Frequency of the 20210 G→A Mutation in the 3′-Untranslated Region of the Prothrombin Gene in 35 Cases of Cerebral Venous Thrombosis

V. Biousse, MD; J. Conard, PhD; C. Brouzes, MD; M.H. Horellou, MD; A. Ameri, MD; M.G. Bousser, MD

Background and Purpose—A novel sequence variation in the 3′-untranslated region of the prothrombin (factor II) gene (nucleotide 20210 G→A) has been recently described as a risk factor for deep vein thrombosis and pulmonary embolism. It is found in ~1% to 4% of healthy subjects. We studied the frequency of this factor II variant in patients with cerebral venous thrombosis.

Methods—The 20210A allele of the prothrombin gene was studied after DNA extraction, polymerase chain reaction amplification, and HindIII digestion in 35 patients with magnetic resonance imaging or angiographically confirmed cerebral venous thrombosis (23 women and 12 men, aged 11 to 71 years).

Results—Two patients (5.7%) had the 20210A allele of the prothrombin gene. Both had other risk factors for thrombosis (use of oral contraceptives and of intrathecal steroids).

Conclusions—The 20210A allele of the prothrombin gene in association with other prothrombic factors may increase the risk of cerebral venous thrombosis, but case-control studies will be necessary to clarify these associations. (Stroke. 1998;29:1398-1400.)

Key Words: cerebral veins ■ venous thrombosis ■ thrombophilia

Numerous causes and predisposing conditions have been associated with cerebral venous thrombosis (CVT). They include all surgical, obstetric, and medical causes of deep vein thrombosis as well as a number of local or regional causes, either infective or noninfective. Despite the continuous description of new causes, the proportion of cases of unknown etiology remains high, from 20% to 35%.1-3 Congenital coagulation disorders, such as protein C, protein S, and antithrombin deficiencies, have been reported in patients with CVT.4 More recently, resistance to activated protein C has been identified as a possible new cause of CVT.4,5 Poort et al8 have described a novel sequence variation in the 3′-untranslated region of the prothrombin (factor II) gene (nucleotide 20210 G→A), which is associated with an increased risk of deep vein thrombosis and pulmonary embolism.8,9 Only 1 case with CVT associated with a 20210A genotype of the prothrombin gene has been reported.9 The aim of our study was to determine the frequency of this factor II polymorphism in a series of 35 patients with CVT and to assess any associated cause or risk factor for CVT.

Subjects and Methods

Subjects
Thirty-five patients (23 women and 12 men) aged 11 to 71 years (mean±SD, 35.6±13.1 years) who were seen from 1989 to 1997 in 2 neurology departments were studied. CVT was diagnosed by MRI, MR, x-ray angiography, or some combination thereof. Thirty of these patients had already participated in a previously published study.4 All gave their fully informed consent to participate in the current study.

Potential causes and risk factors for CVT were systematically investigated by clinical examination, neuroimaging, and hematologic studies including blood cell count, platelets, sedimentation rate, and coagulation (protein C activity, protein S activity and free antigen, antithrombin activity, factor V Leiden mutation, lupus anticoagulant, and anticardiolipin antibodies). The main causes are summarized in the Table.

Prothrombin Gene Polymorphism

The coagulation study was performed in 1997, from 2 weeks to 8 years after CVT. Blood was collected into tubes containing 0.129 mol/L sodium citrate (1:9, vol/vol). After centrifugation at 2000g for 20 minutes, cells were frozen at −30°C. The 20210A allele of the prothrombin gene was studied after DNA extraction, polymerase chain reaction amplification, and HindIII digestion, as described by Poort et al.8

Prothrombin Activity

Prothrombin activity was measured in citrated plasma by using a chronometric method with rabbit thromboplastin and factor II–deficient plasma (Neoplastine, Diagnostica Stago). This test could not be performed in 11 patients who were receiving oral anticoagulants at the time of blood sampling but was possible in 18 patients (no samples were available for the other 6).

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Etiologies of Cerebral Venous Thrombosis in 35 Patients Tested for the 20210A Factor II Polymorphism

<table>
<thead>
<tr>
<th>Etiologies of Cerebral Venous Thrombosis</th>
<th>Number of Patients (Associated Causes or Risk Factors)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral contraceptives alone</td>
<td>6 (1 With systemic lupus erythematosus and factor V Leiden mutation)</td>
</tr>
<tr>
<td>Post partum</td>
<td>6 (With factor V Leiden mutation)</td>
</tr>
<tr>
<td>Post abortum</td>
<td>1</td>
</tr>
<tr>
<td>Behcet’s disease</td>
<td>2</td>
</tr>
<tr>
<td>Nephrotic syndrome</td>
<td>1 (With factor V Leiden mutation)</td>
</tr>
<tr>
<td>Uveomeningitis</td>
<td>2</td>
</tr>
<tr>
<td>Intravenous steroids</td>
<td>1 (With factor V Leiden mutation)</td>
</tr>
<tr>
<td>Post surgery</td>
<td>1 (With oral contraceptives)</td>
</tr>
<tr>
<td>Protein C deficiency</td>
<td>1</td>
</tr>
<tr>
<td>Anemia</td>
<td>1 (With oral contraceptives)</td>
</tr>
<tr>
<td>Primary antiphospholipid syndrome</td>
<td>1 (With factor V Leiden mutation)</td>
</tr>
<tr>
<td>Unknown (&quot;idiopathic&quot;)</td>
<td>10</td>
</tr>
<tr>
<td>20210A factor II polymorphism</td>
<td>2 (1 With oral contraceptives, 1 with intrathecal steroids)</td>
</tr>
</tbody>
</table>

Results

Two patients (5.7%) had the 20210 A allele of the prothrombin gene. None was in the “idiopathic” group of 10 patients. Both had other risk factors for thrombosis (use of oral contraceptives and of intrathecal steroids, respectively; see the Table).

Patient 1 was 50 years old in 1993 when she presented with a history of seizure-associated headache for 3 days. Her neurological examination was normal. CT of the brain showed a spontaneously hyperdense lesion in the left parietal lobe consistent with hemorrhage. MRI demonstrated occlusion of the superior sagittal sinus. She was treated with sodium valproate and hiraparin and then with warfarin for 3 months. She recovered within a few days. Her medical history was unremarkable, and at the time of CVT, she had been taking the same oral contraceptive (ethinyl estradiol 50 μg plus levonorgestrel 0.5 mg) for 20 years. The patient’s mother had had a history of recurrent episodes of deep vein thrombosis when she was 50 years old. She had died at 75 and blood samples were not available for a coagulation work-up. The patient has 1 brother and 1 son, and none had participated in a coagulation study.

Patient 2 was 29 years old in 1994 when he complained of headache, seizures, and transient left hemiparesis. These symptoms occurred a few days after an intrathecal injection of steroids for sciatic neuropathy. CT of the brain was normal. MRI showed occlusion of both lateral sinuses. He was treated with sodium valproate and hiraparin and then with warfarin for 3 months. He recovered within a few days. There was no family history of thrombosis. Factor II activity was 1.18 U/mL in patient 1 but could not be measured in patient 2. Patients without the 20210A polymorphism had factor II activity levels between 0.87 and 1.20 U/mL.

Discussion

In this series of 35 patients with CVT, 2 (5.7%) had the 20210 G→A mutation in the 3′-untranslated region of the prothrombin gene. Prothrombin is the precursor of serine protease thrombin, a key enzyme in the processes of hemostasis and thrombosis, that exhibits procoagulant, anticoagulant, and antifibrinolytic activities. Prothrombin is encoded by a 21-kb-long gene localized on chromosome 11, position 11p11-q12. The prothrombin gene is organized into 14 exons, separated by 13 introns with the 5′-upstream translated region and the 3′-untranslated region, which may play regulatory roles in gene expression. Poort et al8 recently examined the prothrombin gene as a candidate gene for venous thrombosis and found a G→A transition at nucleotide position 20210 in the 3′-untranslated region of the prothrombin gene in 18% of selected Dutch patients with a personal and family history of venous thrombosis, in 6.2% of unselected consecutive patients with a first episode of deep vein thrombosis, and in 2.3% of healthy control subjects. More recently, Hillarp et al9 published similar results. They found the 20210A factor II polymorphism in 7.1% of 99 Swedish patients with deep venous thrombosis and in 1.8% of 282 healthy controls. Bentolila et al11 found this polymorphism in 3.7% of 134 young, healthy French control subjects. Cumming et al12 found the 20210A allele in 5.5% of 219 patients with deep venous thrombosis or pulmonary embolism and in 1.2% of 164 healthy controls. The frequency found in the present series of CVT (5.7%) is thus in accord with the series of deep vein thrombosis,8,11 suggesting that this prothrombin polymorphism may be another risk factor for CVT.

One of our patients had a family history of venous thrombosis, and in both, it was the first personal episode of venous thrombosis. Both had other risk factors for venous thrombosis: 1 had received intrathecal steroids a few days before the onset of CVT, and the other was taking oral contraceptives.3,13–15 It has already been emphasized that in patients with thrombophilia and CVT, there is usually another cause or risk factor.4,7,8,10,13–17 as in our previous series of 40 CVT patients.4 Six had thrombophilia (factor V Leiden mutation in 4, protein S deficiency in 1, and protein C deficiency in 1), and among them, 5 had another risk factor for venous thrombosis (1 with uveomeningitis, 1 with systemic lupus erythematosus, 1 with primary antiphospholipid syndrome, 1 with nephritic syndrome, and 1 who had received high-dose intravenous steroids before the onset of CVT). This suggests that congenital thrombophilia, including prothrombin gene polymorphism, should be investigated in patients with CVT, whether a risk factor is detected or not. In our 2 patients the 20210 polymorphism was the only genetic abnormality. By contrast, the factor V Leiden mutation was present in addition to the 20210A allele in 40% of the patients reported by Poort et al8 and in 34% of the patients reported by Hillarp et al.9 Studies in selected families with venous thrombosis indicate that the association of these 2 mutations increase the frequency of venous thrombosis.8,9,16–18

Poort et al8 found that the factor II activity level was above the highest quartile (>1.15 U/mL) in 87% of the patients with the prothrombin gene mutation. However, the measurement of factor II levels in plasma cannot be used as a screening test. It is not appropriate in patients who are being treated with oral anticoagulants, since factor II is a vitamin K–dependent protein. In addition, factor II may be increased in various
conditions, such as during pregnancy or oral contraception. In our series, the factor II level was similar in patients with (1.18 U/mL) and without (up to 1.20 U/mL) the mutation.

The consequences of detecting the prothrombin gene polymorphism may be important for the treatment and follow-up of patients with CVT, although the optimal duration of anticoagulation in patients with a first episode of venous thrombosis presently remains unknown. As in other thrombophilias, it seems reasonable to avoid the use of oral contraceptives, to eventually start preventive treatment in prothrombotic situations (surgery, pregnancy, etc), and to study other family members, since the mutation is transmitted as an autosomal dominant trait.10

These results suggest that the 20210A allele of the prothrombin gene in association with other prothrombic factors may increase the risk of CVT. However, case-control studies will be necessary to allow more precise determination of the causal relationship and the size of the clinical risk.

References
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