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Objective—To test whether estrogen receptor polymorphisms modify the effects of postmenopausal hormone therapy on biomarkers and on risk of coronary heart disease events, stroke, or venous thromboembolism.

Methods and Results—The design was a nested case-control study in the Women's Health Initiative trials of postmenopausal hormone therapy. The study included all cases in the first 4 years: 359 cases of coronary heart disease, 248 of stroke, and 217 of venous thromboembolism. Six *estrogen receptor-α* polymorphisms and 1 *estrogen receptor-β* polymorphism were genotyped; 8 biomarkers known to be affected by hormone therapy were measured at baseline and 1 year after randomization. The polymorphisms were not associated with risk of vascular events and did not modify the increased risks of coronary heart disease, stroke, or venous thromboembolism due to hormone therapy. However, a reduced response of plasmin-antiplasmin to hormone therapy was noted for *estrogen receptor-1* IVS1 (intron number 1)-354 (interaction $P<0.0001$, corrected for multiple comparisons $P=0.014$) and *estrogen receptor-1* IVS1-1415 (interaction $P<0.0001$, corrected $P=0.014$).

Conclusion—Estrogen receptor polymorphisms reduce the effect of postmenopausal hormone therapy on plasmin-antiplasmin, a marker of coagulation and fibrinolysis. However, screening for estrogen receptor polymorphisms to identify women at less risk of adverse cardiovascular outcomes is not likely to be useful in making decisions about hormone therapy treatment. **(*Arterioscler Thromb Vasc Biol*. 2011;31:464-469.)**

Key Words: blood coagulation ■ clinical trials ■ coronary artery disease ■ epidemiology ■ pulmonary embolism ■ receptors ■ stroke ■ estrogen

Results from the Women's Health Initiative (WHI) and other clinical trials of postmenopausal hormone therapy (HT) for prevention of cardiovascular underline the complexity of the effects of HT. The WHI trials of estrogen (E-alone) and estrogen plus progestin (E+P) found increased risks of stroke and venous thromboembolism (VTE), and in the trial of E+P also an increased risk of coronary heart disease (CHD). In both WHI trials, the risk of CHD and VTE appeared to be higher in the first few years after randomization.[1–8] The effects of HT on CHD may depend on baseline lipoprotein levels, and the effects on stroke may depend on plasmin-antiplasmin (PAP) levels.[9–12] It is not known whether genetic factors modulate clinical cardiovascular responses to HT. However, some studies have suggested that women with the minor allele of IVS1-401 (rs2234693) of the *estrogen receptor-α* (ERα; *estrogen receptor-1* [ESR1]) gene have a 2-fold greater response to HT in some domains of estrogen action such as blood levels of high-density lipoprotein cholesterol (HDL-C) and E-selectin but not of C-reactive protein.[13–15] Sequence variations of the ERα gene or its haplotypes have been reported to be associated with atherosclerosis[16–18] and clinical cardiovascular disease or risk factors in some studies[19–22] but not all.[23–26] Sequence variations in the ERβ (ESR2) gene were associated with extent of atherosclerosis and coronary calcification in an autopsy study, and with clinical cardiovascular disease in women but not men.[27,28] Polymorphism of IVS1-401 (rs2234693) has been associated with VTE independently of other risk factors, such as use of HT,[29,30] but the influence of ERα and ERβ sequence variants on HT-associated risks for clinical arterial cardiovascular events or with VTE has not been tested previously in a clinical trial setting.

In this study, we tested whether several ERα polymorphisms and 1 ERβ polymorphism interacted with treatment assignment on risk of CHD, stroke, and VTE in a nested case-control sample from the WHI E+P and E-alone trials;

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we also tested whether the polymorphisms modify selected biomarker responses to HT.

Methods

Details of the design, recruitment, randomization, data collection, intervention, and outcome ascertainment procedures in the WHI HT trials, including Consolidated Standards of Reporting Trials (CONSORT) diagrams, have been published previously.[1,5]

Study Population and Interventions

The WHI hormone trials enrolled 27 347 postmenopausal women aged 50 to 79 from 1993 to 1998 at 40 US clinical centers based on hysterectomy status: 16 608 without hysterectomy in a trial of E+P and 10 739 with hysterectomy in a trial of E alone. The study was approved by the human subjects review committee at each participating institution, and all participants provided written informed consent. Participants were randomly assigned to take a single daily tablet containing a placebo or active medication: women without hysterectomy took 0.625 mg of conjugated equine estrogens plus 2.5 mg of medroxyprogesterone (Prempro) or placebo, and women with hysterectomy took 0.625 mg of conjugated equine estrogen (Premarin) or placebo.

All centrally adjudicated cases of CHD, stroke, and venous thromboembolism (VTE) occurring during the first 4 years of follow-up were included in biomarker and genotyping studies. Procedures for ascertaining and adjudicating clinical outcomes have been published.[4,8,10,12] Controls were matched on age, randomization date, hysterectomy status, and prevalent cardiovascular disease at baseline. Matching on prevalent disease was specific to the case type, so that cases of CHD were matched on prevalent myocardial infarction, cases of stroke on prevalent stroke, and cases of VTE on prevalent VTE. All controls for the 3 case types were used, after excluding any with incident CHD, stroke, or VTE. The study included 792 cases (CHD=359, stroke=248, VTE=217) and 817 controls. Thirty-two participants experienced more than 1 type of event. Analyses of year 1 biomarker data involved cases who experienced their clinical event after the year 1 visit and corresponding controls; these analyses included 561 women on active treatment and 439 on placebo. Baseline characteristics of the cases and controls included in these analyses and the associations of baseline characteristics with CHD events, ischemic stroke, and VTE have been published previously.[4,8,10,12]

Genetic and Biomarker Analysis

Blood samples were collected from all participants at baseline and 1 year and stored at −70°C using standardized procedures. Analyses were run in single batches including both cases and controls and 10% blind duplicates within 8 years of collection. Biomarkers measured in the case-control studies were hypothesized to modify or mediate HT treatment effects; those included in the current analyses were those previously shown to respond to HT.[10,12] Lipid profiles were analyzed in EDTA plasma with high-density lipoprotein precipitation by heparin manganese (Dade-Behring, Deerfield, Ill). E-selectin, matrix metalloproteinase-9, and homocysteine were measured at Medical Research Laboratories (Highland Heights, Ky). C-reactive protein (N-High Sensitivity CRP, Dade-Behring), fibrinogen (clot rate assay, Diagnostica Stago, Parsippany, NJ), plasminogen activator inhibitor-1 antigen and PAP complex (both by in-house immunoassay), and fibrin D dimer (immunoturbidometric assay, Liatest D-Di) were measured at the Laboratory for Clinical Biochemistry Research, University of Vermont (Burlington, Vt). Genetic polymorphisms frequently reported in the literature[13–30] or identified from prior sequencing[13] were assayed on DNA extracted from buffy coat samples at Wake Forest University (Winston-Salem, NC) Center for Human Genomics using polymerase chain reaction–based methods described previously.[13] Six ERα polymorphisms were studied: ESR1 IVS1-397T>C, also known as -401 and –PvuII (rs2234693); ESR1 exon 1+30T>C (rs2077647); ESR1 351A>G, also known as -345 and –XbaI (rs9340799); ESR1 IVS1-1415A>G (rs9322331); ESR1 IVS1-1505G>A (rs4870056); and ESR1 1989T>C (rs2071454). A single polymorphism for ERβ was studied: ESR2 1730A>G (rs4986938).

Statistical Design and Analysis

In preliminary genetic analyses, the genotype/allele frequencies for all markers were calculated in race/ethnicity strata, and each race stratum was tested separately for Hardy-Weinberg equilibrium. Additive, dominant, and recessive models were examined initially to determine which genetic model best describes the data. Pairwise linkage disequilibrium was examined as described by Devlin and Risch.[31] The association of ER polymorphisms with CHD, stroke, and VTE and their interaction with treatment assignment on these outcomes were described using logistic regression models linear in the number of minor single-nucleotide polymorphism (SNP) alleles while adjusting for covariates known to be associated with the outcomes of interest (age, race/ethnicity, log body mass index, log waist/hip ratio, treated diabetes, smoking status, alcohol consumption, physical activity, history of cardiovascular disease, left ventricular hypertrophy on ECG, systolic blood pressure, history of hypertension, aspirin use, statin use, history of high cholesterol requiring pills, and hormone use at baseline). Covariates were log transformed if they showed a skewed distribution or if log transformation improved model fit. Secondary analyses examined the effects of HT on clinical outcomes by ER polymorphisms within and after the first 2 years following randomization, and other secondary analyses were stratified by age or time since menopause. To assess whether the

Table 1. Association of ER SNPs With CHD, Stroke, and VTE

				CHD (359 Cases)			Stroke (248 Cases)			VTE (217 Cases)		
Polymorphism	SNP ID	Minor/ Major Allele	Minor Allele Frequency, % (n)*	Per-Minor-Allele OR (95% CI)†	Marginal *P*‡	Corrected *P*§	Per-Minor-Allele OR (95% CI)†	Marginal *P*‡	Corrected *P*§	Per-Minor-Allele OR (95% CI)†	Marginal *P*‡	Corrected *P*§
ESR1 IVS1-401	rs2234693	C/T	46.6 (362)	1.12 (0.89, 1.41)	0.343	0.991	0.86 (0.67, 1.11)	0.247	0.963	0.91 (0.70, 1.19)	0.508	1.000
ESR1 exon 1+30	rs2077647	C/T	47.7 (368)	1.06 (0.85, 1.33)	0.601	1.000	0.85 (0.66, 1.09)	0.207	0.923	1.01 (0.79, 1.31)	0.921	1.000
ESR1 IVS1-354	rs9340799	G/A	34.5 (268)	0.97 (0.76, 1.24)	0.804	1.000	0.93 (0.71, 1.22)	0.591	1.000	0.85 (0.64, 1.12)	0.251	0.964
ESR1 IVS1-1415	rs9322331	T/C	31.1 (242)	0.98 (0.76, 1.26)	0.889	1.000	0.84 (0.63, 1.12)	0.239	0.959	0.78 (0.58, 1.05)	0.098	0.712
ESR1 IVS1-1505	rs4870056	A/G	45.7 (355)	1.10 (0.87, 1.39)	0.428	0.997	0.88 (0.68, 1.13)	0.310	0.986	0.90 (0.69, 1.17)	0.438	0.997
ESR1 IVS1-1989	rs2071454	G/T	14.2 (106)	1.16 (0.83, 1.63)	0.379	0.997	1.01 (0.69, 1.47)	0.964	1.000	1.03 (0.69, 1.54)	0.885	1.000
ESR2 1730	rs4986938	T/C	38.0 (294)	0.95 (0.75, 1.21)	0.687	1.000	0.86 (0.65, 1.12)	0.263	0.967	1.08 (0.82, 1.43)	0.581	1.000

*Minor allele frequency in pooled controls (n=817).

†Odds ratio estimate from logistic regression model adjusted for treatment assignment (conjugated equine estrogen, conjugated equine estrogen placebo, conjugated equine estrogen+medroxyprogesterone, conjugated equine estrogen+medroxyprogesterone placebo), age, race/ethnicity, log body mass index, log waist/hip ratio, treated diabetes, smoking status, alcohol use, physical activity, history of cardiovascular disease, left ventricular hypertrophy on electrocardiogram, systolic blood pressure, history of hypertension, aspirin use, statin use, history of high cholesterol requiring pills, and hormone use at baseline.

‡Marginal *P* value was based on 1–degree of freedom test of association between clinical outcome and polymorphisms in the above-mentioned logistic model.

§Multiple comparison permutation adjusted *P* values, based on 1000 permutations. The responses were permuted at each iteration.

Table 2. CHD, Stroke, and VTE Odds Ratio Estimates for Postmenopausal Hormone Therapy vs Placebo According to Number of Minor Alleles of ER SNPs

	No. of Minor SNP Alleles, OR Estimate (95% CI)*				
Polymorphism	0	1	2	Marginal *P*†	Corrected *P*‡
CHD					
ESR1 IVS1-401	2.06 (1.06, 4.01)	0.92 (0.59, 1.42)	1.28 (0.63, 2.58)	0.300	0.993
ESR1 exon 1+30	1.64 (0.87, 3.07)	0.96 (0.61, 1.53)	1.24 (0.63, 2.43)	0.511	1.0000
ESR1 IVS1-354	1.60 (0.97, 2.64)	0.90 (0.56, 1.45)	1.20 (0.46, 3.16)	0.261	0.917
ESR1 IVS1-1415	1.26 (0.79, 2.02)	1.15 (0.70, 1.87)	1.16 (0.41, 3.31)	0.825	1.000
ESR1 IVS1-1505	1.95 (1.02, 3.72)	0.94 (0.60, 1.46)	1.18 (0.58, 2.41)	0.262	0.979
ESR1 IVS1-1989	1.19 (0.81, 1.74)	1.01 (0.52, 1.96)	3.40 (0.42, 27.3)	0.826	1.000
ESR2 1730	1.52 (0.89, 2.59)	1.12 (0.71, 1.77)	0.91 (0.37, 2.22)	0.273	0.999
Stroke					
ESR1 IVS1-401	1.62 (0.86, 3.05)	0.82 (0.49, 1.38)	2.89 (1.21, 6.89)	0.541	1.000
ESR1 exon 1+30	1.49 (0.79, 2.80)	1.01 (0.60, 1.69)	2.36 (1.00, 5.56)	0.563	1.000
ESR1 IVS1-354	1.72 (1.01, 2.95)	0.84 (0.49, 1.43)	3.00 (0.84, 10.7)	0.680	1.000
ESR1 IVS1-1415	1.60 (0.97, 2.64)	0.94 (0.54, 1.65)	2.13 (0.54, 8.33)	0.602	1.000
ESR1 IVS1-1505	1.48 (0.79, 2.76)	0.86 (0.51, 1.45)	3.31 (1.34, 8.15)	0.335	0.995
ESR1 IVS1-1989	1.12 (0.74, 1.70)	1.66 (0.74, 3.76)	5.95 (0.71, 49.9)	0.107	0.835
ESR2 1730	2.20 (1.25, 3.87)	0.99 (0.59, 1.67)	0.71 (0.26, 1.97)	0.019	0.103
VTE					
ESR1 IVS1-401	2.97 (1.43, 6.14)	2.08 (1.22, 3.54)	1.75 (0.77, 3.95)	0.330	1.000
ESR1 exon 1+30	2.74 (1.33, 5.68)	2.24 (1.28, 3.91)	1.65 (0.78, 3.46)	0.330	1.000
ESR1 IVS1-354	4.09 (2.23, 7.52)	1.27 (0.74, 2.19)	2.06 (0.62, 6.86)	0.033	0.661
ESR1 IVS1-1415	3.03 (1.75, 5.26)	1.60 (0.90, 2.83)	1.64 (0.42, 6.42)	0.143	0.930
ESR1 IVS1-1505	3.18 (1.55, 6.53)	1.95 (1.14, 3.34)	1.68 (0.74, 3.85)	0.229	1.000
ESR1 IVS1-1989	2.11 (1.34, 3.34)	2.40 (1.04, 5.55)	5.79 (0.48, 69.8)	0.684	1.000
ESR2 1730	3.67 (1.83, 7.39)	1.73 (1.04, 2.88)	1.91 (0.62, 5.85)	0.160	0.693

*Obtained from a logistic regression model adjusted for hysterectomy status, age, race/ethnicity, body mass index, waist/hip ratio, treated diabetes, smoking status, alcohol use, physical activity, history of cardiovascular disease, left ventricular hypertrophy on electrocardiogram, systolic blood pressure, history of hypertension, aspirin use, statin use, history of high cholesterol requiring pills, and hormone use at baseline.

†One–degree of freedom test of no interaction between treatment assignment (active vs placebo) and polymorphism linear in the number of minor SNP alleles, adjusted for covariates as described above.

‡Multiple comparison permutation adjusted *P* values, based on 1000 permutations. The SNP by treatment interaction was permuted at each iteration.

effect of HT on 1-year change in biomarker levels differ by ER polymorphism, linear regression models were used with individual difference in year 1 and baseline biomarker levels as the outcome; these models included the main effect for treatment assignment (E-alone placebo, E-alone, E+P placebo, E+P), ER polymorphism (coded as continuous according to number of minor SNP alleles), and their interactions while adjusting for the same covariates described above. All the analyses were repeated for whites only to investigate possible population stratification.

Multiple testing was acknowledged using a permutation test. First, the marginal probability values were calculated on the original data set for a group of related hypothesis. Then, data were permuted randomly 1000 times, and probability values were calculated on each permuted data set for the same group of hypothesis, but only the smallest probability value was retained. Finally, the permutation probability value was calculated by counting the times the probability values retained after each permutation was smaller than the one obtained in the original data set and dividing that value by the number of permutations.[32,33] Statistical analyses were performed using SAS statistical software (version 9.2, SAS Institute Inc, Cary, NC) and R (version 2.11.0, R Foundation for Statistical Computing).

Results

Results of analyses including whites only were similar to those for the entire sample and are not shown. Table 1 shows significance levels for association of the 7 ER SNPs with incidence of CHD events, stroke, and VTE. None of the SNPs show evidence of association with these outcomes in this study population. In secondary analyses the association of ESR1 exon 1+30 with CHD varied by years since menopause, with per-allele ORs of 0.51, 0.87, and 1.50 in women <10, 10 to 19, and >20 years since menopause, respectively, (corrected 3-way interaction $P=0.026$); similar analyses by age yielded a nonsignificant $P=0.064$. Table 2 shows significance levels for interaction of each of the SNPs with treatment assignment on the odds ratios for CHD, stroke, and VTE. None of the SNPs appeared to modify the effect of HT on these disease outcomes. In secondary analyses, there were no significant interactions of these SNPs and treatment assignment on risk of CHD, stroke, and VTE during the first

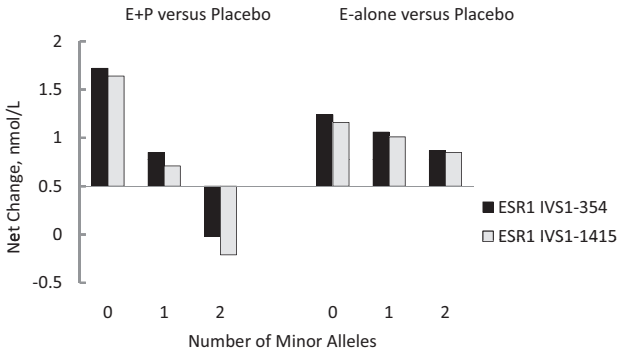

Figure. Postmenopausal HT effects on change in biomarker levels according to number of minor alleles of ERα SNPs. Net change was calculated as change from baseline to year 1 in the active treatment arm minus change from baseline to year 1 in the placebo arm, estimated from regression models that are linear in the number of minor SNP alleles. Models adjusted for potential confounders as in Table 1 and Table 2, except that baseline hysterectomy status interacted with treatments and SNPs, thus allowing the treatment×SNP effect to be different in the E-alone and E+P trials. Multiple comparison permutation adjusted $P=0.014$ for each SNP from 3–degree of freedom test of no interactions among treatment assignment (active versus placebo), hysterectomy status, and polymorphism that was linear in the number of minor alleles, adjusted for covariates as above.

2 years after randomization, or during the subsequent 2 years (data not shown). Similarly, there were no 3-way interactions by age or years since menopause. Analyses of the individual trials did not yield any significant results.

Baseline biomarker levels were not associated significantly with the tested SNPs (data not shown). However, the change in PAP levels on HT compared with placebo varied according to the number of minor alleles of 2 ERα SNPs. ESR1 IVS1-354 and ESR1 IVS1-1415 were significantly (interaction $P<0.0001$, corrected $P=0.014$ for each) and inversely associated with change in levels of PAP (Figure). The modification was consistent across the 2 trials but appeared to be more marked in the E+P trial. These 2 SNPs are in strong linkage disequilibrium ($D'=0.9998$), with a correlation coefficient of 0.8491 (Table 3). The effect of HT on the remaining biomarkers did not appear to be modified by any of the SNPs tested (Supplemental Table I, available online at http://atvb.ahajournals.org). However, there were 3-way interactions of age or years since menopause, ESR1 exon 1+30, and treatment on change in fibrinogen (corrected $P=0.021$ and 0.003, respectively) and of years since menopause, ESR2 1730G, and treatment on change in fibrinogen ($P=0.02$).

Discussion

We found no evidence that ER polymorphisms were associated with risk of CHD, stroke, or VTE, and the polymorphisms tested did not modify the effects of HT on CHD, stroke, and VTE overall. These data do not support the concept that variations in the ER gene and consequent changes in biological effects estrogen to receptors may increase or decrease the adverse effects of HT. Our findings do not necessarily contradict the previously observed associations of estrogen receptor polymorphisms with atherosclerosis,[17–19,27] because the factors leading to precipitation of an acute event in response to HT may differ from those responsible for the buildup of atherosclerosis, but they are in agreement with some other studies in not finding associations of ER polymorphisms with the risks of CHD and stroke.[24,26]

There were some signals that the associations of ESR1 exon 1+30 with CHD risk and fibrinogen varied by age and years since menopause, with higher per-minor allele ORs for CHD but with less favorable changes (decreases) in fibrinogen in younger women and women closer to the menopause. However, the clinical implications are unclear because the treatment effect of HT on CHD risk was not modified by ESR1 exon 1+30 and there was no further interaction with age or years since menopause. Similarly, the clinical implications of the 3-way interaction of ESR2 1730G with treatment and years since menopause on fibrinogen are unclear.

Changes in PAP levels in response to HT were modified by 2 ESR-α polymorphisms (ESR1 IVS1-354 and ESR1 IVS1-1415) in high linkage disequilibrium. PAP was measured in an assay that detects only plasmin in complex with antiplasmin, not free plasmin or antiplasmin.[34] As such, it is a very good marker of active plasmin generation as part of the fibrinolytic response to clot formation. PAP has a low biological variability and an analytic coefficient of variation of 1.7%, thus making it an excellent marker for epidemiological research.[35]

Whereas HT increased the levels of PAP overall,[10,12] the presence of the minor alleles of these polymorphisms was associated with an attenuated response or a reduction in levels of PAP. However, the clinical importance of variation of PAP levels due to receptor polymorphisms in this cohort is

Table 3. Pairwise Linkage Disequilibrium Analysis for ER Polymorphisms*

	D'						
	ESR1 IVS1-401	ESR1 Exon 1+30	ESR1 IVS1-354	ESR1 IVS1 1415	ESR1 IVS1 1505	ESR1 IVS1 1989	ESR2 1730
ESR1 IVS1-401		0.7740	0.9980	0.9997	0.9997	0.6137	0.0022
ESR1 exon 1+30	0.5724		0.8547	0.9232	0.7999	0.9737	0.0206
ESR1 IVS1-354	0.5838	0.4091		0.9998	0.9777	0.5283	0.0053
ESR1 IVS1-1415	0.4977	0.4055	0.8491		0.9930	0.8830	0.0259
ESR1 IVS1-1505	0.9617	0.5883	0.5823	0.5102		0.6215	0.0070
ESR1 IVS1-1989	0.0726	0.1746	0.0245	0.0582	0.0773		0.0930
ESR2 1730	0.0000	0.0003	0.0000	0.0005	0.0000	0.0009	

*Shown in the upper right triangle are Lewontin's D'; shown in the lower left triangle are squares of the Pearson correlation.

unclear. As previously published, for CHD, the baseline levels of PAP were not associated with risk and did not interact with treatment assignment on CHD, and neither did treatment-induced increases in PAP levels.[10] For ischemic stroke, baseline PAP levels were also not associated with risk, and whereas higher baseline levels of PAP interacted with HT treatment to increase the risk of stroke, the HT treatment-induced increases did not increase the risk of stroke.[12] For VTE, baseline levels of PAP were associated with VTE risk, and both baseline PAP and change in PAP levels interacted with HT to further increase the risk of VTE (Cushman M, unpublished data, 2010). The evidence from the WHI trials supports a role for activation of coagulation and fibrinolysis in explaining increased risks of stroke and VTE on HT, but not for the initially increased risk of CHD. However, as noted above, the risks of CHD, stroke, and VTE did not depend on ER polymorphisms, and therefore it is unlikely that variation in PAP levels due to polymorphisms plays an important role in baseline risk or in mediating HT-related risk for these conditions. The current findings, though biologically plausible, need to be replicated in other studies, and in general the relationships of ER polymorphisms to coagulation, fibrinolysis, and HT need further research.

Previous publications based on this data set indicated that higher levels of baseline LDL-cholesterol levels, and possibly lower levels of HDL-C, were associated with greater risk of CHD due to HT.[9–11] The British Women's Heart and Health Study did not find a relationship between ESR1 haplotypes and HDL-C or any other cardiovascular risk factors; however, in the Rochester Family Heart Study, ESR1 polymorphisms were related to increasing levels of apolipoprotein A-1, apolipoprotein A-2, and HDL-C.[23,25] In the current study, we did not find any significant relationships between ER polymorphisms and baseline levels of LDL-C or triglycerides. We also did not replicate a previous finding of increased HDL-C response to E+P in women with the minor allele of ESR1 IVS-401 and several related genotypes.[13] We did not confirm an association between ESR1 IVS-401 alleles and the response of E-selectin to HT, but our findings are in agreement with previous studies in finding no interactions of ER polymorphisms with the response of C-reactive protein to HT treatment.[14,15]

Strengths of the current study include its setting in a randomized controlled clinical trial of HT, which allowed assessment of the interactions of the polymorphisms with HT on vascular disease and biomarkers in addition to the main effects of ER polymorphisms. The standardized and complete ascertainment and classification of clinical outcomes is also a strength. However, the study also has several weaknesses: the number of cases of CHD, stroke, and VTE were relatively small, and it is possible that we may have missed some associations of ER polymorphisms with disease outcomes. Other biomarkers, such as acquired activated protein C resistance and tissue factor pathway inhibitor, were not included. Adherence to HT treatment regimens diminished over time, and this too would have impaired the ability to demonstrate interactions with HT; however, during the first 4 years after randomization included in this analysis, the great majority of participants reported full adherence to study treatment.[1,5] The trials tested only one formulation of estrogen (conjugated equine estrogens), and it is possible that other formulations might have had different effects.

We conclude that ER polymorphisms may influence the procoagulant and subsequent fibrinolytic response to HT as measured by PAP, but we could not demonstrate that these polymorphisms modified the risk of CHD, stroke, or VTE associated with the first 4 years of HT. Therefore, screening for ER polymorphisms to identify women at less risk of adverse cardiovascular outcomes is not likely to be useful for making HT treatment decisions.

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Disclosures

Dr Hsia is currently an employee of AstraZeneca.

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Supplement material

Table I. Postmenopausal Hormone Therapy Effect on Change in Biomarker Levels according to Number of Minor Alleles of Estrogen Receptor SNPs[1]

Biomarker	E+P vs. E+P Placebo (95% CI)			E-alone vs. E-alone Placebo (95% CI)			Marginal p[2]	Corrected p[3]
	Number of minor alleles			Number of minor alleles				
	0	1	2	0	1	2		
LDL-C, mmol/L								
ESR1 IVS-401	-0.51(-0.73,-0.29)	-0.52(-0.71,-0.33)	-0.52(-0.75,-0.29)	-0.73(-0.98,-0.48)	-0.63(-0.83,-0.43)	-0.53(-0.78,-0.28)	.273	1.000
ESR1 Exon 1+30	-0.39(-0.61,-0.18)	-0.46(-0.64,-0.27)	-0.52(-0.74,-0.29)	-0.72(-0.97,-0.47)	-0.56(-0.76,-0.36)	-0.41(-0.66,-0.15)	.115	0.973
ESR1 IVS1-354	-0.41(-0.60,-0.22)	-0.43(-0.60,-0.25)	-0.43(-0.68,-0.19)	-0.62(-0.84,-0.41)	-0.50(-0.69,-0.31)	-0.37(-0.64,-0.11)	.068	0.909
ESR1 IVS1-1415	-0.41(-0.59,-0.23)	-0.43(-0.60,-0.26)	-0.45(-0.71,-0.20)	-0.58(-0.78,-0.37)	-0.50(-0.69,-0.32)	-0.43(-0.71,-0.15)	.016	0.389
ESR1 IVS1-1505	-0.52(-0.73,-0.30)	-0.51(-0.70,-0.33)	-0.50(-0.74,-0.27)	-0.73(-0.98,-0.49)	-0.62(-0.82,-0.42)	-0.50(-0.76,-0.25)	.226	1.000
ESR1 IVS1-1989	-0.48(-0.63,-0.32)	-0.49(-0.68,-0.27)	-0.49(-0.84,-0.14)	-0.66(-0.83,-0.48)	-0.48(-0.71,-0.26)	-0.31(-0.71,0.09)	.285	1.000
ESR2 1730G	-0.62(-0.82,-0.42)	-0.56(-0.73,-0.38)	-0.49(-0.74,-0.25)	-0.67(-0.89,-0.45)	-0.69(-0.88,-0.50)	-0.71(-0.98,-0.44)	.227	0.999
HDL-C, mmol/L								
ESR1 IVS-401	0.13(0.07,0.20)	0.13(0.08,0.19)	0.14(0.06,0.21)	0.23(0.15,0.31)	0.22(0.16,0.29)	0.22(0.14,0.29)	.462	1.000
ESR1 Exon 1+30	0.12(0.06,0.19)	0.11(0.06,0.17)	0.10(0.03,0.17)	0.20(0.13,0.28)	0.20(0.14,0.26)	0.20(0.12,0.28)	.646	1.000
ESR1 IVS1-354	0.12(0.06,0.18)	0.12(0.07,0.18)	0.13(0.05,0.20)	0.23(0.16,0.30)	0.21(0.15,0.26)	0.18(0.10,0.26)	.371	1.000
ESR1 IVS1-1415	0.12(0.06,0.17)	0.11(0.06,0.17)	0.11(0.03,0.19)	0.21(0.15,0.27)	0.20(0.14,0.56)	0.19(0.10,0.28)	.651	1.000
ESR1 IVS1-1505	0.13(0.06,0.20)	0.13(0.07,0.19)	0.13(0.06,0.20)	0.24(0.16,0.31)	0.22(0.16,0.28)	0.20(0.12,0.28)	.415	1.000
ESR1 IVS1-1989	0.10(0.05,0.15)	0.10(0.04,0.16)	0.10(-0.01,0.21)	0.19(0.13,0.24)	0.19(0.12,0.26)	0.19(0.07,0.32)	.990	1.000
ESR2 1730G	0.11(0.05,0.17)	0.10(0.05,0.16)	0.09(0.01,0.16)	0.20(0.13,0.27)	0.19(0.13,0.26)	0.18(0.10,0.27)	.919	1.000
Triglycerides, mmol/L								
ESR1 IVS-401	0.00(-0.22.0.22)	0.00(-0.16,0.23)	0.07(-0.16,0.31)	0.27(0.01,0.52)	0.22(0.02,0.43)	0.19(-0.06,0.44)	.086	0.950
ESR1 Exon 1+30	0.00(-0,21,0.23)	0.06(-0.12,0.25)	0.11(-0.11,0.34)	0.32(0.07,0.57)	0.25(0.05,0.46)	0.19(-0.06,0.44)	.131	0.999
ESR1 IVS1-354	0.10(-0.08,0.30)	0.10(-0.08,0.27)	0.08(-0.16,0.33)	0.31(0.10,0.53)	0.29(0.10,0.48)	0.27(0.00,0.54)	.146	0.998
ESR1 IVS1-1415	0.11(-0.07,0.29)	0.10(-0.07,0.27)	0.09(-0.16,0.34)	0.32(0.12,0.53)	0.29(0.10,0.48)	0.26(-0.02,0.54)	.123	0.998
ESR1 IVS1-1505	0.02(-0.19,0.24)	0.04(-0.15,0.23)	0.06(-0.18,0.29)	0.27(0.02,0.52)	0.24(0.03,0.44)	0.21(-0.05,0.46)	.105	0.994
ESR1 IVS1-1989	0.15(-0.00,0.00)	0.25(0.05,0.46)	0.36(-0.00,0.72)	0.39(0.21,0.57)	0.38(0.15,0.61)	0.37(-0.04,0.78)	.504	1.000
ESR2 1730G	0.18(-0.02,0.38)	0.23(0.05,0.40)	0.27(0.02,0.52)	0.46(0.23,0.68)	0.40(0.21,0.59)	0.35(0.07,0.62)	.828	1.000
C-reactive protein, mg/L								
ESR1 IVS-401	-0.28(-2.90,2.35)	-0.06(-2.34,2.21)	0.15(-2.64,2.95)	0.03(-2.97,3.04)	1.76(-0.67,4.20)	3.50(0.47,6.52)	.244	1.000
ESR1 Exon 1+30	-0.16(-2.76,2.45)	0.39(-1.82,2.59)	0.93(-1.76,3.62)	0.65(-2.32,3.62)	2.32(-0.05,4.70)	4.00(1.00,6.99)	.459	1.000
ESR1 IVS1-354	0.31(-1.97,2.59)	0.46(-1.63,2.56)	0.61(-2.34,3.56)	0.74(-1.80,3.29)	2.78(0.54,5.03)	4.82(1.67,7.97)	.232	1.000

Biomarker	E+P vs. E+P Placebo (95% CI)			E-alone vs. E-alone Placebo (95% CI)			Marginal p[2]	Corrected p[3]
	Number of minor alleles			Number of minor alleles				
	0	1	2	0	1	2		
ESR1 IVS1-1415	0.46(-1.71,2.63)	0.51(-1.58,2.60)	0.56(-2.51,3.63)	1.86(-0.59,4.32)	2.55(0.33,4.78)	3.24(-0.06,6.55)	.889	1.000
ESR1 IVS1-1505	-0.17(-2.76,2.42)	0.09(-2.16,2.35)	0.35(-2.44,3.15)	0.44(-2.51,3.40)	1.99(-0.42,4.40)	3.54(0.49,6.59)	0.374	1.000
ESR1 IVS1-1989	0.53(-1.38,2.43)	1.02(-1.38,3.43)	1.52(-2.67,5.72)	1.99(-0.17,4.14)	4.10(1.39,6.81)	6.21(1.38,11.05)	0.705	1.000
ESR2 1730G	0.23(-2.17,2.64)	1.83(-0.34,4.01)	3.43(0.43,6.43)	3.75(1.10,6.40)	2.96(0.64,5.28)	2.17(-1.12,5.47)	0.280	1.000
E-selectin, mcg/L								
ESR1 IVS-401	-11.4(-14.9,-7.91)	-11.0(-14.1,-7.97)	-10.7(-14.4,-6.87)	-9.35(-13.5,-5.21)	-10.1(-13.4,-6.79)	-10.9(-15.0,-6.71)	0.233	1.000
ESR1 Exon 1+30	-11.3(-14.8,-7.77)	-11.2(-14.1,-8.16)	-11.0(-14.7,-7.36)	-9.11(-13.2,-5.05)	-10.0(-13.2,-6.78)	-10.9(-15.0,-6.78)	0.234	1.000
ESR1 IVS1-354	-10.6(-13.7,-7.49)	-9.49(-12.3,-6.65)	-8.41(-12.4,-4.39)	-8.89(-12.4,-5.37)	-8.80(-11.9,-5.74)	-8.72(-13.0,-4.39)	0.151	0.999
ESR1 IVS1-1415	-9.94(-12.9,-7.00)	-9.13(-12.0,-6.31)	-8.32(-12.5,-4.14)	-7.86(-11.2,-4.50)	-8.71(-11.7,-5.67)	-9.55(-14.1,-5.03)	0.198	1.000
ESR1 IVS1-1505	-11.1(-14.6,-7.69)	-10.7(-13.7,-7.64)	-10.2(-14.0,-6.39)	-8.33(-12.4,-4.30)	-9.72(-13.0,-6.46)	-11.1(-15.3,-6.95)	0.255	1.000
ESR1 IVS1-1989	-9.36(-11.9,-6.79)	-11.6(-15.1,-8.14)	-13.9(-20.1,-7.66)	-8.23(-11.2,-5.29)	-10.0(-13.7,-6.31)	-11.8(-18.4,-5.20)	0.832	1.000
ESR2 1730G	-9.82(-13.1,-6.53)	-8.24(-11.2,-5.29)	-6.66(-10.8,-2.54)	-7.43(-11.1,-3.76)	-7.77(-11.0,-4.57)	-8.11(-12.7,-3.53)	0.738	1.000
Matrix metalloproteinase-9, mcg/L								
ESR1 IVS-401	75.35(20.45,130.3)	60.84(13.26,108.4)	46.34(-12.8,105.5)	33.62(-30.6,97.79)	37.94(-13.4,89.24)	42.26(-22.4,107.0)	0.505	1.000
ESR1 Exon 1+30	41.06(-13.1,95.18)	37.53(-8.23,83.30)	34.01(-22.4,90.45)	17.15(-44.9,79.20)	19.77(-29.6,69.13)	22.39(-41.2,85.97)	0.856	1.000
ESR1 IVS1-354	70.91(22.78,119.0)	56.27(12.14,100.4)	41.63(-21.2,104.5)	31.66(-23.0,86.31)	39.00(-8.50,86.50)	46.35(-21.3,114.0)	0.594	1.000
ESR1 IVS1-1415	72.09(26.26,117.9)	50.12(6.34,93.91)	28.15(-37.1,93.39)	31.67(-20.4,83.73)	37.67(-9.42,84.77)	43.67(-27.0,114.3)	0.436	1.000
ESR1 IVS1-1505	75.49(21.24,129.7)	58.13(11.07,105.2)	40.78(-18.4,99.97)	36.28(-26.3,98.90)	36.46(-14.2,87.11)	36.64(-28.6,101.8)	0.625	1.000
ESR1 IVS1-1989	51.85(12.40,91.31)	47.57(-4.97,100.1)	43.28(-50.5,137.1)	42.51(-2.07,87.10)	24.59(-30.9,80.05)	6.67(-91.5,104.8)	0.891	1.000
ESR2 1730G	112.6(61.72,163.5)	83.03(37.87,128.2)	53.43(-9.64,116.5)	63.41(7.34,119.5)	68.33(19.29,117.4)	73.25(2.62,143.9)	0.035	0.697
Fibrinogen, g/L								
ESR1 IVS-401	-0.26(-0.498,-0.41)	-0.35(-0.54,-0.15)	-0.43(-0.67,-0.19)	-0.37(-0.63,-0.11)	-0.27(-0.47,-0.56)	-0.16(-0.42,0.10)	0.223	1.000
ESR1 Exon 1+30	-0.30(-0.52,-0.74)	-0.32(-0.51,-0.13)	-0.35(-0.58,-0.11)	-0.41(-0.67,-0.16)	-0.24(-0.45,-0.38)	-0.07(-0.33,0.19)	0.072	0.757
ESR1 IVS1-354	-0.25(-0.44,-0.52)	-0.37(-0.54,-0.19)	-0.48(-0.74,-0.23)	-0.31(-0.53,-0.91)	-0.23(-0.42,-0.34)	-0.14(-0.42,0.13)	0.054	0.782
ESR1 IVS1-1415	-0.29(-0.47,-0.10)	-0.39(-0.57,-0.22)	-0.50(-0.76,-0.24)	-0.29(-0.50,-0.84)	-0.26(-0.45,-0.68)	-0.23(-0.51,0.61)	0.199	0.978
ESR1 IVS1-1505	-0.30(-0.52,-0.75)	-0.36(-0.55,-0.17)	-0.42(-0.66,-0.18)	-0.38(-0.63,-0.13)	-0.27(-0.48,-0.65)	-0.16(-0.43,0.10)	0.235	1.000
ESR1 IVS1-1989	-0.31(-0.47,-0.15)	-0.31(-0.52,-0.11)	-0.32(-0.67,0.41)	-0.28(-0.47,-0.10)	-0.88(-0.32,0.14)	0.11(-0.31,0.52)	0.491	1.000
ESR2 1730G	-0.31(-0.52,-0.11)	-0.22(-0.41,-0.36)	-0.13(-0.39,0.13)	-0.18(-0.41,0.54)	-0.18(-0.37,0.25)	-0.17(-0.46,0.11)	0.445	1.000
PAI-1 Ag, mcg/L								
ESR1 IVS-401	-13.9(-27.3,-0.45)	-13.5(-25.1,-1.91)	-13.1(-27.4,1.12)	-22.9(-38.4,-7.42)	-14.5(-27.0,-2.00)	-6.04(-21.7,9.57)	0.203	1.000
ESR1 Exon 1+30	-10.1(-23.6,3.34)	-11.1(-22.5,0.24)	-12.2(-26.0,1.62)	-14.6(-30.1,0.91)	-11.6(-23.9,0.75)	-8.58(-24.4,7.19)	0.789	1.000
ESR1 IVS1-354	-16.6(-28.2,-4.90)	-10.6(-21.3,0.06)	-4.66(-19.7,10.42)	-18.5(-31.8,-5.23)	-12.1(-23.6,-0.56)	-5.66(-22.0,10.70)	0.178	0.999
ESR1 IVS1-1415	-15.5(-26.6,-4.42)	-9.75(-20.4,0.89)	-4.00(-19.7,11.70)	-14.9(-27.7,-2.14)	-12.4(-23.8,-0.90)	-9.77(-27.0,7.46)	0.231	1.000

Biomarker	E+P vs. E+P Placebo (95% CI)			E-alone vs. E-alone Placebo (95% CI)			Marginal p[2]	Corrected p[3]
	Number of minor alleles			Number of minor alleles				
	0	1	2	0	1	2		
ESR1 IVS1-1505	-10.7(-23.9,2.52)	-10.9(-22.3,0.60)	-11.0(-25.3,3.24)	-18.8(-34.1,-3.64)	-11.2(-23.6,1.10)	-3.62(-19.4,12.14)	0.462	1.000
ESR1 IVS1-1989	-3.58(-13.3,6.13)	-13.1(-25.3,-0.93)	-22.6(-43.8,-1.54)	-7.65(-18.7,3.42)	-4.07(-18.2,10.08)	-0.50(-25.8,24.75)	0.073	0.975
ESR2 1730G	1.87(-10.5,14.19)	-6.10(-17.2,4.97)	-14.1(-29.7,1.52)	-3.27(-17.1,10.51)	-5.18(-17.1,6.73)	-7.09(-24.1,9.91)	0.034	0.886
Plasmin-antiplasmin, nmol/L								
ESR1 IVS-401	1.71(1.12,2.30)	1.06(0.55,1.57)	0.41(-0.22,1.04)	1.36(0.68,2.04)	1.08(0.53,1.62)	0.79(0.11,1.48)	0.001	0.296
ESR1 Exon 1+30	1.31(0.71,1.90)	0.93(0.42,1.43)	0.55(-0.06,1.16)	0.90(0.22,1.59)	0.90(0.36,1.45)	0.90(0.21,1.60)	0.118	1.000
ESR1 IVS1-354	1.72(1.21,2.23)	0.85(0.39,1.32)	-0.02(-0.68,0.64)	1.24(0.66,1.82)	1.06(0.55,1.56)	0.87(0.16,1.58)	0.000	0.014
ESR1 IVS1-1415	1.64(1.15,2.12)	0.71(0.25,1.17)	-0.21(-0.90,0.47)	1.16(0.60,1.71)	1.01(0.51,1.50)	0.85(0.10,1.60)	0.000	0.014
ESR1 IVS1-1505	1.73(1.15,2.31)	1.04(0.53,1.54)	0.35(-0.28,0.97)	1.33(0.66,1.99)	1.06(0.52,1.60)	0.80(0.11,1.49)	0.001	0.214
ESR1 IVS1-1989	0.75(0.34,1.16)	1.03(0.51,1.55)	1.31(0.41,2.21)	0.73(0.26,1.20)	0.83(0.23,1.42)	0.92(-0.14,1.99)	0.423	1.000
ESR2 1730G	1.06(0.50,1.61)	0.84(0.34,1.33)	0.62(-0.08,1.33)	0.77(0.15,1.38)	0.90(0.37,1.43)	1.03(0.27,1.79)	0.630	1.000
Homocysteine, mg/L								
ESR1 IVS-401	-0.46(-1.22,0.31)	-0.67(-1.34,0.00)	-0.89(-1.71,-0.07)	-0.60(-1.48,0.29)	-0.66(-1.38,0.06)	-0.72(-1.61,0.18)	0.038	0.754
ESR1 Exon 1+30	-0.66(-1.43,0.10)	-0.88(-1.54,-0.23)	-1.11(-1.90,-0.31)	-1.27(-2.15,-0.38)	-0.88(-1.59,-0.18)	-0.50(-1.39,0.40)	0.059	0.948
ESR1 IVS1-354	-0.23(-0.90,0.44)	-0.39(-1.01,0.22)	-0.56(-1.43,0.32)	-0.19(-0.95,0.56)	-0.42(-1.08,0.25)	-0.64(-1.58,0.30)	0.080	0.995
ESR1 IVS1-1415	-0.18(-0.81,0.46)	-0.37(-0.98,0.25)	-0.55(-1.46,0.35)	-0.18(-0.90,0.55)	-0.37(-1.03,0.29)	-0.56(-1.55,0.42)	0.130	0.979
ESR1 IVS1-1505	-0.43(-1.19,0.33)	-0.64(-1.30,0.02)	-0.85(-1.67,-0.03)	-0.55(-1.41,0.32)	-0.63(-1.34,0.08)	-0.71(-1.62,0.20)	0.043	0.777
ESR1 IVS1-1989	-0.64(-1.20,-0.09)	-0.58(-1.29,0.13)	-0.52(-1.75,0.72)	-0.93(-1.56,-0.30)	-0.23(-1.04,0.57)	0.46(-0.98,1.89)	0.018	0.553
ESR2 1730G	-0.42(-1.13,0.29)	-0.59(-1.22,0.05)	-0.75(-1.63,0.13)	-0.99(-1.78,-0.20)	-0.40(-1.08,0.29)	0.20(-0.78,1.18)	0.091	0.976
D-dimer, mg/L								
ESR1 IVS-401	0.21(0.00,0.42)	0.18(-0.01,0.36)	0.14(-0.09,0.37)	0.01(-0.24,0.26)	0.05(-0.15,0.25)	0.09(-0.16,0.34)	0.867	1.000
ESR1 Exon 1+30	0.20(-0.01,0.42)	0.18(-0.01,0.36)	0.15(-0.07,0.37)	0.05(-0.20,0.29)	0.05(-0.15,0.24)	0.05(-0.20,0.30)	0.876	1.000
ESR1 IVS1-354	0.22(0.04,0.41)	0.24(0.07,0.41)	0.25(0.01,0.49)	0.10(-0.11,0.31)	0.10(-0.08,0.29)	0.10(-0.16,0.36)	0.834	1.000
ESR1 IVS1-1415	0.28(0.11,0.46)	0.22(0.05,0.39)	0.16(-0.09,0.41)	0.14(-0.06,0.34)	0.10(-0.08,0.29)	0.06(-0.21,0.33)	0.252	1.000
ESR1 IVS1-1505	0.24(0.03,0.45)	0.21(0.02,0.39)	0.17(-0.06,0.40)	0.04(-0.20,0.28)	0.09(-0.11,0.28)	0.13(-0.12,0.38)	0.768	1.000
ESR1 IVS1-1989	0.13(-0.02,0.29)	0.20(0.00,0.40)	0.26(-0.09,0.61)	-0.03(-0.20,0.15)	0.11(-0.11,0.33)	0.24(-0.15,0.64)	0.446	1.000
ESR2 1730G	0.24(0.05,0.44)	0.14(-0.03,0.32)	0.04(-0.21,0.28)	0.05(-0.16,0.27)	0.04(-0.15,0.23)	0.02(-0.25,0.29)	0.760	1.000

[1] Change from baseline to year one in active treatment arm - change from baseline to year one in placebo arm, estimated from regression models that are linear in the number of minor SNP alleles, adjusted for potential confounders as in Table 1 and Table 2, except that baseline hysterectomy status interacted with treatments and SNPs, thus allowing the treatment*SNP effect to be different in E-alone and E+P trial.

[2] Three degree of freedom test of no interactions between treatment assignment (active vs. placebo), hysterectomy status and polymorphism that is linear in the number of minor alleles, adjusted for covariates as above.

[3]Multiple comparison permutation adjusted p-values, based on 1,000 permutations. The SNP by treatment interaction was permuted at each iteration.