



ThromboNet System

The **ThromboNet System** enables diagnosis and management of thrombosis associated with congenital predisposing risk factors. **Factor II** (*Prothrombin*) and **Factor V** (*Leiden*) are proteins involved in the blood coagulation cascade. **Methylenetetrahydrofolate Reductase** (*MTHFR*) converts homocysteine to methionine as part of the pathway that converts 5,10- methylenetetrahydrofolate to 5-methyltetrahydrofolate. **Vitamin K Epoxide Reductase subunit 1** (*VKORC1*) is inhibited by warfarin, to exert its anticoagulant effect.

CLINICAL IMPLICATIONS.

Inherited thrombosis is characterized by increased risk of venous thromboembolism (VTE), deep vein thrombosis (DVT), ectopic pregnancy, pulmonary embolism (PE), myocardial infarction (MI), cardiovascular

disease and other complications related to abnormal blood coagulation. Patients with inherited thrombosis are treated with oral anti-coagulant medications such as warfarin (*Coumadin*[®]) and the newer drugs dabigatran (*Predaxa*[®]) and rivaroxaban (*Xarelto*[®]). Patients with *MTHFR* deficiency should be considered for folate supplementation.

High-Priority Profiles for Patients + *ThromboNet System*:

- Women who are Pregnant or on Contraceptives
- History of Deep Vein Thrombosis, Embolism, VTE
- Systemic Lupus Erythematosus
- Long-term Anticoagulation + Antithrombotic Drugs
- Myocardial Infarction or Arterial Restenosis
- Methotrexate Treatment for Cancer, Arthritis

Factor V, Factor II, *MTHFR* and *VKORC1* testing should be considered in patients with any type of venous thrombosis (hepatic, mesenteric, cerebral and recurrent), with a strong family history of thrombotic disease, pregnant women with venous thrombosis, women with history of pregnancy difficulties such as miscarriages, placental abruption, intrauterine fetal growth retardation or still birth, or women taking oral contraceptives and female smokers with myocardial infarction.

MOLECULAR BASIS. The **ThromboNet System** detects the following 5 mutations:

Gene	CPT Code	Genomic Mutation	Amino Acid Alteration
Factor II (<i>Prothrombin</i>)	81240	<i>G20210A</i>	Non-coding (3' UTR)
Factor V (<i>Leiden</i>)	81241	<i>G1691A</i>	Arg 506 Gln
MTHFR	81291	<i>C677T, A1298C</i>	Ala 222 Val, Glu 429 Ala
VKORC1	81355	<i>G3673A</i>	Non-coding (Promoter)

PREVALENCE. The *Factor II G20210A* and *Factor V G1691A* mutations are present in ~2% and ~5% of individuals with North European ancestry respectively. The *MTHFR C677T* and *A1298C* mutations are present in 40% to 50% of individuals with North European ancestry, and the frequency varies considerably in other ethnic groups.



Factor II (*Prothrombin*): ThromboNet System

PATHOPHYSIOLOGY. Clotting Factor II, or prothrombin, is a vitamin K–dependent proenzyme that functions in the blood coagulation cascade. An important disorder of prothrombin is the prothrombin *G20210A* mutation. First reported in 1996 as a familial cause of venous thromboembolism, the prothrombin *G20210A* mutation results in increased levels of plasma prothrombin and a concurrent increased risk for the development of thrombosis. The prothrombin *G20210A* mutation involves the substitution of an adenine for a guanine at position 20210 within the 3' untranslated region of the prothrombin gene. This mutation alters the polyadenylation site of the gene and results in increased mRNA synthesis, with a subsequent increase in protein expression.

CLINICAL IMPLICATIONS. Individuals carrying the prothrombin 20210A mutation have a 2- to 3-fold increased risk for developing thrombosis. One case-control study found evidence of an increased risk of developing an ischemic cerebrovascular event in men aged younger than 60 years with the prothrombin 20210A mutation. A study of cancer patients in the Netherlands found that the presence of the prothrombin 20210A mutation in these patients may increase the risk of venous thrombosis to a level greater than that attributable to the malignancy alone.

Prothrombin *G20210A* is a genetic variant that approximately doubles or triples the risk of forming blood clots in the veins. The variant is commonly associated with the disease venous thromboembolism (VTE), which includes both deep vein thrombosis and pulmonary embolism. Heterozygous carriers who take oral contraceptives are at a 15-fold increased risk of VTE, while carriers also heterozygous with Factor V Leiden have an approximate 20-fold higher risk. Prothrombin *G20210A* is one of the most common genetic risk factors for VTE.

MOLECULAR BASIS. The polymorphism is located in a noncoding region of the prothrombin gene (3' untranslated region nucleotide 20210), replacing guanine with adenine. The position is at or near where the pre-mRNA will have the poly-A tail attached. The variant causes elevated plasma prothrombin levels (hyperprothrombinemia), possibly due to increased pre-mRNA stability. Prothrombin is the precursor to thrombin, which plays a key role in causing blood to clot (blood coagulation). *G20120A* can thus contribute to a state of hypercoagulability, but not particularly with arterial thrombosis. A 2006 meta-analysis showed only a 1.3-fold increased risk for coronary disease.

PREVALENCE. Prothrombin 20210A has an estimated prevalence of 2 to 3% in Caucasian individuals. The mutation is more prevalent in those of southern European descent than in those of northern European descent, and it is rarely seen in Asians or Africans.



Factor V (Leiden): ThromboNet System

PATHOPHYSIOLOGY. Factor V is a clotting protein. People with Factor V Leiden have a genetic mutation that causes the Factor V protein to respond more slowly to being deactivated by the anti-clotting factors. In the normal clotting process, anti-clotting proteins combine to help break up Factor V to keep it from being reused and forming clots when clotting is not needed. The Factor V Leiden mutation keeps the anti-clotting proteins from breaking down Factor V, which maintains it intact and increases the chance of clotting.

In the unaffected person, Factor V functions as a cofactor to allow factor Xa to activate an enzyme called thrombin. Thrombin in turn cleaves fibrinogen to form fibrin, which polymerizes to form the dense meshwork that makes up the majority of a clot. Activated protein C (aPC) is a natural anticoagulant that acts to limit the extent of clotting by cleaving and degrading Factor V.

CLINICAL IMPLICATIONS. Patients with Factor V Leiden who have developed blood clots are treated with oral anti-coagulant medications such as warfarin (*Coumadin*[®]) and the newer drugs dabigatran (*Predaxa*[®]) and rivaroxaban (*Xarelto*[®]). These anticoagulants can lessen the risk of developing additional blood clots and help to avoid potentially serious complications. A blood clot (thrombus) normally forms to stop the bleeding when an artery or vein is damaged, such as when you experience a cut. Clots form as a result of chemical reactions between specialized platelets and clotting factors. Substances in the blood known as anti-clotting factors control excessive formation of blood clots.

Patients with Factor V Leiden, have either inherited one copy of the defective gene (heterozygous), which slightly increases their risk of developing blood clots, or more rarely, inherited two copies, one from each parent (homozygous), which significantly increases the risk of thrombosis.

MOLECULAR BASIS. Factor V Leiden is an autosomal dominant genetic condition that exhibits incomplete dominance, *i.e.* many people carrying the mutation do not suffer any consequences. The condition results in a Factor V variant that cannot be as easily degraded by aPC (activated Protein C). The gene that codes the protein is referred to as *Factor V*. The *Factor V G1691A* missense mutation changes amino acid 506 from Arginine to Glutamine. Since this amino acid is normally the cleavage site for aPC, the mutation prevents efficient inactivation of Factor V. When Factor V remains active, it facilitates overproduction of thrombin leading to generation of excess fibrin and excess clotting.

PREVALENCE. Factor V Leiden is the most common inherited form of thrombophilia. Between 3 to 8% of people with European ancestry carry one copy of the factor V Leiden mutation in each cell, and about 1 in 5,000 people have two copies of the mutation. The mutation is less common in other populations.



MTHFR: ThromboNet System

PATHOPHYSIOLOGY. **Methylenetetrahydrofolate reductase (MTHFR)** is the rate-limiting enzyme in the methyl cycle, and it is encoded by the *MTHFR* gene. Methylenetetrahydrofolate reductase catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine methylation to methionine. Mutations in the *MTHFR* gene are associated with methylenetetrahydrofolate reductase deficiency.

CLINICAL IMPLICATIONS. Genetic variation in this gene may influence susceptibility to occlusive vascular disease, neural tube defects, dementia, mental illness, colon cancer, and acute leukemia. The MTHFR enzyme is involved in folate metabolism. Patients with MTHFR deficiency should be considered for folate supplementation. Because of this, patients who have *MTHFR* mutations and take drugs that affect folate metabolism (e.g. methotrexate), may be more likely to experience toxicity. *MTHFR* testing is valuable for methotrexate prescription to adjust dosages and reduce risk of toxicity.

Low folate intake affects individuals with the 677 *T/T* genotype to a greater extent than those with the 677 *C/C* or 677 *C/T* genotypes. 677 *T/T* individuals with lower plasma folate levels are at risk for elevated plasma homocysteine levels. In studies of human recombinant MTHFR, the protein encoded by 677*T* loses its FAD cofactor three times faster than the functional protein. 677 *T/T* individuals are at an increased risk for certain leukemias and colon cancer. Mutations in the *MTHFR* gene could be one of the factors leading to increased risk of developing mental illness.

Individuals of 677 *T/T* are predisposed to mild hyperhomocysteinemia, because they have less active MTHFR available to produce 5-methyltetrahydrofolate (which is used to decrease homocysteine). Low dietary intake of the vitamin folic acid can also cause mild hyperhomocysteinemia.

MOLECULAR BASIS. The *MTHFR* nucleotide at position 677 in the gene has two variants: *C* (cytosine) or *T* (thymine). *C* at position 677 (leading to an Alanine at amino acid 222) is the normal allele. The 677*T* allele (leading to a Valine substitution at amino acid 222) encodes a deficient, thermolabile enzyme with reduced activity. Individual with two copies of 677*C* (677 *C/C*) have the normal or functional genotype. Individuals with two copies of 677*T* (677 *T/T*, homozygotes) have mild MTHFR deficiency.

At nucleotide 1298 of the *MTHFR* gene, there are two variants: *A* or *C*. 1298*A* (leading to a Glutamic acid at amino acid 429) is the most common while 1298*C* (leading to an Alanine substitution at amino acid 429) is less common. 1298 *A/A* is the normal homozygous, 1298 *A/C* the heterozygous, and 1298 *C/C* the homozygous for the variant.

PREVALENCE. About 10% of the North American population is 677 *T/T* for this polymorphism. Frequency in individuals of Mediterranean ancestry and Hispanics is greater than the frequency in Caucasians, which is greater than in individuals of African descent.