Published in final edited form as:

Ann Neurol. 2013 January ; 73(1): 58–64. doi:10.1002/ana.23735.

Role of Reduced ADAMTS13 in Arterial Ischemic Stroke: A Pediatric Cohort Study

Moritz Lambers, MD^{1,2}, Neil A. Goldenberg, MD³, Gili Kenet, MD⁴, Fenella J. Kirkham, MD⁵, Daniela Manner, MD², Timothy Bernard, MD⁶, Rolf M. Mesters, MD⁷, Ralf Junker, MD², Monika Stoll, PhD⁸, and Ulrike Nowak-Göttl, MD^{1,2}

¹Faculty of Medicine, University of Münster, Münster, Germany

²Center of Thrombosis and Hemostasis, Institute of Clinical Chemistry, University Hospital Schleswig-Holstein, Kiel, Germany

³Section of Hematology/Oncology/Bone Marrow Transplantation, Department of Pediatrics and the Mountain States Regional Hemophilia and Thrombosis Center, University of Colorado and Children's Hospital Colorado, Denver/Aurora, CO

⁴Thrombosis Unit, National Hemophilia Center, Sheba Medical Center, Tel-Hashomer and the Sackler Medical School, Tel Aviv University, Tel Aviv, Israel

⁵Neurosciences Unit, University College London Institute of Child Health, London, United Kingdom

⁶Section of Child Neurology, Department of Pediatrics and the Mountain States Regional Hemophilia and Thrombosis Center, University of Colorado and Children's Hospital Colorado, Denver/Aurora, CO

⁷Department of Internal Medicine, University Hospital of Münster, Münster, Germany

⁸Genetic Epidemiology of Vascular Disorders, Leibniz-Institute for Arteriosclerosis Research at the University of Münster, Münster, Germany.

Abstract

Objective—Previous studies in adults and mice have implicated ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13), also known as von Willebrand factor (VWF)-cleaving protease, as a protective factor for stroke. Here we investigated ADAMTS13 in 208 pediatric patients with arterial ischemic stroke (AIS) and 125 population-based control children in a frequency-matched case-control study.

Methods—The proportion of patients/controls with ADAMTS13 activity levels below and above the 10th percentile was compared. Additionally, in a quintile comparison, the proportion of

© 2012 American Neurological Association

Address correspondence to Dr Nowak-Göttl, Center of Thrombosis and Hemostasis, Institute of Clinical Chemistry, University Hospital Kiel, Arnold-Heller-Str. 3, Building 17, 24105 Kiel, Germany. leagottl@uksh.de.

Authorship The principal study investigators (M.L., M.S., U.N.-G.) act as the guarantors; all other investigators (N.A.G., G.K., F.J.K., D.M., T.B., R.M.M., R.J.) had full access to the data and took part in design, execution, data analysis, and writing the report. F.J.K. confirmed the pediatric stroke subtypes from reviewing imaging and Doppler ultrasound records, and G.K. from the laboratory-hematology aspect. N.A.G. and U.N.-G. were responsible for the statistical analyses.

Potential Conflicts of Interest F.J.K.: employment, University College London; expert testimony, paid into UCL discretionary fund; grants/grants pending, Study of Outcome for Stroke, Sleep Asthma cohort; speaking fees, Brendon's Smile lecture on pediatric stroke; royalties, monies from book on childhood stroke will be forthcoming but negative balance currently; travel expenses, Neonatal and Childhood Stroke (St Gallen, Switzerland), Childhood Stroke (Padua, Italy).

Additional supporting information can be found in the online version of this article.

patients versus controls in the lowest ADAMTS13 quintile was compared to those in the 2nd to 5th quintiles. Adjustment was performed for VWF antigen (VWF:Ag), factor VIII activity (FVIII:C), blood group, and age.

Results—Forty-six of 208 patients (22%) showed ADAMTS13 levels below the 10th percentile, compared with 5 of 125 controls (4%; $p < 0.001$). Odds ratios/95% confidence intervals were 7.30/2.73–19.50 for the lowest percentile and 2.44/1.15–5.16 in the quintile comparison after adjustment for VWF:Ag, FVIII:C, blood group, and age. Comparing the proportion of patients with ADAMTS13 activity below the 10th percentile within the different stroke subtypes (undetermined, cardioembolic, steno-occlusive arteriopathies), no statistically significant differences were found (undetermined, 16 of 89; cardioembolic, 6 of 40; steno-occlusive arteriopathies, 24 of 79; $p = 0.08$). ADAMTS13 levels did not significantly differ among stroke subtypes ($p = 0.29$).

Interpretation—Our findings implicate reduced ADAMTS13 activity as a risk factor for pediatric AIS, and support the concept that ADAMTS13 has a role in the pathogenesis of pediatric AIS.

Stroke in children is a heterogeneous disorder associated with significant morbidity and mortality. As in adults, pediatric stroke is understood to be a multifactorial disease, with an overall estimated yearly incidence of 13 per 100,000 in children.¹ The most frequent reported risk factors for arterial ischemic stroke (AIS) in children include underlying medical conditions such as cardiac disorders, hematologic diseases, cerebrovascular pathologies, and infectious diseases.² In addition, inherited genetic traits have been implicated as risk factors, and multiple genetic and acquired prothrombotic abnormalities have been evaluated in children.^{3–8}

The von Willebrand factor (VWF)-cleaving protease ADAMTS13 also known as a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13, is a 190kDa glycosylated protein that is produced in the liver (hepatic stellate cells), the endothelium, platelets, and brain tissues.⁹ This plasma metalloprotease cleaves ultralarge von Willebrand factor multimers,¹⁰ reduces platelet adhesion and aggregation,^{11,12} and down regulates thrombus formation and inflammation.¹³ ADAMTS13 function is crucial for preventing thrombosis in the microvasculature, as indicated by the occurrence of neurologic deficits in thrombotic thrombocytopenic purpura (TTP). Reduction or absence of ADAMTS13 activity results in impaired degradation of ultralarge VWF multimers, leading to excessive VWF-induced platelet aggregation on the vascular endothelium; in severe ADAMTS13 deficiency (as in TTP), this results in consumption coagulopathy.

The physiological role of ADAMTS13 in regulating VWF multimer size has become appreciated via the study of its deficiency in patients with TTP.^{14,15} Recently it has been suggested that decreased ADAMTS13 activity is associated with premature onset of cardiovascular disease in adults,^{16–18} and hypothesized that ADAMTS13 may protect the brain from ischemia by regulating VWF–platelet interaction after reperfusion.^{19,20} The aim of the present cohort study was to investigate an association of ADAMTS13 activity with risk of pediatric AIS.

Subjects and Methods

This case–control study was performed in accordance with the guidelines of the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) statement.²¹

Ethics

The present study was performed in accordance with the ethical standards established in the updated version of the 1964 Declaration of Helsinki and was approved by the medical ethics committee of the University of Münster, Germany.

Patients

With written parental consent, pediatric patients with a confirmed incident of AIS, aged between 30 days and 18 years, who were consecutively enrolled in the Münster cohort study between January 1, 2004 and December 30, 2010, were included in the present analysis. AIS was confirmed by established standard imaging methods (ie, magnetic resonance imaging and computerized tomography or magnetic resonance angiography).^{2,22–24} AIS was classified according to explicit predefined criteria for children by an independent team of physicians, hematologists and neuroradiologists.^{23,24} Based on clinical and laboratory features and on reviewing the imaging and Doppler ultrasounds performed, stroke subtypes were confirmed by an international experienced pediatric neurologist (F.J.K.) and a pediatric hematologist (G.K.). Both were blinded to the initial diagnosis and were not involved in the patient care. Patients with underlying diseases or treatment regimens that can modify the value or cleavage capacity of ADAMTS13, such as liver disease, anemia, thrombocytopenia, hemolytic uremic syndrome, sepsis, antiphospholipid antibodies, therapeutic anticoagulation (ie, heparin therapy), arterial dissection, or moyamoya prior to revascularization were excluded.^{25–29} Additionally, perinatal stroke cases (age until day 29), babies born prematurely (<36 gestational weeks), and patients older than 18 years at presentation, those lost to follow-up, and those lacking parental consent were excluded. Stroke cases were compared with respect to laboratory testing (described below) to 125 healthy population-based frequency-matched control children, in whom the same exclusion criteria were applied but no neuroimaging studies had been performed. Children in the control group had no history of chronic diseases and were not on medication at the time of recruitment. As described earlier, controls were ascertained as outpatients for evaluation before minor surgery (planned circumcisions and hernias) or bone marrow donation.³⁰

Blood Sample Collection

Blood sample collection from patients and controls was done in the morning after a 12-hour (infants, 4–6-hour) fasting period; samples were drawn by peripheral venipuncture into plastic tubes containing 1/10 by volume of 3.8% trisodium citrate (Sarstedt, Nümbrecht, Germany) and were immediately placed on melting ice. In patients, blood samples were drawn 6 to 12 months after the acute AIS event, and at least 6 weeks after discontinuation of anticoagulation. Platelet-poor plasma was prepared by centrifugation at $3,000 \times g$ and 4°C for 2×20 minutes, aliquoted in polystyrene tubes, stored at -70°C , and thawed immediately before assay.

Laboratory Testing

Factor VIII activity (FVIII:C) was determined by 1-stage activated partial thromboplastin time-based clotting assay, VWF antigen (VWF:Ag) was measured by enzyme-linked immunosorbent assay technique (Asserachrom vWF; Diagnostica Stago, Asnieres-sur-Seine, France), ristocetin cofactor activity (VWF:RCO; subgroup of 23 patients and 47 controls) was measured with the BCS XP analyzer with BC von Willebrand Reagent and VWF:Ag Latex Reagent (Siemens Healthcare Diagnostics, Marburg, Germany), and ADAMTS13 activity was determined by a fluorogenic assay (TECHNOZYM; Technoclone, Vienna, Austria) on the FLUOstar OPTIMA (BMG Labtech, Ortenberg, Germany; intra-assay coefficient of variation, 7.5%; interassay coefficient of variation, 8.0%). Further evaluation

included testing for antiphospholipid antibodies as exclusion criteria (including the lupus anticoagulant at minimum) in all cases. All assays were performed in platelet-poor plasma.

Abnormal FVIII:C, VWF:Ag, VWF:RCo, and antiphospholipid antibody testing values were defined by age-dependent reference ranges 2 standard deviations from the mean derived in a healthy control group distinct from the controls evaluated in the present study.³¹

Reproducibility of abnormal results was confirmed in a second plasma sample 3 months after initial testing.

Statistics

All statistical analyses were performed with the StatView 5 software package (SAS Institute, Cary, NC) and the MedCalc software package (MedCalc, Mariakerke, Belgium). Nonparametric statistics were performed for descriptive analysis (median [minimum–maximum] values; 75th and 25th percentiles; inter-quartile range [IQR]) and intergroup comparisons (Mann–Whitney *U* test; Kruskal–Wallis test). To compare percentile and quintile proportions of ADAMTS13 between patients and controls and to evaluate the interaction between ADAMTS13 and FVIII:C, VWF:Ag, blood group, and age, odds ratios (ORs) with corresponding 95% confidence intervals (CIs) were estimated by multivariate analysis using a logistic regression model. As a standard procedure, the frequencies of patients and controls below the 10th percentile were compared to those above the 10th percentile using age-dependent cutoff values (age adjustment) derived in an independent healthy control cohort (Supplementary Table 1).³⁰ To gain deeper insights into the complex interactions between ADAMTS13 activity and covariates, a quintile comparison was additionally performed. Correlation analysis was performed using the Spearman rank correlation test. For all analyses, a 2-sided *p* value <0.05 was considered statistically significant. Of note, due to the limitation of blood samples, we investigated VWF:RCo, which was strongly correlated with VWF:Ag levels ($\rho = 0.86$), in a subgroup of 23 patients and 47 controls only. Apart from descriptive and correlation analyses, data are shown in the Supplementary Material.

Results

Patient Characteristics

Derivation of the final study population is shown by flow diagram in Figure 1. Characteristics of patients and controls are shown in Table 1. In 89 children AIS was classified as undetermined (including patients with thrombophilia),²³ 40 suffered from cardioembolic stroke,^{2,23,24} and 79 patients were classified as AIS of steno-occlusive arteriopathic origin (including 8 children suffering from stroke of multiple etiologies with arteriopathy as the leading symptom, and 4 children with moyamoya after revascularization operation on aspirin therapy).^{2,22–24}

Descriptive Analysis

In addition to median (minimum–maximum) values in the text, in Table 1 the corresponding 75th and 25th percentiles and IQRs are shown. ADAMTS13 activity beyond the acute AIS onset was significantly lower in patients compared to controls (0.98IU/ml⁻¹ [0.16–1.43] vs 1.03IU/ml⁻¹ [0.60–1.56]; *p* = 0.03; Fig 2). VWF:Ag levels in patients compared to healthy controls did not show a statistically significant difference (1.12IU/ml⁻¹ [0.34–4.35] vs 1.06IU/ml⁻¹ [0.50–4.51]; *p* = 0.65). FVIII:C beyond the acute stroke onset was no different between the 2 groups (1.00IU/ml⁻¹ [0.48–2.05] vs 1.02IU/ml⁻¹ [0.50–3.48]; *p* = 0.44). Platelet count in patients and controls was within the normal age-dependent reference range.

Percentile Comparison

Using age-dependent cutoff values (see Supplementary Table 1) in univariate analysis, 46 of 208 patients with stroke (22%) showed ADAMTS13 activity below the 10th percentile compared with 5 of 125 controls (4%; $p < 0.001$), with an OR/95% CI of 6.70/2.58–17.38. After adjustment for VWF:Ag, FVIII:C, and blood group, OR/95% CI was 7.30/2.73–19.50 (Table 2).

Comparing the proportion of patients with ADAMTS13 activity below the 10th percentile within the different stroke subtypes (undetermined, 16 of 89; cardioembolic, 6 of 40; steno-occlusive arteriopathies, 24 of 79) no statistically significant differences were found ($p = 0.08$). Furthermore, ADAMTS13 activity levels were no different between the stroke subtypes classified ($p = 0.29$).

Quintile Comparison

Cutoff levels used for the quintile comparison are shown in Supplementary Table 2. In univariate and multivariate analyses (Table 3), odds of incident AIS were significantly increased when comparing ADAMTS13 activities in the lowest (1st) quintile with activities in the remaining quintiles; adjusted OR/95% CIs were 2.44/1.15–5.16.

Correlation Analysis

Weak positive correlations were found between ADAMTS13 and (1) blood group ($\rho = 0.18$; $p = 0.002$) and (2) age ($\rho = 0.16$; $p = 0.004$), but not between ADAMTS13 and VWF:Ag, VWF:RCo, or FVIII:C, respectively. In contrast, in the subgroup analysis a strong positive association was found between VWF:RCo and VWF:Ag ($\rho = 0.86$; $p < 0.001$).

Discussion

The findings of the present cohort study revealed that decreased ADAMTS13 activity is a risk factor for AIS in children. These observations are concordant with those in adults, wherein reduced ADAMTS13 levels were associated with premature onset of cardiovascular disease. Specifically, decreased levels of ADAMTS13 have been described in adult patients with myocardial infarction at disease onset as well as beyond the acute phase,^{16,17} and in another cohort in young patients with coronary heart disease.¹⁸ Our findings are also consistent with neurological microvascular involvement in TTP, where the pathogenesis relates to excess of ultralarge VWF multimers due to decreased ADAMTS13 activity. However, our data could not yet clarify whether the reduced ADAMTS13 activity levels in our case-control study are acquired or inherited.

Although high VWF levels have been associated with an increased risk of a first AIS in adults,³¹ VWF levels were not significantly higher in pediatric AIS cases relative to controls in the present analysis, in which children with stroke were investigated 6 to 12 months after stroke onset. Thus, we speculate that different time points of laboratory assessment (ie, acute stroke onset versus analysis clearly beyond the acute phase) may be responsible for the different results. Further investigations on this topic are mandatory to clarify this issue.

Mutations in the *ADAMTS13* gene have been shown to cause familial TTP,^{32–35} clinically associated with brain ischemia with neurologic symptoms due to microthrombi in the microvasculature. In line with this concept, Fujioka et al showed in a reperfusion mouse model that ADAMTS13 gene deletion aggravates ischemic brain damage.²⁰ Recent evidence from a genomewide association study in this cohort³⁶ points toward the involvement of genetic variants in the ADAMTS13 locus and risk for pediatric AIS. However, these results are preliminary in nature and require further validation.

Additionally, it was reported in 2009 that infusion of high-dose ADAMTS13 concentrates administered in mice immediately before reperfusion injury significantly reduces infarct volume and improves functional outcome without evidence of hemorrhage.¹⁹ In addition to familial TTP, acquired TTP has been described in the presence of immunoglobulin (Ig)G, IgA, or IgM antibodies inhibiting ADAMTS13 activity. In these cases, the inhibitory antibody effect could be overridden by administration of recombinant ADAMTS13 concentrate.³⁷ Thus, we speculate that the administration of ADAMTS13 containing fresh frozen plasma or ADAMTS13 concentrate will be a treatment option also for pediatric AIS in subjects with reduced ADAMTS13 activities. Furthermore, because ADAMTS13 is inactivated at the site of thrombus formation via thrombin and factor Xa,³⁸ it is possible that the use of thrombin or anti-factor-Xa inhibitors will indirectly enhance the antithrombotic effect of ADAMTS13.

Limitations of the present study are as follow:

- Due to ethical reasons the amount of plasma that could be drawn in children was restricted. We were unable to perform additional analyses to evaluate VWF:RCo in the entire patient and control cohort and the presence/absence of ultralarge VWF multimers in children with ADAMTS13 levels below the 10th percentile/lowest quintile. However, VWF levels determined by us beyond the acute stroke period did not differ significantly between cases and controls, and we have adjusted for this variable in multivariate analysis, to account for possible confounding of the association of ADAMTS13 activity with AIS risk.
- Although blood samples for the present study were drawn clearly beyond the acute stroke onset (ie, 6–12 months following clinical diagnosis), and patients with liver disease, thrombocytopenia, sepsis, or antiphospholipid antibodies and patients therapeutically anticoagulated with heparin were excluded,^{25–29} it is possible that acquired (including antibody-mediated) ADAMTS13 deficiency could have contributed to AIS risk in some of the children.
- Due to the lack of statistical power, we were not able to perform further subgroup analyses of ADAMTS13 association with AIS risk in specific pediatric AIS subtypes. This should be explored in future collaborative cohort studies.

In summary, our finding of reduced ADAMTS13 activity as a risk factor for incident pediatric AIS supports the concept that ADAMTS13 is involved in AIS pathogenesis in young patients, and builds upon recent literature that suggests the potential of ADAMTS13 as a target for research on future therapeutic strategies in this disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was sponsored by an Interdisziplinäres Zentrum für Klinische Forschung grant (CRA01) from the University of Münster, Germany.

References

1. Mallick AA, O'Callaghan FJK. The epidemiology of childhood stroke. *Eur J Paed Neurol*. 2010; 14:197–205.
2. Kirkham FJ, Prengler M, Hewes DK, Ganesan V. Risk factors for arterial ischemic stroke in children. *J Child Neurol*. 2000; 15:299–307. [PubMed: 10830196]

3. Lanthier S, Carmant L, David M, et al. Stroke in children: the coexistence of multiple risk factors predicts poor outcome. *Neurology*. 2000; 54:371–378. [PubMed: 10668698]
4. Kirkham F, Sébire G, Steinlin M, Sträter R. Arterial ischaemic stroke in children. Review of the literature and strategies for future stroke studies. *Thromb Haemost*. 2004; 92:697–706. [PubMed: 15467898]
5. Munot P, Crow YJ, Ganesan V. Paediatric stroke: genetic insights into disease mechanisms and treatment targets. *Lancet Neurol*. 2011; 10:264–274. [PubMed: 21349441]
6. Haywood S, Liesner R, Pindora S, Ganesan V. Thrombophilia and first arterial ischaemic stroke: a systematic review. *Arch Dis Child*. 2005; 90:402–405. [PubMed: 15781933]
7. Nowak-Göttl U, Langer C, Bergs S, et al. Genetics of hemostasis: differential effects of heritability and household components influencing lipid concentrations and clotting factor levels in 282 pediatric stroke families. *Environ Health Perspect*. 2008; 116:839–843. [PubMed: 18560491]
8. Kenet G, Lütkehoff LK, Albisetti M, et al. Impact of thrombophilia on risk of arterial ischemic stroke or cerebral sinovenous thrombosis in neonates and children: a systematic review and meta-analysis of observational studies. *Circulation*. 2010; 121:1838–1847. [PubMed: 20385928]
9. Dong JF. Structural and functional correlation of ADAMTS13. *Curr Opin Hematol*. 2007; 14:270–276. [PubMed: 17414218]
10. Zheng X, Chung D, Takayama TK, et al. Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura. *J Biol Chem*. 2001; 276:41059–41063. [PubMed: 11557746]
11. Chauhan AK, Motto DG, Lamb CB, et al. Systemic antithrombotic effects of ADAMTS13. *J Exp Med*. 2006; 203:767–776. [PubMed: 16533881]
12. Chauhan AK, Kisucka J, Brill A, et al. ADAMTS13: a new link between thrombosis and inflammation. *J Exp Med*. 2008; 205:2065–2074. [PubMed: 18695007]
13. Motto DG, Chauhan AK, Zhu G, et al. Shigatoxin triggers thrombotic thrombocytopenic purpura in genetically susceptible ADAMTS13-deficient mice. *J Clin Invest*. 2005; 115:2752–2761. [PubMed: 16200209]
14. Furlan M, Robles R, Galbusera M, et al. von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. *N Engl J Med*. 1998; 339:1578–1584. [PubMed: 9828245]
15. George JN. Clinical practice. Thrombotic thrombocytopenic purpura. *N Engl J Med*. 2006; 354:1927–1935. [PubMed: 16672704]
16. Kaikita K, Soejima K, Matsukawa M, et al. Reduced von Willebrand factor-cleaving protease (ADAMTS13) activity in acute myocardial infarction. *J Thromb Haemost*. 2006; 4:2490–2493. [PubMed: 16898955]
17. Crawley JT, Lane DA, Woodward M, et al. Evidence that high von Willebrand factor and low ADAMTS-13 levels independently increase the risk of a non-fatal heart attack. *J Thromb Haemost*. 2008; 6:583–588. [PubMed: 18194418]
18. Bongers TN, de Bruijne EL, Dippel DW, et al. Lower levels of ADAMTS13 are associated with cardiovascular disease in young patients. *Atherosclerosis*. 2009; 207:250–254. [PubMed: 19439298]
19. Zhao BQ, Chauhan AK, Canault M, et al. von Willebrand factor-cleaving protease ADAMTS13 reduces ischemic brain injury in experimental stroke. *Blood*. 2009; 114:3329–3334. [PubMed: 19687510]
20. Fujioka M, Hayakawa K, Mishima K, et al. ADAMTS13 gene deletion aggravates ischemic brain damage: a possible neuroprotective role of ADAMTS13 by ameliorating postischemic hypoperfusion. *Blood*. 2010; 115:1650–1653. [PubMed: 19965676]
21. VonElm E, Altman DG, Egger M, et al. The strengthening the reporting of observational studies in Epidemiology (STROBE) Statement: Guidelines for reporting observational studies. *PLOS Med*. 2007; 4:1623–1627. e296.
22. Williams LS, Garg BP, Cohen M, et al. Subtypes of ischemic stroke in children and young adults. *Neurology*. 1997; 49:1541–1545. [PubMed: 9409343]

23. Sträter R, Becker S, von Eckardstein A, et al. Prospective assessment of risk factors for recurrent stroke during childhood—a 5-year follow-up study. *Lancet*. 2002; 360:1540–1545. [PubMed: 12443591]
24. Wraige E, Pohl KRE, Ganesan V. A proposed classification for subtypes of arterial ischaemic stroke in children. *Dev Med Child Neurol*. 2005; 47:252–256. [PubMed: 15832548]
25. Nguyen TC, Liu A, Liu L, et al. Acquired ADAMTS-13 deficiency in pediatric patients with severe sepsis. *Haematologica*. 2007; 92:121–124. [PubMed: 17229645]
26. Feys HB, Canciani MT, Peyvandi F, et al. ADAMTS13 activity to antigen ratio in physiological and pathological conditions associated with an increased risk of thrombosis. *Br J Haematol*. 2007; 138:534–540. [PubMed: 17608762]
27. Uemura M, Fujimura Y, Matsumoto M, et al. Comprehensive analysis of ADAMTS13 in patients with liver cirrhosis. *Thromb Haemost*. 2008; 99:1019–1029. [PubMed: 18521503]
28. Austin SK, Starke RD, Lawrie AS, et al. The VWF/ADAMTS13 axis in the antiphospholipid syndrome: ADAMTS13 antibodies and ADAMTS13 dysfunction. *Br J Haematol*. 2008; 141:536–544. [PubMed: 18341632]
29. Vomund AN, Elaine M, Majerus EM. ADAMTS13 bound to endothelial cells exhibits enhanced cleavage of von Willebrand factor. *J Biol Chem*. 2009; 284:30925–30932. [PubMed: 19729451]
30. Neuner B, von Mackensen S, Krümpel A, et al. Health-related quality of life in children and adolescents with stroke, self-reports, and parent/proxies reports: cross-sectional investigation. *Ann Neurol*. 2011; 70:70–78. [PubMed: 21425189]
31. Kreuz W, Stoll M, Junker R, et al. Familial elevated factor VIII in children with symptomatic venous thrombosis and post-thrombotic syndrome: results of a multicenter study. *Arterioscler Thromb Vasc Biol*. 2006; 26:1901–1906. [PubMed: 16709943]
32. Bongers TN, de Maat MP, van Goor ML, et al. High von Willebrand factor levels increase the risk of first ischemic stroke: influence of ADAMTS13, inflammation, and genetic variability. *Stroke*. 2006; 37:2672–2677. [PubMed: 16990571]
33. Hanson E, Jood K, Nilsson S, et al. Association between genetic variation at the ADAMT13 locus and ischemic stroke. *J Thromb Haemost*. 2009; 7:2147–2148. [PubMed: 19765208]
34. Levy GG, Nicols WC, Lian EC, et al. Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. *Nature*. 2001; 413:488–494. [PubMed: 11586351]
35. Lotta LA, Garagiola I, Palla R, et al. ADAMTS13 mutations and polymorphisms in congenital thrombotic thrombocytopenic purpura. *Hum Mutat*. 2010; 31:11–19. [PubMed: 19847791]
36. Arning A, Hiersche M, Witten A, et al. A genome wide association study identifies a gene network of ADAMTS genes in the predisposition to pediatric stroke. *Blood*. 2012 in press.
37. Plaimauer B, KremerHovinga JA, Juno C, et al. Recombinant ADAMTS13 normalizes von Willebrand factor-cleaving activity in plasma of acquired TTP patients by overriding inhibitory antibodies. *J Thromb Haemost*. 2011; 9:936–944. [PubMed: 21294825]
38. Shelat SG, Ai J, Zheng XL. Molecular biology of ADAMTS13 and diagnostic utility of ADAMTS13 proteolytic activity and inhibitor assays. *Semin Thromb Hemost*. 2005; 31:659–672. [PubMed: 16388417]

FIGURE 1.
Inclusion and exclusion criteria for patients enrolled in the study are shown.

FIGURE 2.

A box plot of ADAMTS13 activity in patients with arterial ischemic stroke (AIS) and controls is depicted. Median, 75th and 25th percentiles, and individual levels of patient/controls are shown. The difference between controls and children with AIS is statistically significant ($p = 0.03$).

TABLE 1

Patient Demographics and Laboratory Parameters of Interest

Characteristic	Patients	Control Group	<i>p</i>
Total number/% male	208/55	125/51	0.79
Ethnicity, % white	100	100	1.0
Age at AIS onset, ^a median yr (75th-25th percentile; IQR)	2.1 (7.0–0.1; 7.0)	—	—
No. [%] of smoking children >14 years of age	4 of 208 [1.9]	3 of 125 [2.4]	1.0
ADAMTS13, median IU/ml ⁻¹ (75th-25th percentile; IQR)	0.98 (1.11–0.81; 0.30)	1.03 (1.12–0.91; 0.21)	0.03
VWF:Ag, median IU/ml ⁻¹ (75th-25th percentile; IQR)	1.3 (1.33–0.85; 0.48)	1.06 (1.32–0.84; 0.48)	0.65
VWF:RCo, median IU/ml ⁻¹ (75th-25th percentile; IQR)	1.06 (1.39–0.71; 0.69)	0.97 (1.29–0.76; 0.53)	0.66
FVIII, median IU/ml ⁻¹ (75th-25th percentile; IQR)	1.00 (1.17–0.88; 0.29)	1.02 (1.19–0.90; 0.29)	0.44
No. [%] of subjects with ADAMTS13 <10th percentile	46 of 208 [22]	5 of 125 [4]	<0.001

^aPatients were investigated 6 to 12 months beyond acute stroke onset.

AIS = arterial ischemic stroke; FVIII = factor VIII; IQR = interquartile range; VWF:Ag = von Willebrand factor antigen; VWF:RCo = von Willebrand ristocetin cofactor activity.

TABLE 2

Unadjusted and Adjusted Odds of Stroke for Reduced ADAMTS13 Activity Levels, by Univariate and Multivariate Logistic Regression (Percentile Comparison)

ADAMTS13 Analytic Variable ^a	Unadjusted Univariate OR (95% CI)	Adjusted Multivariate OR (95% CI) ^a
<10th percentile ^b	6.70 (2.58–17.38)	7.30 (2.73–19.50)
VWF:Ag per 0.1IU/ml ⁻¹ increase	1.0 (0.99–1.006)	0.99 (0.99–1.004)
FVIII per 0.1IU/ml ⁻¹ increase	1.002 (0.99–1.01)	1.008 (0.99–1.02)
BG O vs A	1.015 (0.60–1.70)	0.85 (0.47–1.54)
BG O vs AB	0.51 (0.16–1.74)	0.44 (0.12–1.56)
BG O vs B	0.74 (0.35–1.58)	0.73 (0.31–1.72)

^a All analytic variables are included in the multivariate analysis.

^b Using age-specific cutoffs (age adjustment) as derived in an independent cohort of healthy pediatric subjects (Supplementary Table 1).²⁸

BG = blood group; CI = confidence interval; FVIII = factor VIII; OR = odds ratio; VWF:Ag = von Willebrand factor antigen.

TABLE 3

Unadjusted and Adjusted Odds of Stroke for Reduced ADAMTS13 Activity Levels (Quintile Comparison)

ADAMTS13 Analytic Variable ^a	Unadjusted Univariate OR (95% CI)	Adjusted Multivariate OR (95% CI) ^a
1st vs 2nd quintile	2.70 (1.21–6.00)	2.01 (0.82–4.90)
1st vs 3rd quintile	2.60 (1.17–5.80)	2.38 (0.98–5.76)
1st vs 4th quintile	3.65 (1.65–8.09)	2.76 (1.13–6.74)
1st vs 5th quintile	2.60 (1.16–5.80)	2.72 (1.11–6.66)
VWF:Ag per 0.1IU/ml ⁻¹ increase	<i>a</i>	0.99 (0.99–1.002)
FVIII per 0.1IU/ml ⁻¹ increase	<i>a</i>	1.006 (0.99–1.016)
BG O vs A	<i>a</i>	0.93 (0.50–1.71)
BG O vs AB	<i>a</i>	0.57 (0.15–2.12)
BG O vs B	<i>a</i>	0.71 (0.30–1.71)
Age increase in months	1.011 (1.007–1.015)	1.01 (1.004–1.013)
1st vs 2nd-5th quintiles	2.87 (1.45–5.64)	2.44 (1.15–5.16)
VWF:Ag per 0.1IU/ml ⁻¹ increase	<i>a</i>	0.99 (0.98–1.002)
FVIII per 0.1IU/ml ⁻¹ increase	<i>a</i>	1.006 (0.99–1.02)
BG O vs A	<i>a</i>	0.95 (0.52–1.73)
BG O vs AB	<i>a</i>	0.60 (0.16–2.23)
BG O vs B	<i>a</i>	0.72 (0.30–1.71)
Age increase in months	<i>b</i>	1.01 (1.005–1.013)

The odds of arterial ischemic stroke were calculated for the lowest quintile (1) versus the 2nd, 3rd, 4th, and 5th quintiles and (2) versus the 2nd to 5th quintiles with adjustment for VWF:Ag, factor VIII activity, blood group, and age in months.

^aAll analytic variables are included in the multivariate analysis.

^bSee Table 2.

BG = blood group; CI = confidence interval; FVIII = factor VIII; OR = odds ratio; VWF:Ag = von Willebrand factor antigen.