Adverse Associations Between CX3CR1 Polymorphisms and Risk of Cardiovascular or Cerebrovascular Disease

Elise Lavergne, Julien Labreuche, Mehdi Daoudi, Patrice Debré, François Cambien, Philippe Deterre, Pierre Amarenco, Christophe Combadière; on Behalf of the GENIC Investigators

Objective—We investigated the role of monocyte-recruiting chemokines in cerebrovascular diseases among the subjects of the GENIC case-control study of brain infarction (BI).

Methods and Results—Of the genotypes tested, only homozygosity for the rare CX3CR1 alleles was more frequent in cases than in controls: the I249 and M280 alleles were associated with an increased risk of BI (OR, 1.66 and OR, 2.62 with \( P<0.05 \), respectively). This effect was independent of other established risk factors and uncorrelated with disease severity. The study confirmed previous reports of a dominant protective association between CX3CR1-I249 allele and the risk of cardiovascular history. The risk of BI associated with homozygosity for the rare CX3CR1 alleles was enhanced in patients with no previous cardiovascular events. Ex vivo studies showed that the number of monocytes adhering to immobilized CX3CL1, the CX3CR1 ligand, increased proportionally to the number of CX3CR1 mutated alleles carried by the individual.

Conclusions—The rare CX3CR1 alleles were associated with an increased risk of BI and with reduced frequency of cardiovascular history. We propose that the extra adhesion of monocytes observed in individuals carrying rare alleles of CX3CR1 may favor mechanisms leading to stroke. (Arterioscler Thromb Vasc Biol. 2005;25:1-7.)

Key Words: cerebral infarction ■ genetics ■ leukocytes ■ chemokines ■ inflammation ■ risk factors

Identifying factors that predispose to atherosclerosis is a major public health challenge for this disease that can have profound clinical effects, including myocardial infarction (MI) and brain infarction (BI), both leading causes of death in developed countries. Environmental factors, disease conditions, and known and yet-to-be-discovered genetic determinants may control the pathogenesis of this disease, which can be considered to be an unusual inflammatory process occurring within the artery wall and leading to leukocyte infiltration into the lesion. Monocyte infiltration and macrophage accumulation beneath the endothelial cell layer appear to be critical events in the genesis of early lesions, in disease progression (chronic lesions), and during acute complications.

Recent studies show that chemokines and their receptors play a key role in atherogenesis. These molecules orchestrate leukocyte migration and thus may directly control the infiltration of monocytes, T cells, and smooth muscle cells into atherosclerotic lesions. Their role in atherogenesis has been inferred from murine models, as well as from the expression of chemokines in human atherosclerotic lesions and of their receptors by inflammatory cells within lesions. Deletion of genes coding for CCR2, its ligand CCL2, CX3CR1, or CXCR2 decreases lesion formation in murine models of atherogenesis. As potent mediators of monocyte migration and macrophage differentiation, chemokines, in particular CCL2, CCL3, CCL13, CCL22, and CX3CL1, have been detected in primary human atherosclerotic lesions. In humans, these chemokines are assumed to enable the selective recruitment of monocytes that express their cognate receptors (such as CCR2 and CX3CR1).

Epidemiological studies have also helped to uncover the critical effect of chemokines and their receptors in cardiovascular disease susceptibility. We identified 2 common single-nucleotide polymorphisms in the open reading frame of the CX3CR1 gene, called V249I and T280, both are associated with a reduced risk of MI. Two studies by McDermott et al report that less common alleles of CX3CR1 are associated with a reduced risk of coronary artery diseases (CAD) in a case-control study and with protection from cardiovascular disease in a population-based study. All of the published studies consistently report these associations between the CX3CR1 polymorphism and the risk of CAD. More recent findings associate rare CX3CR1 alleles with a reduced risk of internal carotid artery occlusive disease and carotid plaque stability. More conflicting or unconfirmed reports connect other chemokine and receptor polymorphisms with CAD as well.
Although many studies have focused on CAD, very few reports examine the potential role of chemokines and their receptors in cerebrovascular pathologies. We decided to extend our investigation of the role of chemokines in these syndromes and therefore evaluated a number of chemokines and their receptors in the GENIC case-control study of BI.

Methods

Study Population
The GENIC ("profi GENétique de l’Infarctus Cérébral") case-control study recruited 510 consecutive patients admitted to 12 French neurological departments. Controls (n=510) with no history of stroke were recruited from individuals hospitalized at the same institutions for any reason other than neurological disease. Stratification by age (±5 years) and sex was used to match case and control patients. Controls with a cardiovascular history other than stroke were eligible if they had 2 white parents. Two neurologists classified patients into etiologic BI subtypes, as previously described.24

Data Collection and Risk Factor Definition
A structured questionnaire was used to collect information about all subjects. We defined hypertension as a history of treated hypertension and a positive cardiovascular history as a history of MI, angioplasty, coronary artery bypass surgery, or lower-limb arterial disease. We classified subjects as having diabetes if they reported being treated for insulin-dependent or noninsulin-dependent diabetes. Use of lipid-lowering drugs was assessed.

CX3CR1, CCR2, CCR5, and CCL2 polymorphisms were identified with Minor Groove Binder polymerase chain reaction-based amplification technology from Perkin Elmer (Applied Biosystems Division, Cheshire, UK). The primer express program (Perkin Elmer) was used to design the probes and primers (Table I, available online at http://atvb.ahajournals.org). Each polymerase chain reaction contained 10 to 200 ng of genomic DNA, 900 nM primers, 200 nM probes, and 6 µL of TaqMan Universal polymerase chain reaction master mix (Perkin Elmer). Amplification took place under the following conditions: 50°C for 2 minutes and 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 62°C for 1 minute. Real-time detection and analysis were performed on an ABI 7700 thermocycler (Perkin Elmer).

Cells
Peripheral blood mononuclear cells were isolated from heparinized venous blood from healthy volunteers by 1-step centrifugation on a Ficoll separating solution (Biochrom KG, Berlin, Germany). Monocytes isolated by a negative procedure (Dynal Biotech ASA, Oslo, Norway) according to the manufacturer’s instructions were 80% to 95% pure (confirmed by flow cytometric analysis). CX3CL1-expressing human embryonic kidney (HEK) cells were obtained as previously described.

Chemotaxis was performed in a 96-well chemotaxis chamber with a filter porosity of 10 µm (NeuroProbe, Cabin John, Md) as previously described.25 Adhesion experiments used the conventional parallel-plate flow technique.25 Briefly, CMFDA-labeled monocytes were allowed to interact with HEK cells in the flow chamber at controlled shear stress and the adherent cells were counted.

Statistical Analysis
Allelic frequencies were calculated by gene counting. Hardy–Weinberg equilibrium was tested with the χ² statistic. To compare the overall distribution of the genotypes of each polymorphism among cases and controls, we used the −2 log-likelihood statistic of a logistic regression model adjusted for matching variables (ie, age, sex, and center). To ensure all genotyped controls and cases were entered in the analysis, we did not use a conditional logistic regression analysis for matched sets. For each CX3CR1 polymorphism, genotypic odds ratios (ORs) for BI were calculated by a logistic regression adjusted for matching variables (ie, assuming a codominant genetic model). No excess risk of BI was associated with heterozygosity for either CX3CR1 polymorphism. ORs were therefore computed on the assumption of a recessive genetic model. The log-likelihood of this model was not significantly different from that of a codominant genetic model. Our analyses concerned the entire study group and were subsequently stratified according to the main BI subtypes (ie, atherothrombotic strokes, lacunar strokes, cardio-embolic strokes, and strokes of unknown cause); in each stratum, cases were compared with their matched controls. Analyses concerning strokes of undetermined cause are not reported, because this is, by definition, a highly heterogeneous group. The homogeneity across the main subtypes of the association between each CX3CR1 polymorphism and the disease was assessed with the Breslow–Day heterogeneity test. Haplotype analysis was performed to better-characterize the contribution of CX3CR1 polymorphisms to the risk of BI. Pair-wise haplotype disequilibrium (LD) was estimated with a log-linear model analysis.26 As previously reported,18–21 the CX3CR1-V249I and T280M polymorphisms were in complete linkage disequilibrium; they generated 6 combined genotypes and 3 haplotypes (VT, IT, and IM). ORs for BI associated with IT and IM by reference to VT were calculated by logistic regression adjusted for matching variables, assuming dominant and recessive models. Polymorphisms and haplotype analysis were adjusted for cerebrovascular risk factors (ie, history of hypertension and diabetes, current smoking, total and low-density lipoprotein cholesterol, and cardiovascular history). The homogeneity of the ORs across cerebrovascular risk factors was tested by introducing the corresponding interaction term into models. Statistical testing was 2-tailed, with α set at 0.05. Data were analyzed with the SAS package (SAS Institute, Cary, NC).

Results

Association Between BI and Established Risk Factors
To evaluate the impact of chemokines and their receptors in stroke, we selected a panel of compelling candidate molecules (those previously reported to have genetic variations involved in atherosclerosis, such as CCR5Δ32, CCR2-164 and its ligand CCL2-G2578, CX3CR1-I249, and CX3CR1-M280) and studied their allelic distribution in the GENIC cohort. Table I summarizes the general characteristics of the

### Table 1. General Characteristics and Conventional Risk Factors for BI in Patients and Controls in the GENIC Study

<table>
<thead>
<tr>
<th></th>
<th>Cases (n=510)</th>
<th>Controls (n=510)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range)</td>
<td>69 (19–85)</td>
<td>68 (20–89)</td>
</tr>
<tr>
<td>Male sex, % (n)</td>
<td>62.4 (318)</td>
<td>62.4 (318)</td>
</tr>
<tr>
<td>BMI, kg/m², mean (SD)</td>
<td>25.5 (4.4)</td>
<td>25.7 (4.8)</td>
</tr>
<tr>
<td>History of hypertension, % (n)</td>
<td>51.2 (260)</td>
<td>36.9 (187)*</td>
</tr>
<tr>
<td>History of diabetes, % (n)</td>
<td>18.1 (92)</td>
<td>12.2 (62)†</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL, mean (SD)</td>
<td>201 (43)</td>
<td>182 (43)*</td>
</tr>
<tr>
<td>LDL cholesterol mg/dL, mean (SD)</td>
<td>125 (0.35)</td>
<td>111 (35)*</td>
</tr>
<tr>
<td>Current smokers, % (n)</td>
<td>29.1 (148)</td>
<td>20.8 (106)†</td>
</tr>
<tr>
<td>Cardiovascular history, % (n)</td>
<td>21.5 (109)</td>
<td>11.9 (60)*</td>
</tr>
<tr>
<td>Stroke history, % (n)</td>
<td>21.2 (108)</td>
<td>(—)</td>
</tr>
</tbody>
</table>

Conditional logistic regression for matched sets were used to compare the general characteristics. Analyses of lipid parameters were also adjusted for lipid-lowering drug use and delay blood sampling. BI indicates brain infarction; BMI, body mass index; LDL, low-density lipoprotein.

*P<0.0001; †P<0.01.
TABLE 2. Distribution of Genotypes for Polymorphisms in Chemokines and Chemokine Receptors in Cases and Controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CX3CR1 V249I</th>
<th>CX3CR1 T280 M280</th>
<th>CCR2 V64I</th>
<th>CCL2 A2578G</th>
<th>CCR5 Δ32</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases (n=458)</td>
<td>Controls (n=468)</td>
<td>Cases (n=460)</td>
<td>Controls (n=469)</td>
<td>Cases (n=455)</td>
</tr>
<tr>
<td>11</td>
<td>51.1 (234)</td>
<td>50.4 (236)</td>
<td>68.2 (314)</td>
<td>72.1 (338)</td>
<td>81.5 (371)</td>
</tr>
<tr>
<td>12</td>
<td>38.9 (178)</td>
<td>43.4 (203)</td>
<td>27.2 (125)</td>
<td>26.2 (123)</td>
<td>17.8 (81)</td>
</tr>
<tr>
<td>22</td>
<td>10.0 (46)</td>
<td>6.2 (29)</td>
<td>4.6 (21)</td>
<td>1.7 (8)</td>
<td>0.7 (3)</td>
</tr>
</tbody>
</table>

Allelic frequency of allele 2

<table>
<thead>
<tr>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.295</td>
<td>0.07</td>
<td>0.04</td>
</tr>
<tr>
<td>0.13</td>
<td>0.45</td>
<td>0.98</td>
</tr>
</tbody>
</table>

1 indicates frequent allele; 2, rare allele.
*Using logistic regression analysis adjusted on matching variables.

study subjects. Case subjects had a higher frequency of cerebrovascular risk factors (smoking, hypertension, hypercholesterolemia, and diabetes) and reported a cardiovascular history more frequently than controls.

Distribution of Chemokine and Chemokine Receptor Polymorphisms in the GENIC Study Population

Table 2 shows the distribution of the various genotypes among the case and control subjects. The allelic frequency of each rare allele fell within the range reported in previous studies. The frequencies of the polymorphisms of chemokine receptors and chemokines did not deviate from Hardy–Weinberg equilibrium among cases or controls (data not shown). The similarity of the genotype distribution of the CCR5Δ32 and CCL2-G2578 alleles among cases or controls (data not shown). The allelic frequency of allele 2 fell within the range reported in previous studies.

TABLE 3. Odd Ratios for BI Associated With CX3CR1 Polymorphisms

<table>
<thead>
<tr>
<th>Genotype</th>
<th>OR (95% CI)</th>
<th>OR (95% CI)*</th>
<th>OR (95% CI)*†</th>
</tr>
</thead>
<tbody>
<tr>
<td>V249I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V V</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>VI</td>
<td>0.89 (0.67–1.16), P=0.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>1.59 (0.97–2.63), P=0.07</td>
<td>1.68 (1.04–2.74), P=0.04</td>
<td>1.66 (1.00–2.76), P=0.05</td>
</tr>
<tr>
<td>T280M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>TM</td>
<td>1.10 (0.82–1.47), P=0.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MM</td>
<td>2.89 (1.26–6.66), P=0.01</td>
<td>2.89 (1.23–6.47), P=0.01</td>
<td>2.62 (1.11–6.18), P=0.03</td>
</tr>
</tbody>
</table>

ORs are computed by using logistic regression analysis after adjustments for matching variables.
*Computed by comparing the frequency of subjects homozygous for the rare allele with the frequency of carriers of the rare allele in cases and controls.
†Additional adjustments for history of hypertension and diabetes, total and LDL cholesterol, current smokers, and cardiovascular history.
Consistently with these previous findings, among cases, cardiovascular history was less frequent in those with at least 1 CX3CR1-I249 allele (26.7% VV, 18.7% VI, 10.9% II; \( P = 0.01 \)). A similar association was observed with the CX3CR1-M280 allele (24.8% TT, 18.6% TM, 4.8% MM; \( P = 0.04 \)). Haplotype analysis sought to identify alleles associated with risk of BI (Table 5). Only the IM haplotype was positively associated with BI under the recessive model (OR, 2.68; 95% CI, 1.16 to 6.19). Similar results were found after adjustment for cerebrovascular risk factors. Because we had found an interaction between CX3CR1 polymorphisms and cardiovascular history (\( P = 0.05 \)), we performed a subgroup analysis according to cardiovascular history. IM homozygosity was associated with an increased risk of BI only among subjects with no cardiovascular history, with an adjusted OR of 4.18 (95% CI, 1.49 to 11.74). In subjects with cardiovascular history, the IM haplotype was associated with a tendency toward reduced risk of BI (OR, 0.09; 95% CI, 0.01 to 1.96). These results were limited, however, by the relatively small number of homozygotes for the rare allele with cardiovascular history.

### CX3CR1 Genotypes and Monocyte Cell Functions

To investigate how CX3CR1 polymorphisms might affect predisposition to BI or CAD, we evaluated the functional impact of these CX3CR1 allelic variants on monocytes, the cells considered to be key players in atherogenesis. An in vitro chemotactic assay tested cells’ response to soluble CX3CL1 and a cell adherence assay tested their response to membrane-anchored CX3CL1. First, we compared the trans-
migration of monocytes from subjects homozygous for the CX3CR1-V249 allele with that of monocytes from those heterozygous and homozygous for the CX3CR1-I249 allele (Figure A). We detected no difference in the migratory capacity of monocytes from individuals carrying these various genotypes. Comparing the chemotactic response of monocytes from individuals with the various CX3CR1 T280 mol/L genotypes yielded similar results (data not shown). We then compared the adhesion of monocytes from individuals with different CX3CR1 genotypes to a monolayer of CX3CL1-expressing HEK cells. Adherent monocytes were counted after each flow change. Monocytes from each individual were tested in duplicate in 2 independent experiments. Numbers of individuals per group are indicated above each bar. *P<0.05 between 2 genotype groups.

Discussion

This study shows that only the rarer CX3CR1 alleles, of all the chemokine and receptor alleles tested, were associated with an increased BI risk and they had no effect on disease severity. We also characterized the impact of mutated CX3CR1 alleles on monocyte functional response and found that the number of monocytes adhering to CX3CL1 increases with the number of I249 alleles that an individual carries. These results support the hypothesis that CX3CR1 plays a pathogenic role in cerebrovascular disease.

A few studies report that some chemokine receptor gene variants are associated with susceptibility to CAD.19–21,27–29 Our study is the first to our knowledge to indicate that the CX3CR1-I249 allele and its genetically linked partner, the CX3CR1-M280 allele, are associated with an increased risk of BI. Because of the linkage disequilibrium between M280 and I249, it is difficult to distinguish the independent contribution of each of these polymorphisms to BI risk. These polymorphisms are not, however, associated with disease severity, as measured by intima-media thickness, handicap scoring, or mortality (data not shown). These results are especially intriguing because previous work, including ours, demonstrated that the rare CX3CR1 allele is associated with a reduced risk of cardiovascular diseases.10–21 The effect in each of these studies was dominant, whereas the association with increased BI risk we describe here appeared to be recessive. This difference suggests that different cellular pathways may underlie the mechanisms of these 2 conditions.

Of the other alleles tested, the 32-bp deletion in the CCR5 open reading frame, which causes the loss of CCR5 receptors on the lymphoid cell surface,30 was not significantly associated with either BI risk or cardiovascular history. This finding is concordant with previous epidemiological data20 as well as with a model of atherogenesis in mice deficient for CCR5 and apolipoprotein B genes.31 Other reports indicate that the CCR5Δ32 allele reduces risk for early MI22 and severe CAD27 by attenuating inflammatory responses. The role of the CCR2/CCL2 axis in atherogenesis is well-established in murine models: both types of deficient mice have fewer and less severe atherosclerotic lesions.8–10 CCL2 antagonist has proven potent in controlling plaque formation in mice.33 In humans, a substitution in the distal regulatory region of the CCL2 gene (CCL2-G2578) has been shown to modulate CCL2 production34 and to be associated with a reduced risk of severe CAD.27 Here, we found no association between the less common CCL2 allele and the risk of BI or cardiovascular history. The situation is even more complex for the CCR2-I64 allele, which has a dominant borderline association with increased BI risk and they had no effect on disease severity. Probably because of this allele’s low frequency, and they do not clarify the initial observation that CCR2-I64 is associated with a reduced risk of MI27 or the subsequent reports that it predisposes its carriers to MI.28,29 Further work is needed to clarify the pathogenic impact of these alleles.

In view of its prognostic power for AIDS18,35 and cardiovascular and cerebrovascular diseases,19–21 understanding the molecular defect caused by CX3CR1 mutations is an important challenge. A recent study reports a global impairment of the CX3CR1-M280 cell response to CX3CL1, ie, ligand
binding, calcium response, and adhesive and chemotactic functions.\textsuperscript{21} We could not confirm these results and obtained opposite results. Monocytes from individuals carrying the CX3CR1-I249-M280 allele were captured in larger numbers than monocytes from those carrying CX3CR1-V249-T280 allele. This set of results was similar to those we obtained with human peripheral blood mononuclear cells of various genotypes and HEK cells transfected with CX3CR1 variants,\textsuperscript{25} and with 2 different techniques of testing adherence. We do not yet have a reasonable explanation for the discrepancies with the previous report from McDermott, but it may be interesting to consider sets of data as reflecting different CX3CR1 functional states. The apparent discrepancies may be artifactual and stem from subtle differences in the techniques or cell environment used. Further work to identify the various transduction steps underlying the CX3CR1-I249-M280 effect might help to clarify these conflicting data.

Monocytes recruited in the intima layer to form atherosclerotic plaque first adhere to and cross the endothelial barrier.\textsuperscript{7,36} Umehara et al suggested, based on an in vitro chemotaxis assay, that membrane-bound CX3CL1 expressed on endothelial cells strengthens monocyte adhesion and thus decreases transmigration of monocytes across endothelial cell monolayers.\textsuperscript{37} This phenomenon may reduce atherosclerosis. The decreased lesion infiltration may mimic the pathological phenotype observed in mice deficient for apolipoprotein E and CX3CR1 genes; the first step of monocyte recruitment is unfavorable in these mice because there are fewer monocyte anchors to the endothelial wall.\textsuperscript{11,38} Platelets that express CX3CR1\textsuperscript{39} play a key role in the pathogenesis of atherothrombotic conditions, eg, acute coronary syndromes and cerebrovascular events. In these disease conditions, overexpression of CX3CL1 by vascular endothelial cells may promote platelet adhesion and activation. It is thus likely that CX3CR1 rare allele may favor proatherogenic conditions. Interestingly, platelet activity as measured by mean platelet volume was positively associated with increased risk of BI but not with major coronary events.\textsuperscript{39} We propose that the level of chemokines produced by different vascular beds may determine the proatherogenic effect of platelets. In addition, Yoneda et al report a positive correlation between NK cell adhesion to endothelial cells and susceptibility to NK cell-mediated cytolysis.\textsuperscript{40} They also found that soluble CX3CL1 markedly suppressed both of these enhanced functions. Thus, CX3CL1, in addition to its monocyte and platelet tethering roles, may favor NK cell–mediated endothelial damage, which can ultimately promote atherothrombotic conditions, as McDermott et al have proposed.\textsuperscript{20} More globally, it is conceivable that the risk of cardiovascular or cerebrovascular disease depends on the relation between the systemic release of CX3CL1 that promotes CX3CR1-expressing cell activation and the competing locally expressed membrane-bound CX3CL1, which promotes CX3CR1-expressing cell adherence. To clarify these issues, the CX3CR1-mediated cellular mechanisms predisposing to BI must be characterized.

Consideration of potential mechanisms whereby the CX3CR1-I249M280 haplotype might predispose carriers to BI is also important. We observed the relation between the CX3CR1 polymorphism and BI only with the homozygous genotype, even though the in vitro data from the adhesion assay were significant with the heterozygous genotype. One way to reconcile these 2 sets of data is to hypothesize that the extra monocyte adhesion is not biologically relevant until it exceeds a threshold reached only by individuals homozygous for these rare alleles. The in vitro assay that revealed the specific functional characteristic of the rare allele may not reflect physiological flow conditions; therefore, the discrimination between heterozygous and homozygous cells may be artifactual.

This work showed that naturally occurring mutations in CX3CR1 have a severe impact on susceptibility to BI and CAD. Because of the complexity of pathologies underlying ischemic stroke, which develop over many years and are likely affected by many different factors, further studies are needed to confirm these findings. This work further revealed that CX3CR1 might be considered a powerful prognostic tool and an excellent therapeutic target for cardiovascular and cerebrovascular diseases.

Acknowledgments

This study was supported by a grant from the Fondation de France.

References


Adverse Associations Between CX3CR1 Polymorphisms and Risk of Cardiovascular or Cerebrovascular Disease

Elise Lavergne, Julien Labreuche, Mehdi Daoudi, Patrice Debré, François Cambien, Philippe Deterre, Pierre Amarenco and Christophe Combadière on Behalf of the GENIC Investigators

Arterioscler Thromb Vasc Biol. published online January 27, 2005;
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/early/2005/01/27/01.ATV.0000157150.23641.36.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/