Factor V Leiden Genotyping Assay: Genetics and Clinical Overview

Test Information (v1_020112):
Factor V Leiden Genotyping Assay (CMGDL test code 4202)
For sample collection, transport and testing information refer to our website www.medgen.med.miami.edu/
See the link for additional test ordering information such as CPT codes, test methodology and limitations.

Background Information
Thrombosis is the leading cause of death worldwide affecting 1 in 1000 individuals annually. Venous thrombosis is triggered by venous stasis, endothelial abnormalities and susceptibility genetic factors. Thrombophilia is defined as the tendency in a disturbance of the plasma coagulation system, and categorized into acquired and inherited. In acquired thrombophilia the abnormal clotting is usually related to a condition that leads to an increased clotting tendency such as recent surgery, trauma, prolonged immobility, pregnancy, obesity, cancer, inflammatory bowel disease, presence of antiphospholipid antibodies, or the use of oral contraceptives or hormone replacement therapy. Inherited Thrombophilia is associated with congenital predisposition factors such as Factor V Leiden mutation, Prothrombin (Factor II) mutation, Methylentetrahydrofolate Reductase (MTHFR) mutation, Protein C deficiency, Protein S deficiency, and Antithrombin III deficiency (Heit, 2007, Dahblack, 2008).

Factor V Leiden, Factor II 20210G>A, and Methylene-tetrahydrofolate Reductase (MTHFR) (667C>T and 1298A>C) mutations are the most common cause of predisposition to inherited thrombophilia (Rosendaal, F.R. 1998).

Factor V Leiden Thrombophilia
Background Information
The Factor V gene spans more than 80 kb on chromosome 1q23 and contains 25 exons. Factor V is converted to the active single-chain glycoprotein (FactorVa) by thrombin. The activated factor V serves as an essential protein in the coagulation cascade and acts as a cofactor for the conversion of prothrombin to thrombin by factor Xa. Factor Va is inactivated by proteolytic activity of Activated Protein C (APC) that cleaves Factor Va at positions R306, R506, and R679. Replacement of arginine (Arg) at position 506 by glutamine (Gln) (1691G>A, R506Q) abolishes the cleavage site of APC at position R506, resulting in >90% resistance to activated protein C. Factor V resistance to APC results in venous thromboembolism (VTE) (Kriffin, 2011, Kujovich, 2010, Nicolas, 2002, Bertina, 1994).

Clinical Significance
Clinical Diagnosis
Clinical suspicion of Factor V Leiden thrombophilia arises with presence of a personal or family history of VTE (deep vein thrombosis or pulmonary embolism), especially if VTE occurred during pregnancy or following the use of oral contraceptives.

Laboratory Diagnosis
Factor V Leiden thrombophilia can be diagnosed either biochemically (APC, Activated Protein C resistance assay), or through molecular detection of F5 mutations, specifically a 1691G>A substitution responsible for the R506Q missense mutation.

Molecular Genetics, inheritance and disease risk
- An autosomal dominant mode characterizes factor V Leiden allele transmission and the corresponding risk for VTE;
- Testing of at risk family members of an affected individual (e.g.. parents and first degree relatives) should be performed for counseling purposes, due to the high prevalence of the F5 gene 1691G>A mutation in the population;
- Heterozygous carriers of Factor V Leiden have a 3-8 fold increased risk of VTE;
- Homozygous individuals of Factor V Leiden have a 9 to 80-fold increased risk of VTE;
- The combination of factor V Leiden allele and thrombophilic disorders (including protein C deficiency, protein S deficiency, and antithrombin
deficiency) has a supra-additive effect on the overall thrombotic risk;  
- Thrombophilia risk increases 20 folds if both a Factor V Leiden mutation and a prothrombin 20210G>A heterozygous allele are present;  
- Factor V Leiden heterozygote individuals have a 4-5 fold increased risk for recurrent VTE if hyperhomo cysteinemia is co-existing versus individuals with a factor V Leiden allele alone [Meinardi et al 2002].  
- A F5 gene 1691G>A mutation carries a 5-to-52-fold increase in thrombotic risk during pregnancy and the puerperium when compared to non-pregnant women without thrombophilia.  
- The risk for VTE is substantially increased in Factor V heterozygote women taking oral contraceptives (Hirsch,1996, Laczkovic, 2007, Kujovich, 2010)  

Thromboembolism risk and Patient management  
Factor V Leiden mutation identification has profound implications for the patient management. In conjunction with F5 gene 1691G>A mutation testing, prothrombin gene mutation analysis (g.20210G>A) and other biochemical tests (such as Homocysteine levels, multiple phospholipid-dependent coagulation assays for a lupus inhibitor; serologic assays for anticardiolipin antibodies and anti-beta2-glycoprotein I antibodies) are helpful in establishing the overall risk of VTE in a patient. Additional testing for protein C, antithrombin and protein S activity may also be considered. Mutation zygosity and presence of additional risk factors may warrant to start anticoagulation therapy (such as prophylactic anticoagulation or long term oral anticoagulation, depending on the clinical situation), refrain from certain medications (such as oral contraceptives and hormonal replacement therapy), and also create awareness of signs and symptoms of VTE in a patient (thus allowing prompt intervention in high risk individuals) (Kujovich, 2010).

Factor V Leiden testing is indicated and recommended if  
- A patient tested positive by APC Protein Resistance. The molecular test helps to distinguish heterozygote from homozygote mutations;  
- A patient has borderline APC resistance assay values. The molecular test helps to establish the genetic status;  
- A patient has very low APC resistance values. The molecular test helps to differentiate heterozygote, homozygote, and pseudo-homozygote genotypes (See Pseudo-homozygous APC resistance”);  
- A first unprovoked VTE presented at any age (especially age is less than 50 years);  
- A history of recurrent VTE is present;  
- Venous thrombosis at unusual sites is present (e.g., cerebral, mesenteric, portal, and hepatic veins);  
- VTE occurs during pregnancy or the puerperium;  
- VTE is associated with use of oral contraceptives or hormone replacement therapy;  
- A first VTE occurs in an individual with a first-degree family member with VTE before age 50 years.

Factor V Leiden testing may be considered in cases of:  
- Women with unexplained fetal loss after ten weeks’ gestation;  
- Women with unexplained severe preeclampsia or "HELLP" (hemolysis, elevated liver enzymes and low platelets), placental abruption, or a fetus with severe intrauterine growth restriction;  
- Female smokers younger than age 50 years with a myocardial infarction or stroke;  
- Individuals older than age 50 years with a first provoked VTE in the absence of malignancy or an intravascular device;  
- Asymptomatic adult family members of probands with a known factor V Leiden mutation, especially those with a strong family history of VTE at a young age;  
- Asymptomatic female family members of probands with known factor V Leiden thrombophilia who are pregnant or are considering oral contraceptive use or pregnancy;  
- Women with recurrent unexplained first-trimester pregnancy losses with or without second- or third-trimester pregnancy losses;  
- Neonates and children with non-catheter related idiopathic VTE or stroke;  
- Individuals younger than age 50 years with unexplained arterial thrombosis;  

Factor V Leiden testing is not recommended as:  
- General population screening or routine test;  
- Prenatal or newborn testing;  
- Prenatal testing is not performed by the CMGDL

Related Tests (visit our website at www.medgen.med.miami.edu/  
Thrombophilia Risk Genotyping Assay (CMGDL test code 4002)  
Factor II G20210A and Factor V Leiden Genotyping Assay (CMGDL test code 4102)  
Factor II G20210A Genotyping Assay (CMGDL test code 4302)  
MTHFR Genotyping Assay (CMGDL test code 4402)
References

#Pseudo-homozygous APC resistance
Pseudohomozygotes are heterozygous for both factor V Leiden and a second mutation causing a factor V deficiency (factor V null mutation). APC resistance of Factor V Leiden pseudohomozygotes is indistinguishable from that of homozygote patients for the Factor V Leiden mutation (Brugge, 2005).