Genetic variants of coagulation factor XIII, postmenopausal estrogen therapy, and risk of nonfatal myocardial infarction

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We hypothesized that possession of either of 2 functional coagulation factor XIII polymorphisms, one within subunit A (Val34Leu) and one within subunit B (His95Arg), might modulate the prothrombotic effects of estrogen and help to explain the variation in incidence of arterial thrombotic events among postmenopausal women using hormone replacement therapy. In a population-based case-control study of 955 postmenopausal women, we assessed the associations of factor XIII genotypes and their interactions with estrogen therapy on risk of nonfatal myocardial infarction (MI). The presence of the factor XIIIA Leu34 allele was associated with a reduced risk of MI (odds ratio [OR] = 0.70, 95% confidence interval [95% CI] = 0.51-0.95). The presence of the factor XIIIB Arg95 allele had little association with MI risk. Neither factor XIII polymorphism alone significantly modified the association between the risk of MI and current estrogen use. In exploratory analyses, however, there was a significant factor XIII subunit gene-gene interaction. Compared to women homozygous for both common factor XIII alleles, the Arg95 variant was associated with a reduced risk of MI in the presence of the Leu34 variant (OR = 0.36, 95% CI = 0.17-0.75) but not in the absence of the Leu34 variant (OR = 1.11, 95% CI = 0.69-1.79). Moreover, among women who had at least 2 copies of the variant factor XIII alleles and were current estrogen users, the risk of MI was reduced by 70% relative to estrogen nonusers with fewer than 2 factor XIII variant alleles (*P* value for interaction = .03). If confirmed, these findings may permit a better assessment of the cardiovascular risks and benefits associated with postmenopausal estrogen therapy. (Blood. 2003;102:25-30)

Introduction

While estrogens may confer protection against cardiovascular disease through several mechanisms,[1] estrogens are also prothrombotic.[2] Recent clinical trials and observational studies of postmenopausal estrogen therapy have suggested a temporal pattern of early harm and late benefit on risk of myocardial infarction (MI).[3-6] The potential role of genetic prothrombotic variants, such as factor V Leiden and prothrombin G20210A, that define a subpopulation of susceptible women who are more likely to experience early thrombotic events has received considerable attention.[7-9] It is equally plausible that genetic variants that reduce thrombotic tendency may define subgroups of postmenopausal women most likely to benefit from estrogen therapy.

Coagulation factor XIII is a component of the blood coagulation system that plays a unique role at the interface of thrombus formation and thrombus dissolution.[10] Factor XIII circulates in blood as a tetramer composed of 2 A subunits and 2 B subunits. The A subunits have enzymatic function. The B subunits, which are noncatalytic, serve a carrier function to stabilize factor XIII in the circulation. Upon proteolytic activation of the A subunits by thrombin, the B subunits dissociate from the factor XIII A_2B_2 tetramer, yielding the active factor XIII enzyme (factor XIIIa) composed of 2 activated A subunits. Factor XIIIa is a transglutaminase that catalyzes the formation of cross-links between fibrin molecules, thus rendering a thrombus more stable and resistant to fibrinolysis. Estrogens not only increase levels of factor XIII but also have multiple other effects on the coagulation and fibrinolytic systems.[2] Thus, common genetic variants of factor XIII that alter procoagulant or fibrinolytic activity may modulate the effect of estrogens on coronary thrombosis and risk of MI.

While the A and B subunits of factor XIII interact as a complex in plasma, the genes that encode them reside on chromosomes 6p and 1q, respectively, and are genetically unlinked. A Val34Leu polymorphism of the factor XIIIA gene located near the thrombin activation site has been associated with an altered rate of factor XIII activation, abnormal fibrin clot structure,[11] and a decreased risk of arterial and venous thrombotic events.[12-15] Komanasin et al recently identified a His95Arg polymorphism of the factor XIIIB gene that is associated with an increased dissociation rate of the factor XIII A_2B_2 tetramer following activation by thrombin,[16] but the role of His95Arg in arterial thrombosis has yet to be examined. We hypothesized that the factor XIIIA Val34Leu and factor XIIIB His95Arg polymorphisms might each be associated with the risk of MI and might each modify the prothrombotic effects of estrogen replacement therapy. Here, we report the associations of these

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Submitted July 30, 2002; accepted November 19, 2002. Prepublished online as *Blood* First Edition Paper, November 27, 2002; DOI 10.1182/blood-2002-07-2308.

Supported in part by grants from the National Institutes of Health (HL43201 and HL60739) and the American Heart Association (9970178N), and a travel grant from the NWO (Nederlandse Organisatie voor Wetenschappelijk Onderzoek), The Hague, the Netherlands.

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2 factor XIII polymorphisms and their interactions with current estrogen use on risk of first myocardial infarction (MI) in postmenopausal women.

Materials and methods

We conducted a population-based case-control study[17,18] in the setting of Group Health Cooperative (GHC), a health maintenance organization with more than 400 000 enrollees. The study was approved by the GHC human subjects committee in accordance with the Helsinki protocol.

Definition of cases and controls

Cases were women aged 30 to 79 who sustained a first nonfatal MI between 1995 and 1998 and were identified from computerized GHC inpatient discharge abstracts and GHC claims databases that include bills for all services provided by non-GHC providers. Controls were a stratified random sample of women without MI matched to cases by age, calendar year, and hypertension status at a ratio of approximately 3:1. Each subject was assigned an index date, defined as the date of hospital admission for MI cases and a computer-generated random date within the same calendar year for controls. Exclusion criteria for both cases and controls included history of prior MI, non–postmenopausal status, and enrollment in GHC for less than 1 year prior to index date.

Data collection

Data collection included a review of GHC medical records, a telephone interview, and a venous blood sample. Information was collected regarding traditional coronary risk factors including blood pressure, height and weight, cholesterol level, family history of MI, smoking, diabetes, and other medical conditions present prior to the index date. Menopause was defined as cessation of ovarian function due to natural menopause or bilateral oopherectomy. Women older than 55 for whom menopausal status at the index date was unclear were assumed to be postmenopausal.

Assessment of hormone use

The GHC computerized pharmacy database includes a record of all prescriptions dispensed to GHC enrollees since 1976.[17,18] Each record includes a patient identifier, the drug type and dose, the date, the quantity dispensed, and dosing instructions. Current users of oral estrogen were defined on the basis of the last hormone prescription prior to the index date. If a subject received enough pills to last until her index date, assuming 80% compliance, she was considered a current user. Thus, for 80% compliance, a woman prescribed 100 pills with instructions to take 1 pill per day was counted as a current user for 125 days (from 100/0.8) after the prescription date.

Genotype analysis

Genomic DNA was extracted from peripheral blood, and genotyping for the factor XIIIA Val34Leu and factor XIIIB His95Arg polymorphisms was performed by polymerase chain reaction (PCR) amplification, followed by restriction enzyme digestion. The forward and reverse amplification primers for factor XIIIA were, respectively, 5′-CCCACAGTGGAGCTTCAGCGC and 5′-CCAGTGGAGACAGAGGATGTTTACCT. The Leu34 allele was detected by the loss of a *Bst*UI restriction site in the PCR product. An exon III nucleotide 8259 A to G transition that results in the factor XIIIB His95Arg substitution[16] was amplified by forward primer 5′-AAAGACAAGCTTAGTTTCATCATT and reverse primer 5′-TCTTCAGTTTAGGAAATGATTCTTAT. The presence of the Arg95 allele alters a recognition site for the restriction enzyme *Nsi*I. The digested PCR products were analyzed on 2.0% agarose gels. Laboratory personnel were blinded to case-control and hormone replacement therapy status. Genotyping for factor V Leiden (Arg506Gln) and prothrombin G20210A was performed as previously described.[7]

Statistical analysis

Clinical and demographic characteristics were compared using the chi-square test or Fisher exact test for categorical variables and Student *t* test for continuous variables. Risk of MI was analyzed using unconditional logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI). Women who were not current estrogen users at the index date and who were homozygous for the respective common factor XIII alleles served as reference groups in primary analyses. For estimating associations with estrogen use as the primary exposure, we included as covariates the matching variables of age, calendar year, and hypertension status, as well as the potential confounding variables of current smoking, diabetes, systolic blood pressure, and total cholesterol.[17,19] Since coagulation gene variants are fixed at birth and unlikely to be confounded by traditional metabolic and life style risk factors, we included only the covariates of age and race when modeling factor XIII genotypes as the primary exposure.

To assess potential interactions, ORs and confidence intervals for MI risk were estimated separately in 2 strata defined by factor XIII genotype. Because of small numbers of subjects homozygous for the less common alleles, these individuals were grouped together with heterozygotes. To formally test for gene-gene or drug-gene interaction on a multiplicative scale, we computed the "synergy index" (SI), a ratio of the odds ratio in one stratum to the odds ratio in the other stratum.[20] *P* values for interaction were derived from the Wald test for the multiplicative interaction term in the logistic regression model. All statistical hypothesis testing was 2-tailed and performed using alpha = 0.05.

Other coagulation factor gene polymorphisms, such as factor V Leiden and prothrombin G20210A, may influence the ability of factor XIII genotype to modulate the prothrombotic effect of estrogen. Therefore, we also explored the effect of factor V Leiden and prothrombin G20210A on risk of MI associated with current estrogen use in relation to coagulation factor XIII genotype. Because the factor V and prothrombin mutations are only prevalent among individuals of European descent, the analysis was confined to white women. In these analyses, the synergy index represents the ratio of the risk of MI for current estrogen use in women carrying the prothrombotic mutation to the risk of MI for current estrogen use in women not carrying the prothrombotic mutation, within each stratum of factor XIII genotype.

Results

The characteristics of the 234 MI cases and 721 female control subjects are presented in Table 1. Overall, 95% of study subjects identified themselves as white. Because nearly 50% of the cases were hypertensive and controls were frequency-matched on hypertension, hypertensive women accounted for half the study subjects. Traditional cardiovascular risk factors differed between cases and controls in the expected manner. Each factor XIII polymorphism was in Hardy-Weinberg equilibrium among the control subjects. As expected from their location on different chromosomes, the factor XIIIA Val34Leu polymorphism and the factor XIIIB His95Arg polymorphism were not in linkage disequilibrium with each other.

The distribution of covariates among controls according to the 3 exposures of primary interest, current estrogen use, Val34Leu genotype, and His95Arg genotype are shown in Table 2.

Control subjects who were current estrogen users were younger and had a lower prevalence of cardiovascular disease and related risk factors than current nonusers of estrogen. The distribution of factor XIIIA Val34Leu genotypes did not differ significantly by racial group. In contrast, the distribution of His95Arg alleles differed greatly between black and nonblack subjects. Among the 15 black controls and the 706 nonblack controls, the frequency distribution of His/His, His/Arg, Arg/Arg genotypes were 7%, 47%, 47%, and 82%, 17%, 2%, respectively ($P < .001$).

The associations of the 3 exposures of primary interest, estrogen replacement therapy, factor XIIIA genotype, and factor XIIIB

Table 1. Characteristics of female MI cases and controls

Characteristic	Cases (N = 234)	Controls (N = 721)
	Mean or %	
Age (years)	67.3	67.5
Black (%)	2.6	2.1
Time enrolled in Group Health Cooperative (years)	19.2	22.5
Current estrogen use (%)	34.2	37.2
Estrogen alone	18.8	20.9
Estrogen plus progestin	15.4	16.2
Hysterectomy (%)	40.2	40.6
Treated hypertension (%)	46.2	53.4
Current smoker (%)	27.4	10.0†
Diabetes (%)	23.5	6.8†
History of angina (%)	17.1	6.8†
Any cardiovascular disease* (%)	28.6	13.2†
Any family history of MI (%)	58.3	46.5†
Body mass index (kg/m^2)	29.2	28.4
Systolic blood pressure (mm Hg)	143.7	138.2†
Diastolic blood pressure (mm Hg)	80.1	79.7
Cholesterol level (mg/dL)	246.3	231.7†
Glucose level (mg/dL)	126.8	106.4†
Factor XIIIA Val34Leu genotype (%)		
Val/Val	65.0	56.3
Val/Leu	29.1	36.1
Leu/Leu	6.0	7.6
Factor XIIIB His95Arg genotype (%)		
His/His	82.5	80.0
His/Arg	16.7	17.3
Arg/Arg	0.9	2.6

*Defined as history of angina, congestive heart failure, stroke, transient ischemic attack, claudication, or surgical treatment for any of these conditions.

†$P < .05$ for the comparison between cases and controls.

genotype with MI risk are shown in Table 3. Current estrogen use had little association with risk of MI. On the other hand, the presence of 1 or 2 copies of the factor XIIIA Leu34 allele was associated with a significantly decreased risk of MI (adjusted OR = 0.70, 95% CI = 0.51-0.95). The presence of a single copy of the factor XIIIB Arg95 allele had little association with MI risk, but the adjusted risk estimate associated with 2 copies of Arg95 was 0.21 (95% CI = 0.04-1.06). There was no evidence that the risk of MI associated with factor XIIIA genotype or with factor XIIIB genotype differed significantly among subgroups defined by other coronary risk factors, including current smoking, obesity, hypertension, diabetes, or hypercholesterolemia (data not shown).

Because our data indicated a strong association between His95Arg genotype and race, we repeated the factor XIIIB genotype analysis separately for black and nonblack women. Among nonblack women, the MI risk estimates associated with carrying a single copy of the Arg95 allele (OR = 0.94, 95% CI = 0.63-1.41) or carrying any copy of Arg95 (OR = 0.86, 95% CI = 0.57-1.28) were similar to the results of the group as a whole. When the His95Arg analysis was restricted to nonblack women, 12 (1.7%) of 706 controls and none of 228 cases were homozygous for Arg95 (OR = 0.00, upper 95% limit = 0.97, Fisher exact $P = .045$). Of the 12 nonblack Arg95/Arg95 homozygous controls, 6 were positive for the factor XIIIA Leu34 variant. Of the 6 black MI cases, 2 were His/His, 2 were His/Arg, and 2 were Arg/Arg. There were too few black subjects to derive meaningful risk estimates for this subgroup.

Potential interactions between estrogen replacement therapy and factor XIII genotype were examined individually for each factor XIII polymorphism. The risk of MI associated with current estrogen use appeared to be slightly lower in carriers (OR = 0.96, 95% CI = 0.55-1.69) than in noncarriers (OR = 1.33, 95% CI = 0.84-2.11) of factor XIIIA Leu34 (synergy index = 0.82, 95% CI = 0.41-1.64). For the factor XIIIB His95Arg polymorphism, the risk of MI appeared to be lower in carriers (OR = 0.77, 95% CI = 0.32-1.86) than in noncarriers (OR = 1.28, 95% CI = 0.87-1.89) of theArg95 allele (synergy index = 0.62, 95% CI = 0.24-1.56). Neither drug-gene interaction was statistically significant on a multiplicative scale. The results were similar when the analyses were restricted to women using unopposed estrogen or combined estrogen/progestin or to nonblack women only (data not shown).

Because of the molecular interaction between the A and B subunits of factor XIII, we also assessed the separate and joint effects of the Leu34 and Arg95 variants on MI risk. Compared to the reference group of women homozygous for both Val34 and His95, women who carried the Leu34 but not the Arg95 allele had an OR of 0.81 (95% CI = 0.58-1.13); those who carried the Arg95 but not the Leu34 allele had an OR of 1.11 (95% CI = 0.69-1.79). However, the combined presence of the Leu34 allele and the Arg95 allele was associated with a substantially decreased risk of MI (OR = 0.36, 95% CI = 0.17-0.75) relative to the reference group. The synergistic interaction between the factor XIIIA Leu34 and factor XIIIB Arg95 alleles on MI risk reduction was statistically significant (synergy index = 0.40, 95% CI = 0.17-0.97; P value for gene-gene interaction = .043).

The factor XIIIA genotype-factor XIIIB genotype interaction is shown in another way in Table 4. When the total number of copies of factor XIII Leu34 and Arg95 alleles per subject were combined into a single variable, there was a progressive decrease in MI risk with increasing number of factor XIII variant alleles (test for trend

Table 2. Characteristics of control subjects according to current estrogen use, factor XIIIA Val34Leu genotype, and factor XIIIB His95Arg genotype

Characteristic	Estrogen use		Factor XIIIA genotype		Factor XIIIB genotype	
	Current (N = 268)	Not current (N = 453)	Leu34$^+$ (N = 315)	Leu34$^-$ (N = 406)	Arg95$^+$ (N = 144)	Arg95$^-$ (N = 577)
	Mean or %		Mean or %		Mean or %	
Age (y)	64.9	68.9	67.6	67.3	66.2	67.8
Black (%)	1.1	2.6	1.6	2.5	9.7	0.2†
Current smoker (%)	10.1	9.9	9.2	10.6	12.5	9.4
Diabetes (%)	3.4	8.8†	6.4	7.1	9.0	6.2
Any CVD* (%)	10.8	14.6	12.4	13.8	11.8	13.5
Body mass index (kg/m^2)	28.5	28.4	28.6	28.3	29.2	28.2
Systolic blood pressure (mm Hg)	135.4	139.9†	138.8	137.8	138.1	138.3
Cholesterol level (mg/dL)	227.9	234.0	230.7	232.5	231.4	231.8
Current estrogen use (%)	100.0	0.0	39.1	35.7	36.8	37.3

*CVD indicates cardiovascular disease.

†$P < .05$ for the comparison between exposed and unexposed.

Table 3. Main effects of estrogen use, factor XIIIA Val34Leu, and factor XIIIB His95Arg on risk of nonfatal myocardial infarction

Exposure	No. of cases	No. of controls	Unadjusted odds ratio (95% CI)*	Adjusted odds ratio (95% CI)†
Estrogen use				
Never	107	266	1.00 (reference)	1.00 (reference)
Past	47	187	0.62 (0.42-0.92)	0.74 (0.48-1.14)
Current	80	268	0.74 (0.53-1.04)	1.05 (0.71-1.54)
Current vs not current			0.88 (0.64-1.20)	1.17 (0.83-1.67)
Factor XIIIA genotype				
Val34/Val34	152	406	1.00 (reference)	1.00 (reference)
Val34/Leu34	68	260	0.70 (0.50-0.97)	0.70 (0.51-0.97)
Leu34/Leu34	14	55	0.68 (0.37-1.26)	0.69 (0.37-1.27)
Any Leu34			0.70 (0.51-0.94)	0.70 (0.51-0.95)
Factor XIIIB genotype				
His95/His95	193	577	1.00 (reference)	1.00 (reference)
His/95/Arg95	39	125	0.93 (0.63-1.38)	0.89 (0.59-1.33)
Arg95/Arg95	2	19	0.31 (0.07-1.36)	0.21 (0.04-1.06)
Any Arg95			0.85 (0.58-1.25)	0.82 (0.55-1.22)

*CI indicates confidence interval.

†Odds ratios for estrogen use were adjusted for matching variables (age, calendar year, and treated hypertension) and potential confounding variables of race, current smoking, diabetes, cholesterol, and systolic blood pressure.[17,19] Odds ratios for factor XIII genotypes were adjusted only for age and race; additional adjustment for smoking status, hypertension, diabetes, or cholesterol level did not appreciably alter the results.

of odds, *P* value = .009). The OR associated with carrying at least 2 variant alleles was 0.54 (95% CI = 0.34-0.86) compared to women who carried less than 2 variant alleles. Of the 13 subjects with 3 or more variant factor XIII alleles, none suffered a nonfatal MI. Based on these findings, we additionally explored the possibility of an interaction between estrogen replacement therapy and combined number of factor XIII variant alleles on the risk of MI (Table 5). In the presence of at least 2 factor XIII variant alleles, estrogen use was associated with a lower risk of MI (OR = 0.43, 95% CI = 0.14-1.30). In the presence of less than 2 factor XIII variant alleles, estrogen use was associated with a higher risk of MI (OR = 1.41, 95% CI = 0.96-2.07). The synergy index was 0.28 (95% CI = 0.09-0.87), which indicates a significant interaction between estrogen therapy and factor XIII variant allele copy number on MI risk (*P* value for estrogen-gene interaction = .027). In sensitivity analyses, the apparent protective interaction between estrogen use and factor XIII variant allele copy number did not differ significantly by hormone therapy type (estrogen alone vs combined estrogen/progestin), dose, duration, or assuming 100% rather than 80% drug compliance (data not shown).

Finally, we examined the MI risk estimates for current estrogen use associated with 0, 1, and 2 or more factor XIII variant allele copies, according to factor V Leiden and prothrombin G20210A status. Because of the relatively small number of subjects carrying factor V Leiden (n = 46) and prothrombin G20210A (n = 29), women carrying either prothrombotic mutation were grouped together. In the absence of any factor XIII variant allele, the OR for MI associated with current estrogen use was 3.7-fold higher (95% CI, 0.8- to 17.5-fold higher) in women carrying factor V Leiden or prothrombin G20210A than in women carrying neither prothrombotic mutation. However, as the number of factor XIII variant alleles increased, the protective effect of factor XIII genotype on risk of MI associated with current estrogen use became more pronounced in factor V Leiden or prothrombin G20210A carriers than in noncarriers. Among women carrying one factor XIII variant allele, the ratio of the estrogen-associated risks of MI in women carrying factor V Leiden or prothrombin G20210A compared to those carrying neither factor V Leiden nor prothrombin G20210A was 0.8 (95% CI, 0.1-6.1). Among women carrying at least 2 factor XIII variant alleles, none of those who also carried factor V Leiden or prothrombin G20210A and were current estrogen users had an MI, and the synergy index was 0 (95% CI, 0-3.5). Although the 95% confidence intervals overlap and the differences are not statistically significant, the synergy indices show a trend toward greater protection against MI by factor XIII variants among current estrogen users who carry factor V Leiden or prothrombin G20210A than among current estrogen users who carry neither prothrombotic mutation. Only one study subject, an estrogen nonuser and current smoker who had no other major risk factors, carried both the factor V Leiden and prothrombin G20210A mutations. She had no copies of either the factor XIIIA Leu34 variant or the factor XIIIB Arg95 variant, and suffered an MI at age 59.

Discussion

In this population-based case-control study of postmenopausal women, the presence of 1 or 2 copies of the Leu34 variant of the

Table 4. Association of total number of copies of factor XIIIA Leu34 and factor XIIIB Arg95 alleles and risk of nonfatal myocardial infarction

No. of factor XIII variant alleles*	No. of cases	No. of controls	Odds ratio (95% CI)†	*P*‡
0	120	329	1.00 (reference)	
1	89	265	0.91 (0.67-1.26)	.58
2	25	114	0.58 (0.35-0.94)	.03
3 or 4	0	13	0 (0.00-0.82)	.03

*Total copy number of factor XIIIA Leu34 and factor XIIIB Arg95 alleles.

†Odds ratios adjusted for age and race. CI indicates confidence interval.

‡*P* value for trend of odds = .009.

Table 5. Interaction between current estrogen use and total number of copies of factor XIIIA Leu34 and factor XIIIB Arg95 alleles on risk of nonfatal myocardial infarction

Estrogen use	No. of factor XIII variant alleles*	No. of cases	No. of controls	Odds ratio (95% CI)	*P*	Measure	Point estimate (95% CI)	*P*
Not current	< 2	135	376	1.00 (reference)		OR for < 2 copies†	1.41 (0.96-2.07)	.07
Current	< 2	74	218	1.40 (0.96-2.04)	.08			
Not current	≥ 2	19	77	0.85 (0.47-1.55)	.59	OR for ≥ 2 copies†	0.43 (0.14-1.30)	.13
Current	≥ 2	6	50	0.33 (0.13-0.85)	.02			

OR indicates odds ratio; CI, confidence interval. Odds ratios for current estrogen use compared to not current (past or never) use were adjusted for age, race, calendar year, treated hypertension status, current smoking, diabetes, systolic blood pressure, and cholesterol.

*Total copy number of factor XIIIA Leu34 and factor XIIIB Arg95 alleles.

†Odds ratio for myocardial infarction associated with current estrogen use among subjects with the indicated number of factor XIII variant alleles.

factor XIIIA gene was associated with decreased risk of nonfatal MI. We also provide the first evidence that a variant of the factor XIIIB gene, His95Arg, may influence cardiovascular risk. By itself, the Arg95 variant was associated with little protection against MI. However, the Arg95 variant reduced significantly the risk of MI in women carrying the Leu34 variant. Similarly, neither factor XIII gene variant alone significantly modified the MI-estrogen association, but, in exploratory analyses, there was a significant drug-gene interaction between the total copy number of factor XIII variant alleles (factor XIIIA Leu34 + factor XIIIB Arg95) and current estrogen use. Among women who had 2 or more copies of the variant factor XIII alleles and were current estrogen users, the risk of nonfatal MI was reduced by nearly 70% relative to estrogen nonusers with less than 2 factor XIII variant alleles.

A primary limitation of this study was the simplicity of the original hypotheses, which included main effects for the 2 factor XIII polymorphisms and interactions between the individual polymorphisms and estrogen replacement therapy. Single nucleotide polymorphisms are not likely to have major effects on non-Mendelian complex disorders such as myocardial infarction. Complex interactions not only between genetic variants but also between genetic variants and environmental factors, including drug therapies such as estrogen, are more plausible biologic mechanisms for variation in the incidence of myocardial infarction. While there was evidence of a graded association between number of copies of factor XIII variant alleles and risk of MI, the sample size and low prevalence of homozygosity for the Leu34 and Arg95 variants limited our ability to assess precisely the effect of allele dosage on risk of MI. Additionally, because of its retrospective nature, the study was confined to case subjects with nonfatal MI, and it is possible that the factor XIII variants may affect case fatality rate rather than disease incidence. Thus, genotypic data from prospective studies involving larger numbers of postmenopausal women will be required to confirm these preliminary findings.

The finding of a reduced risk of MI associated with the Leu34 variant of the factor XIII A subunit in postmenopausal women is consistent with several recent reports that involved mostly men or premenopausal women.[12,14,15] Some of these reports suggested that the modifying effect of common polymorphic variants of factor XIIIA may be confined to susceptible individuals who have metabolic or lifestyle risk factors or who carry other genetic variants that influence coagulation and fibrinolysis.[12,14,15,21] In studies of younger adult men and women, the decreased risk of MI associated with the Leu34 variant was most pronounced in the presence of traditional cardiovascular risk factors such as current smoking, obesity, and hypertension, compared to the absence of these risk factors.[14,15] The absence of any significant modification of the risk of MI associated with factor XIIIA Leu34 by traditional risk factors in the present study of postmenopausal women suggests that such gene-environment interactions may assume greater importance in premenopausal women than postmenopausal women. In other population studies of arterial and venous thrombotic disease, possible gene-gene interactions between coagulation factor XIII and hemostasis proteins involved in fibrin stabilization, such as fibrinogen and plasminogen activator inhibitor-1, have been noted.[12,15,22,23] Similarly, we observed that the apparent thromboprotective effect of the factor XIIIA Leu34 variant was significantly enhanced by the additional presence of the factor XIIIB Arg95 variant. Moreover, we observed a greater modifying effect of factor XIII genotype on lowering the risk of MI associated with current estrogen use in postmenopausal women who carried the factor V Leiden or prothrombin G20210A mutations than in postmenopausal women who did not carry factor V Leiden or prothrombin G20210A.

The Val34Leu amino acid substitution increases the rate of factor XIIIA activation by thrombin and appears to result in an abnormal fibrin clot structure.[11] The His95Arg polymorphism is located within the second sushi domain of the B subunit of factor XIII and increases the rate of dissociation of the factor XIII A_2B_2 tetramer following thrombin activation.[16] Thus, the synergistic reduction in MI risk associated with the combined copy number of Leu34 and Arg95 alleles may result from the cooperative effects of each variant copy in altering the kinetics of plasma factor XIII activation. While these potential gene-gene interactions require confirmation in larger studies, they highlight the importance of examining the joint effects of genes or genotypes that are known to interact at the biochemical or molecular level[24] and the potential role of multilocus genotypes in determining genetic susceptibility to complex disease.[25,26] Additional biochemical experiments will be required to confirm the synergistic effects of these amino acid substitutions on factor XIII procoagulant function.

Until recently, recommendations about estrogen replacement therapy were based largely on observational data indicating that hormone use in postmenopausal women reduces cardiovascular disease risk.[27] Two recently published large, randomized, placebo-controlled trials of primary (Women's Health Initiative [WHI])[28] and secondary (Heart and Estrogen/Progestin Replacement Study [HERS] II)[29] prevention showed no overall cardiovascular benefit of estrogen replacement therapy. It is noteworthy that the lack of overall protection from risk of MI with current estrogen use in the present observational study is consistent with the results of the 2 recent clinical trials,[28,29] but contrary to the reduced MI risk in prior observational studies of hormone replacement therapy.[27] As an observational study, the case-control design may introduce a number of biases, since hormone replacement is not randomly assigned and information

on estrogen use and comorbid conditions must be obtained retrospectively. A recent meta-analysis suggested little association between estrogen replacement therapy and MI when only well-designed observational studies that controlled for important confounders, such as socioeconomic status and major cardiovascular risk factors, were included.[30] Since we obtained detailed information on traditional coronary risk factors and comorbid conditions, we were able to adjust for characteristics known to be associated with MI risk. Another important feature of our study was that information on estrogen use was obtained from prescription refills using a computerized pharmacy database rather than by self-report. While we did not specifically validate estrogen use data obtained in this manner, the validity of centralized pharmacy records as a source of drug compliance information in the setting of population-based studies has been documented previously.[31] Furthermore, sensitivity analyses performed in the present study suggested that the results were not affected by plausible changes in assumptions regarding compliance or characteristics of the hormone prescription.

The excess of arterial thrombotic events shortly after initiating hormone therapy in clinical trials of women with[3,29] or without[28] clinically apparent cardiovascular disease suggests an interaction between postmenopausal estrogen use and other factors that influence thrombosis and fibrinolysis. Several recent studies suggest that prothrombotic mutations including factor V Leiden and prothrombin G20210A may identify individual women who are at particularly high risk of developing arterial or venous thrombotic events following the initiation of estrogen replacement therapy.[7,8,32,33] The findings of the current study suggest that coagulation factor XIII variants may modify the prothrombotic effect of estrogen and thus identify a subgroup of women least likely to experience the early adverse cardiovascular effects of hormone therapy. If these findings are confirmed, screening of postmenopausal women for genetic factors such as factor XIIIA Val34Leu and factor XIIIB His95Arg that interact with estrogen to modulate thrombotic disease susceptibility may guide decisions about use of estrogen replacement therapy.

Acknowledgments

We are indebted to the study participants and their physicians at Group Health Cooperative and to Kerri Wiggins, MS, RD, for data management.

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