Genome and Hormones: Gender Differences in Physiology
Invited Review: Pharmacogenetics of estrogen replacement therapy

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Herrington, David M., and Karen Potvin Klein. Pharmacogenetics of estrogen replacement therapy. J Appl Physiol 91: 2776–2784, 2001.—There are a number of genetic factors that likely modulate both the beneficial and adverse effects of estrogen. An important domain of consideration is the relationship of estrogen and thrombosis risk. Gene polymorphisms among the key elements of the coagulation and fibrinolytic cascade appear to influence the effects of estrogen on risk for venous thromboembolic events and possibly arterial thrombosis as well. Emerging data also suggest that allelic variants in the estrogen receptor-α may modulate estrogen's effects, especially with respect to bone and lipid metabolism.

estrogen receptor-α; polymorphisms; postmenopausal women; thrombosis; risk factors

Hormone Replacement Therapy (HRT) is one of the most frequently prescribed forms of drug therapy in the United States. Approximately 15 million U.S. women take some form of HRT daily. Although approved for treatment of perimenopausal symptoms and osteoporosis, many women and their physicians feel that HRT may also be useful for prevention of a variety of other chronic illnesses, including heart disease. Recently, the results of several randomized clinical trials of HRT for secondary prevention of heart disease have shown that HRT does not appear to slow the clinical or angiographic progression of coronary disease. Furthermore, there is a growing body of evidence that HRT may also be associated with an early increase in risk of arterial and venous thrombotic events (VTEs), perhaps in a subgroup of women who are uniquely at risk for an adverse effect of HRT due to polymorphisms in genes that regulate coagulation and fibrinolysis. These data indicate that the effects of HRT may be far more complex than initially assumed and that a variety of genetic factors may play an important role in modulating the risks and benefits of HRT.

OVERVIEW OF THE HERS TRIAL

The Heart and Estrogen/progestin Replacement Study (HERS) was a randomized, double-blind, placebo-controlled trial of HRT [given as conjugated estrogen (0.625 mg) and medroxyprogesterone acetate (2.5 mg) daily] for prevention of recurrent cardiovascular events in postmenopausal women with established coronary disease. After an average of 4.1 yr of therapy, there was no difference in the rate of primary coronary heart disease (CHD) events (myocardial infarction or CHD death) between active therapy and placebo (52). At baseline, extensive information about cardiovascular disease risk factors, including smoking, diabetes, blood pressure, exercise, and alcohol consumption, were documented with standardized questionnaires. Plasma lipids were measured at baseline and annually during follow-up. Reported clinical cardiovascular events and fractures were confirmed on the basis of review of hospital records and by an independent endpoint committee.

EARLY RISK FOR CHD EVENTS WITH HRT IN HERS AND OTHER STUDIES

In HERS, risk for a nonfatal myocardial infarction (MI) or CHD death was increased by 50% during the first year of follow-up among women on active therapy
THROMBOSIS GENE POLYMORPHISMS AND EARLY RISK WITH ESTROGEN

There are several postulated mechanisms for the pattern of early risk observed in HERS. One leading possibility is that the early increase in coronary events was related to a prothrombotic effect of estrogen. It is well established that both postmenopausal HRT and oral contraceptives increase risk for venous thrombosis (14, 35, 56). The new data from women with established coronary disease in HERS complement previous studies of oral contraceptive use in women with coronary risk factors that have also observed an increased risk for coronary thrombosis (74, 111).

Despite these clinical associations between estrogen use and risk for venous or arterial thrombosis, the exact mechanism remains elusive. Several studies have shown that estrogen promotes generation of both thrombin and fibrin (as evidenced by increased levels of F\textsubscript{1,2}, thrombin-antithrombin complexes, and fibrinopeptide A) (62). This may be related to estrogen-associated increases in factor VII and reductions in protein C leading to higher levels of activated factor V (factor Va; Fig. 1). On the other hand, estrogen also lowers antithrombin III and fibrinogen (the substrate for thrombin), potentially blunting overall fibrin mass. In addition, estrogen appears to augment the fibrinolytic cascade by lowering plasminogen activator inhibitor-1 (PAI-1). The net effect may be that any incipient fibrin that is generated is rapidly degraded, thereby preventing the formation of clinically significant thrombosis.

However, these compensatory effects could be overwhelmed by one or more polymorphisms that alter gene expression of proteins that regulate coagulation or fibrinolysis (Fig. 2). Indeed, there are now preliminary data implicating several thrombosis gene polymorphisms in the setting of estrogen therapy or conditions commonly caused by estrogen. These polymorphisms may be causes for an estrogen-associated increase in risk for thrombotic events seen in the HERS trial.

Selected thrombosis-related gene polymorphisms are discussed in more detail below. Table 1 shows estimated allele and genotype frequencies for these polymorphisms.

Factor V Leiden. One polymorphism of particular interest is the factor V Leiden mutation. This point mutation (Arg 506→Glu) found in 5% of Caucasians in the United States (92) renders the factor V molecule resistant to inactivation by activated protein C (activated protein C (APC) resistance) (13). Case-control and prospective cohort studies have documented a 2- to 7-fold increased risk for venous thromboembolism among factor V Leiden heterozygotes and a 40- to 80-fold increased risk among homozygotes (71). Some (12, 23, 28, 75, 96, 106) but not all (7, 8, 11, 24, 27, 44, 63, 64, 70, 94) studies have suggested that individuals with factor V Leiden or activated protein C resistance are also at increased risk for MI or stroke.

Importantly, risk for venous or arterial thrombosis appears to be greatest among women with the factor V
Leiden mutation who also have increased exposure to endogenous or exogenous estrogen. In a study of consecutive premenopausal women with idiopathic VTE, Vandenbroucke et al. (107) found that women using oral contraceptives had a 3.8-fold increase in risk, whereas those with factor V Leiden had a 7.9-fold increase. In women using oral contraceptives who also had the factor V Leiden mutation, the risk was roughly 35 times greater than noncarriers who were not on oral contraceptives. Several other studies have reported substantially greater APC resistance or prevalence of factor V Leiden in women with VTEs that occurred during pregnancy or while taking oral contraceptives (4, 40, 46, 50, 80). In subjects with factor V Leiden, exogenous estrogen also appears to augment risk for osteonecrosis, a thrombotic complication of bone healing (31).

More recently, Glueck et al. (32) found the factor V Leiden mutation in 12.5% of women who suffered an MI or stroke while on HRT, compared with only 4.3% among women on HRT who had not suffered a coronary or cerebrovascular event. In contrast, rates of factor V Leiden in women with VTEs that occurred during pregnancy or while taking oral contraceptives (4, 40, 46, 50, 80). In subjects with factor V Leiden, exogenous estrogen also appears to augment risk for osteonecrosis, a thrombotic complication of bone healing (31).

Prothrombin (G20210A). Prothrombin is the precursor to thrombin, a key enzyme in thrombosis and hemostasis. In the 3' untranslated region of the prothrombin gene, there is a single nucleotide polymorphism G→A at position 20210 (18). This mutation, which occurs in 2–4% of the general population, is associated with 20% higher levels of prothrombin (89) and a 2.7- to 4.8-fold increased risk for venous thrombosis (21, 49, 69, 75, 89). Several studies have also found an association between this polymorphism and early MI (2, 23, 95, 108) or stroke (21). Some evidence suggests this mutation may further augment the risk for thrombotic events in subjects who also have the factor V Leiden mutation or other inherited thrombophilic conditions (22, 25, 115).

Like factor V Leiden, there are also preliminary data suggesting that risk for venous or arterial thrombosis may be dramatically increased in women with the prothrombin 20210A polymorphism who are taking exogenous estrogen. This possibility was first alluded to in a case report of celiac axis and splenic thrombosis in a woman with the prothrombin 20210A polymorphism who was taking oral contraceptives (34). Subsequently, Martinelli et al. (76) reported an odds ratio of 150 for cerebral vein thrombosis in carriers of the prothrombin mutation who were also on oral contraceptives. In a study of 230 women with hyperlipidemia, Glueck et al. (33) found 86 (37%) had a diagnosis of MI or stroke and 8 (3.5%) were heterozygous for the prothrombin G20210A mutation with respect to risk for MI or stroke (interaction odds ratio = 2.9, 95% CI of 1.4–6.2; P = 0.01).

In a large population-based case-control study conducted in a Seattle-based health-maintenance organization, Psaty et al. (90) found that hypertensive carriers of the prothrombin 20210A variant who were taking HRT had a substantially higher risk of MI than those without the mutation. Compared with nonusers of HRT with the wild-type genotype, women on HRT who were also carriers of the A allele had an 111-fold increase in risk for MI (odds ratio of 10.9; 95% CI of 2.15–55.2).

**Factor VII (R353Q).** Factor VII plays a central role in tissue factor-mediated thrombin generation. Several studies have found factor VII levels to be independently associated with risk for MI (45, 78, 79). Several polymorphisms have been identified in the 12.8-kb factor VII gene, which resides on the long arm of chromosome 13 (83). The R353Q polymorphism refers to a coding change resulting in Arg→Glu at position 353. Individuals who have one or two copies of the Q allele (~20% of the population) have 20–25% lower levels of FVIIc and FVIIag (37). Iacoviello et al. (55) found that individuals who were homozygous for the **Table 1. Estimated allele and genotype frequencies for selected thrombosis-related gene polymorphisms**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Mutant Allele Frequency</th>
<th>Genotype Frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor V</td>
<td>A506→G</td>
<td>0.026</td>
<td>W/W</td>
</tr>
<tr>
<td>Prothrombin</td>
<td>G20210→A</td>
<td>0.026</td>
<td>W/W</td>
</tr>
<tr>
<td>Factor VII</td>
<td>A353→G</td>
<td>0.12</td>
<td>W/w</td>
</tr>
<tr>
<td>PAI-1</td>
<td>4G/5G</td>
<td>0.40</td>
<td>w/w</td>
</tr>
<tr>
<td>β-Fibrinogen</td>
<td>G3/A</td>
<td>0.25</td>
<td>w/w</td>
</tr>
<tr>
<td>GP IIb/IIIa</td>
<td>P1A1/A2</td>
<td>0.15</td>
<td>w/w</td>
</tr>
</tbody>
</table>

GP, glycoprotein; PAI-1, plasminogen activator inhibitor-1.
wild-type allele (R/R) had a 20–25% higher risk of MI than subjects with one or two copies of the mutant allele. However, others have failed to confirm this association (9, 66). Interestingly, among wild-type homozygotes, FVIIc levels correlate with triglyceride levels, whereas in individuals with one or two copies of the mutant allele no such correlation exists (53, 65). This raises the possibility that R/R individuals with estrogen-induced hypertriglyceridemia may also have elevated factor VII levels and increased risk for a thrombotic event.

**PAI-1 (4G/5G).** PAI-1 inhibits the activity of tissue plasminogen antigen and urokinase, thereby inhibiting fibrinolysis. The PAI-1 gene is a 12.3-kb gene with nine exonic regions located in the long arm of chromosome 7 (103). This gene is known to have a promoter region (-675) polymorphism characterized by an additional G residue in a run of four consecutive G. This polymorphism has an allele frequency of 50%. The 4G allele is associated with dramatic increases in PAI-1 secretion in human hepatoma cell lines in response to interleukin-1 (16, 26) and higher circulating levels in vivo, especially in diabetic subjects (73, 85) and subjects with coronary disease (16, 113). This is clinically important, as elevated PAI-1 levels are associated with increased risk for VTE (15) and MI (41, 57), presumably by inhibiting the fibrinolytic cascade.

Of particular relevance for HRT users is the relationship between triglycerides and PAI-1 levels. PAI-1 release is stimulated by very low-density lipoprotein cholesterol in vitro (102). In humans, PAI-1 and triglyceride levels are correlated in normal subjects (59) and even more so in subjects with vascular disease (41, 58). However, this correlation is most apparent in individuals who are homozygous for the 4G allele. Panahloo et al. (85) reported a correlation of 0.65 between PAI-1 and triglycerides among 4G/4G diabetic individuals, with much weaker correlations being evident in 4G/5G and 5G/5G individuals. Whether 4G/4G women (~25% of the population) are at higher risk for venous or arterial thrombotic events in the setting of estrogen-induced hypertriglyceridemia remains unknown.

**Fibrinogen (−455G/A).** Fibrinogen is the precursor of fibrin, a major constituent of thrombus. It also binds to platelet glycoprotein IIb/IIIa on platelets, providing a molecular link that leads to platelet aggregates. Numerous studies have found a significant association between fibrinogen levels and risk for MI (37, 78). Synthesis of the Bβ-chain is the rate-limiting step in determining circulating levels of the mature fibrinogen molecule, which is composed of Aα-, Bβ-, and γ-components (97). There are two promoter region polymorphisms (−455 G/A and −148C/T) in high degree of linkage disequilibrium (105). The mutant allele A (allele frequency of ~19%) is associated with higher levels of fibrinogen (54), progression of coronary (17) and peripheral arterial disease (100), and increased risk of stroke (82). The −148C/T polymorphism is in the interleukin-6 promoter element for the Bβ-molecule, which may explain the higher levels of fibrinogen in −148T smokers (36), since smoking leads to increases in interleukin-6. Recently, it has become clear that estrogen replacement leads to increases in C-reactive protein (10, 93), an acute-phase reactant whose synthesis is regulated by the proinflammatory cytokine interleukin-6. It is possible that estrogen-associated changes in interleukin-6 and C-reactive protein might prove uniquely detrimental in −455A(−148T) women.

**PI²/².** Platelet glycoprotein IIb/IIIa is the fibrinogen receptor that mediates cross-linking of platelets and subsequent thrombus formation. The PI²² polymorphism is a base-pair change resulting in a leucine→proline substitution at residue 33 of the β₃₆₉ subunit of glycoprotein IIb/IIIa receptor protein (81). The PI²² allele, which has an allele frequency of 15% (114), is associated with increased platelet aggregability (29) and has also been associated with risk for premature MI in some (79a, 87, 109) but not other (30, 47, 72, 91) studies. In a recent meta-analysis of PI²/² and risk of MI, the risk of the A² allele was greatest among women (odds ratio = 1.4); however, the CI’s were wide and included unity because of the relatively few data currently available in women (114). Conversely, in platelets from men with the A² allele, incubation with estrogen produces significant inhibition. A¹/A¹ subjects require 1,000-fold higher concentrations of estrogen to achieve the same degree of platelet inhibition (5). These data suggest that gender and estrogen status may have a significant impact on the relationship between PI²/² genotypes and risk for thrombosis.

**OTHER PROMISING AREAS FOR PHARMACOGENETIC RESEARCH WITH HRT**

The estrogen receptors ER-α and ER-β, ligand-activated transcription factors, modulate expression of many proteins responsible for cell function. Several lines of evidence suggest that polymorphisms in ER-α may influence estrogen action. The human ER-α gene, located at 6q24.1, has been cloned, sequenced, and expressed in various cell lines, and site-directed mutagenesis has identified domains that are highly conserved across species and responsible for hormone or DNA binding or transcriptional activation (88). Associations between several naturally occurring ER-α sequence variants and a variety of clinical phenotypes have been examined. The phenotypes include risk (1, 99), age of onset (86), and estrogen receptor status (48, 112) in breast cancer; risk for spontaneous abortion (3, 67); bone mineral density (BMD) (19, 42, 61, 98); body mass index (19); hypertension (68); lipids (60, 77); and coronary atherosclerosis (77). Most of these studies have focused on the IVS1–401 and IVS1–354 polymorphisms. In two clinical studies examining HRT and BMD, HRT had a greater effect on vertebral BMD in women with the IVS1–401 C allele (20, 84).

In human vascular smooth muscle cells, significant heterogeneity in ER-α mRNA transcripts has been reported, including variants with missing exons encoding hormone-binding domain regions (51). The clinical literature includes a case of a man with a premature
stop codon in exon 2 and no functional ER-α receptors (101) who also had low high-density-lipoprotein cholesterol and premature atherosclerosis (104). Work is underway in several laboratories to determine whether there are other ER-α or ER-β polymorphisms that may have important impact on the clinical effects of HRT.

Another more classical area of pharmacogenetic research that remains selectively unexplored involves the genetic regulation of estrogen synthesis and catabolism (Fig. 3). Several of the cytochrome P-450 enzymes are responsible for critical steps in the conversion of estrone to estradiol and its subsequent catabolism. Polymorphisms in several of these genes are known to influence the metabolism of other steroid hormones. However, relatively few data are available concerning the effects of these cytochrome P-450 enzymes on clinical responses to HRT. This is another promising area of our research.

**POTENTIAL IMPORTANCE OF PHARMACOGENETICS OF ESTROGEN**

The significance of these data revolves around three interrelated issues of tremendous public-health importance: prevention of cardiovascular disease in postmenopausal women, safety of HRT, and drug-gene interactions. Cardiovascular disease remains the top killer of postmenopausal women in the United States. Initial enthusiasm for estrogen for primary and secondary prevention of cardiovascular disease has been questioned because of the null results in HERS. However, a real benefit of HRT could have been obscured by an increased risk of cardiovascular disease in a subset of women prone to a thrombotic complication. If verified, this theory suggests that HRT may still be useful to prevent cardiovascular disease in a large number of postmenopausal women still in need of effective pre-

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**Fig. 3.** Pathways of estrogen synthesis and catabolism and the sensitivity of tissue to estrogens. 3β-HSD, 3β-hydroxysteroid dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; DHEA, dehydroepiandrosterone; P-450, cytochrome P-450; SCC, side chain-cleavage enzyme; CYP17, 17β-hydroxylase; CYP21, 21-hydroxylase; CYP11, 11β-hydroxylase; E1, estrone; E2, estradiol; 2-OH-E1, 2-hydroxyestrone; 2-OH-E2, 2-hydroxyestradiol; 2-MeO-E1, 2-methoxyestrone; 2-MeO-E2, 2-methoxyestradiol; 2-OH-3-MeO-E1, 2-hydroxyestrone 3-methyl ether; 2-OH-3-MeO-E2, 2-hydroxyestradiol 3-methyl ether; 4-OH-E1, 4-hydroxyestrone; 4-OH-E2, 4-hydroxyestradiol; 16α-OH-E1, 16α-hydroxyestrone; 16α-OH-E2, 16α-hydroxyestradiol; CYP1A1, cytochrome P-450 1A1; CYP1B1, cytochrome P-450 1B1; COMT, catechol O-methyltransferase. [Reprinted with permission from Clemons M and Goss P. Estrogen and the risk of breast cancer. *N Engl J Med* 344: 276–285, 2001. Copyright 2001 Massachusetts Medical Society.]
preventive strategies, a hypothesis that nevertheless will need to be tested in future clinical studies. Even without unequivocal proof of a cardiovascular benefit, HRT remains one of the most frequently prescribed drugs in the United States, largely for the approved indications to treat perimenopausal symptoms and osteoporosis. However, there may be a subgroup among the 11–15 million U.S. women currently using HRT who are at high risk for a thrombotic complication. Excluding these women could significantly improve the safety of HRT for others hoping to treat or prevent these common conditions. Drug safety is especially important when used in otherwise healthy individuals to prevent future disease. One emerging avenue to improve drug safety and efficacy is through an understanding of drug-genome interactions. This area could become one of the most productive means to improve public health in the next decade. More research is needed to elicit fundamentally important new information about the impact of genomic variation on other effects of estrogen and estrogen antagonists and provide another example of the clinical utility of this broad class of investigational agents.

SUMMARY

Clinical trials of estrogen for secondary prevention of CHD in postmenopausal women have not found the beneficial effects predicted in observational studies and in animal models of atherogenesis. The reasons for this lack of benefit are not yet clear; however, preliminary evidence implicates several thrombosis gene polymorphisms in the setting of estrogen therapy as one possible reason for the disappointing results. Several candidate polymorphisms, among them factor V Leiden, prothrombin G20210A, factor VII (R353Q), PAI-1 (4G/5G), fibrinogen (455G/A), and PI(3)A(2), may have been involved in the estrogen-associated increased risk for thrombotic events observed in the HERS trial. Investigations into polymorphisms of ER-α and ER-β are needed to elucidate how they affect response to estrogen therapy. A better understanding of the complex role of these and other genetic modifiers of estrogen action may help maximize the safety and efficacy of HRT.

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