Letter to the editors-in-chief:

TFPI gene variation and ischemic stroke

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Abbreviations:

BMI, body mass index
CE, cardioembolic
CI, confidence interval
DVT, deep venous thrombosis
GWAS, genome wide association study
IS, ischemic stroke
LSR, The Lund Stroke Register
LVD, large-vessel disease
MAF, minor allele frequency
MDC, Malmö Diet and Cancer study
OR, odds ratio
SAHLSIS, Sahlgrenska Academy Study on Ischemic Stroke
SNP, single nucleotide polymorphism
SVD, small-vessel disease
TF, tissue factor
TFPI, tissue factor pathway inhibitor

Tissue factor pathway inhibitor (TFPI) is the primary inhibitor of the tissue factor (TF)-dependent pathway to thrombus formation after vessel damage. TFPI has therefore been suggested to play a role in the pathogenesis of thrombotic disease. In line with this, a relation between low TFPI levels and venous thromboembolism has been demonstrated [1, 2]. With regard to ischemic stroke (IS), the results are inconsistent: both increased and decreased plasma levels have been reported [3-5]. However, interpreting results on measurements of plasma levels of TFPI is complicated for several reasons. First, TFPI is present in a large endothelial-associated pool as well as in both free and bound plasma pools, and plasma levels of TFPI do not directly reflect the largest, endothelial-associated pool. Second, the circulating levels of total plasma TFPI are low and its normal range is wide, which makes small differences difficult to detect. Analysis of TFPI gene variants may therefore serve as an alternative approach as they could be expected to better reflect local TFPI pools as well as long-term exposure. We therefore investigated whether variation in the TFPI gene is associated with IS.

Materials and Methods

The study population comprises the participants in the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS), the design of which has been reported [6]. Briefly, consecutive patients with first-ever or recurrent acute IS before the age of 70 years (n=844) and matched population controls (n=668) were recruited between 1998 and 2008. Information on the subjects' vascular risk factors was collected as described [6].

Two replication samples were used, The Lund Stroke Register (LSR) and the Malmö Diet and Cancer study (MDC). Sample characteristics, data collection and clinical definitions of these studies have been described [7, 8]. In brief, LSR is a prospective, epidemiologic register initiated in 2001 which consecutively includes all patients with first-ever stroke from the area of Lund University Hospital. Population controls were selected from the same region. MDC is a prospective, population based cohort study which includes 28 449 randomly selected persons at baseline examinations between 1991 and 1996 from which all incident cases of IS up to 2006 were included and matched for age and sex with stroke free control subjects.

Informed consent from all participants or their next-of-kin, and approval by the Ethics Committees of the University of Gothenburg or Lund University were obtained.

Using the "Tagger" program in HaploView, a minimal set of tagSNPs with a minor allele frequency (MAF) >0.1 was captured with r^2>0.8. Fifteen SNPs in the TFPI gene (representative for both TFPI α and β isoforms) were selected for analysis using CEU data from HapMap (Release 23, NCBI B36 assembly). Genotyping was performed with the Golden Gate Assay™ (Illumina Inc., San Diego, CA, USA) and at KBioscience (Hoddesdon, UK).

Assuming a multiplicative genetic model, the odds ratios (ORs) that can be detected with 80% power at the 5% level are in the range of 1.22-1.34 for MAF 0.48-0.11 of the high risk allele for overall IS in SAHLSIS.

Associations between single SNPs and case-control status were investigated using an additive model in binary logistic regression. In a second model the covariates age, sex, hypertension, diabetes mellitus, and smoking were included. Haplotype analysis was performed with a stepwise logistic regression using Helix Tree (Bozeman, MT, USA).

For further details on study populations and genetic analyses please see the online supplement.

Results

Baseline characteristics are shown in Table 1. Genotype distributions did not differ significantly from those predicted by Hardy-Weinberg equilibrium, except for rs2041778, which was excluded from further analysis. The genotyping success rates were 98-100%.

The observed genotype frequencies for the SNPs in the control and overall IS groups, as well as ORs and 95% confidence interval (CI) for overall IS, are presented in Table 2 and in Table S1 in the Supplementary material. The minor alleles of the SNPs rs8176592 (G) and rs8176541 (A), which are in tight linkage (r^2 1.0) in our sample, showed a significant association with a decreased risk of overall IS in SAHLSIS (Table 2). These associations remained after adjustment for age, sex and vascular risk factors (Table 2). Excluding patients with recurrent IS did not alter the results.

To investigate whether the findings could be replicated, rs8176592 and rs8176541 were genotyped in LSR and MDC. No association between the SNPs and overall IS was detected in these samples (Table 2). This was true also when excluding subjects >70 years in the replication sample.

Haplotype analysis did not add any further information.

We also investigated the four main etiologic subtypes of IS separately in SAHLSIS. No subype-specific association was detected in univariate analyses when correction for multiple testing was made. The results are shown in the supplementary material.

Discussion

In the discovery sample SAHLSIS, we detected an association between SNP rs8176592, and the linked SNP rs8176541, and IS. Both SNPs are located in intron 7 of the TFPI gene. This finding is interesting because an effect on transcriptional activity has been reported *in vitro* for rs8176592 and two *TFPI* promoter SNPs [9]. However, we could not validate our finding in the replication sample. Few previous studies have investigated *TFPI* gene variation in IS. In a study by Sayer et al, 162 Caucasian patients with IS and 170 community controls were genotyped for two *TFPI* promoter SNPs (one of which is in strong linkage with rs16829090 included in the present study; r^2=1.0) as well as rs8176592 and rs5940 (also denoted Val264Met). No significant associations were detected [10]. In addition, the *TFPI* mutation C536T and the rs8176592 SNP were included in a Korean study on 271 IS cases and 455 controls, and no association was observed [11]. There are also two small studies that investigated the *TFPI* C536T variant without detecting an association with IS [12, 13]. Smith et al investigated variation in several hemostatic genes in relation to myocardial infarction (MI) and IS. Five *TFPI* SNPs were included, of which one is included in the present study (rs2192824) and one (rs3771059) is in tight linkage (r^2=1.0) with rs8176592 in the present study. No significant associations were found, either with IS or with MI [14]. In line with this, a lack of association between rs8176592 and acute coronary syndromes [15] as well as restenosis after coronary angioplasty [16], has been reported. Likewise, recent genome wide association studies (GWAS) on IS have not reported significant associations for the *TFPI* locus [17, 18]. Thus, the present results, together with the aforementioned studies, do not support the hypothesis that *TFPI* gene variation plays a major role in IS.

The present study has the advantage of a homogenous sample with Caucasian participants from the south-west of Sweden. The sample size is relatively large, although, to detect small ORs an even larger sample size would be needed. Additional limitations are that the design of the three cohorts shows some differences, and that we could not perform subtype-specific analyses in the replication sample.

In conclusion, we found associations between rs8176592 and rs8176541 and overall IS in our discovery sample, but the results could not be replicated. Based on the present study, these variants can therefore not be considered as major contributors to overall IS.

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Conflict of interest statement
None.

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Table 1. Baseline characteristics of the control and overall ischemic stroke groups in SAHLSIS and in the replication sample.

	Discovery sample SAHLSIS		Replication sample (LSR and MDC)	
	Control (n=668)	Ischemic stroke (n=844)	Control (n=1793)	Ischemic stroke (n=3145)
Median age, years (IQR)	58 (50-64)	59 (51-64)	72 (65-79)	74 (66-81)***
Male, n (%)	392 (59)	554 (66)**	999 (56)	1661 (53)*
Hypertension, n (%)	230 (34)	487 (58)***	944 (53)	2143 (69)***
Diabetes mellitus, n (%)	33 (5)	153 (18)***	93 (5)	641 (21)***
Current smoking, n (%)	131 (20)	324 (38)***	282 (16)	725 (24)***

Table 2. Genotype frequencies for the *TFPI* SNPs rs8176592 and rs8176541 in the control and overall ischemic stroke groups in the discovery and replication samples as well as odds ratios and p-values for the association between the genetic variants and overall IS. Genotype frequencies and ORs for the other *TFPI* SNPs are shown in the supplementary material.

		Discovery sample SAHLSIS				Replication sample LSR and MDC		
SNP	Genotype	Control (n=668)	IS (n=844)	OR (95% CI)	Genotype	Control (n=1793)	IS (n=3145)	OR (95% CI)
rs8176592	AA, n (%)	287 (43)	408 (49)	0.83 (0.71-0.97)[a*]	AA, n (%)	822 (47)	1501 (48)	0.97 (0.89-1.06) [a]
	AG, n (%)	310 (47)	363 (43)	0.79 (0.66-0.94)[b**]	AG, n (%)	751 (43)	1273 (41)	1.01 (0.92-1.10) [b]
	GG, n (%)	68 (10)	67 (8)		GG, n (%)	181 (10)	231 (11)	
rs8176541	GG, n (%)	288 (43)	408 (49)	0.83 (0.71-0.98)[a*]	GG, n (%)	840 (48)	1502 (48)	1.00 (0.92-1.10) [a]
	GA, n (%)	310 (47)	365 (43)	0.80 (0.67-0.94)[b**]	GA, n (%)	750 (43)	1272 (41)	1.04 (0.94-1.14) [b]
	AA, n (%)	68 (10)	67 (8)		AA, n (%)	173 (10)	333 (11)	

Table legends

Table 1:
SAHLSIS, the Sahlgrenska Academy Study on Ischemic Stroke; LSR, the Lund Stroke Register; MDC, the Malmö Diet and Cancer study. Data are shown as median and interquartile range (IQR) or number (n) and percentage. Differences between the patients and the controls were examined with the χ^2 test for proportions, and with the Mann-Whitney U-test for continuous variables. *** $p<0.001$, ** $p<0.01$ and * $p<0.05$ compared with the control group.

Table 2:
SAHLSIS, the Sahlgrenska Academy Study on Ischemic Stroke; LSR, the Lund Stroke Register; MDC, the Malmö Diet and Cancer study; IS, ischemic stroke. Odds ratio (OR) with 95% confidence interval (95% CI) for ischemic stroke for the uncommon allele. [a]Unadjusted; [b] adjusted for age, sex, hypertension, diabetes mellitus and smoking. ** $p<0.01$ and * $p<0.05$.