Pregnancy-Associated Plasma Protein A and Its Endogenous Inhibitor, the Proform of Eosinophil Major Basic Protein (proMBP), Are Related to Complex Stenosis Morphology in Patients With Stable Angina Pectoris

Juan Cosin-Sales, MD*; Michael Christiansen, MD*; Paul Kaminski; Claus Oxvig, PhD; Michael T. Overgaard, PhD; Della Cole, BSc, RS; David W. Holt, PhD, DSc; Juan Carlos Kaski, MD, DSc

Background—The metalloproteinase pregnancy-associated plasma protein-A (PAPP-A) has been implicated in coronary plaque disruption. Its endogenous inhibitor, the proform of eosinophil major basic protein (proMBP), may also play a role in this process. Atheromatous plaque disruption often presents as complex angiographic lesions. We sought to assess whether PAPP-A, proMBP, and PAPP-A/proMBP ratio are markers of angiographic plaque complexity in patients with chronic stable angina.

Methods and Results—We studied 396 stable angina patients (age 63 ± 10 years, 230 men) of whom 289 had angiographically documented coronary artery disease (≥75% stenosis). All coronary stenoses ≥30% diameter reduction (n = 531 in 322 patients) were assessed and classified as complex (n = 228) or smooth (n = 303) by previously validated criteria. PAPP-A, proMBP, and C-reactive protein (hs-CRP) serum levels were measured by ELISA. Patients with complex coronary stenoses had a significantly (P < 0.001) higher PAPP-A/proMBP ratio (3.1 ± 1.2 versus 2.7 ± 0.8 × 10⁻³) and PAPP-A levels (5.9 ± 1.6 versus 5.1 ± 1.4 mIU/L) than those without. On univariate analysis, male gender (P < 0.001), age (P < 0.001), previous history of myocardial infarction (P = 0.013), reduced ejection fraction (P < 0.001), severe coronary artery disease (P < 0.001), aspirin treatment (P < 0.001), PAPP-A levels (P < 0.001), and PAPP-A/proMBP ratio (P < 0.001) were correlated with the number of complex stenoses. Multiple regression analysis showed that male gender, age, severe coronary artery disease, and PAPP-A/proMBP ratio were independent predictors of the number of angiographically complex stenoses.

Conclusions—In patients with stable angina, PAPP-A and PAPP-A/proMBP ratio are associated with angiographic plaque complexity. (Circulation. 2004;109:1724-1728.)

Key Words: atherosclerosis ■ coronary disease ■ plaque complexity ■ metalloproteinases

Coronary artery disease (CAD) progression is unpredictable and often cycles in and out of clinically defined phases: asymptomatic, stable angina, progressive angina, and acute coronary syndrome. Clinical stability in patients with chronic stable angina (CSA) is not necessarily indicative of atheromatous plaque stability, ie, absence of inflammation and thrombus formation. In fact, a sizable proportion of atheromatous plaques in CSA patients show inflammatory features and thrombus, and intravascular ultrasound studies have shown coronary plaque rupture in CSA patients and in subjects without cardiovascular symptoms. In recent years, it has become apparent that inflammatory mechanisms involved in the atherogenic process may also lead to plaque disruption, rapid coronary stenosis progression, and acute coronary syndrome (ACS). Macrophages contribute to the disruption of vulnerable atheromatous plaques due to the production of inflammatory mediators and metalloproteinases. We have previously reported a direct relationship between neopterin, a product synthesized by activated macrophages, and the presence of complex coronary stenoses in patients with unstable angina. Initially, Bayes-Genis et al showed that pregnancy-associated plasma protein-A (PAPP-A), a zinc-binding metalloproteinase, was abundantly expressed in ruptured unstable plaques. Its circulating levels were also higher in patients presenting with ACS than in patients with CSA and healthy subjects. PAPP-A levels have recently been shown to represent a potential marker of echocardiographic atherosclerotic lesions in asymptomatic hyperlipidemic pa-

Received August 22, 2003; de novo received December 9, 2003; accepted January 28, 2004.

From the Department of Cardiac and Vascular Sciences (J.C.-S., P.K., D.C., D.W.H., J.C.K.), St George’s Hospital Medical School, London, UK; Department of Clinical Biochemistry (M.C.), Statens Serum Institut, Copenhagen, Denmark; Copenhagen Heart Arrhythmia Research Center (M.C.), Copenhagen, Denmark; and Department of Molecular Biology (C.O., M.T.O.), Science Park, University of Aarhus, Aarhus, Denmark.

*Drs Cosin-Sales and Christiansen contributed equally to this work.

Correspondence to Prof J.C. Kaski, Head, Cardiological Sciences, Department of Cardiac and Vascular Sciences, St George’s Hospital Medical School, Cranmer Terrace, London SW17 0RE, UK. E-mail jkaski@sghms.ac.uk

© 2004 American Heart Association, Inc.

Circulation is available at http://www.circulationaha.org DOI: 10.1161/01.CIR.0000124716.67921.D2
tients with an elevated cardiovascular risk. More recently, Lund et al showed that PAPP-A is a strong independent predictor of both ischemic cardiac events and the need of revascularization in patients with ACS and negative cardiac troponin results.

The proform of eosinophil major basic protein (proMBP) was recently shown to function as the endogenous inhibitor of the proteolytic activity of PAPP-A. We have therefore hypothesized that the PAPP-A/proMBP ratio could be an indicator of the proteolytic activity of PAPP-A and a marker of atheromatous plaque vulnerability in the clinical setting. In this study, we sought to assess whether PAPP-A and the PAPP-A/proMBP ratio are markers of angiographic plaque complexity in patients with CSA.

Methods

Patients
We studied 396 consecutive patients with CSA who underwent routine diagnostic coronary angiography in our institution. CSA was diagnosed in the presence of typical chest pain during exercise, relieved by rest or sublingual nitrates, which remained stable for at least 6 months before study entry. All patients had ≥0.1 mV ST-segment depression during exercise stress testing. The study was approved by the Local Research Ethics Committee, and all patients gave written informed consent before study entry.

Age, gender, height, weight, body mass index, blood pressure levels, cardiovascular risk factors (including systemic hypertension, diabetes mellitus, smoking, family history of CAD, and hyperlipidemia), and cardiac medications were recorded at study entry. Patients with ongoing systemic or cardiac inflammatory processes, liver or renal failure, and neoplastic diseases were not entered in the study.

Blood Sampling, PAPP-A, proMBP, and High-Sensitivity C-Reactive Protein Measurement

Fasting blood samples were obtained from every patient just before diagnostic coronary angiography. Blood was drawn and centrifuged immediately, and serum was then placed into aliquots and stored at −80°C.

Measurements of high-sensitivity C-reactive protein (hs-CRP) were performed on the COBAS Integra (Roche Diagnostics Limited) with the CRP-Latex assay in both the high-sensitivity application (analytic range 0.2 to 12 mg/L) and the normal application (analytic range 2 to 160 mg/L). Analytic precision of the high-sensitivity CRP-Latex assay was 7.6% at a level of 1.02 mg/L, 3.3% at 1.79 mg/L, and 1.3% at 4.36 mg/L. Samples outside the analytic range of the high-sensitivity CRP-Latex assay were analyzed by the CRP-Latex assay in the normal application. The analytic precision of the normal CRP-Latex assay was 2.4% at a level of 29.5 mg/L and 1.3% at a level of 113 mg/L.

PAPP-A levels were determined by means of a biotin-tyramine-amplified enzyme immunoassay with a limit of detection of 0.03 mIU/L. All samples were determined within the assay measuring range. For PAPP-A, the assay range was 0.03 to 10 mIU/L. Interassay variation (CV) was <10% at 0.41, 0.88, and 2.21 mIU/L. For proMBP, the assay range was 0.95 to 15.6 mIU/L with a CV of 11% and 12% at 1.8 and 12 mIU/L, respectively. If the CV of a duplicate determination was >10% for PAPP-A or >15% for proMBP, the samples were rerun. PAPP-A polyclonal antibodies were used for capture, and a combination of monoclonal antibodies was used for detection. The assay was calibrated against the World Health Organization’s international reference standard 78/610, which is the standard for pregnancy-associated proteins.

Total proMBP levels were determined by means of an immunoassay developed at the Statens Serum Institute, Copenhagen. Within the calibrator range used, the interassay CV was <5%.

Angiographic Analyses

Coronary angiography was performed according to the Judkins technique, and images of the coronary tree were obtained in routine, standardized projections with the digital quantitative Philips Integris 3000 system in all patients. Two experienced cardiologists, who had no knowledge of the patients’ clinical characteristics and biochemical results, visually reviewed all angiographic images to characterize the angiographic coronary stenosis morphology.

In every coronary angiogram, we assessed the number of major coronary arteries that showed ≥75% reductions in lumen diameter, and as the main end point of the present study, stenosis morphology was characterized as reported previously in several studies from our group. Briefly, all coronary artery stenoses ≥30% were classified as complex or smooth. Complex stenoses were defined by the following features: (1) irregular morphology or scalloped borders, or both; (2) overhanging or abrupt edges perpendicular to the vessel wall; (3) ulceration (ie, outpouchings within the stenosis); and/or (4) the presence of filling defects consistent with intracoronary thrombus. Coronary stenoses with no complex features were classified as smooth lesions. When discrepancies arose regarding the morphological appearance of a lesion, a third experienced observer was involved, and the lesion was classified by consensus. The reproducibility of the morphological classification was determined by repeating the analysis at intervals >3 month independently by 2 observers who had no knowledge of the stenosis classification obtained at the first reading. Interobserver agreement regarding qualitative morphological analyses of all significant stenoses was 97%.

Statistical Analysis

Results for normally distributed continuous variables are expressed as mean±SD. Comparisons of continuous variables were analyzed with 1-way ANOVA (post hoc Bonferroni test) and unpaired t tests. The Spearman 2-way test was used to assess the relation between 2 quantitative variables with nonnormal distribution. The Pearson 2-way test was used to assess the relationship between 2 quantitative variables with normal distributions. PAPP-A, PAPP-A/proMBP, and hs-CRP serum concentrations had a nonnormal distribution and were therefore transformed logarithmically before multiple regression analysis to fulfill the conditions required for this type of analysis. We assessed independent predictors of complex stenoses using multiple regression analysis, in which the number of complex lesions was the dependent variable. Independent variables that were transformed logarithmically were PAPP-A, PAPP-A/proMBP ratio, hs-CRP levels, number of main coronary vessels that showed a stenosis ≥75%, cardiovascular risk factors, and treatments and other variables that were associated with the number of complex lesions in the univariate analysis. Differences were considered to be statistically significant if the null hypothesis could be rejected with >95% confidence. The SPSS 10.0 statistical software package was used for all calculations.

Results

A total of 396 patients (63±10 years, 230 men) were included. Baseline clinical characteristics and cardiovascular risk factors in these patients are shown in Table 1. Of the 396 patients, 289 had angiographically documented CAD (≥75% stenosis) in at least 1 major coronary artery. Three hundred twenty-two patients had a total of 531 coronary stenoses ≥30% diameter reduction, of which 303 were classified as smooth and 228 as complex. One hundred sixty-eight patients (42%) did not show any complex lesion (Table 2), and 8 patients without stenoses with ≥75% diameter reduction had at least 1 complex lesion. The number of complex lesions correlated significantly with the number of coronary stenoses ≥75% (Spearman r=0.64; P<0.001).

Fewer women had complex lesions (35%) than men (62%), and only 2% of women had >3 coronary stenoses. In contrast, 62% of the males had from 1 to 3 complex coronary
TABLE 1. Baseline Clinical Characteristics, Cardiovascular Risk Factors, and Pharmacological Treatments in 396 Patients With Chronic Stable Angina Pectoris

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>63±10</td>
</tr>
<tr>
<td>Men</td>
<td>230 (58)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.2±4.1</td>
</tr>
<tr>
<td>Previous MI</td>
<td>129 (33)</td>
</tr>
<tr>
<td>CCS class</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>84 (21)</td>
</tr>
<tr>
<td>II</td>
<td>229 (58)</td>
</tr>
<tr>
<td>III</td>
<td>78 (20)</td>
</tr>
<tr>
<td>IV</td>
<td>5 (1)</td>
</tr>
<tr>
<td>Cardiovascular risk factors</td>
<td></td>
</tr>
<tr>
<td>Current smokers</td>
<td>85 (22)</td>
</tr>
<tr>
<td>Previous smokers</td>
<td>193 (49)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>121 (31)</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>167 (42)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>22 (6)</td>
</tr>
<tr>
<td>Family history of MI</td>
<td>217 (55)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.89±2.42</td>
</tr>
</tbody>
</table>

CCS indicates Canadian Cardiovascular Society; MI, myocardial infarction. Values are mean±SD or n (%).

TABLE 2. Coronary Angiography Results

<table>
<thead>
<tr>
<th>Angiography Result</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricular ejection fraction, %</td>
<td>64.9±12.1</td>
</tr>
<tr>
<td>Coronary stenoses ≥75% lumen diameter reduction</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>106 (27)</td>
</tr>
<tr>
<td>1</td>
<td>102 (26)</td>
</tr>
<tr>
<td>2</td>
<td>102 (26)</td>
</tr>
<tr>
<td>3</td>
<td>86 (21)</td>
</tr>
<tr>
<td>Complex coronary stenoses, No. per patient</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>168 (42)</td>
</tr>
<tr>
<td>1–3</td>
<td>198 (50)</td>
</tr>
<tr>
<td>≥3</td>
<td>30 (8)</td>
</tr>
<tr>
<td>Smooth coronary stenoses, No. per patient</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>93 (23)</td>
</tr>
<tr>
<td>1–3</td>
<td>209 (53)</td>
</tr>
<tr>
<td>≥3</td>
<td>94 (24)</td>
</tr>
</tbody>
</table>

Values are mean±SD or n (%).

TABLE 3. PAPP-A, proMBP, hs-CRP Levels, and PAPP-A/proMBP Ratio in Patients With and Without Complex Coronary Stenoses

<table>
<thead>
<tr>
<th>Presence of Complex Coronary Stenoses</th>
<th>Absence of Complex Coronary Stenoses</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPP-A, mIU/L</td>
<td>5.07±1.39</td>
<td>5.89±1.64</td>
</tr>
<tr>
<td>proMBP, mIU/L</td>
<td>2059.3±785.3</td>
<td>2077.5±891.9</td>
</tr>
<tr>
<td>PAPP-A/proMBP, ×10⁻³</td>
<td>2.66±0.82</td>
<td>3.13±1.17</td>
</tr>
<tr>
<td>hs-CRP, mg/L</td>
<td>4.30±7.01</td>
<td>4.65±9.44</td>
</tr>
</tbody>
</table>

PAPP-A/proMBP Ratio was significantly higher in males (2.6±0.8×10⁻³) than in women (3.1±1.6×10⁻³; P<0.001) and in patients with coronary stenosis ≥75% (3.0±1.1×10⁻³) than in patients with normal or nearly normal coronary arteries (2.6±0.9×10⁻³; P=0.001).

PAPP-A/proMBP Ratio, PAPP-A Levels, and Angiographic CAD Complexity

Patients with angiographically complex coronary stenoses had significantly higher PAPP-A levels (5.89±1.64 mIU/L) and PAPP-A/proMBP ratio (3.13±1.17) than those without (5.07±1.39 mIU/L and 2.66±0.82, respectively; P<0.001).

In contrast, proMBP and hs-CRP levels did not differ significantly (P=0.92 and P=0.52, respectively) in patients with and without complex coronary stenoses (Table 3).

Univariate analysis showed that male gender (P<0.001), age (P<0.001), previous history of myocardial infarction (P=0.011), left ventricular ejection fraction (P<0.001), aspirin treatment (P<0.001), number of coronary stenoses ≥75% (P<0.001), logarithm-transformed PAPP-A levels (P<0.001), and logarithm-transformed PAPP-A/proMBP ratio (P<0.001) all correlated with the number of complex lesions. Other variables that showed a trend (P<0.1) toward a correlation were body mass index (P=0.075), systolic (P=0.070) and diastolic (P=0.059) blood pressure, and smoking status (P=0.10). hs-CRP levels did not correlate with the number of complex lesions (P=0.38).

Multiple regression analysis was performed to identify or predict plaque complexity. Of all variables included, male gender, age, number of coronary stenoses ≥75%, and logarithm-transformed PAPP-A/proMBP ratio were independent predictors of the number of complex stenoses in this model (Table 4).

Finally, we assessed, using multiple linear regression

TABLE 4. Univariate and Multiple Regression Analysis With Predictive Variables for Number of Complex Lesions

<table>
<thead>
<tr>
<th>r</th>
<th>P</th>
<th>B (95% CI)</th>
<th>β</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.22</td>
<td>0.001</td>
<td>0.016</td>
<td>0.038</td>
</tr>
<tr>
<td>Male gender</td>
<td>0.38</td>
<td>&lt;0.001</td>
<td>0.54 (0.28–0.80)</td>
<td>0.19</td>
</tr>
<tr>
<td>≥75% stenoses</td>
<td>0.59</td>
<td>&lt;0.001</td>
<td>0.58 (0.46–0.69)</td>
<td>0.46</td>
</tr>
<tr>
<td>LnPAPP-A/proMBP</td>
<td>0.23</td>
<td>&lt;0.001</td>
<td>0.39 (0.05–0.72)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

B indicates increment of the No. of complex lesions with every unit of the independent variable; β value corresponds to the standardized multiple regression coefficient; LnPAPP-A/proMBP, logarithm-transformed PAPP-A/proMBP ratio levels; and ≥75% stenoses, number of main coronary arteries with a ≥75% stenosis.
Discussion

In the present study, we have shown that a significant relationship exists between PAPP-A/proMBP ratio and PAPP-A levels and the presence of complex coronary stenoses in CSA patients. Moreover, in these patients, PAPP-A/proMBP ratio was an independent predictor of plaque complexity. To the best of our knowledge, this is the first study to show that the PAPP-A/proMBP ratio represents a marker of CAD complexity. The present study showed that gender differences exist regarding the presence of complex lesions, PAPP-A/proMBP ratio, and PAPP-A levels. Only a minority of women in the present study had complex stenoses, whereas more than 60% of men had complex lesions, and this is in agreement with previous findings by our group in patients with ACS.7 Similarly, Maehara et al.9 using coronary intravascular ultrasound, studied morphological and angiographic features of coronary plaque ruptures. In their study, of 254 patients with 300 plaque ruptures, only 47 were women. The significance of these findings deserves further study. Of interest, PAPP-A levels and PAPP-A/proMBP ratio were significantly higher in men than in women in the present study.

Although complex lesions are more common in ACS patients than in patients with CSA,22 the presence of complex lesions has been also reported in the latter.4,19 Hangartner et al.19 provided a description of the histology of 448 plaques in coronary arteries of 54 men with CSA and showed that 60% of these plaques were fibrous and 40% had a large lipid core.

The presence of complex stenoses may have prognostic importance, because these lesions tend to progress faster than smooth uncomplicated coronary stenoses1,18,19 and may lead to ACS. Maehara et al.9 showed that complex lesions represent atherogenic, vulnerable plaques prone to disruption or actually disrupted plaques, a finding that supports our previous observations1,18,19 and the hypothesis that plaque rupture may be one of the mechanisms leading to crescendo angina and ACS in patients with CSA.

Plaque vulnerability has been shown to be a function of the increased number of inflammatory cells at the atheromatous site.3,23 Activated macrophages participating in coronary local inflammatory processes secrete metalloproteinases, which may result in weakening of plaque tissue and plaque rupture.8,24 We have previously shown that neopterin, a product secreted by stimulated macrophages, correlated with complex stenoses in patients with unstable angina.7 PAPP-A is a member of the metzincin family of metalloproteinases,25 and initially, Bayes-Genis et al.13 found that PAPP-A was expressed abundantly in both eroded and ruptured plaques but was only minimally expressed in stable plaques of patients who died suddenly of cardiac causes. In their study, circulating PAPP-A levels were significantly higher in ACS patients than in CSA patients and controls, which suggests that the metalloproteinase effect of PAPP-A could have induced plaque disruption and ACS. Recently, these results have been further confirmed by other groups.15,26,27 In addition to PAPP-A measurements, the present study assessed for the first time the potential role of proMBP as a marker of plaque complexity.

The metalloproteolytic activity of PAPP-A is known to be directed toward insulin-like growth factor (IGF) binding protein 4 (IGFBP), causing the release of bound IGF,28,29 which may exert proatherogenic effects.30,31 IGF-I induces macrophage activation, chemotaxis, LDL cholesterol uptake by macrophages, and the release of proinflammatory cytokines by these cells,32,33 which could contribute to plaque disruption. proMBP functions as an inhibitor of the proteolytic activity of PAPP-A.16 Therefore, by preventing cleavage of IGFBPs, proMBP could potentially antagonize the proatherogenic effects of IGF and PAPP-A. Thus, the PAPP-A/proMBP ratio could represent a more comprehensive marker of the activity of PAPP-A.

In our observations in the present study that PAPP-A/proMBP ratio and PAPP-A levels are higher in CSA patients with complex lesions and that the PAPP-A/proMBP ratio is an independent predictor of the number of complex lesions in these patients have physiopathological and clinical relevance. It is somewhat intriguing that in contrast to PAPP-A and PAPP-A/proMBP ratio, hs-CRP levels did not correlate with the presence of complex lesions in the present study. CRP has been found to be a predictor of future coronary events in numerous studies.34,35 In the present investigation, we did not assess clinical outcome, only angiographic characteristics, and this may explain our results. It is conceivable that the use of both CRP and PAPP-A/proMBP may allow more comprehensive patient risk stratification, but this requires further study.

Limitations of the Study

Coronary plaque complexity was assessed by coronary angiography, and this technique has limitations. Coronary angiography provides an assessment of the silhouette of the vessel lumen and often detects macroscopic thrombi, but microscopic thrombi and inflammatory processes developing within the arterial wall are beyond its detection power. However, it has been shown recently that angiographic assessment of complex coronary stenoses strongly correlates with intravascular ultrasound assessment of plaque vulnerability.5 This is an important observation and lends support to our use of angiographic assessment of plaque complexity.

Because the present study was aimed at evaluating the relation among PAPP-A, proMBP, and complex lesions, CRP levels were not used as inclusive or exclusive criteria. Therefore, we did not exclude 11 patients in whom CRP levels were >10 mg/L.

Conclusions

The present study showed that the PAPP-A/proMBP ratio and PAPP-A levels are higher in patients with complex coronary lesions. The PAPP-A/proMBP ratio was independently related to the number of complex stenoses, even after adjustment for confounding factors. Thus, PAPP-A and the PAPP-A/proMBP ratio may be useful serological markers of
atheromatous plaque “vulnerability” in patients with stable angina pectoris.

Acknowledgments
Dr Christiansen was supported by a grant from the Birthe and John Meyer Foundation. Dr Cosin-Sales is the recipient of a research scholarship from the Spanish Society of Cardiology.

References
5. Meyer Foundation. Dr Cosin-Sales is the recipient of a research scholarship from the Spanish Society of Cardiology.
Pregnancy-Associated Plasma Protein A and Its Endogenous Inhibitor, the Proform of Eosinophil Major Basic Protein (proMBP), Are Related to Complex Stenosis Morphology in Patients With Stable Angina Pectoris

Juan Cosin-Sales, Michael Christiansen, Paul Kaminski, Claus Oxvig, Michael T. Overgaard, Della Cole, David W. Holt and Juan Carlos Kaski

_Circulation_. 2004;109:1724-1728; originally published online March 15, 2004; doi: 10.1161/01.CIR.0000124716.67921.D2

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/109/14/1724

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ 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 _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/