# Mammographic breast density as an intermediate phenotype for breast cancer

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**The amount of radiologically dense breast tissue appearing on a mammogram varies between women because of differences in the composition of breast tissue, and is referred to here as mammographic density. This review presents evidence that mammographic density is a strong risk factor for breast cancer, and that risk of breast cancer is four to five times greater in women with density in more than 75% of the breast than in women with little or no density in the breast. Density in more than 50% of the breast could account for about a third of breast cancers. The epidemiology of mammographic density is consistent with its being a marker of susceptibility to breast cancer. Twin studies have shown that the proportion of the breast occupied by density, at a given age, is highly heritable, and inherited factors explain 63% of the variance. Mammographic breast density has the characteristics of a quantitative trait and might be determined by genes that are easier to identify than those for breast cancer itself. The genes that determine breast density might also be associated with risk of breast cancer, and their identification is also likely to provide insights into the biology of the breast and identify potential targets for preventive strategies.**

## Introduction

Breast cancer is the most common cancer in women and a frequent cause of death from cancer in most developed countries.[1] Some cases of breast cancer cluster in families, and the risk of disease is increased two to three times in the first-degree relatives of an affected woman, which suggests that genes are associated with susceptibility to the disease. The genetic factors known to be associated with susceptibility, including mutations in *BRCA1* and *BRCA2*, account for about 25% of familial risk, and perhaps 5% of overall breast-cancer risk.[2–5] The cause of most cases of the disease remain unexplained and are likely to be heterogeneous.

We propose that investigation of the genetic basis of mammographic density, one of the strongest known risk factors for the disease, could help elucidate the genetic factors that contribute to the cause of breast cancer. We summarise evidence that this density is a strong risk factor for breast cancer, independent of age and other risk factors, is highly heritable, and has the properties of a quantitative trait. Thus, mammographic density can be viewed as an intermediate phenotype for breast cancer. The genes that determine this density could be fewer in number and easier to identify than genes that determine susceptibility to breast cancer itself.[6] Identification of the genes that determine mammographic density is also likely to provide insights into the biology of the breast and to identify potential targets for preventive strategies.

## Mammographic density and risk of breast cancer

The radiological appearance of breast tissue differs between individuals because of variations in breast-tissue composition, and differences in the X-ray-attenuation properties of fat, epithelium, and stroma (figure 1).[7] Fat appears dark on a mammogram, whereas epithelium and stroma appear light or white, an appearance that we refer to as mammographic density (figure 2).[8]

## Methods of classification

There are, as yet, no generally accepted standard methods for classification of these variations in the radiological appearance of breast tissue, and the main methods in use include both qualitative and

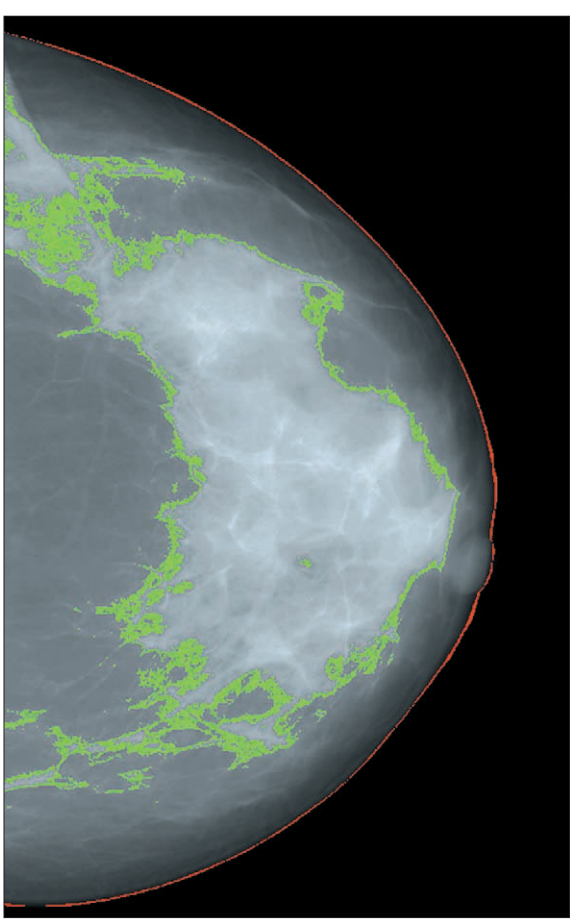

***Figure 1:*** **Computer-assisted measurement of mammographic density**
Red line defines edge of breast; green line shows edge of dense region.

quantitative approaches. In 1976, Wolfe[9,10] first proposed a classification system of four categories that related variations in the appearance of the mammogram to risk of breast cancer. The categories were: N1, in which the breast was mainly fat and risk of breast cancer was lowest; DY, in which the breast was mostly dense and in which risk was highest; and P1 and P2, in which there were linear densities of different extents and in which risks were intermediate. Most well-designed epidemiological studies have found that this classification does identify individuals at different risks of developing breast cancer,[11] although risk gradients have, in general, been smaller than those originally described by Wolfe.

Another qualitative classification, the breast imaging reporting and data systems (BIRADS) has also been used, and has four categories: extremely fatty; scattered density; heterogeneous density; and extremely dense. As yet, few studies have used BIRADS to predict risk, but risk of breast cancer and of tumours both positive and negative for oestrogen receptors is significantly increased in the category of extremely dense.[12,13]

Quantitative approaches that have been used to measure the proportion of density in the breast include estimation by radiologists, planimetry, and computer-assisted methods. Quantitative approaches have, in general, given more consistent results and larger gradients in risk, than qualitative methods.[11] Brisson and colleagues[14] have shown that the addition of a quantitative classification to Wolfe's P2 and DY categories creates substantial gradients in risk, whereas the addition of the Wolfe grades to a quantitative classification provides no additional information about risk. Figure 2 shows examples of categories of the classification that have been used in studies based on estimations by radiologists. Computer-assisted methods are also used for measuring mammographic density. Planimetry and the computer-assisted methods have the advantage over radiologists' classifications (the Wolfe or the BIRADS systems) of generating a continuous rather than a categorical measure and of providing an absolute measure of the projected area of dense tissue and the total area of the breast in the mammogram. Although results generally express the dense area as a proportion of the total area, the area of dense tissue alone is also associated with differences in risk of breast cancer. However, in all three published papers[15–17] that provide risk estimates for both dense area and percentage density, percentage density was associated with larger gradients in risk. The computer-assisted method is highly reproducible, with test-retest reproducibility greater than 0·9 (assessed by the intraclass correlation coefficient) in most studies.[8,16,18]

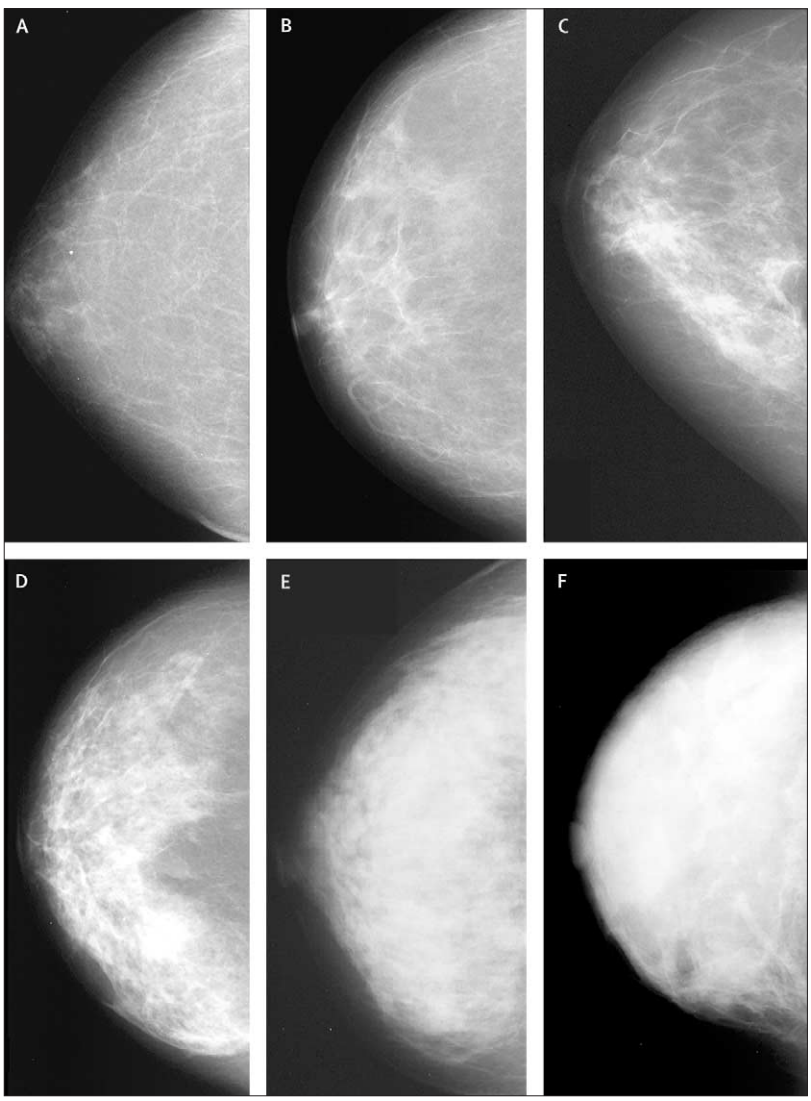


*Figure 2:* **Categories of percentage mammographic density estimated by radiologists**
A=0. B=<10%. C=<25%. D=<50%. E=<75%. F=≥75%. Reproduced with permission from ref 8.

## Quantitative classification

15 independent studies (ten case-control studies and five cohorts or case-control studies nested in cohorts), with a total of 6274 patients with breast cancer and 11 638 controls, have been reported; table 1 shows their methods and results.[15–29] These studies used various methods for measurement of breast density and various definitions of categories of density. Some studies had results for more than one reader or method of measurement, and some assessed more than one type of density. The results shown compare the difference in risk for the highest and lowest categories of density as defined in each study, and for each reader, method of measurement, and type of density, after control for the effects of age, and the other risk factors shown in table 1. All these studies found significantly raised odds ratios of between 1·8 and 6·0, and ten of the 15 found an odds ratio of at least 4·0.

Table 2 shows the results of the four studies[8,16,17,20] shown in table 1 that used much the same definitions of categories of density, and compares risk in patients with more than 75% density with patients with less than 1–10% density. The relative risks found in these studies ranged from 2·82 to 5·99, and estimates of attributable risk for the category of more than 75% density in these studies varied from 3% to 15%, owing mainly to differences in the prevalence of this category between patients. Attributable risk has also been estimated for density in more than 50% of the breast in two of these studies,[15,30] and was 28% and 33%, respectively.

Masking of breast cancer by dense breast tissue does occur and is associated with an increased risk of breast cancer after a negative mammogram.[31] Masking might raise the risks associated with mammographic density in cohort studies, since cancers missed in the first mammogram because of dense tissue would eventually be detected during subsequent follow-up. Masking could, in the short term, increase the risk of breast cancer associated with widespread density, but this effect is expected to disappear with long-term follow-up and repeated screening.[32] The increased risk associated with widespread breast density has been shown to persist in cohort studies without attenuation, for at least 10 years in one study[15] and for at least 7 years in another.[18] Furthermore, risks found in case-control studies, in which the mammogram taken at the time of diagnosis of cancer is used, are not increased by masking.[32] Table 1 shows some case-control studies and have given estimates of risk for widespread breast density that are substantial and significant.

| | Age (years) | n | Type of measurement | Partition* | Odds ratio (95% CI) | Trend† | Adjustments | Ref |
|---|---|---|---|---|---|---|---|---|
| Nested case-control in cohort | NR | 1880 patients 2152 controls | Planimetry | 0 *vs* ⩾75% | 4·3 (3·1–6·1) | Yes | Weight, age at birth of first child, family history, years of education, alcohol use, previous benign biopsy sample, and number of reproductive years | 15 |
| Case-control | 35–64 | 622 patients 443 controls | Computer assisted | <1% *vs* >75% | 5·2 (1·7–16·1) | NR | Age, body-mass index, age at menarche, family history, number of full-term pregnancies, menopausal status, hormone use, and age at first full-term pregnancy | 16 |
| Cohort | 40–80 | 111 patients 3100 controls | Computer assisted | 0·5% *vs* >46% | 3·49 (1·4–5·2) | Yes | Age, education, parity, height, and body-mass index | 17 |
| Nested case-control in cohort | 40–59 | 354 pairs | Estimation by observer and computer assisted | 0 *vs* ⩾75% | 6·0‡ (2·8–13·0)<br>4·0§ (2·1–7·7) | Yes<br>Yes | Age, parity, age at birth of first child, weight, height, number of births, age at menarche, and family history | 18 |
| Case-control | 40–65 | 183 pairs | Estimation by three observers | <10% *vs* ⩾75%<br>2·8‖ (1·4–5·6) | 6·0‖ (2·5–14·1)<br>No family history<br>3·7‖ (1·7–4·1)<br>Yes | Yes | Age at birth of first child, parity, and | 19 |
| Case-control | 20–69 | 408 patients 1021 controls | Estimation by observer | 0 *vs* ⩾60% | 5·4¶ (2·5–11·4)<br>3·8** (1·6–8·7) | Yes<br>Yes | Parity, age at birth of first child, family history, age at menopause, and hormone use | 20 |
| Case-control | NR | 362 patients 686 controls | Estimation by observer | 0 *vs* ⩾60% | 4·4 (2·5–7·9) | Yes | Weight and height | 21 |
| Case-control | 40–67 | 290 patients 645 controls | Estimation by observer | 0 *vs* ⩾60% | 4·6 (2·4–8·5)¶<br>3·2 (1·6–6·5) **<br>5·5 (2·3–13·2)†† | Yes | Age, parity, education, weight, and height | 22 |
| Case-control | 60 (mean) | 647 pairs | Computer assisted | <10% *vs* >50% | 1·8 (1·1–3·0) | No | Age at menarche, menopausal , status parity, age at birth of first child, family history, hormone use, and breast problems | 23 |
| Case-control | <50 | 547 patients 472 controls | Planimetry | <26·7% *vs* >70·3% | 4·4 (3·0–6·7) | NR | Age and study | 24 |
| Case-control | >35 | 108 patients 400 controls | Computerised | < 5% *vs* >25% | 3·3 (1·5–7·2) | NR | Age, year of screening, menopausal status, and body-mass index | 25 |
| Case-control | >35 | 139 patients 553 controls | Computerised | <5% *vs* >25% | 2·9 (1·6–5·6) | NR | Age and parity | 26 |
| Case-control | 30–85 | 160 pairs | Planimetry | <20% *vs* ⩾70% | 4·3 (1·8–10·4) | No | Parity | 27 |
| Nested case-control in cohort | 35–65 | 197 patients 521 controls | Planimetry | Upper *vs* lower‡‡ | 3·6‡‡ (1·7–7·9)<br>2·1§§ (1·1–3·8) | Yes<br>Yes | Body-mass index, parity, and menopause | 28 |
| Nested case-control in cohort | 35–74 | 266 patients 301 controls | Planimetry | <5% *vs* ⩾65% | 4·3 (2·1–8·8) | Yes | Age, weight, and parity | 29 |

NR=Not reported. *Categories of least and most widespread density from which odds ratios were calculate. †Significantly increased risk of breast cancer across all categories of density analysed in study. ‡Area of density estimated by radiologist. §Areas of density calculated by computer-assisted measurement. ||Results from individual observers. ¶Data for homogeneous density. **Data for nodular density. ††Data for total density. ‡‡Data for premenopausal patients. §§Data for postmenopausal participants.

***Table 1:*** **Quantitative blinded studies of breast density and breast-cancer risk: summary of methods and results**

| P | RR (95% CI) | AR | Ref |
|---|---|---|---|
| 19% | 5·49 (2·8–10·8) | 15%* | 8 |
| 10% | 4·35 (3·1–6·1) | 8%* | 16 |
| 3% | 5·23 (1·7–16·1) | 3%† | 17 |
| 17%‡ | 5·99 (2·5–14·1)‡ | 14%*‡ | 20 |
| 18%‡ | 2·82 (1·4–5·6)‡ | 12%*‡ | 20 |
| 14%‡ | 3·74 (1·7–4·1)‡ | 10%*‡ | 20 |

P=prevalence of ⩾75% breast density. RR=relative risk of breast cancer associated with ⩾75% density compared with 1–10% density. AR=P(RR–1)÷RR. *Calculated by original investigators. †Calculated by present investigators. ‡Estimates from same set of images of three different radiologists.

***Table 2:*** **Relative and attributable risks of breast cancer according to breast density**

The studies summarised in table 1 used mammograms from several centres and had highly consistent results, showing that any possible variation between centres in the quality or technical performance of mammography is not a limiting factor in studying risk of breast cancer.

All existing quantitative methods of assessment of mammographic density have limitations. None takes into account the thickness of the breast, and all are based on the area rather than the volume of breast tissue. Computer-assisted methods of measurement require that a dichotomous threshold is placed between dense and non-dense tissue, and do not allow the gradual transition from one to the other that is likely to exist in reality. Attempts to improve methods of measurement by addressing these and other limitations are in progress and could improve risk discrimination and strengthen causal associations.[33,34]

Despite these limitations, the relative risks of breast cancer associated with extensive mammographic density generated by these studies are larger than for most other risk factors for breast cancer, and they persist after adjustment for other risk factors. Although larger relative risks apply to the small proportion of the population who have mutations in *BRCA1* and *BRCA2*,[35,36] the attributable risk associated with these mutations is only about 5%, which is substantially smaller than the attributable risk of about 30% for density in more than 50% of the breast.

## Histology of breast tissue

Ten studies have assessed the relation between the histology of breast tissue and the radiological appearance of the breast.[30] Nine of these studies used breast sections prepared from mastectomy sample or biopsy samples, and most of the studies used qualitative methods to classify density in the breast from which the tissue came. Six of the nine studies that described the epithelium found that epithelial proliferation was associated with mammographic density, and all of the six studies that described stroma found that stromal

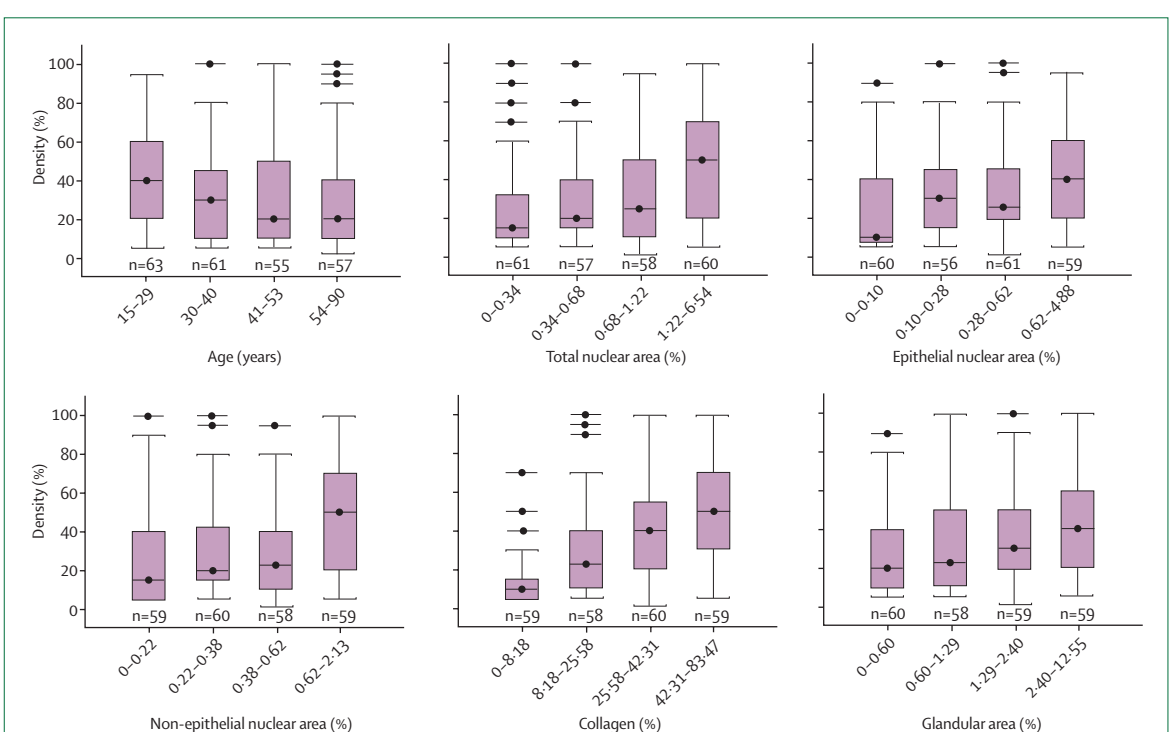


***Figure 3:*** **Boxplots of association between percentage density, age, and histology**
Horizontal line=median. Boxes=IQR. Whiskers=1·5×IQR. Dots=outliers. p values from linear regression, by use of continuous variables adjusted for age, were: age (p=0·04); total nuclear area (p<0·001); epithelial nuclear area (p<0·001); non-epithelial nuclear area (p<0·001); collagen (p<0·001); and glandular area (p<0·001). Reproduced with permission from ref 37.

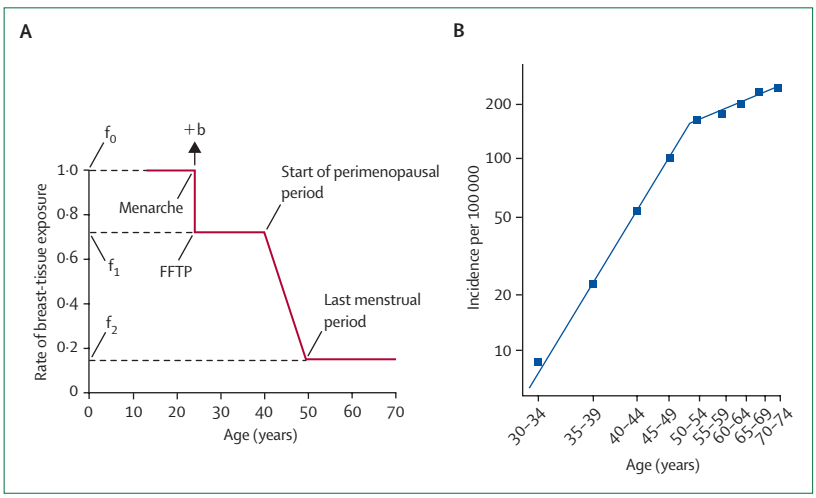


***Figure 4:*** **Pike model of breast-tissue ageing (A) and log-log plot of age-specific incidence of breast cancer in the USA (B)**

FFTP=first full-term pregnancy. b=variable used to calculate age at menarche. $f_0$, $f_1$, and $f_2$ are variables of model. Reproduced with permission from ref 55.

proliferation was associated with density. However, these studies were based on breast tissue removed from women with known or suspected breast disease, and the relation between the histology and radiology of the breast tissue found in these samples might not be representative of women in general.

This potential source of bias was avoided in the study of Li and co-workers,[37] who used breast tissue obtained at forensic autopsy by Bartow and colleagues.[38,39] Randomly selected tissue blocks were taken from slices of breast tissue obtained by subcutaneous mastectomy at the time of forensic autopsy, and quantitative microscopy was used to measure the proportion of the biopsy sample occupied by cells (estimated by nuclear area), glandular structures, and collagen.

Figure 3 shows the association between the measurements of breast tissue made from histological sections (expressed as a percentage of the total area of the section) and the proportion of mammographic density (estimated by a radiologist), in the (faxitron) radiograph of the tissue slice from which the biopsy sample was taken.[37] A high percentage mammographic density was associated with a significantly greater total nuclear area, a greater nuclear area of both epithelial and non-epithelial cells, and a greater proportion of collagen, and a greater area of glandular structures than was found for breasts with less mammographic density. The area of collagen accounted for 29% of the variance in proportion of breast density and the other tissue measurements accounted for between 4% and 7% of the variance in percentage density. Furthermore, age, bodyweight, parity, number of births, and menopausal status, are associated with variations in mammographic density in these and other data, and were associated with differences in one or more of these tissue features.

## Immunohistochemistry of breast tissue

The association of mammographic density with growth factors and stromal-matrix proteins in breast tissue was assessed in 92 formalin-fixed paraffin blocks of breast tissue surrounding benign lesions. Half the samples were from breasts with little or no density, and half were from breasts with widespread density;[40] the two groups were matched for age at the time of biopsy sampling. Sections were stained for cell nuclei, total collagen, the tissue inhibitor of metalloproteinase 3 (TIMP3), which is a stromal-matrix regulatory protein, transforming growth factor β (TGFβ), and insulin-like growth factor I (IGF-I); the area of immunoreactive staining was measured by use of quantitative microscopy. Breast tissue from participants with widespread density had a greater nuclear area (p=0·007), and larger stained areas of total collagen (p=0·003) that did those with little breast density. Moreover, stained areas on immunohistochemistry for TIMP3 (p=0·08) and IGF-I (p=0·02) were greater in women with widespread breast density than in those with less breast density. These differences were greater for those aged 50 years or younger than for older women.

IGF-I is a known mitogen for breast epithelium that is produced in the breast stroma and by the liver, and it is thought to have an important role in mammary carcinogenesis.[41] The proportion of stromal matrix in the breast is determined by the opposing actions of metalloproteinases and their inhibitors, TIMPs. The observed associations of breast density with IGF-I and TIMP3 could show how these factors respectively change cell proliferation and inhibition of matrix degradation.[40]

## Variation in mammographic density

More detailed descriptions of the associations of mammographic density with other risk factors can be found in other reviews.[30,42]

### Age, menstrual, reproductive, and anthropometric variables

Mammographic density has consistently been found to be less widespread in older women, in those who are parous, have had a larger number of livebirths, and have a greater bodyweight.[30,43] Bodyweight and body-mass index are strongly and positively correlated with the total area of the mammogram and the area of non-dense tissue, and weakly and negatively correlated with the area of dense tissue.[44] Therefore, adjustments should be made for body size in analysis of the association between mammographic features and risk of breast cancer.

### Menopause

A longitudinal study[45] of the effect of the menopause on mammographic density, undertaken in a screened population, compared the mammographic density of women who were premenopausal at entry and had undergone

menopause with an age-matched group of women who were also premenopausal at entry, had been followed-up for the same length of time, and had not been through menopause. The menopause was associated with a reduction in the area of tissue that appeared dense on the mammogram, an increase in the area of non-dense tissue, and a decrease in the proportion of density. However, these changes did not account fully for the effects of age on mammographic density seen in cross-sectional data.[45]

### Hormonal interventions

Combined hormone treatment increases mammographic density[46] and is associated with a small increase in risk of breast cancer;[47] effects are not seen with oestrogen alone.[47,48] Tamoxifen[49] and a gonadotropin-hormone-releasing agonist also reduce mammographic density.[50]

### Nutrition

Some cross-sectional studies have found associations between more widespread mammographic density and higher dietary intakes of total, and saturated fat,[22] polyunsaturated fat,[51] and alcohol and a lower intake of vitamin D and calcium.[52] However, few of these associations have been replicated.

The effect of a low-fat, high-carbohydrate diet on mammographic density was examined in a randomised controlled trial[53] in 817 women with density in at least 50% of the mammogram. After 2 years, the area of radiologically dense tissue was reduced by 6·1% in the intervention group compared with 2·1% in the control group (p=0·02), results that could not be accounted for by weight loss, menopausal status, age at entry to the trial, or hormone use. The long-term effects of dietary changes on mammographic density and the effect, if any, that these changes have on the risk of breast cancer, are not yet known.

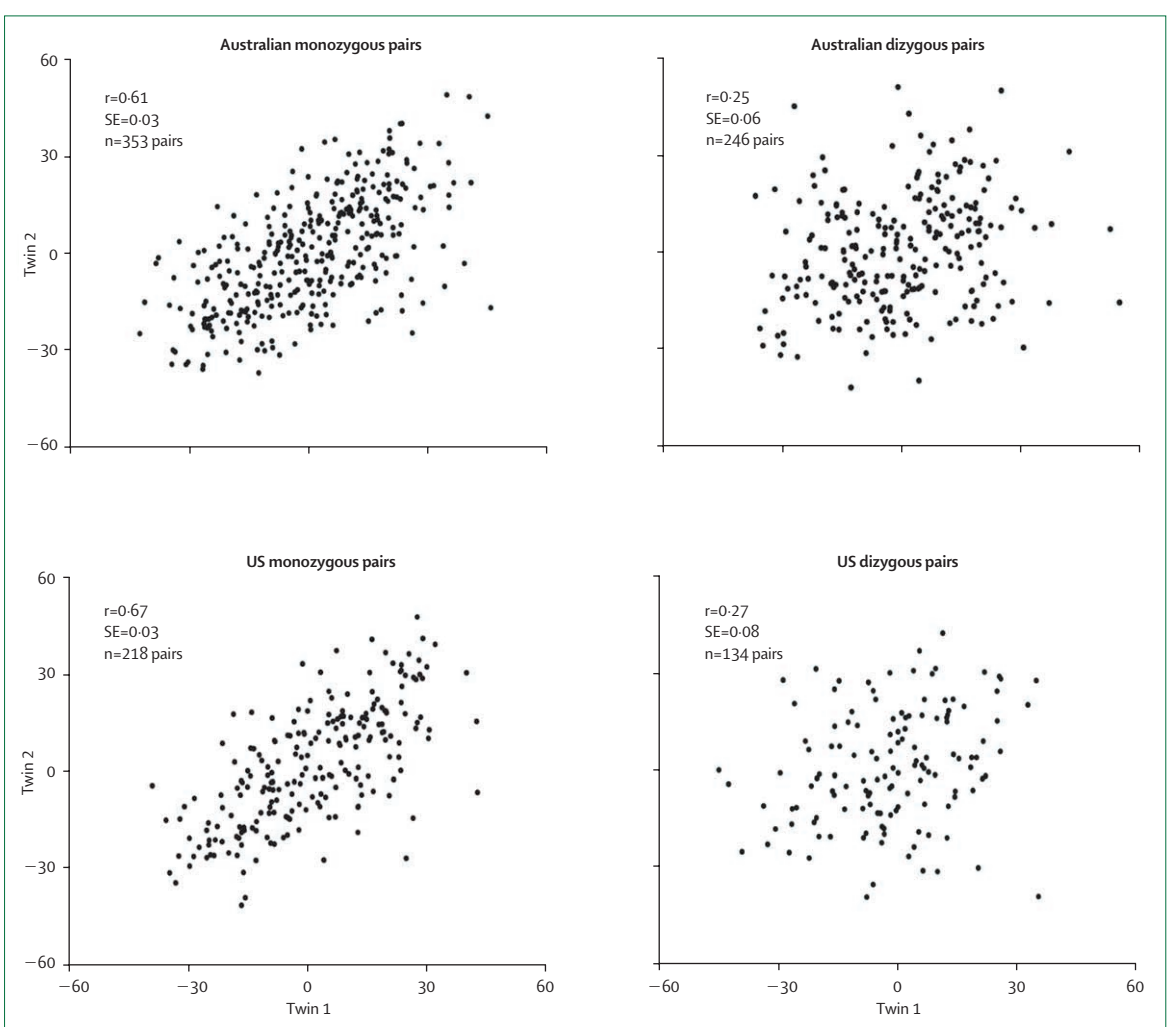


*Figure 5:* **Correlation of percentage mammographic density in twin pairs in Australia and USA and Canada**
Mean percentage of dense tissue was adjusted for body-mass index, age at menarche, menopausal status, parous women, age at first birth, and number of births. Reproduced with permission from ref 8.

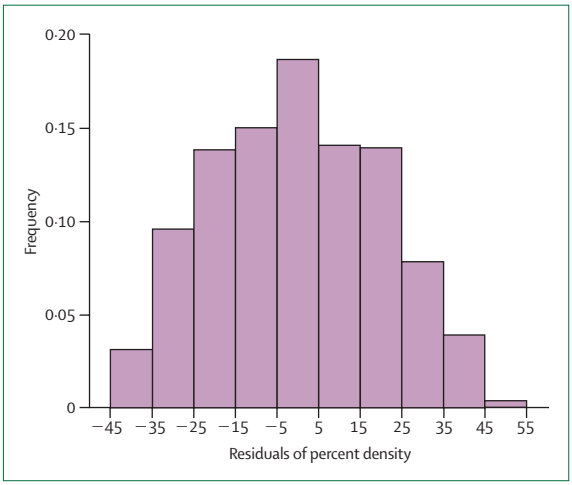


***Figure 6:*** **Distribution of percentage mammographic density in twins after adjustment for age**
Plot shows residuals from linear regression after adjustment for age with all twins. r=correlation coefficient.

Several factors that affect the risk of breast cancer are also associated with variations in one or more histological features of the breast and with mammographic density. All risk factors for breast cancer must ultimately have an effect on the breast, and these findings suggest that, for at least some risk factors, this effect is on the number of cells and the quantity of collagen in the breast that is shown by differences in mammographic density. However, the known risk factors for breast cancer explain only 20–30% of the variance in mammographic density;[44,54] most of the variance is explained by genetic factors.

## Age, mammographic density, and breast cancer

Because the probability of developing breast cancer increases with age, decline in the prevalence of mammographic density that occurs with increasing age could seem a paradox. However, this apparent paradox might be resolved by reference to the model of incidence of breast cancer proposed by Pike and colleagues.[55] Their model is based on the idea that the rate of breast-tissue ageing, rather than chronological age, is the relevant measure for describing the age-specific incidence of breast cancer. Breast-tissue ageing is associated with the effects of hormones on the kinetics of cells in the breast and the accumulation of genetic damage. According to the model, the rate of breast-tissue ageing is most rapid at the time of menarche, slows with each pregnancy, slows further in the perimenopausal period, and is least after the menopause. After fitting suitable numerical values for these features, Pike and colleagues[55] showed that cumulative exposure to breast-tissue ageing, given by the area under the curve in figure 4A, described the age-incidence curve for breast cancer in the USA (figure 4B). The general properties of the model have since been confirmed by observation when applied to the Nurses' Health Study by Rosner and Colditz,[56] who extended the model initially to include the effects of number and spacing of pregnancies, and subsequently to include other, non-reproductive, risk factors.

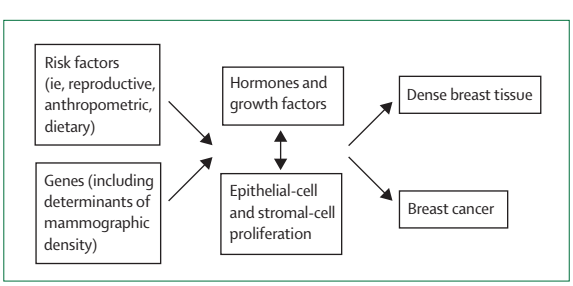


***Figure 7:*** **Biological hypothesis**

Mammographic density shares many of the features of breast-tissue age and is greatest in youth, declines with increasing age, and is reduced by successive pregnancies and menopause. Cumulative exposure to mammographic density could thus reflect cumulative exposure to hormones and growth factors that stimulate cell division in breast stroma and epithelium, and this exposure could be an important factor underlying the age-specific incidence of breast cancer in the population. However, there are important gaps in knowledge about cumulative exposure. Little is known about breast-tissue characteristics or the factors that affect them at early ages. Age-specific absolute risks of breast cancer according to breast density are not yet known and, in particular, whether interventions that reduce cumulative exposure to density will reduce risk of breast cancer also remains unknown.

## Heritability

The factors associated with mammographic density account for only 20–30% of the variation noted in the population.[44,54] Early studies with small numbers of mother-daughter sets[57] and a small twin study[58] suggested that genetic factors might explain a proportion of the variation (ie, the heritability) of mammographic density within a given population.

To address this question, we have done two independent twin studies.[8] Twin pairs aged 40–70 years and living in Australia or the USA and Canada were investigated and information was obtained on the factors known to be associated with variations in mammographic density by use of the same questionnaires. Mammograms were obtained from each member of every twin pair, digitised, and the proportion of density was measured by use of the computer-assisted method by one observer who was unaware of zygosity or pairing. After adjustment for age, age at menarche, parity, number of livebirths, menopausal status, and body-mass index, the variances in the percentage of density were almost identical in both samples. The correlation between twin pairs in the proportion of mammographic

density in Australia and the USA and Canada are shown in figure 5. The classic twin model assumes that variance for a given population can be partitioned into three components representing unmeasured effects, namely additive genetic effects, the effects of environmental factors which are shared by or common to twin pairs, and individual specific environmental effects, that include measurement error. The proportion of the residual variation accounted for by additive genetic factors (heritability) was estimated to be 60% (95% CI 54–66) from Australian twins, 67% (59–75) from twins from the USA and Canada, and 63% (59–67) in the studies combined.

Analysis of the components of variance showed that the best model included only components for the additive genetic factors and person-specific environmental factors.[8] These two twin studies support each other in providing compelling evidence that the wide variation in the proportion of mammographic density among women aged 40–70 years is strongly affected by genetic factors.

After adjustment for age, mammographic density had a symmetrical unimodal distribution (figure 6) much the same as that seen for other quantitative traits such as height. Because mammographic density is a continuous variable, the genes that affect the trait are expected to be associated with variations in its quantity, and the normal distribution after adjustment for age meets the assumptions of the statistical methods used in analysis.

Many of the factors associated with differences in mammographic density, including age at menarche, menopause, and body-mass index, are known to be at least partly heritable, but the estimates of heritability given for mammographic density were generated after adjustment for the effects of these factors. Heritability, however, refers to explanation of variation within a population. Both twin studies were undertaken in populations that are mainly European in origin, so our findings do not exclude the possibility of a greater effect caused by exposure to proportions of environmental factors that lie outside the range usually seen in societies in more developed countries.

Whether mammographic breast-density variation at a given age is determined by many genes or variants in one or more important genes is not known. Pankow and co-workers,[59] showed unadjusted sister–sister relations in breast density that are very similar to the correlation between dizygous pairs noted in our twin studies. Segregation analysis of data from nuclear families, with the assumption of a single mode of inheritance of the risk associated with one or more important genes, could not distinguish between dominant, recessive, or codominant models.

On the basis of the relative risk of breast cancer associated with widespread mammographic density and the recorded correlation between dizygous twin sisters, we have estimated that the familial association in proportion of breast density would explain an increase in risk to first-degree relatives by a factor of 1·05–1·08. Since risk to first-degree relatives of an affected woman is about twice that in other women, this increased risk means that the genes that explain variation in mammographic density could explain 5–8% of familial aggregation on a population basis.[60] Ziv and colleagues,[61] with a qualitative classification of density, showed that women who have first-degree relatives with breast cancer have greater amounts of breast density than do those without affected relatives.

## Growth factors and hormones

Measurements of hormones and growth factors have shown that blood concentrations of growth hormone, IGF-I in premenopausal women, and prolactin in postmenopausal women, all mitogens in the breast, and sex-hormone binding globulin,[62–64] are associated with mammographic density. Oestradiol concentrations are inversely associated with mammographic density in postmenopausal women.[62] Since concentrations of IGF-I and prolactin are associated with an increased risk of breast cancer in premenopausal and postmenopausal women, respectively,[65,66] these findings suggest potential mechanisms for the association of mammographic density with risk of breast cancer.

The search for genes associated with mammographic density is in its infancy and few have been found to date. A preliminary linkage analysis[67] on 68 individuals in 22 families by use of 147 highly polymorphic markers at 30 cM spacing in a genome-wide scan, found weak evidence of linkage to a region on chromosome 6. Variation in catechol-O-methyl transferase (COMT), which is involved in the methylation, conjugation, and inactivation of catechol oestrogens, was associated with mammographic density in two distinct healthy populations,[68,69] and with blood concentrations of IGF-I in one of them.[68] In both, the low-activity variant of COMT was associated with a lower than average proportion of breast density than the high-activity variant in premenopausal women; a third study[70] in women with breast cancer did not find an association of COMT with mammographic density. Other genetic associations that have not yet been replicated include one or several markers in genes for the androgen receptor,[71] insulin-like-growth-factor binding protein,[72] and growth hormone.[73]

## Biological hypothesis

Figure 7 shows our hypothesis that many of the genetic and environmental factors that affect the risk of breast cancer affect the proliferative activity and quantity of stromal and epithelial tissue in the breast, and that these effects are reflected in differences in mammographic density among women of the same age. The evidence shows that the environmental variables include menstrual and reproductive factors, as well as anthropometric variables, but these factors account for only

20–30% of the variance in the proportion of breast density.[44,54] The two twin studies show that most of the variance is explained by as yet unidentified genetic factors.[8]

We propose that these inherited and environmental factors affect breast-tissue composition, at least partly, through effects on the degree of exposure to hormones and growth factors that are mitogens in the breast, including IGF-I and prolactin. These effects result in increased proliferative activity and greater quantities of stromal and epithelial tissue. Greater quantities of cells and proliferative activity than is normal are associated with an increase in susceptibility to carcinogens, and a raised risk of breast cancer.

Consistent with this hypothesis are the findings that risk of proliferative disease without atypia, as well as atypical hyperplasia and cancer-in-situ,[74] are all greater in those with widespread mammographic density than women with less widespread density. Furthermore, widespread breast density increases the probability of invasive cancer developing after a diagnosis of cancer in situ.[75]

The quantity of stromal and epithelial tissue is shown by the dense area of the mammogram. Although the dense area is also related to risk of breast cancer, the proportion of breast density seems to be the stronger risk factor.[15–17] The non-dense area in the mammogram, which shows fat, could also provide information about risk, and most risk factors for breast cancer that affect mammographic density have opposing effects on the dense and non-dense areas of the mammogram.[76] Whether the proportion of breast density is the best possible method of combining the information about risk that is contained in the measured dense and non-dense components of the mammographic image remains to be determined.

## Conclusion

There is detailed evidence that mammographic density is a risk factor for breast cancer, independent of other risk factors, and is associated with large relative and attributable risks for the disease. Mammographic density shows variations in the tissue composition of the breast, and is positively associated with collagen, epithelial cells, and non-epithelial cells, and negatively associated with fat. Widespread breast density is common and estimates of the associated attributable risk suggest that about a third of breast cancer could be explained by density in more than 50% of the breast.

The epidemiology of mammographic density, notably the inverse association with age, is consistent with its being a marker of susceptibility to breast cancer, in a manner similar to the concept of breast-tissue age described by the Pike model. Cumulative exposure to mammographic density could be an important determinant of the age-specific incidence of breast cancer in the population.

As described, mammographic density is highly heritable and thus meets criteria for an intermediate phenotype. Mammographic density is a continuous trait, with a wide unimodal and roughly normal distribution and is likely to be affected by many genes. The genetic variants that affect the tissue composition of the breast are therefore individually likely to have modest effects on risk of breast cancer, but their combined effects could be substantial.

The evidence that mammographic density is a strongly heritable risk factor for breast cancer has implications for our understanding of familial aggregation and of the cause of breast cancer in general. The modest doubling of risk associated with having an affected first-degree relative can only apply if there are strong underlying familial risk factors,[60] and elucidation of the causes of its familial aggregation will be important in understanding the causes of breast cancer.

Classic linkage studies that use multigenerational families could be approaching their limit in identifying common breast-cancer susceptibility genetic variants that have an important effect on breast-cancer risk. Possible reasons for the difficulty in finding evidence for linkage of breast cancer in present genome-wide studies include a high degree of locus (genetic) heterogeneity, low penetrance or low prevalence of alleles that predispose to disease, interactions between genes, and gene–environment interactions (features of many common complex diseases). Another approach, such as doing genetic-mapping studies with a continuously distributed risk factor, such as mammographic density, might be more successful and might identify new genes or pathways involved in breast-cancer susceptibility.

### Search strategy and selection criteria

We searched PubMed using the terms: "mammographic density and breast cancer", "mammographic parenchymal patterns and breast cancer", "breast density and breast cancer" and searched the references in the articles identified in this way. There were no restrictions on language or by date of publication, and published work up to May, 2005, was included. A total of 186 relevant original-research articles were found, of which 45 included estimates of risk of breast cancer associated with mammographic parenchymal patterns or mammographic density. 17 of these were based on quantitative classifications of density, of which 15 were independent studies. 141 papers described causal or other associations with mammographic patterns or density.

Space constraints meant that we could not review or cite all of the published work on this topic. We have therefore focused on approaches to mammographic density that use quantitative methods to assess risk and heritability, and have cited all of the published work in these areas. In other areas, we have, of necessity, been more selective in the research cited, and direct the reader to reviews, where these are available.

Carlson and colleagues[6] have described the potential advantages of intermediate phenotypes in investigating the genetic basis of disease. They pointed out that the occurrence of disease is likely to be the result of many genetic and environmental factors, operating through intermediate phenotypes. The number of such factors that affect any one intermediate phenotype is likely to be smaller than the number that affect the disease itself.

Examples of the application of the use of other highly heritable traits to identify genes associated with diseases that have complex causes include carotid-artery disease and type-2 diabetes.[6,77,78] In the context of mammographic density and breast cancer, this reasoning predicts that fewer genes determine mammographic density than those that determine breast cancer. Some of the genes that define mammographic density could also affect the risk of breast cancer, and the magnitude of their effect on cancer risk remains to be established. However, even if the effect of these genes on risk of breast cancer is small, the identification of the genetic loci associated with mammographic density could provide insights into the biological processes in the breast that determine risk of cancer, and this knowledge could, in turn, suggest potential targets for preventive strategies.

**Conflict of interest**

We declare no conflicts of interest.

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