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# Genetic Considerations in Thrombotic Disorders

This issue of the Genetic Drift is dedicated to the memory of our Montana colleague, Karen Streets, member and avid supporter of the Mountain States Genetics Network for the past 12 years. Karen served on the prenatal committee and her spirit lives on through her many contributions to quality prenatal screening for the network laboratories.

## **From the Editor and Authors:**

The fall 2002 *Genetic Drift* deals with a broad range of topics around the common theme of thrombotic disorders. Primary care providers are already well aware of the importance of adult thrombotic disease and we provide a comprehensive state-of-the-art review by a clinical geneticist who is also an internist. Pediatric thrombotic disorders are increasingly recognized and a complete review, including diagnosis and management, is provided by a pediatric hematologist. Researchers in clinical genetics and child neurology are just beginning to appreciate the role of thrombophilia in fetal loss and neonatal stroke, and recently published investigations in this area are summarized by a pediatric clinical geneticist. The Teratogen Hot Topic for this issue is, naturally, a compilation of the anticoagulants commonly used during pregnancy. The issue ends with discussion of genetic counseling for heterozygosity for the common factor V Leiden mutation.

The articles in this issue were authored by Matthew Taylor, MD (CO), Prasad Mathew, MD (NM), Terri Grebe, MD (AZ), Lynn Martinez (UT), Katherine Berry, MS, CGC (MT) and Elaine Spector, PhD (CO).

Carol L. Clericuzio, MD (NM), Editor

## ***In This Issue***

**Adult Thrombotic Disorders**  
**Pediatric Thrombotic Disorders**

**Fetal and Neonatal Effects of  
Maternal/Fetal Thrombotic Disorders**

**Teratogen Hot Topic: Anticoagulants**  
**Heterozygote Counseling for Factor V  
Leiden mutation**

# Adult Thrombotic Disorders

## Introduction

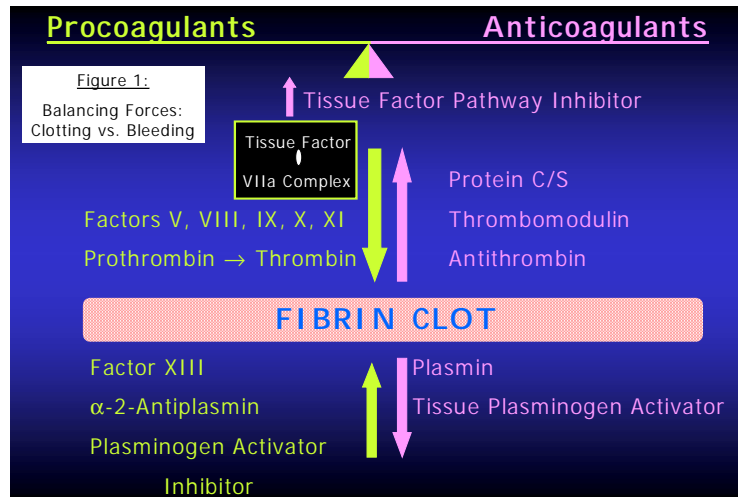
Venous thromboembolism (VTE) is a common condition, affecting an estimated 1.6-1.8 persons per 1,000 annually [Nordstrom 1992] [Hansson 1997]. The disease is familiar to most primary care providers who treat adult patients. Substantial morbidity and mortality result from the development of VTE in both ill and apparently healthy individuals. Pulmonary embolism, the most serious complication of the disease, may affect as many as 600,000 persons in the United States each year, with approximately 60,000 of those dying as a result [Rubinstein 1998]. The so-called post-phlebotic (or post-thrombotic) syndrome is not life threatening but causes substantial disability for many patients. Although VTE does occur in children (estimated 1:100,000 incidence) and certain select populations (e.g. pregnant women, oral contraceptive users, post-surgical patients) the bulk of disease is manifest in older patients (where the incidence approaches 1:100 persons) [Rosendaal 1997]. A large number of acquired and inherited causes of VTE are known (Table 1); and in many cases acquired and inherited risk factors combine to produce disease. The remainder of this article focuses principally on the genetic contributions to VTE risk.

Table 1: Acquired and Inherited Causes of VTE †	
Acquired	Inherited
Neoplastic Conditions	Factor V Leiden mutation
Pregnancy	Prothrombin mutation
Oral Contraceptive Use	MTHFR mutations
Surgery / Trauma	Protein C or S deficiency
prolonged immobilization	Antithrombin III deficiency
Antiphospholipid antibodies	Dysfibrinogenemia
Prior VTE	
Advancing Age	

†: This list is not intended to be exhaustive

## History of Inherited Thrombophilia

Hemostasis is a critical function for complex organisms and involves a delicate balance between procoagulant and anticoagulant factors (Figure 1). The involved proteins in this system are encoded for by a number of genes in the DNA. Historically, genetic factors were felt to be only rare contributors to the risk of developing VTE. Deficiencies in proteins C and S along with antithrombin III deficiency were among the first genetic conditions to be implicated in VTE. They are characterized by autosomal dominant inheritance (present in each generation with 50% risk of transmission to offspring) and may be implicated in only about 5-7% of cases of VTE. In these rare instances, recognition that a genetic cause of VTE was present typically occurred in the setting of recurrent VTE and/or a family history of VTE in close relatives. The documentation of low levels of the various factors confirmed the diagnoses in these cases.



## Increased Recognition of Genetic Factors

From a genetic standpoint, the clinical landscape began to change when, in the early 1990s, researchers began to collect and study families that contained multiple members affected by VTE or recurrent VTE (suggesting a genetic mechanism) but who did not have detectable deficiencies in certain factors. The discovery that a proportion of these patients were resistant to the action of activated protein C was foundation for the discovery, in 1994, of the factor V Leiden mutation (described below). A year later a prothrombin mutation was discovered as another VTE risk factor. These findings, merged with new understanding of the genetic basis of mildly elevated homocysteine levels, elevated genetic considerations into a position of importance in evaluating VTE. Although the absolute risks of VTE in patients with these mutations are modest compared with those in the cases of protein C/S and antithrombin III deficiencies, the impact on VTE is noteworthy as the mutations are relatively common. When the effects of factor V, prothrombin, and homocysteine are considered, a genetic component to the development of VTE can now be identified in 30-45% of unselected thrombotic episodes (Table 2).

<b>Inherited Risk for VTE</b>	<b>Healthy Persons (%)</b>	<b>Persons with VTE (%)</b>	<b>Odds Ratio</b>
Protein S	?	2.3	?
Protein C	0.2-0.4	3.7	9.3-18.5
Antithrombin III deficiency	0.02	1.9	95
Prothrombin mutation	2.7	7.1	2.6
Factor V Leiden mutation	4.8	20	4.2

From: Seligsohn 2001 and Rosendaal 1999

### The Genetic Basis of Factor V Leiden and Prothrombin Mutations

The factor V Leiden and prothrombin gene mutations are the two most common genetic risk factors for VTE, being present in approximately 25% of cases of VTE [Seligsohn 2001]. A detailed explanation of the molecular genetic aspects of these two important mutations is beyond the scope of this review. A familiarity with the fundamental concepts behind these mutations, however, is of value in understanding these mutations in a clinical context. In the 1990s it was recognized that a proportion of VTE patients demonstrated resistance to activated protein C (APC). Protein C functions as an anticoagulant, in part by *inactivating* factor V (which has *procoagulant* activity). In the APC resistant patients the anticoagulant activity of APC on factor V is attenuated ("the factor V is *resistant* to the actions of APC").

In approximately 80% of the APC-resistant patients, an explanation for the "resistance" lies in a mutation in the gene for factor V. The mutation, a single nucleotide change, alters the amino acid sequence of factor V and is called the factor V Leiden mutation (Leiden is the city where the mutation was first detected). The alteration occurs exactly at one of the sites of action for APC, rendering APC less efficient at inactivating the factor V. With effectively more factor V (a procoagulant), an increased tendency to VTE is present.

Persons carrying a factor V mutation are at an increased risk of developing VTE (approximately 20% by mid-adulthood) [Zoller 1997], which represents a 4- to 7-fold risk over non-carriers. This risk is not particularly striking, except for the observation that roughly 5% of the general population carries a single factor V mutation (as high as 15% in Northern Europe). It is the presence of a "modest" VTE risk spread over a large number of individuals that accounts for the importance of this mutation. Homozygotes (individuals with two factor V mutations) have a greatly increased risk of VTE, estimated to be roughly 80-fold over the general population.

## Other Genetic Risk Factors for VTE

The list of other genetic contributors to VTE continues to grow. The genetic basis of protein C and S deficiency and antithrombin III deficiency are now reasonably well established. More recently, attention has focused on mutations in the 5,10-methylenetetrahydrofolate reductase gene (MTHFR) as leading to modest increased levels of homocysteine which is a risk factor for VTE and arterial events. A general algorithm for determining which genetic tests to pursue (and in what order) based on the prevalence of the individual mutations has recently been suggested [Seligsohn 2001]. In this clinically oriented review, Seligsohn and Lubetsky suggest a diagnostic workup based on tests that should be given “high priority” (more common contributors to VTE), “intermediate priority,” and “low priority” (rarely contributing to VTE). The “high priority” tests suggested are listed in Table 3.

Table 3* “High Priority” Tests for VTE
Factor V Leiden Variant <sup>†</sup>
Prothrombin Variant <sup>†</sup>
Serum Homocysteine
Factor VIII (elevated)
Lupus Anticoagulant
*Adapted from Seligsohn 2001
<sup>†</sup> DNA based test

## Multiple Risk Factors for VTE

As more is learned about genetic contributors to VTE it has become increasingly clear that in many cases more than one risk factor may be present. This finding fits well with the concept that the genetic mutations are best thought of as being *risk* factors for VTE, rather than *causative* of VTE. As suggested previously, referring to these genetic changes as “variants” highlights their roles as risk factors rather than powerful mutations that inevitably cause disease. In the case of factor V Leiden mutations, for instance, 20% of mutation carriers develop VTE by mid-adulthood, implying that 80% of the carriers do *not* develop VTE by this age. The interaction of multiple risk factors justifies the approach suggested by Table 3 where a panel of VTE risk factor testing is performed.

## Clinical Application of Genetic Evaluation for VTE

The genetic thrombophilia field continues to evolve and the relatively novelty of these discoveries means that long-term prospective data on the use of this information to promote health and prevent disease is clearly lacking. Not surprisingly an overall consensus on how best to integrate this knowledge into clinical practice has proven to be elusive. The American College of Medical Genetics has addressed the uncertainties in this area with a published statement outlining the reasonable use of factor V Leiden testing (Table 4). These guidelines are intended to assist clinicians in evaluating patients with venous thromboembolism. In many cases the involvement of a geneticist and/or hematologist familiar with the thrombophilias is entirely appropriate.

Table 4: Clinical Testing for Factor V Leiden Variant <sup>†</sup>		
Testing Recommended	Consider Testing	Testing Not Recommended
<ul style="list-style-type: none"> <li>•Age &lt; 50, any venous thrombosis (VT)</li> <li>•Unusual site of VT (hepatic, mesenteric, cerebral veins)</li> <li>•Recurrent VT</li> <li>•VT in pregnancy</li> <li>•VT in oral contraceptive (OCP) users</li> <li>•Relatives of individuals with VT &lt; 50 years</li> <li>•Myocardial infarction in female smokers &lt; 50 years</li> </ul>	<ul style="list-style-type: none"> <li>•Age &gt; 50, any VT</li> <li>•Relatives of persons known to have Factor V mutations where such knowledge may impact management of surgery or pregnancy or use of OCPs</li> <li>•Recurrent pregnancy loss, severe preeclampsia, placental abruption, intrauterine fetal growth retardation, stillbirth</li> </ul>	<ul style="list-style-type: none"> <li>•Random screening of healthy population</li> </ul>

<sup>†</sup> Adapted from Grady et al. 2001

### Thrombophilia and Oral Contraceptive Use

As the factor V mutations and prothrombin gene mutations are relatively common, a consideration of these disorders in women who take oral contraceptives (OCPs) is warranted. There has been (and continues to be) some debate over whether women taking OCPs should undergo counseling and screening for common prothrombotic states. Based on case-control studies it appears that the factor V Leiden mutation greatly increases the risk of thrombosis in women taking OCPs. Estimates of as high as a 35-fold risk of thrombosis is found in women who are both factor V Leiden mutation carriers and taking OCPs [Andersen 1998]. Furthermore, female carriers of factor V mutations who begin OCPs appear to develop thromboembolic disease sooner than non-carriers who initiate OCPs [Bloemenkamp 2000]. These strikingly elevated risks have led some to suggest that universal screening for factor V mutations should be done for all women prior to initiation of OCPs. However, for women with a negative personal or family history of thromboembolic events, this strategy proves to be relatively costly and inefficient. It is estimated that perhaps as many as 500,000 women would need to be screened for factor V mutations to prevent a single excess death from pulmonary embolism [Vandenbroucke 1996]. An additional concern is that a substantial number of women would be identified with factor V Leiden mutations who would *not* have developed thrombosis on OCPs. Potentially denying this group of women OCPs and exposing them to the *risks* associated with pregnancy generates concerns about competing risks for these women. Based on these estimates and concerns it seems more prudent to selectively offer screening only to women who are identified as being at elevated risk from family history data.

### Summary

Hemostasis is a complex balance between thrombosis and bleeding that is coordinated through a number of interacting proteins. Mutations in a number of the genes encoding these proteins have been identified and it is now clear that hereditary factors contribute significantly to thromboembolic disease. The factor V Leiden and prothrombin gene mutations account for as much as 25% of venous thromboembolic disease and merit consideration in the workup of VTE disease. Universal screening for thrombophilias is not currently recommended. However, testing for factor V Leiden mutations has been recommended for thrombosis characterized by: age<50, recurrent event, in pregnancy, in combination with OCPs, in relatives of individuals who had VTE before age 50, in tobacco-using women with myocardial infarctions before age 50, and in the presence of VTE at "unusual sites." In particular, women with factor V Leiden mutations who use OCPs appear to be at high risk of VTE. With an ever-growing number of available

genes/proteins to test, prioritization of “high yield” testing strategies has been recommended. In the near future, the development of inexpensive “thrombophilia panels” may make the evaluation of thrombophilia genes commonplace in clinical practice.

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**Contributed by Matthew Taylor, MD (CO)**

# Pediatric Thrombotic Disorders

The term thrombophilia is used to describe a condition where a person is at increased risk for clot formation (hypercoagulable) in the venous or arterial system. In the late 1800s, Virchow first identified hypercoagulability as a predisposing factor for thromboembolic events. Antithrombin III deficiency was one of the first identified causes for a hypercoagulable state. Since then, several congenital abnormalities of hemostasis that predispose to thromboembolic events have been identified, and in some, the molecular defects delineated.

Childhood thrombosis is receiving increased attention in the past few years. Emerging information regarding coagulation protein structure and function is adding to new understanding of an old disease. In addition, research in vascular biology and atherosclerosis is shedding light on other biochemical pathways that contribute to vascular damage and thrombogenesis. Patients with single gene defects for recognized congenital prothrombotic disorders rarely present with a first thromboembolic event during childhood, unless they also have an acquired risk factor that unmasks the defect. Patients who are homozygous or double-heterozygous for one or more genetic lesions linked to congenital prothrombotic disorder will frequently present with clinical symptoms as newborns or young children.

At the time of initial presentation, a careful history, physical examination, and laboratory evaluations are required. Interpretation of laboratory results in the infants should take into consideration the physiologic low values of coagulants and anticoagulants in this age group. Although abnormal laboratory value results frequently are linked to thromboembolic events, they may not always be causally linked – and consideration must be given to the prevalence and incidence in the general population.

## **Congenital Prothrombotic Disorders Established to Cause a Thromboembolic Event**

Clinically significant relationships have been confirmed between thromboembolic events and deficiencies of protein C, protein S, antithrombin, presence of factor V Leiden (Arg506Gln), prothrombin mutation (G20210A), and some dysfibrinogenemias in adults. These are aberrations in the natural anticoagulant systems that occur in the plasma and at the endothelial cell level. They are usually associated with venous rather than arterial thrombosis, and commonly first occur in the presence of acquired risk factors such as pregnancy, immobilization, surgery, etc. A genetic abnormality predisposing to thromboembolic events can now be identified in up to one-third of unselected adult patients with thromboembolic events, and more than one-half of adult patients with familial thrombosis. The overall increase in risk of thromboembolic events for adults with one of these abnormalities is approximately 10-fold, compared to adults without any abnormality. There is accumulating evidence that multiple coexisting defects are present in adults with the most marked tendency to develop thromboembolic events (Table 1). Hyperhomocysteinemia is now recognized as another important etiology for thrombosis especially of the arterial side. This condition induces its prothrombotic state by adversely affecting the vessel wall, the coagulation system, and the platelet function.

## **Congenital Prothrombotic Disorders That Might Contribute to a Thromboembolic Event**

Congenital prothrombotic disorders that might contribute to a thromboembolic event include abnormalities in the fibrinolytic system, such as plasminogen deficiency, tissue plasminogen activator deficiency, increased plasminogen activator-1, heparin cofactor II deficiency, and decreased factor XII levels (Table 1).

## **Prevalence of Congenital Prothrombotic Disorders in the General Population**

The true prevalence of congenital prothrombotic disorders in the general population is not known. In Caucasians, the prevalence of protein C deficiency is estimated to be 0.3%, antithrombin deficiency is 0.04%, factor V Leiden is 5%, and prothrombin gene polymorphism is 2%. There are no studies of sufficient size to estimate the prevalence of protein S deficiency among healthy adults. Additionally, the prevalence of congenital prothrombotic disorders varies in different ethnic populations; e.g., the prevalence of factor V Leiden varies from approximately 5% in the Caucasian population to <1% in the African and Asian populations. The prevalence of these abnormalities increases in unselected adults with their first thromboembolic event. In those with thrombosis and suspected thrombophilia (positive family history), the prevalences for deficiencies of protein C, protein S, and antithrombin are higher by 5% to 10%. The prevalence for factor V Leiden is approximately 50%, thus emerging as the most frequent congenital prothrombotic risk factor for a thromboembolic event (Table 2).

## **The Incidence of Thromboembolic Events (DVT and PE) in Children**

The incidence of thromboembolic events (DVT [deep vein thrombosis] and PE [pulmonary embolism] ) in children between 1 month and 18 years of age is estimated to be 0.07 cases and 5.3 cases/10,000 hospital admissions, respectively. Two prospective large registry studies reported the incidence of symptomatic neonatal DVT to be 0.24 to 0.26 events per 10,000 births. Comparable incidences of DVT and PE in the adult population are approximately 2.5 to 5%. The incidence in pediatric thrombosis may be increasing secondary to increased utilization of vascular access devices in the intensive care units, and increased use of indwelling catheters for intravenous antibiotics, parenteral nutrition, and chemotherapy. The inherited causes of thrombophilia in children appear to be similar to those in the adult populations. In contrast to adults, in whom thromboembolic events are idiopathic in 40% of patients, only 5% of cases are idiopathic in children. The ratio of venous to arterial thrombosis is approximately 2.5 to 1. The diagnosis of thrombosis is made at a median age of approximately 12 years, with peaks in the neonatal and adolescent periods. Children with homozygous or heterozygous factor V Leiden usually have their first vascular insult following puberty.

## **Acquired Causes of Thrombophilia in Children**

The acquired causes of thrombophilia in children include the antiphospholipid antibody syndrome, use of oral contraceptives, nephrotic syndrome, paroxysmal nocturnal hemoglobinuria (PNH), malignancy and its treatment, especially use of the agent L-Asparaginase in patients with leukemia, dehydration from any cause, malnutrition, and sickle cell disease. The antiphospholipid antibodies (APA) are observed very frequently in children, especially after viral infections, varicella being a very common culprit. But it is infrequent to see thrombosis in children after every viral infection. The APA can be associated with both arterial and venous thromboembolic events, especially stroke. Platelet hyperaggregability as seen in patients with PNH and thrombotic thrombocytopenic purpura (TTP) also confers an increased thrombotic risk. In newborns, there are certain conditions that predispose to neonatal thrombosis, including maternal diabetes mellitus, hypoxia/asphyxia, polycythemia, infections, and the indwelling catheters. Less than 1% of cases of thromboembolic events in neonates are idiopathic. Over 50% of cases in children and over 80% of cases in newborns occur in the upper venous system secondary to the use of indwelling catheters

## **Clinical Presentation of Congenital Prothrombotic Disorders**

Venous thromboembolic events are the most common clinical presentation of single gene congenital prothrombotic disorders and occur in the presence or absence of other acquired risk factors. The most suggestive features for the presence of a congenital prothrombotic disorder include a positive family history, recurrent thromboembolic events, an early age for the first event, the absence of a significant acquired insult, and occurrence in an unusual site. Thromboses in children affect large, central vessels including the atria of the heart, the vena cava, hepatic, renal, and pulmonary vessels. Proximal vessels affected include the subclavian, axillary, jugular, and iliofemoral. Distal thromboses of the calf and arm vessels are uncommon in children.

## **Diagnosis of Thromboembolic Events**

Diagnosis should include use of imaging studies, including, but not limited to ultrasound/doppler, venograms, radionuclide studies and magnetic resonance arteriography/venography. Measurement of d-dimers is gaining importance in the initial screen for the diagnosis of a pulmonary embolus.

## **Complications of Childhood Thrombosis**

Complications of childhood thrombosis include death from thrombosis in 3-5% of patients, pulmonary embolism in about 20%, and the post-phlebotic syndrome in 10-20% of patients.

## **Management of Childhood Thrombosis**

The management of childhood thrombosis follows treatment guidelines in the adult population. These remain the primary source for recommendations in children until more studies in children are completed. The first guidelines for antithrombotic treatment in pediatric patients occurred in 1995, based primarily on adult studies and case studies in children. Data from recent pediatric studies show, however, that extrapolation of adult guidelines to infants and children is suboptimal. But until large scale studies are completed, treatment will follow some of the modified adult guidelines. Treatment includes use of anticoagulation and thrombolytic agents. Indefinite anticoagulation is suggested for patients with 2 or more episodes of spontaneous thromboses, one spontaneous life threatening thrombosis, one spontaneous thrombosis at an unusual site (cerebral, mesenteric vessels) and one spontaneous thrombosis in the presence of more than a single biologic defect. In those with a prothrombotic stimulus, vigorous prophylaxis during high risk situations (e.g. immobilization) is suggested. For venous thromboembolic disease, a minimum of 3-6 months of treatment with anticoagulation is suggested. For a recurrent thromboembolic event that is not related to a central venous catheter, indefinite anticoagulation is suggested. Use of unfractionated heparin in the management of these patients has given way to use of low molecular weight heparins, due to their ease of administration and less frequent monitoring of levels. Oral anticoagulation with use of oral warfarin is still used in some patients after initial heparinization. Fibrinolytic agents like tPA (tissue plasminogen activator) are used at times to manage clots located in areas that be accessed locally or for those situated in dangerous situations like the atrial walls of the heart.

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**Table 1: Odds ratio for prevalence of thrombotic disease in adults with various heterozygotic abnormalities**

Type of abnormality	Number of families	Prevalence of history of thrombosis		
		Heterozygous (%)	Normal (%)	
		Odds ratio		
Antithrombin Def	28	44	2	13.7
Protein C Def	13	44	9	9.8
Protein S Def	15	56	4	10
Dysfibrinogenemia	10	40	0	18
Plaminogen Def	20	1	0	2.6
Impaired fibrinolysis	18	54	33	1.7
Heparin Cofactor II	4	20	13	0.8

**Table 2: Summary of prevalence of inherited thrombophilic disorders in different populations**

Population	Pro C Def (%)	Prot S Def (%)	Anti-thrombin Def (%)	FVLeiden (%)	Pro-thrombin mutation (%)	Hyper-homo-cyteinemia
Normal	0.3	-	0.04	3-8	1	0.3-1.4
Unselected pts with thrombosis	3	1.5	1	13-20	-	4 -10
Pts with thrombosis and thrombophilia	7.9	7.2	5.3	52	7	-

These tables are adapted from references 1 and 5

**Contributed by Prasad Mathew, MD (NM)**

# Fetal and Neonatal Effects of Maternal/Fetal Thrombotic Disorders

The effects of hereditary thrombotic disorders on the developing fetus have not been recognized until recently. Current evidence indicates that maternal/fetal thrombotic disorders play a role in the pathogenesis of several neonatal/pediatric conditions. Maternal (hereditary) thrombophilia has also been implicated as a cause of late and recurrent fetal loss.

## Thrombophilia As a Cause of Fetal/Neonatal Disorders

### Central nervous system defects

Cerebral palsy is one of the most common and debilitating conditions of childhood. Over 300,000 children in the U.S. are affected with this disorder, and in most cases a causal event is not identified. With the advancement of magnetic resonance scanning and other forms of cranial imaging, a subgroup of patients with cerebral palsy have been diagnosed with cerebral infarction, either due to arterial infarction (middle cerebral artery) or cerebral venous thrombosis. Neonatal stroke may be an incompletely ascertained cause of cerebral palsy, mental retardation, seizures, and pediatric death (Curry et al. 2000). Current estimates indicate that ischemic accidents occur rarely in the pediatric population, with an incidence of 2.5/100,000, while the incidence of neonatal stroke ranges from 0.63-1.2/100,000. Approximately 25% of ischemic cerebrovascular disease in children is due to cerebral venous thrombosis. While many risk factors have been associated with the development of stroke, including congenital heart disease, vascular abnormalities, shock, dehydration, sepsis, and metabolic disorders, in an estimated 30% of cases no specific cause can be identified.

Several recent studies provide evidence that genetic thrombotic disorders are significant risk factors for neonatal stroke. Among these disorders are two newly identified genetic mutations in the clotting factor cascade. In 1994, the most common cause of hereditary venous thrombosis in adults was found to be a point mutation in the factor V gene, called the factor V Leiden mutation. Also called Arg506Gln or R506Q, this causes an arginine to glutamine substitution at position 506 in the cleavage site for activated protein C, and thereby reducing the rate of inactivation of factor V. Factor V Leiden has estimated prevalence of 3-5% among the Caucasian population. The second recently identified gene variant is a polymorphism in the 3' untranslated region of the prothrombin gene (factor II), a G to A transition at position 20210, called the prothrombin G 20210 A variant, or P-G20210a-V. This causes an elevated level of prothrombin and a nearly 3-fold increased risk of venous thrombosis in adults.

Mercuri et al. (2001) studied twenty-four infants with perinatal cerebral infarction confirmed by MRI scan and screened for several thrombotic disorders. Twenty-two of these patients had an infarction of the middle cerebral artery, and two had watershed infarctions. Laboratory evaluations included prothrombin time, activated partial thromboplastin time, platelet count, fibrinogen, and vonWillebrand factor antigen. The prothrombotic screen consisted of factor VIIIc, protein C, protein S, and antithrombin. Two DNA mutations, the factor V Leiden mutation and the prothrombin 20210 (G→A transition at position 20210 of the prothrombin gene) were analyzed as well via polymerase chain reaction (PCR). Results were abnormal in 10/24 (42%) of the patients. These included 6 patients with increased factor VIIIc and 5 with heterozygous factor V Leiden. (One patient had both abnormalities.) Parental studies were available for 4/5

children with factor V Leiden, and in all four one parent was heterozygous for this mutation. Of the 6 patients with elevated factor VIIIc, parental studies were normal in 10/11 parents studied. An additional association was found between clinical outcome and abnormal thrombotic profile. In 8/11 (73%) of patients with hemiplegia or developmental delay, an abnormality in the thrombotic profile was detected, compared to 2/13 with normal outcomes who had an abnormal thrombotic profile. There was a significant association between factor V Leiden and the presence of hemiplegia (Fischer's exact test,  $p=.003$ ). All 5 children with factor V Leiden had hemiplegia compared to only 4/20 without this mutation. In addition there was a much higher prevalence of factor V Leiden in this cohort (24%) than in the normal population, estimated to be 2.7-10% in Europe and North America. These results have been confirmed by other studies, including that of Zenz et al. (1998), who studied 33 Austrian children with ischemic stroke and found an 18% incidence of factor V Leiden mutation. This result exceeded the expected prevalence in the Austrian population of 4.6% (Fischer's exact test  $p=.001$ ). Curry et al. (2000) looked at 27 patients with neonatal stroke and found 53% had one or more abnormalities in mother, child, or both. The highest frequency was that of prothrombin G20210A, found in 3 children, 2 with factor V Leiden, and 2 with MTHFR homozygosity. In 46% of mothers an abnormality was found, most commonly 6 with anticardiolipin antibodies, 4 with MTHFR heterozygosity, and 3 with factor V Leiden.

Gunther et al. (2000) looked at the role of genetic and acquired prothrombotic risk factors in symptomatic stroke in 91 full term neonates in a multi-center case-control study in Germany. A total of 62 stroke patients had one or more genetic prothrombotic risk factors compared to the healthy age-matched controls (Fischer's exact test  $p <.001$ ). Most significant among these was lipoprotein (a) level  $> 30\text{mg/dl}$ , found in 20 patients (22%) compared to 10 controls (5.5%) ( $p <.001$ ). Factor V Leiden was again found to be more common among stroke patients than controls (17 vs. 10,  $p = .0016$ ), and protein C deficiency was found in 6 additional patients. These results were in contrast to those of Zenz et al., who found factor V Leiden to be the most significant risk factor. The authors cited differences in study design and the small number of cases as causes for the discrepancy. This report also looked at additional triggering factors including asphyxia, neonatal septicemia, patent foramen ovale, maternal diabetes, antenatal renal venous thrombosis, and fibromuscular dysplasia. Forty-nine patients had an additional triggering factor; most frequently identified were perinatally acquired asphyxia, neonatal septicemia, and patent foramen ovale. Of this subgroup, 33 had at least one prothrombotic risk factor.

The role of thrombotic risk factors has also been studied as a cause of stroke due to cerebral venous thrombosis in children. Carvalho et al. (2001) retrospectively studied 31 patients with cerebral venous thrombosis, including 19 neonates (61.2% of the total). Clinical risk factors were found in 14, including persistent pulmonary hypertension, congenital heart disease, dehydration, meningitis, sepsis, and central nervous system tumor. Radiographic studies showed that 9 had superior sagittal thrombosis, 3 had sigmoid/transverse sinus thrombosis, and 7 had multiple sinus thrombosis. The clinical presentation in these neonates was generally nonspecific with symptoms such as seizures, fever, respiratory distress, lethargy, and decreased oral intake. Eleven of 19 (58%) neonates had stroke; 7 were hemorrhagic and 4 were ischemic. Neurological outcome was also assessed in all 19 patients. Eleven were developmentally delayed, one had a learning disability, and 5 showed no deficits. There were two deaths. Prothrombotic risk factor analysis was performed on 14 patients (although the study does not indicate the percentage of neonates versus pediatric patients). Of this subgroup, 7 (50%) had protein C deficiency, 5 (35%) had antithrombin III deficiency, 3 (33%) had factor V Leiden, 2 had protein S deficiency (14%), and 1 (13%) had antiphospholipid antibodies (13%). The authors concluded that the frequency of prothrombotic risk factors in their patients with cerebral venous

thrombosis indicates that all of these patients, even in the presence of obvious clinical risk factors, should undergo coagulation evaluation.

Finally, Debus et al. (1998) found evidence to suggest that factor V Leiden is also a risk factor in the development of antenatal porencephaly. Among 24 infants with porencephaly, they found 16 with abnormalities of protein C, protein S, factor V Leiden or lipoprotein (a). Three patients had more than one risk factor.

### **Limb defects**

The relationship between genetic thrombotic disorders and other types of congenital anomalies has not been as thoroughly investigated. One recent study found some evidence that there is an excess of thrombophilic disorders in families with a child with a terminal limb defect, and that they may play a role in the etiology of these limb malformations. Hunter (2000) studied 24 mother-child pairs in which the child had a terminal transverse limb defect, ranging from the phalanges up to the elbow/knee, or had Poland anomaly. He looked at an extensive panel of prothrombotic risk factors, including proteins C and S, antithrombin III, factor V Leiden, prothrombin G20210A, lipoprotein (a), homocysteine, maternal anticardiolipin IgG and IgM, and 5,10-methylenetetrahydrofolate reductase (MTHFR), heterozygote and homozygote. (A cytosine to thymine substitution at position 677 in the MTHFR gene [MTHFR C677T] affects MTHFR, the primary methyl donor in the conversion of homocysteine to methionine and results in an elevated plasma homocysteine level.) His results showed an increased frequency over expected in 6 different prothrombotic factors, with statistically significant increases for protein S (maternal and child) and maternal anticardiolipin IgG. The author noted that, while the patient numbers are small, the patients with protein C or S deficiency had transverse defects, typically with "nubbins of distal tissue," while 2 of the 3 children born to mothers with elevated anticardiolipin IgG had Poland anomaly. Although this is only preliminary evidence that thrombophilic disorders play a role in the etiology of limb defects, there are numerous reports in the medical literature citing evidence of a vascular pathogenesis for these types of anomalies. Postulated mechanisms have included subclavian artery supply disruption sequence (SADS), twin-embolization-sequence, *in utero* trauma, partial placental separation as a complication of chorionic villus sampling, and homozygous alpha-thalassemia causing hypoxia. Hunter also postulates that the fetus may be susceptible to maternal thrombotic risk factors, which are not actually inherited by the fetus, but may cause abnormal levels through placental transfer, or by clot or embolus formation at the placental interface.

### **Maternal Thrombophilia As a Cause of Recurrent Fetal Loss**

While the risk of early miscarriage is common in pregnancy, with an incidence approximately 1 in every 10 pregnancies, fetal loss after 20 weeks gestation is less frequent, occurring in 1 in every 200 pregnancies. Placental insufficiency is frequently cited as the cause, and is known to be associated with maternal thrombophilic conditions predisposing to thromboembolism, including antithrombin III deficiency, protein C and S deficiencies, and antiphospholipid-antibody syndrome. Several recent studies have also pointed to the genetic variants of the prothrombotic factors discussed above as risk factors for late fetal loss. The factor V Leiden mutation has been associated with third-trimester fetal loss, as well as first and second trimester losses. Women with the MTHFR C677T variant have also been shown to be at an increased risk for late fetal loss, as well as various for obstetrical complications such as preeclampsia, abruptio placentae, and intrauterine growth retardation.

Martinelli et al. (2000) studied 67 women with a first episode of late fetal loss (after 20 weeks gestation) and 232 women with one or more pregnancies and no late fetal losses. They found

eleven of the 67 women (16%) and 13 of the 232 control women (6%) had either factor V Leiden or prothrombin G20210A mutation. The relative risks of late fetal loss for these women were 3.2 for the factor V Leiden and 3.3 for the prothrombin G20210A (95% confidence interval, 1.0-10.9 and 1.1 -10.3, respectively). Thirteen percent of the women in the late fetal loss group and 20% of the control women had the MTHFR C677T mutation, for a relative risk of 0.8 (95% confidence interval, 0.5-1.2). They concluded that both the factor V Leiden and prothrombin G20210A mutations carry a 3-fold increased risk of late fetal loss.

### **Conclusions**

Recent evidence points to maternal/fetal thrombotic (thrombophilic) disorders as a cause of both neonatal/antenatal disorders of the central nervous system including neonatal stroke, central venous thrombosis, and porencephaly, They may also play an unrecognized role in some cases of cerebral palsy. In addition, these genetic disorders are also implicated as causes of terminal limb malformations and late fetal loss as well as other pregnancy complications. Future studies may implicate this group of disorders in additional types of congenital malformations. A complete laboratory evaluation for these disorders, for both mother and baby, as outlined below, is indicated in cases of neonatal stroke, central venous thrombosis, late fetal loss, and should be considered in patients with unexplained cerebral palsy.

**Suggested laboratory testing for suspected maternal/fetal thrombotic disorders both mother and child or fetus should be tested.**

Protein S  
Protein C  
Antithrombin III  
Factor V Leiden  
Prothrombin G20210A  
Lipoprotein (a)  
MTHFR C677T  
Homocysteine  
Anticardiolipin IgG/IgM (maternal)

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**Contributed by Terri A. Grebe, M.D (AZ)**

## **Teratogen Hot Topic: Anticoagulants**

The use of some anticoagulants during pregnancy can carry a slightly increased risk to the developing embryo and fetus. The list of anticoagulants presented here is not exhaustive, but represents those commonly used to treat thrombotic disorders.

**Warfarin** (also called coumarin derivatives) has been associated with underdevelopment of the nose, stippling of the ends of the long bones and, possibly, developmental delay. The nose grows in length, width as well as away from the face. It is the growth away from the face that can be affected by warfarin, leaving the nose flat against the face. A flatness that, in some cases, can impair breathing and require some reconstructive surgery. Warfarin can also cause stippling at the ends of the long bones (the epiphyses). This stippling shows up on radiographic examination, but has no clinical significance. These effects of warfarin on the nose and long bones are seen with embryonic exposure between weeks 6 and 9 and occur in less than 10% of those exposed. Warfarin has also been associated, very weakly, with a possible increase in developmental delay. This finding has not been substantiated across studies as have the other teratogenic effects of the drug and is, therefore, still in question. If there is an effect on brain development, the risk is very low and the gestational time at which warfarin may induce this finding in the fetus is not known.

**Heparin** has a very large molecular weight and is one of only a few drugs that do not cross the placenta. Because of its size, heparin is considered the treatment of choice for clotting disorders during pregnancy.

**Low molecular weight, oral dosing forms of heparin** are now available for use. These forms of heparin are being closely monitored when used by pregnant women, and, so far, no increased risks for birth defects have been reported. These drugs are used only if patients are unable to use regular heparin.

**Low dose aspirin** (usually 80mg/day) has not been associated with an increased risk to the fetus when used by pregnant women. Aspirin used in regular doses after 26 weeks of pregnancy is sometimes associated with premature closure of the ductus arteriosus, a vessel in the fetal heart. Closure can lead to fetal pulmonary hypertension causing prematurity and, rarely, stillbirth. This effect has not been seen with the low doses of aspirin used to control clotting. However, when pregnant women are treated with aspirin, even in low doses, after 30 weeks gestation, many obstetricians monitor for ductal closure on a regular basis, just to be cautious.

All anticoagulants, of course, carry concern for maternal bleeding during labor and delivery. Discontinuation of therapy near term is done, if possible. The length of time before the mother's due date that therapy is discontinued is dependent upon many variables and decisions about timing are made on a case-by-case basis.

**Contributed by Lynn Martinez (UT)**

# Heterozygote Counseling for the Factor V Leiden Mutation

Heterozygosity for the Leiden mutation in the factor V gene (FVL) is common and may present complex counseling issues. Heterozygosity refers to the situation when an individual has a single Leiden mutation in one of the factor V genes (see Adult Thrombotic Disorders in this issue of the *Genetic Drift*). It is inherited in an autosomal dominant manner, and may be identified in individuals with thrombosis or their family members. Heterozygotes exhibit reduced penetrance, meaning some individuals will never exhibit symptoms. In this condition, at least 90% of heterozygotes are asymptomatic throughout their lives. The heterozygote frequency is 5% of the Caucasian US population, 2% of Hispanic Americans, about 1% of African Americans and Native Americans, and less than 1% of Asian Americans. In the general population, deep vein thrombosis occurs in 1 in 1,000 individuals. Heterozygosity for FVL confers a 4-8-fold increased lifetime risk for deep vein thrombosis, yet thrombosis occurs with other circumstantial factors in 50% of cases. Twenty to sixty percent of Caucasians with thrombosis have FVL, and most of these are heterozygotes. Homozygotes, or those with two copies of the gene, have up to an 80-fold risk for thrombosis. For genetic counseling of heterozygotes, it is useful to divide the asymptomatic from the symptomatic, and adults from children.

Asymptomatic adults should be informed of increased thrombotic risks with oral contraceptives and hormone replacement therapy, surgery, prolonged immobilization, and pregnancy, and puerperium. In addition, they may be tested for coexisting abnormalities such as the prothrombin mutation 20210A variant, functional deficiencies in protein S, C, and antithrombin III, and hyperhomocystinemia, additional defects that significantly increase the risk of thrombosis. Prophylactic use of anticoagulants is not routine, but a short course may be used in the high-risk clinical settings listed above.

Symptomatic adults with FVL who have had a thrombotic event are at a 2-4-fold risk of another event compared with the normal genotype. Testing them for additional risk factors such as those above is recommended and neatly outlined in the American College of Medical Genetics Consensus Statement on factor V Mutation Testing (see Grody et al. 2001). Treatment with anticoagulants is indicated, the duration determined on an individual basis. Women experiencing stillbirth, placental abruption, and severe pre-eclampsia may be heterozygotes exhibiting thrombophilic symptoms. Factor V Leiden is not known to be associated with arterial thrombosis.

Asymptomatic children are unlikely to have complications associated with heterozygosity. The majority who develop thrombosis have an additional genetic and/or environmental risk. Therefore, if heterozygosity is identified, further investigation of coexisting risks and parental counseling regarding risk and possible treatment during surgery and prolonged immobilization is indicated. However, there is no consensus on testing children for the mutation. Care should be taken with considering the issues involved with testing children, such as the benefit of timely medical benefit to the child versus the risk of stigmatization.

Families with FVL and an increased incidence of thrombosis appear to be predisposed to thrombosis beyond the magnitude generally associated with heterozygosity in the general population. Therefore, genetic counseling is frequently useful in evaluating individual risk, making recommendations for management, and addressing psychosocial issues.

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