Estrogen Replacement Therapy, Thrombophilia, and Atherothrombosis

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

In a consecutive case series, cross-sectional study of 401 women referred for hyperlipidemia therapy, (110 [27%] on estrogen replacement therapy [ERT]), we assessed whether ERT-mediated thrombophilia and heritable thrombophilia (20210 G→A prothrombin gene [PTG], Factor V Leiden gene mutation [FV]) interacted as risk factors for atherothrombotic cardiovascular disease (ATCVD). Thirty-eight percent of women (152/401) had ≥ 1 ATCVD event, 57 (14%) had ≥ 2 ATCVD events. Fifteen women (3.7%) were PTG heterozygotes, 24 (6.0%) were FV heterozygotes, (there was 1 double heterozygote [0.25%]); 363 (91%) were wild-type normal for both genes. Of the 152 women with ≥ 1 ATCVD event, 21 (14%) had ≥ 1 thrombophilic gene mutation, versus 17/249 (7%) without events ($X^2 = 5.4$, $P = .02$). In women on ERT and with both genes wild-type normal, 23 of 96 (24%) had ≥ 1 ATCVD event versus 8 of 14 (57%) on ERT and with ≥ 1 thrombophilic mutation, $X^2 = 6.6$, $P = .01$. By stepwise logistic regression, in 401 women (152 with ≥ 1 ATCVD event, 249 no events), positive explanatory variables for ATCVD included FV and/or PTG (risk odds ratio, 2.59, 95% confidence interval [CI] 1.26 to 5.36, $P = .01$) and a PTG*ERT interaction term (risk odds ratio, 2.27, 95% CI 1.36 to 3.79, $P = .0017$). After deleting 23 FV heterozygotes and 14 PTG heterozygotes and 1 double heterozygote from the 401 women, ERT was protective against ATCVD events, with a risk odds ratio of 0.50 and 95% CI of 0.29 to 0.87 $P = .014$. PTG and FV may increase risk for ATCVD, particularly in the presence of ERT, whereas ERT may be protective against ATCVD when PTG and FV are absent.

THE HEART and Estrogen/Progestin Replacement Study (HERS) was the first placebo-controlled clinical trial of estrogen replacement therapy (ERT) in the secondary prevention of coronary heart disease (CHD).[1] In HERS, ERT did not reduce the overall rate of CHD events during 4.1 years of followup in 2,763 women with previous CHD, but increased thromboembolic events by 289%.[1] In HERS, a 50% increase in cardiovascular events was seen in the first year in the ERT group compared with placebo,[1] followed subsequently by fewer events after 3 years of therapy. The Estrogen and Atherosclerosis (ERA) trial[2] was a placebo-controlled trial of ERT in secondary prevention of coronary artery disease. In ERA[2], 309 postmenopausal women with ≥ 1 coronary artery stenosis more than 30% were randomized to Premarin, Premarin plus medroxyprogesterone acetate, or placebo. After an average follow-up of 3.2 years, coronary artery disease progression, measured by change in the mean minimal lumen diameter by quantitative coronary angiography, did not differ among the 3 treatment groups. Like HERS[1] and ERA,[2] preliminary results from a third prospective, placebo-controlled, randomized clinical trial, the Woman's Health Initiative Hormone Replacement Trial (WHI-HRT),[3,4] revealed no cardiovascular benefit from ERT (Lenfant, NHLBI statement, 4/17/00). WHI-HRT included postmenopausal women taking estrogen combined with progestin, estrogen alone, and a placebo group.[3,4] During the first 2 years of the WHI-HRT, there was an increase in the number of myocardial infarctions, strokes, and thromboemboli in women receiving ERT compared with placebos. These increased events, however, did not meet statistical criteria for stopping the trial (Lenfant, NHLBI statement, 4/17/00).

Three recent cross-sectional studies of the association of Factor V Leiden gene mutation (FV)*ERT[5] and prothrombin gene (PTG)*ERT[6,7] interactions with increased atherothrombotic cardiovascular disease (ATCVD) events may provide some insight into the unexpected failure of ERT to reduce CHD in HERS,[1] ERA,[2] and WHI-HRT.[3,4] Approximately 4% of general populations have the FV mutation, and about 4% have the PTG mutation.[5,6] In 423 women referred for hyperlipidemic therapy,[5] we reported an interaction between ERT-mediated thrombophilia and FV for ATCVD.[5] ERT was protective against ATCVD in women without FV.[5] We speculated[5] that when ERT-mediated thrombophilia is superimposed on the heritable thrombophilic FV, ATCVD is promoted, and any putative[1-4] ERT-mediated reduction in ATCVD is overshadowed. We speculated[5] that ERT might reduce ATCVD in women without FV and suggested,[5,6,8,9] as have others,[10-14] that women with the FV mutation not be given ERT, so as to reduce thromboembolic events,[1] and (speculatively) ATCVD.

Recently[6] in a consecutive case series of 275 women, 75 (27%) on ERT at referral for diagnosis and treatment of hyperlipidemia, we reported an interaction between ERT and PTG for ATCVD. By stepwise logistic regression, positive explanatory variables for ATCVD included PTG (risk odds ratio, 5.8, 95% confidence intervals [CI] 1.4 to 30.2, $P = .021$) and a PTG*ERT interaction term (risk odds ratio, 2.70, 95% CI 1.4 to 5.4, $P = .004$).[6] Similar to our conclusions for the FV*ERT interaction,[5] we reported that ERT may be protective against ATCVD when the thrombophilic PTG is absent,[6] whereas the PTG may increase risk for ATCVD, particularly in the presence of ERT. We suggested[6] that the PTG be measured in all women on ERT or before beginning ERT to identify those heterozygous for the PTG mutation (≈ 4%) in whom ERT is contraindicated because of increased risk for ATCVD and thromboembolism, and a second, much larger group of women without PTG (≈ 96%) in whom ERT may reduce risk for ATCVD.

Psaty et al[7] recently assessed risk of first nonfatal myocardial infarction (MI) based on current use of ERT and the presence

From the Cholesterol Center, Jewish Hospital, Cincinnati; and Molecular Diagnostics Laboratories, Cincinnati, OH.

Submitted July 23, 2001; accepted December 2, 2001.

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.

0026-0495/02/5106-0011$35.00/0

doi:10.1053/meta.2002.32729

or absence of the Factor V Leiden and prothrombin 20210 G→A variants in a cross-sectional study of 232 postmenopausal women with MI ([cases] ages, 30 to 79 years) and postmenopausal women without MI (controls) matched to cases by age, calendar year, and hypertension status. The association between ERT use and MI differed between those with and without the prothrombin variant; the investigators concluded: ". . . 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."[7]

The thrombophilic PTG, like the FV, has been associated with increased venous thrombosis,[15-19] and, less commonly, with arterial thrombosis.[6,7,20] In patients with myocardial infarction less than age 50 with no significant coronary artery stenoses on angiography, frequencies of the FV and PTG mutations are increased.[21] Early occlusion of coronary bypasses is associated with the presence of the FV and PTG mutations.[22] Resistance to activated protein C and the FV mutation are common in patients with a history of acute MI or primary hypertension.[23] Risk factors for spontaneous ischemic stroke in childhood[24,25] include FV accompanied by antiphospholipid antibodies, PTG and methylenetetrahydrofolate reductase gene mutations, and lipoprotein (a). In the prospective Bruneck population study, atherothrombosis was identified as a key mechanism in the development of advanced stenotic atherosclerosis, with the FV playing a significant role.[26]

In a consecutive case series, cross-sectional study of 401 women referred for therapy of hyperlipidemia, (110 [27%] on ERT, 152 with ≥ 1 ATCVD event), our specific aim was to determine whether ERT-mediated thrombophilia and heterozygosity for the thrombophilic PTG and/or the thrombophilic FV interacted as risk factors for ATCVD (MI, angioplasty, angina, coronary artery bypass surgery, claudication, ischemic stroke, transient ischemic attack).

MATERIALS AND METHODS

Women

The 401 women were newly referred from midwestern states to the Jewish Hospital Cholesterol Center for outpatient diagnosis and treatment of hyperlipidemia.[5,6] 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, MI, angioplasty, coronary artery bypass surgery, claudication, transient ischemic attack, and ischemic stroke (Table 1). Because of wide variance in referring physicians' and medical records' definitions of stable angina (additional evidence of ATCVD), it was not included in the ATCVD event group. Diagnoses of ATCVD were made both prospectively at entry examination at the Cholesterol Center and retrospectively, as above.

Study Protocol, 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. History was obtained on estrogen and progestin use including dose, duration of therapy, and route of administration. We did not attempt to characterize possible factors, which might have determined which women received or did not receive ERT (history of carcinoma, prior venous thrombosis in the individual or a family member, etc). History on surgical oophorectomy was obtained. A detailed history was taken regarding prescription drug use, as well as vitamins and nutritional supplements.

After an overnight fast, in all 401 women, blood was drawn to genotype PTG and FV mutations.[5,6] Fasting blood was also obtained for measurement of total, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol, triglyceride, lipoprotein (a), homocysteine, methylmalonic acid, and anticardiolipin (ACLA) antibodies IgG and IgM, as previously described.[5,6,27-29]

Of the 401 women, 74 had never previously been studied, and 157 had been included in previous reports of ERT interactions with the Factor V Leiden[5] or PTG[6] mutations. Of 423[5] and 275[6] previously studied women, 170 women in the current report had previous determinations of FV or PTG, but not both, and for the current study, had new measures of FV or PTG so that they now had both thrombophilic genotypes completed.

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 PTG and FV testing; genetic counseling was uniformly done for those heterozygous for the FV and PTG mutations.

Statistical Analysis

To increase the power of the study, ATCVD events involving the carotid, coronary, and peripheral arterial circulations (Table 1) were grouped together into 1 response variable. Several methods were used to determine how ATCVD events were associated with ERT, FV, PTG, and their interactions. Subjects categorized by ≥ 1 of the 7 ATCVD events (n = 152, see Tables 2 and 4) and those with ≥ 2 of the 7 ATCVD events (n = 57, see Tables 3 and 4) were compared with those with no events (n = 249). Group comparisons of ATCVD risk factors were made after covariance adjusting for age and race (see Tables 2 and 3).[30] χ^2 analyses and Fisher's exact tests[30] were used to compare ATCVD events by ERT use and by presence of FV or PTG mutations (see Table 4).

Stepwise logistic regression analysis was performed in 308 women (121 with ≥ 1 ATCVD event, 187 with no event) who had complete data for all of the explanatory variables (panel 1, Table 5). Explanatory variables included PTG, FV, ERT, a PTG*ERT interaction term, a FV*ERT interaction term, a 2 gene term (≥ 1 mutant thrombophilic gene present), a 2 gene*ERT interaction term, total and HDL cholesterol, triglyceride, lipoprotein (a), homocysteine, ACLA IgG and IgM, age, race, hypertension, Quetelet index ($kg/cm^2 \times 1{,}000$, a measure of relative ponderosity), diabetes, cigarette smoking, and relatives' ATCVD ≤ age 55 years. Additional explanatory variables included progesterone, PTG*progesterone and FV*progesterone interaction terms, and a 2 gene*progesterone interaction term. LDL cholesterol could not be calculated in the 50 women (12.5% of the cohort) whose triglycerides were ≥ 400 mg/dL, accounting for inclusion of total cholesterol rather than LDL cholesterol as an explanatory variable in the stepwise logistic regression equations.

Stepwise logistic regression was run separately in all 401 women, 152 with ≥ 1 ATCVD event, 249 with no events, after excluding ACLA antibodies IgG, and IgM from the explanatory variable list (panel 2, Table 5, Fig 1).

Stepwise regression analysis was done in 231 women, 44 with ≥ 2 ATCVD events versus 187 with no events (panel 1, Table 6). Stepwise logistic regression was run separately in 306 women, 57 with ≥ 2 ATCVD events, 249 without events, after excluding ACLA antibodies

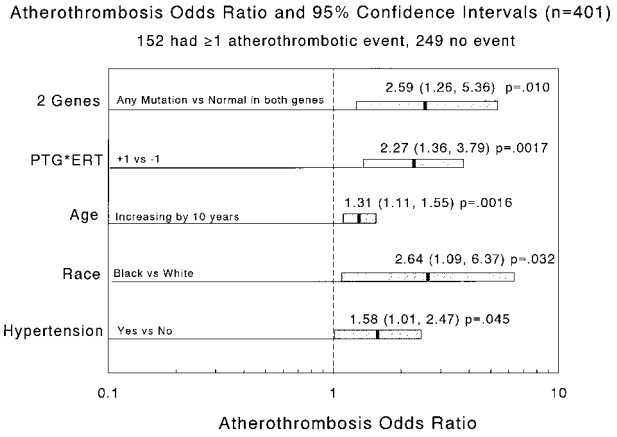

Fig 1. ATCVD odds ratio (95% CI) in 401 women, 152 with ≥ 1 ATCVD event, 249 without events. ATCVD risk panel data excludes ACLA antibody IgG and IgM, but includes the following: 20210G→A prothrombin gene mutation (PTG), Factor V Leiden gene mutation (FV), 2 genes (any PTG and/or FV mutation *v* normal in both genes), ERT, PTG*ERT interaction term, FV*ERT interaction, 2 genes*ERT interaction, total and HDL cholesterol, triglyceride, lipoprotein (a), homocysteine, age, race, hypertension, Quetelet Index, diabetes, cigarette smoking, and relatives' ATCVD ≤ age 55 years.

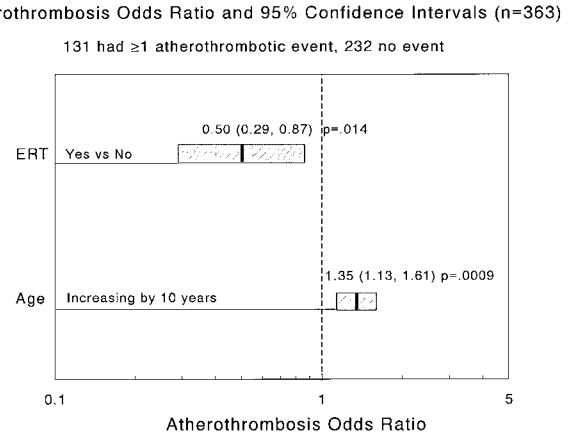

Fig 3. ATCVD odds ratio (95% CI) in 363 women, 131 with ≥ 1 ATCVD event, 232 without events, after removing 23 women heterozygous for FV, 14 heterozygous for PTG, and 1 double heterozygote. ATCVD risk panel data excludes ACLA antibody IgG and IgM, but includes the following: ERT, total and HDL cholesterol, triglyceride, lipoprotein (a), homocysteine, age, race, hypertension, Quetelet Index, diabetes, cigarette smoking, and relatives' ATCVD ≤ age 55 years.

IgG and IgM from the explanatory variable list (panel 2, Table 6, Fig 2).

Stepwise logistic regression was carried out separately in 363 women (131 with ≥ 1 event, 232 event free) after removing the 23 women who were FV heterozygotes, the 14 who were PTG heterozygotes, and 1 double heterozygote (Fig 3).

Stepwise logistic regression was carried out separately in 280 women (48 with ≥ 2 events, 232 event free) after removing the 18 women who were FV heterozygotes, 7 who were PTG heterozygotes, and 1 double heterozygote (Fig 4).

The PTG*ERT interaction term, the FV*ERT interaction term, and the 2 gene*ERT interaction terms were defined as follows: PTG*ERT = 1 for (ERT no and PTG no) or (ERT yes and PTG yes); PTG*ERT = -1 for (ERT no and PTG yes) or (ERT yes and PTG no); FV*ERT = 1 for (ERT no and FV no) or (ERT yes and FV yes); FV*ERT = -1 for (ERT no and FV yes) or (ERT yes and FV no); 2 gene mutation (either PTG or FV)*ERT = 1 for (ERT no and both FV and PTG gene normal) or (ERT yes and either FV or PTG gene yes); 2 gene mutation*ERT = -1 for (ERT no and either FV or PTG gene mutation yes) or (ERT yes and both gene mutations no).

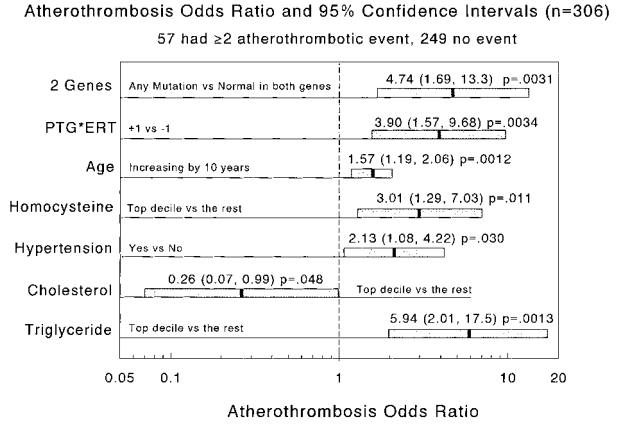

Fig 2. ATCVD odds ratio (95% CI) in 306 women, 57 with ≥ 2 ATCVD events, 249 without events. ATCVD risk panel data excludes ACLA antibody IgG and IgM, but includes the following: 20210G→A prothrombin gene mutation (PTG), Factor V Leiden gene mutation (FV), 2 genes (any PTG and/or FV mutation *v* normal in both genes), ERT, PTG*ERT interaction term, FV*ERT interaction, 2 genes*ERT interaction, total and HDL cholesterol, triglyceride, lipoprotein (a), homocysteine, age, race, hypertension, Quetelet Index, diabetes, cigarette smoking, and relatives' ATCVD ≤ age 55 years.

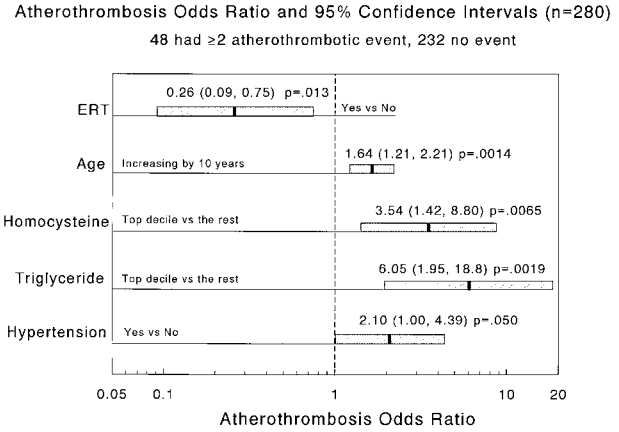

Fig 4. ATCVD odds ratio (95% CI) in 280 women, 48 with ≥ 2 ATCVD events, 232 without events, after removing 18 women heterozygous for FV, 7 heterozygous for PTG, and 1 double heterozygote. ATCVD risk panel data excludes ACLA antibody IgG and IgM, but includes the following: ERT, total and HDL cholesterol, triglyceride, lipoprotein (a), homocysteine, age, race, hypertension, Quetelet Index, diabetes, cigarette smoking, and relatives' ATCVD ≤ age 55 years.

Table 1. ATCVD Events in 152 Women With ≥1 ATCVD Event and in 57 Women With ≥2 Events

	MI	Angioplasty	Angina	CABG	Claud	CVA	TIA
152 women with ≥1 event							
29 with MI		7	10	8	7	4	6
11 with angioplasty	0		6	2	2	0	1
46 with angina	0	0		4	10	0	6
10 with CABG	0	0	0		0	3	2
13 with Claud	0	0	0	0		4	4
18 with CVA	0	0	0	0	0		5
25 with TIA	0	0	0	0	0	0	
57 women with ≥2 events							
20 with MI		7	10	8	7	4	6
7 with angioplasty	0		6	2	2	0	1
14 with angina	0	0		4	10	0	6
4 with CABG	0	0	0		0	3	2
7 with Claud	0	0	0	0		4	4
5 with CVA	0	0	0	0	0		4

Abbreviations: MI, myocardial infarction; CABG, coronary artery bypass graft; Claud, claudication; CVA, ischemic stroke; TIA, transient ischemic attack.

RESULTS

Patient Characteristics

Of the 401 women, 152 (37.9%) had ≥ 1 ATCVD event, 57 (14.2%) had ≥ 2 events, and 249 (62.1%) were event-free (Tables 1, 2, and 3). Among the 152 women with ≥ 1 event, nonoverlapping event categories are displayed in panel 1, Table 1. Among the 57 women with ≥ 2 events, nonoverlapping event categories are displayed in panel 2, Table 1.

The 152 women with ≥ 1 ATCVD events were older, had higher ACLA IgM, and were more likely to be black and hypertensive than the 249 with no events (Table 2).

The 57 women with ≥ 2 ATCVD events were older, had higher ACLA IgM, and were more likely to be hypertensive than the 249 with no events (Table 3).

At the time of referral, 143 of the 401 women (36%) were taking a statin drug, 43 (11%) a fibric acid drug, 13 (3%) both, and 10 (2.5%) other lipid-lowering agents. Of the 401 women, 153 (38%) had therapy to lower cholesterol, 46 (11%) had therapy to lower triglyceride, 16 (4%) had both. Of the 153 women who were taking cholesterol-lowering drugs at study entry, 81 (53%) had an ATCVD event, whereas 71 of 248 women (29%) not taking a cholesterol-lowering drug had an event, $X^2 = 23.8$, $P < .0001$. Patients having an ATCVD event were much more likely than those without an event to enter the study already taking a cholesterol-lowering drug.

Table 2. Risk Factors for ATCVD in 401 Hyperlipidemic Women (152 with event, 249 without event)

	Event (n = 152)			Without Event (n = 249)			Significance of Difference Adjusted for Age and Race
Variable	Mean	SD	Median	Mean	SD	Median	*P*
Age (yr)	59*	12	61	53	15	55	
Cholesterol (mg/dL)	230	68	220	236	66	227	.20
HDLC (mg/dL)	52	16	49	52	17	49	.21
LDLC (mg/dL)	133	50	126 (n = 132)	142	52	133 (n = 219)	.065
TG (mg/dL)	238	230	156	288	480	154	.40
Systolic BP (mm Hg)	126	17	120 (n = 109)	123	15	120 (n = 176)	.44
Diastolic BP (mm Hg)	79	9	76 (n = 109)	77	8	76 (n = 176)	.23
Lp(a) (mg/dL)	39	43	24	31	38	17	.21
Homocysteine (mg/dL)	10	5	9	9	8	8	.46
IgG (GPL)	13.4	9.1	12.0 (n = 121)	12.1	8.2	10.0 (n = 187)	.52
IgM (MPL)	4.9	5.8	3.0 (n = 121)	3.5	2.7	3.0 (n = 187)	.0065
Quetelet (kg/cm^2) × 10^3	2.80	0.54	2.80	2.74	0.65	2.55	.27
Race	14 Black, 138 (91%) White			10 Black, 239 (96%) White			$\chi^2 = 4.5$ $P = .033$
Hypertension	84 No, 68 (45%) Yes			164 No, 85 (34%) Yes			$\chi^2 = 4.5$ $P = .034$
Diabetes	134 No, 18 (12%) Yes			221 No, 28 (11%) Yes			$\chi^2 = 0.0$ $P = .86$
Smoke	125 No, 27 (18%) Yes			216 No, 33 (13%) Yes			$\chi^2 = 1.5$ $P = .22$
Relatives' ATCVD ≤ age 55	73 No, 79 (52%) Yes			127 No, 122 (49%) Yes			$\chi^2 = 0.3$ $P = .56$

Abbreviations: HDLC, high-density lipoprotein-cholesterol; LDLC, low-density lipoprotein-cholesterol; TG, triglycerides; Lp(a), lipoprotein(a); GPL, G-phospholipid; MPL, M-phospholipid.

*$P < .05$.

Table 3. Risk Factors for ATCVD in 306 Hyperlipidemic Women (57 with ≥2 events, 249 without event)

Variable	Event (n = 57)			Without Event (n = 249)			Significance of Difference Adjusted for Age and Race	
	Mean	SD	Median	Mean	SD	Median	*P*	
Age (yr)	62*	10	62	53	15	55		
Cholesterol (mg/dL)	235	82	213	236	66	227	.74	
HDLC (mg/dL)	50	17	48	52	17	49	.13	
LDLC (mg/dL)	126	49	123 (n = 44)	142	52	133 (n = 219)	.051	
TG (mg/dL)	293	281	197	288	480	154	.62	
Systolic BP (mm Hg)	128	19	130 (n = 44)	123	15	120 (n = 176)	.31	
Diastolic BP (mm Hg)	80	11	76 (n = 44)	77	8	76 (n = 176)	.16	
Lp (a) (mg/dL)	42	48	24	31	38	17	.22	
Homocysteine (mg/dL)	11	7	9	9	8	8	.094	
IgG (GPL)	13.8	8.9	11.0 (n = 44)	12.1	8.2	10.0 (n = 187)	.65	
IgM (MPL)	4.7	4.2	3.0 (n = 44)	3.5	2.7	3.0 (n = 187)	.054	
Quetelet (kg/cm^2) × 10^3	2.84	0.56	2.88	2.74	0.65	2.55	.19	
Race	5 Black, 52 (91%) White			10 Black, 239 (96%) White			$\chi^2 = 2.3$	$P = .13$
Hypertension	29 No, 28 (49%) Yes			164 No, 85 (34%) Yes			$\chi^2 = 4.5$	$P = .034$
Diabetes	47 No, 10 (18%) Yes			221 No, 28 (11%) Yes			$\chi^2 = 1.7$	$P = .19$
Smoke	47 No, 10 (18%) Yes			216 No, 33 (13%) Yes			$\chi^2 = 0.7$	$P = .40$
Relatives' ATCVD ≤ age 55	25 No, 32 (56%) Yes			127 No, 122 (49%) Yes			$\chi^2 = 1.0$	$P = .33$

*$P < .05$.

After adjusting for age and race, the 24 women heterozygous for the FV mutation (6% of the cohort) (Table 4) did not differ from the 377 women wild-type normal for the Factor V gene for the cardiovascular risk factors displayed in Tables 2 and 3, except more smoking in FV heterozygotes (33% *v* 14%, $\chi^2 = 6.77$, $P = .01$).

After adjusting for age and race, the 15 women heterozygous for the PTG mutation (3.7% of the cohort) (Table 4) did not differ from the 386 women wild-type normal for the prothrombin gene for the cardiovascular risk factors displayed in Tables 2 and 3, excepting median HDL, 60 mg/dL in PTG heterozygotes versus 49 in wild-type normals, $P = .004$.

After adjusting for age and race, the 110 ERT users (27% of the cohort) differed from the 291 nonusers for lipoprotein (a) (median 12 in the users *v* 22 in nonusers [$P = .015$]) and also for HDL (median 52 in the users *v* 49 in the nonusers [$P = .020$]).

Cross-Tabulation of ERT, Thrombophilic Mutations, and ATCVD Events

In the total cohort of 401 women, 110 (27%) took ERT, 64 unopposed, 46 accompanied by progesterone. Of the 110 women on ERT, 51 had surgical bilateral oophorectomy. Of the 31 women taking ERT who also had ATCVD events, 18 took conjugated equine estrogen (Premarin, Wyeth-Ayerst Pharma-

Table 4. Cross-Tabulation of ATCVD Events, Heterozygosity for the Factor V or Prothrombin Gene Mutations, and ERT

	Of the 152 Patients With ≥1 Event			Of the 57 Patients With ≥2 Events			Of the 249 Patients Without Event		
	n	ERT Yes	ERT No	n	ERT Yes	ERT No	n	ERT Yes	ERT No
Factor V Leiden +	12	4 (33%)	8	7	3 (43%)	4	12	5 (42%)	7
PTG +	9	4 (44%)	5	2	1 (50%)	1	6	1 (17%)	5
V Leiden or PTG at least one +	21	8 (38%)	13	9	4 (44%)	5	17	6 (35%)	11
Both V Leiden and PTG −	131	23 (18%)	108	48	5 (10%)	43	232	73 (31%)	159

	All 401 Women			
	FV or PTG Heterozygote	Wild-Type Normal	Total	
ATCVD event yes	21 (14%)	131 (86%)	152 (100%)	
ATCVD event no	17 (7%)	232 (93%)	249 (100%)	$\chi^2 = 5.37$, $P = .02$
	ERT + (n = 110)			
	ATCVD Event Yes	ATCVD Event No	Total	
FV or PTG heterozygote	8 (57%)	6 (43%)	14 (100%)	
Wild-type normal	23 (24%)	73 (76%)	96 (100%)	$\chi^2 = 6.6$, $P = .01$
	Wild-Type Normal (n = 363)			
	ATCVD Event Yes	ATCVD Event No	Total	
ERT+	23 (24%)	73 (76%)	96 (100%)	
ERT−	108 (40%)	159 (60%)	267 (100%)	$\chi^2 = 8.3$, $P = .004$

Table 5. Significant Independent Determinants of Atherothrombotic Events by Logistic Regression

Dependent Variable	Significant Determinant		Risk Odds Ratio	
	Variable	Sign *P*	Ratio	95% CI
Atherothrombotic event				
(n = 308, 121 events, 187 no events)	G2	+ .0021	3.62	(1.59, 8.24)
	PTG*ERT	+ .0062	2.19	(1.25, 3.83)
Concordant 72%	Age	+ .020	1.27	(1.04 1.55)
Disconcordant 28%	Black race	+ .0007	7.04	(2.27, 21.7)
	IgM	+ .0039	3.32	(1.47, 7.49)
Excluding IgG and IgM from the explanatory variable list				
Atherothrombotic event				
(n = 401, 152 events, 249 no events)	G2	+ .010	2.59	(1.26, 5.36)
	PTG*ERT	+ .0017	2.27	(1.36, 3.79)
Concordant 69%	Age	+ .0016	1.31	(1.11, 1.55)
Disconcordant 31%	Black race	+ .032	2.64	(1.09, 6.37)
	Hypertension	+ .045	1.58	(1.01, 2.47)

NOTE. Stepwise selection on patients' age, top decile of cholesterol, triglycerides, lipoprotein(a), homocysteine, anticardiolipin antibodies IgG, IgM, Quetelet, bottom decile of high-density lipoprotein cholesterol, and categorical variables race, hypertension, diabetes, smoking, relatives' ATCVD events ≤ age 55, prothrombin gene mutation (PTG), Factor V Leiden gene mutation (FV), either of the 2 genes mutation (G2), estrogen replacement therapy (ERT), and ERT interaction with gene mutation terms: PTG*ERT, FV*ERT, G2*ERT.

Event: Yes = 1, No = 0; Race: White = 0, Black/Other = 1; Hypertension: Yes = 1, No = 0; Relatives' ATCVD events (≤ age 55): Yes = 1, No = 0; Smoke: Yes = 1, No = 0; PTG: PN = 1, NN = 0; FV: PN = 1, NN = 0; G2 = 1 if either PTG = 1 or FV = 1, G2 = 0 if both PTG = 0 and FV = 0; ERT: Yes = 1, No = 0.

Risk odds ratio for age was for increasing by 10 years.

ceuticals, Philadelphia, PA) alone (mean [SD] 0.71 [0.21] mg/d), 5 took 0.625 mg Premarin plus 5 mg medroxyprogesterone, 2 Climara (Berlex Laboratories, Wayne, NJ) skin patches (1 mg estradiol), 2 Estratest (Solvay Pharmaceuticals, Marietta, GA) (0.625 mg esterified estrogen plus 2.5 mg methyltestosterone), 2 Estrace (Warner Chilcott, Rockaway, NJ) (1 mg estradiol), and 2 estrogen-progestin oral contraceptives. In these 31 women, ERT was started 8.7 ± 4.7 years before their ATCVD event.

Of the 79 women taking ERT without ATCVD events, 27 took Premarin (0.74 [0.42] mg/d), 26 took 0.625 mg Premarin plus 5 mg medroxyprogesterone, 2 Climara skin patches, 2 Estratest, 6 Estrace, 3 Ogen (0.625 mg estrone), and 13 estrogen-progestin oral contraceptives.

Of the 152 women with ≥ 1 event, 21 (14%) were either FV or PTG heterozygotes versus 17 of 249 (7%) without events ($X^2 = 5.37$, $P = .02$), panel 2, Table 4. In women without either thrombophilic gene mutation, 23 of 96 (24%) on ERT had ATCVD events versus 108 of 267 (40%) not on ERT ($X^2 = 8.3$, $P = .004$), panel 4, Table 4. In those women on ERT and with both genes wild-type normal, 23 of 96 (24%) had ≥ 1 ATCVD event and 73 of 96 (76%) had no event, but in those on ERT and with ≥ 1 thrombophilic mutation, 8 of 14 (57%) had ≥ 1 event and 6 of 14 (43%) had no event, $X^2 = 6.6$, $P = .01$, panel 3, Table 4.

Interactions Between the Two Thrombophilic Gene Mutations and Estrogen Replacement Therapy for ATCVD

By stepwise logistic regression in 308 women with complete ATCVD risk factor data (121 women with ≥ 1 event, 187 no events), significant positive explanatory variables for ATCVD included either FV or PTG positive (risk odds ratio, 3.62, 95% CI 1.59 to 8.24, $P = .0021$), an interaction between PTG and ERT (risk odds ratio, 2.19, 95% CI 1.25 to 3.83, $P = .0062$), age ($P = .020$), race ($P = .0007$), and ACLA IgM ($P = .0039$), panel 1, Table 5.

After excluding ACLA IgG and IgM measures, data was complete in 401 women, 152 with ≥ 1 event, 249 nonevents. Significant positive explanatory variables for ATCVD included either FV or PTG positive (risk odds ratio, 2.59, 95% CI 1.26 to 5.36, $P = .010$), a PTG*ERT interaction term (risk odds ratio, 2.27, 95% CI 1.36 to 3.79, $P = .0017$), age ($P = .0016$), race ($P = .032$), and hypertension ($P = .045$) (panel 2, Table 5, Fig 1).

Progesterone, PTG*progesterone, FV*progesterone, and 2 gene mutation*progesterone interaction terms were not sigificant variables ($P > .05$) in the stepwise logistic regression model.

After deleting 23 FV heterozygotes, 14 PTG heterozygotes, and 1 double heterozygote from the 401 women, 363 remained, 131 with ≥ 1 event, 232 without events (Fig 3). Here, ERT was protective against ATCVD events (risk odds ratio, 0.50, 95% CI 0.29 to 0.87, $P = .014$), Fig 3. Age was positively associated with ATCVD (Fig 3).

By stepwise logistic regression, in 231 women (44 with ≥ 2 ATCVD events and 187 without events) with complete risk factor data, positive explanatory variables for ATCVD included heterozygosity for either FV or PTG (risk odds ratio, 10.7, 95% CI 3.07 to 37.1, $P = .0002$), interaction between PTG and ERT (risk odds ratio, 5.95, 95% CI 1.97 to 18.0, $P = .0016$), top decile ACLA IgM ($P = .029$), age ($P = .0088$), race ($P = .026$), hypertension ($P = .038$), cholesterol ($P = .041$), and triglyceride ($P = .0007$), panel 1, Table 6. The inverse association of cholesterol with ATCVD events is probably attributable to the finding that patients having an ATCVD event at study entry were much more likely than those without events to be already taking a cholesterol-lowering drug (53% *v* 29%).

After excluding ACLA IgG and IgM measures, by stepwise

Table 6. Significant Independent Determinants of Multiple Atherothrombotic Events (≥2 events) by Logistic Regression

Dependent Variable	Significant Determinant		Risk Odds Ratio	
	Variable	Sign *P*	Ratio	95% CI
Atherothrombotic event				
(n = 231, 44 events, 187 no events)	G2	+ .0002	10.7	(3.07, 37.1)
	PTG*ERT	+ .0016	5.95	(1.97, 18.0)
Concordant 85%	IgM	+ .029	3.63	(1.14, 11.5)
Disconcordant 15%	Age	+ .0088	1.57	(1.12, 2.21)
	Black race	+ .026	6.41	(1.24, 33.3)
	Hypertension	+ .038	2.49	(1.05, 5.91)
	Cholesterol	− .041	0.19	(0.04, 0.93)
	Triglyceride	+ .0007	8.77	(2.49, 30.9)
Excluding IgG and IgM from the explanatory variable list				
Atherothrombotic event				
(n = 306, 57 events, 249 no events)	G2	+ .0031	4.74	(1.69, 13.3)
	PTG*ERT	+ .0034	3.90	(1.57, 9.68)
Concordant 79%	Age	+ .0012	1.57	(1.19, 2.06)
Disconcordant 21%	Homocysteine	+ .011	3.01	(1.29, 7.03)
	Hypertension	+ .030	2.13	(1.08, 4.22)
	Cholesterol	− .048	0.26	(0.07, 0.99)
	Triglyceride	+ .0013	5.94	(2.01, 17.5)

NOTE. Stepwise selection on patients' age, top decile of cholesterol, triglycerides, lipoprotein(a), homocysteine, anticardiolipin antibodies IgG, IgM, Quetelet, bottom decile of high-density lipoprotein cholesterol, and categorical variables race, hypertension, diabetes, smoking, relatives' ATCVD events ≤ age 55, prothrombin gene mutation (PTG), Factor V Leiden gene mutation (FV), either of the 2 genes mutation (G2), estrogen replacement therapy (ERT), and ERT interaction with gene mutation terms: PTG*ERT, FV*ERT, G2*ERT.

Event: Yes = 1, No = 0; Race: White = 0, Black/Other = 1; Hypertension: Yes = 1, No = 0; Relatives' ATCVD events (≤ age 55): Yes = 1, No = 0; Smoke: Yes = 1, No = 0; PTG: PN = 1, NN = 0; FV: PN = 1, NN = 0; G2 = 1 if either PTG = 1 or FV = 1, G2 = 0 if both PTG = 0 and FV = 0; ERT: Yes = 1, No = 0.

Risk odds ratio for age was for increasing by 10 years.

logistic regression, in 306 women (57 with ≥ 2 events, 249 without events), positive explanatory variables for ATCVD included being heterozygous for either FV or PTG (risk odds ratio, 4.74, 95% CI 1.69 to 13.3, $P = .0031$), an interaction between PTG and ERT (risk odds ratio, 3.90, 95% CI 1.57 to 9.68, $P = .0034$), age ($P = .0012$), homocysteine ($P = .011$), hypertension ($P = .030$), cholesterol ($P = .048$), and triglyceride ($P = .0013$), (panel 2, Table 6, Fig 2).

After deleting 18 FV heterozygotes, 7 PTG heterozygotes, and 1 double heterozygote from the 306 women (above), 280 remained, 48 with ≥ 2 events, 232 without events (Fig 4). Here, ERT was protective against ATCVD events (risk odds ratio, 0.26, 95% CI 0.09 to 0.75, $P = .013$) (Fig 4). Other significant risk factors included: age ($P = .0014$), hypertension ($P = .050$), homocysteine ($P = .0065$), and triglyceride ($P = .0019$) (Fig 4).

DISCUSSION

HERS,[1] ERA,[2] and the incomplete WHI-HRT[3,4] are, to date, the only prospective, placebo-controlled, randomized clinical trials, which examined whether ERT reduces CHD. These 3 trials suggested that ERT may increase rather than reduce CHD and increase thromboembolism. Women in HERS[1] and ERA[2] had documented CHD at study entry. Our current study and previous reports found that ERT interacts with FV[5] and PTG[6,7] to promote arterial thrombosis[5-7]; ERT also interacts with FV to promote osteonecrosis in women heterozygous for FV.[8,9,31] An interaction between "environmental" factors, such as ERT and the heritable thrombophilic gene mutations,[5-9,20,31] leads to both arterial[13,19,20,32,33] and venous[6,8-12,31,34] thrombosis. Rosendaal et al[20] have reported that cigarette smoking in conjunction with the PTG mutation increases the risk of MI in young women.[20] They found no association between MI and oral contraceptive agents in their cohort and cautioned that their results could not be generalized to older women.[20]

Any associations between ATCVD and thrombophilic gene mutations (V Leiden, PTG)[5-7,14-18,34-36] probably reflect a gene-gender-ERT interaction, since ERT is given to women only.

Although the results of our current study are statistically significant, the absolute number of subjects heterozygous for the FV and PTG was small, 24 for FV (9 on ERT and 15 not on ERT) and 15 for PTG (5 on ERT, 10 not on ERT). We studied women referred because of hyperlipidemia, who may not be representative of unselected 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.

Our current study suggests that PTG and FV may increase risk for ATCVD, particularly in the presence of ERT,[5-7] whereas ERT may be protective against ATCVD when the thrombophilic PTG and FV are absent. Our data support earlier speculations that the 50% increase in cardiovascular events in the ERT group during the first year of HERS,[1] followed by

reduced events in the ERT group after 3 years, reflects an initial high ATCVD event rate in a susceptible cohort[37] of ERT-treated women with FV and/or PTG mutations, with subsequent attrition of this susceptible cohort. Within this frame of reference, the recent, prospective, observational, epidemiologic Nurses Health Study revealed that risk for recurrent major coronary events seemed to increase among short-term ERT users with previous coronary disease, and then decreased with longer-term use.[38]

Our results need to be independently confirmed in larger cohort studies, in non-Caucasians, and in subjects without hyperlipidemia. Both the FV and PTG mutations are much less common in African-Americans than in Caucasians.[29] The most compelling way to study thrombophilic interactions between ERT and both PTG[6,7] and FV mutations[5] would be a randomized, placebo-controlled prospective clinical trial of ERT and CHD. However, the small percentage of women in general populations with V Leiden (4%)[29] and/or PTG mutations (4%)[29] would make a randomized, placebo-controlled, clinical trial astronomically expensive. Prospective case-control studies of thrombophilic interactions between ERT and PTG[6,7] and FV mutations[5] might be done if DNA was archived from patients in HERS,[1] ERA,[2] and WHI-HRT.[3,4]

While the increase in CHD events in the current study appears to be related to an interaction of ERT with the Factor V Leiden and prothrombin gene mutations, other potential adverse cardiovascular effects of ERT include ERT-induced elevation of C-reactive protein,[39] and metalloproteinases.[40] Herrington et al[39] have reported that 0.625 mg oral conjugated estrogen resulted in 65.8% higher levels of C-reactive protein, a marker for endothelial inflammation. Zanger et al[40] reported that 0.625 mg conjugated estrogen plus medroxyprogesterone 2.5 mg/d increased levels of matrix metalloproteinase-9 ($P = .02$), which could increase digestion and weakening of fibrous caps of vulnerable plaques, promoting thrombosis.

If the HERS,[1] ERA[2], WHI,[3,4] findings reflect an interaction between ERT and underlying heritable susceptibility factors, including Factor V Leiden,[5] and/or the prothrombin gene mutations,[6,7] then screening of postmenopausal women may better characterize a woman's expected risk or benefit from ERT for atherothrombotic outcomes. However, Vandenbroucke et al,[41,42] argue that large-scale genetic screening is probably not cost effective. The value of screening for V Leiden and PTG mutations in women before starting ERT will depend on the cost and yield of screening efforts versus the number of clinical events that might possibly be averted.[41,42]

REFERENCES

1. Hulley S, Grady D, Bush T, et al: Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. JAMA 280:605-613, 1998
2. Herrington DM, Reboussin DM, Brosnihan KB, et al: Effects of estrogen replacement on the progression of coronary-artery atherosclerosis. N Engl J Med 343:522-529, 2000
3. McGowan JA, Pottern L: Commentary on the Women's Health Initiative. Maturitas 34:109-112, 2000
4. Design of the Women's Health Initiative clinical trial and observational study. The women's health initiative study group. Control Clin Trials 19:61-109, 1998
5. Glueck CJ, Wang P, Fontaine RN, et al: Effect of exogenous estrogen on atherothrombotic vascular disease risk related to the presence or absence of the Factor V Leiden mutation (resistance to activated protein C). Am J Cardiol 84:549-554, 1999
6. Glueck CJ, Wang P, Fontaine RN, et al: 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. Metabolism 50:360-365, 2001
7. Psaty BM, Smith NL, Lemaitre RN, et al: Hormone replacement therapy, prothrombotic mutations, and the risk of incident nonfatal myocardial infarction in postmenopausal women. JAMA 285:906-913, 2001
8. Glueck CJ, McMahon RE, Bouquot J, et al: Heterozygosity for the Leiden mutation of the Factor V gene, a common pathoetiology for osteonecrosis of the jaw, with thrombophilia augmented by exogenous estrogens. J Lab Clin Med 130:540-543, 1997
9. Glueck CJ, McMahon RE, Bouquot JE, et al: Exogenous estrogen may exacerbate thrombophilia, impair bone healing, and contribute to development of chronic facial pain. Cranio Craniomandibular Practice 16:143-153, 1998
10. Henkens CM, Bom VJ, Seinen AJ, et al: Sensitivity to activated protein C; influence of contraceptives and sex. Thromb Haemost 73: 402-404, 1996
11. Caine YG, Bauer KA, Barzegar S, et al: Coagulation activation following estrogen administration to postmenopausal women. Thromb Haemost 68:392-395, 1992
12. Price DT, Ridker PM: Factor V Leiden mutation and the risks for thromboembolic disease: A clinical perspective. Ann Intern Med 127:895-903, 1997
13. Bauersachs R, Lindhoff-Last E, Erhly AM, et al: Significance of hereditary thrombophilia for risk of thrombosis with oral contraceptives. Zentralbl Gynakol 118:262-270, 1996
14. Rosendaal FR, Siscovick DS, Schwartz SM, et al: Factor V Leiden (resistance to activated protein C) increases the risk of myocardial infarction in young women. Blood 89:2817-2821, 1997
15. Corral J, Gonzalez-Conejero R, Lozano ML, et al: The venous thrombosis risk factor 20210 A allele of the prothrombin gene is not a major risk factor for arterial thrombotic disease. Br J Haematol 2:304-307, 1997
16. Ferraresi P, Marchetti G, Legnani C, et al: The heterozygous 20210A G/A prothrombin genotype is associated with early venous thrombosis in inherited thrombophilias and is not increased in frequency in artery disease. Arterioscler Thromb Vasc Biol 17:2418-2422, 1997
17. Gardemann A, Arsic T, Katz N, et al: The factor II G20210A and Factor V1691 A gene transitions and coronary heart disease. Thromb Haemost 81:208-213, 1999
18. Vargas M, Soto I, Pinto CR, et al: The prothrombin 20210A allele and the Factor V Leiden are associated with venous thrombosis but not with early coronary artery disease. Blood Coagul Fibrinolysis 10:39-41, 1999
19. Redondo M, Watzhe HH, Stucki B, et al: Coagulation factors II, V, VII, and X, prothrombin gene 20210G→A transition, and Factor V Leiden in coronary artery disease: High Factor V clotting activity is an independent risk factor for myocardial infarction. Arterioscler Thromb Vasc Biol 19:1020-1025, 1999
20. Rosendaal FR, Siscovick DS, Schwartz SM, et al: A common prothrombin variant (20210 G to A) increases the risk of myocardial infarction in young women. Blood 90:1747-1750, 1997
21. Van de Water NS, French JK, Lund M, et al: Prevalence of factor V Leiden and prothrombin variant G20210A in patients <50 years with no significant stenoses at angiography three to four weeks after myocardial infarction. J Am Coll Cardiol 36:717-722, 2000

22. Varela ML, Adamczuk YP, Martinuzzo ME, et al: Early occlusion of coronary by-pass associated with the presence of factor V Leiden and the prothrombin 20210A allele: Case report. Blood Coagul Fibrinolysis 10:443-446, 1999

23. Makris TK, Krespi PG, Hatzizacharias AN, et al: Resistance to activated protein C and FV Leiden mutation in patients with a history of acute myocardial infarction or primary hypertension. Am J Hypertens 13:61-65, 2000

24. Kenet G, Sadetzki S, Murad H, et al: Factor V Leiden and antiphospholipid antibodies are significant risk factors for ischemic stroke in children. Stroke 31:1283-1288, 2000

25. Nowak-Gottl U, Strater R, Heinecke A, et al: Lipoprotein (a) and genetic polymorphisms of clotting factor V, prothrombin, and methylenetetrahydrofolate reductase are risk factors of spontaneous ischemic stroke in childhood. Blood 94:3678-3682, 1999

26. Willeit J, Kiechl S, Oberhollenzer F, et al: Distinct risk profiles of early and advanced atherosclerosis: Prospective results from the Bruneck study. Arterioscler Thromb Vasc Biol 20:529-537, 2000

27. Glueck CJ, Shaw P, Lang J, et al: Evidence that homocysteine is an independent risk factor for atherosclerosis in hyperlipidemic patients. Am J Cardiol 75:132-136, 1995

28. Glueck CJ, Glueck HI, Tracy T, et al: Relationships between lipoprotein(a), lipids, apolipoproteins, basal and stimulated fibrinolytic regulators, and D-dimer. Metabolism 42:236-246, 1993

29. Balasa VV, Gruppo R, Glueck CJ, et al: The relationship of mutations in the methylenetetrahydrofolate reductase, prothrombin, and plasminogen activator inhibitor-1 genes to plasma levels of homocysteine, prothrombin, and plasminogen activator inhibitor-1 levels in children and adults. Thromb Haemost 81:739-744, 1999

30. SAS/STAT User's guide: Release 6.03 edition. Cary, NC, SAS Institute, 1988

31. Glueck CJ, Freiberg RA, Fontaine RN, et al: Hypofibrinolysis, thrombophilia, osteonecrosis. Clin Orthop 386:19-33, 2001

32. Bontempo FA, Hasett AC, Faruki H, et al: The Factor V Leiden mutation: spectrum of thrombotic events and laboratory evaluation. J Vasc Surg 25:271-276, 1997

33. Eskandari MK, Bontempo FA, Hassett AC, et al: Arterial thromboembolic events in women with the Factor V Leiden mutation. Am J Surg 176:122-125, 1998

34. Hultin MB, Grimson RC: Factor V Leiden, prothrombin 20210 gene variant, and risk of myocardial infarction. Circulation 99:457-458, 1999

35. Lalouschek W, Aull W, Serles W, et al: C677T MTHFR mutation and Factor V Leiden mutation in patients with TIA/minor stroke: A case-control study. Thromb Res 93:61-69, 1999

36. Zenz W, Bodo Z, Plotho J, et al: Factor V Leiden and prothrombin gene G 20210 A variant in children with ischemic stroke. Thromb Haemost 80:763-766, 1998

37. Herrington DM: The HERS Trial Results: Paradigms Lost? Ann Intern Med 13:463-466, 1999

38. Grodstein F, Manson JE, Stampfer MJ: Postmenopausal hormone use and secondary prevention of coronary events in the Nurses' Health Study. Ann Intern Med 135:1-8, 2001

39. Herrington DM, Brosnihan KB, Pusser BE, et al: Differential effect of E and droloxifene on C-reactive protein and other markers of inflammation in healthy postmenopausal women. J Clin Endocrinol Metab 86:4216-4222, 2001

40. Zanger D, Yang BK, Ardans J, et al: Divergent effects of hormone therapy on serum markers of inflammation in postmenopausal women with coronary artery disease on appropriate medical management. J Am Coll Cardiol 36:1797-1802, 2000

41. Vandenbroucke JP, Rosing J, Bloemenkamp KW, et al: Oral contraceptives and the risk of venous thrombosis. N Engl J Med 344:1527-1535, 2001

42. Vandenbroucke JP, Van der Meer FJ, Helmerhorst FM, et al: Factor V Leiden: Should we screen all oral contraceptive users and pregnant women? BMJ 393:1127-1130, 1996