Elevated α₁-Antitrypsin Is a Risk Factor for Arterial Ischemic Stroke in Childhood

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α₁-Antitrypsin (α₁-AT) is a physiological inhibitor of activated protein C (APC) and therefore decreased APC activity. APC itself causes an anticoagulant effect by inactivating factors Va and VIIIa. The present case-control study was performed to evaluate the role of the elevated α₁-AT concentration in pediatric patients with ischemic stroke (IS). α₁-AT concentrations were measured along with established prothrombotic risk factors 6–12 months after the acute thrombotic onset in 81 Caucasian children with IS aged 1 month to 18 years. The cutoff values defined as age-dependent 90th percentiles were obtained from 229 healthy controls. Median (range) values of α₁-AT were significantly higher in patients compared with control subjects [122.0 mg/dl (61.4–224.0) vs. 114.0 mg/dl (66.8–156.0); p = 0.016]. In addition, 14 of the 81 patients (17.3%) compared with 10 of the 162 controls (6.2%) had α₁-AT concentrations above the 90th age-dependent percentiles (p = 0.012). Multivariate analysis performed in a 1:2 matched case-control setting adjusted for the presence of established prothrombotic risk factors showed a significantly increased odds ratio (OR) and 95% confidence interval (CI) for patients with elevated α₁-AT >90th percentiles and IS (OR/CI: 4.0/1.64–9.92; p = 0.0024). Data shown here give evidence that total α₁-AT concentrations above the 90th age-dependent percentiles independently increase the risk of IS 4.0-fold in Caucasian children.

Key Words
α₁-Antitrypsin · Factor V G1691A · Lipoprotein (a) · Thromboembolism, pediatric

Abstract
α₁-antitrypsin (α₁-AT) is a physiological inhibitor of activated protein C (APC) and therefore decreased APC activity. APC itself causes an anticoagulant effect by inactivating factors Va and VIIIa. The present case-control study was performed to evaluate the role of the elevated α₁-AT concentration in pediatric patients with ischemic stroke (IS). α₁-AT concentrations were measured along with established prothrombotic risk factors 6–12 months after the acute thrombotic onset in 81 Caucasian children with IS aged 1 month to 18 years. The cutoff values defined as age-dependent 90th percentiles were obtained from 229 healthy controls. Median (range) values of α₁-AT were significantly higher in patients compared with control subjects [122.0 mg/dl (61.4–224.0) vs. 114.0 mg/dl (66.8–156.0); p = 0.016]. In addition, 14 of the 81 patients (17.3%) compared with 10 of the 162 controls (6.2%) had α₁-AT concentrations above the 90th age-dependent percentiles (p = 0.012). Multivariate analysis performed in a 1:2 matched case-control setting adjusted for the presence of established prothrombotic risk factors showed a significantly increased odds ratio (OR) and 95% confidence interval (CI) for patients with elevated α₁-AT >90th percentiles and IS (OR/CI: 4.0/1.64–9.92; p = 0.0024). Data shown here give evidence that total α₁-AT concentrations above the 90th age-dependent percentiles independently increase the risk of IS 4.0-fold in Caucasian children.

Introduction
Although venous and arterial thromboses are rare diseases in infancy and childhood, incidences of symptomatic thrombotic manifestations are recorded in 0.07/10,000 children, in 5.3/10,000 admissions of children and 2.4/1,000 admissions of newborns to intensive care units [1, 2]. It has been reported that several clinical and environmental conditions, such as peripartal asphyxia, neonatal infections, fetal diabetes, the use of central lines, trauma or surgery, dehydration, malignant disease, renal disease, autoimmune disease, or the application of oral contraceptives by adolescent girls resulted in elevated thrombin generation with subsequent thrombus formation in infancy and childhood, e.g. venous thrombosis or ischemic stroke (IS) of thromboembolic origin [1, 3–7]. However, before the era of extensive molecular testing in many children the etiopathology of thromboembolic events remained unclear.
Various genetic defects of proteins regulating blood coagulation have been established as risk factors for thromboembolic diseases. Most of them affect the protein C pathway, namely activated protein C (APC) resistance, in the majority of cases due to the factor V mutation, as well as deficiencies of protein C and protein S due to other causes. Prothrombotic states have also been associated with defects in the genes of antithrombin, plasminogen, and fibrinogen [8–10]. In addition, the previously described G20210A variant of the factor II gene also seems to be a common but probably mild risk factor for arterial and venous thromboembolism [11, 12]. Furthermore, prothrombotic states are associated with metabolic diseases such as increased fasting total homocysteine plasma levels [13] as well as elevated concentrations of lipoprotein (a) [Lp(a)] [14]. As recently shown, 40–50% of symptomatic thromboembolism in pediatric Caucasian cohorts are attributable to genetic prothrombotic risk factors.

With the intention to further close the gap between the unclear cases of pediatric stroke with respect to prothrombotic risk factors associated with defects within the protein C pathway, in the present evaluation we focused on a physiological plasma protein that has been described as an inhibitor of APC, namely α1-antitrypsin (α1-AT) [15, 16]. Inhibiting APC, elevated α1-AT could theoretically mimic a ‘protein C-deficient state’ subsequently leading to a prothrombotic situation. To investigate the role of elevated α1-AT as a candidate risk factor for symptomatic IS in children the present case-control study was performed.

Material and Methods

Ethics
The present study was performed in accordance with the ethical standards laid down in the updated relevant version of Declaration of Helsinki and approved by the medical ethics committee at the Westfälische Wilhelms University, Münster, Germany.

Patients
Caucasian pediatric patients with a first symptomatic thromboembolic event were consecutively recruited and screened for hereditary prothrombotic risk factors between January 1996 and June 2003 by the participating centers in the catchment areas of Hamburg, Kiel, Lübeck, Münster, Bielefeld, Düsseldorf, Berlin, Magdeburg, Halle, and Munich.

Inclusion Criteria
Neonates, infants and children aged 1 month to 18 years with the first onset of a thromboembolic stroke event were included. A thromboembolic stroke event had to be confirmed objectively by standard imaging methods, i.e. cerebral CT scanning, MR imaging, MR angiography, or transcranial Doppler ultrasonography.

Exclusion Criteria
Patients older than 18 years, children not of Caucasian origin, patients with incomplete clinical or laboratory workup (established prothrombotic risk factors), and children lost to follow-up or without parental consent were not enrolled in the present study. In addition, children during the acute thromboembolic phase, patients with renal insufficiency, liver disease or malignancies, and adolescent girls taking oral contraceptives were excluded.

Final Study Population
From the ongoing multicenter study a randomly selected subgroup of 81 pediatric patients with a median age of 2.5 years (range: 1 month to 18 years) was enrolled. Randomization into the sub-study – and therefore reduction of the patient number – was performed to achieve standard conditions. For the case-control design a 1:2 matching was performed.

Control Group
A total of 229 healthy children were investigated to determine the age-dependent levels of α1-AT and the age-dependent percentiles. Controls were recruited between January 1996 and June 2003. Control children had no history of chronic disease or thromboembolic events and were medication-free at the time of enrolment. They presented as outpatients for evaluation before minor surgery or were potential bone marrow donors.

Laboratory Tests
With informed parental consent, the factor V G1691A (FV) and factor II G20210A (FII) mutations, concentration of Lp(a), protein C, protein S, antithrombin, total fasting plasma homocysteine levels and total α1-AT levels were investigated in patients and controls 6–12 months after the acute event, using standard laboratory techniques [12–17]. In patients and controls peripheral venipuncture was performed in the morning between 8 and 10 a.m. after a standardized fasting period: at least 4 h for infants below 1 year of age including breast-fed infants, and more than 8 h for children above 1 year of age. Blood samples were collected by peripheral venipuncture into plastic tubes containing 1/10 by volume of 3.8% trisodium citrate (Sarstedt, Nümbrecht, Germany), and placed immediately on melting ice. Platelet-poor plasma was prepared by centrifugation at 2,000 g for 15 min at 4°C 2 times, aliquoted in polystyrene tubes, stored at −70°C, and thawed immediately before the assay procedure. Total α1-AT was investigated with a commercially available antiserum against α1-AT (Dade Behring, Marburg, Germany) in a Behring Nephelometer II, BN II (Dade Behring).

Statistical Analysis
All statistical analyses were performed with the StatView 5 software package (SAS Institute) and the MedCalc software package (MedCalc, Mariakerke, Belgium). Prevalence rates of prothrombotic risk factors in patients and control subjects were calculated by χ2 analysis or by Fisher’s exact test. To compare the overall rate of prothrombotic risk factors between patients and controls and to evaluate the interaction between elevated α1-AT concentrations and established prothrombotic risk factors, odds ratios (ORs) together with 95% confidence intervals (CIs) were estimated from a multivariate analysis using a logistic regression model. The signifi-
cance level was set at 0.05. For variables with a non-Gaussian frequency distribution data were presented as median and ranges. The Mann-Whitney test was used to compare median (range) values obtained in patients and controls, and Spearman’s rank test was used to calculate correlations between total α₁-AT levels and age. p values <0.05 were considered significant.

Results

Distribution of α₁-AT Levels in Healthy Children

The total median concentration of α₁-AT was 114.0 mg/dl (range: 66.8–207.0) in 229 control subjects tested. In addition, in accordance with literature reports and similar to other components of the hemostatic system during childhood [18–20], age dependency was shown (p = 0.045; rho = 0.16). In table 1 age-dependent median (range) levels of α₁-AT concentrations are demonstrated. Infants from 1 to 12 months had a median α₁-AT concentration of 103.5 mg/dl and children from 1.1 to 4.0 years had a median α₁-AT concentration of 112.0 mg/dl, with a further α₁-AT increase noted in older children from 4.1 to 9.0 and up to 18 years (115.0 mg/dl).

Cutoffs Used for Case-Control Study

Similar to median (range) α₁-AT concentrations the upper 90th age-dependent percentiles were determined within the healthy pediatric population. In the youngest age group (1–12 months) the upper 90th percentile was 130.3 mg/dl. Children between 1.1 and 4.0 years showed a cutoff of 144.2 mg/dl, and in subjects between 4.1 and 9.0 years the upper 90th percentile of α₁-AT was 147.2 mg/dl. During late childhood and puberty (9.1–18.0 years) the cutoff for α₁-AT levels decreased towards adult values (140.0 mg/dl).

α₁-AT Concentrations in Patients and Controls

In patients with IS (n = 81) the median level of α₁-AT was 122.0 mg/dl (range 61.4–224.0 mg/dl), whereas in control children (n = 162) the median concentration was significantly lower (114.0 mg/dl, range 66.8–156.0 mg/dl; p = 0.016). In the group of children with stroke, 14 of 81 patients (17.3%) versus 10 of the 162 children (6.2%) in the control group had α₁-AT levels above the age-specific 90th percentile (p = 0.012).

Multivariate Analysis

Logistic regression was performed in a 1:2 matched design (81 patients compared with 162 controls). Table 2 shows the interaction between elevated α₁-AT and established prothrombotic risk factors. Including the overall

<table>
<thead>
<tr>
<th>Age years</th>
<th>α₁-AT levels mg/dl</th>
<th>90th percentiles mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1–1.0</td>
<td>103.5 (79.3–135.0)</td>
<td>130.3</td>
</tr>
<tr>
<td></td>
<td>n = 32</td>
<td></td>
</tr>
<tr>
<td>1.1–4.0</td>
<td>112.0 (69.6–191.0)</td>
<td>144.2</td>
</tr>
<tr>
<td></td>
<td>n = 53</td>
<td></td>
</tr>
<tr>
<td>4.1–9.0</td>
<td>115.0 (66.8–207.0)</td>
<td>147.2</td>
</tr>
<tr>
<td></td>
<td>n = 69</td>
<td></td>
</tr>
<tr>
<td>9.1–18.0</td>
<td>115.0 (76.9–195.0)</td>
<td>140.0</td>
</tr>
<tr>
<td></td>
<td>n = 75</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>114.0 (66.8–207.0)</td>
<td>139.2</td>
</tr>
<tr>
<td></td>
<td>n = 229</td>
<td></td>
</tr>
</tbody>
</table>

α₁-AT levels are given as median with the range in parentheses.

<table>
<thead>
<tr>
<th>Prothrombotic risk factor (total α₁-AT &gt;90th percentiles)</th>
<th>Patients (n = 81)</th>
<th>Controls (n = 162)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With factor V G1691A</td>
<td>16</td>
<td>8</td>
<td>0.01</td>
</tr>
<tr>
<td>With factor II G20210A</td>
<td>6</td>
<td>4</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>With Lp(a) &gt;30 mg/dl</td>
<td>25</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>With protein C type I deficiency</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>With protein S type I deficiency</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>With antithrombin type I deficiency</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Sum of single and combined</td>
<td>62/81 (76.5%)</td>
<td>56/162 (34.6%)</td>
<td>&lt;0.000</td>
</tr>
</tbody>
</table>

Table 2. Distribution of single and combined prothrombotic risk factors in patients and healthy age-matched controls
rate of established prothrombotic risk factors known to be significantly associated with symptomatic thromboembolism in pediatric patients in Germany, e.g. the FV G1691A mutation, the FII G20210A variant, deficiency states of protein C, S or antithrombin, or elevated Lp(a) concentrations with an overall OR/CI of 2.9/1.6–5.3 (p = 0.0004), elevated α1-AT levels greater than the age-specific 90th percentiles retained their statistically significant and independent association (OR/CI 4.0/1.6–9.9; p = 0.002).

Discussion

APC is an anticoagulant serine protease in plasma. It downregulates the coagulation cascade by degrading the procoagulant factors Va and VIIIa by limited proteolysis. Factors Va and VIIIa are obligatory cofactors for thrombin generation. The proteolytic activity of APC in plasma among other factors is regulated by members of the serpin (serine protease inhibitor) family of proteins, e.g. protein C inhibitor, plasminogen activator inhibitor and α1-AT [15, 16, 21, 22]. α1-AT inactivates APC by forming a 1:1 stoichiometric complex [16]. By forming this complex a conformational change occurs in α1-AT, which results in a significant stabilization relative to the noncomplexed serpin α1-AT [23–25]. The inactivation of APC by α1-AT is relatively low, and not influenced by heparin [15, 16]. Despite its low interaction with APC, based on its high concentration in plasma, α1-AT is discussed to be a physiological and important factor for the regulation of APC activity in plasma, especially in the absence of heparin [15, 16, 26, 27]. Furthermore, it has been suggested that α1-AT becomes the major inhibitor of APC when protein C inhibitor is depleted by an increasing APC generation [28, 29].

Data on the possible association between α1-AT concentrations and IS are sparse and controversial. Fletcher et al. [30] described that α1-AT concentrations in adult patients with cerebral infarction were significantly increased in comparison to controls, and Bartosik-Psujek et al. [31] found elevated levels of α1-AT in adult patients with cerebral ischemia. The latter authors determined α1-AT in the acute phase of stroke events within the first 7 days and, therefore, interpret their results as indicating that ischemic necrosis is associated with inflammatory reactions. Furthermore, significantly increased α1-AT levels compared to controls have been described in adult patients with acute ischemic heart disease [32]. Another study that should be mentioned here is the study of Dahl et al. [33], who observed that α1-AT deficiency caused by MZ heterozygosity is associated with a reduced risk of ischemic cerebrovascular disease and ischemic heart disease. Thus, if a low concentration of α1-AT reduces the risk of ischemic vascular disease, then vice versa this would support the hypothesis that an increased α1-AT level is a risk factor for IS. In agreement with these findings Espana et al. [34] demonstrated that the APC:α1-AT complex can be used as a sensitive marker of prothrombotic states.

In contributing to the risk of pediatric thromboembolism the importance of various hereditary hemostatic abnormalities is well established [35]. To date, the rate of single or combined prothrombotic risk factors detected in Caucasian children is approximately 50%, while no thrombophilia has been found in the remaining subjects [12, 14, 17, 36]. Data presented here give evidence that increased α1-AT concentrations greater than age-dependent 90th percentiles further contribute as a significant and independent risk factor to symptomatic IS in Caucasian children, in 17.3% of cases not combined with one of the established risk factors. The OR found was 4.0 for subjects with thromboembolic stroke, i.e. clearly within the range reported for further inherited thrombophilic risk factors in white children, e.g. the heterozygous FV G1691A mutation, the FII G20210A variant or elevated Lp(a) levels.

The results shown here, indicating that α1-AT concentrations greater than age-dependent 90th percentiles are a risk factor for stroke in children, are in accordance with the majority of findings in adults. However, since the blood used to determine the α1-AT level had not been drawn until well after the onset of the acute stroke it is unlikely that the elevation in the α1-AT level could have been caused by the stroke itself. Along with normal values for fibrinogen, D-dimer and prothrombin fragment F1.2 at the time of evaluation (data not shown) we suggest that elevation of α1-AT is more likely a cause rather than a consequence of the stroke event. In addition, since the 1980s information has been available that there are important physiological differences in the hemostatic system in children compared with adults [20]. In contrast to lower levels of the natural anticoagulant inhibitors protein C and protein S in early childhood, α2-macroglobulin and C1-inactivator are elevated until puberty [20, 37], and α1-AT concentrations as earlier reported and established in the healthy cohort investigated here are lower beyond the neonatal period in the first year of life and increase in infants and children during puberty [20, 38, 39]. As a consequence in the last 20 years the need for
age-dependent normal values to correctly classify bleed-
ing as well as thrombotic tendencies in neonates, infants, and children has increasingly been accepted and normal age-dependent values should be applied when conducting risk assessment studies in children.

In summary, besides the established risk factors, an α1-AT concentration greater than the 90th age-dependent percentiles is another risk factor for stroke in Caucasian children, increasing the laboratory rate of detecting risk factors not combined with established thrombophilic risks in 17% of patients. However, the findings of the present study are restricted to white German stroke children, and further studies are recommended to clarify the role of increased α1-AT in pediatric populations not of Caucasian origin. In addition, further longitudinal follow-up studies are necessary to better evaluate and understand the role of α1-AT during the acute and nonacute phase following arterial thromboembolism.

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References


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