Association of Phosphodiesterase 4D Gene With Ischemic Stroke in a Pakistani Population

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Background and Objectives—Identification of STRK1 locus by the deCODE group followed by the discovery of phosphodiesterase 4D (PDE4D) gene in strong association with ischemic stroke patients has provided useful insights toward understanding the genetic etiology of the disease. In this study, we aimed at investigating the association between 3 polymorphisms of the PDE4D gene and ischemic stroke in the Pakistani population.

Methods—Three polymorphisms in PDE4D gene were analyzed in 200 patients of ischemic stroke and 250 controls of Pakistani origin using polymerase chain reaction–restriction fragment length polymorphism method. Data were coded and entered in SPSS Windows (version 12.0). Odds ratios and 95% CIs were calculated using multivariate logistic regression analysis.

Results—Marker SNP83(rs966221) was found significantly associated with ischemic stroke on univariate and multivariate analysis (P<0.005; odds ratio, 1.64 [1.13 to 2.40]). Haplotype analysis for markers in linkage disequilibrium failed to show any association with the disease.

Conclusion—The association of PDE4D variation with ischemic stroke extends to the Pakistani population and supports a role for phosphodiesterases in stroke pathogenesis. (Stroke. 2005;36:2270–2277.)

Key Words: genetics ■ risk factors ■ stroke, ischemic

Stroke is a major cause of morbidity and mortality around the world. According to World Health Organization estimates, 5.5 million people died of stroke in 2002, and roughly 20% of these deaths occurred in South Asia. Although the past 3 decades have seen a decline in the incidence of the disease in the Western population, the burden of the disease in South Asian countries (India, Pakistan, Bangladesh, and Sri Lanka) has inclined and is expected to rise.

Several epidemiologic studies in families and in twins have indicated a distinctive genetic component predisposing to stroke. However, identification of these factors remained elusive until the discovery of STRK1 locus by deCODE group, followed by association of the phosphodiesterase 4D (PDE4D) with ischemic stroke in the Icelandic population.

In this study, we investigated the association between 3 polymorphisms of the PDE4D gene and ischemic stroke in the Pakistani population.

Methods

The study was conducted at the Liaquat National Hospital and Aga Khan University Hospital Karachi in 2001 to 2002. The study population consisted of 200 patients of ischemic stroke and 250 controls. Ischemic stroke was defined as a sudden loss of global or focal cerebral function persisting for >24 hours with corresponding infarction on brain imaging with a probable vascular cause. All patients underwent a complete neurological examination. Data were collected with the help of a pretested and coded data extraction sheet. Controls were free of stroke and were from local population sharing the same environment. The study was approved by the ethics committee of both hospitals.

An individual was classified as having arterial hypertension with a previous diagnosis of hypertension or if systolic or diastolic blood pressure was >140 mm Hg or >90 mm Hg, respectively, on ≥2 different occasions. Subjects were classified as having diabetes mellitus if he or she already had the diagnosis of diabetes mellitus or if his or her fasting plasma glucose was >126 mg/dL. Ischemic heart disease was established on past medical history, review of ECGs, and other relevant clinical information. Sample size of 200 cases and 250 controls based on allele frequencies was calculated for a power of 80% using the software Quanto.

Laboratory Measurement and Techniques

A total of 10 mL of venous blood was collected in an EDTA tube and plain tubes separately for DNA extraction from white blood cells and serum analysis. DNA was extracted using the Wizard genomic DNA purification kit (catalog No. A1125; Promega). Total lipid profile (cholesterol, high-density lipoprotein, low-density lipoprotein, and triglycerides) was studied in a fasting state using the Merck kit (Merck Diagnostica).
Selection of Polymorphisms in \textit{PDE4D} Gene

Three single-nucleotide polymorphisms (SNPs) were selected in the \textit{PDE4D} gene (Table 1). The nomenclature of these markers used by Gretarsdottir et al\textsuperscript{6} is provided in Table 1. All 3 SNPs were found to be associated with ischemic stroke in the Icelandic stroke population.\textsuperscript{6} These polymorphisms are noncoding and are intronic. Respective positions of these SNPs in the \textit{PDE4D} gene have been described previously.\textsuperscript{6}

Nomenclature for sequence variations was based on GenBank accession No. NT_006713.14. Flanking sequences were retrieved from ENSEMBL, and primers were designed using Gene Runner (version 3.05; Hastings Software Inc.) to amplify the region containing these SNPs (Table 1).

Genotyping and Restriction Fragment Length Polymorphism

Polymerase chain reaction (PCR) was performed in a standardized way using the primers listed in Table 1. Aliquots of 5 \( \mu \)L PCR product were subjected to restriction endonuclease digestion for genotyping using the restriction enzymes listed in Table 1. The digested bands were visualized with agarose gel electrophoresis. Positive and negative controls were included on each run.

Statistical Analysis

Biological and clinical variables were compared between cases and controls by Student \( t \) and \( \chi^2 \) tests for continuous and categorical variables, respectively.

Alleles and genotype frequencies were determined by gene counting, and the deviation from Hardy–Weinberg (HW) equilibrium was tested by a \( \chi^2 \) statistic with 1 \( df \). Association of each studied polymorphism and ischemic stroke was assessed by logistic regression analysis adjusting for confounding variables including age, gender, diabetes, and hypertension. Pairwise linkage disequilibrium (LD) coefficients were expressed as \( D' \), which is the ratio of unstandardized coefficient to its minimal/maximal value and estimated by use of the THESIAS program. A value of \( P<0.05 \) was considered statistically significant.

Results

Characteristics of the study population and genotype and allelic distributions of the polymorphisms are given in Tables 2 and 3, respectively.

Homzygosity for T allele at marker SNP83 conferred a significant risk for ischemic stroke on univariate and multivariate analysis. The genotype distribution was in HW equilibrium in cases and controls (\( P=0.1 \)). SNP87 and SNP32 failed to show any association with the disease.

A 2-point haplotype analysis was performed for SNP32 and SNP87 ( \( D' \) value between the 2 SNPs was 0.5; \( P=0.00001 \)) in association with ischemic stroke. However, the global haplotype test for association with the disease was not significant (\( \chi^2=3 \) with 3 \( df; P=0.30 \)). No haplotype was found to be associated with ischemic stroke.

Discussion

In this study, we have shown that SNP83 is associated with an increased risk of ischemic stroke on univariate and multivariate analyses. This association is consistent with the results reported previously by Gretarsdottir et al,\textsuperscript{6} who showed this polymorphism to be significantly associated with the carotid subtype of the disease. A lack of association of SNP32 and SNP87 is not surprising. This is in agreement with the results published by Lohmussar et al,\textsuperscript{8} who failed to find an association of \textit{PDE4D} with ischemic stroke in a central European population. This disparity can be explained by difference in allele and haplotype frequencies and difference in LD pattern between the Pakistani and the Icelandic population. A similar kind of difference was noted by Lohmussar et al,\textsuperscript{8} who observed less pronounced LD between SNPs in their European population compared with the Icelandic population.

Although the role of \textit{PDE4D} in causing stroke is still unclear, it has been speculated that the gene acts by regulating the levels of cAMP and cyclic GMP.\textsuperscript{6} The interaction of phosphodiesterases and these cyclic nucleotides is a part of complex endothelial signaling pathway that has shown to be dysfunctional in cerebrovascular disorders.\textsuperscript{9} cAMP, which is involved in many cellular functions including smooth muscle cell proliferation, is inhibited by \textit{phosphodiesterase 4} in arterial tissues.\textsuperscript{9} Furthermore, cyclic GMP, the production of which is linked with NO production by vascular endothelium,\textsuperscript{10} regulates the levels of these phosphodiesterases.\textsuperscript{10} Recently, it was shown that \textit{phosphodiesterase 4} inhibitor

\begin{table}
\centering
\caption{List of SNPs With Their Corresponding dbSNPrsIDs}
\begin{tabular}{|l|l|l|l|l|l|}
\hline
dbSNPrsID & Base Change & Forward Oligo (5'→3') & Reverse Oligo (5'→3') & Annealing Temperature & Product bp & Wild-Type Allele Variant-Type Allele \\
\hline
SNP32 & g.59832491G>C & ATGAAGAAGAACCTGACC & ATTTGGCCTTGCAATATAC & 62.5°C & MSP A1I & 195, 155+40 \\
SNP83 & g.59538277T>C & TCGTTCAGTTAAGGATGG & ATTTGGCGCTTGCAATATAC & 60°C & Mae II & 492, 204+288 \\
SNP87 & g.59505656C>T & AAGTAGAAGAATCGTAAATGG & ATGAAGAAGAAGATCG & 62.5°C & SSP1 & 307, 209+98 \\
\hline
\end{tabular}
\end{table}

\begin{table}
\centering
\caption{Characteristics of the Study Population}
\begin{tabular}{|l|l|l|l|}
\hline
 & Controls & n=250 & Stroke & n=200 & Test$^\dagger$ \\
\hline
Age, y & 54.1 (8.87) & 62.4 (12.4) & & & \( P<10^{-2} \) \\
Males, % & 65 & 59 & & & 0.13 \\
Hypertension, % & 23 & 74 & & & \( P<10^{-3} \) \\
Diabetes mellitus, % & 16 & 44 & & & \( P<10^{-3} \) \\
Total cholesterol$^\ast$ & 187.0 (49.3) & 169.9 (48.6) & & & 0.01 \\
High-density lipoprotein cholesterol$^\ast$ & 41.2 (10.7) & 40.4 (10.3) & & & 0.03 \\
\hline
\end{tabular}
\end{table}

$^\ast$Variables are expressed in mean (SD); $^\dagger$Variables are expressed in percentages.

$^\dagger$Student \( t \) test was used for variables and $^\ast$\( \chi^2 \) squared tests were used for variables$^\ddagger$. 

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activity was greatly reduced if cGMP synthesis was inhibited or the endothelium removed proving an intricate interlinked interaction of cGMP and phosphodiestrases.9 This could be important because the NO–cGMP pathway is believed to be dysfunctional in stroke.9 A complete understanding of this complex pathway merits further research. This could be therapeutically important as well because phosphodiesterase 4 inhibitors have been thought useful in the prevention of ischemic stroke.10

Confirmation of PDE4D gene association with ischemic stroke in the Pakistani population is important not only because it confirms the association of this gene with the disease, but it is the first genetic risk factor for stroke identified in this population. This study will open new avenues in understanding the pathways underlying this complex disorder.

Acknowledgments
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References


TABLE 3. Genotype and Allelic Distributions in Patients and Controls and Risk of Ischemic Stroke

<table>
<thead>
<tr>
<th>SNP</th>
<th>Cases</th>
<th>Controls</th>
<th>Univariate OR (95% CI)</th>
<th>Multivariate Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Model 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Model 1</td>
</tr>
<tr>
<td>SNP87(rs2910829)</td>
<td></td>
<td></td>
<td></td>
<td>Model 1</td>
</tr>
<tr>
<td>CC*</td>
<td>76 (45)</td>
<td>86 (42)</td>
<td>1.00 (0.73–1.60)</td>
<td>1.20 (0.87–1.69)</td>
</tr>
<tr>
<td>CT</td>
<td>57 (33)</td>
<td>78 (39)</td>
<td>0.82 (0.60–1.20)</td>
<td>1.12 (0.80–1.61)</td>
</tr>
<tr>
<td>TT</td>
<td>37 (22)</td>
<td>39 (19)</td>
<td>1.07 (0.73–1.60)</td>
<td>1.40 (0.92–2.1)</td>
</tr>
<tr>
<td>C allele</td>
<td>104 (61.4)</td>
<td>125 (61.6)</td>
<td>1.00 (0.74–1.35)</td>
<td>1.21 (0.87–1.69)</td>
</tr>
<tr>
<td>T allele</td>
<td>66 (38.5)</td>
<td>78 (38.4)</td>
<td>1.00 (0.74–1.20)</td>
<td>1.40 (0.92–2.1)</td>
</tr>
<tr>
<td>SNP83(rs966221)</td>
<td></td>
<td></td>
<td></td>
<td>Model 1</td>
</tr>
<tr>
<td>CC*</td>
<td>55 (27.8)</td>
<td>49 (19.1)</td>
<td>1.00 (0.74–1.20)</td>
<td>1.20 (0.87–1.69)</td>
</tr>
<tr>
<td>CT</td>
<td>96 (48.5)</td>
<td>139 (54.1)</td>
<td>1.01 (0.74–1.40)</td>
<td>1.24 (0.86–1.78)</td>
</tr>
<tr>
<td>TT</td>
<td>47 (23.7)</td>
<td>69 (26.8)</td>
<td>1.64 (1.13–2.40)†</td>
<td>1.96 (1.28–3.02)†</td>
</tr>
<tr>
<td>T allele</td>
<td>95 (48)</td>
<td>139 (54)</td>
<td>1.26 (0.97–1.64)</td>
<td>1.38 (1.02–1.86)†</td>
</tr>
<tr>
<td>C allele</td>
<td>103 (52)</td>
<td>118 (46)</td>
<td>1.00 (0.74–1.20)</td>
<td>1.40 (0.92–2.1)</td>
</tr>
<tr>
<td>SNP32(rs456009)</td>
<td></td>
<td></td>
<td></td>
<td>Model 1</td>
</tr>
<tr>
<td>AA*</td>
<td>56 (28.6)</td>
<td>69 (26.8)</td>
<td>1.00 (0.74–1.20)</td>
<td>1.20 (0.87–1.69)</td>
</tr>
<tr>
<td>AC</td>
<td>87 (44.4)</td>
<td>139 (54.1)</td>
<td>1.01 (0.73–1.40)</td>
<td>1.23 (0.85–1.78)</td>
</tr>
<tr>
<td>CC</td>
<td>53 (27.0)</td>
<td>49 (19.1)</td>
<td>1.28 (0.88–1.86)</td>
<td>1.19 (0.78–1.8)</td>
</tr>
<tr>
<td>A allele</td>
<td>100 (51)</td>
<td>115 (54)</td>
<td>1.13 (0.86–1.50)</td>
<td>1.11 (0.81–1.51)</td>
</tr>
<tr>
<td>C allele</td>
<td>96 (49)</td>
<td>98 (46)</td>
<td>1.00 (0.74–1.20)</td>
<td>1.40 (0.92–2.1)</td>
</tr>
</tbody>
</table>

In model 1, values are adjusted for age, gender, and ethnicity, whereas values in model 2 are adjusted for age, gender, ethnicity, hypertension, and diabetes mellitus.

Genotypes were taken as reference for a given marker. Odds ratios were calculated under 2 different multivariate models.

P value for odds ratio† is <0.005; and for odds ratio‡, it is <0.05.
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