



Hereditary Anticoagulant Deficiencies

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Keywords

Antithrombin deficiency · Deep venous thrombosis · Protein C deficiency · Protein S deficiency · Purpura fulminans

Abbreviations

APC	Activated protein C
APTT	Activated partial thromboplastin time
AT	Antithrombin
CT	Computed tomography
DIC	Disseminated intravascular coagulation
DVT	Deep venous thrombosis
OR	Odds ratio
PC	Protein C
PIVKaII	Vitamin K absence or antagonists II
PS	Protein S
PT	Prothrombin time
rr	Reference range
VTE	Venous thromboembolism

17.1 Case Report

A full-term Japanese pregnant woman was transferred to a general hospital because of non-reassuring fetal status. She had two healthy siblings and had experienced twice spontaneous abortions. A fetal echogram revealed enlargement of the bilateral cerebral ventricles with periventricular hyper-echoic lesion. An emergency caesarean delivery was performed under the suspicion of fetal intracranial hemorrhage. A male infant was born weighing 2912 g, with APGAR scores 8 and 10 after 1 and 5 min, respectively. No skin lesion was observed at birth. A computed tomography (CT) showed high-density areas in bilateral periventricles. Multiple purpuric lesions appeared a few hours after birth with progressive disseminated intravascular coagulation (DIC). Because of the suspected diagnosis of purpura fulminans, he was transferred to our neonatal intensive care unit 1 day after birth.

On admission, multiple ecchymoses emerged on his extremities despite the plasma transfusion and anticoagulation therapy (Fig. 17.1). Blood tests revealed white blood cells $11.51 \times 10^9/L$, hemoglobin 11.0 g/dL, platelets $101 \times 10^9/L$, fibrinogen 80 mg/dL (reference range (rr), 200–400), prothrombin time (PT) 14.5 sec (rr, 11.6–14.4; PT-% 70%), activated partial thromboplastin time (APTT) 43.3 second (rr, 37.1–48.7), fibrinogen degradation products 142.0 $\mu\text{g/mL}$ (rr, <10), D-dimer 79.7 $\mu\text{g/mL}$ (rr, <1), thrombin–anti-

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Fig. 17.1 The multiple ecchymoses in the dorsum of the left hand (Fig. 17.1) and in the right sole (Fig. 17.1) (on admission)

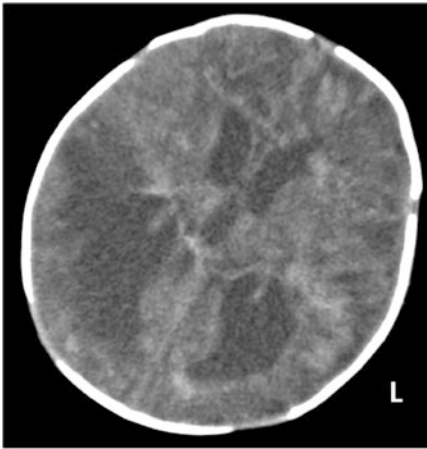


Fig. 17.2 The brain CT scan on admission
Diffuse intracranial hemorrhage indicates cerebral venous sinus thromboses

thrombin complex 7.2 ng/mL (rr, <4), and plasmin α 2–antiplasmin complex 4.7 μ g/mL (rr, <0.8). Protein C (PC) and protein S (PS) activity before the first plasma transfusion were <5% and 86%, respectively. Brain CT scan showed the diffuse intracranial hemorrhage, indicating cerebral venous sinus thromboses (Fig. 17.2).

He was diagnosed as having purpura fulminans caused by PC deficiency. Plasma-derived activated PC (AnactC®, Teijin Pharma, Japan) therapy led to the drastic improvement of his skin lesions and coagulopathy. A gene analysis revealed that he had compound heterozygous PC gene (*PROC*) mutations (c.236G > A, p.Glu49Lys and c.296G > A, p.Glu68Lys). His parents had either heterozygous *PROC* mutation. This patient was discharged from the hospital after receiving ventriculoperitoneal shunt. Unfortunately, he died suddenly at home on 9 months of warfarin therapy.

17.2 Diagnosis

Thromboembolisms occur as a consequence of genetic predispositions, underlying disorders, and direct triggers such as dehydration, infection, or injury. The formation of thrombosis depends on the Virchow's triad: the vessel wall, blood flow, and blood components. Heritable defects in the coagulant and anticoagulant pathways result in the development of venous thromboembolism (VTE) with or without apparent triggers. The genetic risks of VTE include the deficiency of anticoagulant factors of PC, PS, and antithrombin (AT), as well as the variants of coagulation factors of factor V G1691A (factor V Leiden) and prothrombin (factor II) G20210A (Caspers et al. 2012). Hereditary anticoagulant deficiencies are suspected in young adult VTE, neonatal purpura fulminans, patients having recurrent VTE, and/or family history of thrombosis or anticoagulant deficiencies. Hereditary anticoagulant deficiencies are also associated with a tripling risk for the late fetal loss, but no risk for the first-trimester loss. High risk of thrombophilias was reported in association with obstetric complications other than preeclampsia and intrauterine growth retardation.

The hereditary anticoagulant deficiencies are difficult to diagnose in infants and young children without the genetic tests, because these activities are physiologically lower in childhood than in adulthood (Fig. 17.3) (Ichiyama et al. 2016; Takahashi and Yoshioka 1994). Both PC antigen and activity levels increase after birth and reach the lower limit of adult references (~50 IU/dL) during 6 months–1 year of age. Prior to the genetic screening, anticoagulant activity levels

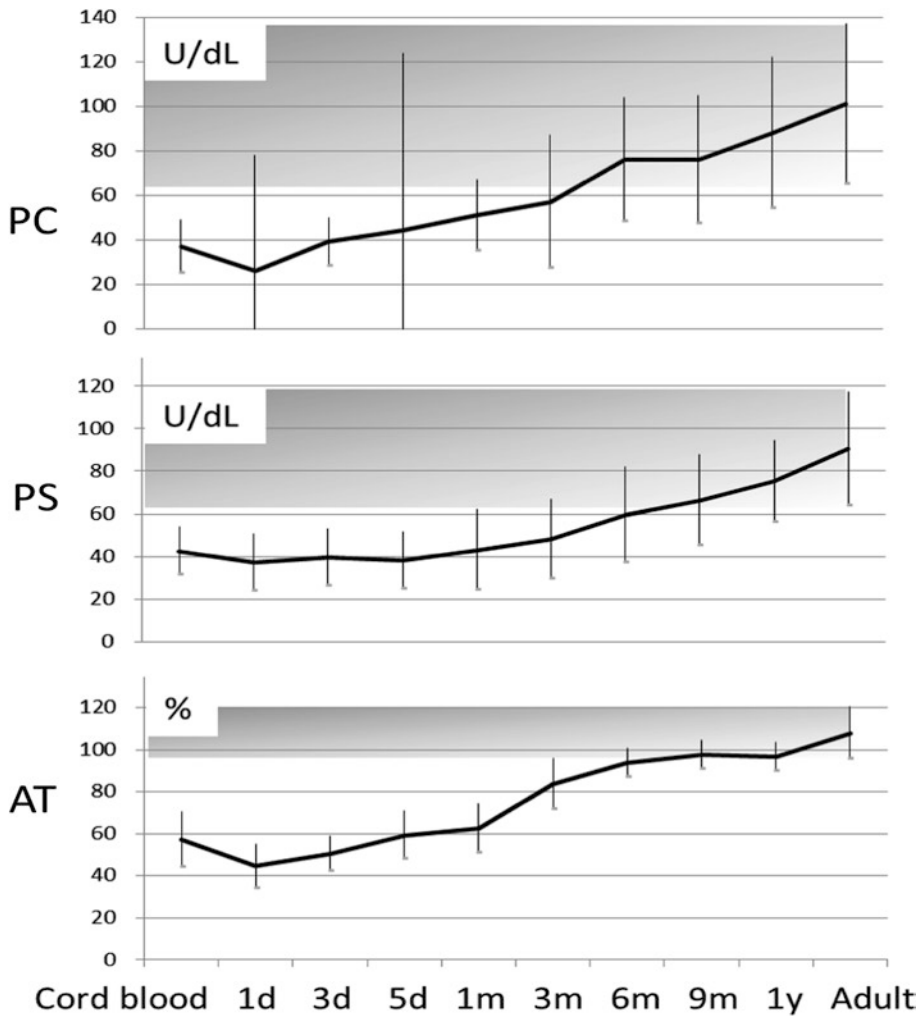


Fig. 17.3 Physiological changes in the plasma activity of protein C (PC), protein S (PS), and antithrombin (AT) in the first year of life. The vertical lines and gray backgrounds show the range of standard deviations of the plasma activity of PC, PS, and

AT and the normal range in adults, respectively. Each measurement was not completed by using the same sample concurrently obtained from the subjects (Ref. Ichiyama et al. 2016; Takahashi and Yoshioka 1994)

are assessed with protein induced by vitamin K absence or antagonists II (PIVKAI), D-dimer, and antiphospholipid antibodies. PC and PS activities are recommended to follow with the concurrent measurements of factor VII, a vitamin K-dependent zymogen with a similar short plasma half-life, to exclude the effects of consumption coagulopathy. Unexplained dissociation among PC, PS, and factor VII activity levels

may portend a diagnosis of heritable PC or PS deficiency (Ichiyama et al. 2016). The coagulation studies by using the age-dependent standards have then high sensitivity in screening for anticoagulant deficiencies. Nevertheless, repeated sampling, family studies, and genetic analyses along with the detailed information of reported cases are essential for the diagnosis for anticoagulant deficiency.

17.3 Biochemical and Molecular Perspectives

17.3.1 Protein C (PC) Deficiency

PC is a vitamin K-dependent anticoagulant factor, which is synthesized by the liver and circulates as a zymogen. It exerts the proper anticoagulant function after the cleavage of the molecule to the serine protease, activated PC (APC). This activation is mediated by thrombin alone, but occurs more efficiently when thrombin is bound to thrombomodulin on the endothelial cell receptor. APC inactivates factor V and factor VIII by cleaving the critical sites of these activated molecules. This reaction is enhanced by PS, factor V, and lipid cofactors of lipoproteins and phospholipids. Reduced concentration of circulating PC fails to control the propagation of thrombin generation by activated factor V and activated factor VIII, even if PC deficiency originates from the decreased production and/or increased consumption. APC has also anti-inflammatory as well as cytoprotective functions (Griffin et al. 2015). These are directly driven by the endothelial and immunocompetent cells via the specific receptor on the given cells. The protective effect of APC in animal models of sepsis depends on its capacity to activate protease-activated receptor-1 and not on its anticoagulant properties. The pleiotropic effects are expected to be beneficial for the treatment of sepsis. Clinical trials of recombinant APC were, however, withdrawn for the risk of bleeding (Ranieri et al. 2012). Refined molecules of APC are being explored for the effective treatment of sepsis and DIC (Quinn et al. 2015).

Plasma levels of PC activity are measured by chromogenic (amidolytic) or coagulometric (clotting) assays. The standard value in healthy adults ranges from 0.65 to 1.35 IU/mL (65–135% of normal). “Mild,” “moderately severe,” and “severe” PC deficiencies are defined as the range of >20% (>0.2 IU/mL), 1–20% (0.01–0.2 IU/mL), and <1% (<0.01 IU/mL), respectively (Ohga et al. 2013a).

PC gene (*PROC*) is located on chromosome 2q13-q14. Heterozygous *PROC* mutation inherits

“mild” or at most “moderately severe” PC deficiency in an autosomal-dominant manner. The first presentation of PC deficiency includes the newborn-onset and the teen-onset modes. Neonatal purpura fulminans and stroke are the distinct manifestations of PC deficiency (Caspers et al. 2012; Ohga et al. 2013b). Thereafter, deep venous thrombosis (DVT) in the legs and pulmonary thromboembolism are commonly affected sites. Approximately 70% of patients first present in teens spontaneously and the remaining 30% with the risk factors. Nonhemorrhagic arterial stroke is also associated with pediatric PC deficiency. Severe PC deficiency is inherited in an autosomal-recessive fashion. The complete defects exclusively arise from biallelic *PROC* mutation, presenting neonatal purpura fulminans and/or intracranial hemorrhage.

The prevalence of heterozygosity for PC deficiency is considered to be 1/200–500 (Tait et al. 1995). Heterozygous PC-deficient adults have a sevenfold increased risk for an initial episode of DVT compared with those having normal PC activity. On the other hand, asymptomatic carriers have low annual incidence of thrombosis at less than 1.0%. The odds ratio (OR) of pediatric thrombosis with PC deficiency is estimated at 7.7 (Table 17.1) (Young G et al. 2008).

Heterozygous PC deficiency is classified into two types. The common form of type I represents an equal reduction in both immunologic and biologic activities. More than 200 mutations and rare deletions were reported. Type II-deficient subjects show normal antigen and decreased activity levels. However, several cases show normal levels of antigen and amidolytic activity, but with reduced levels of clotting activity. It is ascribed to a reduced ability of APC to interact with the platelet membrane or its substrates of activated factor V and activated factor VIII. Recently, K193del has been recognized as the most common variant in Chinese thromboembolisms (Yin and Miyata 2014). On the other hand, it was considered as a polymorphism at first in Japan, because the heterozygotes often show normal activity for PC in resting conditions. It was then reported as PC-Tottori, in the homozygote of a young adult patient with DVT.

Table 17.1 Reported odds ratio and 95% confidence interval [in brackets] for genetic traits associated with a first onset of venous thromboembolism in children and in pediatric patients with recurrent deep venous thrombosis

Genetic predisposition	Cerebrovascular occlusion (first onset)	Deep venous thrombosis	
		First onset	Recurrence
Protein C deficiency	9.3 [4.8–18.0]	7.7 [4.4–13.4]	2.4 [1.2–4.4]
Protein S deficiency	3.2 [1.2–8.4]	5.8 [3.0–11.0]	3.1 [1.5–6.5]
Antithrombin deficiency	7.1 [2.4–22.4]	9.4 [3.3–26.7]	3.0 [1.4–6.3]
Factor V Leiden	3.3 [2.6–4.1]	3.8 [3.0–4.8]	0.6 [0.4–1.2]
Factor II G20210A	2.4 [1.7–3.5]	2.6 [1.6–4.4]	2.1 [1.01–3.5]

Ref. Kenet G et al. 2010; Young G et al. 2008

We experienced that the heterozygote infants for PC-Tottori showed slowly age-dependent increase of PC activity. There is increasing evidence in Asian countries on the double mutants who escaped neonatal thromboses. In Caucasians, factor V Leiden and factor II variant might mask the effects of *PROC* variants on the thrombotic risk of patients (Yin and Miyata 2014).

17.3.2 Protein S (PS) Deficiency

PS is a vitamin K-dependent protein that enhances the anticoagulant effect of APC. This coenzyme is primarily synthesized by hepatocytes and also by endothelial cells, megakaryocytes, and brain cells. It serves as a cofactor for APC in the setting of activated factor V and activated factor VIII inactivation (Fig. 17.4). The inactivation of activated factor V occurs, at first, in the rapid cleavage at Arg506 (factor V Leiden: Gln506) of the molecules, followed by the slower cleavage at Arg306 (second binding site of PS with APC; factor V Cambridge: Thr306) and then Arg679 (Fig. 17.5). PS with APC increases the affinity for phospholipids to enhance activated factor V inactivation. Approximately 40% of circulating PS molecules is in the free form, and the remainder 60% is bound to C4b-binding protein not to interact with APC. Free PS levels are responsible for the direct anticoagulant effects.

Heterozygous mutations in the gene encoding protein S (*PROS1*) on chromosome 3q11 cause autosomal-dominant thrombophilia. Homozygotes or compound heterozygotes for the mutation are rarely found as an autosomal-dominant thrombophilia. However, most cases of

neonatal purpura fulminans had PC but not PS deficiency (Ichiyama et al. 2016; Ohga et al. 2013a). It might account for the relative increase of free PS concentrations (by physiologically low levels of C4b-binding protein), the narrower ranged activity, and/or shorter half-life of PS during the early neonatal period. PS deficiency conveys a risk of thrombosis similar to PC deficiency. The clinical findings in the heterozygotes for PS deficiency are similar to those for AT or PC deficiency. Pulmonary emboli are the common affected lesions. PS-deficient patients experience an initial thrombotic event at approximately 25–30 years of age. Over half of episodes occurred spontaneously, and the remainder had certain risk factors for thrombosis. In pediatric cases, the OR for venous thrombosis due to PS deficiency is estimated to be 5.8 (Table 17.1) (Young G et al. 2008). Several reports suggest an association between PS deficiency and arterial thromboses including ischemic stroke in infants and children (Kenet et al. 2010).

On the other hand, adults with low PS levels but no family history of venous thrombosis have minimal risk of VTE. Low free PS or total PS level (both <0.10th percentile) was not associated with an increased risk of VTE. Young patients with recurrent VTE are associated with double mutations of *PROS1*. Their parents were reportedly asymptomatic although they carried type I PS deficiency. PS deficiency is classified into three types, according to the levels of total and free antigens, along with functional activity. Type I deficiency shows about half levels of normal PS antigen, although free PS antigen and functional activity levels are greatly reduced. Most patients have the missense mutations and base pair

Fig. 17.4 Blood clotting cascade and anticoagulation factors
 Each Roman numeral represents coagulation factor. The “a” means activated form of coagulation factors. *APC* activated protein C, *AT* antithrombin, *PC* protein C, *PS* protein S, *TF* tissue factor, *TM* thrombomodulin

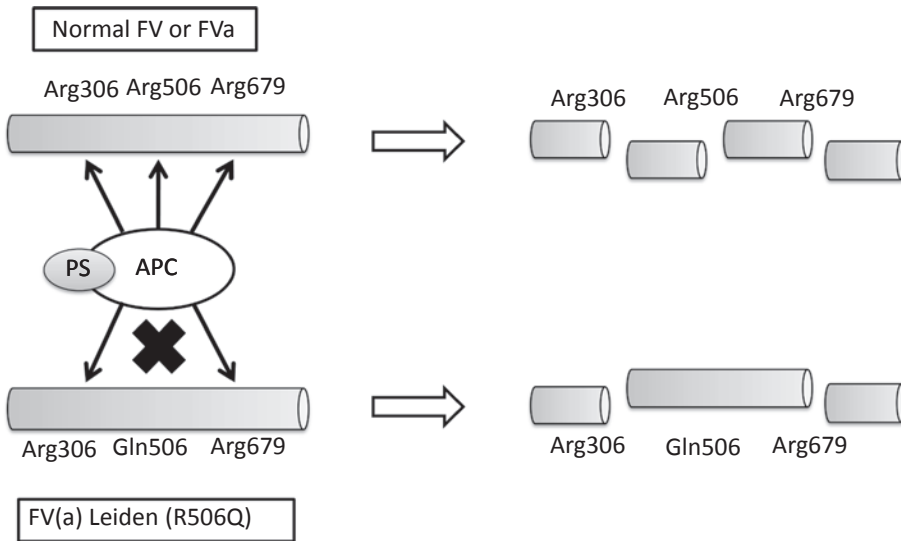
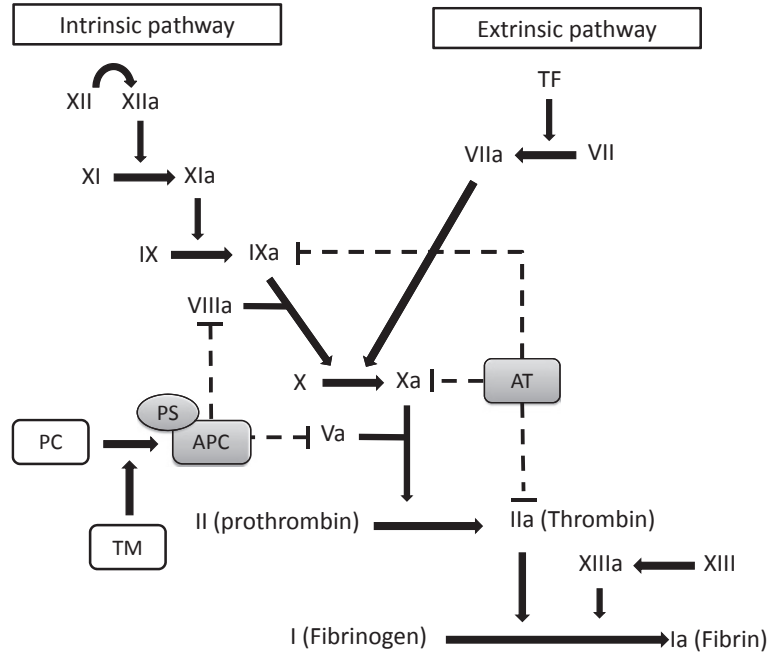


Fig. 17.5 APC resistance of factor V Leiden

Factor V Leiden (Arg506Gln) abolishes the Arg506 cleavage site by APC in factor V and factor Va. *APC* activated protein C, *PS* protein S

insertions. Type II is a qualitative deficiency with normal levels of total antigen and free PS, but impaired function. The rarity of type II-deficient patients indicates the insufficient screening power of functional assays. Type III is characterized by normal levels of total PS antigen, but

reduced levels of free PS and functional activity. Plasma activity levels rather than antigen levels are then preferable for screening PS deficiency. Although the biologic basis of type III PS deficiency remains unclear, *PROS1* mutations were responsible for the type I deficiency, but not the

type III phenotype of age-dependent free PS deficiency (Alhenc-Gelas et al. 2016). The prevalence of PS deficiency depends on the ethnicity. In Asian countries, PC and PS deficiencies are dominantly reported in adults and children with thrombosis. PS-Tokushima (Lys196Glu) is found in 1 per 55 healthy Japanese adults as a common low-risk variant for thrombosis in Japan (Ikejiri et al. 2010).

The lower limit of plasma levels of total and free PS (~65%) in heterozygous deficiency considerably overlaps the ranges of healthy controls (Ichiyama et al. 2016). PS levels are lower in female than male and increase with advanced age. PS, PC, and AT levels are reduced in inflammation. Because C4b-binding protein is an acute phase reactant, it shifts PS to the complexed inactive form leading to the reduced PS activity. On the other hand, PS but not PC levels decrease in pregnancy.

17.3.3 Antithrombin (AT) Deficiency

AT, previously called ATIII, is a plasma protein synthesized by the liver. The anticoagulant factor binds and neutralizes the serine proteases of thrombin, activated factor X, and activated factor IX, which are generated by the coagulation cascade. The AT-mediated effects on the inhibition of these proteases are enhanced by binding to heparin (Fig. 17.4). AT has two major active sites: the reactive center toward the carboxyl terminus and the heparin-binding site at the amino terminus. Thrombin cleaves the reactive site, and the inactive complex molecules are then cleared from circulation. Heterozygous AT deficiencies are found in about 1% of patients having the first episode of DVT. The thrombotic risk of inherited AT-, PS-, or PC-deficient patients is higher than that of factor V Leiden or factor II variant even in infants and children. They have also an eight- to tenfold higher risk for thrombosis than noncarriers (Young G et al. 2008). More than 50% of the first-degree relatives of each deficient patients experience VTE until the first 25 years of life (Holzhauer et al. 2012). The first thrombotic episode of AT deficiency occurs in more than 40% of

patients spontaneously. The remaining patients have some triggers including pregnancy, parturition, contraceptive use, surgery, or trauma. The OR of thrombosis with AT deficiency is estimated to be 9.4, although the risk of thromboembolic events depends on the population selected (Table 17.1) (Young G et al. 2008). Heritable AT deficiency is an autosomal-dominant disease with equal sex distribution. Common affected sites of thrombosis include deep veins in the legs, mesenteric veins, and pulmonary embolism. Complete AT defects due to null mutation lead to the fetal loss. Therefore, neonatal purpura fulminans is not the regular presentation of AT deficiency.

Heritable AT deficiency is classified into two types. Type I-deficient individuals show paralleled reduction of both antigen and activity levels of AT, representing a reduced synthesis of biologically normal protease inhibitor molecules. AT deficiency arises from mostly point mutations of the AT gene (*SERPINC1*) located on chromosome 1q23-25 and rarely a deletion encompassing the gene. Type II-deficient subjects show reduced levels of plasma activity but normal antigen levels of AT, representing a discrete molecular defect. Type II deficiency is subcategorized into three groups assessed by progressive AT assays and heparin-binding assays: the molecular abnormality in reactive site, heparin-binding site, and pleiotropic effect.

17.4 Factor V Leiden

The resistance to APC in an APTT-based clotting assay accounts for familial thrombophilia (Dahlback et al. 1993). The major genotype of APC resistance is factor V Leiden (G1691A, p. Arg506Gln) (Fig. 17.5) (Bertina et al. 1994), being found in more than 80% of APC-resistant patients. In Western countries, factor V Leiden was found in 20–50% of adult patients with VTE. Factor V Leiden is a risk factor for venous and arterial thrombosis. Retrospectively, heterozygosity for factor V Leiden was identified in 12% of patients who had a first episode of DVT or pulmonary embolism and in 6% of controls. In

the elderly (>60 years of age) who suffered from the first VTE without triggers, 26% were heterozygotes for factor V Leiden. Pediatric studies found that factor V Leiden heterozygosity conferred an OR of 3.8 for a first episode of VTE and 0.6 for recurrent VTE in children (Table 17.1) (Young G et al. 2008). The OR for pediatric cerebrovascular occlusion in Factor V Leiden carriers was 3.3 (Kenet G et al. 2010). On the other hand, the annual incidence of VTE in asymptomatic carriers of the factor V Leiden is low (0.58%), which raises questions about the screening asymptomatic family members (Middeldorp et al. 2001). Factor V Leiden was not associated with the risk of myocardial infarction or stroke, but potentially perinatal and pediatric arterial ischemic stroke. Factor V Leiden homozygotes may have the similar high risk of VTE to the heterozygotes for AT, PC, or PS deficiency. As factor V and AT gene are located on chromosome 1p, the coinheritance of factor V Leiden and AT mutations leads to a more severe thrombotic diathesis.

The prevalence of heterozygosity for factor V Leiden ranges 1–9% in Caucasians, but quite rare (<1%) in African blacks, Chinese, Japanese, or Native American ancestries. Factor V Leiden is more prevalent in northern Europe than the southern countries. A single founder allele was suggested among whites of differing ethnic backgrounds. Severe infections might shape the distribution of factor V Leiden, because of the survival advantage inferred by the mouse models. Co-segregation of heterozygous APC resistance due to factor V Leiden and type I factor V deficiency results in severe APC resistance in APTT assays, as found in homozygous factor V Leiden patients (pseudohomozygous). Several polymorphisms in factor V gene include a HR2 haplotype containing the R2 polymorphism (His1299Arg) with mild APC resistance. Some rare factor V mutations other than factor V Leiden showed also APC resistance (factor V Cambridge, Arg306Thr; factor V Liverpool, Ile359Thr; factor V Bonn, Ala512Val; and factor V Nara, Trp1920Arg).

17.4.1 Factor II (Prothrombin) Variant

Factor II (prothrombin)-related thrombophilias are encountered in adults with VTE. The factor II G20210A mutation located at the 3' untranslated region is considered a gain-of-function mutation, which leads to the increase of plasma prothrombin activity level (Soria et al. 2000). For the diagnosis of factor II G20210A, it should be kept in mind that the range of plasma activity level of prothrombin in heterozygotes or homozygotes overlaps the ranges of normal controls. Many individuals heterozygous and homozygous for factor II G20210A develop no thrombosis. Most patients of the heterozygotes also remain asymptomatic until adulthood. On the other hand, the relative risk of thrombosis in the heterozygotes is increased to two- to fivefold in adults and three- to fourfold in children. The recurrent risk for VTE is modest in factor II G20210A carriers. Pregnant loss and obstetric complications in the carriers are similar to those in factor V Leiden carriers.

The diagnosis depends on the genetic study. The factor II variant is not found in Asian ancestries, similarly in factor V Leiden, although factor II Yukuhashi (Arg596Leu) with AT resistance has been recently identified in a Japanese family. Because of the relatively low risk of thrombosis in factor V Leiden and factor II G20210A carriers, the routine screening is not recommended for healthy individuals as long as they have no positive family history of recurrent or young thromboses.

17.5 Prophylaxis and Treatment of Thromboses

Standard management for the first acute thrombosis in adults consists of a course of low-molecular-weight heparin or fondaparinux and concurrent warfarin therapy for at least 5 days on the monitoring for international normalized ratio.

Rivaroxaban, a factor Xa inhibitor, is approved for treatment of acute VTE and prevention of recurrence in adult cases. Factor Xa inhibitors raise no chance of developing heparin-induced thrombocytopenia. However, the pediatric use of factor Xa inhibitors is challenging, because the practical monitoring is on controversy. The standard therapy using tissue plasminogen activator for arterial thrombosis has not been established in the newborn infants.

The concept of anticoagulation therapy depends on the potential risks for (1) VTE recurrence and (2) therapy-related bleeding. Individuals with a spontaneous thrombosis with no identifiable provoking factors and those with persistent risk factors are candidates for the long-term anticoagulation therapy. Three-month treatment is recommended for individuals with transient (reversible) risk factors such as surgery. Graduated compression stockings should be worn for at least 2 years following an acute DVT in adult cases.

No consensus for the use of factor Xa inhibitors alternate for conventional heparin, and warfarin therapy exists on the optimal management for pediatric patients with PC, PS, and AT deficiencies. Fresh frozen plasma should be sufficiently administered to keep at least the lower limit of standard ranges based on the age. PC levels may be required to exceed 20% levels for the effective use of recombinant thrombomodulin, although the effective application is limited to patients with sepsis-induced DIC (Umemura et al. 2016). Thrombomodulin therapy has not been then established in patients with defective PC–PS pathway. Replacement therapy by using plasma-derived or recombinant PC and anti-thrombin concentrates is useful for the treatment of acute thrombosis as well as the prophylaxis for recurrence.

End-of-Chapter Questions

1. What are required to make the correct diagnosis for hereditary anticoagulant deficiencies?
2. Describe the difference of anticoagulant activities between infants and adults.
3. Describe the difference of genetic background between Caucasian and Asian population in hereditary anticoagulant deficiencies.

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