Association between genetic variation at the *ADAMTS13* locus and ischemic stroke

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Published in *Journal of Thrombosis and Haemostasis* 2009;7:2147–2159
http://onlinelibrary.wiley.com/doi/10.1111/j.1538-7836.2009.03617.x/abstract

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von Willebrand factor (VWF) is a large glycoprotein involved in the early stages of hemostasis, where it recruits platelets to sites of vessel injury [1]. Plasma VWF is comprised of multimers. The biological activity of VWF is related to the size of these multimers with larger ones being hemostatically more effective than the smaller forms [2]. VWF multimeric size is modulated by ADAMTS13 (A disintegrin and metalloprotease with thrombospondin motif) [2]. Decreased plasma levels of ADAMTS13 have been reported in patients with myocardial infarction, both acutely [3] and several months after the event [4]. In a recent study on arterial thrombosis at young age, Bongers et al. observed a reduction in plasma levels of ADAMTS13 antigen and activity 1-3 months after the event in patients with coronary heart disease [5].

As reviewed by Dong [2], mutations and polymorphisms in the *ADAMTS13* gene may affect the synthesis, secretion or activity of ADAMTS13. Early studies suggest that ADAMTS13 is derived mainly from hepatic stellate cells [2]. However, the endothelium, platelets and some tissues, including the brain, have also been shown to produce ADAMTS13 [2]. Unlike ADAMTS13 from stellate cells, a significant proportion of that released from endothelial cells and platelets becomes membrane-bound, suggesting that ADAMTS13 from these cells may act locally [2]. Thus, genetic variants of *ADAMTS13* might affect ADAMTS13

membrane interactions in addition to having an effect on plasma levels or activity.

There is limited knowledge on the role of ADAMTS13 in ischemic stroke (IS), especially on possible genetic influences. The aim of the present study was to investigate whether there is an association between genetic variants of *ADAMTS13* and IS and/or any of the IS subtypes.

Details of the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS) have been described elsewhere [6,7]. In summary, this is a case-control study comprising 600 consecutive patients presenting with IS at four stroke units in Western Sweden before the age of 70 years. The upper age limit was chosen based on studies indicating that the genetic contribution is greater in patients suffering stroke at younger age. For each case, one healthy community control, matched for age (±1 year), sex and geographical residence area was randomly selected. All patients underwent neuroimaging. Additional diagnostic work-up was performed as described [6,7]. Each case was classified according to Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria into the etiological subtypes large vessel disease (LVD, n=73), small vessel disease (SVD, n=124), cardioembolic stroke (CE stroke, n=98), and cryptogenic stroke (n=162). Cryptogenic stroke was defined when no cause was identified despite an extensive evaluation. The TOAST categories other determined causes (n=51) and undetermined stroke (n=92) were not included in the subtype analysis. The distribution of traditional risk factors in this sample has been described [6,7]. Assuming a multiplicative genetic model, the odds ratios (ORs) that can be detected with 80% power are in the range 1.22-1.36 depending on the frequency of the high risk allele.

In order to investigate genetic variations at the *ADAMTS13* locus, 6 tag single-nucleotide polymorphisms (SNPs), rs652600, rs739469, rs2073933, rs2285489, rs2301612 and rs4962153, were selected using HapMap Phase II, Data Release 22. Genotyping was performed by TaqMan assays (Applied Biosystems). The assay for rs2073933 was non-functional and could not be replaced. Genotyping was performed blinded to case/control status. Associations between single SNPs/haplotypes and overall IS were investigated using an additive model in conditional logistic regression. To increase the statistical power in IS subtype analyses, the whole control population was used, i.e. unconditional analysis. Thus, in the subtype regression analyses the matching variables age, sex and geographical residence area were included as covariates.

The mean age at index stroke was 56 years, and 36% were women. The genotyping success rate was 99.9%. Three *ADAMTS13* SNPs showed association to IS. One of these was rs4962153 where the uncommon A allele was associated with an increased risk of IS (Table 1). For rs2285489 and rs2301612 the uncommon alleles were associated with a decreased risk (Table 1).

Seven haplotypes with a frequency >1% were found (Table 1). They accounted for 97.2% of the genetic variation. One haplotype, H3, had a higher frequency in cases compared to controls (Table 1).

In contrast to the present results, in the study by Bongers et al. no association was detected between cerebrovascular disease (CVD) and genetic variants [5]. Furthermore, plasma levels of ADAMTS13 were not significantly different in CVD compared to controls. However, the CVD group only included 52 patients with IS and 57 patients with transient ischemic attacks. In the whole sample, one haplotype showed significant association to ADAMTS13 activity. One genetic marker in this haplotype is in strong linkage disequilibrium (LD) (r^2=1.0) with one SNP investigated in the present study, rs734969. The SNP showing strongest association to IS in the present sample, rs4962153, was not investigated by Bongers et al., nor was any SNP in strong LD with this SNP. A limitation of the present study is that plasma levels or activity of ADAMTS13 was not examined. Thus, possible genetic influences on plasma levels or activity of ADAMTS13 need to be investigated further before any speculations can be made on possible mechanisms behind an association between genetic variation at this locus and IS. Furthermore, as discussed above, genetic variants might also affect ADAMTS13 membrane interactions.

In the present study, a specific aim was to investigate genetic associations in the four main IS subtypes, i.e. LVD, SVD, CE stroke and cryptogenic stroke. In this analysis the SNP rs4962153 showed association to cryptogenic stroke (Table 1). This association is interesting in view of the fact that prothrombotic variants have been suggested as risk factors for cryptogenic stroke [8], but there are no studies on ADAMTS13 in this subtype. We found no significant associations for any of the other IS subtypes. However, whether this is due to a subtype-specific effect of *ADAMTS13* gene variation or a lack of statistical power in the smaller subgroups cannot be concluded.

In conclusion, three SNPs in the *ADAMTS13* gene were found to be associated with IS in the present population of relatively young patients from Western Sweden. The SNP rs4962153 showed a significant association both to overall ischemic stroke and to the subtype cryptogenic stroke. To the best of our knowledge, this is the first study investigating *ADAMTS13* gene variants in a large cohort of patients with IS and in different subtypes of IS. Thus, further studies are needed in order to investigate whether the present finding can be replicated, and whether there are associations for other subtypes apart from cryptogenic stroke.

Acknowledgements

This study was supported by the Swedish Research Council (K2008-65X-14605-06-3), the Swedish state (ALFGBG-11206), the Swedish Heart Lung Foundation (20070404) as well as the Rune and Ulla Amlöv, the John and Brit Wennerström, the Per-Olof Ahl, the Yngve Land, the Märta Lundqvist, and the Magnus Bergvall foundations. The authors have no conflict of interest to declare.

Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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Table 1. Genotype frequencies in controls, overall ischemic stroke and the subtype cryptogenic stroke as well as estimated haplotype frequencies in the whole study population.

Genotype*	**Control** n=600	**Ischemic stroke** n=600	**Cryptogenic stroke** n=162
rs652600			
AA, no. (%)	345 (58)	349 (58)	95 (59)
AG, no. (%)	222 (37)	212 (35)	59 (36)
GG, no. (%)	32 (5)	38 (6)	8 (5)
OR (95 % CI)†	Ref	1.02 (0.84-1.23)	0.97 (0.72-1.31)
rs739469			
CC, no. (%)	233 (39)	211 (35)	47 (29)
CG, no. (%)	270 (45)	276 (46)	88 (54)
GG, no. (%)	97 (16)	112 (19)	27 (17)
OR (95 % CI)†	Ref	1.13 (0.96-1.32)	1.23 (0.96-1.58)
rs2285489			
CC, no. (%)	201 (34)	240 (40)	63 (39)
CT, no. (%)	302 (50)	279 (47)	82 (51)
TT, no. (%)	97 (16)	80 (13)	16 (10)
OR (95 % CI)†	Ref	0.82 (0.70-0.97)	0.78 (0.60-1.02)
rs2301612			
CC, no. (%)	161 (27)	191 (32)	44 (27)
CG, no. (%)	302 (50)	290 (48)	97 (60)
GG, no. (%)	137 (23)	118 (20)	21 (13)
OR (95 % CI)†	Ref	0.85 (0.73-1.00)	0.81 (0.63-1.05)
rs4962153			
GG, no. (%)	429 (71)	399 (66)	100 (62)
GA, no. (%)	158 (26)	179 (30)	56 (35)
AA, no. (%)	12 (2)	21 (4)	6 (4)
OR (95 % CI)†	Ref	1.25 (1.01-1.54)	1.50 (1.09-2.07)

Haplotype	**rs2285489**	**rs739469**	**rs2301612**	**rs652600**	**rs4962153**	**Estimated freq (%)**	**OR (95 % CI)‡**
H1	T	C	G	A	G	35.7	Ref
H2	C	G	C	G	G	19.8	1.14 (0.90-1.43)
H3	C	G	C	A	A	15.2	1.33 (1.05-1.69)
H4	C	C	C	A	G	12.9	1.17 (0.90-1.52)
H5	C	C	G	A	G	9.3	1.06 (0.79-1.43)
H6	T	G	C	G	G	2.4	0.72 (0.41-1.28)
H7	C	G	C	A	G	1.9	1.02 (0.57-1.83)

* All genotype distributions were in Hardy-Weinberg equilibrium in controls.
† OR for stroke for the uncommon allele using an additive model. Conditional regression analysis was used for overall ischemic stroke. The whole control group was used also for the subgroup cryptogenic stroke, and thus unconditional regression analysis with the matching variables age, sex and geographical residence area was applied.
‡ OR for overall ischemic stroke using an additive model in conditional regression analysis.