Synergistic Effect of −174 G/C Polymorphism of the Interleukin-6 Gene Promoter and 469 E/K Polymorphism of the Intercellular Adhesion Molecule-1 Gene in Italian Patients With History of Ischemic Stroke

Roberto Pola, MD, PhD; Andrea Flex, MD; Eleonora Gaetani, MD; Roberto Flore, MD; Michele Serricchio, MD; Paolo Pola, MD

Background and Purpose—Interleukin-6 (IL-6) and intercellular adhesion molecule-1 (ICAM-1) are involved in the pathogenetic mechanisms responsible for several ischemic cardiovascular disorders, including cerebral ischemia. IL-6 and ICAM-1 plasma levels and/or function may be genetically influenced. We sought to evaluate distribution and reciprocal interaction of IL-6 G/C gene promoter polymorphism and ICAM-1 E/K gene polymorphism in Italian patients with history of ischemic stroke.

Methods—One hundred nineteen patients with history of ischemic stroke and 133 age- and sex-matched controls were studied. IL-6 and ICAM-1 genotypes were evaluated by polymerase chain reaction and restriction enzyme analysis.

Results—The GG genotype of −174 IL-6 G/C gene polymorphism was significantly associated with history of ischemic stroke at both univariate (P<0.0001) and multivariate analysis (odds ratio [OR], 8.6; P<0.0001). Additionally, the EE genotype of ICAM-1 E/K gene polymorphism was significantly more common in the group of patients with history of ischemic stroke (P=0.003) and was an independent variable associated with stroke history (OR, 4.0; P=0.002). Interestingly, a further increased risk of stroke was found in subjects who concomitantly carry the IL-6 GG and ICAM-1 EE genotypes (IL-6 GG/ICAM-1 EE double-homozygous subjects) (OR, 10.1; P=0.004).

Conclusions—There is a synergistic effect of IL-6 G/C and ICAM-1 E/K gene polymorphisms in patients with stroke history. Reciprocal interactions between genotypes may contribute in determining the risk profile for cardiovascular diseases and may merit further investigation as potential therapeutic targets. (Stroke. 2003;34:XXX—XXXX.)

Key Words: genetics ■ inflammation ■ intercellular adhesion molecule-1 ■ interleukins ■ polymorphism ■ stroke

Interleukin-6 (IL-6) is a prototypical inflammatory cytokine able to damage the endothelium and lead to the initiation of atherosclerosis.1 It is produced by several cell types, including fibroblasts, monocytes, adipocytes, and endothelial cells.2 IL-6 is considered important in the pathogenesis of several ischemic cardiovascular disorders, including unstable angina3 and acute coronary syndromes.4 IL-6 gene expression is also increased in animal models of ischemia/reperfusion brain injury.5 In humans, IL-6 participates in the acute phase response that follows cerebral ischemia, and there is an association between high plasma levels of IL-6 and early neurological worsening after stroke6 and progression of lacunar infarction.7

Intercellular adhesion molecule-1 (ICAM-1) is a member of the immunoglobulin superfamily and the principal ligand for the leukocyte function-associated antigen-1 (LFA-1), a member of the integrin superfamily. One function of the ICAM-1/LFA-1 adhesion system is to assist leukocyte movement into tissue: LFA-1–positive leukocytes are induced to adhere to ICAM-1–positive endothelial surface8,9 and then to pass through the basement membrane into tissue.10,11 LFA-1 is constitutively expressed by brain microglial cells and is upregulated in pathological conditions associated with microglial reaction.12 Both animal and human studies indicate that ICAM-1 may be implicated in the pathogenesis of ischemic cardiovascular disorders, including stroke.13,14 In particular, homozygous null ICAM-1 mice have reduced development of atherosclerotic fatty streaks after a high-fat, high-cholesterol diet and exhibit cerebral protection after occlusion of the middle cerebral artery.13,15 Very recently, it has been shown that elevated plasma concentrations of soluble ICAM-1 are associated with increased risk of future ischemic stroke in patients affected by coronary artery disease.16 Interestingly, there is a reciprocal interaction between ICAM-1 and IL-6, as
ICAM-1 also induces expression of IL-6,17,18 which eventually orchestrates production of other acute phase proteins, amplifying and maintaining the inflammatory phenotype.3

The genes encoding for IL-6 and ICAM-1 are both affected by common, functionally important, genetic polymorphisms. In particular, a guanine/cytosine substitution occurs in position −174 of the IL-6 gene promoter, resulting in 3 possible genotypes (GG, GC, and CC) of the −174 IL-6 G/C polymorphism.19 Another polymorphism occurs in exon 6 of the ICAM-1 gene and results in a change from glutamic acid to lysine in immunoglobulin-like domain 5 of the ICAM-1 protein (469 E/K ICAM-1 gene polymorphism).20 Both of these polymorphisms have been associated with several atherosclerotic and ischemic disorders, including lacunar infarction and vascular dementia.1,21–27

The aim of this study was to evaluate distribution and reciprocal interaction of IL-6 G/C gene promoter polymorphism and ICAM-1 E/K gene polymorphism in Italian patients with history of ischemic stroke.

Subjects and Methods

Patients

Patients and controls were recruited among subjects consecutively admitted to the Department of Medicine of the A. Gemelli University Hospital of Rome, Italy, from January 1, 2001, to April 31, 2002. Subjects who had suffered from an ischemic stroke in the past and had survived this event were enlisted for patients with history of ischemic stroke. The cerebral ischemic event had been documented by CT scan or MRI of the brain. Among these individuals, those with history of cranial trauma, cerebral hemorrhage, atrial fibrillation, other major sources of cardioembolism, coagulation disorders, tumors, chronic inflammatory diseases, and autoimmune diseases were excluded from the study. After exclusion of these cases, a final number of 119 subjects (mean ± SD age, 76.8 ± 8.4 years) were enrolled in the group of patients with history of ischemic stroke. One hundred thirty-three individuals, attending the same Department of Medicine, matched for age and sex, without clinical history or instrumental evidence of cerebrovascular disease or present neurological disturbances, were recruited as controls (mean ± SD age, 76.2 ± 7.1 years). The exclusion criteria were the same as those in the aforementioned patient group. Controls had no relationship with cases and no family history of stroke. All subjects were whites from central and southern Italy and belonged to independent pedigrees.

For all individuals enrolled in the study, a complete medical history was collected and included smoking habits, alcohol consumption, presence of diabetes, and drug treatment. Hypertension was defined as either a systolic blood pressure >140 mm Hg, a diastolic pressure >90 mm Hg, or current treatment with an antihypertensive drug. Hypercholesterolemia was defined as either a need for hypolipidemic drugs or total plasma cholesterol level >5.18 mmol/L. Diabetes was defined as either a fasting plasma glucose level ≥7.8 mmol/L on more than 2 occasions, presence of diabetes, or use of hypoglycemic drugs. Hypertension, diabetes, presence of diabetes, and drug treatment. Hypertension was defined as either a systolic blood pressure >140 mm Hg, a diastolic pressure >90 mm Hg, or current treatment with an antihypertensive drug. Hypercholesterolemia was defined as either a need for hypolipidemic drugs or total plasma cholesterol level >5.18 mmol/L. Diabetes was defined as either a fasting plasma glucose level ≥7.8 mmol/L on more than 2 occasions, presence of diabetes, or use of hypoglycemic drugs. Hypertension, diabetes, history of coronary artery disease, and peripheral artery occlusive disease, were significantly more frequent in patients than controls.

Table 2 shows the distribution of IL-6 and ICAM-1 genotypes and alleles. Genotypes were in Hardy-Weinberg equilibrium. Logistic regression model was used. All analyses were performed with the use of Intercooled STATA 6.0 for Windows (Statistics/Data Analysis, Stata Corporation). Statistical significance was established at P<0.05.

Results

The demographic and clinical data of patients with history of ischemic stroke and controls are shown in Table 1. There were no significant differences between groups in terms of age, sex, smoking habits, and hypercholesterolemia. In contrast, other classic risk factors for ischemic stroke, such as hypertension, diabetes, history of coronary artery disease, and peripheral artery occlusive disease, were significantly more frequent in patients than controls.

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| TABLE 1. Demographic and Clinical Data in Patients With History of Ischemic Stroke and Controls |
|---------------------------------|-------------------|-------------------|
|                                | **HIS Patients** | **Controls** |
|                                | (n=119)          | (n=133)         | **P** |
| Age, y±SD                       | 76.8±8.4         | 76.2±7.1        | 0.48  |
| Male:female ratio               | 57:62            | 62:71           | 0.83  |
| Hypertension                    | 88 (74)          | 69 (52)         | <0.0001 |
| Hypercholesterolemia            | 40 (33)          | 41 (31)         | 0.63  |
| Diabetes                        | 37 (31)          | 24 (18)         | 0.016 |
| CAD                             | 51 (43)          | 35 (26)         | 0.006 |
| PAOD                            | 30 (25)          | 14 (10)         | 0.002 |
| Smoking (current)               | 7 (6)            | 16 (12)         | 0.09  |
| Smoking (former)                | 26 (22)          | 35 (26)         | 0.40  |

HIS indicates history of ischemic stroke; CAD, coronary artery disease; PAOD, peripheral artery occlusive disease. Values in parentheses are percentages.

Statistical Analyses

Demographic and clinical data between groups were compared by χ2 test and t test. Genotype and allele frequencies were compared by χ2 test. Odds ratios (ORs) were calculated with 95% CI. To estimate the association between genotype and history of ischemic stroke, a logistic regression model was used. All analyses were performed with the use of Intercooled STATA 6.0 for Windows (Statistics/Data Analysis, Stata Corporation). Statistical significance was established at P<0.05.
The frequency of the GG genotype in patients with stroke was almost 4 times higher than in controls ($P<0.0001$). In contrast, the frequency of the CC genotype was significantly different between the 2 groups ($P<0.0001$). Likewise, the ratio between the G and C allele frequency was significantly different between the 2 groups ($P<0.0001$).

In addition, the distribution of ICAM-1 genotypes was significantly different between groups (Table 2). In particular, the frequency of the EE genotype in patients with stroke was 2 times higher than in controls ($P=0.003$), while the frequency of the KK genotype was significantly higher in control subjects ($P=0.004$). The ratio between the E and K allele frequency was also significantly different between the 2 groups ($P=0.0003$).

We also evaluated how IL-6 and ICAM-1 genotypes are combined in patients with history of ischemic stroke and controls. Interestingly, we observed that the association between IL-6 GG genotype and ICAM-1 EE genotype (GG/EE or GG/EE subjects) was significantly more common in patients than controls ($P<0.0001$) (Table 3). Indeed, GG/EE and GG/EE subjects constitute almost 40% of patients with history of ischemic stroke and only 11.1% of controls (Table 3). In contrast, the association of IL-6 CC and ICAM-1 KK genotypes (CC/ KK and CC/ KK subjects) was significantly more common in controls than in patients with history of ischemic stroke ($P=0.002$) (Table 3). Similar results were obtained when the combination of IL-6 and ICAM-1 alleles was analyzed. The simultaneous presence of the IL-6 G and ICAM-1 E alleles (G+/E+ subjects) was found in a significantly higher number of patients with history of ischemic stroke than in controls ($P<0.0001$), while the concomitant presence of the IL-6 C and ICAM-1 K alleles (C+/K+ subjects) was significantly more common in control subjects than in stroke patients ($P<0.0001$) (Table 3).

Following these observations, we used a logistic regression model to evaluate whether the GG genotype of the IL-6 gene polymorphism and the EE genotype of the ICAM-1 gene polymorphism are independent variables associated with stroke (Table 4). After adjusting for relevant confounding variables, such as sex, age, hypertension, hypercholesterolemia, diabetes, other cardiovascular diseases, and smoking, we found that both genotypes are significantly associated with history of ischemic stroke. This association is stronger for the GG genotype of the IL-6 gene polymorphism (OR, 8.6; 95% CI, 3.7 to 20.2; $P<0.0001$) but still significant for the EE genotype of the ICAM-1 gene polymorphism (OR, 4.0; 95% CI, 1.7 to 9.5; $P=0.001$) (Table 4). Finally, we evaluated whether the combination of these 2 genotypes (GG/EE subjects) represents an even stronger risk factor for stroke. Again, we used a logistic regression model and

### TABLE 3. Combined Distribution of IL-6 and ICAM-1 Genotypes and Alleles in His Patients and Controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>HIS</th>
<th>Controls</th>
<th>$P$</th>
<th>HIS</th>
<th>Controls</th>
<th>$P$</th>
<th>HIS</th>
<th>Controls</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>KK</td>
<td>12 (10.1)</td>
<td>13 (9.7)</td>
<td>0.93</td>
<td>11 (9.2)</td>
<td>23 (17.2)</td>
<td>0.06</td>
<td>1 (0.8)</td>
<td>13 (9.7)</td>
<td>0.002</td>
</tr>
<tr>
<td>EK</td>
<td>30 (25.2)</td>
<td>13 (9.7)</td>
<td>&lt;0.001</td>
<td>25 (21.0)</td>
<td>29 (21.6)</td>
<td>0.87</td>
<td>8 (6.7)</td>
<td>27 (20.1)</td>
<td>0.002</td>
</tr>
<tr>
<td>EE</td>
<td>17 (14.3)</td>
<td>2 (1.4)</td>
<td>&lt;0.001</td>
<td>12 (10.1)</td>
<td>7 (5.2)</td>
<td>0.14</td>
<td>3 (2.5)</td>
<td>7 (5.2)</td>
<td>0.26</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alleles</th>
<th>HIS</th>
<th>Controls</th>
<th>$P$</th>
<th>Alleles</th>
<th>HIS</th>
<th>Controls</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>G+/E+</td>
<td>84 (70.6)</td>
<td>51 (38.1)</td>
<td>&lt;0.001</td>
<td>C+/K+</td>
<td>45 (37.8)</td>
<td>90 (67.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>G+/E−</td>
<td>23 (19.3)</td>
<td>36 (26.9)</td>
<td>0.14</td>
<td>C+/K−</td>
<td>15 (12.6)</td>
<td>14 (10.4)</td>
<td>0.6</td>
</tr>
<tr>
<td>G−/E+</td>
<td>11 (9.2)</td>
<td>15 (11.2)</td>
<td>0.74</td>
<td>C−/K+</td>
<td>42 (35.3)</td>
<td>26 (19.4)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

HIS indicates history of ischemic stroke. Values in parentheses are percentages.

### TABLE 4. Variables Independently Associated With HIS by Logistic Regression Analysis

<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>CI (95%)</th>
<th>$P$</th>
<th>OR</th>
<th>CI (95%)</th>
<th>$P$</th>
<th>OR</th>
<th>CI (95%)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ICAM-1 EE genotype</td>
<td>4.0</td>
<td>1.7–9.5</td>
<td>0.001</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>IL-6 GG genotype</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>8.6</td>
<td>3.7–20.2</td>
<td>&lt;0.0001</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>GG/EE genotype</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>10.1</td>
<td>2.1–48.5</td>
<td>0.004</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.9</td>
<td>0.5–1.7</td>
<td>0.901</td>
<td>0.9</td>
<td>0.4–1.6</td>
<td>0.718</td>
<td>0.8</td>
<td>0.4–1.3</td>
<td>0.411</td>
</tr>
<tr>
<td>Age</td>
<td>1.0</td>
<td>0.9–1.0</td>
<td>0.611</td>
<td>1.0</td>
<td>0.9–1.0</td>
<td>0.888</td>
<td>1.0</td>
<td>0.9–1.0</td>
<td>0.781</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2.7</td>
<td>1.5–4.9</td>
<td>0.001</td>
<td>2.2</td>
<td>1.2–4.1</td>
<td>0.007</td>
<td>2.3</td>
<td>1.3–4.2</td>
<td>0.003</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>1.2</td>
<td>0.7–2.3</td>
<td>0.395</td>
<td>1.2</td>
<td>0.6–2.1</td>
<td>0.585</td>
<td>1.3</td>
<td>0.7–2.3</td>
<td>0.391</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.7</td>
<td>0.8–3.3</td>
<td>0.102</td>
<td>2.4</td>
<td>1.2–4.9</td>
<td>0.011</td>
<td>1.8</td>
<td>0.9–3.5</td>
<td>0.071</td>
</tr>
<tr>
<td>CAD</td>
<td>1.7</td>
<td>0.9–3.2</td>
<td>0.006</td>
<td>1.6</td>
<td>0.9–3.1</td>
<td>0.104</td>
<td>1.7</td>
<td>0.9–3.1</td>
<td>0.070</td>
</tr>
<tr>
<td>PAOD</td>
<td>2.6</td>
<td>1.2–5.8</td>
<td>0.014</td>
<td>3.8</td>
<td>1.7–8.7</td>
<td>0.001</td>
<td>2.7</td>
<td>1.2–5.9</td>
<td>0.012</td>
</tr>
<tr>
<td>Smoking (current)</td>
<td>0.2</td>
<td>0.1–0.8</td>
<td>0.029</td>
<td>0.2</td>
<td>0.1–0.7</td>
<td>0.015</td>
<td>0.2</td>
<td>0.1–0.8</td>
<td>0.022</td>
</tr>
<tr>
<td>Smoking (former)</td>
<td>0.8</td>
<td>0.4–1.7</td>
<td>0.646</td>
<td>0.8</td>
<td>0.4–1.8</td>
<td>0.729</td>
<td>0.7</td>
<td>0.4–1.8</td>
<td>0.526</td>
</tr>
</tbody>
</table>

HIS indicates history of ischemic stroke; CAD, coronary artery disease; PAOD, peripheral artery occlusive disease.
adjusted for the same confounders considered above. Interestingly, the GG/EE combined homozygosity is more strongly associated with history of ischemic stroke than the 2 separately considered genotypes (OR, 10.1; 95% CI, 2.1 to 48.5; \( P = 0.004 \)) (Table 4).

**Discussion**

This study analyzes for the first time distribution and interaction of IL-6 and ICAM-1 gene polymorphisms in Italian patients with history of stroke. We found that both IL-6 G/C gene polymorphism and ICAM-1 E/K gene polymorphism are significantly associated with history of ischemic stroke. In our population, the presence of the G allele of the IL-6 gene or the E allele of the ICAM-1 gene is significantly associated with stroke history. In particular, occurrence of ischemic stroke is 8.6 times more common in patients homozygous for the G allele compared with CC homozygous individuals. Likewise, stroke occurs 4 times more often in patients carrying the EE genotype of the E/K ICAM-1 gene polymorphism compared with KK homozygous subjects.

A major finding of this study is the discovery that IL-6 and ICAM-1 gene polymorphisms act synergistically in patients with stroke history. Interestingly, the concomitant evaluation of the 2 polymorphisms helps in identifying subjects with history of ischemic stroke. In particular, the highest risk of ischemic stroke is found in IL-6 GG/ICAM-1 EE double-homozygous patients compared with subjects who are homozygous for the IL-6 GG or ICAM-1 EE genotype only (OR, 10.1; 95% CI, 2.1 to 48.5; \( P = 0.004 \)) (Table 4).

The clinical relevance of these gene polymorphisms is based on the fact that plasma levels and/or functional activity of IL-6 and ICAM-1 are strongly influenced by these gene variants. In particular, the GG genotype of the −174 G/C polymorphism of the IL-6 gene promoter correlates with higher circulating IL-6 concentrations, as shown in healthy whites and in Italians undergoing surgical coronary revascularization. However, how the −174 G/C polymorphism modulates IL-6 plasma levels remains controversial because it has been reported that the CC genotype is associated with higher levels of IL-6 in the blood of patients affected by abdominal aortic aneurysm. On the other hand, the 469 E/K polymorphism of the ICAM-1 gene results in a change in the amino acid sequence of the immunoglobulin-like domain 5. This domain is of crucial importance for the activity of the ICAM-1 protein because it modulates the interactions between ICAM-1 and LFA-1 and influences the adhesion of B-cells.

The synergistic effect of these gene polymorphisms mirrors the interaction observed in vitro between IL-6 and ICAM-1 proteins. ICAM-1 induces expression of several proinflammatory cytokines, including IL-1α, IL-1β, tumor necrosis factor-α, and IL-6. In astrocytes, ICAM-1 induces IL-6 expression via the activation of the p38 MAPK and ERK1/2 pathways. On the other hand, IL-6 orchestrates production of many acute phase proteins, promoting and maintaining the inflammatory phenotype. This interaction and amplification process may play an important role in the series of events involved in the pathogenesis and evolution of stroke.

This study has some potential limitations. It is a case-control study, and recruitment and survival bias cannot be excluded. We studied a selected sample of patients admitted to a Department of Medicine for reasons not necessarily related to cerebral ischemia, and this population might be not representative of all patients groups. In addition, our population includes subjects with multiple diseases, and comorbidity may represent a confounding factor. The size of the studied population is relatively small, and therefore our findings need to be confirmed in larger samples and should be tested in groups of different ethnic origins. Finally, we cannot exclude that the observed associations depend on the effect of genes in linkage disequilibrium with the IL-6 or ICAM-1 genes. A strong linkage disequilibrium between the IL-6 gene promoter polymorphism and the variable number of tandem repeat polymorphisms of the 3′ flanking region of the IL-6 gene has been described. Similarly, the ICAM-1 gene is very tightly linked to the LDL receptor gene on chromosome 19.

In conclusion, this study indicates that −174 G/C polymorphism of the IL-6 gene promoter and 469 E/K polymorphism of the ICAM-1 gene are significantly associated with history of ischemic stroke in an Italian population. This association is significantly stronger in IL-6 GG homozygous individuals who also carry the ICAM-1 EE genotype. Considering synergistic effects between genotypes may help in determining the risk profile for cardiovascular diseases.

**References**

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