Retinal Vein Occlusion Associated With Antithrombin Deficiency Secondary to a Novel G9840C Missense Mutation

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Objective: To describe a novel missense mutation in the antithrombin gene associated with antithrombin deficiency type I in a 40-year-old man with retinal vein occlusion.

Design: Investigational case report.

Results: Ophthalmoscopy of the right eye showed hemi-central retinal vein occlusion. The patient’s medical history was negative for glaucoma or cardiovascular risk factors. Screening for thrombophilic disorders revealed antithrombin deficiency type I. Based on a genetic analysis, a novel missense mutation of a transition of guanosine to cytosine at nucleotide position 9840 was detected, predicting the replacement of aspartic acid by histidine encoded by codon 366 (D366H) in exon 5.

Conclusions: Selective screening may be helpful in identifying patients with retinal vein occlusion with thrombophilic defects. When ordering laboratory tests in patients with retinal vein occlusion, antithrombin deficiency type I should be considered in the differential diagnosis.

Clinical Relevance: Our results contribute to a better understanding of the molecular bases of antithrombin deficiency, adding a novel entry for the molecular defects causing antithrombin deficiency type I. Moreover, the identification of this thrombophilic disorder in retinal vein occlusion may be relevant to the issue of the initiation and duration of oral anticoagulant therapy.

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Recently, several studies1-6 have strengthened the hypothesis that coagulation disorders are pathogenic for retinal vein occlusion. Examples include resistance to activated protein C, heparin cofactor II deficiency, hyperhomocysteinemia, factor XII deficiency, antiphospholipid antibody syndrome, and deficiencies in the anticoagulation system (protein C, protein S, or antithrombin).

Antithrombin is the major plasma inhibitor of coagulation proteinases such as thrombin and factor Xa. It is a glycoprotein of 432 amino acids and a member of the large serine protease inhibitor family.7 The antithrombin gene, which has been sequenced and assigned to human chromosome 1q23-25, spans approximately 19 kilobases and consists of 7 exons that code for a signal peptide of 32 amino acids, as well as the mature protein.8-9

Inherited deficiency of antithrombin (Online Mendelian Inheritance in Man code 107300) is associated with a venous thromboembolic tendency at a young age.10 Inheritance follows an autosomal dominant pattern: individuals with deficiency are usually heterozygous.11 The prevalence of inherited deficiency of antithrombin has been estimated at 1:2000 to 1:5000 among the healthy population.12,13 In patients with history of venous thrombosis, its frequency is probably 2% to 5%.13 Most individuals with inherited antithrombin deficiency have decreased levels of immunologically determined antithrombin and reduced functional antithrombin activity. These cases have been classified as type I and represent individuals who produce a diminished quantity of normal antithrombin. Other individuals with antithrombin deficiency have normal or near-normal levels of immunologically determined antithrombin and reduced functional antithrombin activity. A variant antithrombin protein can usually be isolated from plasma, representing about 50% of the total antithrombin (type II).14

Herein, we describe a patient with retinal vein occlusion associated with antithrombin deficiency type I based on a novel...
missense mutation. The mutation involved the single base transition of guanosine to cytosine at nucleotide position 9840 of codon 366 in exon 5. This novel mutation predicts a replacement of an aspartic acid by a histidine at codon position 366 (D366H). Thus far, mutations of the antithrombin gene have not been identified in patients with retinal vein occlusion, to our knowledge.

**METHODS**

We examined a 40-year-old man with hemicentral retinal vein occlusion. Ophthalmologic examination included visual acuity, intraocular pressure, slitlamp biomicroscopy, funduscopy, and fluorescein angiography.

Functional and immunological antithrombin levels were measured, and genetic analysis was subsequently performed. For all functional and immunological assays, blood was collected in tubes with 3.86 wt/vol sodium citrate in an anticoagulant-blood ratio of 0.10.9 vol/vol. Plasma was obtained by centrifugation at 4°C for 15 minutes at 1600 g and stored at −80°C before use. DNA was extracted from peripheral blood collected on EDTA and amplified as described by Saiki et al.15

**ANTITHROMBIN ASSAYS**

Functional antithrombin activity was measured in the presence of heparin (heparin cofactor activity) using reagents with the chromogenic substrate para-nitro-aniline (IL-Test-Antithrombin; Instrumentation Laboratories, Milan, Italy). Immunological antithrombin assays for the quantitative determination of antithrombin in human citrated plasma, using the Laurell technique,26 were performed as previously described (immune serum Assera AT; Diagnostica Stago, Asnieres, France). The plasma was examined by crossed immunoelectrophoresis in the absence and presence of heparin sodium (20 U/mL) to search for any variant form of the protein.17

**SINGLE-STRANDED CONFORMATIONAL POLYMORPHISM ANALYSIS OF THE ANTITHROMBIN GENE**

DNA was extracted from peripheral blood using a previously described protocol.18 All 7 exons of the antithrombin gene were amplified by polymerase chain reaction (PCR)13 using oligonucleotide primer pairs antithrombin 1.5 (5'-GAGATTTAGGAAAGAACC-3') and antithrombin 1.3 (5'-TTGAGGCTAICTGCTGTAGCTC-3') for exon 1, P1 and P2 for exon 2,19 antithrombin 3.3 (5'-ACCACCATGTTAAC- TAGGC-3') and antithrombin 3.5 (5'-CTCCAGCAGTCTCTACGAGC-3') for exon 3A, E3-5 and E3-3 for exon 3B,20 E4-5 and E4-3 for exon 4,20 E5-5 and E5-3 for exon 5,20 and P511C and P512B for exon 6.20 DNA amplification was performed as previously described.21

For single-stranded conformational polymorphism (SSCP) analysis,24 a previously described protocol was used.19 Nondeuterated and denatured samples were loaded on SSCP gels. After electrophoresis, the gels were fixed and dried on filter paper. Autoradiography was performed for 24 hours.

**DNA SEQUENCING OF THE 7 ANTITHROMBIN EXONS**

The 7 antithrombin exons were PCR amplified in a total volume of 100 µL as described for SSCP analysis except that 1 µg of genomic DNA, 200 µmol/L of each deoxyribonucleotide triphosphate, and 100 pmol of each primer were used. Radioactive nucleotides were omitted from the reaction mixtures. After amplification, the PCR products were purified on a Centri-100 microconcentrator (Amicon, Beverly, Mass) and sequenced in both directions by dideoxynucleotide chain termination with the PCR primers, using the Sequenase Version 2.0 kit (US Biochemical, Beverly) as described.23

**RESULTS**

**REPORT OF A CASE**

A 40-year-old man was initially seen after 4 weeks of blurred vision in his right eye. There was no pain, headache, or jaw claudication. On the initial examination, visual acuity was 20/200 OD and 20/20 OS. Anterior segment examination, pupillary reaction test results, and intraocular pressure were unremarkable in both eyes. Ophthalmoscopy of the right eye revealed venous dilatation and tortuosity, with scattered intraretinal hemorrhages in both superior quadrants. Ophthalmoscopy of the unaffected eye showed no abnormalities. Fluorescein angiography of the right eye disclosed leakage of the venule walls, with delayed filling of the retinal veins.

Based on these findings, the diagnosis of hemicentral retinal vein occlusion of the right eye was made, and the patient was started on oral antiplatelet therapy with acetylsalicylic acid, 100 mg/d. In the further course, visual acuity in the right eye improved progressively and was 20/60. By this time, the patient had developed optic disc swelling and retinal edema of the posterior pole (Figure 1). The patient’s medical history was negative for glaucoma, arterial hypertension, diabetes mellitus, atherosclerotic disease, hyperlipidemia, or cigarette abuse, and he did not receive ocular or systemic medications. Physical examination findings that included 24-hour ambulatory blood pressure monitoring, color duplex sonography of the carotid arteries, and fasting plasma glucose test results were unremarkable. His family history was positive for stroke and central retinal vein occlusion.

Further investigation into possible causes included comprehensive thrombophilia screening. Normal re-
Results were obtained for platelet count, prothrombin time, activated partial thromboplastin time, factor VII activity and antigen, von Willebrand factor antigen, ristocetin cofactor, factor II, factor VIII coagulant and factor XII activity, protein C and protein S activity, heparin cofactor II, plasma homocysteine level, lupus anticoagulant, anticardiolipin antibodies, activated protein C response, plasminogen activity, fibrinogen, tissue plasminogen activator antigen and plasminogen activator inhibitor activity, protein C and protein S activity, heparin cofactor II, plasma homocysteine level, lupus anticoagulant, anticardiolipin antibodies, activated protein C response, plasminogen activity, fibrinogen, tissue plasminogen activator antigen and plasminogen activator inhibitor activity, and antigen and plasma histidine-rich glycoprotein levels. However, antithrombin activity levels were recurrently found to be decreased at 65% to 67% (reference range, 86%-122%, functional test as already mentioned), and antithrombin antigen levels were recurrently mildly decreased at 16 to 17 mg/dL (reference range, 21-30 mg/dL). Crossed immunoelectrophoresis of the plasma in the absence or presence of heparin did not reveal any variant form of the inhibitor. Based on these laboratory findings, the diagnosis of antithrombin deficiency type I was made.

DNA ANALYSIS

Single-stranded conformational polymorphism analysis of the 7 exons showed normal patterns in exons 1 to 4 and a modified pattern in exon 5. The exon 5 fragment was PCR amplified, purified, and sequenced. Sequencing of the amplified exon 5 DNA revealed a substitution mutation of guanosine to cytosine of nucleotide position 9840, codon 366 of the antithrombin gene complementary DNA (Figure 2), predicting the change from an aspartic acid to a histidine in the mutated protein. DNA sequencing of the remaining antithrombin exons did not show any abnormalities compared with the published antithrombin sequence.

COMMENT

There is rapidly growing body of literature on the relationship between coagulation disorders and systemic thromboembolism. Most of this literature concerns variation in the genes for blood coagulation factors, inhibitors, fibrinolytic factors, and platelet membrane receptors. The relationship between factor V G1691A polymorphism and the intermediate phenotype, resistance to activated protein C, has been broadly established. In 1996, a common prothrombin polymorphism (G20210A substitution) that increases the risk for venous thrombosis was identified. Hyperhomocysteinemia has been shown to be associated with a common polymorphism, C677T, in the methylene tetrahydrofolate reductase gene, which can potentially interact with hemostatic gene polymorphisms.

Results of other studies have strengthened the hypothesis that coagulation disorders are pathogenic for retinal vein occlusion. Examples include resistance to activated protein C, hyperhomocysteinemia, heparin cofactor II deficiency, and antiphospholipid antibody syndrome. However, there are scant data in the literature relating antithrombin III deficiency and retinal vein or artery occlusion. Results of several studies on major thromboembolism and coagulation disorders indicate that antithrombin deficiency type I was made.

Figure 1. Fundus photography of the right eye 4 weeks after onset of symptoms, showing optic disc swelling, venous dilatation and tortuosity, and scattered intraretinal hemorrhages and retinal edema in both superior quadrants, consistent with hemicentral retinal vein occlusion.

Figure 2. Detailed picture of the sequenced exon 5 DNA reveals a substitution mutation (guanosine to cytosine) of nucleotide position 9840 (arrow), codon 366 of the antithrombin gene. The substitution of guanosine for cytosine predicts the change from an aspartic acid to a histidine (coded by CAT) in the mutated protein.
bin III deficiency tends to be of low prevalence. Among patients with a history of venous thromboembolism, the frequency is 1.1% to 4%. The prevalence of this disorder has been estimated at 1:2000 to 1:5000 among the healthy population. However, this low prevalence is coupled with a high risk for major thromboembolic events and affected patients typically manifest recurrent venous thrombosis, usually beginning in adolescence or early adulthood.

Although environmental effects such as cardiovascular risk factors are undoubtedly important, coagulation disorders can play a crucial role in and may lead to apparently spontaneous retinal venous occlusions. The present case indicates that individuals 45 years or younger who manifest retinal vein occlusion in the absence of cardiovascular risk factors should be evaluated for the possibility of an underlying thrombotic tendency. In particular, the diagnosis should be considered in patients with a personal or family history of thromboembolism. This conclusion compares favorably with the results of previous studies on the role of coagulation disorders in retinal vein occlusion. A negative family history, however, does not exclude the possibility of inherited thrombophilia, as these defects have a low penetrance and spontaneous mutations can occur.

Several studies of patients referred for evaluation of major thromboembolic events have identified molecular defects leading to antithrombin deficiency. However, we are unaware of any previous reports on an association between mutations of the antithrombin gene and retinal vein occlusion. Herein, we identified a novel missense mutation in the antithrombin gene involving the single base transition of guanosine to cytosine at nucleotide position 9840 of codon 366. This novel mutation results in the replacement of an aspartic acid by a histidine at codon position 366 (D366H) in exon 5, leading to the phenotype of antithrombin deficiency type I. To the best of our knowledge, this represents the first report of this missense mutation in the medical literature. However, our genetic findings compare favorably with the results of other recent work. The genomic basis for most type I deficiencies reported to date has been point changes within the antithrombin gene, producing termination codons, frame shifts with premature termination, RNA processing defects, or amino acid deletions or substitutions.

The question of whether patients with retinal vein occlusion with a genetic thrombophilic abnormality such as antithrombin deficiency should receive anticoagulants remains controversial. Although oral anticoagulant therapy with coumarin is the standard of care in patients with major venous thromboses, there are insufficient data to support a similar therapeutic approach in retinal vascular occlusive disease. Therefore, the risks and potential benefits of antithrombotic therapy in each individual must be considered carefully. If a hereditary defect is found, referral should be made to a hematologist and consideration given to screening of family members to prevent further thrombotic episodes.

In summary, ophthalmologists should be aware of antithrombin deficiency as a rare but potential risk factor in patients with retinal vein occlusion. A multidisciplinary evaluation is required.

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REFERENCES


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