Soluble Urokinase Plasminogen Activator Receptor
A Risk Factor for Carotid Plaque, Stroke, and Coronary Artery Disease

Margaretha Persson, PhD; Gerd Östling, MSc; Gustav Smith, MD, PhD; Viktor Hamrefors, MD; Olle Melander, MD, PhD; Bo Hedblad, MD, PhD; Gunnar Engström, MD, PhD

Background and Purpose—Recent studies indicate that the urokinase system could have an important role in atherogenesis and plaque rupture. The relationships among the soluble urokinase plasminogen activator receptor (suPAR), carotid plaque, and incidence of ischemic stroke and coronary artery disease (CAD) events were studied in a prospective cohort.

Methods—Occurrence of carotid plaque and plasma levels of suPAR were assessed in 5166 men and women, aged 45 to 68 years, participating in the Malmö Diet and Cancer study. Incidences of ischemic stroke and CAD were monitored during a mean follow-up of 15 years.

Results—Subjects with carotid plaque had significantly higher levels of suPAR compared with those without carotid plaque. suPAR was associated with increased incidence of ischemic stroke (hazard ratio [HR] for third versus first tertile, 1.50; 95% confidence interval [CI], 1.06–2.11) and CAD (HR, 1.55; 95% CI, 1.13–2.13) after adjustment for risk factors. The risk factor–adjusted HR for ischemic stroke was 2.21 (95% CI, 1.52–3.22) in subjects with carotid plaque and high suPAR (ie, third tertile) and 1.51 (95% CI, 1.05–2.17) in subjects with carotid plaque and low suPAR (reference). High levels of suPAR significantly increased the risk of ischemic stroke and CAD in subjects with carotid plaque.

Conclusions—suPAR is associated with increased occurrence of carotid plaque and increased incidence of ischemic stroke and CAD. Presence of both elevated levels of suPAR and carotid plaque increases the risk of ischemic stroke in an additive way. (Stroke. 2014;45:18-23.)

Key Words: carotid artery plaque ■ coronary artery disease

Recent studies have shown that urokinase-type plasminogen activator (uPA) and its receptor (uPAR) play important roles in the pathogenesis of vascular diseases.1,2 Besides the role of uPA and uPAR in fibrinolysis, uPAR has also been associated with cell migration and matrix degradation.3 uPA and uPAR are expressed by inflammatory cells, such as monocytes, neutrophils, and macrophages.4 High uPAR levels have been reported in ruptured atherosclerotic plaque or segments with severe atherosclerosis.5,6 During acute and chronic inflammation, uPAR is released from the cell surface to form the soluble bioactive form suPAR. suPAR has emerged as a novel inflammatory biomarker that could reflect the overall status of the immune system.4

Recent studies have reported relationships between increased suPAR and incidence of cardiovascular disease (CVD), type 2 diabetes mellitus (DM), and cancer.7-9 Elevated levels of suPAR in plasma and carotid plaque have been associated with symptoms of cerebrovascular disease (ie, amaurosis fugax, transient ischemic attack, or stroke).5,10 It has also been shown that patients with ischemic stroke express higher levels of suPAR compared with patients with amaurosis fugax.11 However, to date, no association has been found between suPAR in plasma and suPAR in carotid plaque.11

The aim of the present study was to examine the associations among suPAR, carotid plaque, and incidence of ischemic stroke and coronary artery disease (CAD), respectively, in a large population-based cohort study.

Materials and Methods

Subjects
The Malmö Diet and Cancer Study (MDCS) is a population-based prospective cohort study designed to explore the associations between dietary habits and health outcomes.12 All men and women aged 45 to 69 years living in Malmö, Sweden, were eligible for participation. At baseline, anthropometry, blood pressure (BP), and lifestyle factors were determined, and whole blood samples were collected. Between November 1991 and February 1994, every other participant was invited to participate in a substudy exploring the associations between cardiovascular risk factors and ultrasound-determined degree of carotid atherosclerosis, thus comprising the MDCS cardiovascular cohort. This cohort consisted of 6103 subjects (3531 women and 2572 men) and has previously been described in detail.13 Age at baseline examination was between 46 and 68 years (mean, 58 years). Participants were asked to return and donate whole blood samples in...
a fasting state. Of the total 6103 subjects, 5540 returned and sufficient plasma volumes for the purpose of measuring suPAR were available for 5378 subjects. Individuals without information regarding carotid plaque (n=212) were also excluded. Thus, the present study included 5166 participants. Those excluded from the analysis did not differ significantly from those included in the study with regard to age or sex. The MDCS, including the cardiovascular cohort, was approved by the Ethics Committee of Lund University, Lund, Sweden (MDC LU 51–90). All participants provided informed consent.

### Measurements

At the baseline examination, each subject was examined by a nurse for measurements of anthropometry, supine BP, nonfasting blood sampling, and administration of questionnaires assessing smoking habits, medical history, and lifestyle factors. During this visit, ultrasonic examination of the right carotid artery was performed. The carotid artery was imaged for the existence of atherosclerotic plaque, defined as focal thickening of the intima-media layer >1.2 mm. Intima-media thickness was determined in the far wall of the distal common carotid artery and in carotid bifurcation according to the leading edge principle and using a specially designed computer-assisted image analysis system. The examination procedure and image analysis were performed by specially trained and certified sonographers. The reproducibility was monitored during the study by analyzing interobserver and intraobserver variations in the measurement of intima-media thickness.

DM was defined as self-reported physician diagnosis per questionnaire, current antidiabetic treatment, or fasting whole blood glucose ≥6.1 mmol/L. Smoking habits were classified as current smoker, ex-smoker, and never smoker. BP (mm Hg) was measured once after a 10-minute rest in the supine position. Body mass index was calculated as kg/m². Total cholesterol and high-density lipoprotein (HDL) cholesterol were measured from fasting blood samples and white blood cell (WBC) count from nonfasting samples according to standard procedures at the Department of Clinical Chemistry, University Hospital Malmö. suPAR and high-sensitivity C-reactive protein (hsCRP) were measured from frozen samples (−80°C). hsCRP (mg/L) was analyzed using the Tina-quant CRP latex high-sensitivity assay (Roche Diagnostics, Basel, Switzerland) on an ADVIA 1650 Chemistry System (Bayer Healthcare, NY). suPAR was measured using the commercial ELISA suPARnostic kit (ViroGates, Copenhagen, Denmark). The interassay coefficient of variation was 5%, and the intra-assay coefficient of variation was 3.05%. suPAR has shown high stability through several freezing and thawing cycles.

### Classification of CVD, Coronary Artery Disease, and Ischemic Stroke

Record linkage with the Swedish Hospital Discharge Register, the Stroke Register of Malmö, and cause-of-death Register was used to obtain information on events. The ascertainment of cases and validity of these registries have been shown to be good. All subjects included in the study were follow-up from the baseline examination until first occurring CAD, ischemic stroke, emigration from Sweden, or death until June 30, 2009.

CAD was defined as fatal or nonfatal myocardial infarction, death from ischemic heart disease, coronary artery bypass graft surgery, or percutaneous coronary intervention. Myocardial infarction was defined on the basis of International Classification of Diseases, Ninth and Tenth Revisions (ICD9 and ICD10), codes 410 and I21, respectively, in the Swedish hospital discharge register or cause-of-death register. Death caused by ischemic heart disease was defined on the basis of codes 412 and 414 (ICD9) or I22 to I23 and I25 (ICD10) in the cause-of-death register. Coronary artery bypass graft surgery was identified from the hospital discharge register based on national Swedish classification systems of surgical procedures. Percutaneous coronary intervention procedures were identified from a nationwide register, which includes all consecutive patients at all 29 centers that perform coronary angiography or percutaneous coronary intervention in Sweden.

Ischemic stroke (ICD10 code I63) was diagnosed when computed tomography, MRI, or autopsy could verify the infarction or exclude other causes of the stroke symptoms. Register linkage to nationwide registers was performed based on the unique 10-digit personal identification number of each Swedish citizen.

### Statistics

All statistical analyses were performed with SPSS version 21.0. Baseline characteristics were compared between subjects with and without plaque using Student t test for continuous variables and χ² test for dichotomous variables and presented as mean±SD, median with interquartile range (IQR), or percentages. suPAR and hsCRP were markedly skewed and, therefore, log-transformed. To compare the mean value of suPAR in subjects with and without carotid plaque, a linear regression model was used and adjusted for age, sex, smoking, cholesterol, HDL, systolic BP, antihypertensive treatment, DM, hsCRP, and WBC count. Kaplan–Meier survival plots were used to study the cumulative event-free survival in relation to sex-specific tertiles of suPAR. Cox regression models were used to analyze the relationship between incidence of CAD and ischemic stroke.

### Table 1. Baseline Characteristics of Subjects With and Without Carotid Plaque

<table>
<thead>
<tr>
<th>Variable, n (%)</th>
<th>No Carotid Plaque n=2878</th>
<th>Carotid Plaque n=2288 (44.3)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>56.1 (5.9)</td>
<td>59.4 (5.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smokers, %</td>
<td>22.6</td>
<td>30.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.6 (3.9)</td>
<td>25.5 (3.8)</td>
<td>0.268</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>1.41 (0.37)</td>
<td>1.35 (0.37)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>6.06 (1.10)</td>
<td>6.31 (1.07)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>6.6</td>
<td>10.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Syst BP, mmHg</td>
<td>138 (18)</td>
<td>146 (19)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diast BP, mmHg</td>
<td>86 (9)</td>
<td>88 (9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>55.8</td>
<td>73.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hsCRP, mg/L</td>
<td>1.3 (0.6–2.6)</td>
<td>1.5 (0.7–3.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WBC count, 10^9/L</td>
<td>6.0 (2.1)</td>
<td>6.2 (1.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>suPAR, ng/mL</td>
<td>2.74 (2.37–3.29)</td>
<td>2.96 (2.51–3.57)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD, median (interquartile range), or percentages. BMI indicates body mass index; Diast BP, diastolic blood pressure; HDL, high-density cholesterol; hsCRP, high-sensitivity C-reactive protein; suPAR, soluble urokinase plasminogen activator receptor; Syst BP, systolic blood pressure; and WBC, white blood cell.
respectively. The first model was adjusted for age and sex; in the second model, smoking, cholesterol, HDL, systolic BP, antihypertensive treatment, DM were included; and in the third model, WBC count, those with a carotid plaque had significantly higher suPAR levels (P<0.008).

Incidence of CAD and Ischemic Stroke
During a mean follow-up period of 14.9 years (76,973 person-years), 367 CAD cases (4.8 per 1000 person-years) and 309 ischemic strokes (4.0 per 1000 person-years) occurred. The cumulative incidence rates of CAD and ischemic strokes were higher for subjects with high plasma concentrations of suPAR (Figure 1A and 1B).

The age- and sex-adjusted hazard ratio (HR) for CAD was 2.42 (95% confidence interval [CI], 1.82–3.23) when comparing the highest with the lowest tertile. The risk increase remained statistically significant (HR, 1.55; 95% CI, 1.13–2.13) after adjustment for risk factors (Table 2). The HR per 1 SD log suPAR in a fully adjusted model was 1.14 (95% CI, 1.01–1.27).

The age- and sex-adjusted HR for ischemic stroke was 2.20 (1.63–2.97) when comparing the highest with the lowest tertile of suPAR. The risk increase was attenuated but statistically significant (HR, 1.50; 95% CI, 1.06–2.11) after adjustment for risk factors (Table 2). The HR of ischemic stroke in a fully adjusted model was 1.15 (95% CI, 1.02–1.30) per 1 SD log suPAR.

We investigated the association with incidence of CAD and ischemic stroke, respectively, with mutual adjustments for suPAR (in tertiles) and presence of carotid plaque in the same model adjusted for age, sex, smoking, systolic BP, antihypertensive treatment, DM, cholesterol, HDL, hsCRP, and WBC count. The HR for ischemic stroke associated with the presence of carotid plaque was 1.51 (95% CI, 1.16–1.96), and the HR for CAD was 1.48 (95% CI, 1.16–1.89). The corresponding figures for upper tertile of suPAR were as follows: HR=1.48 (95% CI, 1.15–1.95) and HR=1.46 (95% CI, 1.05–1.10) for ischemic stroke and CAD, respectively. There was no evidence of any interaction between suPAR and carotid plaque.

Joint Effects of suPAR and Carotid Plaque
Subjects with both increased levels of suPAR and presence of carotid plaque had an increased incidence of CAD (HR, 3.05; 95% CI, 1.57–5.91) in an age- and sex-adjusted model. In a fully adjusted model, the HR was 1.70 (95% CI, 1.20–2.41; Table 3).

For subjects with both elevated levels of suPAR and presence of carotid plaque, the age-adjusted and sex-adjusted HR for incidence of ischemic stroke was 3.49 (95% CI, 2.51–4.84) compared with subjects with low levels of suPAR and without carotid plaque. The risk was attenuated but statistically

**Results**

The proportion of participants with carotid plaque was 44.3%. Baseline characteristics among subjects with and without carotid plaque are shown in Table 1. Median suPAR was 2.92 (IQR, 2.50–3.49) ng/mL for women and 2.72 (IQR, 2.35–3.33) for men.

**suPAR and Carotid Plaque**

Both women and men with carotid plaque had significantly higher levels of suPAR compared with those without carotid plaque (women: 3.05 [IQR, 2.59–3.65] versus 2.82 [IQR, 2.43–3.37] ng/mL; P<0.001; men: 2.82 [IQR, 2.42–3.46] versus 2.62 [IQR, 2.27–3.15] ng/mL; P<0.001). In a linear regression analysis adjusted for age, sex, smoking, systolic BP, antihypertensive treatment, DM, HDL, cholesterol, hsCRP, and WBC count, those with a carotid plaque had significantly higher suPAR levels (P=0.008).
suPAR is a novel biomarker of inflammation, and it has been shown that suPAR is expressed in carotid plaque. In this study, we showed that elevated levels of suPAR were significantly associated with an increased risk of both CAD and ischemic stroke. Plasma levels of suPAR were increased in subjects with carotid plaque, and presence of both carotid plaque and elevated suPAR levels substantially increased risk of ischemic stroke and CAD.

Previous studies have shown that elevated levels of suPAR are related to increased risk of CVD. In a prospective study of 2315 healthy individuals, elevated suPAR, in combination with elevated hsCRP, improved the risk prediction beyond the Framingham risk score. To our knowledge, there are no studies that have explored the relationship with ischemic stroke.

There are several possible links between suPAR and atherosclerotic disease and, to date, there is no consensus about the causal role of the urokinase system in atherosclerosis. uPAR and its ligand are involved in many pathogenic pathways, including plasminogen activation and fibrinolysis, pericellular proteolysis and matrix degradation, cell adhesion, cell migration, and proliferation.

Discussion

suPAR is a novel biomarker of inflammation, and it has been shown that suPAR is expressed in carotid plaque. In this study, we showed that elevated levels of suPAR were significantly associated with an increased risk of both CAD and ischemic stroke. Plasma levels of suPAR were increased in subjects with carotid plaque, and presence of both carotid plaque and elevated suPAR levels substantially increased risk of ischemic stroke and CAD.

Previous studies have shown that elevated levels of suPAR are related to increased risk of CVD. Our results are in accordance with a previous study that showed that suPAR in plasma was associated with carotid plaque and that subjects with elevated levels of suPAR had an increased risk of CVD. In a prospective study of 2315 healthy individuals, elevated suPAR, in combination with elevated hsCRP, improved the risk prediction beyond the Framingham risk score. To our knowledge, there are no studies that have explored the relationship with ischemic stroke.

Table 2. Incidence of Ischemic Stroke and Coronary Artery Disease During 15-Year Follow-up by Sex-Specific Tertiles of suPAR

<table>
<thead>
<tr>
<th>suPAR, ng/mL</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median value</td>
<td>2.26</td>
<td>2.83</td>
<td>3.74</td>
</tr>
<tr>
<td>n=5130</td>
<td>1710</td>
<td>1710</td>
<td>1710</td>
</tr>
<tr>
<td>Stroke event</td>
<td>62</td>
<td>88</td>
<td>159</td>
</tr>
<tr>
<td>Model 1</td>
<td>Reference</td>
<td>1.18 (0.85–1.64)</td>
<td>2.20 (1.63–2.97)</td>
</tr>
<tr>
<td>Model 2</td>
<td>Reference</td>
<td>0.97 (0.69–1.36)</td>
<td>1.56 (1.13–2.15)</td>
</tr>
<tr>
<td>Model 3</td>
<td>Reference</td>
<td>1.02 (0.71–1.46)</td>
<td>1.50 (1.06–2.11)</td>
</tr>
</tbody>
</table>

Table 3. Incidence of Ischemic Stroke and Coronary Artery Disease in Categories of High or Low suPAR and Presence or Absence of Carotid Plaque

<table>
<thead>
<tr>
<th>Low suPAR/No Plaque</th>
<th>High suPAR/No Plaque</th>
<th>Low suPAR/Plaque</th>
<th>High suPAR/Plaque</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=5130</td>
<td>2060</td>
<td>805</td>
<td>1385</td>
</tr>
<tr>
<td>Stroke event</td>
<td>58</td>
<td>51</td>
<td>93</td>
</tr>
<tr>
<td>Model 1</td>
<td>Reference</td>
<td>2.07 (1.42–3.02)</td>
<td>1.84 (1.32–2.57)</td>
</tr>
<tr>
<td>Model 2</td>
<td>Reference</td>
<td>1.68 (1.13–2.49)</td>
<td>1.57 (1.11–2.23)</td>
</tr>
<tr>
<td>Model 3</td>
<td>Reference</td>
<td>1.49 (0.98–2.26)</td>
<td>1.51 (1.05–2.17)</td>
</tr>
</tbody>
</table>

| n=5091 | 2059 | 804 | 1374 | 854 |
| CAD event | 78 | 55 | 121 | 113 |
| Model 1 | Reference | 1.77 (1.25–2.50) | 1.88 (1.41–2.52) | 3.05 (2.27–4.11) |
| Model 2 | Reference | 1.29 (0.89–1.86) | 1.54 (1.14–2.09) | 1.79 (1.28–2.48) |
| Model 3 | Reference | 1.17 (0.79–1.74) | 1.50 (1.09–2.06) | 1.70 (1.20–2.41) |
Increased in atherosclerotic lesions. It has been shown that uPAR is highly expressed in macrophages and symptomatic carotid atherosclerotic plaques, especially in ruptured plaque segments. This supports a role for uPAR in plaque rupture. Studies reported that the cleaved receptor could alter the chemokine-dependent cell migration. It has also been suggested that suPAR could downregulate the immune defense. It was recently reported that suPAR inhibits neutrophil effec
torycytosis, whereas membrane-bound uPAR has been shown to facilitate phagocytosis of bacteria. Therefore, it has been suggested that cleavage of uPAR and formation of suPAR could reflect a functional impairment of the host defense rather than a surrogate marker of inflammation, which might explain the higher prognostic value of suPAR compared with other biological markers. Together, these data suggest that suPAR could be a factor that modulates cellular immunity.

Strength and Limitations
The large population-based cohort, which was investigated at 1 single unit, is a major strength of the study. The assessment of carotid plaque was performed by well-trained and certified sonographers, and the reproducibility has been shown to be good. The end points were retrieved from nationwide and local registers, and the case validity has been found to be high for myocardial infarction. Most ischemic strokes were validated by review of hospital records.

There are also several limitations. In this study, 44.3% had a carotid plaque, defined as a focal thickening of intima-media wall >1.2 mm. The prevalence of carotid plaque depends on the definition. Today, there is no universal consensus definition of carotid plaque. In the study by Lyngbeak et al, carotid plaque was defined as a local thickening of the intima-media layer of >50% or a local sharp increase in echo density with shadowing. It is unknown what effect different definitions could have on the relationships with suPAR. The measurement of suPAR was performed in plasma samples taken at baseline and stored at −80°C. However, it has been shown that suPAR is stable during cycles of thawing and freezing. Another limitation is that the ultrasound measurement was provided only for right carotid artery; however, even so, the plaque prevalence is comparable with other cohorts measuring both sides or only right carotid artery.

In conclusion, elevated levels of suPAR are significantly associated with increased risk of CAD and ischemic stroke, respectively. Individuals with carotid plaque have, on average, higher suPAR concentrations, and presence of both elevated levels of suPAR and carotid plaque substantially increases the risk of ischemic stroke and CAD.

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Disclosures
None.

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