The Relative Importance of Genetics and Environment on Mammographic Density

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Abstract

Background: **Although several environmental factors predict mammographic density, estimates of its heritability have been quite high. We investigated whether part of the presumed heritability might be attributed to differential sharing of modifiable risk factors in monozygotic (MZ) and dizygotic (DZ) twins.**
Methods: **We measured percent and absolute mammographic density using mammograms from 257 MZ and 296 DZ twin pairs. The correlation of intrapair mammographic density was compared according to zygosity across strata of modifiable risk factors. Portions of variance attributable to additive genetic factors, shared environment, and individual environment were calculated using a variance component methodology in the entire set, and within twin pairs stratified by environmental trait similarity.**
Results: **Both percent density and absolute mammographic density were more highly correlated between MZ twins than DZ twins, but the correlations varied across strata. Body mass index (BMI) and parity strongly predicted differences in mammographic density within MZ twin pairs. After adjusting for covariates, 53% of the total variance in percent density and 59% of that in absolute density seemed attributable to genetic effects, but these estimates varied greatly by stratum. For twins dissimilar on BMI (difference >2.5 kg/m^2), the additive genetic component of absolute density was estimated at only 20% (±19%), and the common and individual environment at 21% (±14%) and 49%, respectively (*P* value for heterogeneity across BMI = 0.0001).**
Conclusion: **Our results confirm that the genome is an important determinant of mammographic density but suggest that an unknown portion of the mammographic density effect attributed to the genome may be due to shared modifiable environmental factors.** (Cancer Epidemiol Biomarkers Prev 2009;18(1):102–12)

Introduction

Mammographic density is a strong, independent risk factor for breast cancer (1-10); risk is 4- to 6-fold higher in women with very high density compared with those with very low density. Although most studies use percent mammographic density, the absolute amount of densities is also associated with breast cancer risk (5). Observational studies indicate that percent mammographic density is associated with reproductive factors and lifestyle events (11-13). Clinical trials show an increase in percent mammographic density after estrogen-progestin therapy (14, 15) and a decrease after suppression of ovulation (16, 17).

Mammographic density also is heritable. A small twin study first showed mammographic parenchymal patterns to be more similar between monozygotic (MZ) than dizygotic (DZ) twins (18). A study of familial aggregation found sisters, but not mothers, to be correlated on the fraction of the breast volume assessed by a radiologist to contain radiographically dense tissue (19). A subsequent analyses of twins from North America and Australia reported that the correlation of percent dense tissue was twice as high within MZ twin pairs (0.63) as within DZ pairs (0.27) after adjustment for such covariates as age, body mass index (BMI), age at menarche, cessation of menstruation, parity, number of live births, and age at first birth (20). Using a classic twin covariance model, and making the ''equal environment'' assumption (21) that the effects of shared nongenetic factors are identical for MZ and DZ twin pairs, ''heritability'' (''the proportion of variance attributable to additive genetic factors'') was estimated to be in the range of 63% [95% confidence interval (95% CI), 59-67%].

We sought to confirm the finding that percent mammographic density is determined largely by genetic factors, and to reassess the equal environment assumption, by examining the proposition that the members of identical and fraternal twin pairs are equally likely to share environmental determinants of density. If part of the presumed genetic component is due to sharing of other modifiable risk factors, then this would be important to determine for genetic research projects trying to identify the genetic basis for mammographic density.

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Materials and Methods

The California Twin Program (22) was created by linking a file from the California Department of Vital Statistics with records of live multiple births between 1908 and 1982 to a file of active California drivers' licenses, identifying 271,047 native California twins, among whom valid addresses were available for 115,733. A 16-page questionnaire covering perceived zygosity (23) and reproductive, familial, lifestyle, and medical characteristics (including history of mammography) was sent to each twin. A total of 51,609 individuals, representing ~32,000 pairs, completed and returned the questionnaire. Participants were representative of native California twins by age according to both zygosity and geographic location of residence (22). The response rate for female twins born in 1948 to 1977 was 47.9%, based on 21,362 respondents. Throughout this article, we use the term ''zygosity'' to indicate perceived zygosity, which was assessed based on responses to validated questions (23).

During the years 1997 to 2000, we identified and sent informed consents and mammogram release forms to the 3,246 members of doubly respondent female MZ or like-sex DZ twin pairs from which at least one twin reported having had a mammogram and who reported no history of breast cancer. We interviewed all willing participants by telephone and obtained information about breast cancer risk factors, reproductive variables, hormone therapy (HT) use (brand name, period of use, and dose), and the approximate date and place of all mammograms taken over the past 5 y.

Of the 3,246 women, 981 could not be reached (629 with inadequate contact information, 166 with incorrect telephone numbers, and 186 who failed to answer after five attempts). We successfully contacted or located 2,265 individuals, of whom 1,726 agreed to participate (30 had died, 213 had no available mammograms, and 296 were either uninterested in participation or had a co-twin lacking either interest or a mammogram). The signed consent forms of 70 of the 1726 women were never received. Thus, among those who were eligible for the study (2,022) and were located, we had 1,656 participate for a response rate of 81.9%.

We contacted the women's health care provider or mammogram facility, and borrowed and scanned a total of 2,881 mammograms, of which 24 were of unreadable quality. The 2,857 usable mammograms represented both members of 295 MZ pairs and 331 DZ pairs (1,252 individuals). For each pair of twins, we selected the most recent mammograms that had been obtained closely together in time. If more than one matched set was available from the same year, we chose the most recent.

Selecting the craniocaudal mammograms of the same breast for each pair (left by default, right if a left breast had been biopsied), each film was digitized using a Cobrascan CX312T scanner (Radiographic Digital Imaging) at a resolution of 150 pixels/inch (59 dots/cm). Films were read in batches with mammograms from paired twins in random sequence in the same batch, and with the reader unaware of pair identity or zygosity. Density was quantified using the Madena assessment method (17). After a reader (trained by G.U.) outlined the total area of the breast using a computerized tool, software was used to count the number of pixels within the outline. Mammographic density was then assessed (by G.U.), first, by identifying the region of interest, incorporating all dense areas excepting those representing the pectoralis muscle and other scanning artifacts, and then applying a yellow tint to all pixels within the region of interest shaded at or above a threshold intensity of gray. The software then counted the tinted pixels, which represent the area of absolute density. Percent density equals the amount of absolute density divided by the total breast area. Test-retest reliability was 0.95 for absolute density and 0.94 for percent density.

Women reporting hormone use at the time of mammogram were considered to be ''hormone users,'' regardless of their menopausal status. Current nonusers either were considered postmenopausal if their menstrual periods had ''completely'' stopped naturally, surgically, or pharmacologically or, if not, were considered ''premenopausal or perimenopausal.''

For women who had undergone hysterectomy and were not aware of their menopausal status, we considered them as postmenopausal if they were age $\geq$55 or had used hormone therapy (HT) at the time of mammogram.

Ideally, we would have wanted a measure of how closely the environment was shared when the twins were young. If the equal environment hypothesis was wrong, we would have expected ''sharing of the environment'' to be as strong a predictor of similarities in densities as zygosity. We had no such variable, but instead, we classified twins on current social ''closeness'' (24) using the following question: ''How often do you see, call, or write each other? Daily, weekly, monthly, every few months, at least yearly, less than yearly?'' Those communicating at least weekly were considered ''close'' and the remainder ''not close.'' A similar dichotomy has been used previously (25).

Statistical Analysis. To obtain a consistent number of women for the analyses, we made the following exclusions: Among the 1,252 participants, 32 women had previously been diagnosed with breast cancer and 1 woman had previously used tamoxifen. Exclusion of these women and their co-twins resulted in the exclusion of 31 pairs (n = 62 individuals). Further, we excluded women (and their co-twins) who had missing information on key covariates such as age (18 pairs), body mass index (BMI) (4 pairs), parity (15 pairs), and menarche (5 pairs), leaving 1,106 participants for our analyses.

We compared the mean values of continuous risk factors according to zygosity using t tests. As a measure of twin concordance in density, we correlated the density values (using Spearman's correlation coefficients with corresponding 95% CIs) between paired MZ and DZ twins and then repeated the estimates after stratifying the twins on the following characteristics: BMI, menopausal status, parity, and, for identical pairs, intrapair social closeness.

We used generalized estimation equations to obtain adjusted regression coefficients for various covariates using percent and absolute density as the dependent variable. These coefficients take into account the correlation between twins.

Additionally, using a standard matched case-control analysis with conditional logistic regression, we calculated the odds ratio (OR) for having the more dense mammogram within a MZ pair in relation to the members' difference in environmental experience. For

Table 1. Comparison of selected risk factors between MZ and DZ individual twin participants in the current mammographic density study and all other twins in the California Twin Study

	MZ		DZ	
	Participants	Other California twins	Participants	Other California twins
Age on 12/31/99				
<51	208 (40.5%)	1,126 (73.5%)	284 (48.0%)	1,036 (68.8%)
51-60	288 (56.0%)	340 (22.2%)	278 (47.0%)	408 (27.1%)
>60	18 (3.5%)	66 (4.3%)	30 (5.1%)	62 (4.1%)
*P**		<0.001		<0.001
BMI †				
<26	331 (64.5%)	959 (63.5%)	380 (64.6%)	972 (65.5%)
26-29	73 (14.2%)	264 (17.5%)	89 (15.1%)	221 (14.9%)
≥30	109 (21.3%)	287 (19.0%)	119 (20.2%)	291 (19.6%)
*P**		0.18		0.93
Hip/waist ratio				
Hips > waist	467 (91.0%)	1,292 (85.5%)	538 (91.5%)	1,290 (87.0%)
Hips = waist or hips < waist	46 (9.0%)	220 (14.6%)	50 (8.5%)	192 (13.0%)
*P**		0.001		0.005
Alcohol use monthly				
0-2 drinks	251 (50.2%)	848 (56.5%)	282 (48.8%)	826 (56.3%)
>2 drinks	249 (49.8%)	654 (43.5%)	296 (51.2%)	641 (43.7%)
*P**		0.015		0.002
Age of menarche				
≤11	117 (22.8%)	295 (19.5%)	129 (21.8%)	328 (22.1%)
12	140 (27.2%)	459 (30.3%)	176 (29.7%)	420 (28.2%)
13	159 (30.9%)	482 (31.8%)	199 (33.6%)	459 (30.8%)
≥14	98 (19.1%)	279 (18.4%)	88 (14.9%)	282 (18.9%)
*P**		0.33		0.15
No. live births				
0	132 (25.8%)	410 (27.3%)	116 (20.0%)	395 (26.8%)
1-2	292 (57.0%)	796 (53.0%)	339 (57.6%)	764 (51.8%)
≥3	88 (17.2%)	297 (20.0%)	134 (22.8%)	316 (21.4%)
*P**		0.24		0.003
Menopausal status †				
Premenopausal	368 (91.8%)	1,280 (94.8)	436 (92.8%)	1,229 (94.8%)
Postmenopausal	33 (8.2%)	70 (5.2)	34 (7.2%)	68 (5.2%)
*P**		0.023		0.11

*χ^2 *P* value.
† At the time of original twin questionnaire.

example, we estimated the OR of being the twin with the densest mammogram (outcome) associated with being the twin who had the highest level of each environmental exposure among twins discordant on that exposure. Each environmental factor was examined thrice using three different definitions of density change: (*a*) for all pairs differing in mammographic density regardless of the magnitude, (*b*) for pairs with a mammographic density difference of ≥5% (thus, excluding pairs with less than a 5% difference in density), and (*c*) for pairs with a mammographic density difference of ≥10% (excluding those with less than a 10% difference). These analyses were adjusted for age and BMI. Further adjustment for parity or menopausal status/HT use did not change the results, and these results are therefore not presented in this report.

We used a variance component methodology to calculate the variation in mammographic density attributable to (A) additive genetic effects (''heritability''), (C) common (shared) environment, and (E) unique individual twin environment. We chose a priori to include the C term because certain known determinants of breast cancer (and presumed determinants of mammographic density), including weight gain, parity (family size), and alcohol/exogenous hormone usage, are likely to be influenced by parents or peers. All variance components were estimated using the ''Sequential Oligogenic Linkage Analysis Routines'' (SOLAR) package[8] (26). The variance component algorithm was previously available as Fisher. Three models were run for each measure of density: a crude model, a model adjusted for age at mammogram only, and a model adjusted for all reported covariates (BMI, age at menarche, age at mammogram, parity, and menopausal status/hormone use). In each analysis, variance components were estimated as the fraction of residual variation explained and then recalculated based on a new total variance obtained by including the additional variance attributable to covariates (COV). The fitted models constrained A, C, and E to be nonnegative. The variances associated with A, C, and COV were then subtracted from unity to infer the proportion of variance attributable to E. When C was estimated to be equal to 0, it was dropped from the model.

We also ran analyses where the variance estimates were allowed to vary across strata of important mammographic density risk factors (parity, BMI, etc.). We ran a likelihood ratio test where we compared the log

[8] http://www.sfbr.org/solar/

likelihood of the model combining the strata with the sum of the log likelihoods when we let the variable vary across strata. The degrees of freedom were estimated as (number of strata − 1) * (variance component + number of covariates − 1). The *P* values from this likelihood ratio test indicate whether the estimated components of the variance varied across strata.

Results

Participants in the California Twin Study have previously been found to be representative of native California twins with respect to age, sex, race, and geographic residence (22). The few differences between subjects in the current study and all female twins of the original cohort are summarized in Table 1. Women included in this study, having had mammograms, were slightly older and more likely to be postmenopausal than other women in the cohort. They also drank more alcohol and were somewhat more likely to have a higher hip/waist ratio than nonparticipants. Participating MZ and DZ twins were generally similar except that DZ twins had given birth slightly more often than nonparticipants. We examined the distribution of time intervals between paired mammograms and found no differences between those for MZ and DZ twins. Although two thirds of paired mammograms were obtained in different calendar years, the intrapair difference in density was independent of the length of the interval between them (data not shown).

Table 2 shows the zygosity-specific differences in risk factors for high mammographic density. On average,

Table 2. Breast cancer risk factors and social closeness in 1,106 individual twins by zygosity

Characteristic	MZ (n = 514; 257 pairs)	DZ (n = 592; 296 pairs)	P*
	Mean (SD)	Mean (SD)	
Age at interview (y)	51.6 (4.9)	51.3 (5.2)	0.30
Range	43-69	43-75	
Age at mammography (y)	50.5 (5.0)	50.0 (5.3)	0.15
Range	40-67	39-74	
Interval between interview and mammography (y)	1.2 (1.2)	1.3 (1.2)	0.22
No. mammograms in last 5 y	3.4 (1.6)	3.4 (1.5)	0.96
BMI	25.9 (5.8)	25.8 (5.6)	0.78
Age at menarche (y)	12.4 (1.2)	12.4 (1.2)	0.45
No. live births (among parous women) †	2.7 (1.3)	2.8 (1.4)	0.21
Percentage of dense tissue (percent mammographic density)	29.7 (20.1)	29.8 (21.6)	0.94
Difference in percent mammographic density between twins ‡	10.7 (9.4)	18.7 (15.0)	<0.0001
Absolute dense tissue (cm^2)	31.9 (24.7)	32.9 (26.6)	0.52
Difference in absolute dense tissue between twins (cm^2) ‡	13.0 (14.4)	20.4 (20.2)	<0.0001
Absolute nondensity (cm^2)	91.7 (57.6)	95.2 (64.7)	0.34
Difference in absolute nondense tissue between twins (cm^2) ‡	29.5 (30.2)	53.1 (48.1)	<0.0001
	n with characteristic (%)	n with characteristic (%)	
European ethnicity	502 (97.7%)	574 (97.0%)	0.47
Parous	419 (81.5%)	516 (87.2%)	0.010
Alcohol, >2 drinks in the last month	238 (52.5%)	278 (54.3%)	0.58
Smoking, smoked within last 6 mo	49 (9.5%)	93 (15.7%)	0.002
Postmenopausal			0.16
Premenopausal	186 (36.2%)	242 (40.9%)	
Postmenopausal	288 (56.0%)	314 (53.0%)	
Unknown§	40 (7.8%)	36 (6.1%)	
Ever hormone use (among postmenopausal women)	241 (83.7%)	257 (82.6%)	0.73
Type of hormone (among ever hormone users)			
% most recent contained E only	124 (53.7%)	117 (48.0%)	0.40
% most recent contained P only	17 (7.4%)	17 (7.0%)	
% most recent contained E+P	90 (39.0%)	110 (45.1%)	
Hormone use at mammogram (among postmenopausal women)	197 (71.9%)	207 (68.1%)	0.32
Type of hormone (among current hormone users)			
% E only	102 (52.6%)	101 (50.3%)	0.50
% P only	14 (7.2%)	10 (5.0%)	
% E+P	78 (40.2%)	90 (44.8%)	
Closeness, no. twin pairs report being close			
Both report being close	173 (67.8%)	139 (47.9%)	
One report being close	28 (11.0%)	40 (13.8%)	
Both report not being close	54 (21.2%)	111 (38.3%)	<0.0001

NOTE: Numbers do not add up due to missing information on number of mammograms in the last 5 y (1 MZ and 1 DZ individual), alcohol use (61 MZ and 80 DZ individuals), ever hormone use (3 DZ individuals), type of most recent hormone (9 MZ and 13 DZ individuals), hormone use at mammogram (14 MZ and 10 DZ individuals), type of current hormone (3 MZ and 6 DZ individuals), and closeness (2 MZ pairs and 6 DZ pairs).

*P value from t test to compare means or χ^2 to compare frequencies.

† Among 419 MZ and 516 DZ parous individuals.

‡ Absolute difference in percent and absolute mammographic density and absolute nondense tissue (mammographic density in twin with highest density minus mammographic density in twin with lowest density).

§ Unknown category is excluded in the χ^2 test.

Table 3. Association between breast cancer risk factor, zygosity, and frequency of contact between twins

Variable	Zygosity		Closeness	
	MZ	DZ	Weekly contact	Less often
BMI absolute difference				
≤2.5	168 (65.4%)	132 (44.6%)	202 (56.9%)	98 (49.5%)
≥2.6	89 (34.6%)	164 (55.4%)	153 (43.1%)	100 (50.5%)
*P**		<0.0001		0.094
Alcohol use				
Both >2 drinks monthly or ≤2 drinks monthly	143 (70.1%)	131 (58.0%)	189 (68.7%)	85 (54.8%)
Discordant	61 (29.9%)	95 (42.0%)	86 (31.3%)	70 (45.2%)
*P**		0.009		0.004
Smoking				
Both smoke or both do not smoke	232 (90.3%)	231 (78.0%)	305 (85.9%)	158 (79.8%)
Discordant	25 (9.73%)	65 (22.0%)	50 (14.1%)	40 (20.2%)
*P**		0.0001		0.062
Menarche				
Within 1 y	133 (51.8%)	81 (27.4%)	144 (40.6%)	70 (35.4%)
≥1 y difference	124 (48.2%)	215 (72.6%)	211 (59.4%)	128 (64.6%)
*P**		<0.0001		0.23
Nulliparity				
Both nulliparous or both parous	190 (73.9%)	230 (77.7%)	261 (73.5%)	159 (80.3%)
Discordant for parity	67 (26.1%)	66 (22.3%)	94 (26.5%)	39 (19.7%)
*P**		0.30		0.074
Parity (among parous pairs) †				
Both 1-2 or both ≥3	93 (52.8%)	125 (55.6%)	141 (57.6%)	77 (49.4%)
Discordant	83 (47.2%)	100 (44.4%)	104 (42.4%)	79 (50.6%)
*P**		0.59		0.11
Menopause				
Both pre or post	178 (69.3%)	197 (66.6%)	239 (67.3%)	136 (68.7%)
Discordant	42 (16.3%)	66 (22.3%)	67 (18.9%)	41 (20.7%)
Any unknown ‡	37 (14.4%)	33 (11.1%)	49 (13.8%)	21 (10.6%)
*P**		0.11		0.75
Ever use of HT (among postmenopausal pairs)§				
Both never or both ever users	96 (86.5%)	83 (72.2%)	114 (78.6%)	65 (80.2%)
Discordant	15 (13.5%)	32 (27.8%)	31 (21.4%)	16 (19.8%)
*P**		0.008		0.77

NOTE: Numbers do not add up due to missing information on alcohol use (53 MZ pairs and 70 DZ pairs) and hormone use (2 DZ pairs).
*χ^2 *P* value.
†Among 176 MZ and 225 DZ parous pairs.
‡Unknown categories are excluded in the χ^2 tests.
§Among 111 MZ and 117 DZ postmenopausal pairs.

fewer MZ twins had smoked within 6 months before mammogram compared with DZ twins ($P = 0.002$), and MZ twins were less likely to be parous ($P = 0.01$), although among the parous, the average parity did not differ by zygosity. The average percent mammographic density in MZ and DZ twins was essentially identical (29.7% and 29.8%). The average absolute density was very similar between MZ and DZ twins (MZ = 31.9 cm^2, DZ = 32.9 cm^2), as was the nondense area (MZ = 91.7 cm^2, DZ = 95.2 cm^2). DZ pairs tended to differ more than MZ pairs with respect to absolute dense area, nondense area, and percent density between paired twins ($P < 0.0001$).

MZ twins were more likely to be concordant for BMI, alcohol consumption, smoking, age at menarche, and hormone use than DZ twins (Table 3). We made similar comparisons using the ''social closeness'' variable. For some variables such as alcohol intake and parity, the propensity for twins to share the same risk factor level differed as much or more according to the frequency of social contact as it did by zygosity. Closeness, however, could measure many different risk factors, and in the following, we therefore proceeded with strata of more defined risk factors known to influence mammographic density.

The correlation between both the percent and the absolute mammographic density of paired twins was much higher for MZ pairs than for DZ pairs (Table 4). However, these unadjusted correlations were higher within twin pairs with similar risk characteristics on parity, BMI, and smoking than within pairs discordant for the same factor, especially within DZ pairs. For instance, the within-pair correlations in percent density among twins with similar BMI (<1.1 kg/m^2 difference) were 0.73 for percent density in MZ twins and 0.54 for DZ twins, whereas they were 0.43 for MZ twins and 0.16 for DZ twins discrepant for BMI (>5.0 kg/m^2).

In a regression analysis that takes into account the correlation between twins, the cofactors that most strongly predicted percent mammographic density were BMI, parity, menopausal status/HT use, as well as age and age at menarche (Table 5). For absolute density, the most important factors were BMI and menopausal status/HT use.

To evaluate the effect of modifiable environmental or nonheritable factors independent of any confounding by genetic factors, we made comparisons within pairs of identical twins. Table 6 provides the results of conditional logistic regressions between paired MZ twins using the twin with the more dense mammogram as the

''case.'' The results show strong and significant associations between various risk factors and mammographic density among MZ twins. For instance, MZ twins who were 2.5 kg/m^2 heavier than their matched twin were at 41% to 63% significantly reduced risk of having the highest percent density compared with their twin (depending on the definition used for the most dense mammogram). Women who were nulliparous were at least twice as likely to have the highest percent density as their twin. The results were essentially unchanged when adjusted for additional risk factors (results not shown).

Table 7 provides the results of the standard twin ANOVAs based on the ''equal environment'' assumption that differences in exposure to nonheritable factors between the members of MZ twin pairs are similar to those between the members of DZ twin pairs. With adjustment for age only, 75% of the variance in percent density, and 63% of the variance in absolute density, was attributed to additive genetic effects (i.e., due to differences in zygosity). However, after adjustment for exposure variables that are known to influence mammographic density, and presumed to be nonheritable, the genetic or ''heritable'' component of the total variance was reduced to 53% for percent density and 59% for absolute density. In other words, there was a 22% reduction in the presumed genetic component of the total variance for percent density after adjusting for these various covariates. Thirty-one percent of the variance in absolute nondensity was attributed to the genetic component after adjustments (results not shown). After adjustments, the remaining components of variance in percent density were individual environment (25%) and covariates (20%). Of this 20% total variance due to covariates, BMI explained ~14% of the variance in percent mammographic density, whereas the other factors parity, alcohol, age, age at menarche, hormone use, and menopausal status each explained <1%. The heritable component was similar, although slightly lower in postmenopausal than in premenopausal women.

If the equal environment assumption were to hold, the results should be similar across strata defined by environmental risk factors. However, the variance components varied significantly across strata of BMI, parity, and menopausal status/hormone use (all likelihood ratio Ps $\leq$ 0.01) for both percent and absolute

Table 4. Pairwise correlations of mammographic density by zygosity and breast cancer risk factors (n = 1,106; 553 pairs)

	N	N	Percent density		Absolute density	
	MZ	DZ	MZ Sp R (95% CI)	DZ Sp R (95% CI)	MZ Sp R (95% CI)	DZ Sp R (95% CI)
All twins	257	296	0.74 (0.68-0.79)	0.38 (0.28-0.47)	0.69 (0.61-0.75)	0.47 (0.37-0.55)
BMI absolute difference						
<1.1	90	55	0.73 (0.62-0.81)	0.54 (0.31-0.70)	0.78 (0.68-0.85)	0.59 (0.38-0.74)
1.1-2.5	78	77	0.81 (0.72-0.87)	0.45 (0.25-0.61)	0.76 (0.64-0.84)	0.51 (0.32-0.66)
2.6-5.0	51	77	0.72 (0.55-0.83)	0.29 (0.07-0.48)	0.69 (0.50-0.81)	0.41 (0.21-0.58)
>5.0	38	87	0.43 (0.12-0.66)	0.16 (−0.06-0.36)	0.36 (0.04-0.61)	0.38 (0.18-0.55)
Age at menarche						
Within 1 y	133	81	0.72 (0.63-0.79)	0.28 (0.07-0.47)	0.71 (0.61-0.78)	0.44 (0.25-0.60)
≥1 y difference	124	215	0.76 (0.67-0.82)	0.41 (0.29-0.51)	0.67 (0.55-0.75)	0.48 (0.37-0.57)
Nulliparity						
Both nulliparous	14	5	0.82 (0.50-0.94)	1	0.78 (0.39-0.92)	1
Both parous	176	225	0.73 (0.65-0.79)	0.42 (0.31-0.52)	0.67 (0.58-0.75)	0.50 (0.39-0.59)
Discordant	67	66	0.73 (0.59-0.82)	0.12 (−0.13 to 0.35)	0.65 (0.48-0.77)	0.29 (0.05-0.49)
Parity (among parous pairs)						
Both 1-2 pregnancies	47	62	0.73 (0.55-0.84)	0.51 (0.30-0.67)	0.67 (0.47-0.80)	0.53 (0.32-0.69)
Both ≥3 pregnancies	46	63	0.78 (0.63-0.87)	0.36 (0.12-0.56)	0.67 (0.46-0.80)	0.42 (0.19-0.60)
Discordant	83	100	0.70 (0.57-0.80)	0.40 (0.22-0.55)	0.66 (0.52-0.77)	0.53 (0.37-0.66)
Alcohol use*						
Both >2 drinks monthly	79	80	0.80 (0.71-0.87)	0.34 (0.13-0.52)	0.82 (0.73-0.88)	0.44 (0.24-0.60)
Both ≤2 drinks monthly	64	51	0.72 (0.58-0.82)	0.40 (0.14-0.61)	0.66 (0.48-0.77)	0.46 (0.20-0.65)
Discordant	61	95	0.71 (0.56-0.82)	0.36 (0.17-0.52)	0.56 (0.35-0.71)	0.41 (0.23-0.56)
Smoking						
Both current smoker	12	14	0.79 (0.37-0.93)	0.60 (0.09-0.85)	0.78 (0.33-0.93)	0.42 (−0.15 to 0.77)
Both do not smoke	220	217	0.75 (0.68-0.80)	0.37 (0.25-0.48)	0.69 (0.62-0.76)	0.49 (0.38-0.58)
Discordant	25	65	0.58 (0.23-0.79)	0.30 (0.06-0.51)	0.50 (0.12-0.74)	0.37 (0.14-0.56)
Menopause						
Both pre	67	80	0.66 (0.49-0.77)	0.35 (0.14-0.53)	0.56 (0.37-0.71)	0.47 (0.27-0.62)
Both post	111	117	0.71 (0.61-0.79)	0.38 (0.22-0.53)	0.68 (0.56-0.77)	0.40 (0.23-0.54)
Discordant	42	66	0.72 (0.52-0.84)	0.33 (0.10-0.53)	0.76 (0.58-0.86)	0.51 (0.30-0.66)
Any unknown	37	33	0.70 (0.48-0.83)	0.33 (−0.02 to 0.60)	0.70 (0.48-0.83)	0.34 (−0.01 to 0.61)
HT use (among postmenopausal pairs)						
Both current HT users	64	52	0.64 (0.47-0.77)	0.40 (0.14-0.60)	0.60 (0.42-0.74)	0.39 (0.12-0.60)
Both nonusers	17	13	0.59 (0.13-0.83)	0.74 (0.28-0.91)	0.65 (0.23-0.86)	0.74 (0.28-0.91)
Discordant for current HT use	23	46	0.85 (0.66-0.93)	0.25 (−0.05 to 0.50)	0.87 (0.70-0.94)	0.33 (0.04-0.56)
Any unknown	7	6	0.86 (0.23-0.98)	0.54 (−0.52 to 0.93)	0.64 (−0.26 to 0.93)	0.94 (0.49-0.99)

Abbreviation: Sp R, Spearman's correlation coefficient.
*Fifty-three MZ pairs and 70 DZ pairs were excluded due to missing information on their alcohol use or their co-twin's alcohol use.

Table 5. Multivariable regression analysis (generalized estimating equation) to determine effect of various risk factors on mammographic density after adjustment for twin status (n = 1,106)

Variable	Percent density			Absolute density		
	β	SE	P*	β	SE	P*
Age at mammogram	−0.23	0.13	0.072	−0.18	0.17	0.28
BMI	−1.57	0.10	<0.0001	−0.64	0.15	<0.0001
Parous (y/n)	−4.25	1.45	0.003	−2.66	2.17	0.22
Number of additional births	−0.99	0.41	0.016	−1.02	0.55	0.063
Age at menarche	0.89	0.47	0.060	0.17	0.63	0.79
Menopausal status/current HT use						
Premenopausal, no current HT use	Reference			Reference		
Postmenopausal, not current HT user	−6.74	1.76	0.0001	−9.58	2.38	<0.0001
Postmenopausal, current HT user	−5.75	1.34	<0.0001	−7.58	1.89	<0.0001
Unknown	−1.03	2.00	0.61	−2.29	2.87	0.43

*P values are based on Z test in the generalized estimating equation model.

density, and for absolute density, the estimates also varied across strata of menarche and alcohol (Table 7). Among twins that were very similar on environmental factors, such as BMI (<1.1 unit difference between the twins), parity (both nulliparous or both had one to two children), and hormone use (both being nonusers), there was a strong contribution of ''common'' environmental factors to percent mammographic density, and the ''heritable'' component of mammographic density tended to be lower. On the other hand, when twins were known to be very discordant for an environmental determinant of mammographic density, the role of the unique environment increased substantially, and in some of these strata, the heritable component was lower. This contribution of the individual environment was particularly striking for absolute density for women discordant on alcohol intake, BMI, or nulliparity. For twins with a BMI difference of ≤1.1 kg/m^2, the estimate of heritability in absolute density was 57% (±19%), whereas for those with differential BMI (difference >2.5 kg/m^2), the estimate was 20% (±19%).

If we adjust for the 18 comparisons made in this table using a Bonferroni adjustment, the P values for alcohol and parity (nulliparous, parous) for absolute density and BMI, menarche, menopausal status, and HT use for both absolute and percent density would still have been statistically significant [$P < 0.0028$ (=0.05/18)].

Discussion

Here, we have observed, as did Boyd and colleagues (20), that mammographic density correlates more strongly between the members of identical than the members of fraternal twin pairs. Our measure of an overall estimate for the heritable component of 53% for percent and 59% for absolute mammographic density is similar to their estimates of 63% (20) and 65% (27). If we were to ignore the part of the variance explained by the covariates, and estimate the fraction of A divided by the sum of A, C, and E, this would yield 67%, again similar to the previously published result of 63%. Accordingly, the proportion of the variance in the heritability measure among twins suggests that the level of mammographic density is under strong genetic control. However, our results also suggest that a portion of this apparently heritable variation may be due to variation in potentially modifiable factors. We show that BMI and parity, two factors long recognized to predict mammographic density (11), actually do so even within identical twin pairs. Moreover, both the intra-twin correlation between individual levels of density and the proportion of variance attributable to zygosity (and therefore heritability) are shown to vary according to how similar the twins are on presumed nonheritable factors. These inconsistencies are especially true for absolute density and among DZ twins.

The assumption of equal environment implies that greater similarity between the members of identical twin pairs, relative to the members of fraternal pairs, is a reflection of the more complete identity of their genomes (21). There is evidence that MZ twins, even during childhood, are treated differently by their parents, teachers, and peers than DZ twins (28). However, even if the experience of the home environment during childhood was equitable among DZ and MZ twins, fraternal twins are likely to grow dissimilar more rapidly than identical twins as they mature, and as their distinct identities become more apparent to themselves and to others. In contrast, the likeness of identical twins is inevitably recognized by the twins themselves and is reinforced by others, leading to behavioral dissimilarity between paired twins according to zygosity. For example, whereas the identical co-twin of a smoker is 40% more likely to initiate smoking (24) than the same-sex fraternal twin of a smoker, the identical twin who remains in close contact with a smoking co-twin is 30% more likely to initiate smoking than one who remains socially distant, and identical female twins are 20% more likely to remain close than fraternal female twins (24). Thus, known behavioral predictors of a disease are likely to be shared more often by identical than fraternal twins. As indicated by the findings in Tables 2 and 3, reproductive and lifestyle characteristics in this population follow this prediction.

It has previously been pointed out that there is no powerful way to distinguish common environment from additive genetic factors without twins reared apart and extensive environmental variables (29). We suggest that an unknown portion of the effect conventionally attributable to the common genome of identical twins may therefore be due to common modifiable factors. We showed a large reduction in the proportion of the variance attributed to heritability when we controlled

for known environmental factors (for percent density, the proportion of the variance attributed to heritability declined from 75% in the model adjusted only for age to 53% in the model adjusted for other known risk factors, whereas for absolute density the adjusted estimate was 59%). Further, we observed that factors known to predict mammographic density, such as parity and BMI, also did so within MZ pairs. Finally, when we ran stratified analyses, restricting these to twin pairs extremely dissimilar on BMI, the heritability estimate for percent density was 42% and absolute density was as low as 20%. Our findings suggest that ''adjustments'' in the heritability model cannot be perfect. The inevitable misclassifications and deviations from average dose, together with the assumptions required by the model, probably combine to prevent complete elimination of the confounding due to environment (30). Although the true proportion of variance attributable to the genome is substantial, it cannot be accurately measured, and may be well under 50%.

The heritability estimates varied according to strata of environmental factors for both absolute and percent density, but there were some differences in our results for these two measures of mammographic density. The difference was particularly striking for BMI, but this is as expected because percent density already has partially adjusted for the confounding effect of BMI by dividing by the breast size as it appears on the mammogram. Although percent density is the most commonly used measure of mammographic density, absolute density is also associated with breast cancer risk (5, 7, 31). Given that absolute density may be a marker of not only the amount of stroma but also the number of epithelial cells in the breast (32), it is possible that absolute density is the most important etiologic variable.

Certain of the phenotypic factors we found to be environmentally determined may be at least partly determined genetically. BMI is one example. Although the trends in obesity over time are clearly an environmental phenomenon, there are well known genes that

Table 6. OR of being the MZ twin with the highest mammographic density (defined as any fraction of a % or cm^2 higher, 5% higher, or 10% higher) associated with various risk factors

Risk factors/traits the MZ pairs were discordant for* (definition of highest mammographic density)	Pairs	Greater percent density	Greater absolute density
		OR (95% CI)	OR (95% CI)
BMI >2.5 greater			
Any difference in mammographic density †	88	0.59 (0.39-0.92)	0.68 (0.45-1.05)
At least 5% difference ‡	57	0.50 (0.29-0.86)	0.61 (0.36-1.05)
At least 10% difference ‡	41	0.37 (0.18-0.73)	0.41 (0.21-0.81)
Menarche >1 y earlier			
Any difference †	122	1.02 (0.71-1.47)	1.00 (0.70-1.44)
At least 5% difference ‡	78	0.90 (0.55-1.45)	1.06 (0.67-1.69)
At least 10% difference ‡	49	0.73 (0.37-1.41)	0.84 (0.43-1.62)
Nulliparous vs parous			
Any difference †	66	2.13 (1.23-3.68)	1.99 (1.18-3.34)
At least 5% difference ‡	42	2.91 (1.25-6.81)	3.58 (1.59-8.04)
At least 10% difference ‡	28	6.48 (1.37-30.7)	3.85 (1.22-12.1)
Both parous (parity 1-2 vs ≥3)			
Any difference †	83	1.44 (0.92-2.24)	1.79 (1.14-2.84)
At least 5% difference ‡	56	2.30 (1.30-4.08)	2.19 (1.23-3.90)
At least 10% difference ‡	35	2.33 (1.10-4.93)	2.33 (1.10-4.93)
Alcohol use (>2 drinks/mo vs ≤2 drinks/mo)			
Any difference †	60	0.59 (0.34-1.02)	0.50 (0.29-0.87)
At least 5% difference ‡	43	0.53 (0.28-1.03)	0.47 (0.24-0.91)
At least 10% difference ‡	28	0.49 (0.21-1.13)	0.47 (0.20-1.08)
Smoke currently vs do not smoke			
Any difference †	25	0.99 (0.40-2.47)	0.64 (0.25-1.66)
At least 5% difference ‡	18	0.94 (0.33-2.67)	0.61 (0.21-1.78)
At least 10% difference ‡	13	0.89 (0.28-2.82)	0.62 (0.18-2.08)
Premenopause vs postmenopause			
Any difference †	41	1.69 (0.80-3.55)	1.40 (0.66-2.99)
At least 5% difference ‡	24	1.60 (0.59-4.34)	1.60 (0.59-4.34)
At least 10% difference ‡,§	15	1.60 (0.33-7.82)	1.60 (0.33-7.82)
Both menopausal (current HT use vs none)			
Any difference †	23	1.55 (0.66-3.67)	1.52 (0.63-3.66)
At least 5% difference ‡	17	2.34 (0.73-7.52)	2.12 (0.70-6.45)
At least 10% difference ‡,§	8	1.87 (0.28-12.6)	0.57 (0.10-3.27)
Both menopausal (current EPT use vs none)‖			
Any difference †	38	0.76 (0.37-1.54)	1.15 (0.59-2.27)
At least 5% difference ‡	27	0.59 (0.23-1.55)	1.08 (0.44-2.67)
At least 10% difference ‡	18	0.41 (0.10-1.72)	1.11 (0.31-3.95)

NOTE: Analyses restricted to members of identical twin pairs.

*Twin pairs that were concordant (or not different by the defined quantity of each factor) were excluded from each analysis. All models are adjusted for age at mammogram and BMI except that analyses for BMI were only adjusted for age.

† Any difference in mammographic density means that the OR is the OR for having the highest mammographic density within the twin pair.

‡ Difference in mammographic density of 5% (or 10%) means that the OR is for having at least 5% (or 10%) higher mammographic density than the co-twin.

§Age at mammogram was excluded in the models due to unstable estimates.

‖None includes E only or P only current users.

Table 7. Contribution of additive genetic effects (A), common environment (C), and unique environment (E) to variance in percent and absolute mammographic density

Strata of twins	*n*	Percent mammographic density					Absolute mammographic density				
		A (SE)*	C (SE)*	COV*	E†	*P*‡	A (SE)*	C (SE)*	COV	E†	*P*‡
All twins (adjusted for age only)	1,106	0.75 (0.02)	0	0.02	0.23		0.63 (0.10)	0.07 (0.09)	0.02	0.29	
All twins (adjusted for multiple factors)§	1,106	0.53 (0.09)	0.01 (0.08)	0.20	0.25		0.59 (0.10)	0.08 (0.09)	0.06	0.28	
Both alcohol use ≤2 drinks/mo§	318	0.65 (0.03)	0	0.17	0.18		0.84 (0.02)	0	0.04	0.12	
Both alcohol use >2 drinks/mo§	230	0.53 (0.06)	0	0.20	0.27		0.67 (0.06)	0	0.08	0.26	
Alcohol use discordant§	312	0.53 (0.16)	0.02 (0.14)	0.22	0.24	0.20	0.08 (0.23)	0.39 (0.17)	0.05	0.48	<0.0001
Alcohol use unknown for at least one twin§	246	0.24 (0.19)	0.21 (0.16)	0.23	0.31		0.25 (0.17)	0.40 (0.15)	0.08	0.26	
BMI difference <1.1§	290	0.34 (0.16)	0.28 (0.15)	0.16	0.22		0.57 (0.19)	0.21 (0.18)	0.07	0.16	
BMI difference ≥1.1§	816	0.51 (0.03)	0	0.22	0.27	0.025	0.47 (0.12)	0.11 (0.10)	0.07	0.35	0.0002
BMI difference ≤2.5§	600	0.47 (0.11)	0.15 (0.11)	0.17	0.21		0.69 (0.12)	0.12 (0.12)	0.03	0.17	
BMI difference >2.5§	506	0.42 (0.06)	0	0.22	0.36	0.0009	0.20 (0.19)	0.21 (0.14)	0.10	0.49	0.0001
Menarche within 1 y§	428	0.51 (0.04)	0	0.18	0.30		0.71 (0.03)	0	0.07	0.23	
Menarche >1 y apart§	678	0.57 (0.10)	0.01 (0.09)	0.22	0.19	0.003	0.48 (0.13)	0.14 (0.11)	0.07	0.32	0.0004
Both nulliparous§	38	0	0.45 (0.09)	0.33	0.22		0	0.80 (0.04)	0.10	0.10	
Both parous§	802	0.47 (0.10)	0.07 (0.09)	0.19	0.27		0.62 (0.11)	0.07 (0.10)	0.07	0.24	
Discordant for nulliparity§	266	0.51 (0.05)	0	0.25	0.23	0.004	0.53 (0.07)	0	0.09	0.39	0.0006
Both 1-2 births§	218	0.19 (0.15)	0.34 (0.13)	0.23	0.24		0.43 (0.18)	0.22 (0.16)	0.11	0.23	
Both ≥3 births§	218	0.51 (0.19)	0.03 (0.17)	0.21	0.25		0.70 (0.05)	0	0.11	0.20	
Both parous, discordant 1-2 vs ≥3 births§	366	0.54 (0.05)	0	0.19	0.27	0.009	0.64 (0.17)	0.06 (0.15)	0.07	0.23	0.004
Both premenopausal§	294	0.57 (0.05)	0	0.18	0.25		0.48 (0.18)	0.23 (0.16)	0.01	0.28	
Both postmenopausal§	456	0.51 (0.14)	0.03 (0.12)	0.19	0.27		0.44 (0.16)	0.20 (0.14)	0.04	0.32	
Discordant§	216	0.66 (0.17)	0.04 (0.17)	0.15	0.16	0.002	0.75 (0.06)	0	0.01	0.25	<0.0001
At least one twin unknown	140	0.40 (0.10)	0	0.23	0.37		0.70 (0.08)	0	0.05	0.25	
Menopausal pairs§											
Both current HT users§	232	0.33 (0.20)	0.20 (0.17)	0.17	0.31		0.42 (0.25)	0.16 (0.21)	0.01	0.40	
Both nonusers§	60	0	0.68 (0.06)	0.15	0.17		0	0.69 (0.09)	0.05	0.26	
Discordant§	138	0.49 (0.09)	0	0.27	0.24	0.0006	0.75 (0.27)	0.02 (0.27)	0.09	0.13	0.0002
At least one twin unknown	26	0.38 (0.24)	0	0.24	0.38		0.37 (0.24)	0	0.20	0.43	
Close as adults§	710	0.45 (0.11)	0.09 (0.10)	0.21	0.25		0.62 (0.12)	0.08 (0.12)	0.05	0.24	
Not close as adults§	396	0.55 (0.05)	0	0.21	0.25	0.15	0.40 (0.19)	0.15 (0.15)	0.08	0.38	0.053

*Proportion of total variance due to A = additive genetic effects, C = common environment, and COV = covariates as estimated by taking the proportion of residual variance due to A or C and the proportion of variance due to covariates over the total variance (1 + proportion of variance due to covariates). C was automatically dropped from model in those instances it was estimated to be equal to 0.

†Proportion of total variance due to E = individual environment as inferred from the equation A + C + COV + E = 1.

‡*P* value from likelihood ratio test comparing the log likelihood of model with all twins combined to models allowing the estimates to vary by stratum. Unknown categories were excluded from *P* value calculations.

§Model adjusted for the following covariates: BMI (continuous), age at menarche (continuous), age at mammogram (continuous), parity (continuous), and menopausal status/hormone use.

increase the risk of obesity (33). There may also be genetic determinants of traits such as alcohol consumption or reproductive ability. However, this makes our findings even more important for research projects trying to identify the genetic factors that determine mammographic density because to identify the pure ''density'' genetic component, one would need to take into account such other factors that influence mammographic density. An example of this is the genome-wide linkage scan recently published by Vachon et al. (34). In this analysis, BMI seemed to mask the signal from the locus on chromosome 5, and only after adjustment for BMI was there clear evidence for linkage with log odds for linkage scores of 3 or more.

Vachon and colleagues (35) reported findings suggesting that the heritability estimates of mammographic density may be lower in postmenopausal than premenopausal women. They found estimates of 59% in premenopausal, but only 19% in postmenopausal women, again suggesting that nonheritable factors may play an important role. Although our results could not confirm a substantially lower heritability estimate in postmenopausal women, they suggest that it is difficult to distinguish the effects of common environment from the genetic effects across strata defined by menopausal status and hormone use.

Even an accurate heritability estimate is difficult to interpret. As pointed out as early as 1974 by Lewontin

(36, 37), the partitioning of variance by models used in classic twin analysis is based on an assumption of mutually exclusive genetic and environmental causation, although it is likely that environmental cofactors are necessary for the pathogenesis of almost every disease, even those assumed to be highly ''genetic.'' Manipulation of the environment in the form of diet, for example, can prevent heritable phenylketonuria. Differences in disease concordance (or mammographic density concordance) between identical and fraternal twins are likely to be due to interaction with inequitably distributed nonheritable factors.

Our findings may have implications with respect to the biology of mammographic density. Even within twin pairs, generally of comparable stature, large differences in BMI were associated with differences in absolute density, with higher BMI being associated with lower absolute density, although additional fat would not be expected to materially alter radiographic opacity. The finding remained even when pairs discordant for extreme obesity (BMI in excess of 35) were excluded. Obesity may therefore produce changes in other histologic elements, some of which may have the effect of reducing the absolute amount of mammographic density. This hypothesis is consistent with an early report by Boyd and colleagues (38) of a weak inverse association between absolute density and measures of obesity among premenopausal women.

In conclusion, our results confirm the importance of genetic factors in mammographic density, but they also suggest that an unknown portion of the presumed heritable effect may be due to shared modifiable or even nonheritable factors in identical twins. The results of classic twin variance analyses may be confounded by differential shared intrapair behavior and environmental exposures associated with zygosity, a violation of the equal environment assumption. Furthermore, these modifiable factors must be taken into account by studies aiming to identify genetic factors for mammographic density. Finally, we should not ignore mammographic density as a potentially modifiable risk factor.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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