Increased Lipoprotein(a) Is an Important Risk Factor for Venous Thromboembolism in Childhood

Ulrike Nowak-Göttl, MD; Ralf Junker, MD; Marion Hartmeier, MD; Hans-Georg Koch, MD; Nicole Münchow, MD; Gerd Assmann, MD; Arnold von Eckardstein, MD

Background—Serum levels of lipoprotein(a) [Lp(a)] are determined largely by genetic variation in the gene encoding for apolipoprotein(a) [apo(a)], the specific protein component of Lp(a) that is very homologous to plasminogen. High plasma levels of Lp(a) increase the risk for premature atherosclerotic vessel diseases. We investigated the little-characterized role of Lp(a) as a risk factor for venous thromboembolic diseases, alone and in conjunction with established thrombophilic risk factors of proteins regulating blood coagulation and fibrinolysis.

Methods and Results—Serum levels of Lp(a) and lipids, protein C, protein S, and antithrombin, as well as the size of apo(a) isoforms and the presence of the factor V:Q506 mutation, were determined in 186 consecutively admitted children from neonates to 18 years old with a history of venous thrombosis and in 186 age- and disease-matched control subjects. Children with a history of venous thrombosis had a significantly higher median Lp(a) level (19 versus 4.4 mg/dL) than control subjects. The risk for thromboembolic events in children with Lp(a) levels in the upper quartile, ie, >30 mg/dL, was 7.2 (95% CI, 3.7 to 14.5). The size of apo(a) isoforms was inversely related to Lp(a) levels and to the risk for thromboembolic events. Compared with the highest quartile of the apo(a) size distribution, the lowest quartile was associated with a risk of 8.2. In addition, multivariate statistical analysis gives evidence that the factor V:Q506 mutation (OR/CI, 2.8/1.6 to 4.9), protein C (OR/CI, 6.5/2.1 to 19), and antithrombin deficiency (OR/CI, 10.4/1.2 to 90) were independent risk factors of childhood venous thrombosis. Coincidence of elevated Lp(a) with factor V:Q506 mutation or deficiencies of protein C or antithrombin further increased the risk for thromboembolic events to 8.4.

Conclusions—Lp(a) >30 mg/dL is a risk factor for venous thromboembolism in childhood. Lp(a) measurements should be included in the screening of causal factors in children with venous thromboembolic events. (Circulation. 1999;100:743-748.)

Key Words: lipoproteins ■ thrombus ■ embolism ■ pediatrics ■ genetics

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, deficiencies of protein C and protein S as well as the factor V mutation FV:Q506, which underlies the resistance of activated protein C. Prothrombotic states have also been associated with defects in the genes of antithrombin, plasminogen, and fibrinogen.1–3 Finally, the previously described 20210GA variant of the prothrombin gene also seems to be a common but probably mild risk factor for arterial and venous thromboembolism.4

Many prospective and case-control studies identified elevated levels of lipoprotein(a) [Lp(a)] as a risk factor for premature myocardial infarction and stroke.5–13 Lp(a) resembles LDL in its high cholesterol content and in the presence of 1 molecule of apolipoprotein (apo) B. The additional protein in Lp(a) is called apo(a) and contains a protease domain, a kringle V–like domain, and a variable number of kringle IV repeats, all of which have strong structural homologies to plasminogen.14,15 The genetically determined variation in the number of kringle IV repeats leads to the synthesis of apo(a) isoforms whose molecular weight varies between 200 and 800 kDa and is inversely correlated to Lp(a) plasma levels.14,16 The size polymorphism and additional genetic variation of the apo(a) gene largely control Lp(a) levels so that Lp(a) concentrations have intraindividual and interindividual variability, with serum levels ranging between 0 and 300 mg/dL.14,16

Both in vitro and in vivo, Lp(a) has antifibrinolytic properties15,17–19 that have in part been made responsible for its association with cardiovascular disease. In this respect, it is surprising that only few data are available on the role of Lp(a) as a risk factor for venous thrombosis and thromboembolic stroke of venous origin. Some small-scale studies and
single case reports have indicated that elevated Lp(a) levels may further increase the risk of thrombotic events in patients suffering from an acquired prothrombotic state due to rheumatic diseases, polycythemia vera, or nephrotic syndrome. In 35 children who were consecutively referred to us because of venous thrombosis, we previously observed an increased prevalence of Lp(a) >50 mg/dL. To investigate whether elevated Lp(a) levels are associated with the occurrence of thromboembolic events in childhood, we have now conducted a controlled multicenter case-control study. The frequency distribution of apo(a) isoforms was also analyzed to rule out the possibility that differences in Lp(a) levels among case and control subjects are secondary to the thromboembolic events or their treatment. Finally, we compared the relative risks associated with elevated Lp(a) or established thrombophilic risk factors and determined their cooperative effects on the occurrence of thromboembolic events.

Methods

Study Population

The present multicenter case-control study was performed in accordance with the ethical standards laid down in the relevant version of the 1964 Declaration of Helsinki and approved by the medical ethics committee at the Westfälische Wilhelms-University, Münster, Germany.

Inclusion in this study was subject to the following criteria: (1) thrombotic events had to occur before the age of 18 years; (2) thromboembolism had to be confirmed objectively by standard imaging methods, ie, duplex sonography, venography, CT and MR imaging for the diagnosis of venous thromboembolism, and cerebral CT scanning, MR imaging, MR angiography, or transcranial Doppler ultrasonography for the diagnosis of thromboembolic ischemic stroke; and (3) to prevent confounding effects of acute reactive processes or oral anticoagulation, ≥3 months had to pass between the last thrombotic episode and blood sample collection for the quantitative assays described below. Patients with abnormal quantitative laboratory test results were followed up for a minimum of 6 weeks after the first examination to obtain ≥1 further blood sample for reanalysis.

Since 1996, the above criteria were fulfilled by 186 newly admitted patients 0 to 18 years old (median age at first thrombotic event 5.5 years) from different geographic areas of Northwest Germany. The thrombotic manifestations included isolated deep vein thrombosis (n=42), central nervous thrombosis (n=32), renal venous thrombosis (n=10), axillary vein thrombosis (n=8), subclavian vein thrombosis (n=9), superior caval vein thrombosis (n=11), inferior caval vein thrombosis (n=12), portal vein thrombosis (n=12), hepatic vein thrombosis (n=1), veno-occlusive disease (n=5), deep vein thrombosis with pulmonary embolism (n=2), pulmonary embolism (n=10), retinal venous thrombosis (n=2), and thromboembolic stroke of venous origin (patient foramen ovale: n=28). With informed parental consent, samples from 186 age- and disease-matched children from the same geographic areas were investigated as controls. Underlying diseases of patients and control subjects are shown in Table 1.

Laboratory Methods

Blood Sampling

With informed parental consent, blood samples were collected by peripheral venipuncture into 3.8% trisodium citrate (1 part anticoagulant, 9 parts blood; Sarstedt tubes) and placed immediately on melting ice. Platelet-poor plasma was prepared by centrifugation at 3000g for 20 minutes at 4°C, divided into aliquots in polystyrene tubes, stored at −70°C, and thawed immediately before the assay. For gene analysis, we obtained venous blood in EDTA-treated tubes, stored at −70°C, and thawed immediately before the assay.

Measurement of Lipids, Lipoproteins, and Lp(a)

Total cholesterol, triglycerides, and HDL cholesterol were measured with enzymatic assays and (for HDL cholesterol) a precipitation method from Boehringer Mannheim on a Hitachi autoanalyzer. LDL cholesterol was calculated with the Friedewald formula. Lp(a) plasma concentrations were measured with an ELISA technique.

Table 1. Underlying Diseases in Patients (n=186) and Control Subjects (n=186)

<table>
<thead>
<tr>
<th>Disease</th>
<th>Patients</th>
<th>Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>5 (0–18)</td>
<td>5.5 (0–18)</td>
</tr>
<tr>
<td>Male:female ratio</td>
<td>1:1.1</td>
<td>1:1.1</td>
</tr>
<tr>
<td>No. of underlying diseases</td>
<td>74</td>
<td>77</td>
</tr>
<tr>
<td>Central lines†</td>
<td>28</td>
<td>25</td>
</tr>
<tr>
<td>Severe bacterial/viral infection†</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>Staphylococci</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Pneumococci</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Varicella-zoster virus</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Malignancy†</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>Acute leukemia</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Non-Hodgkins lymphoma</td>
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<td>2</td>
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<tr>
<td>Hodgkins lymphoma</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Neuroblastoma</td>
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<td>2</td>
</tr>
<tr>
<td>Nephroblastoma</td>
<td>3</td>
<td>3</td>
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<tr>
<td>Rheumatic diseases‡</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Cardiac diseases‡†</td>
<td>28</td>
<td>30</td>
</tr>
<tr>
<td>TGA</td>
<td>4</td>
<td>4</td>
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<tr>
<td>TrA</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>HLHS</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>TAC</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>TOF</td>
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<td>1</td>
</tr>
<tr>
<td>VSD</td>
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<td>8</td>
</tr>
<tr>
<td>ASD</td>
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<td>4</td>
</tr>
<tr>
<td>DCM</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Ebstein anomaly</td>
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<td>1</td>
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<tr>
<td>Peripartal asphyxiast†</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Acute dehydration†</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

TGA indicates transposition of the great arteries; TrA, tricuspid atresia; HLHS, hypoplastic left heart syndrome; TAC, truncus arteriosus; TOF, tetralogy of Fallot; VSD, ventricular septal defect; ASD, atrial septal defect; and DCM, dilated cardiomyopathy.

*Median and range values.
†Absolute numbers of patients or control subjects.
‡Hemi-Fontan correction was performed in 2 patients and 3 control subjects; operation according to Fontan in a further 2 patients and 2 children of the control group.
using mouse monoclonal anti-apo(a) capture antibodies and sheep polyclonal anti-apoB detection antibodies [COALIZA Lp(a), Chromogenix]. Standards and controls were purchased from Immuno. Intra-assay/interassay coefficients of variation are 4.5%/3.6% at 6 mg/dL and 7.0%/4.2% at 40 mg/dL. The lower detection limit of this assay was 0.001 mg/dL.

**Determination of Apo(a) Size Polymorphism**

In a randomly selected subgroup of 51 patients and 46 control subjects with detectable Lp(a) levels on immunoassay, we determined the apo(a) size polymorphism as a phenotype by agarose gel electrophoresis of plasma and subsequent anti-apo(a) immunoblotting, principally as described by Marcovina et al. After electrophoresis and electrotransfer onto nitrocellulose membranes, apo(a) isoforms were immunodetected by successive incubation of the nitrocellulose membranes with a polyclonal rabbit anti-apo(a) antiserum (Behringwerke), biotinylated donkey antibodies against rabbit IgG (Amersham), streptavidin-conjugated horseradish peroxidase (Amersham), and a chemiluminescence blotting substrate (Boehringer Mannheim). All incubations were performed as recommended by the supplier of the chemiluminescence blotting substrate (Boehringer Mannheim). The chemiluminescent immunoreaction was visualized by photoimaging with the BAS1500 (Fuji, Photo Film). The number of kringle IV repeats of the apo(a) isoforms was estimated by their relative electrophoretic mobility in comparison with standard apo(a) isoforms, which were purchased from Immuno, and by means of the TINA program (Raytest), which supports the BAS 1500 photoimager. In most cases, only 1 of 2 possible isoforms was immunodetectable, either because of true homozygosity or because of the presence of undetectable null alleles, which are the most frequent ones in white populations. In a few cases in which 2 isoforms were immunodetectable, we considered only the smaller one for statistical evaluation.

**Assays of Hemostatic Factors**

The FV:Q506 mutation, amidolytic protein C activity, and antithrombin activity in both patients, chromogenic substrates from Chromogenix (Asserachrom, Stago), and anticardiolipin IgM and IgG antibodies (ELISA, Chromogenix) were measured as described previously. For all quantitative plasma-based assays, a prothrombotic defect was diagnosed only when the plasma level of a protein was outside the limits of its normal range in at least 2 independent samples. A heterozygous type I deficiency state was diagnosed when functional plasma activity and immunological antigen concentration of a protein were <50% of normal of the lower age-related limit. A homozygous state was defined by activity levels and antigen concentrations <10% of normal.

**Statistical Analysis**

An exploratory analysis was performed using the Statistical Package for the Social Sciences (SPSS-X). Mean values and SDs were compared by Student’s t test. Because of their non-Gaussian frequency distributions, data on Lp(a) and triglycerides are presented as frequency distributions, data on Lp(a) and triglycerides are presented as means and were evaluated statistically after logarithmic transformation. Correlations between apo(a) size and Lp(a) levels were calculated by the Spearman rank test. P values <0.05 were considered significant. Multivariate logistic procedure (logistic regression: maximum likelihood ratio) was applied to identify independent risk factors for venous thrombosis. Variables tested for inclusion in the model were Lp(a), FV:Q506, protein C, protein S, and antithrombin. The level of significance for differences in prevalences was calculated by the Wald χ² test.

**Results**

**Lipids, Lipoproteins, and Lp(a)**

Children (n=186) who had suffered venous thrombosis differed significantly from 186 age- and disease-matched control subjects by a higher median/range of Lp(a) (19/0 to 170 mg/dL versus 4.4/0 to 125 mg/dL) but not by plasma levels of cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol. As known from many studies in white populations, the frequency distribution of Lp(a) levels was skewed to lower concentrations in both patients and control subjects (Figure, panel A). However, very high levels of Lp(a) were much more frequent in patients than in control subjects: After stratification for quartiles of Lp(a), the risk for thromboembolic events did not differ within the 3 lowest quartiles, in which Lp(a) levels were ≤30 mg/dL, but was increased by a factor of 7.7 in the highest quartile compared with the lowest quartile as reference group (Table 2). Lp(a) levels >30 mg/dL, which is also the commonly accepted cardiovascular risk, occurred with a prevalence of 42.0% among patients compared with a prevalence of only 10.3% among control subjects (odds ratio 7.2, with a 95% CI of 3.7 to 14.5) (Table 3).

**Subgroup Analysis of Lp(a)**

Results of a subgroup analysis of Lp(a) in 74 patients without underlying diseases and 74 healthy matched control subjects did not differ significantly from results obtained from the entire study population: Children with spontaneous thrombosis differed significantly from 74 age-and sex-matched healthy control subjects by a higher median/range of Lp(a) (17/0 to 145 mg/dL versus 4/0 to 80 mg/dL; P=0.001). The odds ratio/95% CI of Lp(a) >30 mg/dL was 7.1/2.7 to 18.6.

**Apo(a) Size Polymorphism**

Lp(a) levels are determined largely by variation of the apo(a) gene and are little influenced by environmental factors. Nevertheless, to rule out the possibility of high Lp(a) levels in patients being secondary to the thromboembolic events or treatment modalities, we determined the apo(a) size poly-
TABLE 2. Odds Ratio of Thromboembolic Diseases in Children According to Quartiles of Lp(a) Levels and Quartiles of Estimated Numbers of Apo(a) Kringle IV Repeats

<table>
<thead>
<tr>
<th>Quartile</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lp(a), mg/dL</td>
<td>0–2</td>
<td>3–7</td>
<td>7–30</td>
<td>&gt;30</td>
</tr>
<tr>
<td>Odds ratios according to quartiles of Lp(a) (95% CI)</td>
<td>1</td>
<td>0.78</td>
<td>1.07</td>
<td>7.68*</td>
</tr>
<tr>
<td>Odds ratios according to quartiles of apo(a) isoforms (95% CI)</td>
<td>8.21*</td>
<td>2.69</td>
<td>1.30</td>
<td>1</td>
</tr>
</tbody>
</table>

Quartiles of Lp(a) levels were defined in 186 children with thromboembolic events (patients) and 186 age- and disease-matched control subjects; quartiles of kringle IV repeats were defined in a randomly selected subgroup of 51 patients and 46 control subjects. Odds ratios presented here indicate the risk multiplicator associated with Lp(a) levels of the lowest quartile as reference group and the number of kringle IV repeats of the highest quartile, respectively.

*P<0.001 (χ² test).

morphism in the randomly selected subgroup of 51 patients and 46 control subjects with immunodetectable Lp(a) levels. As expected, the estimated number of kringle IV repeats was negatively correlated with Lp(a) levels in both patients (r = −0.775; P < 0.001) and control subjects (r = −0.360; P = 0.0169). Small isoforms were more frequently present in the plasma of patients with thromboembolic events than in that of control subjects (Figure, panel B). Compared with the highest quartile as reference group of the number of kringle IV repeats, the lowest quartile was associated with an 8.2-fold increased risk for thromboembolic events (Table 2).

Established Prothrombotic Risk Factors
Lp(a) >30 mg/dL, the FV:Q506 mutation, protein C, protein S, and antithrombin were analyzed by a multivariate logistic procedure to determine their independent contributions to the risk of venous thrombosis. In addition to Lp(a), the FV:Q506 mutation, protein C, and antithrombin deficiency were found to be independent risk factors of childhood venous vascular occlusion (Table 3). In contrast, protein S deficiency was not different between patients and control subjects. None of the patients or control subjects had elevated titers of IgM or IgG anticardiolipin antibodies.

Increased Lp(a) in Combination With Established Prothrombotic Risk Factors
Based on results obtained from multivariate analysis that Lp(a) >30 mg/dL, the heterozygous FV:Q506 mutation, protein C, and antithrombin deficiency were independent risk factors for venous thrombosis in childhood, an intensification of risks in the presence of >1 factor was observed: In 1.6% of the control subjects but in 12.2% of the patients, Lp(a) levels >30 mg/dL were coincident with ≥1 of the above-mentioned genetic hemostatic disorders (OR/CI, 8.4/2.5 to 28.5). Increased Lp(a) was most frequently combined with the heterozygous FV:Q506 mutation, which was found in 1.6% of control subjects compared with 9.6% of patients.

Discussion
In the multicenter study presented here, elevated Lp(a) serum concentrations, clearly correlated with the presence of small apo(a) isoforms, were identified as risk factors for the occurrence of venous thromboembolic events in childhood. Lp(a) levels located in the upper quartile of this white population exceeded 30 mg/dL and increased the risk of a thromboembolic event in childhood by factor of 7.2. This odds ratio is higher than that found in children heterozygous for the FV:Q506 mutation, which in the white population has been considered the most important single risk factor for thrombotic events and which was shown to have a prevalence similar to that of elevated Lp(a).2

In vitro, Lp(a) inhibits the activation of plasminogen by streptokinase and tissue plasminogen activator (tPA) and competes with plasminogen for binding to fibrin as well as for binding to annexin II, the plasminogen/tPA receptor on endothelial cells and platelets.15,17–19,31–34 Because of these properties and the great structural homology between Lp(a) and plasminogen, it has been hypothesized that Lp(a) inhibits fibrinolysis. In fact, apo(a) transgenic animals were resistant to tPA-dependent lysis of artificially induced fibrin thrombi.18 These antifibrinolytic properties of Lp(a) have been made responsible in part for the association of elevated Lp(a) and risk for atherosclerotic vessel diseases.5–16,17–19 Surprisingly, much less attention has been paid to the role of elevated Lp(a) as a risk factor for venous thromboembolic diseases. Some small-scale studies and single case reports have indicated that elevated Lp(a) levels may further increase the risk of thrombotic events in patients suffering from an acquired prothrombotic state due to rheumatic diseases, polycythemia vera, or nephrotic syndrome.20–25 Studies in unselected adult patients
with venous thrombosis or embolism as well as in women practicing hormonal contraception revealed no association between Lp(a) and thromboembolic diseases.\textsuperscript{35,36}

With an annual incidence of 0.07/10 000, thromboembolic disease in childhood is rare and probably develops through concomitant hemodynamic disturbances in the cardiovascular system and hereditary disturbances of the hemostatic system.\textsuperscript{37,38} In this regard, children with thromboembolic events represent a high-risk population similar to patients with rheumatic diseases, nephrotic syndrome, or polycythemia vera. Interestingly, elevated Lp(a) has previously evolved as a prothrombotic risk factor in these selected high-risk cohorts\textsuperscript{20–25} but not in unselected cohorts with sporadic thromboembolic events.\textsuperscript{39} We therefore hypothesize that Lp(a) acts as an additional important risk factor for the precipitation of venous thromboembolic events in the white population studied here. Furthermore, on the basis of results obtained from multivariate analysis that Lp(a) > 30 mg/dL, the heterozygous FV:Q\textsuperscript{506} mutation, protein C, and antithrombin deficiency were independent risk factors of venous thrombosis in childhood, an intensification of risks in the presence of > 1 factor was observed. Although the findings presented here in a white childhood thrombosis population are not generalizable to other racial and ethnic groups, in particular Americans of African origin, measurement of Lp(a) should be included in screening programs performed in young patients suffering from venous thromboembolism, in addition to FV:Q\textsuperscript{506}, protein C, protein S, and antithrombin. Currently, no specific Lp(a)-lowering therapy is available. However, elevated Lp(a) levels may be an indication for the initiation of anticoagulant therapy, just as other prothrombotic factors currently serve as an indication for this treatment. Further studies are necessary to determine whether anticoagulant treatment reduces the risk of future events in these high-risk patients.

Appendix
Coinvestigators were as follows (alphabetical order within different institutions): A. Heinecke (Institute of Medical Statistics, University Hospital Münster, Germany); B. Ziegler (University Children’s Hospital Munich, Germany); K. Auberger (University Children’s Hospital Munich, Germany); A. Claviez, R. Schneppenheim (University Children’s Hospital Kiel, Germany); U. Göbel, C. Mauz-Körholz (University Children’s Hospital Düsseldorf, Germany); C. Wermes (University Children’s Hospital Hannover, Germany); and B. Ziegler (Scripps Research Institute, La Jolla, Calif).

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References


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