Role of the Interleukin-6 −174 G>C Gene Polymorphism in Retinal Artery Occlusion

Martin Weger, MD; Iris Steinbrugger, MD; Anton Haas, MD; Winfried März, MD; Yousef El-Shabrawi, MD; Wolfgang Weger, MD; Otto Schmut, PhD; Wilfried Renner, PhD

Background and Purpose—Proinflammatory cytokines including interleukin-6 (IL-6) are supposed to play a pivotal role in the development of atherosclerosis. A common polymorphism in the promoter of the IL-6 gene (IL-6 −174G>C) affects plasma IL-6 concentrations and has been suggested as a risk factor for cardiovascular disease. The aim of the present case-control study was to investigate the role of this polymorphism for retinal artery occlusion (RAO).

Methods—One hundred eighty-two patients with RAO and 307 control subjects were genotyped for the IL-6 −174G>C polymorphism. Genotypes were determined by fluorogenic exonuclease (TaqMan) assay.

Results—The prevalence of the CC genotype was significantly lower in patients with RAO than in control subjects (10.4% versus 19.9%; \( P=0.006 \)). Homozygosity for the C allele was associated with an odds ratio of 0.50 (95% CI, 0.28 to 0.89) for RAO.

Conclusions—The CC genotype of the IL-6 −174G>C polymorphism may be associated with a protective effect against RAO. (Stroke. 2005;36:249-252.)

Key Words: atherosclerosis | genetics | interleukin-6 | ophthalmology | retinal artery occlusion | risk factors

Atherosclerosis is a low-grade chronic inflammatory disease.1 Numerous cytokines including interleukin 6 (IL-6) have been suggested to play an essential role in atherogenesis.2 IL-6 is a pleiotropic cytokine and is synthesized by several different cell types, including monocytes and vascular endothelial cells.3 Immunohistochemical studies found both increased IL-6 protein and mRNA concentrations in atherosclerotic plaques.4–6 Further evidence for its role in atherogenesis comes from animal experiments, showing that IL-6 promotes the development of early atherosclerotic lesions.7 In humans, increased plasma IL-6 levels have been associated with unstable angina and have predicted future cardiovascular events.8–12

In 1998, a functional polymorphism in the promoter region of the IL-6 gene at position −174 (−174G>C) was identified.13 An in vitro study using transfected human cell line cells reported higher baseline IL-6 levels in cells with the G construct compared with those transfected with the C allele.13 Stimulation with lipopolysaccharides or IL-1 resulted in a significantly increased IL-6 transcription rate; this effect, however, was restricted to cells with the G allele. In another in vitro study using anti-CD3/CD28–stimulated peripheral blood lymphocytes, IL-6 concentrations were 3× higher among carriers of the G allele.14 Additionally, some but not all in vivo studies found higher plasma IL-6 concentrations in subjects with the GG genotype than among homozygotes for the C allele.13,15,16

Recently, the −174G>C gene polymorphism has been suggested as a risk factor for coronary heart disease, carotid atherosclerosis and stroke.17–25 Its role in retinal artery occlusion (RAO), however, has not yet been determined.

RAO is a common cause for a severe visual loss in patients >50 years. Impaired blood flow in the central retinal artery or one of its branches leads to infarction of the affected retinal tissue. Embolization and hemorrhage under an atherosclerotic plaque have been shown to play a major role in the pathogenesis of RAO.26,27 Consequently, arterial hypertension, hyperhomocysteinemia, and arteriosclerosis as well as diabetes mellitus have been identified as risk factors.28–30 Yet only a fraction of cases can currently be explained by the known risk factors alone. The purpose of the present study was therefore to investigate an hypothesized association between the IL-6 −174G>C gene polymorphism and the presence of RAO.

Subjects and Methods

The study was designed as a retrospective case-control study to analyze the role of genetic risk factors for RAO. Patients and control subjects were seen at the local department of ophthalmology between September 1996 and June 2003. The study was performed according to the guidelines of the National Gene Technology Act and the local Ethics Committee. Written informed consent was obtained from all participants before enrolment.

All participants underwent ophthalmological examination, including visual acuity testing, slit-lamp, and fundus examination. RAO
was diagnosed by ophthalmoscopic fundus examination revealing superficial retinal whitening in the distribution of the involved artery. Accordingly, occlusion of the central retinal artery, involving the entire retina, was classified as central retinal artery occlusion (CRAO, n=89), whereas the involvement of one of its branches was classified as branch retinal artery occlusion (BRAO, n=93). Exclusion criteria for patients with RAO comprised giant cell arteritis and other types of vasculitis. The control group consisted of 307 subjects who were referred to our department for other reasons than retinal vascular occlusion. Subjects with any ophthalmological evidence or history of retinal vascular occlusion, anterior ischemic optic neuropathy, or vasculitis were not eligible as controls.

Arterial hypertension was defined by a systolic blood pressure ≥160 mm Hg, diastolic blood pressure ≥90 mm Hg, or the current use of antihypertensive drugs. Subjects were classified as diabetics when being treated for insulin-dependent or noninsulin-dependent diabetes mellitus. According to their smoking status, subjects were defined as non- or current smokers. Hypercholesterolemia was defined by the intake of lipid-lowering drugs or a fasting plasma cholesterol level ≥200 mg/dL.

Genotype Determination
Genomic DNA was isolated from peripheral blood lymphocytes by standard methods and stored at −20°C. Genotyping for the IL6 −174G>C polymorphism was done by a 5'-nuclease assay (TaqMan). Primer and probe sets were designed and manufactured using Applied Biosystems’ Assay-by-Design custom service. The polymerase chain reaction was performed in a Primus 96 plus thermal cycler (MWG Biotech AG) using a total volume of 5 μL containing 2.5 μL SuperHot Master Mix (Bioron GmbH, Germany), 0.125 μL Assay-by-design Mix (Applied Biosystems), 0.375 μL H2O, and 2 μL DNA. Reactions were overlaid with 15 μL mineral oil. Cycling parameters were 1 minute at 94°C for primary denaturation, followed by 45 cycles of 15 s at 92°C and 1 minute at 60°C. Fluorescence was measured in a VICTOR fluorescence plate reader (HVD Life Sciences) using excitation/emission filters of 485 nm/520 nm for FAM-labeled probes (−174C allele) and 530 nm/572 nm for VIC-labeled probes (−174G allele). The data were exported into Excel format and depicted and analyzed as scatter plot.

Statistics
SPSS for Windows (release 10.0.5; SPSS, Inc) was used for statistical analyses. Continuous variables were analyzed by t test and are presented as means±SE. Categorical variables are presented as percentages and are compared by χ2 test. Odds ratios and 95% CIs were determined by logistic regression analysis. The PS program was used for Power calculation.30 The criterion for statistical significance was P<0.05.

Results
Baseline parameters and clinical characteristics of patients and controls are shown in Table 1. As expected, arterial hypertension, hypercholesterolemia, and current smoking status were found significantly more often in patients than in control subjects.

Genotypes were determined successfully in all participants and did not deviate from the Hardy–Weinberg equilibrium. Table 2 shows genotype distribution of the IL6 −174G>C genotypes and the C allele frequencies in patients with RAO and controls. The prevalence of the CC genotype was significantly lower in patients with RAO than in control subjects (P=0.006). In a logistic regression analysis adjusted for arterial hypertension, hypercholesterolemia, smoking status, and history of myocardial infarction and stroke, homozygosity for the C allele was associated with an odds ratio of 0.50 (95% CI, 0.28 to 0.89) for RAO (Table 3).

Frequencies of the GG, GC, and CC genotypes did not significantly differ between patients with CRAO (28.0%, 59.6%, and 12.4%, respectively) or BRAO (41.9%, 49.5%, and 8.6%, respectively).

The prevalences of the CC genotype and C allele in the control group were similar to those previously reported by others.13,31–34 This study had a statistical power of 0.80 to detect an odds ratio of 0.47 for carriers of the homozygous CC genotype.

Discussion
The IL-6 −174G>C gene polymorphism has been previously suggested as a risk factor for cardiovascular disease.17–25 This study, however, is the first to investigate the role of this polymorphism in RAO. Genotypes were determined in 182 patients with RAO and 307 control subjects, showing a significantly lower prevalence of the CC genotype among patients with RAO. Homozygosity for the C allele was associated with an odds ratio of 0.50 for RAO. In addition, a subgroup analysis comparing genotype distribution among patients with CRAO and BRAO revealed no significant difference. Thus our data suggest a protective role of the CC genotype against both types of RAO. Considering the results from in vitro studies demonstrating lower expression of IL-6 by the −174C variant, this observation is biologically plausible.13,14 Among other cytokines, IL-6 has been shown to play an essential role in atherogenesis by inducing endothelial dysfunction, enhanced expression of adhesion molecules, proliferation of smooth muscle cells, and matrix degeneration.35–37 Furthermore, synthesis of coagulation factors is stimulated by IL-6.38–39 Although the precise mechanism is

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### Table 1. Baseline Characteristics of Patients and Controls

<table>
<thead>
<tr>
<th></th>
<th>Patients With RAO (n=182)</th>
<th>Controls (n=307)</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Males, (%)</td>
<td>105 (57.7)</td>
<td>172 (66.0)</td>
<td></td>
</tr>
<tr>
<td>Females, (%)</td>
<td>77 (42.3)</td>
<td>135 (44.0)</td>
<td></td>
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<tr>
<td>Mean age, years ± SD</td>
<td>69.1 ± 11.3</td>
<td>70.9 ± 11.4</td>
<td></td>
</tr>
<tr>
<td>Arterial hypertension, (%)</td>
<td>123 (67.6)</td>
<td>142 (46.3)†</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus, (%)</td>
<td>31 (17.0)</td>
<td>56 (18.2)</td>
<td></td>
</tr>
<tr>
<td>Hypercholesterolemia, (%)</td>
<td>140 (76.9)</td>
<td>202 (65.8)*</td>
<td></td>
</tr>
<tr>
<td>Current smoker, (%)</td>
<td>37 (20.3)</td>
<td>32 (10.4)†</td>
<td></td>
</tr>
<tr>
<td>History of myocardial infarction, (%)</td>
<td>22 (12.1)</td>
<td>21 (6.8)*</td>
<td></td>
</tr>
<tr>
<td>History of stroke, (%)</td>
<td>30 (16.5)</td>
<td>19 (6.2)‡</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05
†P<0.01
‡P<0.001

Data were compared by χ² test.

GC vs GG
CC vs GC+GG

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### Table 2. Distribution of IL6 −174G>C Genotypes

<table>
<thead>
<tr>
<th></th>
<th>Patients With RAO (n=182)</th>
<th>Controls (n=307)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG, (%)</td>
<td>64 (35.2)</td>
<td>107 (34.9)</td>
<td></td>
</tr>
<tr>
<td>GC, (%)</td>
<td>99 (54.4)</td>
<td>139 (45.3)</td>
<td>0.46*</td>
</tr>
<tr>
<td>CC, (%)</td>
<td>19 (10.4)</td>
<td>61 (19.9)</td>
<td>0.006†</td>
</tr>
<tr>
<td>C allele frequency</td>
<td>0.376</td>
<td>0.421</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Data were compared by χ² test.

*GC vs GG
†CC vs GC+GG
not yet known, we therefore suggest that the genetic influences on IL-6 levels may contribute to RAO risk through inflammatory pathways.

A limitation of our study is that plasma IL-6 concentrations were not determined. We were thus unable to investigate a correlation between genotypes of the IL-6 −174G>C polymorphism and IL-6 plasma levels. Previous studies reported conflicting results. Differences in study design and lack of haplotype analysis may at least in part account for these discrepant findings. Some studies used patients with apparent atherosclerotic disease to investigate the relationship between genotypes of the IL-6 −174G>C polymorphism and IL-6 plasma concentrations. However, atherosclerosis itself has already been associated with increased plasma IL-6 levels. Thus the presence of atherosclerosis may have confounded any influence of the IL-6 −174G>C polymorphism on IL-6 levels.

Finally, other functional polymorphisms of the IL-6 gene have been identified. These polymorphisms have been suggested to exert a synergistic effect on IL-6 transcription. The individual contribution of each polymorphism to the IL-6 transcription rate is therefore difficult to assess. Analysis of combined polymorphisms, so called haplotypes, will very likely provide more insight into the complex relations between IL-6 gene variants and plasma levels.

In conclusion, our study suggests that homozygosity for the C allele of the IL-6 −174G>C gene polymorphism is associated with a protective effect against RAO. Nevertheless, our results warrant confirmation by large prospective studies.

Acknowledgments
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References


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