

Research Article

The Heritability of Mammographic Breast Density and Circulating Sex-Hormone Levels: Two Independent Breast Cancer Risk Factors

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Abstract

Background: Mammographic breast density and endogenous sex-hormone levels are both strong risk factors for breast cancer. This study investigated whether there is evidence for a shared genetic basis between these risk factors.

Methods: Using data on 1,286 women from 617 families, we estimated the heritabilities of serum estradiol, testosterone, and sex-hormone binding globulin (SHBG) levels and of three measures of breast density (dense area, nondense area, and percentage density). We tested for associations between hormone levels and density measures and estimated the genetic and environmental correlations between pairs of traits using variance and covariance components models and pedigree-based maximum likelihood methods.

Results: We found no significant associations between estradiol, testosterone, or SHBG levels and any of the three density measures, after adjusting for body mass index (BMI).

The estimated heritabilities were 63%, 66%, and 65% for square root-transformed adjusted percentage density, dense area, and nondense area, respectively, and 40%, 25%, and 58% for log-transformed-adjusted estradiol, testosterone, and SHBG. We found no evidence of a shared genetic basis between any hormone levels and any measure of density, after adjusting for BMI. The negative genetic correlation between dense and nondense areas remained significant even after adjustment for BMI and other covariates ($\rho = -0.34$; SE = 0.08; $P = 0.0005$).

Conclusions: Breast density and sex hormones can be considered as independent sets of traits.

Impact: Breast density and sex hormones can be used as intermediate phenotypes in the search for breast cancer susceptibility loci. *Cancer Epidemiol Biomarkers Prev*; 21(12); 2167–75. ©2012 AACR.

Introduction

Higher circulating estradiol (E2) and testosterone (T) levels are known to increase the risk of postmenopausal breast cancer, whereas risk is inversely related to the levels of sex-hormone binding globulin (SHBG), which binds estradiol and testosterone with high affinity, effectively limiting their bioavailability (1–3). It has

been hypothesized that the hormone levels affect risk mainly through their proliferative effects on mammary tissue (2, 4). This proliferative activity may lead to not only greater areas of fibroglandular tissue at risk of developing cancer but also to a higher risk of mutations as higher mitotic rates increase susceptibility to mutations (5). Fibroglandular tissues within breasts are radiodense and appear lighter than fat on a mammogram, and this dense area is referred to as mammographic density. Women with dense tissue in more than 75% of their breasts are known to be at 4- to 6-fold increased risk of breast cancer relative to women with less than 10% dense tissue, after taking into account other known risk factors for the disease (6). Most studies to date have focused on percentage mammographic density, but there is evidence that the absolute dense area may be more relevant for breast cancer risk (7), whereas the role of the nondense area remains unclear (8).

The mechanism behind this association is not known, however, several hormonal breast cancer risk factors are known to influence mammographic density (9–11). This raises the possibility that some of the association between

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levels of circulating hormones and breast cancer risk may be mediated through an increase in mammographically dense area.

Circulating levels of sex hormones are thought to have an inherited component, but there have been few attempts to estimate the heritability in females. A study of postmenopausal twins estimated heritabilities of 0.14, 0.39, and 0.56 for estradiol, testosterone, and SHBG levels, respectively, whereas a study of postmenopausal women from the Framlingham Heart Study found an identical heritability for SHBG but a slightly lower estimate for testosterone levels (0.26; refs. 12, 13).

Twin studies estimate that between 50% and 70% of the variance of percentage mammographic density is genetically determined (14–17). A recent study used a polygenic risk score approach to show that percentage mammographic density and breast cancer have a shared genetic basis that is mediated through a large number of common variants (18). In addition, common genetic variants within the *ZNF365*, *LSP1*, and *RAD51L1* genes, which were initially identified as being associated with breast cancer risk have been subsequently shown to be also associated with breast density, in the direction consistent with their effect on risk (19, 20).

Given that mammographic density and endogenous sex-hormone levels are both strong risk factors for breast cancer and may each share a common genetic basis with breast cancer, we wished to examine the association between these 2 traits and any evidence for a shared genetic basis.

Materials and Methods

Study population

SIBS. The Sisters in Breast Screening Study (SIBS) was designed to study the genetic basis of quantitative phenotypes related to breast cancer, in particular breast density and sex-hormone levels (21). Women who attended the local Cambridge and Huntingdon Breast Screening Services to have mammographic examinations under the National Health Service (NHS) Breast Screening Program were identified and invited to participate in the study if they had 1 or more female blood relatives (sisters, half-sisters, first cousins, aunts, or nieces) who had also undergone mammographic screening. Women with relatives who could have screening within 2 years were also included. Families were also ascertained through newspaper and radio advertisements.

A letter, blood kit, and questionnaire covering information on family relationships, reproductive and hormonal factors, lifestyle factors, oral contraceptive and hormone replacement therapy (HRT) use, and medical history, such as cancer and benign breast disease, were sent to the participants. Twin status of participants was collected by the study coordinator and verified by comparing genome-wide genetic data. Anthropometric measurements and blood samples were taken at the general practices by trained staff (21). The study was approved by the Eastern Multicentre Research Ethics Committee.

Recruitment began in 2000; the analyses reported here included women who had enrolled in the study up to May 2010.

Mammographic density estimation

Mammograms were undertaken as part of the UK NHS Breast Screening Program in which women, ages 50 to 64 years, are screened every 3 years by a 2-view (mediolateral and craniocaudal) mammogram. All available mammograms for each participant were retrieved from the local screening service and digitized using the Array 2905 Laser Film Digitizer and the program DICOM ScanPro Plus Version 1.3E (Array Corp), with 50- μ m pixel resolution and 12-bit digitization, and an absorbance of 4.7. Density readings were conducted using a computer-assisted program, CUMULUS (22). The dense area, the nondense area, and hence the percentage density (dense area expressed as a percentage of the total breast area) were estimated for each film as continuous variables. Mediolateral views of both breasts were used as they were more widely available and breast density measured by the mediolateral and craniocaudal views are known to be highly correlated. All films were assessed by a single trained reader (J. Brown). Mammograms were analyzed in a random order. All readings were made independently for the right and the left breast. Ten percent of the films were rescored by the same reader for a different study and the intrareader repeatability was 0.84. The interreader reproducibility was examined in the earlier study by comparing 10% of the readings with those of a radiologist (R.M.L. Warren) was 0.88 (21).

Hormone measurements

SIBS women who were more than 55 years of age, were self-reported as being postmenopausal for at least 2 years and not on HRT at the time of blood sampling were selected for sex-hormone measurements. Serum estradiol concentrations were measured using an in-house RIA using a highly specific rabbit antiserum, which had been raised against an estradiol-6-carboxymethylxime-bovine serum albumin conjugate and estradiol-6-carboxymethylxime-[2-¹²⁵I] iodohistamine (23). SHBG was measured using Immulite chemiluminescent immunoassay (Seimens Healthcare Diagnostic), and serum testosterone levels were measured using a Coat-a-Count Solid Phase RIA Kit (TKTT2 Seimens Healthcare Diagnostic). The use of both methods has been described previously (24, 25).

For estradiol at a concentration of 25 pmol/L, the within assay variation was 6.5% and the between assay variation was 16% ($n = 18$). For testosterone, at a concentration of 2.5 nmol/L, the within assay variation was 7% and the between assay variation was 16% ($n = 28$). For SHBG at a concentration of 50 nmol/L, the within assay variation was 5.8% and the between assay variation was 6.6% ($n = 7$).

Two women with extreme estradiol levels (>1,000 pmol/L) were excluded from the estradiol analyses.

Twenty women (2.7%) with estradiol levels below the level of detection (<3.0 pmol/L) and 33 women (4.4%) with testosterone levels below detection levels (<0.14 nmol/L) were assigned the value of the minimum level of detection.

Statistical analysis

Pedigree information was available for 617 families, comprising 1,286 SIBS participants and 2,402 imputed relatives. The relatives were only included to provide the correct pedigree structures for the heritability estimations; no phenotype information was available for them. The 1,286 SIBS women were genotyped using the Illumina HumanCytoSNP-12 platform as part of a genome-wide association study (26). Single-nucleotide polymorphism (SNP) assays with call rates less than 95% [or <99% for minor allele frequency (MAF) 1%–5%], SNPs with a MAF less than 1%, or Hardy–Weinberg equilibrium $P < 10^{-4}$ were removed for a total of 255,051 genotyped SNPs. Duplicate concordance was more than 99.9% ($n = 10$). All samples had a call rate more than 99% and heterozygosity 30% to 38%.

A subset of 8,236 uncorrelated SNPs ($r^2 < 0.1$) were used to construct a kinship coefficient matrix (GenABEL software; ref. 27), which was used to verify the reported relationships between the 1,286 SIBS women. Where necessary, the pedigree was corrected on the basis of the genetic data, in consultation with the study coordinator. Ten individuals from 4 families were excluded as having less than 85% estimated European ancestry, and 8 individuals from 3 families were excluded where the relationships could not be clarified. After exclusions, 1,268 women from 614 families with genome-wide genetic data were included in the analyses, of which 810 women had hormone data, 1,140 women had breast density data, and 682 women had both density and hormone measurements.

Multiple linear regression was used to investigate the association between mammographic density and sex-hormone levels. Density readings from the mammogram closest to the time of blood sampling were taken and the mean of measures between the right and left breast readings was used (unless only 1 side was available, in which case the readings from that side were used). The hormone measures were log-transformed and the density measures were square root-transformed to better approximate to a normal distribution.

In the regression model, each density measure (percentage dense, dense area, and nondense area) was taken as the outcome variable. Estradiol, testosterone, and SHBG levels were the exposure variables. The final multivariate model included age at mammogram, time between mammogram and blood sampling, ages at menarche, menopause, parity and first pregnancy, body mass index (BMI), waist-to-hip ratio (WHR), and under bust measurements (cm). HRT use was categorized into "never" and "former" users. Analyses were done using STATA 10.0 software package (Stata Corp.).

Heritability estimation

Mammograms of both breasts taken at 2 time points were available for most women, and therefore each woman included in the heritability estimation had up to 4 readings of density measures. Square root-transformed density measures were adjusted for age at each mammogram. All other covariates were obtained at only 1 time point. The mean of residuals generated through multivariate regression was used to assign an average adjusted density value for each woman.

Stepwise linear regression was carried out to determine the best model for each of the hormones. Log-transformed estradiol levels were adjusted for age at blood draw, BMI, previous HRT use, and batch. Log-transformed SHBG levels were adjusted for the above variables together with WHR. Log-transformed testosterone levels were additionally adjusted for previous HRT use, bilateral oophorectomy, and age at menopause.

To estimate the heritability of each trait and to estimate the genetic and nongenetic correlations between pairs of traits, we use variance and covariance components models and pedigree-based maximum likelihood methods as implemented in SOLAR (28, 29).

Each phenotype was modeled as a linear function of the form:

$$P_i = \mu + \sum_{j=1}^M \beta_j v_{ij} + g_i + e_i$$

in which P_i is the phenotype in an individual i , μ is the mean of the trait, β_j the regression coefficient of the j th of M covariates with value v_{ij} in the i th individual, g_i and e_i represent deviations from μ that are due to additive genetic effects and unmeasured environmental effects, respectively. The effects of g_i and e_i were assumed to be uncorrelated with each other and normally distributed with a mean of 0 and variances σ_g^2 and σ_e^2 , respectively (28).

Given this model, the covariance between any 2 relatives (Ω) can be expressed as a function of the additive genetic variance and random environmental variance. To include the extended pedigree structures of families within SIBS, each of the variances was multiplied by a structuring matrix within SOLAR. The structuring matrix for the additive genetic variance is 2 times the matrix of kinship coefficients (ϕ), whereas the structuring matrix for the environmental variance is an identity matrix (I), under the assumption of no correlation in the environmental component among individuals. Thus,

$$\Omega = 2\phi\sigma_g^2 + I\sigma_e^2.$$

Once the expected mean and covariance matrix for each pedigree were defined, the likelihood of a pedigree was evaluated using the multivariate normal density function and summed over all pedigrees to obtain multiple likelihood estimates (MLE) of σ_e and σ_g (28), and hence the heritability (h^2) of each trait was estimated by calculating the ratio of the variance attributed to the additive genetics

effects, σ_g^2 , and the phenotypic variance, $\sigma_g^2 + \sigma_e^2$. The P values for the heritability calculations were obtained through standard likelihood ratio tests.

Bivariate variance components model

To evaluate evidence for common genetic effects influencing 2 phenotypes, for example percentage density and estradiol levels, bivariate variance components models were used (30). The phenotypic correlations between 2 traits were divided into the component explained by shared additive genetic effects (ρ_G) and the component explained by shared environmental (nongenetic) effects (ρ_E). On the basis of the heritabilities (h_1^2 and h_2^2) calculated for the 2 traits, the phenotypic correlation can be expressed as the weighted sum of their genetic and environmental correlations (30);

$$\rho_P = \rho_G [(h_1^2 h_2^2)]^{1/2} + \rho_E [(1 - h_1^2)(1 - h_2^2)]^{1/2}.$$

The genetic correlations (ρ_G) captures the extent to which the same genetic factors influence both traits, and the environmental correlations (ρ_E) captures the extent to which the same environmental factors influence both traits. Two hypotheses were tested using likelihood ratio tests. The first hypothesis was whether the genetic correlation between the 2 traits was 0 ($\rho_G = 0$); rejection of this hypothesis would suggest the existence of 1 or more genetic factors that influence both traits. The second hypothesis tested whether the environmental correlation between the 2 traits was 0 ($\rho_E = 0$); rejection of this hypothesis would suggest that 1 or more unmeasured or unadjusted common environmental factors influence both traits. The statistical tests for the association between breast density and sex-hormone levels were 2-sided, whereas the tests for heritability were necessarily 1-sided.

Results

The mean age at mammogram of the 1,286 women included in the analyses was 61.7 years (range, 50–78 years) with an average BMI of 27 kg/m² (Table 1). The majority of women were parous (89.5%) with an average of 2 live births. The mean percentage density was 15.0% \pm 11.9%. The geometric mean levels (\pm SE) of estradiol, testosterone, and SHBG levels in the cohort were 14.3 \pm 2.2 pmol/L, 0.66 \pm 1.8 nmol/L, and 49.4 \pm 1.6 nmol/L, respectively (Table 1). Most of the relative pairs in the study were sisters, with cousins being the next most common relationship (Table 2). None of the women included in the estimation of heritability of hormone measures were using HRT at the time of sampling. Eleven women included in the heritability estimation of density measures reported using HRT.

Square root-transformed dense area and percentage density were negatively associated with age at mammogram and BMI and positively associated with ages at first pregnancy and menarche, whereas the nondense area was positively associated with age and BMI (all $P < 0.05$;

Table 1. Characteristics of SIBS women that were included in this study ($n = 1,286$)

| Characteristic | Mean | SD |
|---|-------------|-------|
| Age, y | 61.7 | 5.4 |
| Interval between the mammogram examination and blood sampling, mo | 7 | 7 |
| Percentage density, % | 15.0 | 11.9 |
| Dense area, cm ² | 67.0 | 58.8 |
| Nondense area, cm ² | 171.8 | 115.2 |
| Estradiol, pmol/L ^a | 14.3 | 2.2 |
| Testosterone, nmol/L ^a | 0.66 | 1.8 |
| SHBG, nmol/L ^a | 49.4 | 1.6 |
| BMI, kg/m ² | 27.1 | 5.2 |
| WHR | 0.8 | 0.06 |
| Chest circumference under the bust, cm | 34.1 | 3.7 |
| Age at menarche, y | 12.8 | 1.6 |
| Age at menopause, y | 48.7 | 6.7 |
| Age at first child birth, y | 23.6 | 4.5 |
| Parity | 2.1 | 1.1 |
| HRT use | | |
| Never users | 653 (50.8%) | |
| Past users | 621 (48.3%) | |
| Current users ^b | 11 (0.01%) | |

^aGeometric mean values.

^bIncluded only in the calculations for density measures.

Supplementary Table S1). Testosterone levels were positively associated with BMI, whereas estradiol levels were positively associated with BMI and negatively associated with age. SHBG levels were negatively associated with BMI and positively associated with age ($P < 0.05$; Supplementary Table S2).

Association between mammographic density measures and hormone measurements

Levels of estradiol and testosterone adjusted for age were negatively associated with percentage density ($P < 0.001$ for both) and positively associated with nondense area ($P < 0.001$ for both; Table 3). A negative association was seen between estradiol level and absolute dense area ($P = 0.001$) and a borderline negative association was seen with testosterone levels ($P = 0.03$). Age-adjusted SHBG levels were positively associated with percentage density and negatively associated with the nondense area of the breasts ($P < 0.001$ for both). After additional adjustment for BMI, none of these associations remained statistically significant, allowing for multiple testing. The inclusion of WHR, chest circumference, age at menarche and menopause, parity, HRT use (never/past users), and age at first live birth, made little difference to the results (data not shown).

Table 2. Numbers of relative pairs by degree of relationship among the 1,286 SIBS women included in heritability analyses

| Relationship pairs | | Number of pairs |
|--------------------|----------------------|-----------------|
| First degree | Sisters | 644 |
| | Mother and daughters | 23 |
| Second degree | Half-sisters | 9 |
| | Aunts and nieces | 25 |
| Third degree | Cousins | 185 |

Heritability estimation. After adjusting for age at mammographic examination and menopausal status, the heritability estimates for dense area, percentage density, and the nondense area of the breasts were 68%, 70%, and 77%, respectively (Table 4). On further adjustment for BMI, WHR, HRT use, age at menarche, and age at menopause, the heritability estimates reduced to 66%, 63%, and 65% for the 3 measures respectively.

Heritability estimates for hormone levels adjusted for age at blood draw and batch were 52%, 59%, and 27% for estradiol, SHBG, and testosterone levels, respectively. On further adjustment for BMI, WHR, HRT use, bilateral oophorectomy, and age at menopause, the heritability estimates were 40%, 58%, and 25%, respectively (Table 4).

Genetic and environmental correlations between density measures and circulating sex-hormone levels

Estimates of genetic (ρ_G) and environmental (ρ_E) correlations between each density measure and each sex-hormone level are given in Table 5. After adjustment for age and menopausal status, nondense area was positively genetically correlated with age-adjusted testosterone levels ($P = 0.001$ for test of $\rho_G = 0$). This positive association was seen to drive the negative genetic association between percentage density and

testosterone levels (as the nondense area forms part of the denominator of percentage density). The results were similar for estradiol levels. The nondense area and SHBG levels were negatively genetically correlated with each other, whereas percentage density and SHBG levels were positively genetically correlated ($P = 0.03$ for test of $\rho_G = 0$ for both traits; Table 5).

After multivariate adjustment for BMI, WHR, and other risk factors, there was no significant evidence of shared genetic influences between hormone and density measures ($H_0: \rho_G = 0$), with the exception of a borderline association between nondense area and testosterone ($P = 0.04$). There was no evidence of shared environmental factors in any of the analyses ($H_0: \rho_E = 0$; Table 5).

Genetic and environmental correlations among density measures, sex-hormone levels and body mass index

To explore this further, we tested for genetic and/or environmental sharing between BMI and each trait of interest, adjusting for age and menopausal status. The results provide strong evidence of common genetic factors influencing the nondense area and BMI in the same direction ($P = 6.6 \times 10^{-14}$), and hence affecting percentage density and BMI in opposite directions ($P = 5.2 \times 10^{-7}$). There was, however, no evidence of a shared genetic component to BMI and dense area. Estradiol was positively genetically correlated with BMI, whereas SHBG levels showed inverse genetic correlation; testosterone was not genetically correlated with BMI (Table 6).

The dense and nondense areas of breasts adjusted for age at mammogram and menopausal status showed significant negative genetic correlations ($P = 8.0 \times 10^{-5}$; Table 7), suggesting the presence of common genetic factors exerting opposite effects on the dense and nondense areas of the breasts. Adjusting for BMI made very little difference to the results. There was no evidence of shared environmental factors between dense and nondense areas ($P = 0.16$; Table 7).

Table 3. Association between mammographic density measures and serum sex-hormone levels

| | Covariates in model | Percentage density | | | Absolute dense area | | | Nondense area | | |
|---------------------------------|---------------------|--------------------|------|---------|---------------------|------|---------|---------------|------|---------|
| | | β | SE | P value | β | SE | P value | β | SE | P value |
| Serum estradiol (n = 671) | Age and batch | -0.50 | 0.08 | <0.001 | -57.9 | 17.8 | 0.001 | 228.6 | 28.7 | <0.001 |
| | +BMI ^a | -0.15 | 0.08 | 0.047 | -39.3 | 19.9 | 0.049 | 15.4 | 20.8 | 0.46 |
| Serum testosterone (n = 682) | Age and batch | -0.32 | 0.09 | <0.001 | -49.2 | 22.2 | 0.03 | 125.2 | 31.7 | <0.001 |
| | +BMI ^a | -0.16 | 0.08 | 0.064 | -38.6 | 22.4 | 0.09 | 31.4 | 22.8 | 0.17 |
| Serum SHBG (n = 682) | Age and batch | 0.84 | 0.14 | <0.001 | 56.2 | 32.3 | 0.08 | -435.9 | 45.9 | <0.001 |
| | +BMI ^a | 0.19 | 0.15 | 0.19 | 7.82 | 36.7 | 0.83 | -68.1 | 41.8 | 0.10 |

^aBMI was added to the model that adjusts for age and batch.

Table 4. Heritability estimates (h^2) for breast density measures ($n = 1,140$) and serum sex-hormone levels ($n = 810$)

| Trait | Age and menopausal status adjusted | Multivariate adjusted |
|---------------------|------------------------------------|-----------------------|
| | $h^2 \pm SE$ | $h^2 \pm SE$ |
| Density measures | | |
| Percentage density | 0.70 \pm 0.08 | 0.63 \pm 0.08 |
| Absolute dense area | 0.68 \pm 0.08 | 0.66 \pm 0.08 |
| Nondense area | 0.77 \pm 0.06 | 0.65 \pm 0.07 |
| Hormone levels | | |
| Estradiol | 0.52 \pm 0.10 | 0.40 \pm 0.10 |
| Testosterone | 0.27 \pm 0.10 | 0.25 \pm 0.10 |
| SHBG | 0.59 \pm 0.09 | 0.58 \pm 0.10 |

Discussion

Mammographic density is known to respond to changes in a woman's hormonal milieu. It has been hypothesized that some of the increased risk of breast cancer caused by endogenous hormones through cell proliferation may be reflected as a change in mammographic density (5).

In our cross-sectional study of 682 postmenopausal women, we found no strong associations between estradiol, testosterone, and SHBG levels and any of the 3 density measures, after adjusting for adiposity (BMI), in line with most, but not all, of the previous studies in this area (31–38). The majority of the previous studies were relatively small in size ($n < 300$) and/or considered only percentage density, rather than also looking at the absolute dense and nondense areas. A study of 722 postmenopausal Norwegian women found significant positive correlations between SHBG levels and both percentage and absolute density even after adjusting for BMI (39), but may not have completely corrected for adiposity. Our results support those from the Nurses Health Study, which found circulating levels of estradiol and testosterone to be strongly associated with breast cancer risk independent of percentage mammographic density, and *vice versa* (40). This absence of association is perhaps surprising given the known association between HRT use and density. It may be that the levels of endogenous sex hormones in postmenopausal women are too low to cause a detectable effect on mammographic density, or that the variability in the levels and/or densities are too small to provide sufficient statistical power. It is known that there is a much stronger relationship between combined estrogen and progesterone HRT and breast density than there is with just estrogen-HRT and breast density (41–43). The very low progesterone levels in postmenopausal women may be why these analyses found little relationship between estradiol levels and mammographic density. The mean age at mammogram in our study was 61.7 years, thus we cannot rule out the possibility that significant

associations may be evident in a cohort of younger women. Indeed, it may be that mammographic density is determined more by long-term hormonal exposure and is less affected by variations in postmenopausal hormones.

All 6 variables seem to have substantial inherited components; the estimated heritabilities were 63%, 66%, and 65% for square root-transformed adjusted percentage-density, dense area, and nondense area, respectively, and 40%, 25%, and 58% for log-transformed-adjusted estradiol, testosterone, and SHBG, respectively. It is interesting to note that the density heritabilities are almost identical to those from a large North American/Australian twin study, despite the different study designs and the different statistical methodologies used (14, 15). Our estimated heritabilities are a little lower than those from a Korean study of twins and first-degree relatives (16) but higher than those from a Californian twin study, especially for nondense area (31%; ref. 17). However, the results from a sibling study among women belonging to the Old Amish population in Pennsylvania (30) were somewhat different, with heritability estimates of 35% for log percentage density, 39% for log dense area, and 71% for 0.3 power-transformed nondense area.

Our estimated heritability for log SHBG levels was very similar to those estimated in a twin study from Australia (0.56; ref. 13) and in families from the Framingham Heart Study (also 0.56; ref. 12). Our heritability for log testosterone was similar to the Framingham study (0.25) but a little lower than the twin study estimate (0.39 for square root-transformed testosterone), whereas our heritability for log estradiol levels (0.40) was somewhat higher than in the twin study (0.14).

Heritability estimates are population specific, as variations in additive and nonadditive genetic variances as well as environmental variances can vary between populations (44). For example, the specific reproductive norms within the Amish population meant that parity was higher than in most Western populations, which could affect the total variance of density measures such that the proportion of variance explained by additive genetic factors may be lower (30). In our study, healthy women recruited through a population-based screening program give heritability estimates that are more generalizable to a Western population.

This is the first study to look for a shared genetic basis between breast density and sex-hormone levels. The apparent genetic associations for testosterone and SHBG levels with nondense area (and hence percentage density) were attenuated and became nonsignificant after adjusting for BMI. The role of BMI as a confounder was confirmed by identifying a positive genetic correlation between BMI and nondense area (and hence a negative correlation with percentage density) and genetic correlations between BMI and estradiol (positive) and SHBG (negative). However, we did identify a significant negative genetic correlation between dense and nondense areas, which remained significant even after adjustment

Table 5. Genetic and environmental correlations (ρ_G and ρ_E , respectively) between breast density measures and endogenous sex-hormone levels, $n = 1,268$

| Trait 1 (breast density) | Trait 2 (sex hormones) | ρ_P^a | $\rho_G \pm SE$ | $\rho_G = 0^b$ | | $\rho_E = 0$ | |
|------------------------------------|---------------------------|------------|------------------|----------------|-------------------|----------------|----------------|
| | | | | <i>P</i> value | $\rho_E \pm SE$ | <i>P</i> value | <i>P</i> value |
| Age and menopausal status adjusted | | | | | | | |
| Percentage density | Estradiol | -0.27 | -0.24 \pm 0.13 | 0.09 | -0.33 \pm 0.17 | 0.08 | |
| | Testosterone | -0.15 | -0.41 \pm 0.18 | 0.03 | 0.07 \pm 0.16 | 0.68 | |
| | SHBG | 0.27 | 0.28 \pm 0.12 | 0.03 | 0.26 \pm 0.18 | 0.18 | |
| Dense area | Estradiol | -0.14 | -0.05 \pm 0.15 | 0.75 | -0.27 \pm 0.18 | 0.15 | |
| | Testosterone | -0.10 | -0.16 \pm 0.20 | 0.44 | -0.07 \pm 0.15 | 0.66 | |
| | SHBG | 0.08 | 0.04 \pm 0.13 | 0.75 | 0.15 \pm 0.19 | 0.43 | |
| Nondense area | Estradiol | 0.34 | 0.49 \pm 0.11 | 0.0001 | 0.12 \pm 0.18 | 0.52 | |
| | Testosterone | 0.16 | 0.55 \pm 0.16 | 0.001 | -0.24 \pm 0.18 | 0.16 | |
| | SHBG | -0.38 | -0.42 \pm 0.09 | 0.0002 | -0.31 \pm 0.17 | 0.09 | |
| Multivariate adjusted | | | | | | | |
| Percentage density ^c | Estradiol ^{d,e} | -0.06 | -0.06 \pm 0.17 | 0.75 | -0.07 \pm 0.17 | 0.66 | |
| | Testosterone ^f | -0.06 | -0.30 \pm 0.21 | 0.15 | 0.11 \pm 0.15 | 0.45 | |
| | SHBG ^g | 0.05 | 0.01 \pm 0.14 | 0.93 | 0.11 \pm 0.20 | 0.57 | |
| Dense area ^c | Estradiol ^{d,e} | -0.07 | -0.02 \pm 0.17 | 0.91 | -0.12 \pm 0.17 | 0.48 | |
| | Testosterone ^f | -0.06 | -0.14 \pm 0.21 | 0.52 | -0.006 \pm 0.15 | 0.97 | |
| | SHBG ^g | 0.01 | -0.12 \pm 0.14 | 0.41 | 0.22 \pm 0.20 | 0.27 | |
| Nondense area ^c | Estradiol ^{d,e} | 0.02 | 0.17 \pm 0.17 | 0.30 | -0.14 \pm 0.16 | 0.38 | |
| | Testosterone ^f | 0.05 | 0.40 \pm 0.20 | 0.04 | -0.22 \pm 0.15 | 0.12 | |
| | SHBG ^g | -0.06 | -0.11 \pm 0.13 | 0.43 | 0.02 \pm 0.19 | 0.90 | |

NOTE: Values significant with a *P* value less than 0.05 have been highlighted in bold.^aPhenotypic correlation estimate.^b*P* value for test of null hypothesis that correlation due to additive genetic factors is 0 ($\rho_G = 0$).^cDensity measures were square root-transformed and adjusted for age, previous HRT use, BMI, WHR, age at first birth, menarche, and menopause.^dEstradiol levels were log-transformed and adjusted for age at blood draw, BMI, HRT use, and batch.^eThe sample size for estradiol levels and density measures correlation estimation was 1,284.^fTestosterone levels were log-transformed and adjusted for age at blood draw, BMI, HRT use, bilateral oophorectomy, age at menopause, and batch.^gSHBG levels were log-transformed and adjusted for age at blood draw, BMI, WHR, HRT use, and batch.

for BMI and other covariates ($\rho = -0.34$; $P = 0.0005$). This correlation is close to the estimates reported by Stone and colleagues (15) and Sung and colleagues (16) in their twin studies ($\rho = -0.30$, and -0.25 , respectively), pointing to

the existence of genetic loci with opposing effects on dense and nondense tissue. We did not find any such correlation between the nongenetic determinants of the 2 traits ($P = 0.13$), making it unlikely that the observed genetic

Table 6. Genetic and environmental correlations (ρ_G and ρ_E , respectively) between breast density measures, sex-hormone levels and BMI, $n = 1,268$

| Trait 1 | Trait 2 | ρ_P | $\rho_G \pm SE$ | <i>P</i> value ($\rho_G = 0$) | $\rho_E \pm SE$ | <i>P</i> value ($\rho_E = 0$) |
|---------------------|---------|----------|------------------|---|------------------|---------------------------------|
| Density measures | | | | | | |
| Percentage density | BMI | -0.46 | -0.51 \pm 0.07 | 5.2×10^{-7} | -0.33 \pm 0.16 | 0.09 |
| Dense area | BMI | -0.17 | -0.18 \pm 0.09 | 0.08 | -0.17 \pm 0.18 | 0.35 |
| Nondense area | BMI | 0.69 | 0.75 \pm 0.04 | 6.6×10^{-14} | 0.53 \pm 0.13 | 0.003 |
| Hormone measurement | | | | | | |
| Estradiol | BMI | 0.46 | 0.57 \pm 0.10 | 1.3×10^{-5} | 0.33 \pm 0.15 | 0.08 |
| Testosterone | BMI | 0.14 | 0.15 \pm 0.17 | 0.37 | 0.16 \pm 0.14 | 0.27 |
| SHBG | BMI | -0.45 | -0.41 \pm 0.09 | 3.0×10^{-4} | -0.53 \pm 0.12 | 0.002 |

Table 7. Genetic (ρ_G) and environmental (ρ_E) correlations between absolute dense area and nondense area of breasts, $n = 1,140$

| Breast density | | ρ_P^a | $\rho_G \pm SE$ | P value ($\rho_G = 0$) ^b | $\rho_E \pm SE$ | P value ($\rho_E = 0$) |
|---|--|------------|------------------|--|------------------|--------------------------|
| Trait 1 | Trait 2 | | | | | |
| Dense area ^c | Nondense area ^c | -0.33 | -0.37 \pm 0.07 | 8.0 $\times 10^{-5}$ | -0.24 \pm 0.16 | 0.16 |
| Dense area + BMI ^d | Nondense area + BMI ^d | -0.29 | -0.32 \pm 0.08 | 0.001 | -0.23 \pm 0.13 | 0.10 |
| Multivariate adjusted dense area ^e | Multivariate adjusted nondense area ^e | -0.29 | -0.34 \pm 0.08 | 5.0 $\times 10^{-4}$ | -0.21 \pm 0.13 | 0.13 |

^aPhenotypic correlation between absolute dense area and the nondense area.

^bP value for test of null hypothesis of no genetic correlations ($\rho_G = 0$).

^cSquare root-transformed dense and nondense area adjusted for age at mammogram and menopausal status alone.

^dSquare root-transformed dense and nondense areas adjusted for age at mammogram, menopausal status and BMI.

^eSquare root-transformed dense and nondense areas were adjusted for age at mammogram, HRT use, BMI, WHR, age at first birth, age at menarche, and menopause.

correlation is an artifact of the way dense and nondense areas are measured.

A unique strength of this study is the ability to verify family relations reported in questionnaires using kinship coefficients calculated using genome-wide SNP data. This led to several corrections and exclusions of individuals from the study. Correlations among parents and siblings can be affected by common environmental influences, which could lead to heritability estimates being overestimated (13). The inclusion of more distant relatives may help, as their correlations are less likely to be biased by nongenetic factors, however, heritability estimates based on distant relatives alone would have high sampling errors. Families with extended pedigrees were included in the present study using a statistical method that was designed to use information from such a structure (28).

Conclusions

In this population-based study of families with extended pedigrees, high heritability estimates obtained from twin studies for density measures were confirmed. The dense and the nondense areas of the breasts were shown to be influenced partly by the same genetic factors but in opposite directions, whereas BMI and the nondense breast area were positively genetically correlated. High heritability estimates were also found for estradiol and SHBG. After adjustment for BMI, there was no evidence for a shared genetic basis between the density measures and sex hormones included in this study, hence the established relationships between endogenous sex-hor-

mone levels and breast cancer risk do not seem to be explained by variations in breast density in this age group. Breast density and sex hormones can thus be considered as independent sets of traits, each of which can be used as intermediate phenotypes in the search for breast cancer susceptibility loci.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Leyland, T. Audley, R.M.L. Warren, D.F. Easton
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The Heritability of Mammographic Breast Density and Circulating Sex-Hormone Levels: Two Independent Breast Cancer Risk Factors

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