Interaction of Estrogen Replacement Therapy With the Thrombophilic 20210 G/A Prothrombin Gene Mutation for Atherothrombotic Vascular Disease: A Cross-Sectional Study of 275 Hyperlipidemic Women

Charles J. Glueck, Ping Wang, Robert N. Fontaine, Luann Sieve-Smith, and James E. Lang

In a consecutive case series, cross-sectional study of 275 women referred for therapy of hyperlipidemia, (75 [27%] on estrogen replacement therapy [ERT]), our specific aim was to determine whether ERT-mediated thrombophilia and heterozygosity for the thrombophilic 20210 G/A prothrombin gene mutation interacted as risk factors for atherothrombotic cardiovascular disease (ATCVD). Of the 275 women, 100 (36%) had ATCVD; 10 (3.6%) were heterozygous for the 20210 G/A prothrombin gene mutation. In women without the 20210 G/A prothrombin gene mutation, 15 of 71 (21%) on ERT had ATCVD versus 78 of 194 (40%) not on ERT ($X^2 = 8.31$, $P = .004$). By stepwise logistic regression, in 261 women with ATCVD risk factor data, positive explanatory variables for ATCVD included the 20210 G/A prothrombin mutation (risk odds ratio, 5.8; 95% confidence intervals [CI], 1.4 to 30.2; $P = .021$) and a 20210 G/A prothrombin gene mutation*ERT interaction term (risk odds ratio, 2.70; 95% CI, 1.4 to 5.4; $P = .004$). ATCVD events were more likely in 2 subgroups of women (ERT minus [−] and 20210 G/A prothrombin gene mutation −) or (ERT plus [+] and 20210 G/A prothrombin gene mutation +), $P = .004$. Other positive explanatory variables for ATCVD events included age ($P = .004$), triglycerides ($P = .012$), lipoprotein (a) ($P = .03$), and homocysteine ($P = .032$). ERT may be protective against ATCVD when the thrombophilic 20210 G/A prothrombin gene mutation is absent, whereas the 20210 G/A prothrombin gene mutation may increase risk for ATCVD, particularly in the presence of ERT. We suggest that the 20210 G/A prothrombin gene mutation be measured in all women on ERT or before beginning ERT to identify those heterozygous for the thrombophilic prothrombin gene mutation (4%) in whom ERT is contraindicated because of increased risk for ATCVD and thromboembolism, and a second, much larger group of women without the 20210 G/A prothrombin gene mutation (96%) in whom ERT may possibly reduce risk for ATCVD.

RECENTLY, THE FIRST major, placebo-controlled clinical trial of estrogen replacement therapy (ERT) in the secondary prevention of coronary heart disease (CHD) has been reported (the Heartland Estrogen/Progestin Replacement Study [HERS] study).[1] Over an average 4.1 years of followup, ERT failed to reduce the overall rate of CHD events, but increased thromboembolic events by 289% and gall bladder disease by 38%.[1] In the Estrogen and Atherosclerosis (ERA) trial,[2] 309 postmenopausal women with at least 1 coronary artery stenosis greater than 30% were randomized to premarin 0.625 mg/d (n = 100), premarin plus medroxyprogesterone acetate 2.5 mg/day (n = 104), or placebo (n = 105). After mean followup of 3.2 years, there was no difference between the 3 groups in coronary artery disease progression measured by change in the mean minimal lumen diameter by quantitative coronary angiography. Moreover, the change in mean minimal lumen diameter from baseline to follow-up, a measure of disease progression, did not differ significantly among the 3 treatment groups. Preliminary data from the data and safety monitoring board of a third prospective, placebo-controlled, randomized clinical trial, the Woman's Health Initiative Hormone Replacement Trial (WHI-HRT),[3,4] like HERS[1] and ERA,[2] showed no cardiovascular benefit from ERT (Lenfant C, National Heart, Lung, and Blood Institute [NHLBI] statement, 4/17/00). The WHI-HRT included 16,609 postmenopausal women with a uterus taking estrogen combined with progestin, 10,739 women with a hysterectomy taking estrogen alone, and a placebo group.[3,4] During the first 2 years of the WHI-HRT, there was a small increase in the number of myocardial infarctions, strokes, and thromboemboli in women taking active hormones compared with placebos. These increased events, however, did not meet statistical criteria for stopping the trial (Lenfant, NHLBI statement, 4/17/00). Along with HERS[1] and ERA,[2] WHI-HRT[3,4] was the third prospective, placebo-controlled, randomized clinical trial, which suggested that ERT is not cardioprotective in postmenopausal women with CHD, and substantially increases thromboembolism.

Our recent cross-sectional study[5] of interactions between the thrombophilic Factor V Leiden gene mutation, ERT, and atherothrombotic cardiovascular disease (ATCVD) in 423 women referred for hyperlipidemic therapy may provide some insight into the unexpected failure of ERT to reduce CHD in the HERS,[1] ERA,[2] and WHI-HRT[3,4] studies. We reported an interaction between ERT-mediated thrombophilia and the thrombophilic Factor V Leiden mutation for ATCVD.[5] Independent of other risk factors for ATCVD, ATCVD events were more likely in 2 subgroups of women, not taking ERT (ERT minus [−]) and without the Factor V Leiden gene mutation (Leiden gene mutation minus [−]) or ERT users (ERT plus [+]) and Leiden gene mutation present (Leiden plus [+]).[5] ERT was protective against ATCVD in Leiden gene mutation − women; 24% of Factor V Leiden gene mutation − women on ERT had sustained ATCVD versus 43% of those not on ERT ($P = .001$).[5] We speculated[5] that when ERT-mediated thrombophilia is superimposed on the heritable thrombophilic Factor V Leiden mutation, ATCVD is promoted, and any putative[1-4] ERT-associated reduction in ATCVD is overshadowed. We speculated[5] that ERT might reduce ATCVD in

From the Cholesterol Center, Jewish Hospital, Cincinnati, OH.
Submitted June 7, 2000; accepted September 21, 2000.

Supported in part by the Jewish Hospital Medical Research Council and by the Lipoprotein Research Fund of the Jewish Hospital.
Address reprint requests to Charles J. Glueck MD, The Cholesterol Center, Alliance Business Center, 3200 Burnet Ave, Cincinnati OH 45229.

0026-0495/01/5003-0007$35.00/0
doi:10.1053/meta.2001.21020

women without the Factor V Leiden gene mutation and suggested,[5-7] as have others,[8-12] that women with the Factor V Leiden gene mutation not be given ERT, so as to reduce thromboembolic events,[1] and (speculatively) ATCVD.

The thrombophilic 20210 G/A mutation in the prothrombin gene, like the Factor V Leiden gene mutation, has been associated with increased venous thrombosis[13-17] and, less commonly, with arterial thrombosis.[18] However, none of the studies[13-18] have examined the interaction of ERT with the thrombophilic prothrombin gene mutation for arterial[5] as well as venous thrombosis.

In a consecutive case series of 275 women, 75 (27%) on ERT at referral for diagnosis and treatment of hyperlipidemia, our specific aim in a cross-sectional study was to determine whether there was an interaction between thrombophilic heterozygosity for the 20210 G/A prothrombin gene mutation and ERT as risk factors for ATCVD.

MATERIALS AND METHODS

Women

The 275 women were newly referred from midwestern states as outpatients to the Jewish Hospital Cholesterol Center for diagnosis and treatment of hyperlipidemia.[19] They were studied as a consecutive case series in the temporal sequence of their referral without any selection bias and with no exclusions.

In each woman, by history, physical examination, and review of the referring doctors' and hospital records, ATCVD was characterized by ≥1 of the following events: unstable angina, myocardial infarction, angioplasty, coronary artery bypass surgery, claudication, transient ischemic attack, and ischemic stroke. Stable angina, additional evidence of ATCVD, was arbitrarily not included in the ATCVD event group. The diagnoses were made both prospectively at entry examination at the Cholesterol Center and retrospectively using chart review, as above. ATCVD events were grouped together into 1 response variable to increase the power of the study.

Study Protocol and Laboratory Methods

At the initial visit, information was obtained regarding age, race, height and weight, hypertension, diabetes, cigarette smoking, and first degree relatives' ATCVD ≤ age 55. The diagnosis of diabetes was determined by the referring physicians' use of oral therapy or insulin for diabetes. Glucose intolerance and family history of diabetes were not examined as potential confounding variables. A detailed history was taken regarding prescription drug use, as well as vitamins and nutritional supplements. After an overnight fast, blood was drawn for measurement of total, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, triglyceride, lipoprotein (a), homocysteine, methylmalonic acid, and anticardiolipin antibodies IgG and IgM, as previously described.[20-22]

Genotyping for the prothrombin 20210 G/A and Factor V Leiden mutations was performed by polymerase chain reaction (PCR) as previously described.[5,23] The genetic information was safeguarded in password-restricted, off-line computer files, with results provided only to the patients and their physician of record, and not otherwise released except by written request from the patient. Patients were provided with the results of their prothrombin and Factor V Leiden gene testing; genetic counseling was uniformly given for those positive for the 20210 G/A prothrombin and Factor V Leiden gene mutations.

Statistical Analysis

Several methods were used to determine how ATCVD risk odds were associated with ERT, the 20210 G/A prothrombin gene mutation, and their interactions. ATCVD risk factors were compared in women with and without ATCVD events after covariance adjusting for age and race (Table 1).[24] Similarly, comparisons of ATCVD risk factors were made between the 10 patients heterozygous for the 20210 G/A prothrombin gene mutation (+) and the 265 women who were wild-type normal for the 20210 G/A prothrombin gene mutation (−). Comparisons were also made between the 75 women on ERT (ERT +) and 200 without ERT (ERT −). Second, χ^2 analysis was used to examine cross tabulation of ATCVD events and nonevents by ERT and by prothrombin gene status (Table 2, Fig 1). Third, stepwise logistic regression analysis was performed in 192 women who had complete data for all of the explanatory variables: 20210 G/A prothrombin gene mutation, ERT, a 20210 G/A prothrombin gene mutation*ERT interaction term, total and HDL cholesterol, triglyceride, lipoprotein (a), homocysteine, anticardiolipin antibodies IgG and IgM, age, race, hypertension, Quetelet index (kg/cm^2 × 1,000, a measure of relative ponderosity), diabetes, cigarette smoking, and relatives' ATCVD ≤ age 55 years (panel 1, Table 3).

The 20210 G/A prothrombin gene mutation*ERT interaction term was defined as follows: 20210 G/A prothrombin gene mutation*ERT = 1 for (ERT − and 20210 G/A prothrombin gene mutation −) or (ERT + and 20210 G/A prothrombin gene mutation +); 20210 G/A prothrombin gene mutation*ERT = −1 for (ERT − and 20210 G/A prothrombin gene mutation +) or (ERT + and 20210 G/A prothrombin gene mutation −).

Stepwise logistic regression was run separately in 261 women, excluding anticardiolipin antibodies IgG and IgM from the explanatory variable list (panel 2, Table 3). Stepwise logistic regression was also run in these 261 women, adding statin (n = 69), fibric acid (n = 18), and statin plus fibric acid (n = 9) use at study entry as an additional explanatory variable. Stepwise logistic regression was also run in 251 women, excluding the 10 women heterozygous for the prothrombin gene mutation.

RESULTS

Patient Characteristics

The 275 women were predominantly white; 100 (36%) had ATCVD events and 175 (64%) were event-free (Table 1). Many of the 100 women with an ATCVD event had ≥1 event. Of the 100 women with ATCVD events, 10 had angioplasty (1 angioplasty alone), 16 had myocardial infarction (4 alone), 18 had coronary artery bypass grafts (3 alone), 20 had ischemic stroke (6 alone), 26 had claudication (5 alone), 34 had transient ischemic attacks (14 alone), and 45 had unstable angina (24 alone). Women with an ATCVD event were older than event-free women ($P = .0009$), had lower LDL cholesterol, reflecting increased statin drug use ($P = .002$), had marginally higher homocysteine ($P = .098$), and had marginally higher lipoprotein (a) ($P = .06$) (Table 1).

At the time of referral, 69 of the 275 women (25%) were taking a statin drug, 18 (7%) a fibric acid drug, and 9 (3%) both.

Risk factors for ATCVD, as in Table 1, did not differ ($P > .05$) in the 10 women heterozygous for the 20210 G/A prothrombin gene mutation compared with the 265 women wild-type normal for the gene, except for higher HDL cholesterol in the mutation group ($P = .01$), median 60 *v* 49 mg/dL.

Irrespective of ATCVD, after covariance adjusting for age and race, ERT users (n = 75) differed from nonusers (n = 200) having lower anticardiolipin antibody IgG ($P = .018$, median 8 *v* 11 G-phospholipid [GPL]), higher HDL ($P = .017$, median 53 *v* 47 mg/dL), and lower lipoprotein (a) ($P = .003$, median 10

Table 1. Risk Factors for ATCVD in 275 Hyperlipidermic Women (100 with an event, 175 without an event)

Variable	Event (n = 100)			Without Event (n = 175)			Significance of Difference Adjusted for Age and Race
	Mean	SD	Median	Mean	SD	Median	*P*
Age	58	12	59	52	14	54	
Cholesterol (mg/dL)	236	74	236	241	56	237	.14
HDL (mg/dL)	50	17	48	52	17	49	.26
LDL (mg/dL)	133	49	128 (n = 81)	150	50	145 (n = 151)	.002
TG (mg/dL)	275	245	210	249	352	160	.50
Lp(a) (mg/dL)	40	44	23	29	39	14	.06
Homocysteine (mg/dL)	10.4	6.1	8.7	9.0	4.4	8.3	.10
IgG (GPL)	13.4	9.7	11.0	11.4	6.9	10.0	.15
IgM (MPL)	04.2	3.9	3	3.5	4.4	3.0	.26
Quetelet (kg/cm^2) $\times$ 10^3	2.78	0.52	2.84	2.73	0.65	2.51	.42
Race	8 Black, 92 (92%) White			18 Black, 157 (90%) White			$\chi^2 = 0.39$ $P = .53$
Hypertension	58 No, 42 (42%) Yes			117 No, 56 (32%) Yes			$\chi^2 = 2.55$ $P = .11$
Diabetes	88 No, 12 (12%) Yes			160 No, 15 (9%) Yes			$\chi^2 = 0.85$ $P = .36$
Smoke	84 No, 16 (16%) Yes			153 No, 21 (12%) Yes			$\chi^2 = 0.84$ $P = .36$
Relatives' ATCVD $\leq$ age 55	44 No, 53 (55%) Yes			84 No, 68 (45%) Yes			$\chi^2 = 2.32$ $P = .13$

Abbreviations: TG, triglycerides; Lp(a), lipoprotein(a); GPL, G-phospholipid. MPL, M-phospholipid.

v 21 mg/dL). Triglyceride levels did not differ by ERT use ($P = 0.53$).

Associations of ATCVD With the 20210 G/A Prothrombin Gene Mutation and ERT

Cross-tabulating ATCVD events by ERT and by the 20210 G/A prothrombin gene mutation showed that ERT was protective against ATCVD in 20210 G/A prothrombin gene mutation − women ($\chi^2 = 15.8$; $df = 3$; $P = .001$, Table 2). Of women on ERT, without the 20210 G/A prothrombin gene mutation, 21% had sustained ATCVD versus 40% of those not on ERT ($\chi^2 = 8.31$; $df = 1$; $P = .004$) (Fig 1).

Interactions Between the 20210 G/A Prothrombin Gene Mutation and ERT for ATCVD

By stepwise logistic regression in 192 women (in which data was complete for all explanatory variables), positive explanatory variables for ATCVD included the 20210 G/A prothrombin gene mutation ($P = .032$) and a 20210 G/A prothrombin gene mutation*ERT interaction term ($P = .006$) (panel 1, Table 3). The positive 20210 G/A prothrombin gene mutation*ERT interaction term indicated that, independent of other risk factors, ATCVD events were more likely in 2 subgroups of women, ERT − and 20210 G/A prothrombin gene mutation −, or ERT + and 20210 G/A prothrombin gene mutation + (panel 1, Table 3). The risk odds ratio of having ATCVD in those having the prothrombin gene 20210 G/A mutation versus those who were wild-type normal for the prothrombin gene was 6.5, 95% confidence interval (CI), 1.33 to 47.6; $P = .032$ (Table 3). The risk odds ratio for the ERT*prothrombin gene mutation was 2.75, 95% CI, 1.36 to 5.83 ($P = .006$) (panel 1, Table 3). Age ($P = .019$) and anticardiolipin antibody (ACLA) IgM ($P = .009$) were also positively related to development of ATCVD (Table 3).

After excluding anticardiolipin IgG and IgM from the explanatory variables list to maximize sample size, 261 women were included in the stepwise logistic regression (panel 2, Table 3, Fig 2). Positive explanatory variables for ATCVD included prothrombin gene 20210 G/A heterozygosity ($P = .021$) and a 20210 G/A prothrombin gene mutation*ERT interaction term ($P = .004$) (panel 2, Table 3, Fig 2). The positive 20210 G/A prothrombin gene mutation*ERT interaction term indicated that, independent of other risk factors, ATCVD events were more likely in 2 subgroups of women, ERT − and 20210 G/A prothrombin gene mutation − or ERT + and 20210 G/A prothrombin gene mutation + (panel 2, Table 3). The risk odds ratio for the ERT*prothrombin gene

Table 2. Cross-Tabulation of Atherothrombotic Events by ERT and Heterozygosity for the 20210 G/A Prothrombin Gene Mutation

	Atherothrombosis + (%)	Atherothrombosis − (%)	All (%)
ERT+, PTG−	15 (21)	56 (79)	71 (100)
ERT−, PTG−	78 (40)	116 (60)	194 (100)
ERT+, PTG+	4 (100)	0 (0)	4 (100)
ERT−, PTG+	3 (50)	3 (50)	6 (100)
All	100 (36)	175 (64)	

NOTE. $\chi^2 = 15.8$; $df = 3$; $P = .001$; exact test $P = .0005$.

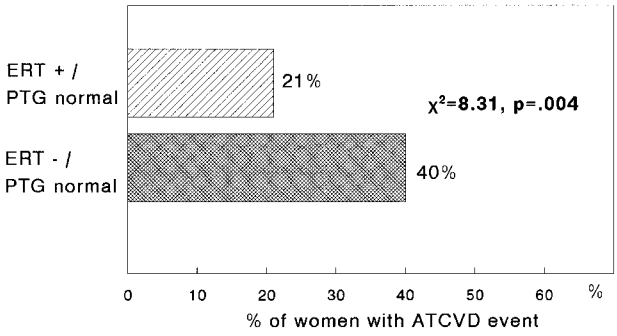

Fig 1. Percentage of 265 women (all with the wild-type normal prothrombin gene) with an ATCVD event, cross-tabulated by estrogen replacement therapy (ERT + [n = 71], ERT − [n = 194]).

Table 3. Significant Independent Determinants of Atherothrombotic Events by Logistic Regression

Dependent Variable	Significant Determinant			Risk Odds Ratio	
	Variable	Sign	*P*	Ratio	95% CI
Atherothrombotic event (81 events, 111 no events)	PTG	+	.032	6.48	(1.33, 47.6)
	PTG*ERT	+	.006	2.75	(1.36, 5.83)
Concordant 71%	Age	+	.019	1.34	(1.06, 1.73)
Disconcordant 29%	IgM	+	.009	3.54	(1.40, 9.64)
Excluding IgG and IgM from the explanatory variable list					
Atherothrombotic event (100 events, 161 no events	PTG	+	.021	5.80	(1.40, 30.2)
	PTG*ERT	+	.004	2.70	(1.41, 5.40)
Concordant 72%	Age	+	.004	1.38	(1.11, 1.72)
Disconcordant 27%	Triglycerides	+	.012	4.17	(1.43, 13.6)
	Lipoprotein (a)	+	.030	2.71	(1.12, 6.89)
	Homocysteine	+	.032	2.79	(1.11, 7.35)

NOTE. Stepwise selection on patients' age, top decile of cholesterol, triglycerides, lipoprotein(a), homocysteine, anticardiolipin antibodies IgG, IgM, Quetelet, bottom decile of HDL cholesterol, and categorical variables race, hypertension, smoking, relatives' ATCVD events ≤ age 55, prothrombin gene mutation (PTG), estrogen replacement therapy (ERT), a PTG and ERT interaction (PTG*ERT).

Event, Yes = 1, No = 0; race, white = 1, black/other = 0; hypertension, yes = 1, no = 0; relatives' ATCVD events (≤ age 55), yes = 1, no = 0; SMK, yes = 1, no = 0; PTG, PN = 1, NN = 0; ERT, yes = 1, no = 0.

Risk odds ratio for IgM was for top decile (≥7 MPL) *v* the rest; risk odds ratio for triglycerides was for top decile (≥539 mg/dL) *v* the rest; risk odds ratio for lipoprotein(a) was for top decile (≥84 mg/dL) *v* the rest; risk odds ratio for homocysteine was for top decile (≥13.9 μmol/L) *v* the rest; risk odds ratio for age was for increasing by 10 years.

Abbreviations: SMK, smoking yes, no; PN, heterozygous for the prothrombin gene mutation; NN, wildtype normal for the prothrombin gene.

mutation was 2.70, 95% CI, 1.41 to 5.40 (panel 2, Table 3, Fig 2). Other positive explanatory variables for ATCVD events included age, triglycerides, lipoprotein (a), and homocysteine (Table 3, Fig 2).

After removing the 10 women who had the prothrombin gene mutation from the above 261, and rerunning the stepwise logistic regression model, ERT was marginally protective against ATCVD events ($P = .055$; odds ratio, 0.51; 95% CI, 0.25 to 0.97).

After adding statin and fibric acid use at study entry as an additional explanatory variable in the above 261 women, statin use was associated with increased ATCVD events ($P = .002$; odds ratio, 2.74; 95% CI, 1.55 to 4.88).

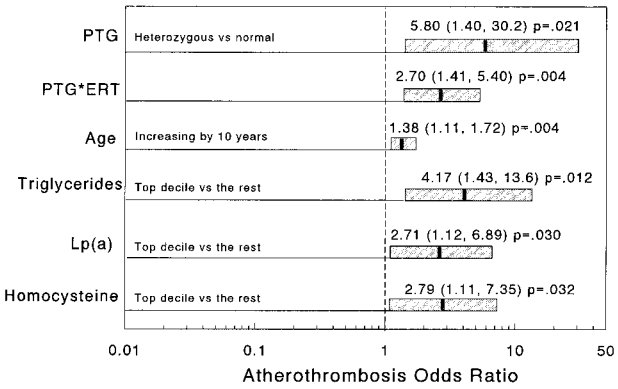

Fig 2. ATCVD odds ratio (95% CI) in 261 women, 100 with an ATCVD event, 161 without events. ATCVD risk panel data excludes anticardiolipin antibody IgG and IgM, but includes the following: 20210 G/A prothrombin gene mutation, ERT, prothrombin gene * ERT interaction term, total and HDL cholesterol, triglyceride, lipoprotein (a), homocysteine, age, race, hypertension, Quetelet Index, diabetes, cigarette smoking, and relatives' ATCVD ≤ age 55 years.

In women with the prothrombin gene mutation, ATCVD events occurred at a median of 3 months after starting ERT.

Study Limitations

Although the results of the study are statistically significant, the absolute number of subjects heterozygous for the 20210 G/A prothrombin gene mutation was small, 10 (4 on ERT and 6 not on ERT). The study relies on a population referred because of hyperlipidemia, which may not be representative of the overall group of postmenopausal women. To provide adequate statistical power, ATCVD events involving the carotid, coronary, and peripheral arterial circulations were pooled. Pooling necessarily lumps acute and chronic thrombotic and atherosclerotic events, which may, speculatively, reflect how ATCVD events present clinically. A very much larger study would be required to focus on individual ATCVD events, assuming that events could be categorized as thrombotic alone or atherosclerotic alone, without overlap.

DISCUSSION

HERS,[1] ERA,[2] and WHI-HRT[3,4] are the only prospective, placebo-controlled, randomized clinical trials, which examined whether ERT reduces CHD. Although the WHI-HRT[3,4] results are preliminary and incomplete,[3,4] these 3 trials uniformly suggest that ERT does not reduce CHD, but increases thromboembolism. The present study and our recent cross-sectional report[5] of interactions between the thrombophilic Factor V Leiden gene mutation, ERT, and ATCVD in 423 women referred for hyperlipidemia therapy may provide some insight into the unexpected[1-4] failure of ERT to reduce CHD events in the HERS,[1] ERA,[2] and WHI[3,4] studies. The present study and our previous reports that ERT promotes arterial thrombosis[5] and osteonecrosis in women heterozygous for the thrombophilic Factor V Leiden gene mutation[6,7] are not the first to

suggest that an interaction between "environmental" factors and the heritable thrombophilic gene mutations[18] leads to both arterial[12,25,26] and venous[6-11,27] thrombosis. Rosendaal et al[18] have reported that cigarette smoking in conjunction with the 20210 G/A prothrombin gene mutation substantially increases the risk of myocardial infarction in young women. Rosendaal et al[18] concluded that the risk of myocardial infarction in young women heterozgyous for the prothrombin gene mutation ". . . seemed limited to those with other risk factors", such as smoking. In the present study, the "other risk factors" cited by Rosendaal et al[18] appeared to encompass ERT.

ATCVD outcome differences between previous reports[13-17,27-29] and women in the present study may, speculatively, be determined by gender, with an interaction between ERT (never given to men) and genetic makeup (thrombophilic 20210 G/A prothrombin gene mutation) of the women.

ERT may amplify the positive association of the thrombophilic 20210 G/A prothrombin gene mutation with ATCVD, and ERT-mediated protection against ATCVD is most marked in women with the normal, "wild-type" prothrombin gene. In the current study, after excluding women with the prothrombin gene mutation, ERT was marginally protective against ATCVD events ($P = .055$). If these findings can be confirmed in larger, prospective studies, then we suggest that 20210 G/A prothrombin gene mutation be determined in all women on ERT or before starting ERT. This would identify the 4% of Caucasian women with the 20210 G/A prothrombin gene mutation[23] in whom ERT is contraindicated because of increased risk for ATCVD and thromboembolism and a second, much larger (96%) group of women without the 20210 G/A prothrombin gene mutation in whom ERT may, speculatively, reduce risk for ATCVD.

In the present cross-sectional study, we speculate that the positive association of statin drug use with ATCVD events at study entry suggests that referring physicians were more likely to give statins to patients who already had an ATCVD event.

Our results need to be independently confirmed in larger cohort studies, in non-Caucasians, and in subjects without hyperlipidemia. The most compelling way to study thrombophilic interactions between ERT and both the 20210 G/A prothrombin gene mutation and the Factor V Leiden mutation[5] would be a randomized, placebo-controlled prospective clinical trial of ERT and CHD. Prospective case-control studies of thrombophilic interactions between ERT and the 20210 G/A prothrombin gene and the Factor V Leiden mutation[5] might be done if DNA was archived from patients in HERS,[1] ERA,[2] and WHI-HRT.[3,4] Future clinical trials of ERT should include 20210 G/A prothrombin gene and Factor V Leiden gene[5] mutation screening with outcomes assessors blinded to the genotype data.

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