Influence of Estrogen Receptor α and Progesterone Receptor Polymorphisms on the Effects of Hormone Therapy on Mammographic Density

Fränzel J.B. van Duijnhoven,[1] Petra H.M. Peeters,[1] Ruth M.L. Warren,[2] Sheila A. Bingham,[3] André G. Uitterlinden,[4] Paulus A.H. van Noord,[1] Evelyn M. Monninkhof,[1] Diederick E. Grobbee,[1] and Carla H. van Gils[1]

[1]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands; [2]Department of Radiology, Addenbrooke's Hospital, Cambridge Breast Unit; [3]Medical Research Council Dunn Human Nutrition Unit, Cambridge, United Kingdom; and [4]Department of Internal Medicine, Erasmus Medical Center, Rotterdam, the Netherlands

Abstract

Postmenopausal hormone therapy increases mammographic density, a strong breast cancer risk factor, but effects vary across women. We investigated whether the effect of hormone therapy use is modified by polymorphisms in the estrogen receptor (*ESR1*) and progesterone receptor (*PGR*) genes in the Dutch Prospect-EPIC and the English EPIC-Norfolk cohorts. Information on hormone therapy use was obtained through questionnaires at recruitment and after 5 years. Blood samples were collected and consecutive mammograms were available through breast cancer screening programs. For 795 hormone therapy users, one mammogram before and a second mammogram during hormone therapy use was included. For 781 never hormone therapy users, mammograms with similar time intervals were included. Mammographic density was assessed using a computer-assisted method. Changes in density were analyzed using linear regression. A statistically significant difference in percentage density change between hormone therapy users and never users was seen in women with the *ESR1 Pvu*II *Pp* or *pp* genotype (2.24%; $P < 0.01$), but not in those with the *PP* genotype (0.90%; $P = 0.47$). Similarly, effects of hormone therapy on percentage density were observed in women with the *ESR1 Xba*I *Xx* or *xx* genotype (2.20%; $P < 0.01$), but not in those with the *XX* genotype (−0.65%; $P = 0.70$). Also, effects were seen in women with the *PGR +331 GG* genotype (2.04%; $P < 0.01$), but not in those with the *GA* or *AA* genotype (0.98%; $P = 0.53$). The *PGR PROGINS* polymorphism did not seem to make women more susceptible to the effects of hormone therapy use. In conclusion, our results suggest that specific polymorphisms in the *ESR1* and *PGR* genes may make women more susceptible to the effects of hormone therapy use on mammographic density. (Cancer Epidemiol Biomarkers Prev 2006;15(3):462–7)

Introduction

Epidemiologic studies provide strong evidence that the use of postmenopausal hormone therapy is associated with an increased breast cancer risk (1-3). This observed increase may be explained by the effects of hormone therapy use on mammographic density, a strong breast cancer risk factor (4, 5). Another study from our group[5] shows that the absolute mean decline in percentage density between mammograms, which were on average 3 years apart, was 7.36% for never hormone therapy users and 5.58% for hormone therapy users (difference between groups 1.78%; $P < 0.01$). This suggests that postmenopausal hormone therapy use slows down the natural reduction in mammographic density. Other studies have shown that postmenopausal hormone therapy use even increases breast density (6-15). These effects, however, are not seen in all women who use hormone therapy (6-13).

Hormone receptors in the breast could influence susceptibility to the effects of hormone therapy use as estrogens and progesterone exert their effects through the estrogen and progesterone receptors (16, 17). Some polymorphisms in the genes coding for these receptors may change the expression of the receptors and may, therefore, modify the effect of hormone therapy use on mammographic density. Within the estrogen receptor α (*ESR1*) gene, the *Pvu*II (also known as c.454-397T→C, IVS1-397 T/C, or rs2234693) polymorphism and the *Xba*I (also known as c.454-351A→G, IVS1-351 A/G, or rs9340799) polymorphism were selected. These polymorphisms have been associated with mammographic density (18) as well as breast cancer risk (19-23).

Within the progesterone receptor (*PGR*) gene, the *+331 G/A* polymorphism and the *C/T Hist770Hist* polymorphism (also known as rs1042839) were selected. The *+331 G/A* polymorphism is located in the promoter region and the *C/T Hist770Hist* polymorphism in exon 5 of the *PGR*. The latter polymorphism is in complete linkage disequilibrium with an *C/T Val660Leu* polymorphism in exon 4 and an Alu insertion in intron 7 and together they are called the *PROGINS* complex (24). Both the *+331 G/A* polymorphism and the *PROGINS* polymorphism have been investigated in relation with breast cancer risk but the results were inconclusive (25-28).

In this study, we investigated whether polymorphisms in the *ESR1* and *PGR* genes modify the effect of hormone therapy use on mammographic density.

Materials and Methods

Study Population. Women were selected from the Prospect-EPIC (29) and the EPIC-Norfolk (30) studies, a Dutch and an English cohort, both participating in the European Prospective

Received 9/27/05; revised 12/9/05; accepted 1/10/06.

Grant support: Dutch Cancer Society grant UU 2002-2716.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Carla H. van Gils, Julius Center for Health Sciences and Primary Care, University Medical Center, Utrecht, Mail-drop Str. 6.131, P.O. Box 85500, 3508 GA Utrecht, the Netherlands. Phone: 31-30-2503014; Fax: 31-30-2505485. E-mail: C.vangils@umcutrecht.nl

Copyright © 2006 American Association for Cancer Research.

doi:10.1158/1055-9965.EPI-05-0754

[5]F.J.B. van Duijnhoven, et al. Postmenopausal hormone therapy and changes in mammographic density, submitted for publication.

Investigation into Cancer and Nutrition (EPIC; ref. 31). Between 1993 and 1997, participants had a medical examination, filled out lifestyle and food frequency questionnaires (32, 33), and donated a blood sample. At the end of the inclusion period, 17,357 women were included in Prospect-EPIC and 16,744 women and 13,698 men were included in EPIC-Norfolk. All participants gave written or oral informed consent and the studies were approved by the local ethical committees. Approximately 5 years after recruitment, participants from both cohorts filled out a follow-up questionnaire. Besides questionnaire data, consecutive mammograms were available for women through the regional breast cancer screening programs.

The baseline as well as the follow-up questionnaire was used to obtain information on postmenopausal hormone therapy use. Hormone therapy use was defined as the use of hormones for menopausal complaints. Both questionnaires comprised questions on the age at which a woman started and stopped using hormone therapy. Hormone therapy users were defined as those women who reported at either the baseline or the follow-up questionnaire that they had ever used hormone therapy. Never hormone therapy users were defined as those women who reported at both questionnaires that they never used hormone therapy.

Ever hormone therapy users were eligible if one screening mammogram before hormone therapy use and a second mammogram during hormone therapy use could be obtained. Never hormone therapy users were matched to hormone therapy users on year of mammogram, duration of interval between mammograms, and year of birth, and, thus, two mammograms with approximately the same time interval were collected for never hormone therapy users. Hormone therapy users as well as never hormone therapy users were excluded when they used oral contraceptives at the first mammogram or when they were diagnosed with breast cancer before or within 2 years of the second mammogram. The reason for this is that the hormones in oral contraceptives or the presence of a tumor may influence the density on a mammogram. Mammograms were selected for 620 hormone therapy users and 620 never hormone therapy users from Prospect-EPIC and for 175 hormone therapy users and 161 never hormone therapy users from EPIC-Norfolk. Blood samples were available for all these women, except for 18 hormone therapy users and 15 never hormone therapy users from EPIC-Norfolk.

Genotyping Analysis. In the Prospect-EPIC cohort, genomic DNA was extracted from WBC using the QIAamp DNA Blood Mini kit (Qiagen Benelux B.V., Venlo, the Netherlands) according to the instructions of the manufacturer.

In the EPIC-Norfolk cohort, genomic DNA was extracted from whole blood samples using the phenol-chloroform method by Whatman International (Ely, United Kingdom). DNA yields from both cohorts were quantified using a fluorescent stain (PicoGreen, Molecular Probes Inc., Eugene, OR) and samples were diluted to a final DNA concentration of 5 ng/μL (Prospect-EPIC) or 4 ng/μL (EPIC-Norfolk).

Genotyping was done using the Taqman SNP genotyping technology (Applied Biosystems, Foster City, CA). Primer and probe design was done by the manufacturer (sequences available on request), and reactions were done according to the protocol of the manufacturer. Samples were classified as *PP, Pp,* or *pp* (representing the *CC, CT,* and *TT* genotypes of *Pvu*II, respectively); *XX, Xx,* or *xx* (representing the *GG, GA,* and *AA* genotypes of *Xba*I, respectively); *GG, GA,* or *AA* for the *+331 G/A* genotype; and *CC, CT,* or *TT* for the *PROGINS* genotype. The assay was repeated for samples with initial missing genotypes. After repeating the PCR, the percentage of undetermined samples was 0.5% (7 of 1,543) for the *Pvu*II genotype, 0.2% (3 of 1,543) for the *Xba*I genotype, 2.9% (45 of 1,543) for the *+331 G/A* genotype, and 2.4% (37 of 1,543) for the *PROGINS* genotype.

Mammographic Density Analysis. Mammographic density was assessed using the mediolateral oblique mammogram, which is the routine view for breast cancer screening in the Netherlands and the United Kingdom. It has previously been shown that the proportions of mammographic density on craniocaudal views and mediolateral oblique views and on left and right views are strongly correlated and that representative information on mammographic density is provided in a single view (34). Mammographic density was assessed on the left view for all women.

After digitizing the films using a laser film scanner (Lumiscan 50, Lumisys for Prospect-EPIC and Lumiscan 85, Lumisys for EPIC-Norfolk), mammographic density was quantified using a computer-assisted method based on gray levels in the digitized mammogram (35). For each image, the reader first sets a threshold to determine the outside edge of the breast and to discriminate between the dark area outside the breast and the lighter area within the breast. Another threshold is set to determine the area of dense tissue within the breast, which is the lightest tissue visible on the mammogram. The program then determines the amount of pixels within the total breast area and within the dense area and calculates the percentage of dense tissue in the breast, which is the dense area divided by the total breast area multiplied by 100. In literature, the percentage of dense tissue, which is a relative measure of dense tissue, is mostly used. It may, however, be more relevant to study the absolute amount of dense tissue, which consists of connective and epithelial tissue and is regarded as the target tissue for breast cancer. We, therefore, present results on both the relative and absolute measure of mammographic density, which we refer to as ''percentage density'' and ''dense area,'' respectively.

The mammograms from Prospect-EPIC (the Netherlands) and EPIC-Norfolk (United Kingdom) were read separately, but both by the same observer (F.J.B. van Duijnhoven). They were read in sets of 68 (United Kingdom) and 70 (the Netherlands) images composed of both mammograms from 34 or 35 women in random order. To assess the reliability of the reader, two library sets of 68 (United Kingdom) and 70 (the Netherlands) images were made, which consisted of randomly chosen mammograms. This same library set was read before the first set, after the last set, and at several time points between sets, which were blinded for the reader. The images in the library set were randomly ordered every time they were read to prevent the observer from recognizing this set. An average intraclass correlation coefficient of 0.88 (range 0.82-0.93) for dense area and 0.94 (range 0.91-0.95) for percentage density was reached between repeated readings. These results are comparable with previous studies using the same method (35).

Data Analysis. Distributions of breast cancer risk factors at baseline are given for hormone therapy users and never hormone therapy users in Prospect-EPIC and EPIC-Norfolk together, using means with SDs, medians with range or frequencies (where appropriate). Differences were tested by the Student's t test, Mann-Whitney test, or χ^2 analysis. Menopausal status was categorized in premenopausal together with perimenopausal versus postmenopausal. Women were counted as premenopausal women when they were still menstruating and were not using oral contraceptives or other hormones. Women were counted as postmenopausal women when they experienced at least 12 consecutive months of amenorrhea. All other women were counted as perimenopausal women. Family history of breast cancer was defined as having at least a mother or a sister diagnosed with breast cancer. Smoking was categorized in pack-years, which is

defined as the mean number of cigarettes per day divided by 25 and multiplied by years of smoking. Current alcohol intake was measured in grams of ethanol per day and categorized in approximate tertiles. Physical activity was categorized in a four-level index, which was derived by combining occupational physical activity together with time participating in cycling and other recreational physical exercise (36).

The observed genotype distributions were compared with those expected under Hardy-Weinberg equilibrium using a goodness-of-fit χ^2 test with 1 degree of freedom.

The absolute change in percentage density and dense area was compared between hormone therapy users and never hormone therapy users by linear regression analysis. To determine the change, mammographic density at the second mammogram was used as the outcome variable and mammographic density at the first mammogram was included as a covariate in the unadjusted model. The mean mammographic density at the second mammogram was calculated from the model and the change in density was computed by subtracting the mean mammographic density at the first mammogram from this number for hormone therapy users and never hormone therapy users separately. To investigate potential effect modification, results were stratified by *Pvu*II genotype (*PP* versus *Pp* with *pp*), *Xba*I genotype (*XX* versus *Xx* with *xx*), *+331 G/A* genotype (*GG* versus *GA* with *AA*), and *PROGINS* genotype (*CC* versus *CT* with *TT*). Women with one or two copies of the *p* allele (*Pvu*II) and those with one or two copies of the *x* allele (*Xba*I) were combined into one stratum, because these alleles have been associated with higher breast densities (18) and higher breast cancer risk (21). In addition, women with one or two copies of the *A* allele (*+331 G/A*) or the *T* allele (*PROGINS*) were combined into one stratum due to small numbers. Interaction was tested by adding the product term of hormone therapy use (no/yes) and these characteristics (in above-mentioned categories) to the linear regression model.

In the adjusted models, in addition to hormone therapy use (no/yes) and mammographic density at the first mammogram, age, body mass index, age at menarche, parity (nulliparous/parous), menopausal status (premenopausal and perimenopausal/postmenopausal), family history of breast cancer (no/yes), previous oral contraceptive use (no/yes), smoking (0, <5, 5-15 and >15 pack-years), alcohol consumption (tertiles), physical activity (inactive, moderately inactive, moderately active, active), and study population (Prospect-EPIC/EPIC-Norfolk) were included as covariates.

All analyses were done with SPSS version 12.0.

Results

Distribution of breast cancer risk factors at baseline are shown for women who started hormone therapy use and for women who never used hormone therapy (Table 1). Percentage density at the first mammogram was lower for never hormone therapy users (37.0%) than for those who would start to use hormone therapy after the first mammogram (40.5%). Similarly, the dense area at the first mammogram was lower for never hormone therapy users (40.6 cm^2) than for hormone therapy users (47.2 cm^2). Never hormone therapy users more often were postmenopausal (74.8% versus 68.3%) and had less often used oral contraceptives in the past (63.4% versus 67.2%) compared with hormone therapy users. Never hormone therapy users less frequently used alcohol than hormone therapy users.

Data from our previous study[5] showed that the absolute mean decline in percentage density between mammograms was 7.36% for never hormone therapy users and 5.58% for hormone therapy users (difference between groups 1.78%; $P < 0.01$).

In this study, the absolute change in percentage density by hormone therapy use was stratified by genotype (Table 2). Genotype distributions of *Pvu*II, *Xba*I, *+331 G/A,* and *PROGINS* were in Hardy-Weinberg equilibrium (*P* values were 0.42, 0.33, 0.22, and 0.07, respectively). Corresponding to our overall results[5], the decline in mammographic density was larger for never hormone therapy users than for hormone therapy users in every stratum except for the stratum of women with a *XX* genotype. A statistically significant difference in percentage density change between hormone therapy users and never users was seen in women with the *ESR1 Pvu*II *Pp* or *pp* genotype (2.24%; $P < 0.01$) but not in those with the *PP* genotype (0.90%; $P = 0.47$). Similarly, effects of hormone therapy on percentage density were observed in women with the *ESR1 Xba*I *Xx* or *xx* genotype (2.20%; $P < 0.01$), but not in those with the *XX* genotype (−0.65%; $P = 0.70$). Also, effects were seen in women with the *PGR +331 GG* genotype (2.04%; $P < 0.01$), but not in those with the *GA* or *AA* genotype (0.98%; $P = 0.53$). The difference in change was statistically significant in both strata of the *PROGINS* polymorphisms. None of the interactions between hormone therapy use and the characteristics in Table 2 were statistically significant.

The absolute changes by hormone therapy use and by these strata were also assessed for dense area instead of percentage density. Similar results were seen for the strata of the genotypes (data not shown).

Discussion

The results of this study suggest that hormone therapy use does not affect mammographic density in women with the *ESR1 PP* or *XX* genotype, but that it slows down the breast involution process in women with one or two copies of the *p* allele or *x* allele. Similarly, hormone therapy use does not seem to have an effect on mammographic density in women with one or two copies of the *+331 A* allele, whereas it does in women with the *GG* genotype. The *PROGINS* polymorphism in the *PGR* do not seem to make women more susceptible to the effects of hormone therapy use on mammographic density.

In this study, we focused on genes that may make women more susceptible to the effects of hormone therapy use on mammographic density. Only one other study has reported results on polymorphisms in this respect. Lord et al. (37) found a statistically significant interaction between combined hormone therapy use and the *CYP1B1* polymorphism as well as the *AKR1C4* polymorphism in relation to changes in mammographic density. The polymorphisms in this study were located in genes that are involved in hormone metabolism, whereas the polymorphisms in our study are located in genes that code for hormone receptors. One other study investigated the interaction of hormone therapy use with androgen receptor genotypes in relation to mammographic density (38). However, this study did not address changes in density, but merely measured density at one point in time. Lillie et al. (38) reported that in users of combined hormone therapy, women with a higher number of *CAG* repeat lengths had a statistically significantly higher mean percentage of density than women with a lower number of these repeats. The results from this study, together with our results, indicate that variants of hormone receptor genes may make women more susceptible to the effects of hormone therapy use on mammographic density.

The molecular explanation for our findings is not entirely clear. Two studies have suggested that the *ESR1 Pvu*II *P* allele increases *ESR1* transcription (39, 40), whereas another study suggested that the *Pvu*II *p* allele and the *Xba*I *x* allele increases transcription of *ESR1* (41). These results indicate that *Pvu*II and *Xba*I polymorphisms are involved in the production of *ESR1*, but the exact function needs to be clarified.

The *+331 A* allele (24) and the *PROGINS T* allele (42) have been reported to increase the transcription of the *PGR*. In theory, one would expect that the effect of hormone therapy use would be largest in women with variants that result in a higher expression of the receptor. The results for the *+331 G/A* polymorphism, however, show that the effect of hormone therapy use is only seen in women with the *G* allele, which has been related to a lower expression of the *PGR*. In addition, the *PROGINS* polymorphism did not seem to make women more susceptible to the effects of hormone therapy use. Our results do not seem to correspond with what would be expected on the basis of the above-mentioned functionality studies. However, it should be kept in mind that the functionality of the *+331 G/A* polymorphism as well as the *PROGINS* polymorphism has only been investigated in one study. Therefore, further molecular research is needed to provide an explanation for the findings of our study.

Strengths of our study include the longitudinal design and the use of a continuous density measurement method. Furthermore, our observational study gives us the opportunity to investigate whether characteristics influence the effects of hormone therapy use on mammographic density in women who use hormone therapy in real life. As opposed to clinical trials, where women are randomly assigned to receive placebo or a hormone therapy regimen, the women in our observational study are prescribed hormone therapy use because of a medical indication. Because of their different backgrounds, effects of hormone therapy may not be the same in these women (43).

Our study, however, also has certain limitations, which may have reduced the opportunity to show genetic effect

Table 1. Breast cancer risk factors according to hormone therapy use

Breast cancer risk factors	No hormone therapy use (n = 781)	Hormone therapy use (n = 795)	P
	Mean (SD)/median (range)	Mean (SD)/median (range)	
Age at baseline (y)	55.4 (4.3)	55.7 (4.3)	0.22*
Body mass index (kg/m^2)	25.7 (3.7)	25.5 (3.7)	0.16*
Age at menarche (y)	13.2 (1.5)	13.5 (2.1)	<0.01*
Percentage density at first mammogram (%)	37.0 (0.3-88.2)	40.5 (0.1-88.8)	<0.01†
Dense area at first mammogram (cm^2)	40.6 (0.2-173.9)	47.2 (0.5-199.6)	<0.01†
	n (%)	n (%)	P ‡
Parity			
Nulliparous	80 (10.3)	88 (11.1)	
Parous	700 (89.7)	707 (88.9)	0.60
Menopausal status			
Premenopausal/perimenopausal	197 (25.2)	252 (31.7)	
Postmenopausal	584 (74.8)	543 (68.3)	<0.01
Family history of breast cancer			
No	673 (87.6)	704 (89.7)	
Yes	95 (12.4)	81 (10.3)	0.20
Previous oral contraceptive use			
Never	285 (36.6)	261 (32.8)	
Ever	494 (63.4)	534 (67.2)	0.12
Smoking (pack-years)			
0	399 (52.4)	375 (48.6)	
0.01-4.99	131 (17.2)	149 (19.3)	
5.00-14.99	131 (17.2)	134 (17.4)	
≥15.00	101 (13.3)	114 (14.8)	0.45
Alcohol consumption (g/d)			
<1.70	293 (37.8)	237 (29.9)	
1.70-9.99	240 (31.0)	269 (33.9)	
≥10.00	242 (31.2)	287 (36.2)	<0.01
Physical activity			
Inactive	54 (6.9)	58 (7.3)	
Moderately inactive	221 (28.3)	233 (29.3)	
Moderately active	227 (29.1)	237 (29.8)	
Active	279 (35.7)	267 (33.6)	0.85
PvuII			
PP	166 (21.4)	183 (23.6)	
Pp	402 (51.8)	385 (49.7)	
pp	208 (26.8)	207 (26.7)	0.55
XbaI			
XX	98 (12.6)	96 (12.4)	
Xx	362 (46.4)	371 (47.9)	
xx	320 (41.0)	308 (39.7)	0.84
+331 G/A			
GG	661 (86.7)	672 (89.6)	
GA	100 (13.1)	76 (10.1)	
AA	1 (0.1)	2 (0.3)	0.09§
PROGINS			
CC	560 (73.2)	539 (71.3)	
CT	185 (24.2)	191 (25.3)	
TT	20 (2.6)	26 (3.4)	0.54

*Student's t test.
†Mann-Whitney test.
‡χ^2 test.
§Because of low expected numbers in some cells, we combined *GA* and *AA* in one category.

Table 2. Absolute density change in percentage density by hormone therapy use and stratified by genotype

	$\beta^{\dagger}$	*P*	No hormone therapy use (*n* = 713)*	Hormone therapy use (*n* = 721)*
			Density change (*n*)	Density change (*n*)
Estrogen receptor				
*Pvu*II				
PP	0.90	0.47	−7.47 (147)	−6.57 (167)
Pp/pp	2.24	<0.01	−7.40 (563)	−5.16 (554)
*Xba*I				
XX	−0.65	0.70	−6.70 (87)	−7.35 (87)
Xx/xx	2.20	<0.01	−7.48 (626)	−5.28 (634)
Progesterone receptor				
+331 G/A				
GG	2.04	<0.01	−7.48 (601)	−5.44 (623)
GA/AA	0.98	0.53	−7.12 (95)	−6.14 (74)
PROGINS				
CC	1.58	0.02	−7.20 (512)	−5.63 (503)
CT/TT	2.67	0.02	−8.26 (187)	−5.59 (200)

*Total numbers are lower than in Table 1, because of loss of some or all of the potential confounders.
†Model adjusted for density at first mammogram, age, body mass index, age at menarche, parity (nulliparous/parous), menopausal status (premenopausal and perimenopausal/postmenopausal), family history of breast cancer (no/yes), previous oral contraceptive use (no/yes), smoking (0, <5, 5-15, and ≥15 pack-years), alcohol consumption (tertiles), physical activity (inactive, moderately inactive, moderately active, active), and study population (Prospect-EPIC/EPIC-Norfolk).

modification. The age at which women started and stopped the use of hormone therapy was assessed on the basis of a questionnaire and the mammograms before and during hormone therapy use were selected accordingly. If women made a mistake in answering the age at start or stop of hormone therapy, misclassification may have influenced our results. Although this misclassification is nondifferential, if present, it may have lead to an underestimation of the effect.

Another possibility is that effect modification is mainly present in a specific type of hormone therapy use. The study by Lord et al. (37), for example, only showed statistical interactions of polymorphisms with the use of combined hormone therapy and not with unopposed estrogen therapy. The exact type of hormone therapy preparation was known for a subset of the Prospect-EPIC women (*n* = 231), but we chose not to make a distinction between the different types of hormone therapy use because sample sizes would then be too small.

Another limitation of our study may be that we did not have information about changes in body mass index and that we, therefore, could not adjust for this in our analyses. Body mass index, however, seems to only have an effect on nondense tissue in the breast (44). Adjusting for changes in body mass index will, therefore, only have an influence on results for the relative measure of mammographic density (percentage density). The results in this study for the absolute measure of mammographic density (dense area) will remain the same.

Although not proven yet, it is very likely that women who have an adverse effect of hormone therapy use on mammographic density are the ones who are at higher risk for breast cancer when they use hormone therapy. However, even if this is not the case, the adverse effects on breast density may result in a reduced sensitivity and specificity of mammographic breast cancer screening (45, 46). Identifying subgroups that are most susceptible to the effect of hormone therapy on mammographic density is, therefore, important. When these subgroups are established, specific action could be taken toward the women with highest susceptibility. For instance, these women could be advised against the use of hormone therapy or when they are using hormone therapy could be closely monitored to see how the breast tissue responds.

In conclusion, our results suggest that polymorphisms in the *ESR1* and *PGR* genes may modify the relation between hormone therapy use and mammographic density. This may provide an explanation for the observed interindividual differences in effects of hormone therapy use on mammographic density and supposedly breast cancer risk.

Acknowledgments

We thank Dr. Norman Boyd for the training in mammographic density assessment and Prof. Martin Yaffe and his department for the technical support of the computer-assisted software to determine mammographic density; Stichting Preventicon, the ''Vereniging van Vriendinnen van het DOM project,'' and the Norwich Breast Screening Unit for making the mammograms from all participants available; Robert Luben and Bernard Slotboom for assisting in selecting the study populations; Iqbal Warsi, José Drijvers, Joke Metselaar-van den Bos, Bert Rodenburg, and Jelmer Hoefakker for assisting in identifying and collecting the mammograms; Arjan Barendrecht for assisting in the DNA concentration measurements; and Pascal Arp for his assistance in the Taqman PCR assays.

References

1. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Collaborative Group on Hormonal Factors in Breast Cancer. Lancet 1997;350:1047–59.
2. Chlebowski RT, Hendrix SL, Langer RD, et al. Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausal women: the Women's Health Initiative Randomized Trial. JAMA 2003;289:3243–53.
3. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. JAMA 2002;288:321–33.
4. Boyd NF, Byng JW, Jong RA, et al. Quantitative classification of mammographic densities and breast cancer risk: results from the Canadian National Breast Screening Study. J Natl Cancer Inst 1995;87:670–5.
5. Byrne C, Schairer C, Wolfe J, et al. Mammographic features and breast cancer risk: effects with time, age, and menopause status. J Natl Cancer Inst 1995;87:1622–9.
6. Christodoulakos GE, Lambrinoudaki IV, Panoulis KP, et al. The effect of various regimens of hormone replacement therapy on mammographic breast density. Maturitas 2003;45:109–18.
7. Greendale GA, Reboussin BA, Sie A, et al. Effects of estrogen and estrogen-progestin on mammographic parenchymal density. Postmenopausal Estrogen/Progestin Interventions (PEPI) Investigators. Ann Intern Med 1999;130:262–9.
8. Kilicdag EB, Bagis T, Pourbagher A, Tarim E. Hormone replacement therapy and mammographic density. Int J Gynaecol Obstet 2004;86:56–8.
9. Lundstrom E, Christow A, Kersemaekers W, et al. Effects of tibolone and continuous combined hormone replacement therapy on mammographic breast density. Am J Obstet Gynecol 2002;186:717–22.
10. McNicholas MM, Heneghan JP, Milner MH, Tunney T, Hourihane JB, MacErlaine DP. Pain and increased mammographic density in women receiving hormone replacement therapy: a prospective study. Am J Roentgenol 1994;163:311–5.
11. Ozdemir A, Konus O, Nas T, Erbas G, Cosar S, Isik S. Mammographic and ultrasonographic study of changes in the breast related to HRT. Int J Gynaecol Obstet 1999;67:23–32.
12. Persson I, Thurfjell E, Holmberg L. Effect of estrogen and estrogen-progestin

replacement regimens on mammographic breast parenchymal density. J Clin Oncol 1997;15:3201–7.
13. Rutter CM, Mandelson MT, Laya MB, Seger DJ, Taplin S. Changes in breast density associated with initiation, discontinuation, and continuing use of hormone replacement therapy. JAMA 2001;285:171–6.
14. Freedman M, San Martin J, O'Gorman J, et al. Digitized mammography: a clinical trial of postmenopausal women randomly assigned to receive raloxifene, estrogen, or placebo. J Natl Cancer Inst 2001;93:51–6.
15. Greendale GA, Reboussin BA, Slone S, Wasilauskas C, Pike MC, Ursin G. Postmenopausal hormone therapy and change in mammographic density. J Natl Cancer Inst 2003;95:30–7.
16. Katzenellenbogen BS. Estrogen receptors: bioactivities and interactions with cell signaling pathways. Biol Reprod 1996;54:287–93.
17. Leonhardt SA, Boonyaratanakornkit V, Edwards DP. Progesterone receptor transcription and non-transcription signaling mechanisms. Steroids 2003;68:761–70.
18. van Duijnhoven FJ, Bezemer ID, Peeters PH, et al. Polymorphisms in the estrogen receptor {α} gene and mammographic density. Cancer Epidemiol Biomarkers Prev 2005;14:2655–60.
19. Andersen TI, Heimdal KR, Skrede M, Tveit K, Berg K, Borresen AL. Oestrogen receptor (ESR) polymorphisms and breast cancer susceptibility. Hum Genet 1994;94:665–70.
20. Cai Q, Shu XO, Jin F, et al. Genetic polymorphisms in the estrogen receptor α gene and risk of breast cancer: results from the Shanghai Breast Cancer Study. Cancer Epidemiol Biomarkers Prev 2003;12:853–9.
21. Onland-Moret NC, van Gils CH, Roest M, Grobbee DE, Peeters PH. The estrogen receptor α gene and breast cancer risk (the Netherlands). Cancer Causes Control 2005;16:1195–202.
22. Shin A, Kang D, Nishio H, et al. Estrogen receptor α gene polymorphisms and breast cancer risk. Breast Cancer Res Treat 2003;80:127–31.
23. Wedren S, Lovmar L, Humphreys K, et al. Oestrogen receptor α gene haplotype and postmenopausal breast cancer risk: a case control study. Breast Cancer Res 2004;6:R437–49.
24. de Vivo I, Huggins GS, Hankinson SE, et al. A functional polymorphism in the promoter of the progesterone receptor gene associated with endometrial cancer risk. Proc Natl Acad Sci U S A 2002;99:12263–8.
25. de Vivo I, Hankinson SE, Colditz GA, Hunter DJ. A functional polymorphism in the progesterone receptor gene is associated with an increase in breast cancer risk. Cancer Res 2003;63:5236–8.
26. Feigelson HS, Rodriguez C, Jacobs EJ, Diver WR, Thun MJ, Calle EE. No association between the progesterone receptor gene +331G/A polymorphism and breast cancer. Cancer Epidemiol Biomarkers Prev 2004;13:1084–5.
27. Wang-Gohrke S, Chang-Claude J, Becher H, Kieback DG, Runnebaum IB. Progesterone receptor gene polymorphism is associated with decreased risk for breast cancer by age 50. Cancer Res 2000;60:2348–50.
28. Spurdle AB, Hopper JL, Chen X, et al. The progesterone receptor exon 4 Val660Leu G/T polymorphism and risk of breast cancer in Australian women. Cancer Epidemiol Biomarkers Prev 2002;11:439–43.
29. Boker LK, van Noord PA, van der Schouw YT, et al. Prospect-EPIC Utrecht: study design and characteristics of the cohort population. European Prospective Investigation into Cancer and Nutrition. Eur J Epidemiol 2001;17:1047–53.
30. Day N, Oakes S, Luben R, et al. EPIC-Norfolk: study design and characteristics of the cohort. European Prospective Investigation of Cancer. Br J Cancer 1999;80 Suppl 1:95–103.
31. Riboli E, Kaaks R. The EPIC Project: rationale and study design. European Prospective Investigation into Cancer and Nutrition. Int J Epidemiol 1997;26:S6–14.
32. Ocke MC, Bueno-de-Mesquita HB, Goddijn HE, et al. The Dutch EPIC food frequency questionnaire. I. Description of the questionnaire, and relative validity and reproducibility for food groups. Int J Epidemiol 1997;26:S37–48.
33. Ocke MC, Bueno-de-Mesquita HB, Pols MA, Smit HA, Van Staveren WA, Kromhout D. The Dutch EPIC food frequency questionnaire. II. Relative validity and reproducibility for nutrients. Int J Epidemiol 1997;26:S49–58.
34. Byng JW, Boyd NF, Little L, et al. Symmetry of projection in the quantitative analysis of mammographic images. Eur J Cancer Prev 1996;5:319–27.
35. Byng JW, Boyd NF, Fishell E, Jong RA, Yaffe MJ. The quantitative analysis of mammographic densities. Phys Med Biol 1994;39:1629–38.
36. Wareham NJ, Jakes RW, Rennie KL, et al. Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Public Health Nutr 2003;6:407–13.
37. Lord SJ, Mack WJ, Van Den BD, et al. Polymorphisms in genes involved in estrogen and progesterone metabolism and mammographic density changes in women randomized to postmenopausal hormone therapy: results from a pilot study. Breast Cancer Res 2005;7:R336–44.
38. Lillie EO, Bernstein L, Ingles SA, et al. Polymorphism in the androgen receptor and mammographic density in women taking and not taking estrogen and progestin therapy. Cancer Res 2004;64:1237–41.
39. Herrington DM, Howard TD, Brosnihan KB, et al. Common estrogen receptor polymorphism augments effects of hormone replacement therapy on E-selectin but not C-reactive protein. Circulation 2002;105:1879–82.
40. Schuit SC, Oei HH, Witteman JC, et al. Estrogen receptor α gene polymorphisms and risk of myocardial infarction. JAMA 2004;291:2969–77.
41. Maruyama H, Toji H, Harrington CR, et al. Lack of an association of estrogen receptor α gene polymorphisms and transcriptional activity with Alzheimer disease. Arch Neurol 2000;57:236–40.
42. Agoulnik IU, Tong XW, Fischer DC, et al. A germline variation in the progesterone receptor gene increases transcriptional activity and may modify ovarian cancer risk. J Clin Endocrinol Metab 2004;89:6340–7.
43. van der Schouw YT, Grobbee DE. Menopausal complaints, oestrogens, and heart disease risk: an explanation for discrepant findings on the benefits of post-menopausal hormone therapy. Eur Heart J 2005;26:1358–61.
44. Haars G, van Noord PA, van Gils CH, Grobbee DE, Peeters PH. Measurements of breast density; no ratio for a ratio. Cancer Epidemiol Biomarkers Prev 2005;14:2634–40.
45. Carney PA, Miglioretti DL, Yankaskas BC, et al. Individual and combined effects of age, breast density, and hormone replacement therapy use on the accuracy of screening mammography. Ann Intern Med 2003;138:168–75.
46. Kerlikowske K, Grady D, Barclay J, Sickles EA, Ernster V. Effect of age, breast density, and family history on the sensitivity of first screening mammography. JAMA 1996;276:33–8.