Convalescent plasma levels of TAFI activation peptide predict death and recurrent vascular events in ischemic stroke survivors

Katarina Jood[1], Petra Redfors[1], Ann Gils[2], Christian Blomstrand[1], Paul J. Declerck[2,] and Christina Jern[1]

[1]Institute of Neuroscience and Physiology, the Sahlgrenska Academy at Gothenburg University, Gothenburg, Sweden

[2]Laboratory for Pharmaceutical Biology, Faculty of Pharmaceutical Sciences, Katholieke Universiteit Leuven, Leuven, Belgium

Correspondence to Katarina Jood, Institute of Neuroscience and Physiology, Department of Clinical Neuroscience and Rehabilitation, the Sahlgrenska Academy at Gothenburg University, Per Dubbsg 14, S-413 45 Gothenburg, Sweden.

Tel: +46-31-3429052. Fax: +46-31-842160. e-mail: katarina.jood@neuro.gu.se

Published in: Journal of Thrombosis and Haemostasis, 2012 10 (4), pp. 725-727.
www.schattauer.de/en

Thrombin Activatable Fibrinolysis Inhibitor (TAFI) is a zymogen present in human plasma. Upon activation by trypsin-like enzymes such as thrombin, plasmin or the thrombin/thrombomodulin complex, the activation peptide (TAFI-AP) is released from the catalytic domain (TAFIa). TAFIa operates by continuously removing C-terminal lysine residues on plasmin-modified partially degraded fibrin, thus attenuating the rate of plasminogen activation and fibrinolysis [1].

We have investigated plasma levels of intact TAFI and TAFI-AP, as measured by two genotype-independent ELISAs [2], in a large case-control study of ischemic stroke (IS), the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS) [3]. Both TAFI measures showed association to IS independently of vascular risk factors. The association was stronger for TAFI-AP than for intact TAFI, and was present in all major etiologic IS subtypes, indicating that TAFI activation may contribute to the development of IS irrespective of the underlying etiology. In the present study, we used data from a prospective follow-up of the SAHLSIS cohort to investigate the possible association between TAFI levels and prognosis after stroke with respect to future death and/or recurrent vascular events.

Details of SAHLSIS have been described elsewhere [4]. In brief, 600 patients with acute ischemic stroke, aged 18-69 years, were consecutively recruited at Stroke Units in Western Sweden. Each case was classified according to modified "TOAST" criteria [4]. Stroke severity was scored using the Scandinavian Stroke Scale. Risk factors were defined as described [4]. All participants provided written informed consent. This study was approved by the Ethics Committee of the University of Gothenburg.

Blood was drawn under standardized conditions three months after the index event (median 102 days, range 85-125 days) [3]. Plasma levels of intact TAFI and released AP were measured by sandwich-type ELISAs as described [2,3]. High-sensitivity C-reactive protein (hsCRP) was analyzed as described [5].

Two years after inclusion in the study, the survival rates of patients were assessed using data from the Swedish population register. Surviving patients were contacted for a telephone interview that involved questions on recurrent stroke, transient ischemic attacks (TIA) and coronary events (CE). If the patient was unable to provide answers, a relative or caregiver was interviewed. In addition, the Swedish Hospital Discharge register was screened for all patients. Reported vascular events were confirmed by review of the medical records. Given that some early recurrent events may have gone undetected using this approach, all of the medical records from hospitals and primary care units collated in the three months after the index stroke were reviewed by two neurologists (P.R. and K.J.). Recurrent stroke was defined as a new sudden neurologic deficit or a deterioration of the previous deficit occurring after >24 hours of stability, and not attributable to edema, hemorrhagic transformation, or concomitant illness. For TIA, the neurologic deficit had to have resolved completely within 24 hours. CE was defined as a myocardial infarction, hospitalization for unstable angina, coronary-artery bypass grafting or percutaneous coronary intervention.

To avoid influence of an acute vascular event on convalescent plasma TAFI measures [6], patients suffering from a recurrent vascular event before follow-up were excluded (n=42). Measurements of convalescent TAFI plasma levels were missing in 38. Thus, the present analysis was performed on 520 IS patients surviving free from recurrent vascular events at follow-up after three months.

The small number of events precluded separate analyses of the prespecified endpoints of death, recurrent stroke/TIA and CE. Instead, associations with the composite endpoint of death, recurrent stroke/TIA and/or CE were investigated.

Plasma TAFI measures and hsCRP were logarithmically transformed. We compared baseline characteristics using standard statistical tests. We used Cox regression to obtain univariate and multivariable hazard ratios (HRs) for the composite outcome. Multivariable analyses were performed using the backward elimination procedure including age, sex, hypertension, smoking, diabetes, hyperlipidemia, stroke severity, TOAST subtype, anticoagulant treatment, intact TAFI, TAFI-AP and hsCRP in the initial model. Plasma levels were standardized so that reported HRs represent an increase by one standard deviation (SD) (0.245 for logarithmically transformed intact TAFI and 0.242 for TAFI-AP). Data were analyzed using SPSS 18.0, and statistical analyses were performed in a two-tailed fashion. $P<0.05$ was considered significant.

Two-year follow-up data were obtained for 517 (99.5%) patients. During follow-up 12 deaths, 19 fatal or non-fatal recurrent strokes, 8 TIAs, and 3 CEs occurred, resulting in 37 composite endpoints. Patients who developed a composite endpoint during follow-up were older ($p<0.01$), had more severe index strokes ($p<0.01$), and had higher convalescent plasma concentrations of released TAFI-AP ($p<0.05$) at baseline as compared to patients surviving free from vascular events (Table S1). The univariate HR of the composite endpoint for one SD increase of TAFI-AP was 1.44 (95% CI 1.05-1.97) and this association remained after adjustment for confounders (Table 1). Similar results were obtained when excluding CE from the analysis (data not shown). In contrast and interestingly, there was no significant association between the composite endpoint and plasma concentration of intact TAFI (Table 1). We also went on to investigate the possible predictive value of the TAFI-AP/ intact TAFI ratio. However, no significant associations to the composite endpoint were detected (univariate HR 1.97, 95% CI 0.83-4.14).

Prospective data from clinical studies using genotype-independent assays of plasma TAFI are limited. However, in line with results from our study, no association between total TAFI and the risk of a future cardiovascular event in healthy individuals was observed in the PRIME study [7]. Likewise, in the AtheroGene study, a prospective study of patients with coronary artery disease, no association between plasma levels of total TAFI and future cardiovascular death was observed [8]. However, in that study, TAFIa/TAFIai, another measure of plasma activated TAFI, but not TAFI-AP, did predict future cardiovascular death. Whether the discrepant findings with respect to TAFI-AP in the AtheroGene and our study represents biological differences between IS and coronary artery disease remains to be elucidated, as the number of events in both studies was small. Nevertheless, taken together, findings from clinical prospective studies suggest an association between plasma levels of activated, but not intact, TAFI and arterial thrombotic disease. This finding is biological plausible as experimental data show that it is the amount of activated TAFI, and not the total amount, that plays a crucial role in retarding fibrinolysis [9]. Alternatively, the association between markers for plasma activated TAFI and arterial thrombotic disease is mediated by an increased thrombin generation, as TAFI activation is dependent on the thrombin and thrombomodulin concentrations.

The strengths of our study are a comprehensive follow-up with few patients lost to follow-up, standardized blood sampling, and genotype-independent assays. However, this study also has important limitations, including limited statistical power due to the small number of events. Moreover, it would be of interest to investigate markers of thrombin generation in SAHLSIS as well as in other longitudinal studies of IS.

In conclusion, in this prospective study of IS survivors, we found that

convalescent plasma TAFI-AP, but not intact TAFI, showed association to future death and/or recurrent vascular events in IS survivors, independently of conventional vascular risk factors. Whether this finding represent a causal role of plasma activated TAFI in the prognosis after IS cannot be concluded, and further studies are thus warranted.

REFERENCES

1. Boffa MB, Koschinsky ML. Curiouser and curiouser: recent advances in measurement of thrombin-activatable fibrinolysis inhibitor (TAFI) and in understanding its molecular genetics, gene regulation, and biological roles. Clin Biochem 2007;40:431-42.
2. Ceresa E, Brouwers E, Peeters M, Jern C, Declerck PJ, Gils A. Development of ELISAs measuring the extent of TAFI activation. Arterioscler Thromb Vasc Biol 2006;26:423-428.
3. Ladenvall C, Gils A, Jood K, Blomstrand C, Declerck PJ, Jern C. Thrombin activatable fibrinolysis inhibitor activation peptide shows association with all major subtypes of ischemic stroke and with TAFI gene variation. Arterioscler Thromb Vasc Biol 2007;27:955-62.
4. Jood K, Ladenvall C, Rosengren A, Blomstrand C, Jern C. Family history in ischemic stroke before 70 years of age: the Sahlgrenska Academy Study on Ischemic Stroke. Stroke 2005;36:1383-1387.
5. Ladenvall C, Jood K, Blomstrand C, Nilsson S, Jern C, Ladenvall P. Serum C-reactive protein concentration and genotype in relation to ischemic stroke subtype. Stroke 2006;37:2018-2023.
6. Brouns R, Heylen E, Willemse JL, Sheorajpanday R, De Surgeloose D, Verkerk R, De Deyn PP, Hendriks DF. The decrease in procarboxypeptidase U (TAFI) concentration in acute ischemic stroke correlates with stroke severity, evolution and outcome. J Thromb Haemost 2010;8:75-80.
7. Morange PE, Tregouet DA, Frere C, Luc G, Arveiler D, Ferrieres J, Amouyel P, Evans A, Ducimetiere P, Cambien F, Tiret L, Juhan-Vague I; Prime Study Group. TAFI gene haplotypes, TAFI plasma levels and future risk of coronary heart disease: the PRIME Study. J Thromb Haemost 2005;3:1503-10.
8. Tregouet DA, Schnabel R, Alessi MC, Godefroy T, Declerck PJ, Nicaud V, Munzel T, Bickel C, Rupprecht HJ, Lubos E, Zeller T, Juhan-Vague I, Blankenberg S, Tiret L, Morange PE; AtheroGene Investigators. Activated thrombin activatable fibrinolysis inhibitor levels are associated with the risk of cardiovascular death in patients with coronary artery disease: the AtheroGene study J Thromb Haemost 2009;7:49-57.
9. Leurs J, Nerme V, Sim Y, Hendriks D. Carboxypeptidase U (TAFIa) prevents lysis from proceeding into the propagation phase through a threshold-dependent mechanism. J Thromb Haemost 2004;2:416-23

ACKNOWLEDGMENTS
The authors thank collaborators within the SAHLSIS group. The authors thank Miet Peeters for excellent laboratory assistance.

SOURCES OF FUNDING
This study was supported by the Swedish Research Council (K2011-65X-14605-09-6), the Swedish state (ALFBGB-148861), the Swedish Heart and Lung Foundation (20100256), the Swedish Stroke Association, the Yngve Land Foundation for Neurological Research, the Rune and Ulla Amlövs Foundation for Neurological Research, the John and Brit Wennerström Foundation for Neurological Research, the Per-Olof Ahl Foundation for Neurological Research, and the Research Grant G.0676.10 of the Fund for Scientific Research-Flanders.

DISCLOSURE
The authors have no conflict of interest to declare.

TABLE 1. Hazard ratios and 95% confidence intervals of a composite endpoint during follow-up for 1 SD increase in logarithmically transformed convalescent plasma TAFI concentration

	Univariate HR	Multivariable HR
Intact TAFI	1.20 (0.87-1.64)	0.87 (0.58-1.31)
TAFI -AP	1.44 (1.05-1.97)	1.51 (1.08-2.12)

Cox proportional hazards regression models. Multivariable analyses were performed using the backward elimination procedure including age, sex, hypertension, smoking, diabetes, hyperlipidemia, stroke severity, TOAST subtype, anticoagulant treatment, intact TAFI, TAFI-AP and hsCRP in the initial model. Age, TAFI-AP, hyperlipidemia and stroke subtype were included in the final model.