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Inherited thrombophilia and adverse pregnancy outcomes: What the evidence shows

Recent findings suggest that inherited thrombophilias lead to habitual fetal loss, preeclampsia, and placental abruption. Here, an expert examines the role of gene polymorphism-related clotting abnormalities in these outcomes and reviews studies of promising interventions.

When obstetric histories of habitual or late fetal loss, preeclampsia, intrauterine growth retardation, and placental abruption or infarct suggest placental compromise, a search for thrombophilia is warranted. This article examines:

• evidence implicating inherited thrombophilia polymorphisms in these adverse outcomes

• promising findings of 2 interventional studies of pregnancy outcomes in thrombophilia carriers: enoxaparin treatment for women experiencing habitual late fetal loss, and folic acid treatment for severe preeclampsia.

Polymorphisms linked to thrombotic risk

The adverse pregnancy outcomes described above represent a spectrum of disease with considerable overlap in etiology. The risk of each is increased in the presence of even 1 of the other complications.

A similar placental pathology is common to these outcomes, suggesting aberrant maternal/fetal perfusion as a unifying feature. The causes of such perfusion abnormalities are diverse, although placental infarct and

KEY POINTS

■ Because polymorphisms affecting thrombotic potential are frequent (2% to 20% of the general population), even a slightly increased risk of thrombosis affects a far greater number of pregnancies than complete deficiencies of clotting factor proteins S and C or antithrombin III.

■ Observational studies suggest a role for heparin or folate supplementation in women with thrombophilia polymorphisms and prior adverse pregnancy outcomes.

■ Hyperhomocysteinemia, which sometimes develops from the influences of a polymorphism, has been linked to preeclampsia and may contribute to placental abruption and infarct.

■ The Leiden polymorphism of the factor V, with its greater propensity for thrombosis, occurs at increased frequency in women who experience habitual miscarriages.

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microthrombi suggest an underlying perturbation of the clotting cascade.

Although clotting factor deficiencies including protein S, protein C, and antithrombin III have long been known to increase thrombotic disease risk, these hereditary deficiencies occur in less than 1% of the general population, and thus contribute minimally to placental compromise and adverse outcomes.[1]

Of greater clinical significance are changes in DNA sequences collectively known as gene polymorphisms. These gene sequence changes occur with variable population frequency and impact on protein function.

Now recognized are several polymorphic genes which modulate the balance between the opposing prothrombotic and antithrombotic actions of the clotting cascade. Polymorphisms of clotting-cascade proteins occur in 1% to 4% of the population, with variable impact on thrombotic risk.

Polymorphisms linked to endothelial damage

Gene polymorphisms outside the clotting cascade also can increase the risk of thrombosis. Inflammation of the endothelial lining due to elevated homocysteine is associated with an increased risk of both venous and arterial thrombosis. In the conversion of homocysteine to methionine, highly polymorphic gene sequences of the methylene tetrahydrofolate reductase enzyme (MTHFR) can slow the pathway, resulting in hyperhomocysteinemia. Polymorphisms influencing homocysteine metabolism occur in up to 20% of individuals in some populations.

The power of polymorphisms

Genetic polymorphisms do not necessarily imply a disease state. These DNA changes are preserved in populations, and their effects on the proteins they code are variable, even of benefit in some populations. (That may explain the continued presence of polymorphisms despite their negative clinical impact in other populations.) The effect of a genetic polymorphism on the quantitative or qualitative function of a protein is variable, and often occurs in combination with other genetic, physiologic, or environmental changes.

The frequency of a specific polymorphism varies from population to population, reflecting ancient adaptation to specific environments. Given the complex interactions required for modulation of the opposing thrombotic and antithrombotic processes of the clotting cascade, genetic polymorphisms affecting key receptors, enzymes, and cofactors all contribute to functional control of clotting.

Without this precise control, 1 mL of blood can convert the total body volume of fibrinogen to fibrin—and clot formation—in 10 to 15 seconds.[2] The process leading to the fibrin plugs of pathologic thrombi differs little from the appropriate hemostasis of a laceration, leading to the description of thrombosis as "fibrin plugs in the wrong place or at the wrong time."[2]

The major inhibitors of thrombosis are antithrombin III (ATIII), protein C, and protein S, which form a complex to prevent excessive clotting. Typically, this complex binds with factors V and VIII, rendering them inactive and thus limiting the progression of clot formation. Loss of interaction between this antithrombotic complex (ATIII-protein S-protein C) and the clotting-cascade factors leads to unregulated progression of the clotting cascade and excessive thrombosis formation.

Polymorphisms identified in clotting abnormalities

Resistance to activated protein C. Occurring in 2% to 5% of the general population, resistance to activated protein C (APC) results in loss of clot inhibition and increased thrombotic potential of the clotting cascade, as reflected in the striking 20% to 60% incidence of APC resistance among individuals with thrombotic disease. While APC resistance can be acquired, such as through antiphospholipid antibody syndrome, in the

majority of cases an inherited polymorphism of factor V is responsible.

Factor V Leiden. Polymorphism factor V Leiden, a substitution of glutamine for arginine at amino acid 506 of factor V, exemplifies the role of DNA polymorphisms in clotting abnormalities. The Leiden polymorphism renders factor V resistant to interaction with protein C. Even with adequate qualitative and quantitative function of protein S, protein C, and ATIII, the antithrombotic regulatory complex is rendered ineffective by factor V resistance to protein C binding.

The relative risk of thrombosis in the presence of factor V Leiden follows a dose-response curve. In some families, heterozygotic carriers of factor V Leiden with 1 copy of the polymorphism have a 10-fold increased risk of venous throbosis. Homozygotes with 2 aberrant copies face a 91-fold increase.

Prothrombin 20210A. Recognition of polymorphisms at other sites along the clotting cascade is emerging. The clotting-cascade step of conversion of prothrombin to thrombin is central to thrombus formation. A change of G to A at position 20210 in prothrombin (prothrombin 20210A) elevates baseline prothrombin levels and thrombin formation.

Heterozygotic carriers of prothrombin 20210A are 3 times more prevalent among individuals with venous thrombosis and 9 times more prevalent in individuals with familial thrombosis.

Hyperhomocysteinemia. A frequent condition in the general population, hyperhomocysteinemia is also an important contributor to the overall risk of thrombotic disease.[3] Although the exact mechanism for this is unclear, mild to moderate increases in homocysteine levels are associated with an increased relative risk of thrombosis (2.5).

FIGURE

Factor V Leiden among women with habitual pregnancy loss
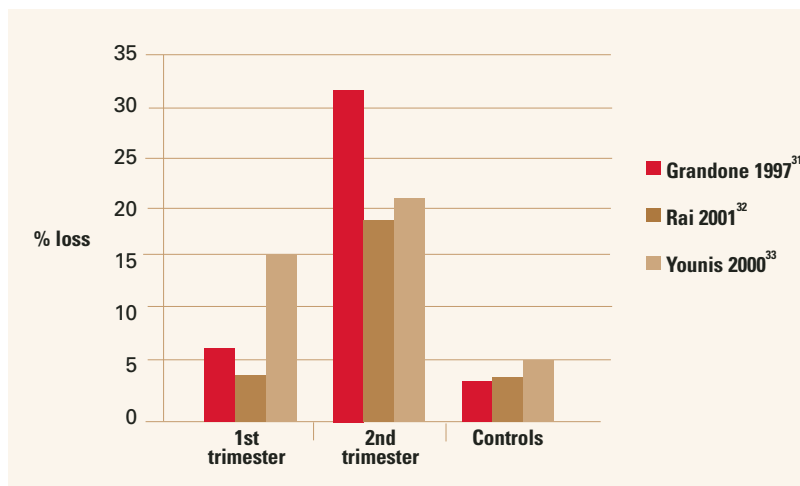
Dietary restriction of folate and vitamin B_{12} remains the most common cause.

However, in at least a quarter of cases, DNA polymorphisms of the homocysteine-methionine pathway are associated with hyperhomocysteinemia and a mild to moderate increase in thrombosis. The most common inherited cause, a C to T change at position 677 of MTHFR, produces a thermolabile enzyme with reduced catalytic capacity and lowered conversion of homocysteine. The diminished conversion of homocysteine to methionine is further slowed by folate deficiency.[4] Other MTHFR polymorphisms also may contribute to diminished catalytic capacity of this pathway, with resultant increased levels of homocysteine.[5]

Polymorphisms identified in obstetric complications

Fetal loss and habitual miscarriage. Among women who experience habitual miscarriage, factor V Leiden contributes primarily to the subgroup of losses that occur during the second trimester. The association between factor V Leiden and early first-trimester fetal loss is not as robust (**FIGURE**).[6]

In the first prospective analysis of obstetric outcomes in carriers of factor V Leiden, infertility or miscarriage was 1.5 times greater than controls (95% confidence interval [CI], 1.2–2.7). In carriers who had experienced 2 or more fetal losses, factor V Leiden was 2.5 times greater than expected (95% CI, 1.2–5.13).[7] Conversely, however, among families with multiple individuals with thrombotic disease, habitual early losses are not more common.[8]

Limited evaluations of the prothrombin gene polymorphism likewise both support and refute an association with early or late fetal loss.[9] Given the relatively low incidence of late fetal loss with either polymorphism, the majority of women with factor V Leiden or a prothrombin polymorphism will have successful pregnancies.[10]

A variety of studies support a link between mild to moderate hyperhomocysteinemia and preeclamptic toxemia.

With respect to the methionine-homocysteine pathway, both folate deficiency and a MTHFR polymorphism play a role—alone or in combination.[6] In fact, 1 study reported that hyperhomocysteinemia alone did not display a significant association with early fetal loss, suggesting that folate deficiency and MTHFR polymorphisms may operate through additional, unidentified variables. However, in a meta-analysis, hyperhomocysteinemia alone had a pooled risk of habitual pregnancy loss of 2.7 (95% CI, 1.4–5.2) and 4.2 (95% CI, 2.0–8.8) for fasting and postmethionine levels, respectively.[11] The relatively weak association with hyperhomocysteinemia, the diversity in definition of habitual pregnancy loss, and the variability inherent in homocysteine testing likely contribute to these conflicting findings.

Preeclampsia. Several studies have confirmed an association between APC resistance and preeclamptic toxemia. In fact, the American College of Obstetricians and Gynecologists reports a 2.4-fold increase in factor V Leiden, a major determinant of APC resistance, among women with severe preeclamptic toxemia.[12] However, analyses in non-Caucasian populations typically do not identify factor V Leiden as a risk factor.[13,14]

An association between the prothrombin 20210A polymorphism and severe preeclampsia is variable and not confirmed in all populations. The lower background frequency of this polymorphism may limit a sufficient sample size for detection of a significant association.

With regard to homocysteine, a variety of studies support a link between mild to moderate elevations and preeclamptic toxemia. Homocysteine is mildly elevated during pregnancies complicated by preeclamptic toxemia, and this elevation persists postpartum.[15,16] Homocysteine elevations are also present in the second trimester before blood pressure increases occur.[16] Even at 15 weeks' gestation, an elevated homocysteine level indicates an almost 3-fold increased risk of severe preeclampsia.[17] Based on a meta-analysis of 5 studies, mild to moderate increases in homocysteine alone are significantly associated with a risk for severe preeclampsia, while folate deficiency alone is not (**TABLE 1**). The hyperhomocysteinemia is explained only in part by the increased rate of MTHFR polymorphism, with other contributors unidentified.[18]

Placental abruption and infarct. Hyperhomocysteinemia, folate deficiency, and MTHFR polymorphisms may also contribute to abruption and placental infarct. Hyperhomocysteinemia is 3 times more common in study patients versus controls (31% versus 9%), with fasting levels more significantly increased than postmethionine levels.[19] Likewise, MTHFR polymorphisms have been significantly associated with abruption and fetal growth restriction. The combination of heterozygosity for 2 MTHFR polymorphisms, C677T and A1298C, occurs in almost a quarter of placental abruptions.[20]

TABLE 1

Meta-analysis of the association between preeclampsia and selected metabolic defects[6]

METABOLIC DEFECT	ODDS RATIO	95% CI
Folate deficiency	1.2	0.5–2.7
Hyperhomocysteinemia	20.9	3.6–121.6
Methylene tetrahydrofolate reductase polymorphisms	2.6	1.4–5.1

Other contributors to placental thrombosis

Several factors reflecting normal physiologic changes of pregnancy also contribute to the increased thrombotic risk when thrombophilia polymorphisms are present during pregnancy. The low vascular resistance of the placenta, key to the adequate perfusion of the fetus, conversely sets the stage for fibrin deposition and clot formation. The normal increase in the levels of fibrinogen and clotting proteins during pregnancy also contribute to clot formation. These physiologic changes of pregnancy may be modulated by diverse environmental changes. When the most common polymorphism of MTHFR is present, the rate of this enzyme's functioning is further impaired by low folate, a condition often present due to physiologic decreases of folate during pregnancy. Suppression of available folate associated with cigarette smoking may further compound the deleterious effects of the MTHFR polymorphism on homocysteine levels.

Multiple thrombophilias increase risk

In a similar additive fashion, an individual with multiple thrombophilia polymorphisms faces even greater increases in thrombotic risk. For example, factor V Leiden working concomitantly with protein C or protein S deficiency, or factor V Leiden or prothrombin 20210A paired with hyperhomocysteinemia carry relative risks of venous thrombosis

greater than any of these elements alone. For patients with a history of thrombotic disease from the European Prospective Cohort on Thrombophilia (EPCOT) study, the highest odds for a stillbirth (odds ratio [OR], 14.3; 95% CI, 2.4–86.0) occurred in women with combined thrombophilia abnormalities.[8]

Multiple inherited thrombophilias also may interact at the maternal-fetal interface. Consistent with Mendelian inheritance, the fetus will inherit 1 of the maternal alleles at each gene of the clotting-cascade proteins. Chronologically, the fetal arterial supply is established as maternal spiral arteries perfuse the intervillous spaces, with the maternal and fetal blood supply of the placenta present 3 to 4 weeks after conception. Histologically, evidence of placental ischemia can be found on either the maternal or fetal side. It is unknown whether the risk of placental compromise is greater in the presence of maternal or fetal thrombophilia, alone or in combination. Initial study of factor V Leiden from spontaneous miscarriages suggests a slight skewing toward increased fetal inheritance of the maternal polymorphism, suggesting a further contributory role of the fetus to overall risk.[21] Interestingly, MTHFR polymorphisms in combination (C677T and A1298C) occur significantly more often among spontaneous losses than in fetal bloods at delivery, suggesting a decreased potential for viability among such fetuses.[22] In populations with no association between preeclampsia and maternal factor V Leiden, the MTHFR polymorphism or

Folate supplementation decreases homocysteine levels in women*[30]

TIME OF ASSESSMENT	MEAN FASTING HOMOCYSTEINE (UMOL/L)	95% CI	MEAN POST-LOAD HOMOCYSTEINE (UMOL/L)	95% CI
Presupplementation	16.6	13.0–20.1	68.5	60.8–76.2
Postsupplementation	6.1	5.6–6.7	29.3	25.6–33.0

CI=confidence interval
*All subjects had a history of preeclampsia.

prothrombin polymorphism fetal studies are likewise unrevealing.[23]

Promising results of enoxaparin, folate studies

Inherited thrombophilias may be amenable to treatment with baby aspirin, heparin, folic acid, intravenous immunoglobulin G (IgG), or factor concentrates. Currently, no randomized, controlled intervention trials have been conducted. Further, since initial adverse pregnancy outcomes in inherited thrombophilia patients are relatively rare, it will be difficult to identify a sufficiently sizable population at risk.

For inherited thrombophilia carriers with recurrent adverse pregnancy outcomes, 2 areas for intervention deserve greater attention: enoxaparin treatment for women experiencing habitual late fetal loss[24] and folic acid treatment for severe preeclampsia.[30]

Enoxaparin and recurrent fetal loss. The Brenner enoxaparin trial examined 96 women who had experienced recurrent pregnancy loss (3 or more in the first trimester [7-12 weeks], 2 or more in the second trimester, or 1 in the third trimester). Only losses with a fetal pole previously documented by ultrasound were counted. Approximately 66.6% of the participants had a thrombophilia polymorphism identified.

Subsequent pregnancies among these women were treated with 40 mg enoxaparin daily (80 mg daily for women with combined abnormalities). A significant difference in pregnancy outcome was noted when these patients were compared with their historical outcomes, although no differential in risk for solitary versus combined defects (83% versus 69%, $P=.37$) could be determined. Results appeared equally efficacious with either 40 mg or 80 mg enoxaparin a day.

Although these findings are encouraging, the use of historical controls for pregnancy outcome studies is notorious for overestimating the benefit of any single intervention. More complete analysis of the benefits of enoxaparin must await randomized controlled trials.

Several other studies have addressed the use of preconception aspirin (81 mg per day) and postconception unfractionated heparin among women with a history of habitual miscarriage. In 1 investigation involving 149 women whose recurrent miscarriages were due to a variety of procoagulant defects as well as factor V Leiden, fewer than 1% failed such therapy.[25] Among a smaller population (n=34), treatment with postconception heparin resulted in decreased fetal loss, although the drug had no effect on other obstetric complications.[26] In addition, among women with a combination of infertility, miscarriage, and thrombophilia, who experienced an 85% historical rate of early-pregnancy loss, that rate declined to 15% when either preconception enoxaparin (history of infertility) or post-conception enoxaparin (miscarriage alone) was administered.[27]

Folic acid supplementation and preeclamptic toxemia. Among all individuals—pregnant or not—folate supplementa-

Folic acid supplementation in women with a history of severe preeclampsia[30]

TIME OF ASSESSMENT	GESTATIONAL AGE AT DELIVERY (WEEKS)	BIRTH WEIGHT (G)
Presupplementation	29.5 ± 3.7	1,088 ± 570
Postsupplementation	36.7 ± 2.2	2,867 ± 648

tion significantly lowers homocysteine levels. Efficacy is greatest at the highest pretreatment homocysteine levels and least at the lowest pretreatment levels.[28]

Decreases in homocysteine levels are seen with 0.5 mg to 5 mg folic acid daily plus vitamin B_6. No additional benefit is obtained with supplemental vitamin B_{12}. Folate appears most beneficial in lowering homocysteine in the presence of MTHFR polymorphisms, as such individuals have higher homocysteine levels at given folate deficiencies than individuals without this polymorphism.[29]

Based on these observations, folic acid supplements have been examined for their role in preeclampsia and intrauterine growth retardation (IUGR).[30] In 1 study, women with a history of preeclampsia; hemolysis, elevated liver enzymes and low platelet count (HELLP) syndrome; or eclampsia (n=181) and IUGR (n=26) were identified following delivery. Assessment in the nonpregnant state—by measurement of both fasting and post–methionine-load levels— indicated positive results (above the 97.5 percentile in healthy premenopausal females) in 17.7% of preeclamptic toxemia cases and 19.2% of IUGR cases.

Of this group, 27 women proceeded to daily supplementation with folate (5 mg) and vitamin B_6 (250 mg) for a minimum of 10 weeks and achieved significantly decreased homocysteine levels (**TABLE 2**). (Interestingly,

analysis by fasting levels alone would have missed more than 50% of the hyperhomocysteinemic women; their latent metabolic insufficiencies were documented only following load to the enzymatic system with methionine.) The supplementation protocol was continued preconception in 14 eligible women (11 had been hospitalized for preeclamptic toxemia and 3 had a history of IUGR), with a baby aspirin added daily at 10 to 12 weeks. Although the recurrence rate (64%) was unaltered from the expected, disease onset occurred later in pregnancy, resulting in infants delivered at significantly later gestational ages with better birth weights. These results anticipate reduced neonatal mortality and morbidity (**TABLE 3**).

Summary

With rapid expansion of human genome sequencing and low-cost assays for the detection of DNA sequence changes, the panel of suspect polymorphisms will likely increase. Randomized, controlled studies are needed to optimize the treatment choices. ■

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