

# Hormone Replacement Therapy, Prothrombotic Mutations, and the Risk of Incident Nonfatal Myocardial Infarction in Postmenopausal Women

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FOR MANY YEARS, RECOMMENDATIONS about the use of hormone replacement therapy (HRT) in postmenopausal women have been based largely on observational studies, which suggest that HRT reduces the risk of coronary heart disease.<sup>1-3</sup> The likely mechanisms are numerous<sup>4</sup> and include the beneficial effects of estrogens on lipids.<sup>5</sup> But estrogens are also known to be prothrombotic.<sup>6</sup> In men with prostate cancer or cardiovascular disease<sup>7,8</sup> and in women on oral contraceptives,<sup>9</sup> high doses or potent formulations of estrogens are associated with thrombotic complications, including myocardial infarction (MI), stroke, and venous thrombosis. In postmenopausal women, HRT is also a risk factor for venous thrombosis.<sup>10-13</sup>

The results of the Heart and Estrogen/progestin Replacement Study (HERS) have renewed interest in the potential adverse effects of HRT.<sup>12,13</sup> In this randomized clinical trial of secondary prevention, combined HRT was no better than placebo at preventing coronary events in postmenopausal women (relative risk [RR], 0.99; 95% confidence interval [CI], 0.81-1.22).

**Context** Estrogens are known to be prothrombotic, and findings from the Heart and Estrogen/progestin Replacement Study suggest that in women with clinically recognized heart disease, hormone replacement therapy (HRT) may be associated with early harm and late benefit in terms of coronary events.

**Objective** To assess whether, as hypothesized, prothrombotic mutations modify the association between HRT use and incidence of first myocardial infarction (MI).

**Design and Setting** Population-based, case-control study conducted in a Seattle-based health maintenance organization.

**Participants** Cases were 232 postmenopausal women aged 30 to 79 years who had their first nonfatal MI between 1995 and 1998. Controls were a stratified random sample of 723 postmenopausal women without MI who were frequency-matched to cases by age, calendar year, and hypertension status.

**Main Outcome Measure** Risk of first nonfatal MI based on current use of HRT and the presence or absence of coagulation factor V Leiden and prothrombin 20210 G→A variants among cases and controls, stratified by hypertension.

**Results** One hundred eight MI cases and 387 controls had hypertension and 124 MI cases and 336 controls did not. Among hypertensive women, the prothrombin variant was a risk factor for MI (odds ratio [OR], 4.32; 95% confidence interval [CI], 1.52-12.1) and, in this stratum, there was also a significant interaction between use of HRT and presence of the prothrombin variant on risk of MI. Compared with nonusers of HRT with wild-type genotype, women who were current users and who had the prothrombin variant (n=8) had a nearly 11-fold increase in risk of a nonfatal MI (OR, 10.9; 95% CI, 2.15-55.2). The interaction with the prothrombin variant was more pronounced in analyses assuming 100% compliance than in those assuming 80% compliance with HRT. The interaction was absent among nonhypertensive women and was less pronounced if hypertensive and nonhypertensive women were combined into 1 group. No interaction was found for factor V Leiden in either hypertensive or nonhypertensive women. Among hypertensive women, the estimates were affected only in trivial ways by adjustment, and the interaction with the prothrombin variant was specific to HRT.

**Conclusions** Our results suggest that among postmenopausal hypertensive women, the association between HRT use and MI risk differed between those with and without the prothrombin 20210 G→A variant. If these findings are confirmed in other studies, screening for the prothrombin variant may permit a better assessment of the risks and benefits associated with HRT in postmenopausal women.

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terval [CI], 0.81-1.22). In post-hoc analyses, treatment was associated with early harm during the first year of follow-up (RR, 1.52; 95% CI, 1.01-2.29)

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and a late benefit during follow-up years 4 and 5 (RR, 0.75; 95% CI, 0.50-1.13). One broad hypothesis offered to explain this pattern of risks was the possibility of "an immediate prothrombotic, proarrhythmic, or proischemic effect of treatment that is gradually outweighed by a beneficial effect on the underlying progression of atherosclerosis."<sup>12,13</sup> According to this interpretation, a subgroup—perhaps defined by a clinical characteristic, an environmental exposure, or a genetic trait—is susceptible to an early adverse effect of estrogens while the rest of the population benefits from estrogen therapy. When a therapy produces effects that differ by more than chance variation in 2 subgroups, an interaction is present.

Genetic variants are excellent candidates for such interactions. Vandembroucke et al<sup>14</sup> have described an interaction between oral contraceptives and factor V Leiden on the risk of deep venous thrombosis. While factor V Leiden and the prothrombin 20210 G→A variant are clearly associated with the risk of venous thrombosis,<sup>15</sup> reports of an association between these prothrombotic variants and coronary heart disease have been inconsistent, some finding an increased risk,<sup>16-19</sup> while others do not.<sup>20-24</sup> Whether HRT places women with either of these 2 prothrombotic variants at an especially high risk of MI remains unknown. Before the results of HERS were published or known to us, we initiated a population-based, case-control study to assess this interaction as an *a priori* hypothesis.

## METHODS

### Setting

The setting for this project was Group Health Cooperative (GHC), a Seattle, Wash.-based health maintenance organization with an enrollment of more than 400 000. The methods have been described previously<sup>25,26</sup> and will be summarized only briefly here. The study was reviewed and approved by human subjects committees at both GHC and the University of Washington, Seattle. All subjects gave written

informed consent before initiating the study. During the period of this study, the preferred oral estrogens at the GHC were esterified estrogens.

### Identification of Cases and Controls

Cases were female GHC enrollees who survived an incident MI between January 1995 and December 1998. Potential cases were identified from 2 sources: (1) the computerized discharge abstracts for the 2 GHC hospitals; and (2) the GHC claims databases, which include bills for all services provided by non-GHC physicians and health care facilities. We have used and evaluated similar methods in previous case-control studies.<sup>25-28</sup> Due to different funding sources, cases were stratified on hypertension status as assessed by the computerized pharmacy database. Controls were a stratified random sample of postmenopausal female GHC enrollees sampled from the GHC computerized enrollment files on the basis of person-time.<sup>29</sup> Controls were frequency matched to the cases by age (within decade), calendar year, and hypertension status at a ratio of approximately 3 to 1. Controls met the same eligibility criteria as the cases, but they had not had an MI. Grants from the National Institutes of Health and the American Heart Association funded primary data collection on cases with and without hypertension, respectively.

### Index Dates and Eligibility

All women had an index date. For the cases, the index date was the date of admission for the first acute MI; and for the controls, the index date was a computer-generated random date within the same calendar year for which they had been chosen as controls. For all women, we only collected risk factor data available before the index date. This approach ensured that cases and controls met the same eligibility criteria. All women were aged 30 to 79 years at their index dates. For all subjects, we excluded women who were members of GHC for less than 1 year or who did not have at least 4 physician visits prior to

their index dates; women who had had a prior MI; and women who were not postmenopausal. Additionally, we excluded cases whose index event was a complication of a procedure or a surgery.

### Data Collection and Definition of HRT Use

Data collection included a review of the GHC outpatient medical record, a telephone interview, and a venous blood sample from women who consented to participate. Based on the medical record, research assistants determined eligibility and collected information about the following traditional risk factors for coronary heart disease: blood pressure and pulse; height and weight; cholesterol level, smoking status, family history, hysterectomy status, marital status, and use of health services; medical conditions such as angina, hypertension, diabetes, congestive heart failure, stroke, and peripheral vascular disease. Cardiovascular disease was defined as a history of angina, stroke, claudication, or vascular procedures, including coronary bypass, angioplasty, carotid endarterectomy, or peripheral vascular bypass. Research assistants were not blinded to case-control status.

The GHC computerized pharmacy database was used to assess current HRT use at the index date. Since 1976, the GHC pharmacy database has included a record for all prescriptions dispensed to GHC enrollees. Each pharmacy record includes a patient identifier, the drug type and dose, the date, the quantity dispensed, and dosing instructions. For determining current use, we searched the pharmacy data for the hormone prescription immediately preceding the reference date. When a woman (who was at least 80% compliant) received enough pills to last until her index date, she was counted as a current user; otherwise, she was counted as a nonuser. For 80% compliance, a woman who received 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 dispensing date. Current progestin use was de-

fined in the same way. In preplanned sensitivity analyses, we reanalyzed data that defined current HRT use assuming 100% rather than 80% compliance. For 100% compliance, a woman who received 100 pills with instructions to take 1 pill per day was counted as a current user for 100 days after the prescription dispensing date. Only users of oral estrogens with or without progestins were classified as users.

### Blood Collection and Laboratory Analysis

A blood specimen was drawn from the antecubital vein into tubes containing edetic acid and kept at 4°C until the

blood was initially processed. Buffy coats were prepared, washed in saline, and stored at -70°C. Specimens were shipped on dry ice to the laboratory in Leiden, the Netherlands. The DNA was extracted from the white blood cells using standard salting-out procedures.<sup>30</sup> The status of the prothrombin variant (20210 G→A) was assessed by the presence of a *HindIII* restriction site in the polymerase chain reaction fragment.<sup>31</sup> The presence of factor V Leiden (1691 G→A) was assessed by the loss of an *MnlI* restriction site as originally described by Bertina et al.<sup>32</sup> Laboratory personnel were blinded to case-control status and to HRT status.

### Statistical Analysis

In comparing case and control characteristics, we used the *t* test for continuous variables, the  $\chi^2$  test for categorical variables, and the Fisher exact test. Because of the stratified sampling, the analysis was stratified on hypertension status. Odds ratios (ORs) and CIs were estimated in the standard way,<sup>33,34</sup> and logistic regression was used for multivariable analysis. The ORs estimating the association between HRT and MI were also calculated separately in the 2 strata defined by genotype: (1) women with a prothrombotic variant (susceptible women); and (2) women with the wild-type (normal) genotype (nonsusceptible women). All statistical tests were 2-tailed.

For the primary analysis, we classified women as current or not current users of HRT at their index dates by the 80% compliance method. The current users were compared with the noncurrent users. Women with a prothrombotic variant were compared with women with the wild-type genotype. Formal tests for interaction were carried out with both case-control and case-only methods,<sup>35,36</sup> which are more efficient and powerful than case-control methods.<sup>37</sup> Both methods estimate the synergy index (SI), which is a ratio of the OR in the susceptible women to the OR in the nonsusceptible women. An SI of 1 means that the ORs in the 2 subgroups were the same and that there was no interaction on the multiplicative scale; a SI of greater than 1 means that the joint effect of gene and the drug were supramultiplicative compared with their expected effect, which is the product of their individual effects.

### RESULTS

Of the 955 women, 108 cases and 387 controls had hypertension, and 124 cases and 336 controls were in the stratum without hypertension. The control-to-case matching ratio was higher in the hypertensive (3.6:1) than in the nonhypertensive (2.7:1) women. Genotype assays were available for 950 women for factor V Leiden and for 953 women for the prothrombin variant. Factor V

**Table 1.** Characteristics of Cases and Controls Stratified on the Presence of Treated Hypertension

Characteristics*	With Hypertension		Without Hypertension	
	Cases	Controls	Cases	Controls
No. of persons	108	387	124	336
Age, y†	67.8	67.7	67.1	67.0
Blacks	4.6	6.5	0.8	6.5§
Current smokers	24.1	9.8§	29.8	8.9§
Nonsedentary	59.3	73.4§	64.5	73.8
Married	59.3	64.6	46.8	62.2§
Hysterectomy	40.7	38.5	29.0	32.7
Diabetes	29.6	9.6§	17.7	3.9§
Angina	26.9	10.3§	14.5	6.0§
Any cardiovascular disease	37.0	16.3§	20.2	8.3§
History of coronary bypass surgery or angioplasty	7.4	1.6§	1.6	1.2
Family history of myocardial infarction	46.3	29.2§	37.9	20.8§
Height, m†	1.6	1.6	1.6	1.6
Weight, kg†	79.0	78.6	76.1	71.7§
Time enrolled in Group Health Cooperative, y†	19.9	22.2	18.9	23.4§
No. of visits in prior year	7.5	6.0§	5.1	4.8
Blood pressure†				
Systolic, mm Hg	145.2	144.0	142.6	131.7§
Diastolic, mm Hg	78.9	81.7§	81.1	77.4§
Cholesterol, mg/dL (mmol/L)†	250.3 (6.5)	234.5 (6.1)§	243.1 (6.3)	225.4 (5.8)§
High-density lipoprotein cholesterol, mg/dL (mmol/L)†	48.2 (1.3)	55.1 (1.4)§	51.4 (1.3)	58.6 (1.5)§
Glucose, mg/dL (mmol/L)†	134.9 (7.5)	110.8 (6.2)§	117.8 (6.5)	101.5 (5.6)§
Potassium, mmol/L†	4.2	4.1	4.3	4.3
Creatinine, mg/dL (μmol/L)†	1.0 (88)	1.0 (84)	0.9 (80)	0.9 (80)
Prothrombin‡	7.4	1.8§	4.0	3.3
Factor V Leiden‡	3.7	4.9	4.0	6.0
Current estrogen use	38.0	37.5	31.5	36.9

\*Values are expressed as percentages unless otherwise indicated.

†Values are expressed as means.

‡Heterozygous or homozygous for the variant allele.

§*P* < .05 for the comparison between cases and controls separately for those with and without hypertension.

Leiden was present in 23 hypertensive and 25 nonhypertensive women, and no homozygotes were present. The prothrombin variant was present in 15 hypertensive and 15 nonhypertensive women. One additional nonhypertensive control was homozygous for the variant prothrombin allele.

Within each stratum, frequency matching produced a control group with a mean age close to that of the cases (TABLE 1). In both the hypertensive and the nonhypertensive strata, diabetes, smoking, physical activity, cholesterol, high-density lipoprotein cholesterol, glucose, family history, and a history of angina differed between cases and controls in the expected manner. Among nonhypertensive women, weight and systolic and diastolic blood pressure also differed significantly between cases and controls. Among the hypertensive women, weight, systolic blood pressure, and mean time enrolled in GHC were similar in the cases and controls.

TABLE 2 summarizes the main effects of HRT, factor V Leiden, and the prothrombin variant on MI risk. Among hypertensive women, current HRT use and factor V Leiden were only weakly associated with MI risk. On the other

hand, the prothrombin variant was associated with an increased risk of MI in hypertensive women (OR, 4.32, 95% CI, 1.52-12.1), after adjustment for covariates, the OR increased to 7.02 (95% CI, 2.27-21.8). Among nonhypertensive

women, there was little association of HRT or either prothrombotic variant with case-control status, before or after adjustment for covariates.

TABLE 3 summarizes the stratified analyses for the prothrombin variant.

**Table 2.** Association of Hormone Replacement Therapy, Factor V Leiden, and the Prothrombin Variant With First Nonfatal Myocardial Infarction

Characteristic	No. of Cases	No. of Controls	Odds Ratio (95% Confidence Interval)	
			Unadjusted	Adjusted*
With Hypertension				
Hormone use				
Not current	67	242	1.00	1.00
Current	41	145	1.02 (0.66-1.59)	1.28 (0.78-2.09)
Factor V Leiden				
Wild type	104	363	1.00	1.00
Variant	4	19	0.67 (0.31-1.48)	0.61 (0.23-1.61)
Prothrombin				
Wild type	100	378	1.00	1.00
Variant	8	7	4.32 (1.52-12.1)	7.02 (2.27-21.8)
Without Hypertension				
Hormone use				
Not current	85	212	1.00	1.00
Current	39	124	0.78 (0.51-1.22)	1.06 (0.63-1.78)
Factor V Leiden				
Wild type	119	316	1.00	1.00
Variant	5	20	0.66 (0.24-1.81)	0.46 (0.13-1.59)
Prothrombin				
Wild type	119	325	1.00	1.00
Variant	5	11	1.24 (0.42-3.65)	0.79 (0.23-2.70)

\*Adjusted for age, calendar year, smoking, cardiovascular disease, systolic blood pressure, total cholesterol, and diabetes.

**Table 3.** Association Among Hormone Use, Prothrombin Variant, and Risk of First Nonfatal Myocardial Infarction\*

Hormone Use	Prothrombin	No. of Cases	No. of Controls	Odds Ratio (95% Confidence Interval)	P Value	Measure	No. of Subjects	Estimate (95% Confidence Interval)	P Value
<b>With Hypertension, 80% Compliance</b>									
Not current	Wild type	65	236	1.00		Nonsusceptible OR	478	0.89 (0.56-1.42)	.64
Current	Wild type	35	142	0.89 (0.56-1.42)	.64	Susceptible OR	15	7.50 (0.76-74.2)	.10†
Not current	Variant	2	5	1.45 (0.28-7.66)	.66	Case-control SI	496	8.38 (0.81-86.8)	.06‡
Current	Variant	6	2	10.9 (2.15-55.2)	.002†	Case-only SI	108	5.57 (1.07-29.1)	.03†
<b>With Hypertension, 100% Compliance</b>									
Not current	Wild type	70	241	1.00		Nonsusceptible OR	478	0.75 (0.47-1.21)	.24
Current	Wild type	30	137	0.75 (0.47-1.21)	.24	Susceptible OR	15	18.0 (1.27-256)	.03†
Not current	Variant	2	6	1.15 (0.23-5.81)	.87	Case-control SI	496	23.9 (1.61-354)	.008‡
Current	Variant	6	1	20.7 (2.45-174)	.001†	Case-only SI	108	7.0 (1.34-36.7)	.02†
<b>Without Hypertension</b>									
Not current	Wild type	80	205	1.00		NA		NA	NA
Current	Wild type	39	120	0.83 (0.53-1.30)	.42	NA		NA	NA
Not current	Variant	5	7	1.83 (0.56-5.94)	.31	NA		NA	NA
Current	Variant	0	4	0	.21	NA		NA	NA

\*OR indicates odds ratio; SI, synergy index; and NA, not applicable.

†Calculated by Fisher exact test.

‡Calculated by the likelihood ratio method.

**Table 4.** Association Among Hormone Use, Factor V Leiden, and Risk of First Nonfatal Myocardial Infarction

Hormone Use	Factor V Leiden	No. of Cases	No. of Controls	Odds Ratio (95% Confidence Interval)	P Value	Measure	No. of Subjects	Estimate (95% Confidence Interval)	P Value
<b>With Hypertension</b>									
Not current	Wild type	64	227	1.00		Nonsusceptible OR	467	1.04 (0.67-1.63)	.85
Current	Wild type	40	136	1.04 (0.67-1.63)	.85	Susceptible OR	23	0.57 (0.05-6.61)	.65
Not current	Variant	3	12	0.89 (0.24-3.24)	.86	Case-control SI	490	0.55 (0.05-6.60)	.64
Current	Variant	1	7	0.51 (0.06-4.19)	.52	Case-only SI	108	0.53 (0.05-5.31)	.59
<b>Without Hypertension</b>									
Not current	Wild type	81	198	1.00		Nonsusceptible OR	435	0.79 (0.50-1.23)	.29
Current	Wild type	38	118	0.79 (0.50-1.23)	.29	Susceptible OR	25	0.58 (0.05-6.37)	.66
Not current	Variant	4	14	0.70 (0.22-2.19)	.54	Case-control SI	460	0.74 (0.07-8.44)	.81
Current	Variant	1	6	0.41 (0.05-3.44)	.39	Case-only SI	124	0.53 (0.06-4.93)	.57

**Table 5.** Effect of Adjustment on the Association of Hormone Use and Prothrombin Variant With the Risk of First Nonfatal Myocardial Infarction in Women With Hypertension\*

Adjustment	Hormone Use	Prothrombin Variant	Both
No covariates	0.89 (0.56-1.42)	1.45 (0.28-7.66)	10.9 (2.15-55.2)
Age	0.88 (0.56-1.41)	1.49 (0.28-7.86)	11.0 (2.17-55.9)
Calendar year	0.87 (0.54-1.38)	1.37 (0.25-7.38)	11.6 (2.27-59.6)
Race	0.89 (0.56-1.41)	1.43 (0.27-7.53)	10.7 (2.11-54.3)
Current smoking	0.90 (0.56-1.43)	1.74 (0.33-9.24)	11.8 (2.29-60.6)
Diabetes	1.07 (0.66-1.74)	2.04 (0.38-10.9)	13.5 (2.59-70.2)
Cardiovascular disease	0.92 (0.58-1.48)	1.35 (0.24-7.48)	10.4 (1.97-54.4)
Systolic blood pressure	0.90 (0.57-1.44)	1.48 (0.28-7.81)	10.8 (2.14-55.0)
Cholesterol	0.92 (0.58-1.47)	1.74 (0.32-9.39)	13.3 (2.58-68.1)

\*Values are expressed as odds ratio (95% confidence interval).

Hypertensive women who had the wild-type genotype for prothrombin and who were not current users of HRT served as the reference group. Among women with the wild-type genotype, HRT use was associated with a small reduction in the risk of nonfatal MI (OR, 0.89; 95% CI, 0.56-1.42). Compared with the reference group, women who had the prothrombin variant and who were not current users of HRT had only a small increase in the OR to 1.45 (95% CI, 0.28-7.66). In the absence of an interaction (on a multiplicative scale), the expected joint effects of the prothrombin variant and current HRT use would be 1.29. Compared with the reference group, the 8 women who had the prothrombin variant and who were current users of HRT had an almost 11-fold increase in the risk of MI (OR, 10.9; 95% CI, 2.15-55.2;  $P=.002$ ).

The analyses stratified on susceptibility also appear in Table 3. Among the 478 nonsusceptible, hypertensive

women (with prothrombin wild type), HRT use was associated with a slight decrease in the risk of MI (OR, 0.89). Among the 15 susceptible, hypertensive women (with prothrombin variant), HRT use was associated with an increased risk of MI (OR, 7.50; 95% CI, 0.76-74.2;  $P=.10$ ). The case-control estimate of the SI was 8.38 (95% CI, 0.81-86.8;  $P=.06$ ). The case-only estimate of the SI was similar at 5.57 (95% CI, 1.07-29.1;  $P=.03$ ) and indicated a significant interaction between HRT and the prothrombin variant on the risk of nonfatal MI among hypertensive women. When the analysis was restricted to whites only (data not shown), the case-control SI was 8.62 (95% CI, 0.83-89.4); the corresponding case-only SI was 5.38 (95% CI, 1.03-28.15). When we assumed 100% rather than 80% compliance, the evidence of an interaction became more pronounced with a case-control SI of 23.9 (95% CI, 1.61-354;  $P=.008$ ).

Among women without hypertension, no cases had the prothrombin variant and used HRT. There was no evidence of an interaction. Combining estimates from these 2 strata, defined by hypertension status, did not appear to be appropriate. If hypertensive and nonhypertensive women were combined into a single group, the interaction would have been less pronounced (SI, 2.0; 95% CI, 0.45-8.95).

TABLE 4 summarizes the data for factor V Leiden in the same format used for the prothrombin variant. Again, women who were not current users by the 80% compliance method and who did not have factor V Leiden served as the reference group. Individually or jointly, there was little association between HRT use or factor V Leiden and MI risk. None of the CIs for these associations excluded the null hypothesis. The SIs estimated by both the case-control and the case-only methods were small and did not differ from the null hypothesis of no interaction.

The small number of hypertensive women with the prothrombin variant ( $n=15$ ) limited the ability to adjust for potential confounding factors. TABLE 5 summarizes the effect of adjustment for a number of covariates one at a time. The unadjusted OR estimates are the same as in Table 3: 0.89 for HRT use, 1.45 for the prothrombin variant, and 10.9 for the combination of HRT use and the prothrombin variant. Adjustments for age, calendar year, race, smoking, diabetes, cardiovascular dis-

ease, systolic blood pressure, or cholesterol each had little effect on the point estimates. The lowest adjusted OR for the combination of HRT use and the prothrombin variant was 10.4, and the lower limit of the 95% CIs never went below 1.97.

TABLE 6 summarizes the findings of a series of analyses that attempted to identify other potential interactions with the prothrombin variant on the risk of MI, primarily among women with hypertension. The SIs for HRT use at 80% and 100% compliance were 8.38 (95% CI, 0.81-86.8) and 23.9 (95% CI, 1.61-354), respectively. No other characteristic was associated with such an elevated SI. For most of the characteristics, the ORs for MI risk were similar among those with and without the prothrombin variant. For the association between cardiovascular disease (such as angina) and MI risk, the OR was higher among those with the prothrombin variant (OR, 6.00, 95% CI, 0.48-75.4) than among those without the prothrombin variant (OR, 2.92, 95% CI, 1.79-4.78). The SI for cardiovascular disease was 2.05 (95% CI, 0.16-27.0). Zero entries precluded the ability to examine an interaction for smoking and for diabetes in the hypertensive women, but there was no evidence of an interaction in the population as a whole.

Among the controls, 85.7% of HRT users were taking esterified estrogens, and most of the others were taking conjugated estrogens. Estrogen patches were rarely used at GHC (2 cases and 2 controls). Among the 8 hypertensive women currently taking HRT and having the prothrombin variant, the 6 cases had a slightly smaller mean number of lifetime prescriptions for estrogens than the 2 controls (31 vs 39,  $P = .71$ ). One of the 6 cases had started HRT 7 months prior to her MI while the other 5 had been regular users for at least 1 year before their MI event. Among the 6 hypertensive cases who had the prothrombin variant and who were current users of HRT, all 6 were using esterified estrogens, 5 of the 6 were taking estrogen alone rather than in combination

**Table 6.** Candidate Characteristics for Interaction With the Prothrombin Variant and Case-Control Status\*

Characteristic	Prothrombin Variant		Case-Control Synergy Index†
	Without	With	
	Subjects With Hypertension		
Hormone use %			
80	0.89 (0.56-1.42)	7.50 (0.76-74.2)	8.38 (0.81-86.8)
100	0.75 (0.47-1.21)	18.0 (1.27-256)	23.9 (1.61-354)
Cardiovascular disease	2.92 (1.79-4.78)	6.00 (0.48-75.4)	2.05 (0.16-27.0)
Family history	2.19 (1.39-3.44)	1.50 (0.17-13.2)	0.69 (0.07-6.33)
Nonsedentary	0.51 (0.32-0.81)	0.50 (0.04-7.10)	0.98 (0.07-14.5)
High systolic blood pressure	0.93 (0.60-1.45)	0.80 (0.10-6.35)	0.86 (0.10-7.16)
High cholesterol	2.23 (1.41-3.53)	3.60 (0.28-46.4)	1.62 (0.12-21.7)
All Subjects			
Diabetes	4.14 (2.70-6.36)	5.10 (0.47-55.9)	1.23 (0.11-14.0)
Current smoking	3.66 (2.49-5.40)	3.09 (0.25-38.3)	0.84 (0.07-10.8)

\*Values are expressed as odds ratio (95% confidence interval) unless otherwise indicated  
†Values are expressed as synergy index (95% confidence interval)

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with a progestin, and 5 were taking 0.625 mg/d and 1 was taking 0.3 mg/d. The antihypertensive medications used by these 6 women were similar to the agents typically used at GHC, and 3 of the 6 were taking atenolol.

## COMMENT

In this population-based, case-control study, the prothrombin 20210 G→A variant was a risk factor for MI among hypertensive women. There was also a significant interaction between the use of HRT and the prothrombin variant on the risk of MI among women with hypertension. Compared with the reference group, the 8 women who were current HRT users and who had the prothrombin variant had a nearly 11-fold increase in the risk of a nonfatal MI (OR, 10.9, 95% CI, 2.15-55.2). The 95% CI of the SI as assessed by the more powerful case-only method, a formal test for a multiplicative interaction, excluded the null hypothesis (Table 3). The interaction was more pronounced for the 100% compliance method than for the 80% compliance method of defining current HRT use (Table 3). No such interaction was found for nonhypertensive women (Table 3) or for factor V Leiden in either the hypertensive or the nonhypertensive women (Table 4). Among the hypertensive women, the estimates were affected only in trivial ways by ad-

justment for potential confounding factors (Table 5). The interaction with the prothrombin variant was specific to HRT use and not to other characteristics (Table 6).

The prothrombin variant has been associated with an increased incidence of venous thrombosis and with elevated levels of prothrombin in plasma.<sup>31</sup> In other observational studies, the prothrombin variant has been associated with the incidence of MI in some studies<sup>17,19</sup> but not in others.<sup>20,22,24</sup> While previous studies have identified potential interactions of oral contraceptive use with factor V Leiden<sup>14</sup> and of HRT use with activated protein C resistance<sup>38</sup> on the incidence of venous thrombosis, there are no previous epidemiologic reports of an interaction between HRT use and prothrombotic variants on the risk of MI. The only cross-sectional analysis, conducted in women with hyperlipidemia, assessed HRT use at the time of the clinic visit rather than at the time of the cardiovascular event.<sup>39</sup>

This study had a number of limitations. Due to the low prevalence of the prothrombin variant, it was not possible to adjust simultaneously for multiple potential confounding factors in a single model.<sup>40</sup> While the healthy user effect is often invoked to explain the association between HRT use and a decreased risk of cardiovascular events,<sup>41,42</sup>

such biases are less likely to be important in studies of genetic variants as risk factors. In addition to the possibility of confounding, potential alternative explanations for the findings of genetic association studies include linkage disequilibrium and population admixture. The results were also based on a small number of women with the genetic variant, so there remained considerable statistical uncertainty around the risk estimates reported in this study.

For both the prothrombin variant and factor V Leiden, we had expected, but did not find, similar interactions with HRT use. The cases in this study all represent survivors of an MI, and it is possible that the interaction may affect mortality rather than disease incidence. If, for instance, the joint effects of factor V Leiden and HRT use are associated with a high case-fatality rate, a case-control study of nonfatal MI would fail to detect an interaction. On the other hand, survival bias is unlikely to induce the interaction seen with the prothrombin variant and HRT use in this study.

For the prothrombin variant, the findings of an interaction among those with hypertension were not confirmed by the findings among those without hypertension. Several explanations, including chance, are possible. Three of the 6 hypertensive cases who had the prothrombin variant and who were HRT users were also taking the  $\beta$ -blocker atenolol, which may have improved their post-MI survival. In hypertensive patients, moreover, the presence and the severity of target organ disease is strongly associated with several prothrombotic abnormalities, including elevated levels of D-dimer.<sup>43,44</sup> As we have previously hypothesized,<sup>6</sup> the presence of subclinical or clinical cardiovascular disease, such as angina or hypertensive disease, may be important in initiating the prothrombotic effects of estrogens, including any potential interaction with the prothrombin variant. Rosendaal<sup>15</sup> has described the risk of venous thrombosis in terms of multiple interacting causes, and a similar model may be relevant for arterial thrombotic disease.

In this case-control study, which excluded women with a previous MI, the interaction with the prothrombin variant was associated with current use of HRT rather than with recent initiation of therapy as in HERS. If a prothrombotic third factor such as coronary atherosclerosis or hypertensive target organ disease is also required,<sup>43,44</sup> the onset of the thrombotic event may be delayed until that third factor progresses far enough or becomes severe enough, in the presence of the prothrombin variant and HRT use, to precipitate an MI. If this third-factor hypothesis is true, the findings of this study would be expected to differ from those of HERS in terms of the time relationship between MI events and the initiation of HRT. HERS was a secondary prevention trial that enrolled only women with clinical coronary disease so that the hypothetical third factor was already present from the outset when participants were randomized and began taking HRT.

Among hypertensive controls, the prothrombin variant was present in only 1.8% of women. An uncommon susceptibility factor, such as the prothrombin variant or another associated with a high risk of events among HRT users, might account in part for the pattern of early harm and late benefit seen in the HERS trials.<sup>12,13</sup> Simulation studies suggest that the putative unknown susceptibility factor has to exhibit both a prevalence of 3% to 5% and a risk ratio of 13 to 25 in HRT users to reproduce the pattern of early harm and late benefit seen in HERS. While the prothrombin variant is a good candidate, other susceptibility factors are also likely to be important.

The long-term goal of research in the area of pharmacogenetics is to help clinicians individualize treatment for their patients and select drug therapies that maximize either effectiveness, or safety, or both. If the HERS findings are indeed the result of an interaction between HRT and a susceptibility factor, there is an urgent public health need to identify the putative susceptibility factor. Based on their biology and preva-

lence, the prothrombin variant and factor V Leiden were both reasonable candidates. The findings of this study suggest the possibility of an interaction between the prothrombin variant and HRT use on the incidence of MI among women with hypertension, but they need to be confirmed in other settings. If the findings are confirmed, or if other drug-gene interactions are identified, clinicians may eventually screen postmenopausal women for selected genetic variants that help characterize a woman's expected risk or benefit from HRT for a variety of outcomes, including MI, stroke, and venous thrombosis.

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