverse). PCR assays were carried out using 1.25 U of Ampli*Taq* Polymerase Gold (Applied Biosystems, CA, U.S.A.), 100 ng of genomic DNA, 2.0-2.5 mM of $MgCl_2$, and 10 μM of primer. The amplification conditions were as follows: an initial denaturation cycle at 95°C for 5 min, followed by 35 amplification cycles (denaturation at 95°C for 30 sec, annealing at 62°C for 30 sec, and extension at 72°C for 1 min), and a final extension at 72°C for 7 min. The PCR products were electrophoresed in a 1.2% agarose gel, and the amplified genomic DNA fragments were extracted from the gel and purified using a QIAquick® gel extraction kit (Quiagen, Hilden, Germany) according to the manufacturer's instructions. Direct sequencing of both strands was performed using BigDye terminator kits (PE Biosystems, CA, U.S.A.) to determine the individual genotypes.

Statistical analysis

The distribution of *PDE4D* SNP 41 among controls was tested for Hardy-Weinberg equilibrium using an exact test. Continuous and categorical variables were tested using a t test and chi-square analysis. Single-marker associations between phenotype and genotype were tested by chi-square analysis, and the relative risks for ischemic stroke associated with each genotype were calculated by logistic regression analysis (SPSS 14.OK for Windows; SPSS, Chicago, IL, U.S.A.). Differences were considered significant for p values of ≤ 0.05.

RESULTS

The baseline characteristics of the cases and controls are shown in Table 1. Compared with the controls, the cases were significantly older and, as expected, had a higher prevalence of conventional vascular risk factors at baseline. The cases consisted of 113 patients with SVD and 92 patients with LAD. The genotype frequencies for *PDE4D* SNP 41 were in Hardy-Weinberg equilibrium in the control group (p=0.936)

Using a single-marker chi-square analysis, no significant

Table 1. Baseline characteristics of cases and controls

	Cases (n=205)	Controls (n=103)	*p* value
Age (yr)	64.8±11.6	61.8±10.4	0.025
Gender (female %)	47.3	54.4	0.18
Hypertension (%)	60.5	31.1	<0.001
Diabetes (%)	28.3	14.6	0.01
Smoking (%)	20	5.8	0.001

Values are mean±SD or %.

Table 2. Distribution of *PDE4D* SNP41 genotypes

Genotype	Cases (n=205)			Controls (n=103)
	SVD (n=113)	LAD (n=92)	SVD+LAD	
GG	48 (42.5)	41 (44.6)	89 (43.4)	52 (50.5)
GA	51 (45.1)	42 (45.7)	93 (45.4)	43 (41.7)
AA	14 (12.4)	9 (9.8)	23 (11.2)	8 (7.8)
p value	0.847		0.333	

All results were expressed as number (% of total). p value for χ^2 test.
SVD, small vessel disease; LAD, large artery disease.

Table 3. The odds ratio of *PDE4D7* SNP41 genotypes for ischemic stroke

	AOR* (95% CI)	*p* value
GG vs. GA	1.258 (0.743-2.130)	0.39
GG vs. AA	1.454 (0.590-3.585)	0.42
GG vs. GA/AA	1.427 (0.848-2.403)	0.18

*AOR; adjusted odds ratio (adjusted for age, hypertension, diabetes and smoking status).
95% CI, 95% confidence interval.

difference was observed between SVD and LAD and between the cases (SVD+LAD) and controls in the distribution of the *PDE4D* SNP 41 genotypes (Table 2). The results from the adjusted conditional logistic regression analysis (adjusted for age, hypertension, diabetes and smoking status) showed no significant association between the *PDE4D* SNP 41 genotypes and an increased risk of noncardiogenic ischemic stroke (Table 3).

DISCUSSION

A recent case-control study reported an association between risk of ischemic stroke and genetic variation of the *PDE4D* gene in an Icelandic population (5). However, there is a major problem with combining individuals with cardiogenic and carotid stroke into one subgroup for genetic analysis. Although the authors insist that cardiogenic and carotid strokes are most clearly related to atherosclerosis (5), only 25% of cardiogenic strokes directly result from complications of coronary atherosclerosis (18). Therefore, the combined subgroup is highly heterogeneous in terms of the underlying pathomechanism of stroke and therefore is not suitable for genetic analysis. To avoid this problem in present study, only patients with noncardiogenic ischemic stroke were selected as cases.

The original Icelandic study reported that *PDE4D* SNP 41 was one of the variants most significantly associated with ischemic stroke (5). Furthermore, a meta-analysis of nine case-control studies consisting of 3,808 stroke cases and 4,377 controls also reported a significant association between stroke and *PDE4D* SNP 41 (17). However, no association was found between *PDE4D* SNP 41 and noncardiogenic ischemic stroke in a Korean population in the present study. This result adds to the growing body of recent evidence suggesting that variants in the *PDE4D* gene do not constitute a major risk factor for ischemic stroke (6, 7). The most likely explanation for this discrepancy is that the genetic variants tested are not causally associated with stroke, but rather are in linkage disequilibrium with the causative alleles, which may differ across populations.

A Japanese group genotyped 35 genetic markers in *STRK1*, a candidate locus for stroke identified in the original Icelandic study (19), and found that two haplotype blocks were significantly associated with noncardiogenic ischemic stroke in a Japanese population (9). Interestingly, one of the blocks was located in the region of the *PDE4D* gene, including SNP 41, but the other block, which was more significantly associated with noncardiogenic ischemic stroke, was located beyond the region of the *PDE4D* gene in the *STRK1* locus. The authors of the Japanese study suggested that the region of the *PDE4D* gene might be the susceptibility region for cardioembolic stroke and that the region identified in their study might be the susceptibility region for noncardiogenic ischemic stroke (9). This suggestion is reinforced by a non-Icelandic European study in which a possible association between the *PDE4D* gene and cardioembolic stroke, rather than with ischemic stroke on the whole, was identified (6). Our study also strengthens the Japanese scenario that there may be a susceptibility region for noncardiogenic ischemic stroke other than that of the *PDE4D* gene within the *STRK1* locus. Although the logic of their hypothesis concerning the susceptibility region for cardioembolic stroke is not clear, the lesson from the Japanese study is obvious: the causative genetic variants of ischemic stroke may differ across populations. Further studies are needed to identify the causative genetic variant in the *PDE4D* gene and to define the region of susceptibility for ischemic stroke in a Korean population.

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