Insulin Resistance and Elevated Levels of Tissue Plasminogen Activator in First-Degree Relatives of South Asian Patients With Ischemic Cerebrovascular Disease

Kirti Kain, MRCP; Andrew J. Catto, MRCP, PhD; John Young, MSc, FRCP; John Bamford, MD, FRCP; John Bavington, MRCPsych; Peter J. Grant, MD, FRCP

Background and Purpose—South Asians in the United Kingdom suffer from an increased mortality from cerebrovascular disease compared with whites. Evidence suggests that the relatives of white stroke patients are at increased risk of vascular disease. The aim of this study was to investigate atherothrombotic risk factors in the first-degree relatives of South Asian patients suffering from ischemic cerebrovascular disease and to compare them with South Asian subjects free from clinically detectable cerebrovascular disease.

Methods—We compared 143 relatives of South Asians with ischemic stroke (South Asian relatives group) with 146 South Asian control subjects from West Yorkshire, UK.

Results—The ages and ethnic and sex distributions of South Asian relatives and South Asian controls were similar. There were no significant differences in body mass index, waist-hip ratio, number of current smokers, and past medical history of hypertension, diabetes mellitus, or myocardial infarction between the 2 groups. Fasting blood glucose, glycosylated hemoglobin (HbA1c), total cholesterol, triglycerides, and HDL cholesterol were similar in the 2 groups. Fasting insulin (South Asian relatives, 12.0; South Asian controls, 8.5 mU/L; \( P < 0.0001 \)) (independent of tissue plasminogen activator) and insulin resistance (derived by Homeostasis Model Assessment) (South Asian relatives, 2.7; South Asian controls, 1.9; \( P = 0.001 \)) were significantly raised in stroke relatives. Stroke relatives showed elevated levels of tissue plasminogen activator (South Asian relatives, 11.6; South Asian controls, 8.4 ng/mL; \( P < 0.0001 \)), which was independent of plasma insulin. There were no differences in plasminogen activator inhibitor antigen or activity between the groups.

Conclusions—South Asians stroke relatives exhibit hyperinsulinemia, increased insulin resistance, and increased tissue plasminogen activator levels. These observations might account for increased susceptibility to atherothrombotic disease in this ethnic group. (Stroke. 2001;32:1069-1073.)

Key Words: case-control studies ■ ethnic groups ■ fibrinolysis ■ insulin

A therothrombotic stroke is one of the leading causes of mortality in South Asians residing in the United Kingdom. During 1989–1992, the standardized mortality ratio for cerebrovascular disease was 155 for South Asian males and 141 for South Asian females compared with whites (taken as 100). The precise mechanisms that account for the excess cerebrovascular mortality among South Asians have not been adequately explained. However, several studies indicate higher plasma insulin levels and insulin resistance in South Asians than in their white counterparts. Consequently, South Asians tend to have more abnormalities in those risk factors clustering with hyperinsulinemia and insulin resistance, specifically, higher waist-hip ratio (WHR), hypertriglyceridemia, lower HDL cholesterol, and an increased prevalence of hypertension. These observations either alone or in consort might account for some of the excess risk of cerebrovascular disease in South Asians. This is of interest because several, but not all, prospective studies in whites demonstrate that hyperinsulinemia is associated with an increased risk of ischemic stroke. Furthermore, hemostatic factors and fibrinolytic factors in particular, such as plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA), might also be of interest in this context, since these factors have also been associated with insulin resistance. For example, in a prospective study of middle-aged white men, increased tPA levels independently predict risk of ischemic cerebrovascular disease.

In white populations, first-degree relatives of subjects with cerebrovascular disease are at increased risk of developing cardiovascular and cerebrovascular disease. The determin-
nants of the increased risk in whites are unknown. No information is available on atherothrombotic vascular risk factors in the first-degree relatives of South Asian subjects with ischemic stroke. Therefore, the aim of this study was to investigate atherothrombotic risk factors, particularly those risk factors clustering with hyperinsulinemia and insulin resistance and fibrinolytic factors, in the first-degree relatives of South Asian ischemic stroke patients and comparable South Asian control subjects without a personal or family history of stroke.

Subjects and Methods

Study Subjects

A history of ischemic stroke was confirmed in South Asian patients from clinical records and the results of a noncontrast cranial CT scan. One hundred forty-three South Asian first-degree relatives (mother, father, or siblings) of confirmed stroke patients were recruited. Only one relative of each stroke subject was recruited, and therefore relatives were derived from different families. Relatives of subjects with subarachnoid hemorrhage were not included. One hundred forty-six South Asian control subjects were recruited from general practitioners' lists from West Yorkshire and were considered free from a personal or family history of cerebrovascular disease. All subjects were of South Asian origin (India, Pakistan, or Bangladesh), and their 4 grandparents were from one of these countries. Subjects with mixed parentage were not recruited. All the subjects gave written informed consent according to a protocol approved by the local research ethics committee.

Assessment of Anthropometric and Clinical Risk Factors

Height and weight were recorded without shoes and in light clothing. Body mass index (BMI) was calculated from weight in kilograms divided by height squared in meters. Blood pressure was taken after an overnight fast of at least 10 hours and rest for 20 minutes, at maximal protrusion of the hips at the level of symphysis pubica, abdominal girth to the nearest 0.5 cm and dividing it by measurement also to the nearest 2 mm Hg. Smoking history was classified into subjects who never smoked and smokers (ex-smokers or current smokers). WHR was calculated by taking the measurement at minimum abdominal girth to the nearest 0.5 cm and dividing it by measurement at maximal protrusion of the hips at the level of symphysis pubica, also to the nearest 0.5 cm.

Biochemical Measurements

After an overnight fast of at least 10 hours and rest for 20 minutes, fasting blood samples without venous stasis, were drawn from an antecubital vein with a 19-gauge needle. Blood was taken into 0.9% iced citrate (pH 8.8) at a ratio of 9 parts blood to 1 part citrate for assay of tPA, PAI-1 antigen, and PAI-1 activity and into iced lithium heparin for the determination of insulin levels. Samples were centrifuged at 2560g and 4°C for 30 minutes. Aliquots of plasma were snap-frozen in liquid nitrogen and stored at −40°C until assay. PAI-1 activity was measured by chromogenic assay; PAI-1 antigen, tPA, and plasma insulin were measured by the enzyme-linked immunosorbent assay method.

The interassay and intra-assay coefficients of variation were 9.5% and 7% for tPA, 8.0% and 6.3% for PAI-1 activity, 9.7% and 6.0% for PAI-1 antigen, and 5.6% and 5.3% at 18 μIU/mL and 9.8% and 3.0% at 84 μIU/mL for insulin, respectively.

Values for insulin resistance were calculated from the Homeostasis Model Assessment (HOMA), which assumes that normal-weight, healthy subjects aged <35 years have 100% β-cell function and an insulin resistance of unity. 17 HOMA was expressed as a product of fasting insulin and glucose levels divided by 22.5.

Measurements of plasma glucose (by glucose oxidase method), cholesterol, and triglycerides were determined by Hitachi 747 autoanalyzer (Boehringer Mannheim). HDL cholesterol was measured by Hitachi 717 autoanalyzer after removal of chylomicrons and LDL by precipitation with phosphotungstic acid and magnesium chloride. Glycosylated hemoglobin (HbA1c) was measured by Glycomat autoanalyzer (Ciba Corning), with a reference range of 4.5% to 6.5%.

Statistical Methods

Values for BMI, WHR, fasting blood glucose, HbA1c, triglycerides, HDL cholesterol, LDL cholesterol, insulin, PAI-1 antigen, and PAI-1 activity were log-transformed to achieve near normal distribution. Differences in continuous variables between the groups were assessed by independent sample t test. Differences in categorical data between the 2 groups were assessed by χ² test. Partial age-adjusted correlation was used to assess the relationship of levels of insulin and tPA with other continuous variables. Logistic regression analysis was performed to determine significant differences in atherothrombotic risk factors between South Asian relatives and controls. ANOVA was used to study the differences in mean levels of insulin and tPA between the 2 groups. Statistical significance was taken as P<0.05. All statistical analyses were performed with SPSS for Windows version 9.0 (SPSS Inc).

Results

The ethnic distribution of South Asian subjects from India, Pakistan, and Bangladesh in the 2 groups was the same (data not shown). The demographic and clinical characteristics of the relatives of South Asians with ischemic stroke (South Asian relatives group) and South Asian controls are presented in Table 1. There was no difference in age, sex, and anthropometric measurements between the 2 groups. The frequencies of hypertension, diabetes mellitus, and myocardial infarctions were similar in both groups. No difference was observed in fasting lipid levels or HbA1c.

Insulin levels and insulin resistance (derived from the HOMA estimation) was greater in South Asian relatives than in South Asian controls (Table 1). Age-adjusted insulin correlated with BMI (South Asian relatives, r=0.38, P=0.0001; South Asian controls, r=0.41, P=0.0001), HDL cholesterol (South Asian relatives, r=−0.20, P=0.02; South Asian controls, r=−0.46, P=0.0001), triglycerides (South Asian relatives, r=0.20, P=0.03; South Asian controls, r=0.37, P=0.0001), and tPA (Table 2) in both groups. Fasting insulin (age adjusted) also correlated with systolic blood pressure (r=0.21, P=0.02) in South Asian relatives and with WHR (r=0.42, P=0.0001) and diastolic blood pressure (r=0.23, P=0.008) in South Asian controls.

Fasting insulin was higher in South Asian relatives even after adjustment for age, sex, systolic blood pressure, and ethnic origin (12.02 versus 8.32 mU/L; P=0.0001). When we added fasting blood glucose, triglycerides, HDL cholesterol, WHR, and BMI to the ANCOVA model, the differences remained significant (12.0 versus 9.02 mU/L; P=0.001). Finally, with tPA in the model, insulin remained significantly higher in South Asian relatives (11.78 versus 9.60 mU/L; P=0.03).

Unadjusted tPA levels were significantly elevated in South Asian relatives, the mean difference being 3.2 ng/mL (P<0.00001) (Table 1). tPA correlated with the features of insulin resistance, namely, WHR, fasting blood glucose, triglycerides, HDL cholesterol, and insulin in both South Asian relatives and South Asian controls and with diastolic blood pressure and BMI in South Asian controls (Table 2).
tPA levels were elevated in South Asian relatives compared with South Asian controls, even after adjustment for age, sex, systolic blood pressure, and ethnic origin (11.6 versus 8.5 ng/mL; \( P < 0.0001 \)). When insulin was added to the aforementioned factors, mean tPA values were 11.4 versus 8.7 ng/mL (\( P < 0.0001 \)). With insulin resistance included in the model (but with insulin excluded), mean values were 11.4 versus 8.7 ng/mL (\( P < 0.0001 \)). Finally, after adjustment for all the insulin resistance risk factors, including fasting blood glucose, fasting insulin, triglycerides, HDL cholesterol, WHR, and BMI, in addition to the aforementioned factors, tPA was persistently elevated in South Asian relatives compared with South Asian controls (11.2 versus 8.7 ng/mL, respectively; \( P < 0.0001 \)).

When we used a logistic regression model with the group (relative versus control) as the dependent variable and tPA, age, sex, systolic blood pressure, insulin, fasting blood glucose, triglycerides, HDL cholesterol, WHR, and BMI, the variables that were significantly different between South Asian relatives and South Asian controls were tPA, fasting blood glucose, and insulin. The odds ratio for 1-SD change in insulin (mU/L) was 1.5 (95% CI, 1.06 to 2.17; \( P = 0.01 \)). The odds ratio for 1-SD change in tPA (ng/mL) was 3.7 (95% CI, 2.36 to 5.96; \( P < 0.0001 \)).

### Discussion

South Asians exhibit significantly increased mortality from coronary artery disease and cerebrovascular disease.\(^1\) The adverse conventional risk factor profile and features of the insulin resistance syndrome in South Asians do not fully account for the increased mortality.\(^2\)^\(^4\)^\(^5\) We know of no study of hemostatic risk factors for ischemic stroke in South Asians. There are only limited data on the heritability of circulating risk factors in South Asian relatives, although Shaukat et al\(^18\) demonstrated that insulin levels in the male offspring of South Asian subjects awaiting coronary angiography were elevated compared with whites.

#### TABLE 1. Demographics, Clinical Characteristics, and Risk Factor Levels in First-Degree Relatives of South Asian Stroke Patients and Control Subjects

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Stroke Relatives (n=143)</th>
<th>Controls (n=146)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male:female</td>
<td>75:68</td>
<td>75:71</td>
<td>NS</td>
</tr>
<tr>
<td>Age, y (range)</td>
<td>40 (19–80)</td>
<td>40 (17–81)</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>26 (1.1)</td>
<td>25 (1.2)</td>
<td>NS</td>
</tr>
<tr>
<td>WHR</td>
<td>1.1 (1.1)</td>
<td>1.1 (1.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Current smokers:nonsmokers, %</td>
<td>11.0</td>
<td>9.0</td>
<td>NS</td>
</tr>
<tr>
<td>History, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>12.0</td>
<td>13.0</td>
<td>NS</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>2.0</td>
<td>3.0</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>6.0</td>
<td>7.0</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>130 (23)</td>
<td>128 (22)</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>85 (13)</td>
<td>82 (13)</td>
<td>0.06</td>
</tr>
<tr>
<td>Fasting blood glucose, mmol/L</td>
<td>5.1 (1.2)</td>
<td>5.1 (1.3)</td>
<td>NS</td>
</tr>
<tr>
<td>HbA(_1)c, %</td>
<td>5.3 (1.1)</td>
<td>5.3 (1.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.2 (1.0)</td>
<td>5.1 (1.1)</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.1 (1.3)</td>
<td>1.1 (1.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.5 (1.8)</td>
<td>1.4 (1.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting insulin, mU/L</td>
<td>12 (1.8)</td>
<td>8.5 (2.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin resistance*</td>
<td>2.7 (1.9)</td>
<td>1.9 (2.7)</td>
<td>0.001</td>
</tr>
<tr>
<td>tPA, ng/mL</td>
<td>11.6 (4.0)</td>
<td>8.4 (3.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PAI-1 activity, U/mL</td>
<td>16.7 (2.1)</td>
<td>15.5 (2.2)</td>
<td>NS</td>
</tr>
<tr>
<td>PAI-1 antigen, ng/mL</td>
<td>12.2 (2.3)</td>
<td>11.1 (2.8)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean (SD) unless indicated otherwise.
*Calculated by HOMA.

#### TABLE 2. Age-Adjusted Partial Correlation Coefficients of tPA With Features of Insulin Resistance in First-Degree Relatives of South Asian Stroke Patients and Control Subjects

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Relatives</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.07</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td>0.14</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>Fasting blood glucose</td>
<td>0.24</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.43</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>−0.39</td>
<td>−0.50</td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>0.25</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.04</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.14</td>
<td>0.26</td>
<td></td>
</tr>
</tbody>
</table>

\( P < 0.05 \) when \( r > 0.11 \); \( P < 0.01 \) when \( r > 0.21 \).
The established metabolic and environmental risk factors for stroke in whites are age, hypertension, diabetes mellitus, and smoking. The hemostatic risk factors prospectively linked to ischemic cerebrovascular disease are tPA and fibrinogen.14,19–21 Fibrinolytic factors also contribute to ischemic cerebrovascular disease through a strong association with insulin resistance. Moreover, a family history of coronary artery disease and cerebrovascular disease increases the individual’s risk of vascular morbidity, especially at a younger age; this has been verified in studies of white families with coronary artery and cerebrovascular disease.15,16

The results from our study demonstrate, for the first time, that first-degree relatives of South Asian stroke patients have increased levels of tPA, along with increased plasma insulin levels and increased HOMA index for insulin resistance. The elevation in tPA levels in South Asian stroke relatives was independent of plasma insulin and the HOMA index of insulin resistance. In white subjects, there is already firm evidence that elevated tPA levels are predictive of risk of first stroke.14 In the US Physicians Health Study, 88 subjects who developed a stroke within 5 years of follow-up, compared with 471 disease-free controls, had significantly elevated baseline tPA levels (11.1 versus 9.6 ng/mL). This increase in tPA is comparable to that seen in the South Asian relatives in the present study and indicates that elevated tPA may be a risk factor in this asymptomatic at-risk population. There is still some controversy as to why tPA is one of the most consistent risk factors for vascular disease, when functionally elevated tPA is intuitively related to the maintenance of vascular patency. One suggestion has been that it merely reflects elevated levels of the inhibitor PAI-1, although there is no evidence to support this in the present study, since PAI-1 concentrations were the same in the 2 groups. Alternatively, tPA may represent an early association with underlying insulin resistance, and the relatives exhibited some degree of fasting hyperinsulinemia with normoglycemia to support this view. There is also evidence from white stroke subjects that insulin resistance may play a role in the pathogenesis of ischemic stroke.6 These findings indicate that elevated tPA, in addition to insulin resistance, may be an early factor of atherothrombotic risk in high-risk South Asian subjects.

Insulin resistance is a hallmark of atherosclerosis; it is associated with compensatory hyperinsulinemia and dyslipidemia and predicts both stroke and ischemic heart disease in whites.6,7,22 There is also an increased prevalence of hyperinsulinemia and insulin resistance in South Asians compared with whites.4 The present study demonstrates that South Asian relatives of stroke patients exhibit hyperinsulinemia (independent of tPA) and increased insulin resistance compared with the South Asian controls, putting them at increased risk of both coronary artery disease and ischemic stroke.

This study also indicates considerable similarities in the risk factor profile of South Asian relatives and controls. In particular, the prevalence of diabetes and hypertension in the 2 groups was similar. However, the increased insulin resistance in relatives might be the primary underlying factor, which may lead in some subjects to diabetes and in some others to hypertension and which has some connection with the development of elevated tPA levels. Our study design did not include oral glucose tolerance tests, and thus we cannot exclude the possibility that despite similar fasting glucose levels, South Asian relatives of stroke patients would have a higher prevalence of milder abnormalities of glucose tolerance than South Asian control subjects.

Our main finding is that South Asian relatives of stroke patients are more insulin resistant than South Asian controls, and they have elevated levels of tPA (independent of plasma insulin levels or HOMA index of insulin resistance). However, these findings do not allow inferences on causality because of the cross-sectional nature of the study. Therefore, a prospective study in the South Asian population is needed to confirm our findings.

**Acknowledgments**

This study was supported by a grant from the Stroke Association.

**References**

12. Thogersen AM, Jansson JH, Boman K, Nilsson TK, Weinheil L, Huhtasaari F, Hallmans G. High plasminogen activator inhibitor and tissue plasminogen activator levels in plasma precede a first acute myocardial infarction in both men and women: evidence for the...


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*Stroke.* 2001;32:1069-1073
doi: 10.1161/01.STR.32.5.1069

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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