Homozygous Antithrombin Deficiency in Adolescents Presenting With Lower Extremity Thrombosis and Renal Complications
Two Case Reports From Turkey

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Summary: We present 2 cases of lower extremity deep venous thrombosis in 2 gypsy adolescents from related families. The patients had low antithrombin activity levels and inherited homozygous antithrombin deficiency was confirmed by molecular analysis (Leu131Phe mutation). One patient had a history of nephrectomy at the age of 9 due to nonfunctioning kidney and 2 siblings died in the third trimester. The other propositus had an elder sister who suffered from postpartum deep vein thrombosis and pulmonary embolism. Heterozygous mutation was demonstrated in both parents.

Key Words: antithrombin deficiency, thrombosis, heparin-binding site defects, Leu131Phe mutation

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Hereditary antithrombin (AT) deficiency is an autosomal-dominant disorder predisposing to venous thromboembolism (VTE). Its prevalence in the general healthy population is about 1 in 5000 and in patients with history of thrombosis 1 in 500.1 Relative risk of VTE was estimated to be increased by 25- to 50-fold in patients with AT deficiency.2 Homozygosity is usually not compatible with life. Heparin-binding site (HBS) defects are, however, associated with milder thrombotic phenotype compared with other types of AT deficiencies and can therefore be present in homozygous states.3

It has been reported that homozygous children of asymptomatic carriers can develop severe venous or arterial thrombosis in association with very low plasma AT activity levels.4,5 Very few patients with homozygous AT HBS mutations are reported. We report the clinical characteristics and the molecular AT analyses in 2 adolescents with inherited homozygous AT deficiency with complications of pregnancy within their relatives.

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CASE REPORTS

In 2011 and 2012, 2 adolescent patients with deep venous thrombosis (DVT) of lower extremities, confirmed by Doppler ultrasound imaging, were referred to our center. Echocardiography showed no pathology. Hemoglobin electrophoresis ruled out sickle cell disease or trait, and coagulation assays were performed. Genetic analysis for factor V Leiden, Prothrombin G20210A, MTHFR C677T, MTHFR A1298C, APO B, and APO E polymorphisms were performed by real-time PCR. Because of low AT activity levels (chromogenic assay STA-Stachrom AT III; Diagnostica Stago, France), DNA samples of the patients and some of the family members were sent to the University Hospital of Brussels in Belgium for genetic study of AT gene mutations. In both index patients, sequencing of all exons and intron-exon junctions of AT gene SERPINC1 was performed. The patients were from related gypsy families living in the north-west of Turkey.

Case 1

A 13-year-old gypsy girl was referred to our center from a local hospital for treatment of VTE and investigations for thrombophilia. She was admitted to the local hospital 3 days ago with abdominal pain, fever, diarrhea, and swelling of the left leg. Doppler ultrasound examination of the lower limbs revealed thrombosis extending from the left iliac vein to the left femoral and popliteal vein. Diarrhea resolved in a few days. The parents reported consanguineous marriage; they were both 37 years old and had no history of thrombosis. Her elder sister developed postpartum lower extremity DVT and pulmonary embolism at the age of 19. There were 3 more siblings aged 18, 17, and 4 years without any history of thrombosis (Fig. 1). Their uncle died of myocardial infarction at the age of 34.

Laboratory Tests

PT 40.4 s, aPTT 24.2 s, INR 1.41, fibrinogen 7.3 g/L, D-dimer 7.84 mg/mL, protein S 72% (60% to 140%), protein C 92% (70% to 130%), functional plasma level of AT 21% (80% to 120%), factor VIII 300% (60% to 150%), homocysteine 9.9 μmol/L, antinuclear antibodies negative (<12 U/mL), antithrombin III activity 30% (<80% to 120%), factor XIII 369% (60% to 150%), platelet count 115 (100 to 300) × 109/L, haptoglobin 1 mg/dL (1 to 2 mg/dL), prothrombin time 17 s (11.5 to 15.5 s), fibrinogen level 7.3 g/L, fibrinogen degradation products 1.9 mg/mL (0.5 to 1.5 mg/mL), protein C 92% (70% to 130%), protein S 72% (60% to 140%), AT activity 21% (80% to 120%), factor VIII 300% (60% to 150%), homocysteine 9.9 μmol/L, antithrombin III activity 30% (<12 U/mL), antithrombin III activity 30% (<12 U/mL), and homoglobin electrophoresis normal. Genetic studies for thrombophilia revealed homozygous A1298C MTHFR polymorphism.

Thrombolysis with recombinant tissue plasminogen activator was refused by the parents considering the risk of hemorrhage. In the referring hospital, enoxaparin treatment was started. Effective plasma factor anti-Xa level was not achieved even at a dose of 80 mg twice daily (approximately 2 mg/kg/d). On the 7 to 9 days of enoxaparin treatment, AT concentrate was administered (1 x 1000 U/d Human AT III; Kybernin-P Farma-Tek). On day 15, Coumadin was added (0.1 mg/kg/d) and enoxaparin was stopped on day 20. Recanalization of the occluded veins was not achieved.

Etiology of the fever was not clear. Blood and urine cultures remained sterile. Abdominal ultrasound, thorax x-ray, and
In 8 Pedigree of the AT-deficient family. The arrows indicate the 2 index patients. Patients homozygous for the p.Leu131Phe mutation are depicted by filled squares/circles, whereas heterozygous individuals are depicted by half-filled squares/circles. Percentages are AT activity levels. Age at first thrombotic is indicated where appropriate. AT indicates antithrombin; ND, AT activity not determined.

Lifelong Coumadin treatment and compression stockings were recommended. Homozygous AT p.Leu131Phe mutation was detected in the patient and in the symptomatic elder sister. Both parents were heterozygous for the same mutation. The patient was discharged on the day 43. Thrombosis persisted in the left iliac and femoral veins. During the 15-month follow-up, she has post-thrombotic syndrome (persistent left lower extremity swelling) but experienced no new thrombotic event.

Case 2

A 16-year-old gypsy boy developed pain and swelling in his left leg after breaking woods with his foot soles. His admission to a local hospital was 1 month after the event. Doppler ultrasound study revealed subacute thrombosis in the left femoral, popliteal, and great saphenous vein. Coumadin was started and he was discharged on the day 43. Thrombosis persisted in the left iliac and femoral veins. During the 15-month follow-up, she has post-thrombotic syndrome (persistent left lower extremity swelling) but experienced no new thrombotic event.

FIGURE 1. Pedigree of the AT-deficient family. The arrows indicate the 2 index patients. Patients homozygous for the p.Leu131Phe mutation are depicted by filled squares/circles, whereas heterozygous individuals are depicted by half-filled squares/circles. Percentages are AT activity levels. Age at first thrombotic is indicated where appropriate. AT indicates antithrombin; ND, AT activity not determined.

LABORATORY TESTS

- PLT 429 × 10^3/μL, PT 14.6 seconds, aPTT 24.2 seconds, INR 1.2, fibrinogen 2.5 g/L, D-dimer 3.09 mg/mL, protein S 84%, protein C 110%, functional plasma level of AT 17%, factor VIII 176%, homocysteine 12.45 μmol/L, nucleic acids negative, anti-cardiolipin IgG and IgM negative, hemoglobin electrophoresis normal, and heterozygous MTHFR C677T polymorphism. Coumadin treatment (0.1 mg/kg) was continued with target INR between 2 and 3. The same homozygous AT mutation as in the first patient was detected. The father was heterozygous for the same mutation but the mother could not be investigated (Fig. 1). During the 26-month follow-up the patient experienced no new thrombotic event.

DISCUSSION

Unprovoked VTE in patients with a family history of thrombosis requires testing for heritable thrombophilia. In a recent study, mutation analysis of 150 patients (aged below 40 y) with a history of VTE revealed 5 patients (3.5%) with AT mutations. In these patients, AT activity and antigen levels were within the normal range and only genetic testing was diagnostic. Prevalence of inherited AT mutations in patients with thrombosis was reported to be higher than previously estimated.

The missense mutation detected in the presented patients is located in exon 2 of the AT gene and affects amino acid 131 with transition of leucine to phenylalanine (numbered p.Leu131Phe according to HGVS nomenclature, formerly p.Leu99Phe). This mutation, causing a type II HBS deficiency, has originally been described as AT Budapest III in a few patients from the Balkan region with severe thrombosis in childhood. AT is the main physiological inhibitor of blood coagulation and it inactivates thrombin and several serine proteases including factors IXa, Xa, XIa, and XIIa. AT consists of 2 major domains responsible for the interaction with heparin and thrombin. Inherited AT deficiency is a rare and heterogeneous disorder. In type I deficiency, both the functional activity and antigenic levels are proportionally reduced (quantitative deficiency). In type II AT deficiency, there is normal antigen level but reduced AT activity due to a dysfunctional protein. Among symptomatic patients, type I is much more prevalent, often representing up to 80% of total cases. Most patients with inherited heterozygous AT deficiency have AT activity levels in the range of 40% to 60%. Today > 200 different AT mutations are described. Prevalence of VTE in patients with type II heparin-binding deficiency is ~6%. Homozygous AT deficiency is probably lethal as no homozygous deficiencies have been reported, except for the HBS subtypes. The 2 index cases presented here are homozygously affected with HBS p.Leu131Phe mutation. Heterozygous individuals like the parents of our patients have lower thrombotic risk. The reduced thrombotic risk could be explained by the fact that the β-glycoform of AT (representing 10% of the circulating AT) does not lose heparin affinity due to homozygous p.Leu131Phe mutation and therefore might compensate the effect of this mutation.
The clinical presentations of AT deficiency are typically VTE of lower extremities and pulmonary embolism. Thrombosis at unusual sites such as cerebral sinuses, mesenteric, hepatic, renal, and retinal veins can also occur but association with arterial thrombosis is rare. Approximately 60% of VTE in inherited AT deficiency is unprovoked.\(^1\)

Our first patient had an episode of fever which might have provoked VTE. The proposed mechanism is that fever accelerates the conversion of AT to a more inactive latent form.\(^3\) A second hypothesis has also been recently suggested: the mutant monomer might form polymers, mainly intracellularly in a reaction to stress (like a fever episode). These polymers are retained inside the cell, and the secretion of monomers is therefore impaired, reducing antithrombotic potential.\(^9\)

There was kidney loss in the second patient. Renal involvement is not a typical presentation in inherited AT deficiency. Few reports are available on that matter. Hara and Naito\(^10\) described renal disease in patients with inherited AT deficiency due to fibrin deposition in the kidney glomeruli or renal vein thrombosis. Miura et al\(^11\) described obstruction of the renal artery, renovascular hypertension, and segmental infarction of the right kidney after surgery of intussusception in a 1-year-old Japanese boy with genetically confirmed type I AT deficiency. Thrombosis of the renal veins is an important complication that can lead to infarction of the kidney and even kidney loss. Renal complications of inherited AT deficiencies need further support.

The initial management of VTE in a patient with AT deficiency is usually not different from VTE in any other patient. Initial management consists of administration of low-molecular weight heparin with or without prior thrombolytic therapy. Vitamin K antagonists can be used in the maintenance therapy.\(^1\) Patients with AT deficiency can show resistance to heparin and additional AT concentrate infusion is then recommended.\(^12,13\) Fischer et al\(^7\) reported some patients with AT mutations that needed high dosage of dalteparin to achieve therapeutic levels of anti-factor-Xa. AT concentrate was also used in our first patient due to subtherapeutic anti-Xa levels.

In this study, in both families, pregnancy complications or bad pregnancy outcomes were present. In the first family, the elder sister had postpartum life-threatening DVT and pulmonary embolism. The second patient’s mother experienced third trimester fetal losses, probably due to homozygous AT deficiency of the fetuses. Homozygosity is known to be often fatal in utero.\(^1\) Women with inherited thrombophilia are known to have an increased risk of miscarriage.\(^14\) Thromboprophylaxis in AT-deficient women—anteprtum and postpartum—with heparin (mainly low-molecular weight heparin) and compression stockings is recommended.\(^15,16\)

In conclusion, we can state that homozygous AT type II HBS deficiency is associated with high risk for VTE, even in adolescents. On the basis of the family history of our 2 propositi, homozygous HBS deficiency is also responsible for pregnancy-related complications and bad pregnancy outcome. Identification of the mutation underlying the deficiency provides information on thrombotic risks and allows discrimination between heterozygous and homozygous individuals. The genetic data are important for justification of lifelong anticoagulation in homozygous patients and administration of prophylactic treatment in risk situations (pregnancy, surgery) in heterozygous carriers.

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