No evidence for an association between genetic variation at the *SERPINI1* locus and ischemic stroke

A. TJÄRNLUND-WOLF, S. OLSSON, K. JOOD, C. BLOMSTRAND, and C. JERN

Institute of Neuroscience and Physiology, Section for Clinical Neuroscience and Rehabilitation, the Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden.

Correspondence: Prof Christina Jern, Address: Institute of Neuroscience and Physiology, the Sahlgrenska Academy at University of Gothenburg, Per Dubbsgatan 14, 413 45 Gothenburg, Sweden. Tel: +46 31 343 57 20; fax: +46 31 84 21 60. E-mail: christina.jern@neuro.gu.se

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Sirs:
Neuroserpin, primarily expressed by neurons, is an important inhibitor of tissue-type plasminogen activator (t-PA). t-PA has a multifaceted role in the brain and is involved in several physiologic and pathologic processes. Its beneficial role on the vascular side is attributed to its thrombolytic function. Moreover, recombinant t-PA is the only FDA-approved pharmacological treatment of acute ischemic stroke (IS) [1]. In the brain there are several cell-types that express t-PA besides the cerebrovascular endothelial cells, *e.g.* neurons, microglia and astrocytes [2]. Under physiological conditions t-PA is involved in synaptic plasticity and memory-related processes [3,4]. However, during pathological conditions, such as in the acute phase of IS, excess release of t-PA may trigger proteolytic cascades, subsequently leading to excitotoxicity and neurodegeneration [5,6]. In this context, t-PA has been associated with increased stroke volume [7] and increased cerebrovascular permeability [8-10]. Experimental models have also shown that treatment with neuroserpin or neuroserpin gene overexpression results in a reduced infarct volume and prevents t-PA-induced increase in blood-brain barrier permeability [8,11,12].

To date, there is only one published study addressing the potential association between common genetic variants at the neuroserpin (*SERPINI1*) locus and IS. In the Stroke Prevention in Young Women Study 2 (SPYW2), Cole and colleagues detected an association between a single nucleotide polymorphism (SNP) located in intron 1 of the neuroserpin gene (rs6797312) and IS, and this association was restricted to Caucasian women (n=95) in an age-adjusted dominant model [13]. Aim of the present study was to investigate the association between genetic variants at the *SERPINI1* locus and IS in a Swedish sample of relatively young (≤70 years) individuals.

The study population comprised patients (n=844) and population-based, healthy controls (n=668) from the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS), the design of which has been reported [14,15]. All participants were Caucasian. Patients had presented with first-ever or recurrent acute IS before

reaching the age of 70 years. The upper age limit was chosen based on studies indicating that the genetic contribution is greater in patients suffering from stroke at younger age. Written informed consent from participants, and approval by the Ethics Committee of the University of Gothenburg were obtained. Baseline characteristics are presented in Table 1.

In addition to rs6797312 [13], 23 haplotype tagging SNPs at the *SERPINI1* locus were selected using HapMap (Data release 22/PhaseII on NCBI B36 assembly). Genotyping was performed at the SNP technology platform at Uppsala University (http://genotyping.se) using the Golden Gate assay (Illumina Inc. San Diego, CA, USA). Four of the SNPs (rs2055026, rs1553221, rs13064973, rs1973360) were excluded due to technical difficulties in the assay design and three (rs1192448, rs3792297, rs3792298) were excluded due to low sample call rates (<90%). The average call rate for the SNPs that passed the quality control was 99%. Associations between single SNPs and case/control status were investigated using univariate binary logistic regression primarily adjusted for age and sex. Haplotype analysis was conducted using the software Helix Tree (Golden Helix Inc. Bozeman, MT, USA). Our study has 80% power to detect an OR of 1.25 at the 5% level for SNPs with minor allele frequencies (MAF)=0.3.

No significant association was observed between IS and the analysed SNPs (Table 2). Adjustment for age and sex did not alter the results (Table 2), nor did adjustments for vascular risk factors (smoking, hypertension and diabetes mellitus, data not shown). Haplotype analysis did not provide any additional information (data not shown). When analysing men and women separately, no significant association was observed in either subgroup (Table 2). All genotype distributions conformed to Hardy-Weinberg equilibrium. MAF (Table 2) were in agreement with frequencies seen for the same SNPs in the CEU population in the HapMap project (rs6797312 has no HapMap frequency data). MAF of rs6797312 in a European population was 0.44 in the study by Cole and colleagues [13], which is in good agreement with our results (MAF 0.41).

In conclusion, our study did not provide any evidence for an association between genetic variation at the *SERPINI1* locus and IS, either in the whole group or in the female subgroup. We were thus unable to replicate the finding of Cole and colleagues, which was based on a rather small study population [13]. Our results further underscore the importance of independent replication of novel genetic findings.

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Conflict of interest
The authors declare that they have no conflict of interest.

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Table 1. Baseline characteristics of the control, and overall ischemic stroke groups.

	Control (*n*=668)	Ischemic stroke (*n*=844)
Mean age, years (SD)	56 (10)	56 (11)
Male, *n* (%)	392 (59)	554 (66)*
Hypertension, *n* (%)	230 (34)	487 (58)**
Diabetes mellitus, *n* (%)	33 (5)	153 (18)**
Current smoking, *n* (%)	131 (20)	324 (39)**

SD, standard deviation; *n*, numbers. *$p<0.01$ and **$p<0.001$, compared with the control group. 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.

Table 2. Minor allele frequencies and odds ratios for the association between genetic variants at the neuroserpin (*SERPINI1*) locus and ischemic stroke.

SNP	Minor allele	MAF Controls	SNP Cases	Ischemic stroke OR (95% CI)* *P*-value	Women[a] OR (95% CI)** *P*-value	Men[b] OR (95% CI)** *P*-value
rs6771578	G	0.28	0.28	0.99 (0.84-1.15) 0.85	0.90 (0.70-1.16) 0.42	1.05 (0.86-1.29) 0.64
rs2420034	T	0.49	0.50	1.05 (0.91-1.21) 0.48	1.00 (0.79-1.26) 0.97	1.08 (0.91-1.30) 0.38
rs13090569	A	0.27	0.29	1.11 (0.94-1.30) 0.21	1.10 (0.86-1.42) 0.44	1.11 (0.91-1.37) 0.30
rs9856551	G	0.21	0.24	1.18 (0.98-1.42) 0.08	1.14 (0.87-1.51) 0.34	1.20 (0.94-1.53) 0.14
rs9859639	T	0.32	0.32	0.99 (0.85-1.16) 0.94	0.95 (0.74-1.21) 0.66	1.03 (0.84-1.26) 0.81
rs6797312	A	0.41	0.41	1.02 (0.88-1.18) 0.81	1.04 (0.83-1.31) 0.73	1.00 (0.83-1.21) 0.97
rs13090836	G	0.41	0.41	1.02 (0.88-1.18) 0.79	1.05 (0.83-1.32) 0.69	1.00 (0.83-1.21) 0.97
rs13093535	G	0.29	0.28	0.94 (0.80-1.10) 0.43	0.78 (0.60-1.02) 0.07	1.04 (0.85-1.27) 0.70
rs13071510	G	0.50	0.49	1.00 (0.87-1.16) 0.98	1.14 (0.90-1.44) 0.28	0.93 (0.78-1.12) 0.45
rs3804617	G	0.22	0.23	1.07 (0.90-1.27) 0.46	1.08 (0.82-1.41) 0.59	1.06 (0.84-1.33) 0.64
rs11706323	C	0.14	0.12	0.87 (0.70-1.07) 0.19	0.84 (0.58-1.20) 0.33	0.88 (0.68-1.15) 0.34
rs11919881	A	0.19	0.17	0.89 (0.74-1.07) 0.22	0.82 (0.60-1.12) 0.21	0.93 (0.74-1.18) 0.56
rs16851498	G	0.24	0.26	1.11 (0.94-1.31) 0.22	1.12 (0.91-1.39) 0.29	1.12 (0.91-1.39) 0.29
rs7641394	G	0.15	0.16	1.08 (0.89-1.32) 0.43	0.98 (0.71-1.36) 0.91	1.15 (0.89.1.48) 0.29
rs6785838	C	0.21	0.21	1.04 (0.87-1.25) 0.65	0.92 (0.68-1.24) 0.58	1.11 (0.89-1.40) 0.35
rs11707934	A	0.21	0.22	1.02 (0.85-1.22) 0.83	0.88 (0.65-1.17) 0.38	1.11 (0.89-1.39) 0.36
rs720958	G	0.13	0.13	0.98 (0.79-1.21) 0.84	0.88 (0.62-1.25) 0.48	1.04 (0.79-1.37) 0.78

Odds ratio (OR) for stroke for the uncommon allele using an additive model in logistic regression. MAF denotes minor allele frequency. Cases *n*=844, controls *n*=668. *age and sex adjusted; **age adjusted; [a]cases *n*=290, controls *n*=276; [b]cases *n*=554, controls *n*=392.