# Prothrombin G20210A Gene Mutation and Further Prothrombotic Risk Factors in Childhood Thrombophilia

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***Abstract***—Risk factors for venous thrombosis in adults are the prothrombin G20210A and the factor V (FV) G1691A mutations and hereditary deficiencies of protein C, protein S and antithrombin. However, data are limited on the relevance of these risk factors for thrombosis in children and adolescents. We therefore investigated 261 patients aged 0 to 18 (median 5.7 years, 48% male) with venous thrombosis and controls (n=370) for the presence of prothrombotic risk factors including the prothrombin G20210A mutation. The following frequencies of hereditary risk factors (patients versus controls), odds ratios (OR) and 95% confidence intervals (CI), or results of Fisher's exact test, respectively, were found: prothrombin G20210A, 4.2% versus 1.1%, OR/CI 4.1/1.3 to 12.8; FV G1691A, 31.8% versus 4.1%, OR/CI 11.0/6.2 to 19.7; protein C deficiency, 9.2% versus 0.8%, OR/CI 12.4/3.7 to 41.6, protein S deficiency, 5.7% versus 0.8%, OR/CI 7.5/2.1 to 26.0; antithrombin deficiency in 3.4% in the patients, but not in the controls, $P=0.0003$. The prothrombin mutation was combined with the heterozygous FV G1691A mutation (2.3%) or protein C deficiency (0.3%) in the patients, but not in the controls (prothrombin and FV mutation, $P=0.0048$; prothrombin and protein C deficiency, not significant). The carrier frequencies and ORs of all hereditary risk factors showed a non-significant trend toward higher prevalences in patients suffering spontaneous thrombosis, compared with those with an additional underlying disease. In conclusion, the prothrombin G20210A and the FV G1691A mutation, deficiencies of protein C, protein S, and antithrombin are important risk factors for venous thrombosis during childhood and adolescence. **(*Arterioscler Thromb Vasc Biol*. 1999;19:2568-2572.)**

**Key Words:** thrombosis ■ hemostasis ■ pediatrics ■ factor V G1691A mutation ■ protein C ■ protein S ■ antithrombin

Disorders of the hemostatic system are major causes of thrombophilia in adults. Various genetic defects of proteins regulating blood coagulation and fibrinolysis predispose to thrombosis, for example, deep venous thrombosis, pulmonary embolism, and cerebrovascular disease.[1,2] The factor V (FV) G1691A mutation leads to a loss of a cleavage site of the protein and therefore to increased thrombin generation, whereas defects within the genetic coding for protein C, protein S, and antithrombin lead to a lower expression or a loss of function of the protein and therefore a hypercoagulable state.[3–10] Carriers of the FV G1691A mutation, or protein S, protein C, and antithrombin deficiency, are at an increased risk for thromboembolic events, especially in cases of a homozygous carrier state.[1–9] Moreover, the recently described G20210A mutation within the 3′-untranslated region of the prothrombin gene is a common but probably mild risk factor of venous thrombosis.[11–16] However, because of the low incidence of childhood vascular accidents, the role of such genetic defects in the childhood population is unclear. On the one hand, thrombosis in pediatric patients is described as a multifactorial disorder, frequently discussed as being due to nongenetic endogenous or exogenous trigger mechanisms (bacterial/viral infections, cancer and polychemotherapy, rheumatic diseases, cardiac malformations, immobilization, trauma, use of central lines). On the other hand, several cases have been published showing the relevance of risk factors within the hemostatic system for thromboembolic episodes during childhood and adolescence.[17–21]

In this report we present the results of a multicenter case-control study on pediatric patients with venous thrombosis with regard to the prothrombin G20210A mutation and further hereditary risk factors.

## Methods

### Study Design and Subjects

At the onset of this case-control study, the following criteria were defined for the inclusion of patients: age at first thrombotic onset up to 18 years; objective confirmation of thrombosis by standard

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**TABLE 1. Thrombotic Manifestations in Patients Investigated**

| Manifestation | n |
|---|---|
| Deep vein thrombosis | 118 |
| Cerebral venous thrombosis | 29 |
| Renal venous thrombosis | 36 |
| Superior caval vein thrombosis | 32 |
| Inferior caval vein thrombosis | 15 |
| Portal vein thrombosis | 9 |
| Mesenteric vein thrombosis | 2 |
| Multiple sites of venous thromboses | 8 |
| Isolated pulmonary embolism | 12 |

imaging methods; to prevent results from being affected by an acute reactive process or oral anticoagulation, the time period between the last thrombotic episode or end of oral anticoagulation therapy and blood sample collection for coagulation assays had to be at least 3 months; patients found to have an abnormal protein-based laboratory test result (protein C, protein S, antithrombin) were followed up with at least 1 additional blood sample 6 or more weeks after the first examination. Only those patients who had a second abnormal blood test were defined as having a protein deficiency, whereas those who had a normal result in the second test were defined as having no protein deficiency.

All symptomatic patients admitted to the participating study centers were included in the study. Hence, from 1996 onward, 261 patients (median age at thrombosis 5.7 years, range 0 to 18 years, male, n=124, female, n=137) were recruited. The thrombotic manifestations reported are shown in Table 1. Duplex sonography, venography, computed tomography, and magnetic resonance imaging were performed to diagnose venous thrombosis.

The control group consisted of nonthrombotic patients hospitalized for the same underlying diseases as the thrombosis patients (n=220). Moreover, 150 healthy control subjects were recruited. Control subjects were matched for sex and age and consisted of 154 male subjects and 216 female subjects (Table 2).

## Blood Sampling

Blood samples were obtained by peripheral venipuncture into plastic tubes containing one-tenth volume of 3.8% trisodium citrate (Sarstedt) and placed immediately on melting ice. Platelet-poor plasma was prepared by being centrifuged at 3000*g* for 20 minutes at 4°C, 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) from which cells were separated by centrifuging at 3000*g* for 15 minutes. The buffy coat layer was then removed and stored at −70°C, pending DNA extraction by standard techniques.

**TABLE 2. Underlying Diseases Found in Patients and Control Subjects**

| | Patients (n=261) | Control Subjects (n=370) |
|---|---|---|
| Age (median and range), y | 5.7 (0–18) | 6.0 (0–18) |
| Male/female | 124/137 | 154/216 |
| No underlying disease* | 100 | 150 |
| Bacterial/viral infection | 30 | 49 |
| Central line | 31 | 38 |
| Malignancy | 36 | 42 |
| Rheumatic diseases | 9 | 12 |
| Cardiac diseases | 30 | 44 |
| Trauma | 10 | 12 |
| Asphyxia | 11 | 19 |
| Dehydration | 4 | 4 |

*Patients with spontaneous thrombosis and healthy control subjects.

Informed parental consent were obtained from both patients and control subjects after they were informed in detail on the aims of the study. Patients' blood samples were obtained by venipuncture performed for routine diagnostics; therefore no additional venipuncture was necessary for study purposes.

## Laboratory Analysis

Genetic analysis (prothrombin G20210A mutation and FV G1691A) was performed with the use of methods already described.[12,17,22] Amidolytic protein C and antithrombin activities were measured on an ACL 300 analyzer (Instrumentation Laboratory) with the use of chromogenic substrates (Chromogenix). Free protein S antigen, total protein S, and protein C antigen were measured with the use of commercially available ELISA assay kits (Stago). Partigen plates (radial immunodiffusion) used to determine antithrombin concentrations were purchased from Behring Diagnostics. In addition, crossed immunoelectrophoresis (Behring Diagnostics and Dako) was performed in patients with antithrombin deficiency. Details of measurement were described earlier.[17]

A heterozygous type I deficiency state of protein C and antithrombin was diagnosed when functional plasma activity and immunologic antigen concentration of a protein were <50% of normal of the lower age-related limit.[22] A homozygous state was defined if activity levels and antigen concentrations were <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.

## Statistical Analysis

Prevalences of prothrombotic risk factors in patients and control subjects were compared by $\chi^2$ analysis or Fisher's exact test if necessary. The significance level was set at 0.05. In addition, odds ratios (OR) and 95% confidence intervals (CI) were calculated. All statistical analyses were performed with the use of the MedCalc software package).

The current study 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.

# Results

## Total Study Population

As shown in Table 3, a single inherited coagulation defect was found in 142 (54.4%) of the 261 patients and in 25 (6.8%) of the 370 control subjects ($\chi^2$ analysis, $P<0.0001$; OR 19.8, 95% CI 12.2 to 32.0). Combinations of the prothrombin mutation and a further genetic risk factor appeared in 7 (2.7%) patients but not in the control group (Fisher's exact test, $P=0.0020$). The highest risk of occurrence of a thrombotic event was found in protein C–deficient patients (patients vs control subjects, 9.2% vs 0.8%; Fisher's exact test, $P<0.0001$; OR 12.4, 95% CI 3.7 to 41.6), followed by homozygous or heterozygous carriers of the FV mutation (patients vs control subjects, 31.8% vs 4.1%; $\chi^2$ analysis, $P<0.0001$; OR 11.0, 95% CI 6.2 to 19.7). Carriers of the prothrombin G20210A mutation had the lowest risk (patients vs control subjects, 4.2% vs 1.1%; Fisher's exact test, $P<0.0152$; OR 4.1, 95% CI 1.3 to 12.8). No homozygous carrier of the prothrombin mutation or a protein deficiency was identified in the populations investigated.

**TABLE 3. Distribution of Hereditary Prothrombotic Risk Factors in Patients (n=261) and Control Subjects (n=370)**

| Risk Factors | Control Subjects | Patients | OR (95% CI) | *P* |
|---|---|---|---|---|
| Single | | | | |
| Prothrombin G20210A | 4 (1.1%) | 11 (4.2%) | 4.1 (1.3–12.8) | 0.0152 |
| FV G1691A (total) | 15 (4.1%) | 83 (31.8%) | 11.0 (6.2–19.7) | <0.0001* |
| Heterozygous | 14 (3.8%) | 77 (29.5%) | 10.6 (5.9–19.3) | <0.0001* |
| Homozygous | 1 (0.3%) | 6 (2.3%) | 8.7 (1.0–72.6) | 0.0220 |
| Protein C deficiency | 3 (0.8%) | 24 (9.2%) | 12.4 (3.7–41.6) | <0.0001 |
| Protein S deficiency | 3 (0.8%) | 15 (5.7%) | 7.5 (2.1–26.0) | 0.0003 |
| Antithrombin deficiency | 0 | 9 (3.4%) | ... | 0.0003 |
| Combinations | | | | |
| Prothrombin G20210A and protein C deficiency | 0 | 1 (0.4%) | ... | NS |
| Prothrombin G20210A and FV G1691A | 0 | 6 (2.3%) | ... | 0.0048 |
| Total | 25 (6.8%) | 149 (57.1%) | 18.4 (11.4–29.5) | <0.0001* |

Numbers of subjects are shown with percentage frequencies in parentheses.
*$\chi^2$ analysis. Other values by Fisher's exact test.

## Subgroup Analysis

A subgroup analysis was performed in which the entire patient population was divided into subgroups of patients with spontaneous thrombosis (n=100) and patients with an additional underlying disease (n=161). Because no difference of carrier frequencies of prothrombotic defects was found between control subjects with underlying disease and those without, each patient group was compared with the entire control group. For all prothrombotic mutations, the carrier frequencies and therefore ORs were higher in patients with spontaneous thrombosis compared with those with an underlying disease. However, in no case did the distribution show a significant difference between the subgroups presented here. The total number of prothrombotic defects was 64 (64.0%) in the patients with spontaneous thrombosis compared with 85 (52.8%) of the 161 patients with an additional underlying disease ($\chi^2$ analysis, not significant) (Table 4).

## Discussion

In recent years, the relations of various hereditary hemostatic abnormalities contributing to the risk of venous thrombosis—in particular the prothrombin G20210A and the FV G1691A mutations, a deficiency or functional loss or modification of protein C, protein S, and antithrombin—have been well established. However, most studies have focused on thrombotic events in adults. We therefore performed the current study to assess the risk of thrombosis in childhood and adolescence with respect to prothrombotic risk factors within the hemostatic system.

The overall frequency of the heterozygous prothrombin G20210A variant was 4.2% in the patients investigated compared with a prevalence of 1.1% in the control group, showing the importance of this mutation for childhood thrombosis. This result is in accordance with a number of reports from adult studies showing heterozygous G20210A

**TABLE 4. Distribution of Hereditary Prothrombotic Risk Factors in Patients With Spontaneous Thrombosis (n=100) and Those With Underlying Disease (n=161)**

| Risk Factors | Patients (Spontaneous Thrombosis) | OR (95% CI) | *P* | Patients (Underlying Disease) | OR (95% CI) | *P* |
|---|---|---|---|---|---|---|
| Single | | | | | | |
| Prothrombin G20210A | 5 (5.0%) | 4.8 (1.3–18.3) | 0.0241 | 6 (3.7%) | 3.5 (1.0–12.7) | NS |
| FV G1691A (total) | 35 (35.0%) | 12.7 (6.6–24.7) | <0.0001* | 48 (29.8%) | 10.1 (5.4–18.6) | <0.0001* |
| Heterozygous | 32 (32.0%) | 12.0 (6.1–23.6) | <0.0001* | 45 (28.0%) | 9.8 (5.3–18.6) | <0.0001* |
| Homozygous | 3 (3.0%) | 11.4 (1.2–110.9) | 0.0318 | 3 (1.9%) | 6.8 (0.7–66.0) | NS |
| Protein C deficiency | 13 (13.0%) | 18.3 (5.1–65.5) | <0.0001 | 11 (6.8%) | 9.0 (2.5–32.6) | 0.0002 |
| Protein S deficiency | 7 (7.0%) | 9.2 (2.3–36.3) | 0.0011 | 8 (5.0%) | 6.4 (1.7–24.4) | 0.0042 |
| Antithrombin deficiency | 4 (4.0%) | ... | 0.0020 | 5 (3.1%) | ... | NS |
| Combinations | | | | | | |
| Prothrombin G20210A and protein C deficiency | ... | ... | ... | 1 (0.6%) | ... | NS |
| Prothrombin G20210A and FV G1691A | ... | ... | ... | 6 (3.7%) | ... | <0.0001 |
| Total | 64.0 (64%) | 24.5 (13.8–43.6) | <0.0001* | 85 (52.8%) | 15.4 (9.3–25.7) | <0.0001* |

Numbers of subjects are shown with percentage frequencies in parentheses.
*$\chi^2$ analysis. Other values by Fisher's exact test.
Also see Table 3.

carrier rates from 4.0% to 20.0% in thrombosis patients and from 1.0% to 4.0% in healthy control subjects.[11] Besides, heterozygosity or homozygosity for the FV G1691A mutation as well as protein S, protein C, or antithrombin deficiency are clearly associated with an increased risk of thromboembolic events during childhood. In the current study, the carrier frequencies of these prothrombotic defects were each in the same range as for adult patients and confirm earlier reports of smaller studies on childhood thrombophilia.[1,2,12–14,17–21,23–26] The only exception from this general finding was the carrier frequency of protein C deficiency, which was found to be relatively high in the patients investigated here, especially in patients with spontaneous thrombosis. On the one hand, this may be attributed to the small number of patients; on the other hand, according to the trend toward higher carrier frequencies of all risk factors in the spontaneous thrombosis group, this finding may reflect the high importance of protein C deficiency for young patients.

Combinations of the prothrombin G20210A mutation and further genetic defects (FV G1691A or protein C deficiency) were found in 7 patients but not in the control subjects. Because only 1 patient could be identified as a carrier of the prothrombin mutation and protein C deficiency, the difference between patients and control subjects reached a clear significance only for the combination of the prothrombin and the FV mutation. However, these observations suggest that combined defects of the prothrombin G20210A variant and further prothrombotic risk factors play an important role not only in adult carriers but also in young patients.[12,15,16]

Thrombosis during childhood is frequently discussed as being due to nongenetic endogenous or exogenous trigger mechanisms.[17–20] Because in our study ≈62% of patients with thrombosis had an underlying disease, we can support these findings. Moreover, the frequencies of hereditary risk factors in patients with spontaneous thrombosis were not significantly different from the frequencies found in patients with underlying disease, but there was a trend toward higher carrier frequencies in spontaneous thrombosis. Thus the role of hereditary defects may be exceeded by acquired risk factors.

In conclusion, carriers of the prothrombin 20210A allele, the FV G1691A mutation, or protein C, protein S, or antithrombin deficiency are at high risk of occurrence of thrombotic events during childhood and early adolescence. Data presented here suggest that the combination of the prothrombin G20210A variant and further prothrombotic risk factors increases the risk of thrombosis, especially with the presence of the FV G1691A mutation.

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## Appendix

### Members of the Childhood Thrombophilia Study Group

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