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Prothrombotic Risk Factors in Children with Spontaneous Venous Thrombosis and Their Asymptomatic Parents: A Family Study

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Abstract

The present study was designed to assess to what extent single and combined clotting abnormalities influence spontaneous vascular accidents in pediatric patients, and how the children affected differ in their prothrombotic risk profiles from their biological first-degree family members. In addition, this study was performed to investigate if relatively mild thrombophilic polymorphisms not leading to thrombosis in the parents cause severe clinical expression when coinherited with an established prothrombotic risk factor. The factor V (FV) G1691A mutation, the prothrombin (PT) G20210A variant, the methylenetetrahydrofolate reductase (MTHFR) T677T genotype, the plasminogen activator inhibitor (PAI)-1 promoter polymorphism, lipoprotein (Lp)(a), antithrombin, protein C, and protein S were investigated in 48 childhood patients aged neonate to <18 years (median 0.5 years) with spontaneous venous thromboembolism (SVT) compared with the carrier status of their first-degree family members. In 19 of the 48 patients (39.6%), one prothrombotic risk factor was diagnosed, and in 27 of the 48 subjects (56.3%) at least two prothrombotic defects/alleles. In the majority of cases with SVT, the FV G1691A mutation was involved either with a second mutated allele or combined with elevated Lp(a), the 4G/4G genotype of the PAI -1 promoter polymorphism, and the T677T MTHFR genotype. The rate of combined prothrombotic risk factors was significantly higher in childhood patients compared with their parents ($p=0.0004$). In conclusion, based on the data presented here we suggest that early-onset SVT in childhood patients is mainly caused by combinations of at least two prothrombotic risk factors.
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Key Words: Spontaneous venous thrombosis; Prothrombotic risk profiles; Children; Parents

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Since the recent discovery of activated protein C resistance [1], in the majority of cases due to the factor (FV) G1691A gene mutation [2], evidence has been accumulating that venous thromboembolism is a multigenetic disorder [3]. Besides antithrombin deficiency and deficiencies within the protein C pathway, the 20210A allele within the 3′-untranslated region of the prothrombin (PT) gene is discussed as a common but mild risk factor of venous thrombosis in children and adults [4,5]. Finally, the C677T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene facilitates the manifestation of hyperhomocysteinemia especially in individuals with low folate intake. Its involvement in arterial vascular disease as well as venous thromboembolism has been discussed [6,7]. The latter is still a matter of controverse discussion [8]. Combinations between the established prothrombotic risk factors mentioned greatly increase the risk of thrombosis in adults [7,9–13].

Pediatric venous thromboembolism is being increasingly viewed as a multifactorial disorder as well [14]; however, information on the role of combined hemostatic defects in childhood thrombosis is limited. Very recently we have shown elevated lipoprotein (Lp)(a), the heterozygous state for the FV G1691A mutation and the prothrombin G20210A mutation, deficiencies of protein C and antithrombin to be risk factors of childhood thromboembolism [5,14–17]. A decreased fibrinolysis due to enhanced levels of the plasminogen activator inhibitor (PAI)-1 concomitant with low activity of tissue-type plasminogen activator has moreover been shown in childhood carriers of the FV G1691A gene mutation who suffered a thrombotic event [18]. In addition, evidence shows that elevated PAI-1 levels are associated with the 4G/4G genotype of the recently described deletion/insertion 4G/5G polymorphism in the PAI-1 gene [19–21].

The present multicenter study has been undertaken to unravel the hypothesis that genetic prothrombotic risk profiles in children with early onset spontaneous venous thromboembolism (SVT) differ from the carrier status of their first-degree family members, especially if relatively mild thrombophilic polymorphisms not leading to thrombosis in the parents cause a more severe clinical expression when coinherited with an established prothrombotic risk factor. This question not only raises a scientific issue. It is also of interest with respect to ethical backgrounds and to what extent a screening of genetic factors is justified in symptomatic patients during childhood and adolescence not associated with a positive family history of a disease. Of particular interest is whether this screening should be performed also in siblings not suffering from a symptomatic disease to date.

1. Patients, Materials, and Methods

1.1. Ethics

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

1.2. Patients and First-degree Family Members

Inclusion criteria: Infants and children aged neonate to <18 years with first onset SVT confirmed by standard imaging methods (venography, compression sonography, computed tomography, magnetic resonance imaging), and whose biological parents were both available for a complete diagnostic work-up including prothrombotic risk factors, were defined as study patients. From September 1995–September 1999, 48 consecutive Caucasian patients (median age at first thrombotic onset 0.5 years, range neonate to <18 years, male/female ratio 1:1.1) with clinically and imaging-confirmed SVT were prospectively recruited from different geographic catchment areas of Germany [5,16].

Exclusion criteria: Childhood patients with potential triggering-acquired risk factors (i.e., malignancies, central venous lines, immobilization, surgery, trauma, plaster casts, rheumatic diseases, severe infections, and use of oral contraceptives) were not enrolled in the present study.

At acute onset of SVT the majority of patients presented with isolated vascular occlusion of the inferior caval vein (n=9), combined femoral and iliac vein thrombosis (n=8), cerebral vein throm-

bosis (n=8), isolated calf thrombosis (n=5), renal venous thrombosis (n=5), femoral vein thrombosis concomitant with pulmonary embolism (n=4), intracardial thrombus formation (n=4), femoral vein thrombosis (n=3), and vascular thrombosis of the portal vein (n=2), respectively.

Ninety-six biological parents with a median (range) age of 32 years (23–51 years) were investigated with their informed consent. No thromboembolism has occurred so far in 70 of the 72 first-degree family members with at least one prothrombotic gene mutation or thrombophilic polymorphism. However, in a 32-year-old woman with elevated Lp(a) and protein C deficiency type I, first venous thrombosis occurred during pregnancy. Deep venous thrombosis with pulmonary embolism was noted in a 23-year-old male with antithrombin deficiency type I concomitant with immobilization and severe nicotine abuse.

1.3. Blood Samples

With informed parental consent, blood samples were collected 6 weeks—3 months after the acute SVT by peripheral venipuncture into plastic tubes containing 1/10 by volume of 3.8% trisodium citrate or into plastic tubes without additives, respectively (Sarstedt, Nümbrecht, Germany). Citrated blood was placed immediately on melting ice. Platelet poor plasma and serum were prepared by centrifugation at 3000 g for 20 minutes at 4°C, or at room temperature, respectively, aliquoted in polystyrene tubes, stored at –70°C, and thawed immediately before the assay procedure. For genetic analysis we obtained venous blood in EDTA-treated sample tubes (Sarstedt, Nümbrecht, Germany), from which cells were separated by centrifugation at 3000 g for 15 minutes. The buffy coat layer was then removed and stored at –70°C pending DNA extraction by a spin column procedure (Quiagen, Hilden, Germany).

1.4. Laboratory Analyses

The MTHFR C677T, the FV G1691A, and the prothrombin G20210A genotypes were determined by polymerase chain reaction and analysis of restriction fragments as previously reported [2,4,6]. The PAI-1 4G/5G genotype was detected by allele-specific polymerase chain reaction [20].

Amidolytic protein C and antithrombin activities were measured on an ACL 300 analyzer (Instrumentation Laboratory, Munich, Germany) using chromogenic substrates (Chromogenix, Mølndal, Sweden). Free protein S antigen, total protein S, and protein C antigen were measured using commercially available ELISA assay kits (Stago, Asnières-sur-Seine, France). Partigen plates (radial immunodiffusion) used to determine antithrombin concentrations were purchased from Behring Diagnostics, Marburg, Germany. In addition, crossed immunoelectrophoresis (Behring Diagnostics, Marburg, Germany; Dako, Denmark) was performed in patients with antithrombin deficiency. Lp(a) was determined with the COALIZA Lp(a) assay kit (Chromogenix, Mølndal, Sweden). The laboratory staff was blind to whether the blood samples originated from a patient or from a first-degree family member.

A heterozygous type I deficiency state (protein C, antithrombin) was diagnosed when functional plasma activity and immunological antigen concentration of a protein were below 50% of normal of the lower age-related limit [14]. A homozygous state was defined if activity levels and antigen concentrations were less than 10% of normal. A type II deficiency was diagnosed with repeatedly low functional activity levels along with normal antigen concentrations. The diagnosis of protein S deficiency was based on reduced free protein S antigen levels combined with decreased or normal total protein S antigen concentrations, respectively. Criteria for the hereditary nature of a haemostatic defect were its presence in at least one further first- or second-degree family member and/or the identification of a causative gene mutation [4,14,16].

1.5. Statistics

Prevalences of prothrombotic risk factors in childhood patients and first-degree relatives were compared by the chi-square test. The significance level was set at 0.05. All statistical analyses were performed using the MedCalc software package (MedCalc, Mariakerke, Belgium).

2. Results

Forty-six of the 48 symptomatic childhood patients were carriers of prothrombotic risk factors, whereas

Table 1. Single prothrombotic risk factors in childhood patients and their first-degree family members. In all cases, protein deficiencies were type I

Prothrombotic risk factor	Patients $n=48$	Father $n=48$	Mother $n=48$	Both Parents $n=96$
Factor V G1691A	3 (6.3%)	5 (10.4%)	6 (12.5%)	11 (11.5%)
Prothrombin G20210A	2 (4.2%)	2 (4.2%)	1 (2.1%)	3 (3.1%)
PAI-1 4G/4G	3 (6.3%)	8 (16.7%)	4 (8.3%)	12 (12.5%)
MTHFR T677T	1 (2.1%)	5 (10.4%)	2 (4.2%)	7 (7.3%)
Lipoprotein (a)>30 mg/dl	10 (20.8%)	7 (14.6%)	11 (23%)	18 (18.8%)
Protein C deficiency	—	1 (2.1%)	1 (2.1%)	2 (2.1%)
Protein S deficiency	—	—	—	—
Antithrombin deficiency	—	1 (2.1%)	1 (2.1%)	2 (2.1%)
Total	19 (39.6%)	29 (60.4%)	26 (54.2%)	55 (57.3%)

no thrombophilia could be detected in two of them so far. In 19 of the 48 childhood patients (39.6%), one single risk factor was found compared with 55 subjects of the 96 first-degree family members (57.3%). Single prothrombotic risk factors in childhood patients and their first-degree family members are shown in Table 1.

Twenty-seven of the 48 patients (56.3%) had two or more genetic risk factors compared with 17 double defects found in the 96 first-degree family members (17.7%). More than two defective alleles (i.e., the homozygous FV A1691A genotype combined with the PT G20210A variant, elevated Lp(a)> 30 mg/dl along with the PT G20210A variant and the MTHFR T677T genotype, and the homozygous PT A20210A genotype with the MTHFR T677T variant, elevated Lp(a) and protein S type I deficiency) were found in 3 further symptomatic subjects. The overall rate of two or more prothrombotic risk factors/polymorphisms (Table 2) was significantly higher in symptomatic childhood patients compared with their parents ($p=0.0004$).

3. Discussion

Results of the present family study show that symptomatic children with spontaneous venous thromboembolism have a significantly higher rate of combined genetic prothrombotic risk factors than their first-degree family members. Established prothrombotic risk factors such as the factor V gene G1691A mutation, the prothrombin G20210A variant, deficiency states of protein C, protein S or antithrombin, and increased levels of Lp(a) were found along with further genetic polymorphisms (i.e., the homozygous MTHFR 677TT variant) and the PAI-1 4G/4G genotype. We wish to point out that 13 of the 27 combined risk profiles (48%) were due to different combinations within established defects of the protein C pathway, the PT gene variant, antithrombin deficiency, or elevated Lp(a). The latter recently was shown to be an inherited and independent risk factor of childhood venous thrombosis [16,22]. In contrast, only 6 out of 17 asymptomatic first-degree family members (35.3%) had a prothrombotic risk profile of two defects within the protein C pathway, the PT gene mutation and elevated Lp(a). Only two healthy adults showed a profile similar to the affected children. However, no first-degree family member had more than two combined thrombophilias. A smaller proportion of children with SVT, 39.6%, was suffering from only one prothrombotic risk factor. However, no isolated deficiencies of protein C, protein S, or antithrombin were diagnosed in the symptomatic childhood population studied.

Based on various trials, evidence is accumulating that familial thrombophilia, defined as a genetically determined tendency to thrombosis, may be due to a combination of clotting defects [3,7,9–14]. In particular, evidence shows that inheritance of FV G1691A combined with deficiencies of protein C [10], protein S, and antithrombin [9–11], as well as with the PT G20210A variant [13], the MTHFR T677T genotype (in some studies [7,12]), the PAI-1 4G/4G variant [19], or enhanced Lp(a) concentrations [15,16] further increases the manifestation

of early vascular accidents in children and young adults. We suggest from the data presented here that the combination of genetic polymorphisms which are not necessarily independent risk factors for venous thrombosis (such as the MTHFR T577T genotype or the homozygous PAI-1 4G/4G polymorphism) and established prothrombotic risk factors further increase the risk of symptomatic vascular accidents in the subjects affected [7,19]. This is similar to childhood patients suffering at least one established thrombophilia along with acquired risk factors (i.e., central venous lines or chemotherapy in childhood leukemia) [5,16,23,24].

Children not only differ from adults anatomically and physiologically, they also differ in the types of diseases from which they suffer and the manifestation of diseases they have in common with adults. Therefore, it is not only a scientific question but also an ethical issue of whether a comprehensive screening of genetic factors is justified in symptomatic childhood patients with a negative family history of a disease, and particularly if this screening is also recommended in non-symptomatic siblings. Data presented here along with results from recently published controlled studies in childhood thrombophilia give evidence [16,17,19] that the risk of a symptomatic thrombotic event during childhood is enhanced in these patients carrying at least one prothrombotic risk factor. Based on the Mendelian theory of inheritance, siblings of a symptomatic childhood patient affected with a combined prothrombotic defect carry in approximately 50% of cases a single thrombophilia and two or more gene mutations or polymorphisms in 25% of cases, respectively.

We clearly wish to point out that it is not justified from the data presented here to recommend long-term anticoagulant therapy in asymptomatic family members carrying combined prothrombotic risk factors. However, a short-term primary anticoagulant prophylaxis in situations known to increase the rate of symptomatic vascular accidents in children (i.e., immobilization, cancer, central venous lines, congenital heart disease, or rheumatic diseases) [5,16,23,24] should be discussed on an individual basis in offsprings of symptomatic childhood patients, especially when the prothrombotic risk profile is similar to the affected propositus.

Table 2. Combined or homozygous prothrombotic risk factors in childhood patients and their first-degree family members. In all cases, protein deficiencies were type I.

Prothrombotic risk factors	**Patients** *n*=48	**Father** *n*=48	**Mother** *n*=48	**Both Parents** *n*=96
Factor V G1691A and:				
Lipoprotein (a)>30 mg/dl	6 (12.5%)	—	2 (4.2%)	2 (2.1%)
PAI-1 4G/4G	3 (6.3%)	4 (8.3%)	2 (4.2%)	6 (6.3%)
MTHFR T677T	2 (4.2%)	—	—	—
Prothrombin G20210A	—	—	1 (2.1%)	1 (1%)
Factor V A1691A (homozygous) and:				
no further risk factor	2 (4.2%)	—	—	—
Prothrombin G20210A	1 (2.1%)	—	—	—
Lipoprotein (a)>30 mg/dl and:				
PAI-1 4G/4G	6 (12.5%)	1 (2.1%)	2 (4.2%)	3 (3.1%)
MTHFR T677T	2 (4.2%)	—	1 (2.1%)	1 (1%)
Antithrombin deficiency	1 (2.1%)	—	—	—
Protein C deficiency	1 (2.1%)	—	—	—
Prothrombin G20210A	—	—	2 (4.2%)	2 (2.1%)
Protein S deficiency	—	1 (2.1%)	—	1 (1%)
Prothrombin G20210A & MTHFR T677T	1 (2.1%)	—	—	—
Prothrombin A20210A & MTHFR T677T & Protein S deficiency	1 (2.1%)	—	—	—
PAI-1 4G/4G and:				
Prothrombin G20210A	—	—	1 (2.1%)	1 (1%)
MTHFR T677T	1 (2.1%)	—	—	—
Total	27 (56.3%)	6 (12.5%)	11 (23.1%)	17 (17.6%)

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References

1. Dahlbäck B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C. Proc Natl Acad Sci USA 1993;90:1004–8.
2. Bertina RM, Koeleman BPC, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velde PA, Reitsma PH. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994;369:64–7.
3. Seligsohn U, Zivelin A. Thrombophilia as a multigenic disorder. Thromb Haemost 1997; 78:297–301.
4. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3′-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 1996;88:3698–03.
5. Junker R, Koch HG, Auberger K, Münchow N, Ehrenforth S, Nowak-Göttl U. Prothrombin G20210A gene mutation and further prothrombotic risk factors in childhood thrombophilia. Arterioscler Thromb Vasc Biol 1999;19:2568–72.
6. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJH, den Heijer M, Kluitmans LAJ, van den Heuvel LP, Rozen R. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nature Genetics 1995;10:111–3.
7. Salomon O, Steinberg DM, Zivelin A, Gitel S, Dardik R, Rosenberg N, Berliner S, Inbal A, Many A, Lubetsky A, Varon D, Martinowitz U, Seligsohn U. Single and combined prothrombotic factors in patients with idiopathic venous thromboembolism—Prevalence and risk assessment. Arteriol Thromb Vasc Biol 1999;19:511–8.
8. Alenc-Gelas, M, Arnaud E, Nicaud V, Aubry ML, Fiessinger JN, Aiach M, Emmrich J. Venous thromboembolic disease and the prothrombin, methylene tetrahydrofolate reductase and factor V genes. Thromb Haemost 1999;81:506–10.
9. Boven van HA, Reitsma PH, Rosendaal FR, Baiston TA, Chowdhury V, Bauer KA, Scharrer I, Conard J, Lane DA. Factor V Leiden (FVR506Q) in families with inherited antithrombin deficiency. Thromb Haemost 1996; 75:417–21.
10. Koelemann BPC, Reitsma PH, Allaart CF, Bertina RM. Activated protein C resistance as an additional risk factor for thrombosis in protein C-deficient families. Blood 1994;84: 1031–5.
11. Zöller B, Berntsdotter A, Frutos de PG, Dahlbäck B: Resistance to activated protein C as an additional genetic risk factor in hereditary deficiency of protein S. Blood 1995;85:3518–23.
12. Cattaneo M, Tsai MY, Bucciarelli P, Taioli E, Zighetti ML, Bignell M, Mannucci PM. A common mutation in the methylenetetrahydrofolate reductase gene (C677T) increases the risk for deep-vein thrombosis in patients with mutant factor V (Factor V:Q506). Arterioscler Thromb Vasc Biol 1997;17:1662–6.
13. Ehrenforth S, von Depka Prondsinski M, Aygören-Pürsün E, Nowak-Göttl U, Scharrer I, Ganser A. Study of the prothrombin gene 20210 GA variant in FV:Q506 carriers in relationship to the presence or absence of juvenile venous thromboembolism. Arterioscler Thromb Vasc Biol 1999;19:276–80.
14. Ehrenforth S, Junker R, Koch HG, Kreuz W, Münchow N, Scharrer I, Nowak-Göttl U. Multicenter evaluation of combined prothrombotic defects associated with thrombophilia in childhood. Eur J Pediatr 1999;158:S97–S04.
15. Nowak-Göttl U, Debus O, Findeisen M, Kassenböhmer R, Koch HG, Pollmann H, Postler C, Weber P, Vielhaber H. Lipoprotein (a): its role in childhood thromboembolism. Pediatrics 1997;99(6). URL: http://www.pediatrics.org/cgi/content/full/99/6/e11.
16. Nowak-Göttl U, Junker R, Hartmeier M, Koch HG, Münchow N, Assmann G, von Eckardstein A. Increased lipoprotein (a) is an important risk factor for venous thrombosis in childhood. Circulation 1999;100:743–8.
17. Nowak-Göttl U, Sträter R, Heinecke A, Junker R, Koch HG, Schuierer G, von Eckardstein

A. Lipoprotein (a) and genetic polymorphisms of clotting factor V, prothrombin, and methylenetetrahydrofolate reductase are risk factors of spontaneous ischemic stroke in childhood. Blood 1999;94:3678–82.
18. Nowak-Göttl U, Binder M, Dübbers A, Kehrel B, Koch HG, Veltmann H, Vielhaber H. Arg to Gln mutation in the factor V gene causes poor fibrinolytic response in children after venous occlusion. Thromb Haemost 1997;78: 1115–8.
19. Junker R, Bäumer R, Koch HG, Schettler C, Weber D, Nowak-Göttl U. Heterozygous FV R506Q mutation in childhood thrombosis: a possible link to the plasminogen activator inhibitor-1 (PAI-1) 4G/4G genotype and elevated lipoprotein (a) – preliminary results of a single center pilot study. Fibrinolysis & Proteolysis 1999;13:26–9.
20. Falk G, Almquist A, Nordenhem A, Svensson H, Wiman B. Allele specific PCR for detection of a sequence polymorphism in the promoter region of the plasminogen activator inhibitor-1 (PAI-1) gene. Fibrinolysis 1995;9:170–4.
21. Dawson SJ, Wiman B, Hamsten A, Green F, Humphries S, Henney AM. The two allele sequences of a common polymorphism in the promoter of the plasminogen activator inhibitor (PAI-1) gene respond differently to interleukin-1 in HepG2 cells. J Chem Biol 1993; 268:10739–45.
22. Boerwinkle E, Leffert CC, Lin J, Lackner C, Chiesa G, Hobbs HH. Apolipoprotein (a) gene accounts for greater than 90% of the variation in plasma lipoprotein (a) concentrations. J Clin Invest 1992;90:52–60.
23. Nowak-Göttl U, Dübbers A, Kececioglu D, Koch HG, Kotthoff S, Runde J, Vielhaber H. Factor V Leiden, protein C, and lipoprotein (a) in catheter-related thrombosis in childhood: a prospective study. J Pediatr 1997;131:608–12.
24. Nowak-Göttl U, Wermes C, Junker R, Koch HG, Schobess R, Fleischhack G, Schwabe D, Ehrenforth S. Prospective evaluation of the thrombotic risk in children with acute lymphoblastic leukemia carrying the MTHFR TT677 genotype, the prothrombin G20210A variant, and further prothrombotic risk factors. Blood 1999;93:1595–99.