Mammographic Breast Density—Evidence for Genetic Correlations with Established Breast Cancer Risk Factors

Julie A. Douglas,[1] Marie-Hélène Roy-Gagnon,[1] Chuan Zhou,[2] Braxton D. Mitchell,[3] Alan R. Shuldiner,[3] Heang-Ping Chan,[2] and Mark A. Helvie[2]

[1]Departments of Human Genetics and [2]Radiology, University of Michigan Medical School, Ann Arbor, Michigan; and [3]Department of Medicine, University of Maryland Medical School, Baltimore, Maryland

Abstract

Previous twin and family studies indicate that the familial aggregation of breast density is due (in part) to genetic factors. Whether these genetic influences are shared with other breast cancer risk factors, however, is not known. Using standard film-screen mammography, we screened 550 women, including 611 pairs of sisters, from the Old Order Amish population of Lancaster County, Pennsylvania. We digitized mammograms and quantified the dense and nondense areas of the breast using a computer-assisted method. Information about other breast cancer risk factors was collected via questionnaires and a physical exam. Using pedigree-based variance component methods, we estimated the genetic contributions to several breast cancer risk factors, including breast density, and evaluated the evidence for shared genetic influences between them. After adjusting for covariates, genetic effects accounted for >33% of the total variance of each risk factor ($P < 0.001$), including breast density, and the dense and nondense areas of the breast were significantly genetically correlated with parity [genetic correlation (ρ_G) = -0.47; $P = 0.013$] and age at menarche (ρ_G = -0.38; $P = 0.008$), respectively. The nondense area of the breast and, in turn, breast density, expressed as a ratio of dense area to total area, were also genetically correlated with most measures of adiposity but in opposite directions ($\rho_G \geq 0.75$; $P < 10^{-7}$ for nondense area). We conclude that the genetic components that influence breast density are not independent of the genetic components that influence other breast cancer risk factors. This shared genetic architecture should be considered in future genetic studies of breast density. (Cancer Epidemiol Biomarkers Prev 2008;17(12):3509–16)

Introduction

Breast cancer is the most common cause of cancer-related mortality in women worldwide (1). Among breast cancer risk factors, increased breast density, as measured from a mammogram, is one of the strongest but perhaps least understood (2). Mammographic breast density refers to the radiographic dense areas on a mammogram and is a measure of the amount of fibroglandular tissue in the breast. Studies have repeatedly shown that women with dense tissue in >75% of the breast are at a 4- to 6-fold increased risk of developing breast cancer compared with women with little to no breast density (3, 4). Some studies also suggest that breast cancer risk is directly associated with (4-7) and may be even better predicted by (8) the absolute amount of dense tissue. At present, however, the most commonly used quantitative measure of breast density is the ratio of dense area to total area. While breast density (measured as a ratio) may be a useful prognostic indicator of breast cancer risk, there are several undesirable consequences of using it in the context of etiologic research (9). For example, ratios can be difficult to interpret because of the potential confounding due to the nondense component of the denominator, which reflects the amount of fat in the breast. Still, only a few studies have separately measured and analyzed the dense and nondense components, and even fewer have compared the inferences made from absolute versus relative measures of breast density (10, 11).

Twin and family studies have established evidence for a significant genetic influence on breast density. For example, in a study on 571 monozygotic and 380 dizygotic twin pairs from the United States and Australia, unmeasured genes accounted for >60% of the variation in percent (12) [and absolute (13)] breast density after adjustment for age and other covariates. Although the mode of inheritance of breast density is likely to be complex, Vachon and colleagues (14) previously implicated the transmission of a major gene for percent breast density in a study of 1,370 women from 258 multigenerational breast cancer families. In a subsequent genomewide scan based on 583 women from 89 of these families, Vachon et al. (15) also recently reported significant evidence of linkage for percent breast density on chromosome 5p. Although ~45 candidate genes are located within the 1-LOD (log of odds) support interval surrounding their chromosome

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Requests for reprints: Julie A. Douglas, Room 5912, Buhl Building, 1241 E. Catherine Street, Ann Arbor, MI 48109-5618. Phone: 734-615-2616; Fax: 734-763-2784. E-mail: jddoug@umich.edu

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5p peak, to our knowledge, none have been tested for association with breast density. Indeed, candidate gene studies of breast density are still in their infancy, with a relatively small number of genes examined and few, if any, clear associations.

In addition to having a documented genetic component, breast density is known to vary with age, reproductive and menstrual history, and measures of body size. Studies have consistently shown that breast density is inversely associated with age and, among women of the same age, is lower in those who are parous, have had a larger number of live births, or are postmenopausal (16). As a ratio, breast density is also inversely associated with several measures of body size, including body mass index (BMI) and weight (16). Finally, observational studies and clinical trials indicate that breast density is higher in women who are using combination (estrogen plus progestin) hormone replacement therapy (17, 18). Many of these breast density–associated traits are also well-recognized breast cancer risk factors, with several exhibiting associations in the same direction as their effects on breast density. For example, nulliparity and hormone replacement therapy use are associated with increased breast density and breast cancer risk (16-19). Although these relationships are thought to reflect hormonal exposures, the actual biological pathways by which these risk factors operate on breast density (and breast cancer risk) are not known.

Because breast density is associated with several breast cancer risk factors that also have significant heritable components, we hypothesized that some of the observed phenotypic correlations between them are attributable (in part) to genetic factors. We address these hypotheses here in the context of an ongoing family-based genetic study of breast density in women from the Old Order Amish population of Lancaster County, Pennsylvania. Although the overall goal of our study is to identify genes that influence breast density, the aim of the current investigation is to estimate the genetic contributions to individual differences in breast density and associated breast cancer risk factors and assess the evidence for shared genetic influences between them. Whether shared genetic factors influence these traits is relevant to ongoing and future genetic studies of breast density, including our own.

Materials and Methods

Overall Study Design. We recruited women from the Old Order Amish population, with an emphasis on pairs of sisters, as part of an ongoing study to identify the genetic factors that influence mammographic breast density. Participants were identified by word-of-mouth and door-to-door interviews. Between June 2005 and October 2007, we had approached a total of 1,024 women, including 568 (55%) who had participated, 314 (31%) who had declined, 134 (13%) who did not meet our eligibility criteria, and 8 (1%) who withdrew after consenting. For the present investigation, our final analysis-ready sample included 550 women. All Old Order Amish women were eligible to participate if they were ≥40 years of age at the time of interview and had at least one living sister who was also ≥40 years of age. Women were excluded if they (*a*) were pregnant or lactating in the previous 6 months, (*b*) had ever been diagnosed with breast or ovarian cancer, (*c*) had one or both ovaries removed, or (*d*) used exogenous sex steroid hormones in the previous 6 months. To study natural variation in breast density, it was necessary to exclude women who had taken exogenous hormones and/or whose endogenous hormone production may have been medically altered. Although suspension of exogenous hormone use for ~3 weeks seems to reverse the mammographic breast density increase associated with its use (20), we elected to apply the more conservative 6-month exclusion criteria. The impact of using this more stringent threshold is likely minimal since <10% of our participants reported having ever used exogenous hormones.

Measurement of Mammographic Breast Density. Women who had not had a mammogram in the previous 12 months were screened via standard, two-view film screen mammography at a Mammography Qualified Standard Act–approved site (n = 538). For women who had had a mammogram in the previous 12 months (n = 12), we requested medical record release of their most recent mammogram and report. Craniocaudal views were digitized with a LUMISYS 85 laser film scanner at a pixel size of 0.05 × 0.05 mm and 4,096 gray levels. A single radiologist (M.A.H.) measured total breast area and absolute dense area from a digitized craniocaudal view of the right and/or left breast using interactive thresholding and our computer-assisted program Mammographic Density ESTimator (21). Additional technical details of our approach (21), including an evaluation study (22), are described elsewhere. For comparative purposes, relative density was calculated as the ratio of the dense area to the total area, and the nondense area was calculated as the difference between the total and dense areas. Based on data from the first 155 women, agreement between percent breast density estimates from the left and right craniocaudal views was high, with a mean absolute difference of 2.8%, a root mean squared error of 4%, and a within-individual r of 0.92. When we rescored an ~10% random sample of films distributed across the range of percent breast density (58 right craniocaudal views and 2 left craniocaudal views), the intrareader variability was equally low, with a mean absolute difference of 3.5%, a root mean squared error of 4.5%, and a within-individual r of 0.96 for percent density. Agreement between estimates for left and right craniocaudal views and intrareader variability were comparable for absolute dense area. Because our measurements of breast density were highly reproducible and differences in estimates from both breasts did not exceed differences from repeated readings of the same breast, we present results below using the right craniocaudal view for 465 women and (for technical reasons) the left craniocaudal view for the remaining 85 women.

Questionnaires and Anthropometric Measurements. Information on medical, reproductive, menstrual and family history, and medication use was collected using standardized questionnaires adapted for use in this population. Medical history information included physician-diagnosed cancer, osteoporosis, diabetes, heart attack, stroke, and surgeries. Reproductive or menstrual information included current and past frequency of

menstrual bleeding, childbearing and breastfeeding history, and ages at menarche, first birth, and menopause. Family history was limited to the number of first-degree relatives by relationship type, history of breast or ovarian cancer, age at diagnosis, and, if deceased, age and cause of death. History of breast or ovarian cancer in paternal and maternal grandmothers and age at diagnosis were also sought. Because smoking (especially among women) and alcohol consumption are infrequently practiced among the Amish (23), we did not collect this information. Trained nurses measured height and weight using a stadiometer and calibrated scale, with shoes removed and in light clothing. BMI was calculated as weight (kilogram) divided by the square of height (square meter). Waist circumference was measured at the level of the umbilicus, and hip circumference was measured at the widest protuberance across the pelvis. We defined participants as postmenopausal if they reported having natural menopause and no menstrual bleeding in the previous 12 months, and we defined women as premenopausal if they reported having menstrual bleeding in the previous 12 months. Women who reported a hysterectomy (n = 45) were defined as post- and premenopausal if they were >51 and ≤51 years of age, respectively, the ages at which natural menopause had occurred in ~90% and ~10% of participants. In other words, after excluding participants who reported a hysterectomy, natural menopause had occurred in ~90% of women who were >51 years of age (269 of 309) and in ~10% of women who were ≤51 years of age (23 of 196). Participants reported no other form of surgical menopause besides hysterectomy (without oophorectomy).

Statistical Analysis. In total, we analyzed three breast measures (dense area, nondense area, and percent density), four reproductive or menstrual traits (number of live births and ages at menarche, first birth, and menopause), and four measures of body size (height, weight, BMI, and waist circumference). Before conducting the quantitative genetic analyses described below, we assessed the distributions of all traits and, where necessary, transformed them to approximate univariate normality. A logarithm transformation was applied to the dense area of the breast, percent breast density, age at menarche, BMI, weight, and waist circumference, and power transformations were applied to the nondense area of the breast (0.3) and age at menopause (2). All other variables were left untransformed. We used standard variance and covariance component models and pedigree-based maximum likelihood methods (24, 25) as implemented in SOLAR (Sequential Oligogenic Linkage Analysis Routines) (26) to estimate trait heritabilities and to investigate the genetic and environmental correlations between pairs of traits. Pedigree relationships were determined from the Anabaptist Genealogy Database (version 4.0; ref. 27) by including genealogic information on the parents and grandparents of the study participants.

To estimate heritability, we partitioned variation in each trait, for example, dense area of the breast, into a component due to individual-specific covariates, including age and menopausal status, the additive genetic variance (σ_a^2), which captures the effects of unmeasured genes, and an individual-specific environmental component or residual error. The heritability (h^2) of each trait was estimated by the ratio of the variance attributable to the additive genetic effects (σ_a^2) and the phenotypic variance after adjustment for covariates (σ_{adj}^2). We assessed the significance of particular components, for example, σ_a^2, using standard likelihood ratio tests, that is, by comparing the likelihood of a model in which the component was estimated to the likelihood of a model in which the component was constrained to be zero. Given our sibling pair design, we were unable to distinguish and therefore estimate genetic dominance and shared sibling environment. We estimated the proportion of the total phenotypic variance explained by the additive genetic variance as the product of the heritability estimate and 1 minus the proportion of the variance explained by the covariates (σ_c^2), that is, $(1 - \sigma_c^2)$ (h^2).

To evaluate the evidence for genetic effects jointly influencing breast density and other breast cancer risk factors, we used bivariate variance component models to partition the phenotypic correlation (ρ_P) between each pair of traits, for example, dense area and number of live births, into components attributable to the same additive genetic effects (ρ_G or genetic correlation) and the same environmental effects (ρ_E or environmental correlation). Briefly, based on the heritabilities of the two traits (h_1^2 and h_2^2), the phenotypic correlation between the traits can be expressed as a weighted sum of their genetic and environmental correlations, namely, $\rho_P = \rho_G [(h_1^2 h_2^2)]^{1/2} + \rho_E [(1 - h_1^2)(1 - h_2^2)]^{1/2}$. The genetic correlation (ρ_G) captures the extent to which the same genes influence both traits, whereas the environmental correlation (ρ_E) captures the extent to which the same environmental factors influence both traits. Because significant genetic and/or environmental correlations can arise from nonsignificant phenotypic correlations, for example, when the genetic and environmental correlations have opposite signs, we analyzed each pair of traits without regard to their overall phenotypic correlation. Using likelihood ratio tests, we evaluated two hypotheses involving the genetic correlation. First, we tested whether the genetic correlation was zero ($\rho_G = 0$). Rejection of this hypothesis suggests that one or more of the same genetic factors influence both traits. Second, we tested whether the genetic correlation was 1 or -1 ($\rho_G = 1$ or -1). Rejection of this hypothesis suggests that there exist one or more unique genetic factors that influences one trait but not the other. Lastly, we also tested whether the environmental correlation was zero ($\rho_E = 0$). Rejection of this hypothesis suggests that one or more of the same environmental factors (unmeasured or unadjusted for) influence both traits.

All statistical tests were necessarily one sided, and P values < 0.05 were considered statistically significant. No adjustments for multiple comparisons were made. We assessed the impact of outliers on the estimates of heritability and genetic and environmental correlation by examining the change in estimates after excluding extreme values, which we defined by >3 SDs from the mean. All analyses (except where noted previously) were conducted using version 8.2 of the Statistical Analysis System programming language (SAS Institute).

Human Subjects Approval. The institutional review boards at the Universities of Michigan and Maryland approved all aspects of the protocol, and all participants gave written informed consent, including permission to release their medical records.

Results

For this investigation, our sample included 550 women from 212 distinct sibships, with 1 to 9 women per sibship. Of these sibships, 41%, 23%, and 20% were composed of two, three, and four or more participants, respectively. Table 1 summarizes the number of pairwise relationships among all 550 women after merging in genealogical information on their parents and grandparents. In total, there were 643 pairs of first-degree relatives, including 611 sister-sister and 32 mother-daughter pairs, and 3,391 more distantly related pairs. Table 2 describes selected characteristics of the 550 participants. All women were between the ages of 40 and 88 years, with a mean of 56 years. There were 218 and 332 pre- and postmenopausal women, respectively. After excluding the 40 postmenopausal women who reported previous surgical removal of their uteri, the average age at natural menopause was 49 ± 4 years (±SD). Fewer than 10% of all participants reported previous use of exogenous hormones, and none had taken hormones in the previous 6 months (per our exclusion criteria). Most women were parous (91%), with an average of 8 live births.

Mean (±SD) dense area, nondense area, and proportion of dense area were 15 ± 10 cm^2, 96 ± 50 cm^2, and 0.16 ± 0.11, respectively. As expected, breast density was higher in premenopausal women than in postmenopausal women (data not shown) and was inversely associated with age (Fig. 1). Age and menopausal status were significantly correlated with log-transformed dense area and percent density and accounted for 15% and 17% of the variation in each trait, respectively (Table 3). After adjusting for these covariates, the heritability of dense area was 39% and was significantly different from zero ($P < 10^{-4}$). Of the total variation in dense area, age and menopausal status accounted for 15%, additive genetic factors accounted for 33% [(1 - 0.15)(0.39)], and 52% remained unexplained. Similarly, of the total variation in percent density, covariates explained 17%, additive genetic factors accounted for 29%, and 54% remained unexplained. The heritability of the nondense area of the breast was 71% ($P < 10^{-14}$) after adjusting for covariates.

After transformation and adjustment for age and menopausal status, the dense and nondense areas were both genetically and environmentally correlated. The genetic and environmental correlations (±SE) were 0.38 ± 0.17 and -0.42 ± 0.17, respectively, and both were significantly greater and less than zero (P = 0.036 and 0.008, respectively), suggesting the presence of shared genetic and environmental factors exerting similar and opposite effects, respectively, on the dense and nondense areas of the breast. At the same time, the genetic correlation between these two areas was significantly <1, suggesting that there also exist unique genetic factors influencing each of these traits. Based on these estimates and estimates of the trait heritabilities, the corresponding phenotypic correlation between the dense and nondense areas was close to zero (0.02), consistent with the within-individual partial r (data not shown).

Table 1. Number of pairwise relationships among study participants

Relationship pair	No. of pairs
Sister-sister	611
Mother-daughter	32
Aunt-niece	209
Double first cousins	40
First cousins	2,254
First cousins, once removed	548
Half first cousins	30
Half first cousins, once removed	76
Second cousins	218
Half second cousins	16

NOTE: Pedigree relationships were determined by merging in the parents and grandparents of the study participants (n = 550).

Table 2. Selected characteristics of study participants (n = 550)

	Mean ± SD	Range
Age (y)	56 ± 9	40-88
Premenopausal*	218 (40)	
Age at menarche (y)	13 ± 1	10-18
Age at natural menopause (y)	49 ± 4	34-58
Ever used hormones*	46 (8)	
Reproductive factors		
Parous*	502 (91)	
Number of live births	8 ± 3 †	1-15
Age at first birth (y)	22 ± 3 †	17-37
Ever breast fed*	486 (96) †	
Body size measures		
Height (cm)	160 ± 6	135-178
Weight (kg)	75 ± 16	38-139
BMI (kg/m^2)	29 ± 6	16-57
Waist circumference (cm)	90 ± 11	63-127

*Number (and percentage).
†Based on 502 parous women.

Heritability estimates for the other breast cancer risk factors were also significantly different from zero, with 34% to 66% of the total variation in each trait attributable to additive genetic effects (Table 3). Estimates of the genetic correlation between each of these traits and each breast measure are given in Table 4 (after transformation and adjustment for age and menopausal status). All genetic correlations were significantly different from ±1 (data not shown). In addition, the dense area of the breast was inversely related to and significantly genetically correlated with the number of live births (-0.47 ± 0.16; ±SE; P = 0.013 for test of $\rho_G = 0$). Thus, the inverse correlation between the dense area of the breast and live birth number may be attributable (in part) to genetic factors. Similarly, the nondense area of the breast was significantly genetically correlated with age at menarche (-0.38 ± 0.13; P = 0.008 for test of $\rho_G = 0$). As expected, the nondense area of the breast was positively and strongly genetically correlated with most body size measures ($P < 10^{-7}$ for test of $\rho_G = 0$), including weight (ρ_G = 0.75), BMI (ρ_G = 0.80), and waist circumference (ρ_G = 0.81). Similar genetic correlations were observed for percent breast density but in the opposite direction. The genetic correlations between the remaining pairs of traits were low and not significantly different from zero.

Estimates of the environmental correlation between each breast measure and each of the other breast cancer risk factors are also given in Table 4 (after transformation and adjustment for age and menopausal status). The dense area of the breast was positively and significantly environmentally correlated with age at menarche (0.38 ± 0.14; P = 0.005) and height (0.34 ± 0.16; P = 0.032) and negatively and significantly

environmentally correlated with BMI (-0.26 ± 0.12; $P = 0.034$). Similarly, the nondense area of the breast was positively and significantly environmentally correlated with the number of live births (0.44 ± 0.18; $P = 0.012$), as well as most body size measures, including weight (0.83 ± 0.07; $P = 0.002$), BMI (0.80 ± 0.06; $P = 0.005$), and waist circumference (0.81 ± 0.06; $P = 0.002$). The environmental correlations between percent breast density were similar and in the same (opposite) direction as they were for the dense (nondense) area. The environmental correlations between the remaining pairs of traits were low and not significantly different from zero. Together, these results suggest that there exist individual-specific but unmeasured environmental factors that contribute to the correlations between several pairs of these traits.

We repeated all heritability and genetic and environmental correlation analyses after removing individuals with extreme values. With the exception of the environmental correlation between percent breast density and age at first birth, all heritability and correlation estimates from these analyses were within 1 SE of the original estimates.

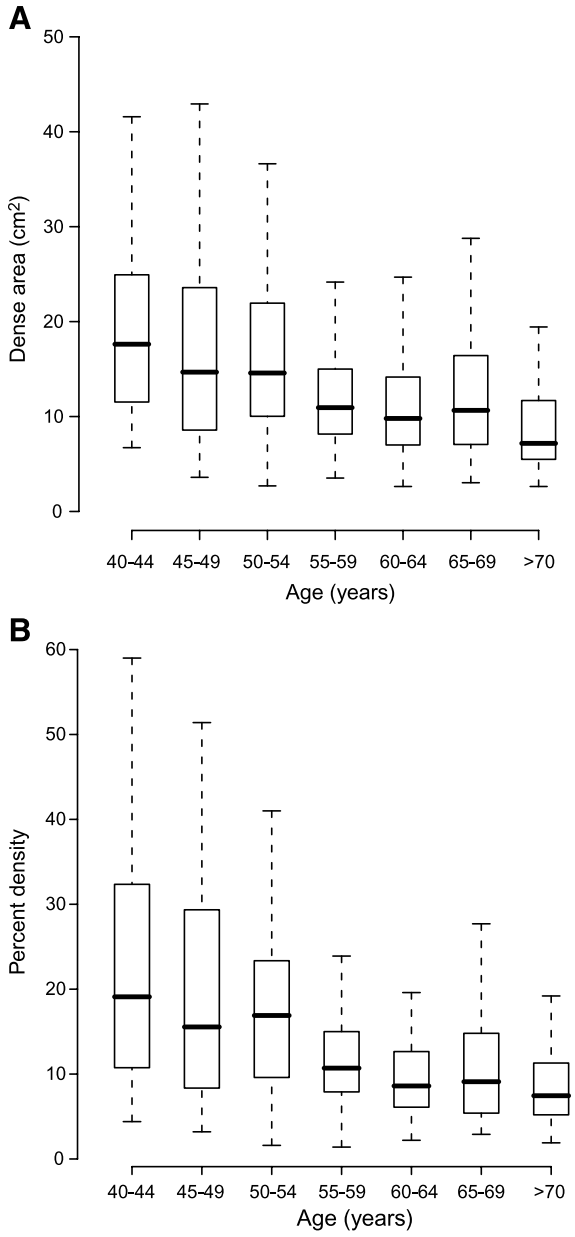

Figure 1. Interindividual variability in dense area (**A**) and percent breast density (**B**) by age ($n = 550$). *Horizontal black bars*, median; *boxes*, interquartile range; *whiskers*, 1.5 times the interquartile range.

Discussion

With their unique cultural customs and relatively similar environmental exposures, a well-defined, genetically closed population structure, and extensive genealogic records, the Old Order Amish provide an ideal context in which to study the genetic contributions to breast density. Of particular relevance to studying breast density, the Old Order Amish population is characterized by a very low prevalence of exogenous hormone use, including oral contraceptives and hormone replacement therapy, and high parity. Still, our results suggest that breast density varies widely in the Old Order Amish population, with values that are comparable with other highly parous populations. For example, in a sample of 294 Hispanic women (two thirds of whom were postmenopausal and three fourths of whom reported three or more live births), Lopez et al. (28) reported an overall mean of 17.7% for percent breast density, with a range of 1.9% to 54.6%. Similarly, in our sample of women (approximately two thirds of whom were also postmenopausal and three fourths of whom reported five or more live births), the mean and range of percent breast density were 15.8% and 1.4% to 59%, respectively.

To our knowledge, our study is the first non-twin study to estimate the genetic contributions to the dense and nondense areas of the breast and the first study to examine the contribution of genetic factors to the correlation between breast density and other breast cancer risk factors. We found that the dense and nondense areas of the breast were significantly heritable in our sample, with 33% and 68% of the total variance, respectively, attributable to additive genetic effects. Although these estimates are consistent with the significant genetic influences reported by Stone et al. (13), comparisons of heritability are always ill advised. For example, with respect to the environmental factors that impact breast density, the women in this sample likely share relatively similar environments. Thus, all one can infer from a relatively higher (or lower) estimate of heritability is that there is less (or more) environmental variation relative to the genetic variation in this sample. We note that screening and adjusting for other significant covariates (in addition to age and menopausal status) did not meaningfully alter our estimates of the heritability of absolute breast density. In fact, age at menarche and number of live births were the only other covariates significantly correlated with log-transformed dense area, and together, they explained no more than an additional 8% of the variation in this trait. After including all four covariates in our model, the heritability of the dense area of the breast was 36% (versus 39% with adjustment for age and menopausal status only).

Table 3. Heritability estimates (h^2) for breast measures and other breast cancer risk factors

Trait	$h^2 \pm$ SE	P	Proportion of total variance explained by	
			Covariates	Genes
Dense area	0.39 ± 0.11	1.8×10^{-5}	0.15	0.33
Percent density	0.35 ± 0.11	1.2×10^{-4}	0.17	0.29
Nondense area	0.71 ± 0.10	1.7×10^{-15}	0.04	0.68
Age at menarche	0.58 ± 0.10	1.9×10^{-12}	<0.01	0.58
Number of live births	0.49 ± 0.11	1.4×10^{-8}	<0.01	0.49
Age at first birth	0.34 ± 0.11	3.4×10^{-4}	<0.01	0.34
Age at natural menopause	0.58 ± 0.17	1.3×10^{-4}	0.02	0.56
Height	0.70 ± 0.10	5.3×10^{-17}	0.06	0.66
Weight	0.58 ± 0.10	4.1×10^{-12}	0.04	0.56
BMI	0.47 ± 0.10	3.6×10^{-8}	0.01	0.46
Waist circumference	0.57 ± 0.11	1.8×10^{-10}	<0.01	0.57

NOTE: Data in second column are after adjustment for age and menopausal status. Dense area, percent density, age at menarche, weight, BMI, and waist circumference were log transformed. Nondense area and age at natural menopause were power transformed (0.3 and 2, respectively). Other variables were not transformed.

We also found that other breast cancer risk factors were significantly heritable and that some of their associations with breast density were due to an underlying structure of shared genetic (and environmental) effects. Particularly noteworthy is our finding that breast density and live birth number are genetically correlated. It has been commonly hypothesized that the inverse association between these two traits is due to a decrease in the proliferative activity of the parous epithelium and, in turn, that the subsequent decreased risk for breast cancer is due to the differentiation (during pregnancy) of lobular type 1 to 4 cells, which are assumed to be less susceptible to malignant transformation. Our results are consistent with this hypothesis and suggest that a significant component of this association may be due to genetic factors that influence breast density and live birth number (or fertility) in opposite directions. Because the Old Order Amish discourage the use of contraceptives and share a relatively uniform socio-cultural and -economic background, we were in a unique position to study this relationship. Of note, our finding of a significant genetic component to fertility is also consistent with a recent report by Pluzhnikov et al. (29), who studied both components of reproductive fitness (fertility

Table 4. Genetic and environmental correlations (ρ_G and ρ_E, respectively) between breast measures and other breast cancer risk factors

Trait 1	Trait 2	$\rho_G \pm$ SE	$\rho_E \pm$ SE
Dense area	Age at menarche	-0.26 ± 0.18	0.38 ± 0.14*
	Number of live births	-0.47 ± 0.16 †	-0.12 ± 0.13
	Age at first birth	-0.02 ± 0.23	0.17 ± 0.12
	Age at natural menopause	0.30 ± 0.25	-0.07 ± 0.21
	Height	-0.06 ± 0.17	0.34 ± 0.16 †
	Weight	0.20 ± 0.18	-0.19 ± 0.14
	BMI	0.27 ± 0.20	-0.26 ± 0.12 †
	Waist circumference	0.10 ± 0.19	-0.18 ± 0.14
Percent density	Age at menarche	0.19 ± 0.18	0.19 ± 0.13
	Number of live births	-0.02 ± 0.22	-0.32 ± 0.11 †
	Age at first birth	-0.18 ± 0.26	0.23 ± 0.11
	Age at natural menopause	0.15 ± 0.28	-0.07 ± 0.20
	Height	-0.19 ± 0.18	0.32 ± 0.15 †
	Weight	-0.59 ± 0.13*	-0.52 ± 0.10 ‡
	BMI	-0.61 ± 0.14*	-0.55 ± 0.08 ‡
	Waist circumference	-0.72 ± 0.11 ‡	-0.51 ± 0.09*
Nondense area	Age at menarche	-0.38 ± 0.13*	0.12 ± 0.20
	Number of live births	-0.30 ± 0.17	0.44 ± 0.18 †
	Age at first birth	0.04 ± 0.19	-0.11 ± 0.17
	Age at natural menopause	0.10 ± 0.21	0.03 ± 0.24
	Height	0.11 ± 0.13	-0.13 ± 0.22
	Weight	0.75 ± 0.06 ‡	0.83 ± 0.07*
	BMI	0.80 ± 0.06 ‡	0.80 ± 0.06 ‡
	Waist circumference	0.81 ± 0.05 ‡	0.81 ± 0.06*

NOTE: Data are adjusted for age and menopausal status. Dense area, percent density, age at menarche, weight, BMI, and waist circumference were log transformed. Nondense area and age at menopause were power transformed (0.3 and 2, respectively).

*$P < 0.01$ for test of null hypothesis that $\rho_G = 0$ (or $\rho_E = 0$), that is, correlation due to additive genetic factors is zero (or correlation due to unmeasured environmental factors is zero).

†$P < 0.05$ for test of null hypothesis that $\rho_G = 0$ (or $\rho_E = 0$), that is, correlation due to additive genetic factors is zero (or correlation due to unmeasured environmental factors is zero).

‡$P < 0.001$ for test of null hypothesis that $\rho_G = 0$ (or $\rho_E = 0$), that is, correlation due to additive genetic factors is zero (or correlation due to unmeasured environmental factors is zero).

and mortality) in the Hutterites and found significant familial correlations in family size. At present, however, the genes that influence fertility in human populations are unknown, partly owing to the difficulty of controlling for the influence of nongenetic factors. Our results suggest it may be ill advised to adjust for live birth number in the genetic analysis of breast density given the strong genetic correlation between them.

Based on samples of unrelated women, Boyd et al. (10) and Haars et al. (9) previously showed that the inverse correlations of various measures of adiposity with breast density, expressed as a percentage of total breast area, are due to positive correlations with the nondense area of the breast. Our data are consistent with these observations and suggest that many of these correlations may have a common and strong genetic basis. Specifically, in our sample, several measures of body size exhibited strong and significant positive genetic correlations with the nondense (but not dense) area of the breast. For example, approximately two thirds of the phenotypic correlation between the nondense area of the breast and weight (0.77) was due to the same genetic factors after adjusting for age and menopausal status. Thus, any genetic analysis of percent breast density will be strongly confounded by adiposity. One such example is provided by Vachon et al. (15), who recently reported that their linkage evidence on chromosome 5p for percent breast density nearly doubled after adjustment for BMI. Although Vachon et al. (15) recognized that percent breast density was genetically correlated with BMI in their sample (0.71), they were unable to analyze the dense and nondense areas separately because only percent density was characterized.

In our sample, the nondense area of the breast was also significantly (negatively) genetically correlated with age at menarche. Age at menarche was, in turn, significantly (negatively) genetically correlated with each of the adiposity measures described above (data not shown). Together, these correlations are consistent with findings from a recent study by Wang et al. (30), who reported significant negative genetic correlations between several obesity phenotypes, including BMI, and age at menarche. As described by Wang et al. (30), these findings are biologically consistent with documented differences in hormonal concentrations and fat distribution in women who experience early versus late menarche.

In addition to identifying significant genetic correlations between the dense and nondense areas of the breast and other breast cancer risk factors, we also found that the environmental correlations were significantly different from zero for several trait pairs. For example, the dense area of the breast was positively environmentally correlated with age at menarche and height. These findings imply the existence of other important covariates that were either not included in our models or, more likely, not measured in our study and are consistent with the individual-specific effects noted in our univariate analyses. For example, ~50% of the total variability in the dense area of the breast was unexplained by measured covariates and unmeasured additive genetic factors. Factors that may have contributed to this unexplained variation (and environmental correlation with other traits) include exposures that may have occurred earlier in life, for example, dietary intake and hormones. Indeed, some of the hormonal factors that influence height also seem to regulate mammary gland development (31).

Based on an analysis of monozygotic and dizygotic twins, Stone et al. (13) previously reported a negative genetic correlation between the dense and nondense areas of the breast [-0.30 $\pm$ 0.04 ($\pm$SE) after a logarithm transformation and adjustment for covariates]. In our sample, however, the genetic correlation between these areas was positive (0.38 $\pm$ 0.17 after transformation and adjustment for covariates). In other words, data from Stone et al. (13) suggest that there exist common genetic influences that act in opposite directions on the dense and nondense areas, whereas the data presented here suggest that these shared genetic influences operate in the same direction. It is interesting to note that the within-individual correlation between the dense and nondense areas was also remarkably different between our two studies [after adjustment for age, 0.002 in our sample versus -0.35 in the sample of Stone et al. (13)] but consistent with our study-specific environmental correlations, which were similar in sign and magnitude (-0.42 $\pm$ 0.17 in our sample and -0.31 $\pm$ 0.04 in their sample). Because our parameterizations, populations of inference, and study designs are not directly comparable, it is difficult to reconcile these differences.

Data from the present study add to the accumulating evidence that breast density has a strong heritable component and provide new evidence that part of this heritable component is shared with other breast cancer risk factors. Still, we acknowledge several study limitations. First, given our study design, we were unable to examine the influence of shared environments. For example, to the extent that shared childhood environments contribute to correlations in breast density between sisters, we may have overestimated the genetic contributions to individual differences in (and correlations between) breast density and other breast cancer risk factors. Second, our findings may not generalize to other populations, particularly given the unique reproductive practices of the Old Order Amish. Despite this, our study participants were similar in many other ways to the U.S. female Caucasian population as determined by our analysis of age-matched data from the 2001-2002 National Health and Nutrition Examination Surveys (data not shown). Third, we were unable to examine (with confidence) the relationship between breast density and an important breast cancer risk factor, namely, family history of breast cancer. Irregular medical care practices in this population make it difficult to obtain and/or verify information on family cancer history. Fourth, with our modest sample size, we were underpowered to examine the extent to which genetic variances and correlations were menopausal specific. Tentative examination of menopausal-specific estimates of heritability and genetic and environmental correlations, however, suggests that the relative contributions of genetic and nongenetic factors were similar in pre- and postmenopausal women (data not shown).

In summary, our results indicate that breast density varies widely in the Old Order Amish population and is strongly influenced by genetic factors. Our results also suggest that the genetic and environmental factors that influence breast density are not independent of the genetic and environmental factors that influence other breast cancer risk factors. These findings are being used

to inform our ongoing genetic investigation of breast density in the Old Order Amish. The evidence presented here for shared genetic influences on breast density and other breast cancer risk factors may lead to more powerful searches for the loci and genes that influence breast density. Indeed, the power to identify loci that influence breast density may be increased by jointly analyzing genetically correlated traits (32, 33).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Kamangar F, Dores GM, Anderson WF. Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. J Clin Oncol 2006;24:2137–50.
2. Couzin J. Breast cancer. Dissecting a hidden breast cancer risk. Science 2005;309:1664–6.
3. 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.
4. 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.
5. Kato I, Beinart C, Bleich A, Su S, Kim M, Toniolo PG. A nested case-control study of mammographic patterns, breast volume, and breast cancer (New York City, NY, United States). Cancer Causes Control 1995;6:431–8.
6. Ursin G, Ma H, Wu AH, et al. Mammographic density and breast cancer in three ethnic groups. Cancer Epidemiol Biomarkers Prev 2003;12:332–8.
7. Maskarinec G, Pagano I, Lurie G, Wilkens LR, Kolonel LN. Mammographic density and breast cancer risk: the multiethnic cohort study. Am J Epidemiol 2005;162:743–52.
8. Torres-Mejia G, De SB, Allen DS, et al. Mammographic features and subsequent risk of breast cancer: a comparison of qualitative and quantitative evaluations in the Guernsey prospective studies. Cancer Epidemiol Biomarkers Prev 2005;14:1052–9.
9. 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.
10. Boyd NF, Lockwood GA, Byng JW, Little LE, Yaffe MJ, Tritchler DL. The relationship of anthropometric measures to radiological features of the breast in premenopausal women. Br J Cancer 1998; 78:1233–8.
11. Heng D, Gao F, Jong R, et al. Risk factors for breast cancer associated with mammographic features in Singaporean Chinese women. Cancer Epidemiol Biomarkers Prev 2004;13:1751–8.
12. Boyd NF, Dite GS, Stone J, et al. Heritability of mammographic density, a risk factor for breast cancer. N Engl J Med 2002;347: 886–94.
13. Stone J, Dite GS, Gunasekara A, et al. The heritability of mammographically dense and nondense breast tissue. Cancer Epidemiol Biomarkers Prev 2006;15:612–7.
14. Pankow JS, Vachon CM, Kuni CC, et al. Genetic analysis of mammographic breast density in adult women: evidence of a gene effect. J Natl Cancer Inst 1997;89:549–56.
15. Vachon CM, Sellers TA, Carlson EE, et al. Strong evidence of a genetic determinant for mammographic density, a major risk factor for breast cancer. Cancer Res 2007;67:8412–8.
16. Boyd NF, Lockwood GA, Byng JW, Tritchler DL, Yaffe MJ. Mammographic densities and breast cancer risk. Cancer Epidemiol Biomarkers Prev 1998;7:1133–44.
17. 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.
18. 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.
19. Dumitrescu RG, Cotarla I. Understanding breast cancer risk—where do we stand in 2005? J Cell Mol Med 2005;9:208–21.
20. Colacurci N, Fornaro F, De FP, Mele D, Palermo M, del VW. Effects of a short-term suspension of hormone replacement therapy on mammographic density. Fertil Steril 2001;76:451–5.
21. Zhou C, Chan HP, Petrick N, et al. Computerized image analysis: estimation of breast density on mammograms. Med Phys 2001;28: 1056–69.
22. Martin KE, Helvie MA, Zhou C, et al. Mammographic density measured with quantitative computer-aided method: comparison with radiologists' estimates and BI-RADS categories. Radiology 2006; 240:656–65.
23. Hsueh WC, Mitchell BD, Aburomia R, et al. Diabetes in the Old Order Amish: characterization and heritability analysis of the Amish Family Diabetes Study. Diabetes Care 2000;23:595–601.
24. Hopper JL, Mathews JD. Extensions to multivariate normal models for pedigree analysis. Ann Hum Genet 1982;46:373–83.
25. Lange K, Boehnke M. Extensions to pedigree analysis. IV. Covariance components models for multivariate traits. Am J Med Genet 1983;14: 513–24.
26. Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet 1998;62:1198–211.
27. Agarwala R, Biesecker LG, Schaffer AA. Anabaptist genealogy database. Am J Med Genet C Semin Med Genet 2003;121:32–7.
28. Lopez P, Van HL, Colangelo LA, Wolfman JA, Hendrick RE, Gapstur SM. Physical inactivity and percent breast density among Hispanic women. Int J Cancer 2003;107:1012–6.
29. Pluzhnikov A, Nolan DK, Tan Z, McPeek MS, Ober C. Correlation of intergenerational family sizes suggests a genetic component of reproductive fitness. Am J Hum Genet 2007;81:165–9.
30. Wang W, Zhao LJ, Liu YZ, Recker RR, Deng HW. Genetic and environmental correlations between obesity phenotypes and age at menarche. Int J Obes 2006;30:1595–600.
31. Hovey RC, Trott JF, Vonderhaar BK. Establishing a framework for the functional mammary gland: from endocrinology to morphology. J Mammary Gland Biol Neoplasia 2002;7:17–38.
32. Almasy L, Dyer TD, Blangero J. Bivariate quantitative trait linkage analysis: pleiotropy versus co-incident linkages. Genet Epidemiol 1997;14:953–8.
33. Klei L, Luca D, Devlin B, Roeder K. Pleiotropy and principal components of heritability combine to increase power for association analysis. Genet Epidemiol 2008;32:9–19.

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