

## ESTROGEN-RECEPTOR POLYMORPHISMS AND EFFECTS OF ESTROGEN REPLACEMENT ON HIGH-DENSITY LIPOPROTEIN CHOLESTEROL IN WOMEN WITH CORONARY DISEASE

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### ABSTRACT

**Background** Sequence variants in the gene encoding estrogen receptor  $\alpha$  ( $ER-\alpha$ ) may modify the effects of hormone-replacement therapy on levels of high-density lipoprotein (HDL) cholesterol and other outcomes related to estrogen treatment in postmenopausal women.

**Methods** We characterized 309 women with coronary artery disease who were enrolled in the Estrogen Replacement and Atherosclerosis trial with respect to eight previously described and two newly identified  $ER-\alpha$  polymorphisms, and we examined the association between these polymorphisms and the response of HDL cholesterol and other lipids to treatment with estrogen alone or estrogen plus progestin.

**Results** After adjustment for age, race, diabetes status, body-mass index, smoking status, alcohol intake, and frequency of exercise, the 18.9 percent of the women who had the IVS1-401 C/C genotype (i.e., with C on both chromosomes in intervening sequence 1 at position 401 before exon 2) had an increase in the HDL cholesterol level with hormone-replacement therapy that was more than twice the increase observed in the other women (13.1 mg per deciliter vs. 6.0 mg per deciliter,  $P$  for treatment-by-genotype interaction = 0.004); this effect was limited to changes in the HDL subfraction 3 (HDL<sub>3</sub>) ( $P$  for interaction = 0.04). Similar patterns of response were observed for three other highly linked  $ER-\alpha$  intron 1 polymorphisms close to the IVS1-401 site (range of  $P$  values for interaction = 0.07 to 0.005). The pattern of increased response of HDL cholesterol in women with the IVS1-401 C/C genotype was evident in both the women receiving estrogen and those receiving estrogen plus progestin, was preserved across racial and ethnic groups, and was significant among women who were compliant with the study medication ( $P < 0.001$ ).

**Conclusions** Postmenopausal women with coronary disease who have the  $ER-\alpha$  IVS1-401 C/C genotype, or several other closely related genotypes, have an augmented response of HDL cholesterol to hormone-replacement therapy. (N Engl J Med 2002;346:967-74.)

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ESTROGEN raises plasma levels of high-density lipoprotein (HDL) cholesterol, an effect often cited as an explanation for the lower rates of heart disease in premenopausal women and in postmenopausal women receiving estrogen-replacement therapy.<sup>1,2</sup> However, HDL cholesterol levels in women vary considerably in response to endogenous or exogenous estrogen. Family studies suggest that a significant portion of the variability in HDL cholesterol levels can be attributed to genetic factors,<sup>3,4</sup> although the specific genes involved are not yet well defined. Allelic variants of the gene encoding estrogen receptor  $\alpha$  ( $ER-\alpha$ , also known as estrogen receptor 1 [ $ESR1$ ]) that alter its expression or function could account for some of the genetic variability, especially in women. This would not be surprising, since functionally important variants in other genes encoding steroid receptors, including receptors for androgens,<sup>5</sup> mineralocorticoids,<sup>6</sup> vitamin D,<sup>7</sup> and glucocorticoids,<sup>8</sup> have already been identified. If common  $ER-\alpha$  polymorphisms modify the effects of estrogen on HDL cholesterol or other factors, identifying and characterizing these might help patients and physicians assess the risks and benefits of hormone replacement and could lead to new understanding about estrogen action and the regulation of HDL cholesterol.

We measured the association between 10 sequence variants in  $ER-\alpha$ , including two novel polymorphisms, and the response of the HDL cholesterol level to hormone-replacement therapy among women in the Estrogen Replacement and Atherosclerosis trial.

### METHODS

#### Study Subjects

The study population, design, and main results of the Estrogen Replacement and Atherosclerosis trial were described previously.<sup>9,10</sup> A total of 309 unrelated postmenopausal women with established coronary artery disease were randomly assigned to receive 0.625 mg of oral conjugated equine estrogen per day, estrogen plus 2.5 mg of medroxyprogesterone acetate (progestin) per day, or placebo and were followed for an average of 3.2 years for progression of angiographically defined coronary disease. Plasma specimens obtained

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after overnight fasting were collected at base line and annually thereafter for the determination of lipid and lipoprotein levels. Buffy coats were separated from base-line plasma specimens and frozen at  $-80^{\circ}\text{C}$ . Data on factors likely to influence plasma HDL cholesterol levels — including age, race or ethnic group, diabetes status, body-mass index (defined as the weight in kilograms divided by the square of the height in meters), smoking status, frequency of exercise, and alcohol intake — were collected with the use of standardized questionnaires and procedures.<sup>9</sup> The study protocol was approved by the institutional review boards at the participating sites, and all subjects gave written informed consent.

### Analyses of Lipids and Hepatic Lipase

Cholesterol and triglyceride levels were measured on an autoanalyzer (Technicon RA-1000, Tarrytown, N.Y.) with the use of previously described techniques.<sup>11-14</sup> HDL cholesterol levels were measured with the use of heparin–manganese precipitation.<sup>15,16</sup> HDL subfraction 2 (HDL<sub>2</sub>) and subfraction 3 (HDL<sub>3</sub>) levels were measured with the use of dextran sulfate precipitation.<sup>17</sup> Low-density lipoprotein (LDL) cholesterol levels were calculated with the use of the Friedewald formula.<sup>18</sup> Apolipoprotein A-I and apolipoprotein B levels were measured in duplicate plasma samples with the use of a centrifugal analyzer (Cobas Fara, Roche Diagnostics, Indianapolis) and antibodies against human apolipoprotein A-I and apolipoprotein B. At the end of the trial, blood specimens for hepatic lipase assays were collected 15 minutes after an intravenous bolus infusion of 60 U of heparin per kilogram of body weight. Hepatic lipase activity was assayed with the use of a [<sup>3</sup>H]triolein Triton X-100 emulsion, as described previously.<sup>19</sup>

### DNA Isolation and Genotyping

DNA was isolated from stored buffy coats with the use of a standard guanidine thiocyanate procedure. Genotyping was performed for each single-nucleotide polymorphism with the use of the polymerase chain reaction (PCR) followed by PCR restriction-fragment–length polymorphism analysis, allele-specific PCR, or capillary electrophoresis (3700 DNA Analyzer, Applied Biosystems, Foster City, Calif.).<sup>20-25</sup> For the allele-specific PCR assays, a PCR reaction was performed with the external primer pair, followed by a second reaction with the allele-specific primer pair. PCR fragments from allele-specific PCR and PCR restriction-fragment–length polymorphism assays were separated on 2 percent agarose gels and genotyped according to the size of the resulting fragments. For the capillary electrophoresis assays, fluorescently labeled PCR fragments were diluted in water and run on 3700 DNA Analyzers. Genotypes were determined with the use of Genotyper software (Applied Biosystems). Single-nucleotide polymorphisms located in coding regions are denoted by the nucleotide number counted from the translation start site based on the Genbank reference sequence XM\_045967.

### DNA Sequencing and Analysis

In DNA from the 96 women with the highest and lowest responses of HDL cholesterol to hormone-replacement therapy, segments of the promoter region and the 5' and 3' regions of the first intervening sequence (IVS1) were amplified by PCR and sequenced (Big Dye Terminator sequencing kit, Applied Biosystems). Sequencing products were analyzed on a 3700 DNA Analyzer. DNA sequencing data were aligned and analyzed with the use of DNA-analysis software (Sequencher, Gene Codes, Ann Arbor, Mich.). Details about the sequencing primers and the PCR and sequencing conditions are included in Supplementary Appendixes 1 and 2 (available with the full text of this article at <http://www.nejm.org>).

### Statistical Analysis

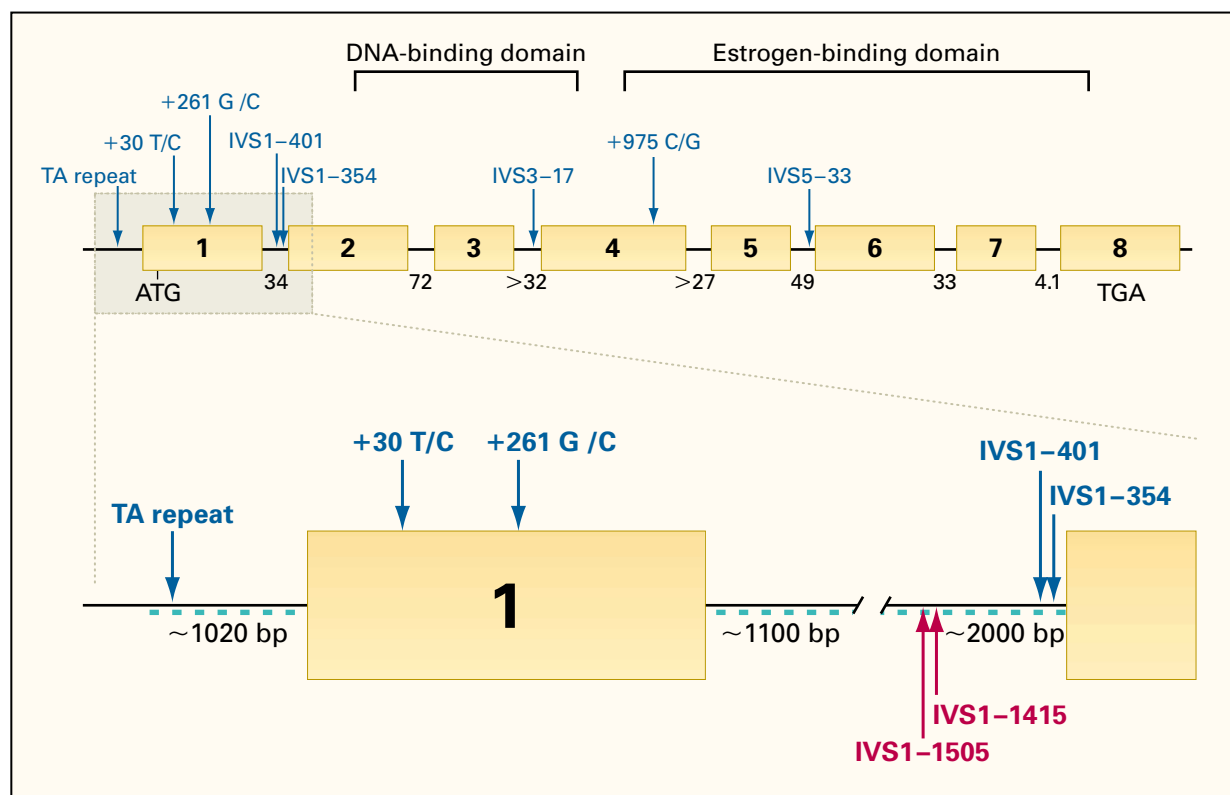
The chi-square test was used to test for significant departures from Hardy–Weinberg equilibrium. Shuffling tests<sup>26</sup> were performed to determine linkage disequilibrium between pairs of loci (Genetic

Data Analysis, version 1.0215, University of Connecticut, Storrs). Lipid and lipoprotein levels during the trial were calculated as mean follow-up measurements obtained annually and at the end of the trial. Generalized linear models were used to describe relations among mean plasma lipid levels during the trial, estrogen treatment, and various genotypes after adjusting for lipid values at base line, age, race (black, white, or other), diabetes status (requiring medication or not), body-mass index, smoking status at base line (yes or no), frequency of exercise (never, seldom [one or two days per week], sometimes [three to five days per week], or often [five to seven days per week]), and alcohol intake (yes or no). Evidence for interaction was based on the nominal two-sided P values from the F test for the term for the treatment-by-genotype interaction. Exploratory data analyses that used additive, dominant, and recessive models revealed that homozygous and heterozygous carriers of the more common allele for intron 1 polymorphisms responded similarly with respect to HDL cholesterol and were therefore combined for some analyses. Effects of estrogen treatment were analyzed according to the intention-to-treat principle, unless otherwise indicated. Exploratory data analyses revealed that the effect of genotype on lipid values during the trial did not differ between the two active-treatment groups; therefore, data from these groups were combined unless otherwise indicated.

## RESULTS

Figure 1 indicates the location of each polymorphism within *ER-α*, including two novel single-nucleotide polymorphisms in intron 1 that were identified as a result of resequencing. The nine single-nucleotide polymorphisms and the TA-repeat dinucleotide polymorphism were in Hardy–Weinberg equilibrium. Frequencies for the variant single-nucleotide polymorphism alleles ranged from 7.3 percent to 47.8 percent (Table 1). The number of TA repeats in the promoter-region microsatellite ranged from 18 to 34 (median, 26). The four single-nucleotide polymorphisms in the 2-kb region 5' of exon 2 were in linkage disequilibrium with each other and with the two single-nucleotide polymorphisms in exon 1 ( $P < 0.001$ ) but not with single-nucleotide polymorphisms located in intron 3, exon 4, or intron 5. (Additional data on linkage disequilibrium among nine single-nucleotide polymorphisms in *ER-α* are available as Supplementary Appendix 3 with the full text of this article at <http://www.nejm.org>.) The pattern of linkage disequilibrium was similar among white and black participants.

Increases in the HDL cholesterol level with hormone-replacement therapy were greatest in women who were homozygous for the less common alleles for the intron 1 polymorphisms. P values for the treatment-by-genotype interactions with the use of dominant models ranged from 0.004 to 0.07 (Table 2). When values were expressed as the percent change from base-line values, homozygotes for the less common alleles for the intron 1 polymorphisms had a 24 to 33 percent increase in HDL cholesterol levels with hormone-replacement therapy, as compared with a 13 percent increase among carriers of the more common alleles. HDL cholesterol levels at base line were slightly higher in women with IVS1–401 C/C and



**Figure 1.** The Gene Encoding Human Estrogen Receptor  $\alpha$  ( $ER-\alpha$ ).

Numbered yellow boxes indicate exons. Vertical arrows indicate single-nucleotide polymorphisms and the TA-repeat polymorphism (blue indicates previously recognized variants, and red new variants identified in this study). Portions resequenced for detection of novel single-nucleotide polymorphisms are indicated by dashed lines. Intronic regions (solid black lines) are not drawn to scale. The numbers under each intron indicate the estimated size of the intron, expressed in kilobase pairs.

**TABLE 1.** ALLELE AND GENOTYPE PREVALENCES OF EACH OF THE HUMAN ESTROGEN RECEPTOR  $\alpha$  POLYMORPHISMS.\*

ALLELE OR GENOTYPE	POLYMORPHISM								
	+30 T→C (EXON 1) (N=290)	+261 G→C (EXON 1) (N=289)	IVS1-1505 A→G (N=298)	IVS1-1415 C→T (N=301)	IVS1-401 T→C (N=305)	IVS1-354 A→G (N=305)	IVS3-17 T10→T9† (N=269)	+975 C→G (N=301)	IVS5-33 T5→T4† (N=284)
	percent								
P1	47.8	7.3	43.6	30.7	45.2	34.3	38.1	26.1	8.3
P2	52.2	92.7	56.4	69.3	54.8	65.7	61.9	73.9	91.7
P1/P1	21.7	1.0	17.4	7.6	18.9	9.3	15.2	7.3	0.7
P1/P2	52.1	12.5	53.8	46.2	52.3	49.7	45.7	37.5	15.1
P2/P2	26.2	86.5	30.2	46.2	28.8	41.1	39.0	55.1	84.2

\*P1 denotes the less common allele for each polymorphism, and P2 the more common allele. Because of rounding, not all percentages total 100.

†Numerals indicate the number of thymidine nucleotides present in the T-repeat regions of each allele.

IVS1-1505 G/G genotypes (i.e., with C or G on both chromosomes in intervening sequence 1 at position 401 or position 1505 before exon 2, respectively) than in women with IVS1-401 T/T and IVS1-1505 A/A genotypes, respectively (P=0.05 and P=0.06).

Among the four single-nucleotide polymorphisms in intron 1, the evidence of an interaction was greatest for the IVS1-401 polymorphism (Fig. 2). Women with the IVS1-401 C/C genotype who were assigned to hormone-replacement therapy had an increase of 13.1 mg per deciliter (0.34 mmol per liter) in HDL cholesterol, as compared with an increase of 6.0 mg per deciliter (0.16 mmol per liter) in women with the C/T or T/T genotype (P value for treatment-by-IVS1-401 interaction=0.004). In analyses limited to women who took at least 80 percent of their study medication, the interaction was even more pronounced (P<0.001). Evidence of an interaction was also present within each of the two active-treatment groups (change in HDL cholesterol levels according to treatment and IVS1-401 status: estrogen and C/C, 26.0 percent; estrogen and C/T or T/T, 14.9 percent; estrogen plus progestin and C/C, 29.0 percent; estrogen plus progestin and C/T or T/T, 11.1 percent; P for interaction=0.03 and 0.007, respectively). When subjects for whom genotypes had been determined were stratified according to race and ethnic group into groups of 221 non-Hispanic whites, 33 blacks, and 11 others, the pattern was preserved in all three groups, although only non-Hispanic whites were sufficiently numerous to support an inference of interaction with confidence (P=0.02 for IVS1-401 C/C vs. C/T or T/T).

In women receiving hormone-replacement therapy who had the IVS1-401 C/C genotype, HDL<sub>3</sub> cholesterol levels increased by 13.6 mg per deciliter (0.35 mmol per liter), as compared with 8.2 mg per deciliter (0.21 mmol per liter) in women with the C/T or T/T genotype (P for interaction=0.04) (Fig. 3). There was no effect of the IVS1-401 genotype on the response of HDL<sub>2</sub> cholesterol to hormone-replacement therapy. Increases in apolipoprotein A-I associated with hormone-replacement therapy were also greatest for women with the IVS1-401 C/C genotype (Fig. 3); however, this increase was not significantly different from the increase observed in women with the C/T or the T/T genotype (36 mg per deciliter vs. 28 mg per deciliter; P for interaction=0.68). Similarly, the numerically greater reductions in levels of LDL cholesterol and apolipoprotein B among women with the IVS1-401 C/C genotype were not sufficiently large to support an inference of interaction (data not shown).

At the end of the trial, hepatic lipase levels were slightly lower in women with the IVS1-401 C/C genotype than in women with the C/T or T/T genotype (P=0.06). However, there was no evidence of interaction with hormone-replacement therapy (change in hepatic lipase levels with hormone-replacement therapy as compared with placebo: C/C, 1.4 U per milliliter; C/T or T/T, 2.0 U per milliliter; P for interaction=0.78). On the other hand, examination of another estrogen-sensitive protein produced in the liver, sex hormone-binding globulin, also revealed a significant treatment-by-IVS1-401 interaction (P for interaction=0.02) (Fig. 3).

Despite the favorable effects on HDL cholesterol

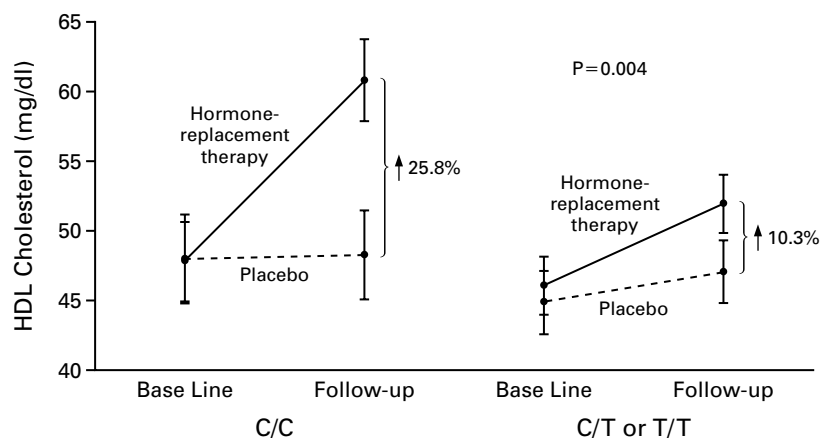
**TABLE 2.** MEAN HIGH-DENSITY LIPOPROTEIN CHOLESTEROL LEVELS AT BASE LINE AND FOLLOW-UP ACCORDING TO TREATMENT GROUP AND GENOTYPE IN INTRON 1 OF HUMAN ESTROGEN RECEPTOR  $\alpha$ .\*

GENOTYPE	TREATMENT GROUP	IVS1-1505		IVS1-1415		IVS1-401		IVS1-354	
		BASE LINE	FOLLOW-UP	BASE LINE	FOLLOW-UP	BASE LINE	FOLLOW-UP	BASE LINE	FOLLOW-UP
mg/dl									
P1/P1	Hormone-replacement therapy	48.9±3.0	60.7±3.0	45.0±4.0	59.4±4.0	47.4±2.9	60.4±2.9	45.1±3.5	59.8±3.5
	Placebo	48.6±3.3	48.6±3.4	48.1±4.7	48.5±5.0	47.6±3.1	47.9±3.2	46.9±4.3	48.1±4.5
P1/P2	Hormone-replacement therapy	46.2±2.2	52.6±2.2	48.5±2.3	55.1±2.3	47.2±2.1	53.1±2.2	48.3±2.2	54.6±2.3
	Placebo	44.8±2.6	47.2±2.7	44.2±2.7	46.2±2.8	44.4±2.6	47.7±2.6	45.5±2.6	48.2±2.6
P2/P2	Hormone-replacement therapy	44.8±2.5	50.2±2.5	45.1±2.2	51.4±2.3	42.8±2.5	48.8±2.5	44.9±2.3	51.1±2.3
	Placebo	44.3±3.0	45.5±3.2	47.7±2.6	49.0±2.7	44.6±3.0	45.2±3.1	46.1±2.8	47.0±2.8
P values for interactions									
Additive model†		0.02		0.20		0.009		0.05	
Dominant model‡		0.005		0.07		0.004		0.02	

\*Plus-minus values are means ±SE, with adjustment for age, race or ethnic group, body-mass index, diabetes status, smoking status, frequency of exercise, and alcohol intake. To convert values for cholesterol to millimoles per liter, multiply by 0.02586.

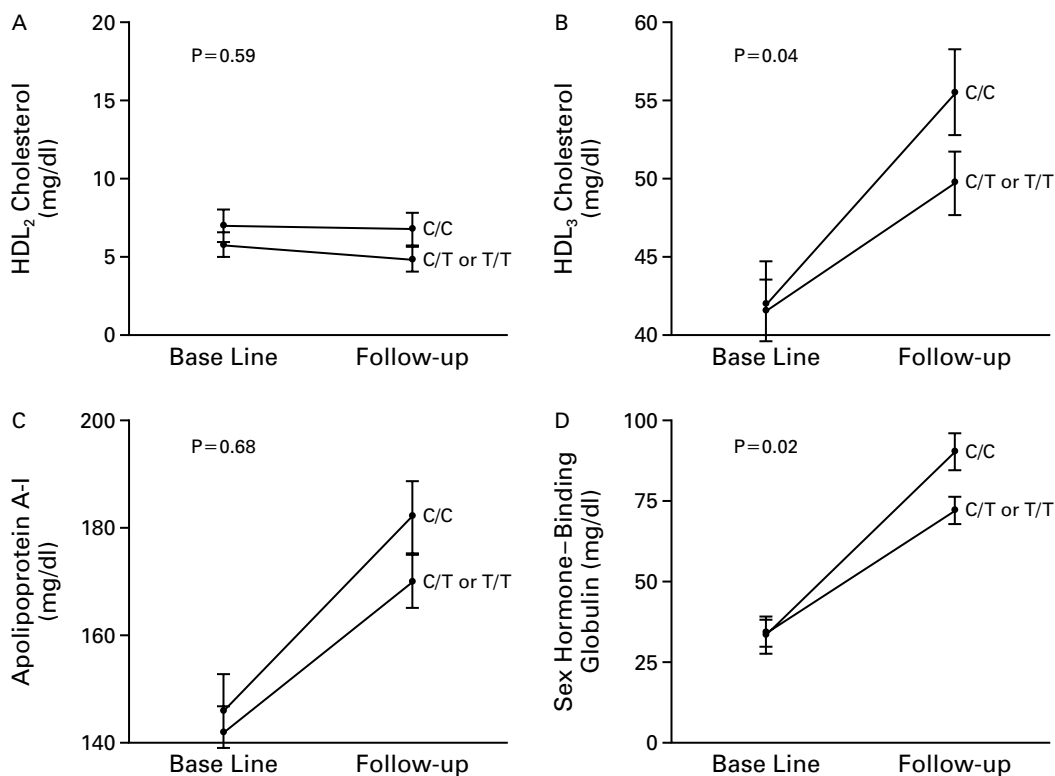
†The additive model was used to test for an interaction between treatment group and P1/P1, P1/P2, and P2/P2.

‡The dominant model was used to test for an interaction between treatment group and P1/P1, P1/P2, and P2/P2. In this model, P2 was treated as a dominant allele.



**Figure 2.** Mean ( $\pm$ SE) High-Density Lipoprotein (HDL) Cholesterol Levels at Base Line and Follow-up among Women in the Estrogen Replacement and Atherosclerosis Trial According to Study Group and Human Estrogen Receptor  $\alpha$  IVS1-401 Genotype, with Adjustment for Age, Race or Ethnic Group, Body-Mass Index, Diabetes Status, Smoking Status, Frequency of Exercise, and Alcohol Intake.

The P value is for the treatment-by-genotype interaction. To convert values for cholesterol to millimoles per liter, multiply by 0.02586.



**Figure 3.** Levels of High-Density Lipoprotein (HDL) Subfraction 2 (HDL<sub>2</sub>) (Panel A), HDL Subfraction 3 (HDL<sub>3</sub>) (Panel B), Apolipoprotein A-I (Panel C), and Sex Hormone-Binding Globulin (Panel D) at Base Line and Follow-up in Women Receiving Active Therapy, According to IVS1-401 Genotype.

The results for women receiving placebo are not shown. Results are expressed as group means, with the error bars indicating standard errors. P values are for the treatment-by-genotype interaction after adjustment for potential confounders. To convert values for cholesterol to millimoles per liter, multiply by 0.02586.

levels, progression of angiographically defined coronary disease did not differ significantly between women with the IVS1-401 C/C genotype who were assigned to hormone-replacement therapy and the other women assigned to hormone-replacement therapy; however, the power to detect such an interaction for the angiographic end point was extremely limited. None of the other *ER-α* polymorphisms examined, including several variants of the promoter TA repeat, were associated with a change in the response of HDL cholesterol levels to hormone-replacement therapy.

### DISCUSSION

The human *ER-α* gene, located at 6q24.1, has been cloned, sequenced, and expressed in various cell lines, and site-directed mutagenesis has identified highly conserved domains responsible for hormone or DNA binding or transcriptional activation.<sup>27</sup> Associations between numerous naturally occurring sequence variants of human *ER-α* and several clinical phenotypes have been studied, including risk,<sup>23,28</sup> age at onset,<sup>29</sup> and estrogen-receptor status<sup>30,31</sup> in breast cancer; risk of spontaneous abortion<sup>32,33</sup>; bone mineral density<sup>34-37</sup>; body-mass index<sup>35</sup>; hypertension<sup>38</sup>; lipid levels<sup>39,40</sup>; and coronary atherosclerosis.<sup>39</sup> Most reports have focused on the IVS1-401 and IVS1-354 polymorphisms. In general, only null, weak, or inconsistent relations with clinical phenotypes were evident in these observational studies.

In contrast, we examined the effect of various *ER-α* polymorphisms on the response to treatment with hormone-replacement therapy. Postmenopausal women with the IVS1-401 C/C genotype, and several other closely linked intron 1 polymorphisms, had an increase in HDL cholesterol levels with hormone-replacement therapy that was more than twice the increase observed in other women. This effect was dominated by significantly greater increases in HDL<sub>3</sub>, the subfraction most strongly associated with coronary events<sup>41</sup> and progression of coronary atherosclerosis.<sup>42-44</sup> Women with these genotypes also had slightly higher levels of HDL cholesterol at base line, possibly because of low, but still present, levels of circulating estradiol before randomization. On the basis of data from observational studies<sup>45</sup> and clinical trials of derivatives of fibric acid,<sup>41,46</sup> the observed increase of 13.1 mg per deciliter in HDL cholesterol levels with hormone-replacement therapy in women with the IVS1-401 C/C genotype might be expected to lower the risk of coronary events by 26 to 39 percent. However, in the Heart and Estrogen/Progestin Replacement Study, an increase of 4 mg per deciliter (0.10 mmol per liter) in HDL cholesterol levels with hormone-replacement therapy had no effect on the risk of coronary heart disease in women with established disease. Thus, the magnitude of the effect of

hormone-replacement therapy on the risk of coronary events in women with the IVS1-401 C/C genotype remains uncertain.

Metabolic pathways that determine the number and cholesterol content of HDL particles include the synthesis of apolipoprotein A-I and hepatic lipase-mediated clearance of free cholesterol from HDL to the liver for excretion. Synthesis of apolipoprotein A-I and hepatic lipase activity are known to be modified by estrogen.<sup>47</sup> The effect of the IVS1-401 polymorphism on estrogen activity in other tissues is not known. In two small clinical trials of the effects of estrogen on bone mineral density, the IVS1-401 C allele was associated with greater effects of estrogen on the bone mineral density of vertebrae.<sup>48,49</sup> However, a study of 248 Korean women found no such association.<sup>50</sup>

The molecular mechanism by which the IVS1-401 C allele is associated with augmented estrogen action with respect to HDL cholesterol remains unclear. It is possible that the IVS1 single-nucleotide polymorphisms are merely linked to another as-yet-unidentified causative sequence variant. However, if there is another linked causative variant, it does not appear to be one of the previously identified single-nucleotide polymorphisms in exon 1 or the TA repeat in the promoter region. Nor does it appear that there are other previously unrecognized polymorphisms in the first 1000 bp of the promoter region that can account for the observed interaction.

Intronic enhancer regions that augment gene transcription have been reported for other genes<sup>51</sup>; however, we are unaware of such reports for *ER-α*. A search of transcription factor binding sites revealed that the IVS1-401 C allele produces a potential binding site for the myb family of transcription factors. Expression of myb is itself activated by estrogen.<sup>52</sup> Whether the IVS1-401 C allele leads to transcription of *ER-α* augmented by myb is not known. Intronic polymorphisms are also known to modify the splicing of messenger RNA (mRNA) transcripts, resulting in significant changes in gene function.<sup>53,54</sup> Studies of smooth-muscle cells from human vascular tissue have documented significant heterogeneity in *ER-α* mRNA transcripts, including variants with deletions of exons encoding regions of the hormone-binding domain.<sup>55</sup> In one highly informative case, a man with a premature stop codon in exon 2 had no functional *ER-α* receptors.<sup>56</sup> Interestingly, he also had low HDL cholesterol levels and premature atherosclerosis.<sup>57</sup>

A limitation of genetic-association studies concerns the difficulty of precisely defining the phenotypes of interest. With respect to HDL cholesterol, levels are determined by multiple genetic and environmental factors. Identical HDL cholesterol levels may occur because of the influence of entirely unrelated genotypes. However, in this study, the phenotype of interest was

not simply the HDL cholesterol level but, rather, the change in that level in response to a specific, uniformly applied environmental factor — estrogen replacement. That the results were also significant in each of the two groups randomly assigned to active treatment and that the pattern was preserved in separate racial and ethnic subgroups provide additional evidence of the validity of the observations. No formal efforts were made to adjust for multiple comparisons in these hypothesis-generating analyses. However, the level of statistical significance for the main finding and the internal consistency of results across several outcomes and subgroups reduce the likelihood that the results were due to chance alone.

Nevertheless, more steps are required before the importance of this treatment-by-gene interaction can be fully ascertained. First, data from other clinical studies are needed to confirm the initial observation. Second, a greater understanding of the molecular mechanisms responsible for the association among the IVS1–401 polymorphism, estrogen, and HDL cholesterol is required. Further research should focus on verification that the IVS1–401 C allele is not simply linked to another as-yet-unidentified causative sequence variant, examination of the effect of the IVS1–401 polymorphism on the quantity and quality of *ER-α* transcripts in the liver or other relevant target tissues, examination of possible interactions between *myb* and *ER-α*, and determination of the steps in the regulation of HDL metabolism that are subsequently modified. Finally, larger clinical studies examining clinical end points are required to determine if the observed treatment-by-gene interaction with respect to HDL cholesterol translates into important differences in the risk of clinical cardiovascular and other events in women receiving estrogen replacement.

In summary, the data presented here indicate that women with the *ER-α* IVS1–401 C/C genotype or several other closely linked genotypes have an augmented response of HDL cholesterol to estrogen replacement. The effects of these genotypes on the risk of clinical cardiovascular events in the setting of hormone replacement are not yet established. Furthermore, their influence on other clinical effects of estrogen, including the relief of perimenopausal symptoms and the effects on the risk of osteoporosis, venous thrombosis, and breast cancer, must also be evaluated before a judgment about their clinical role can be made. Nonetheless, these data point to the possibility of using genetic screening to tailor decisions about hormone-replacement therapy to maximize the health and well-being of postmenopausal women.

Part of this work has appeared in abstract form (Circulation 2001;103:1353).

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