The Heritability of Mammographically Dense and Nondense Breast Tissue

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Abstract

Background: **Percent mammographic density (PMD) is a risk factor for breast cancer. Our previous twin study showed that the heritability of PMD was 63%. This study determined the heritabilities of the components of PMD, the areas of dense and nondense tissue in the mammogram.**
Methods: **We combined two twin studies comprising 571 monozygous and 380 dizygous twin pairs recruited from Australia and North America. Dense and nondense areas were measured using a computer-assisted method, and information about potential determinants was obtained by questionnaire. Under the assumptions of the classic twin model, we estimated the heritability of the log dense area and log nondense area and the genetic and environmental contributions to the covariance between the two traits, using maximum likelihood theory and the statistical package FISHER.**
Results: **After adjusting for measured determinants, for each of the log dense area and the log nondense area, the monozygous correlations were greater than the dizygous correlations. Heritability was estimated to be 65% (95% confidence interval, 60-70%) for dense area and 66% (95% confidence interval, 61-71%) for nondense area. The correlations (SE) between the two adjusted traits were −0.35 (0.023) in the same individual, −0.26 (0.026) across monozygous pairs, and −0.14 (0.034) across dizygous pairs.**
Conclusion: **Genetic factors may play a large role in explaining variation in the mammographic areas of both dense and nondense tissue. About two thirds of the negative correlation between dense and nondense area is explained by the same genetic factors influencing both traits, but in opposite directions.** (Cancer Epidemiol Biomarkers Prev 2006;15(4):612–7)

Introduction

Mammographic density has been shown to be a strong predictor of breast cancer risk that is independent of other known risk factors (1). The radiologically dense areas that appear light on a mammogram represent the connective and epithelial tissue in the breast, and the radiologically nondense areas that appear dark represent fat. Examples of a dense and nondense breast are given in Fig. 1. Most recent studies express mammographic density as the proportion of the total breast area that is radiologically dense, typically expressed as the percent mammographic density (PMD; refs. 1, 2). PMD has been assessed by a variety of methods, including estimation by an observer; however, the computer-assisted method that we have used in previous work generates a continuous quantitative measure of the area of the breast and the area of dense tissue. This allows separate examination of the factors associated with the absolute areas of dense and nondense areas (total area minus dense area) in the mammogram (3). These associations are of interest because the association of PMD with risk of breast cancer must be explained by associations of one or both of the dense and nondense areas with risk.

There is evidence that the area of dense tissue area is associated with risk of breast cancer. Byrne et al. found in a large nested case-control study a 3-fold gradient in risk of breast cancer across six equally spaced categories of dense tissue area, independent of age and other measured risk factors (2). The association of nondense tissue area and breast cancer, independent of dense tissue area, was not described.

An early study of mother-daughter pairs by Wolfe et al. (4) and a small twin study (5) suggested that genetic factors might explain a proportion of the variation (i.e., the heritability) of mammographic density. Pankow et al. (6) and Vachon et al. (7) provided further evidence of a likely genetic etiology via a family study that calculated correlations between mother-daughter and sister pairs and conducted a segregation analysis and a linkage analysis.

Previous analysis of our large twin study in both Australia and North America showed that the heritability of PMD seems to be about 60%, after adjustment for age and other covariates (8). It is not known, however, whether the *absolute* dense tissue area and/or the *absolute* nondense tissue area are heritable. The fact that PMD is heritable does not imply that one or both of these measures are heritable. Furthermore, if both measures have a heritable component, it is not known if the same genes are involved.

The purpose of this study was to estimate the heritability of absolute dense tissue area (henceforth dense area) and absolute nondense tissue area (henceforth nondense area). In addition, by analyzing the components of PMD separately, we have also assessed the extent to which any correlation between dense and nondense areas was due to the same genes being associated with variation in both traits.

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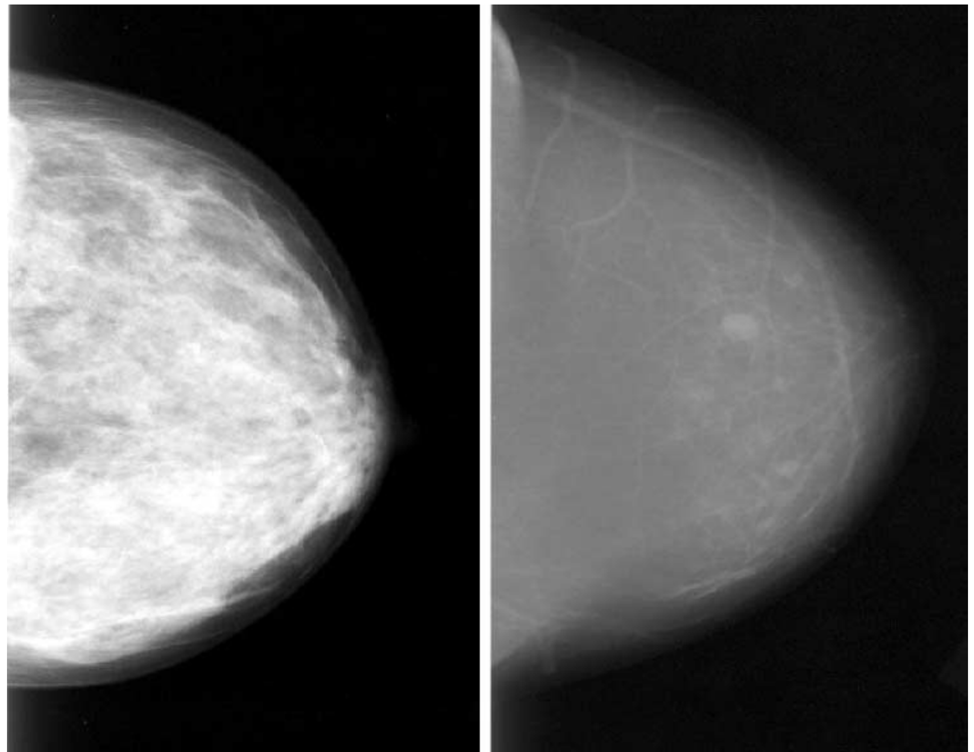

Figure 1. Examples of a dense breast (*left*) and a nondense breast (*right*).

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Materials and Methods

As previously reported (8), we obtained questionnaire and mammographic data from two samples of monozygous and dizygous pairs of female twins, one from Australia and the other from Canada and the United States. Subjects were aged between 40 and 70 years at interview, and mammograms of twins in the same pair were taken within 36 months of each other and within 36 months of the time of questionnaire administration. Pairs in which one or both twins had breast cancer, or a breast augmentation or reduction mammoplasty, before the date of the mammogram were excluded.

Australian twins were recruited through the Australian Twin Registry (9), which sent a letter to the twins from the principal investigator that explained the aims of the study, invited participation, and included consent forms for participation and release of mammograms. Twins who agreed to participate were contacted by a research assistant and, if they had not undergone mammography within the previous 2 years, were given information on how to make an appointment with a state-run mammographic screening program. In North America, twins were recruited through print and electronic media; the annual Twins Days Festival held in Twinsburg, OH; mammography units of the Ontario Breast Screening Program; and the Twins Foundation (a Rhode Island–based nonprofit organization with a resource center containing information on twins).

All twins who participated provided written informed consent for participation and permission to release their most recent mammogram. Films from Australian twins were digitized in one center in Melbourne and sent on compact disc to Toronto, whereas films from North American twins were all digitized in Toronto. For each subject, one craniocaudal view for one breast was measured by one observer (N.F.B.) using a previously described interactive thresholding technique (3), in which the total area of the breast appearing on the mammogram and the area of dense tissue appearing on the mammogram were measured. The area of nondense tissue was then calculated as the total area minus the dense area.

A questionnaire was completed for each participating twin and included demographic information, weight, height, physical activity, smoking history, alcohol consumption, reproductive history, cessation of periods, use of oral contraceptives and hormone replacement therapy, breast examination, and family history of cancer. Questionnaires were given by telephone interview in Australia and were self-administered in North America with telephone interviews used only to clarify incomplete or ambiguous responses. There were some differences between Australia and North America in questions related to menopausal status; thus, we derived a common variable for cessation of periods and used this in analyses.

For North American twins, zygosity was determined using questions and methods of classifying responses that have been shown to give 95% agreement with zygosity based on blood typing in middle-aged adults (10-12). In addition, zygosity was examined using laboratory methods. A blood sample was requested from a random sample of 10% of the North American twins, and from all twin pairs in whom members of the pair gave conflicting responses to the Torgersen questions and zygosity was ambiguous. Testing was carried out in Dr. Hegele's laboratory where six markers for non-tandem repeats were assessed (D2S44, D17S79, D1S7, D4S139, D16S85, and D14S13). Blood was requested from 70 pairs of twins and received from 56 pairs, of whom 37 had been sampled randomly, and 19 were of ambiguous zygosity. Of the random sample, 3 of the 37 pairs (8%) had incorrectly considered they were monozygous by their answers to the questionnaire. Of the 19 ''ambiguous'' pairs, 8 proved to be monozygous, and 11 proved to be dizygous. Australian twins were read a description of the differences between identical and nonidentical twins and then asked if they thought they were identical based on this description. Those whose answers contradicted each other, or who were unsure, were telephoned and asked the same set of questions used in North America and classified using the same methods outlined by Torgersen.

Statistical Methods. As described in Boyd et al. (8), we fitted fixed and random effects to log-transformed dense area and log-transformed nondense area, under the assumptions of

Table 1. Characteristics of monozygous and dizygous female twin subjects

	Monozygous (n = 571), mean (SE)	Dizygous (n = 380), mean (SE)
Age at interview (y)	50.72 (0.33)	53.03 (0.38)
Age at mammogram (y)	50.65 (0.32)	52.55 (0.38)
Time between interview and mammogram (mo)	6.29 (0.23)	6.14 (0.28)
Weight (kg)	66.18 (0.50)	67.87 (0.61)
Height (cm)	162.00 (0.26)	162.97 (0.32)
Age at menarche (y)	13.04 (0.06)	12.93 (0.07)
Age at first birth (y; parous women only)	24.88 (0.14)	25.07 (0.17)
No. live births	2.31 (0.05)	2.42 (0.06)
Years of oral contraceptive use	6.04 (0.22)	5.87 (0.27)
Years of hormone replacement therapy	2.46 (0.17)	2.05 (0.21)
Pack-years of smoking	5.58 (0.43)	6.97 (0.52)
PMD	37.82 (0.79)	35.69 (0.96)
Absolute dense area of mammographic tissue (cm^2)	40.50 (0.96)	38.33 (1.17)
Absolute nondense area of mammographic tissue (cm^2)	78.60 (2.03)	84.78 (2.47)
Total area of the breast (cm^2)	119.14 (1.98)	123.05 (2.40)
Parous (% yes)	84.6	85.9
Cessation of periods (% stopped)	59.3	55.0

the multivariate normal model for pedigree analysis. The statistical package FISHER was used to fit all models by maximum likelihood (13) and to test model assumptions (14). Statistical inference and choice of parsimonious models were based on standard asymptotic likelihood theory and Akaike's information criterion (15). All quoted *P*s are nominal and two sided.

The fixed effects were estimated as linear functions of measured covariates, including age centered on 40 years. A quadratic term for age was not found to be statistically significant for either trait and was omitted. We modeled the random effects in several ways. Initial descriptive analyses involved estimating the residual variance (σ^2) and, for monozygous and dizygous pairs, either separate covariances ($\sigma_{\text{monozygous}}{}^2$ and $\sigma_{\text{dizygous}}{}^2$) or separate correlations ($\rho_{\text{monozygous}}$ and ρ_{dizygous}). These analyses were then extended by allowing the random effects variables to vary as a linear function of age (again centering on 40 years).

We fitted variance components models under the assumptions of the classic twin method, which assumes that the total residual variance of each trait can be partitioned into $\sigma_a{}^2$ representing the independent and additive effects, both within and between genes, of genetic variation at presumably multiple loci (A); $\sigma_c{}^2$ representing the effects of environmental factors common to twins within the same pair (C); and $\sigma_e{}^2$ representing the effects of individual specific factors that are independent within a pair (and hence not due to genetic factors), and includes the effects of any measurement error (E; ref. 16). The key assumption of this model is that the effects of environmental factors common to the twins are the same for monozygous pairs and dizygous pairs. Under the further assumption that the three factors A, C, and E have independent and additive effects on the trait variance, the total residual variance is the sum of the additive, common environment, and individual specific variance components. That is, $\sigma^2 = \sigma_a{}^2 + \sigma_c{}^2 + + \sigma_e{}^2$. Given that monozygous pairs share all genetic variants, whereas dizygous pairs share on average half their genetic variants, under this model, the covariance for monozygous pairs is the sum of the additive and the common environment variance components, whereas the covariance for dizygous pairs is the sum of half the additive variance component plus all the common environmental variance component (16). The heritability, or proportion of residual variance attributed to additive genetic factors, is the ratio of the additive component and the total variance (i.e., $\sigma_a{}^2/\sigma^2$).

Just as the classic twin model can be used to partition the residual variance of specific traits in genetic and environmental components, it can also be used to partition the covariance between two traits (17). The cross-trait correlation is the correlation between the two traits in the same individual. The cross-trait cross-twin correlation is the correlation between one trait in one twin and the other trait in the other twin. A comparison of monozygous and dizygous cross-trait correlations provides an estimate of the degree to which the covariation between two traits can be attributed to the *same* genetic factors causing variation in *both* traits. Under the assumptions of the classic twin model, the covariance between the two traits is at least partly attributable to genetic factors if the cross-trait correlation is greater in monozygous pairs than in dizygous pairs. The underlying idea behind partitioning of

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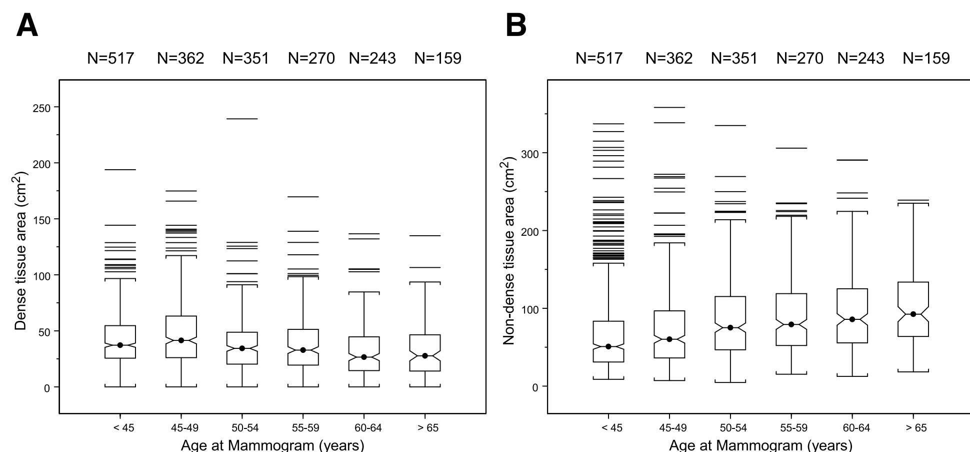

Figure 2. Distribution of the absolute area of dense breast tissue (**A**) and absolute area of nondense breast tissue (**B**) by age at mammogram. *Black dot,* median; *box widths,* 1st and 3rd quartiles; *whiskers,* 1.5 times the interquartile range; *lines outside the whiskers,* outliers. If the notches on two boxes do not overlap, this indicates a difference in a location at a rough 5% significance level.

the covariance into genetic and environmental components is the same as above for partitioning the variance. The covariance between two traits (ω) is the sum of the components of covariance ($\omega_a + \omega_c + \omega_e$) with the subscripts referring to the three factors A, C, and E, described above. The monozygous and dizygous cross-trait correlations are derived the same way as above, and under this model, the correlation within an individual is $\omega/\sigma_1\sigma_2$, and the twin pair cross-trait correlations are $[\omega_a + \omega_c]/\sigma_1\sigma_2$ and $[1/2(\omega_a) + \omega_c]/\sigma_1\sigma_2$ for monozygous and dizygous pairs, respectively. The proportion of the covariance attributable to genetic factors is the ratio of the additive component and the total cross-trait covariance (i.e., ω_a/ω; refs. 17, 18). The correlation between the A components for the two measures is $\omega_a/\sigma_{a1}\sigma_{a2}$, where σ_{a1}^2 and σ_{a2}^2 are the additive genetic variances of the two measures. Similarly, the correlation between the C components and the E components are $\omega_c/\sigma_{c1}\sigma_{c2}$ and $\omega_e/\sigma_{e1}\sigma_{e2}$, respectively, where σ_{c1}^2 and σ_{c2}^2 are the common environment variances of the two measures, and σ_{e1}^2 and σ_{e2}^2 are the individual specific variances of the two measures.

Results

Table 1 shows that monozygous and dizygous twins were similar on most of the listed characteristics (all $P > 0.05$) with the exception that monozygous twins were >2 years younger than dizygous twins at time of mammogram and at time of interview (both $P < 0.0001$), were almost 2 kg lighter ($P = 0.03$), 1 cm shorter ($P = 0.02$), and smoked less ($P = 0.04$). Australian and North American twins were similar on the listed characteristics (8).

Figure 2A shows that the cross-sectional medians of dense area decreased slightly with increasing age. The variance of dense area increased with age, whereas the variance of nondense area decreased with age (see Fig. 2B). Due to the skewed distributions of both dense and nondense area, log-transformed dense and nondense areas were used in all subsequent analyses. The distributions of (log) dense area and (log) nondense area and the model fits below were all similar for Australian and North American twins (graphs and data not shown).

For both dense area and nondense area, the best fitting variance components models included an additive genetic component and an individual specific environmental component only (henceforth referred to as the AE model), whether adjusted for age alone or for age and other covariates. That is, the estimate of the common environment component was the lower bound zero in models containing all three components (ACE model), and in all instances, the AE model gave a far better fit than the model containing the common environment and individual specific environment components only (CE model).

Table 2 shows the residual variance (σ^2) for each model of log dense and nondense area, respectively, the monozygous and dizygous covariances ($\sigma_{\text{monozygous}}^2$ and $\sigma_{\text{dizygous}}^2$, respectively) and the correlations ($\rho_{\text{monozygous}}$ and ρ_{dizygous}, respectively). Each column of Table 2 provides the estimates from the fit of a separate model. In the first model, the mean of each measure is adjusted for age only, and in the second model, the mean is adjusted for age and other covariates. For both these models, the variance and variance components are assumed to be constants with respect to age. In the third model, the mean is as in the second model but the residual variance, and either the monozygous and dizygous covariances or the monozygous and dizygous correlations, are all allowed to vary linearly with age. The estimates presented in column three are the estimates at age 40 and a term to represent the change per year after age 40.

Dense area was negatively associated with age, weight, number of live births, years of oral contraceptive use, and cessation of periods and positively associated with height, age at menarche, and years of hormone replacement therapy use (all $P < 0.05$). There was no evidence of an independent statistically significant association with age at first birth, pack-years of smoking, or years of alcohol consumption. From Table 2, the decrease in the residual variance (σ^2) from 0.63 in column 1 to 0.60 in column 2 indicates that adjusting for the additional significant variables accounted for <5% of the age-adjusted variance in dense area.

Nondense area was positively associated with age, weight, number of live births, and pack-years of smoking and negatively associated with height, age at menarche, and age at first birth (data not shown). These covariates accounted for ~50% of the mean age-adjusted variance in nondense area, and most of this was due to weight. There was no evidence of an independent association of nondense area with years of oral contraceptive use, years of hormone replacement therapy, years of alcohol consumption, and cessation of periods.

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Table 2. Estimates (SE) of residual variance (σ^2), of monozygous and dizygous covariances ($\sigma_{\text{monozygous}}^2$ and $\sigma_{\text{dizygous}}^2$, respectively), of monozygous and dizygous correlations ($\rho_{\text{monozygous}}$ and ρ_{dizygous}, respectively), and of heritability (H) under the AE model on log dense area and log dense area, separately

Estimate	Age adjusted for mean	Mean adjusted for age and covariates*	Random effect a function of age†
Log dense area			
σ^2	0.63 (0.023)	0.60 (0.022)	0.43 + 0.015 (age 40)
$\sigma_{\text{monozygous}}^2$	0.43 (0.025)	0.39 (0.024)	0.31 + 0.007 (age 40)
$\sigma_{\text{dizygous}}^2$	0.16 (0.030)	0.13 (0.029)	0.09 + 0.004 (age 40)
$\rho_{\text{monozygous}}$	0.67 (0.021)	0.66 (0.022)	0.73 − 0.005 (age 40)
ρ_{dizygous}	0.25 (0.046)	0.21 (0.047)	0.21 + 0.001 (age 40)
H	0.66 (0.022)	0.65 (0.024)	0.71 (0.039)
−2 Log-likelihood	668.1	585.8	531.8
Log nondense area			
σ^2	0.45 (0.017)	0.24 (0.0089)	0.28 − 0.004 (age 40)
$\sigma_{\text{monozygous}}^2$	0.34 (0.018)	0.16 (0.0096)	0.19 − 0.002 (age 40)
$\sigma_{\text{dizygous}}^2$	0.15 (0.021)	0.05 (0.012)	0.06 − 0.001 (age 40)
$\rho_{\text{monozygous}}$	0.76 (0.017)	0.67 (0.022)	0.67 − 0.0002 (age 40)
ρ_{dizygous}	0.34 (0.042)	0.21 (0.044)	0.23 − 0.002 (age 40)
H	0.75 (0.017)	0.66 (0.023)	0.67 (0.036)
−2 Log-likelihood	−162.7	−1,165.3	−1,178.8

*Adjusted covariates for mean log dense area were age, height, weight, age at menarche, number of live births, years of oral contraception use, years of hormone replacement therapy use, menopausal status, and population (Australian vs North American). Adjusted covariates for mean log nondense area were age, height, weight, age at menarche, number of live births, age at first birth, pack years of smoking, and population (Australian vs North American).
†These analyses allowed the random effects variables to vary as a linear function of age (centered on 40 y).

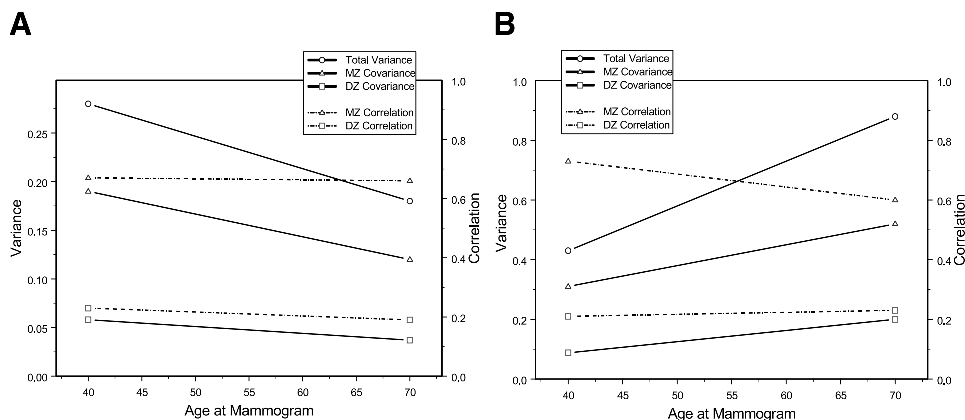

Figure 3. A. Variance of log of dense breast tissue area (*left y axis*) and estimates of correlation of the log of dense breast tissue area for MZ and DZ pairs (*right y axis*) by age at mammogram. **B.** Variance of log of nondense breast tissue area (*left y axis*) and estimates of correlation of the log of nondense breast tissue area for MZ and DZ pairs (*right y axis*) by age at mammogram.

Table 2 shows that for dense area, the correlations and covariances were greater for monozygous pairs than for dizygous pairs when adjusted for age alone (all $P < 0.001$). After additional adjustment for the measured significant covariates, the correlation for monozygous pairs was 0.66 (95% confidence interval, 0.62-0.70) and for dizygous pairs was 0.21 (95% confidence interval, 0.12-0.30). The ratio of monozygous/dizygous correlations was significantly greater than 2:1 ($P = 0.01$).

Table 2 also shows that for nondense area, the correlations and covariances were greater for monozygous pairs than for dizygous pairs when adjusted for age alone (all $P < 0.001$). After additional adjustment for the measured significant covariates, the correlation for monozygous pairs was 0.67 (95% confidence interval, 0.63-0.71) and for dizygous pairs was 0.21 (95% confidence interval, 0.12, 0.30). The ratio of monozygous/dizygous correlations was significantly greater than 2:1 ($P = 0.007$).

From the AE model, the estimates of heritability for dense area and nondense area were 66% and 75%, respectively, when adjusting for age alone and 65% and 66%, respectively, when adjusting for age and the other covariates (Table 2). The continuous lines in Fig. 3A and B show that based on column 3 of Table 2, for dense area, the total variance and the monozygous covariance increased with age ($P < 0.001$ and $P = 0.03$, respectively), and for nondense area, the total variance decreased with age ($P = 0.007$). The dotted lines, however, show that the monozygous correlations and the dizygous correlations did not change significantly with age (all $P > 0.05$). That is, as can be calculated from Table 2, the estimates of heritability under the AE model ranged from 0.71 at age 40 to 0.57 at age 70 for dense area, and from 0.67 at age 40 to 0.66 at age 70 for nondense area, but none of the changes in heritability estimates with age were statistically significant.

Table 3 shows that within the same individual, dense and nondense areas were negatively correlated, and these correlation estimates were similar whether the measures were adjusted for age alone (−0.35) or for age and other covariates (−0.31). The absolute age and covariate-adjusted cross-trait correlation was greater in monozygous pairs than in dizygous pairs (0.20 versus 0.08; $P = 0.009$), and the ratio of absolute correlations was not significantly different from 2:1 ($P = 0.08$). Under the classic twin model, this is consistent with additive genetic factors that influence both traits, but in opposite directions. These additive genetic factors explain the monozygous correlation of −0.2, which is about two thirds of the correlation of −0.3 within an individual; that is, these factors explain about two thirds of the covariance between the two traits. The correlation between the additive genetic factors that influence dense area and the additive genetic factors that influence nondense area was −0.30 (SE = 0.04). The correlation between the individual specific environmental factors that influence dense area and the individual specific environmental factors that influence nondense area was −0.31 (SE = 0.04).

Discussion

This study of 571 monozygous and 380 dizygous female twin pairs has shown that the mammographic areas of dense tissue and nondense tissue both have high heritability of about 65%; the same as has been previously reported for PMD. These findings pertained to both age-adjusted, and age- and covariate-adjusted mammographic measures. The two mammographic measures were negatively correlated within the same individual. The magnitude of the negative correlation between the two measures across twin pairs was greater in monozygous than dizygous pairs, suggesting that genetic factors explain two thirds of the within-individual correlation. These findings were consistent and similar whether using the Australian or North American samples.

The findings above have been derived under the assumptions of the classic twin model, which attributes greater correlations in monozygous pairs than dizygous pairs solely to genetic factors. This model makes the critical assumption that the strength of nongenetic factors common to twins within the same pair is independent of zygosity. To address this assumption, we have measured by questionnaire those factors known or thought to influence mammographic density, some of which are known to be more correlated within monozygous pairs than dizygous pairs. Adjusting for these in the analyses had minimal effect on the estimates of correlations, whether they be within-pair on the same trait, within individual on the same trait, or across traits on different individuals within a

Table 3. Estimates (SE) of the cross-trait correlation between dense and nondense area in the same individual and the cross-trait cross-twin correlation between dense area in one twin and nondense area in the other twin

Bivariate model: log dense and log nondense	Correlation in same individual	Cross-correlation	
		Monozygous pairs	Dizygous pairs
Mean adjusted for age	−0.35 (0.023)	−0.26 (0.026)	−0.14 (0.034)
Mean adjusted for age and covariates	−0.31 (0.023)	−0.20 (0.027)	−0.08 (0.036)

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twin pair. In no case did adjustment change the general conclusion that monozygous pairs had a statistically significant and greater (absolute) correlation than dizygous pairs. This does not preclude, however, the existence of unmeasured nongenetic factors that influence mammographic density and are more correlated in monozygous pairs.

The effect of any misclassification of monozygous and dizygous pairs due to errors in the twins' self-report of zygosity would decrease the observed monozygous pair correlation and increase the observed dizygous pair correlation. This would result in reduced power to detect a greater monozygous pair correlation and hence attenuate estimates of heritability towards the null. Our study of genetic markers found that, as have other studies (12), that misclassification is about 5%. Therefore, if the assumptions of the classic twin model are true, we may have slightly underestimated heritability.

The means and variances of both log dense area and log nondense area were found to depend on age. Mean dense area decreased with age, and after adjusting for this, the variance increased with age. Mean nondense area increased with age, and after adjusting for this, the variance decreased with age. The monozygous and dizygous pair covariances showed similar patterns with age as did the variances (see Fig. 3). Consequently, the twin pair correlations did not change substantially with age after allowing for the above age-related effects on both means and variances; hence, we ignored these age-related effects on second order statistics in the modeling of cross-trait correlations. These age-related effects, however, could be a consequence of interactions between the effects of genetic and environmental factors on dense area. This is because should the effect of genetic factors depend on environmental factors that change as an individual ages, the genetic variance will increase with age (19). For nondense area, however, the decrease in within pair covariances could be reflecting the dissipation as the twins' age of the effects (we have not measured) of shared childhood environment that may have been stronger within monozygous pairs than within dizygous pairs. That is, the evidence for genetic influences on variation with age is perhaps stronger for dense area than for nondense area. It is also possible, given that for both dense and nondense area the monozygous pair correlation was more than twice the dizygous pair correlation, there may also exist nonadditive genetic factors (e.g., dominance or epistasis effects) for these traits, although it is difficult to tease apart the effects of additive and nonadditive genetic factors due to them being so strongly confounded (20).

Results from the bivariate analyses provide further evidence of a strong genetic component in both mammographic measures. There was an inverse relationship between dense and nondense area; although both measures are related to the total area of the breast on a mammogram, one cannot presume that women with larger breasts would have both greater dense area and greater nondense area. Most of this inverse relationship was due to same genetic factors influencing both measures, but in opposite directions. The additive genetic factors for both measures were negatively correlated ($r = -0.30$), as were the individual specific environmental factors ($r = -0.31$).

There is wide variation in both dense and nondense area that is as yet unexplained. Most of this is likely to have a genetic etiology, a proportion due to different genes influencing the two measures. There may, however, be unmeasured genetic factors that have opposite effects on dense and nondense area and therefore have a compounded effect on PMD, a strong risk factor for breast cancer. Efforts to identify the genes involved with mammographic density should involve analysis of both dense and nondense areas and PMD. They should also take into account the genetic architecture revealed by this twin study, searching also for the genes that have opposite effects on dense and nondense areas.

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