Research Article

Localized Fibroglandular Tissue as a Predictor of Future Tumor Location within the Breast

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Abstract

Background: Mammographic density (MD) is a strong marker of breast cancer risk, but it is unclear whether tumors arise specifically within dense tissue.

Methods: In 231 British women diagnosed with breast cancer after at least one negative annual screening during a mammographic screening trial, we assessed whether tumor location was related to localized MD 5 years prior to diagnosis. Radiologists identified tumor locations on digitised films. We used a validated algorithm to align serial images from the same woman to locate the corresponding point on the prediagnostic film. A virtual 1 cm square grid was overlaid on prediagnostic films and MD calculated for each square within a woman's breast (mean = 271 squares/film). Conditional logistic regression, matching on a woman's breast, was used to estimate the odds of a tumor arising in a square in relation to its prediagnostic square-specific MD.

Results: Median (interquartile range) prediagnostic MD was 98.2% (46.8%–100%) in 1 cm-squares that subsequently contained the tumor and 41.0% (31.5%–53.9%) for the whole breast. The odds of a tumor arising in a 1 cm-square were, respectively, 6.1 (95% CI: 1.9–20.1), 16.6 (5.2–53.2), and 25.5-fold (8.1–80.3) higher for squares in the second, third, and fourth quartiles of prediagnostic MD relative to those in the lowest quartile within that breast ($P_{trend} < 0.001$). The corresponding odds ratios were 2.3 (1.3–4.0), 3.9 (2.3–6.4), and 4.6 (2.8–7.6) if a 3 cm-square grid was used.

Conclusion: Tumors arise predominantly within the radiodense breast tissue.

Impact: Localized MD may be used as a predictor of subsequent tumor location within the breast. *Cancer Epidemiol Biomarkers Prev; 20(8); 1718–25. ©2011 AACR.*

Introduction

Mammographic density (MD), which reflects the amount of radiodense fibroglandular tissue in the breast, is one of the strongest known markers of susceptibility to breast cancer. High MD (i.e., dense tissue occupying >75% of the breast) is associated with a 4- to 6-fold increased breast cancer risk relative to women with little density (<5%; refs. 1, 2), with a third of breast cancers in high-risk populations being attributable to MDs over 50%

(2, 3). Although high MD is associated with a greater subsequent breast cancer risk, it is not known whether MD is directly related to risk, with tumors arising within the radiodense tissue itself, or simply a marker of susceptibility, that is both MD and breast cancer risk have common determinants (e.g., parity), but MD is not an intermediate factor between such determinants and cancer. Reductions in MD have been shown to be associated with reduced breast cancer risk, although not consistently (4–6). Clarification of whether MD is directly related to risk, or simply a correlate of its determinants, will enhance our understanding of the pathogenesis of breast cancer and will provide information on the value of using localized MD as a predictor of subsequent tumor location, possibly opening up new avenues for the clinical management of high-risk women.

Two previous studies have assessed whether tumors develop within radiodense areas of the breast, but produced conflicting findings (7, 8). By using diagnostic films of 22 patients with a solitary ductal carcinoma *in situ* (DCIS), Ursin and colleagues concluded that 21 of 22 tumors were located within dense tissue (7). However, temporality could not be established and thus the results might have arisen from dense tissue being more likely to undergo carcinogenic transformation or from

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proliferation of the dense stromal tissue as a consequence of developing a tumor. Vachon and colleagues examined prediagnostic mammograms of 350 breast cancer cases (invasive and DCIS) and, in contrast, found no association between the breast quadrant with highest density either 3 or 7 years prior to diagnosis and the quadrant location of the subsequent tumor (8). Both studies relied on a quadrant approach to examining localized breast density. Densities can be either diffuse or focal in nature (9), thus, using too large a region, such as a quadrant, might not yield an accurate measure of density in the specific area where the tumor arises.

The present study aims to determine whether, in women who subsequently develop breast cancer, localized dense areas of the breast several years prior to diagnosis were at a greater risk of being the location of the subsequent tumor than less dense areas within that same breast. To achieve this, we used a novel approach (10) to align a woman's prediagnostic and diagnostic mammograms and measure localized MD for much smaller areas than previously (e.g., 1 cm^2 squares) in mammograms taken several years prior to cancer diagnosis.

Material and Methods

Study population

The study was conducted within the Age Trial, a British trial of annual mammographic screening (11). Women randomized to the intervention arm (~54,000) between 1991 and 1997 were offered annual breast screening from age 39–41 to age 48 years. From age 50 onwards, they were invited for screening every 3 years as part of the UK National Health Service (NHS) Breast Screening Programme (BSP). Screening in the trial was by 2-view X-ray mammography, craniocaudal and mediolateral oblique (MLO) views at the first screen, and by MLO view thereafter. Women were followed up through the BSP and the NHS Central Register to ascertain cancer incidence. Participants in the present study were women in the intervention arm of the trial who were diagnosed with breast cancer before December 31, 2007 after having undergone at least 1 negative screening round. The study was approved by the U.K. South East Research Ethics Committee. Participants provided written informed consent.

Tumor location on diagnostic films

Diagnostic and prediagnostic mammograms were retrieved from screening centers and hospitals and digitized on an Array 2905 laser digitizer (optical density 0–4.0, 12-bit resolution, pixel length 50 μm; Array Corporation Europe). On the digitized diagnostic MLO image displayed on a normal computer screen in a darkened room, the tumor location was marked by 1 of 2 radiologists (CR and LW; Fig. 1A), with a randomly selected 6% independently marked by both radiologists twice. Histology, pathology, and radiology reports, available for all but 2 cases, were provided. Within-radiologist mean

distance (95% CI) between tumor points independently marked were 1.04 (0.52–2.08) mm and 1.71 (1.44–2.03) mm for the 2 radiologists. The median (interquartile range, IQR) between-radiologist difference between tumor points was 1.73 (1.52–7.70) mm.

Identification of the point on prediagnostic films where the tumor originated

The MLO film 5 years prior to diagnosis, or nearest to this date, was selected throughout. The point on the prediagnostic film (of the same breast) that corresponded to the identified tumor location point was found by using an automated affine registration method to align serial (time separated) mammograms from the same woman. This point on the prediagnostic image was treated as the point at which the subsequent lesion develops (Fig. 1B). Variations in the imaging process (e.g., positioning and degree of breast compression) cause the breast to appear at different positions in serial mammograms (Fig. 1A). We had previously optimized 3 intensity-based automated registration methods—affine, fluid, and free-form deformation—specifically to provide accurate alignment of serial X-ray mammograms. We evaluated the accuracy of these optimized registration algorithms against 5 film readers who independently identified landmarks (i.e., tumor and normal features such as nipples and ligaments) on 52 pairs of diagnostic and corresponding prediagnostic digitized images. Registration errors for each 1 of the 3 algorithms were calculated as the distance between the registered point (i.e., the film reader's feature location on the diagnostic image transformed onto the prediagnostic image) and the corresponding feature location on the prediagnostic image identified by the film readers, the latter taken as the "gold standard" (10). The affine method had the highest accuracy, similar to that between independent film readers, with 80% of affine registration errors being less than 1 cm and 100% being less than 2 cm (10).

As the affine registration is an intensity-based method (i.e., an intensity defined similarity measure of corresponding pixels is maximized), we excluded a circle around the tumor center on the diagnostic image to avoid the tumor being aligned to prediagnostic dense tissue thus creating a biased positive association. The circle's diameter was the tumor size from the pathology report, if available ($n = 129, 56\%$), or diameters of 18 mm (if only an invasive component was present) or 20 mm (otherwise; Table 1).

Whole breast and square-specific density readings

MD readings were carried out on the prediagnostic MLO films by a single reader (VM), blinded to subsequent tumor location, using the interactive threshold Cumulus method (12). This method dichotomizes pixels on a digitized mammogram according to their intensities into dense and nondense using the threshold defined by the user, automatically estimating dense and total breast areas (in cm^2) and their ratio (as percent MD). Thirty-five
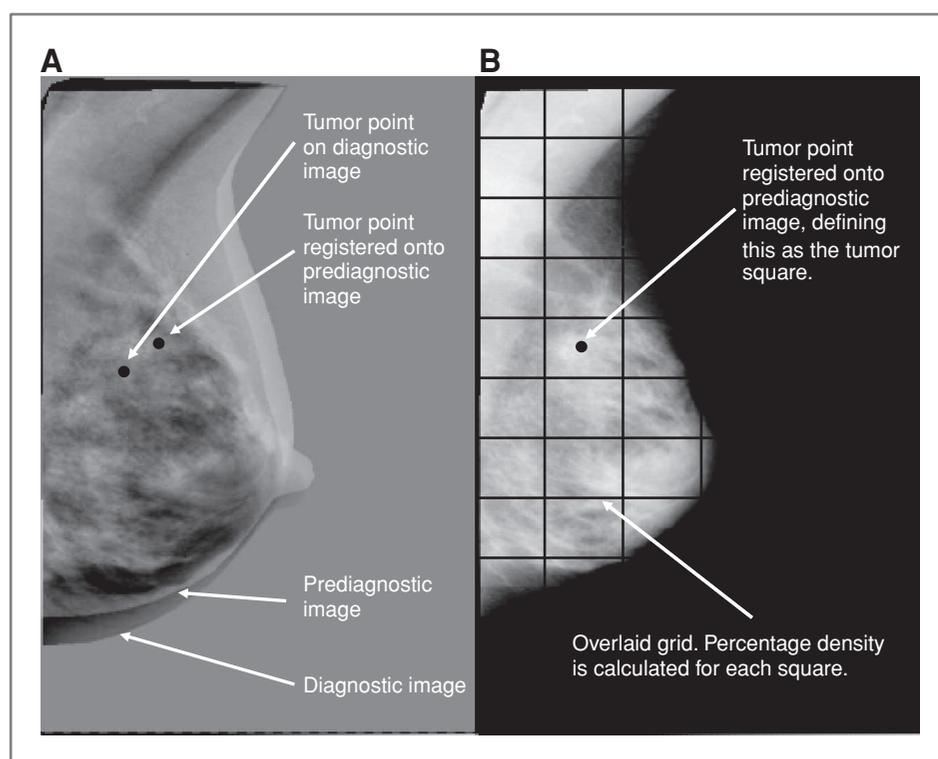
Figure 1. Image registration and tumor location. A, diagnostic and prediagnostic images overlaid on each other, with tumor point identified on diagnostic image. B, prediagnostic image with registered tumor point located on it and overlaid square grid.

(9%) films were read twice, giving a high repeatability (intraclass correlation coefficient for percent MD 0.97; 95% CI 0.95–0.98). An investigation of these breast cancer cases and of controls randomly selected among breast cancer-free Age Trial participants—closely matched to each case on screening centre, date of birth (± 1 year) and date of mammogram (± 1 year)—showed a strong association between prediagnostic whole breast percent MD and subsequent breast cancer risk (MD > 75% was associated with a 6.6-fold (95% CI 2.1–20.5) increase in risk relative to MD < 25%).

A semiautomated method was specifically developed to overlay a square grid on the prediagnostic image such that the square containing the tumor location was centered on that point (Fig. 1B). The coordinate points of the grid squares were then fed into the "mask" access database sheet of Cumulus to generate a series of images from the same breast that sequentially showed each square of the breast, 1 at a time. Thereafter, using the same intensity threshold selected to determine whole-breast percent MD, we calculated the percent MD in each grid square, that is, square-specific MD, by using the Cumulus software. For the same grid size, percent MD in a given square is directly proportional to absolute dense area. A grid size with squares of 4-cm length was initially used as affine registration errors are less than 2 cm either side of the location identified by the film readers (gold standard; ref. 10). As this grid size minimized misclassification in identifying the tumor square but reduced the accuracy of the localized MD measurements, we also estimated square-specific MD

for grid sizes of 3, 2, and 1 cm. Finally, we repeated MD measurements (whole breast and square specific) by using a higher intensity threshold to capture a more-specific small area of extremely dense tissue. This was done to capture the highest concentration of dense tissue, mimicking what would be the largest thicknesses of dense tissue in a volumetric method. Whole-breast mean percent density (SD) 5 years prior to cancer diagnosis was 42.4% (16.3%) with the usual intensity threshold, but only 7.7% (4.6%) with the "extremely high dense" one.

Statistical methods

The study design lends itself to a matched case–control analysis, in which the matching set is a woman's breast, the square in which the tumor is subsequently located is the "tumor square," and all other squares are "tumor-free squares." Along the edge of the breast, some squares were smaller than the defined grid size because any non-breast features located within them (e.g., pectoral muscle) were masked and excluded. Thus, only tumor-free squares whose areas were within 10% of the tumor square area were included. If no tumor-free squares were within this size range, the case–control set was excluded ($n = 3$ and 8 for the 1 and 3 cm grids, respectively).

Conditional logistic regression was used to estimate the odds of a square later becoming the tumor square versus remaining tumor free in relation to the square-specific MD and within-breast quartiles of square-specific MD. The analysis was repeated by using predefined categories of square-specific MD (i.e., <10%, 10%–29%,

Table 1. Prediagnostic mammographic measures and tumor characteristics among women with breast cancer (*n* = 231)

Prediagnostic mammographic measures		Mean (SD)
	Age at screen (y)	42.5 (2.3)
Whole breast	Breast area (cm^2)	140.4 (51.7)
	Dense area (cm^2)	56.4 (25.1)
	Percent MD (%)	42.4 (16.3)
Square specific (1 cm)	No. of squares per film	271 (81)

Tumor characteristics		*N* (%)
Age at diagnosis [y, mean (SD)]		46.8 (3.1)
Laterality	Left	116 (50.2)
Grade[a]	1	39 (22.0)
	2	80 (45.2)
	3	58 (32.8)
Whole tumor diameter (mm, *n* = 129)		18 (13, 27)[b]
Noninvasive component present[c]		172 (76.2)
of which:	Ductal carcinoma *in situ*	159 (92.4)
	Lobular carcinoma *in situ*	18 (10.5)
	Other *in situ*	1 (0.6)
	Maximum diameter (mm, *n* = 42)	19 (12, 25)[b]
Invasive component present		182 (78.8)
of which:	Ductal	149 (81.9)
	Lobular	28 (15.4)
	Tubular/cribriform	14 (7.7)
	Medullary, mucoid, other	7 (3.8)
	Maximum diameter (mm, *n* = 164)	18 (13, 27)[b]

[a]54 missing, percentage out of non-missing.
[b]Median (IQR).
[c]No. of exclusively noninvasive lesions = 43; no. of cancers with both invasive and noninvasive components = 182; no. with missing information = 6. Percentage out of nonmissing.

30%–79%, and ≥80%). As analyses were matched on the woman's breast, woman level variables (e.g., age) did not vary within a set/breast so could not, as a main effect, influence the outcome within a woman. Effect modification by these variables was, however, examined.

Results

Radiologists viewed diagnostic films for 284 breast cancer cases and were able to identify a tumor on 236 (83%). This analysis comprised 231 cases (5 were excluded because of different sized prediagnostic/diagnostic films) diagnosed during 1994–2007. Characteristics of the participants' prediagnostic films, and of their tumors, are shown in Table 1. By design, the prediagnostic images were taken, on average, 5 years (median = 4.9, IQR = 3.4–5.1) prior to diagnosis. A similar number of

tumors occurred in the left and right breasts (Table 1), most located in the upper region.
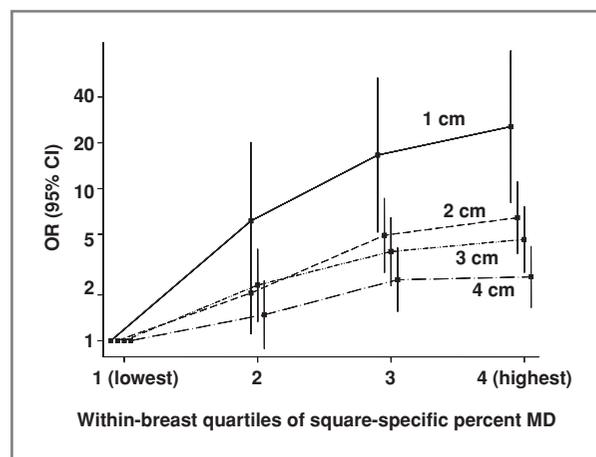
Prediagnostic mean percent MD was 42.4% (SD = 16.3%) for the whole breast. In contrast, prediagnostic mean percent MD for the tumor square was 73.8% [SD = 36.2%, highly skewed (median = 98.2%, IQR = 46.8%–100%)] by using a 1-cm grid and 68.5% [SD = 31.4, (median = 78.1%, IQR = 47.3%–96.8%)] for a 3-cm grid. The higher average percent MD in tumor squares compared with the whole breast is illustrated in Figure 2A for a 1-cm grid, where most points lie above the equality line; many tumor squares were entirely dense, with 64% having MD > 80%, such that the distribution of MD in tumor squares was entirely shifted upward with little overlap with the distribution of whole breast MD (Fig. 2B). The distribution of percentiles of within-woman square-specific MD for tumor squares was right skewed. In 25% of
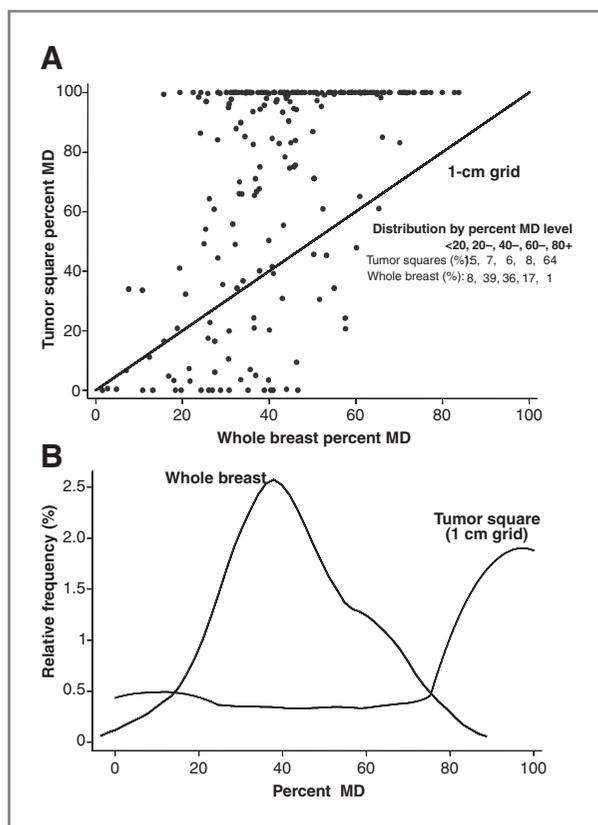
Figure 2. Whole breast versus tumor square-specific MD in prediagnostic images. A, tumor square-specific percent density plotted against the corresponding whole-breast density for the same breast (the straight line represents the line of equality). B, relative distribution of whole breast and tumor square density values among all study participants.

Figure 3. Odds of a tumor developing in a given square in relation to its prediagnostic square-specific MD, by grid length.

women, density of the tumor square for the smallest grid size (1 cm) was, prediagnostically, within the top 10% of most dense squares, whereas in 75% of women, the tumor square was within the top 35% of most dense squares. The corresponding figures for the other grid sizes were of a similar magnitude (e.g., respectively, within the top 9% and top 32% for a 3-cm grid).

There was a dose–response relationship between the within-woman square-specific MD quartile and the odds of that square being the location of the subsequent tumor (Fig. 3, Table 2). For the smallest grid size (1 cm), 57% of squares that were to become tumor squares had MD in the top quartile of the within-breast MD distribution; only 15% had MD in the bottom half (Table 2). Correspondingly, the odds (95% CI) of a tumor being located within a given square were, respectively, 6.1 (1.9–20.1), 16.6 (5.2–53.2), and 25.5 (8.1–80.3) times higher in squares in the second, third, and fourth quartiles of within-breast MD distributions relative to those in the lowest quartile (Table 2). The magnitude of the association was strongest for the 1-cm grid, albeit with wider CIs because of very few tumors arising in the reference (lowest density) category, and was attenuated for larger grid sizes

(Fig. 3, Table 2). Taking the more stable highest quartile as the reference category yielded ORs of breast cancer in the third, second, and first quartiles of 0.65 (0.47–0.90), 0.24 (0.16–0.36), and 0.04 (0.01–0.12), respectively, for a 1-cm grid. Alternatively, for the predefined categories of square-specific MD, ORs were 2.35 (1.18–4.65), 5.00 (3.01–8.31), and 9.28 (5.88–14.65) for percent MD of 10%–29%, 30%–79%, and $\geq$80% relative to <10%.

The association between within-woman square-specific MD quartiles and subsequent tumor location was not modified by prediagnostic whole-breast MD (0%, 20%–, 40%–, and 60%+), time between prediagnostic film, and cancer diagnosis (<5 and $\geq$5 years), or the nature (exclusively noninvasive vs. invasive/noninvasive), grade (1/2 vs. 3), and size (<20 vs. $\geq$20 mm) of the tumor (P for interaction $\geq$ 0.12 in all for a 3-cm grid; $P \geq$ 0.28 in all for a 1-cm grid).

By using the "extremely high dense" threshold, ORs (95% CI) were 1, 1.2 (0.5–3.0), 1.7 (1.0–2.8), and 4.7 (3.5–6.3) for percent of extremely high density in the square being less than 2.5% (reference category), 2.5%–4.9%, 5.0%–19.9%, and $\geq$20.0%, respectively. Thirty-six percent of tumor squares, but only 1.7% whole breasts, had squares with $\geq$20% extremely high density.

Discussion

This study shows that breast tumors arise predominantly within prediagnostic radiodense tissue. The probability of an area subsequently developing into a tumor increased as the amount of radiodense tissue in that area increased, strongly suggesting that it is specifically concentrations of radiodense fibroglandular tissue that are at risk of undergoing malignant transformation.

Strengths and limitations
Previous studies (7, 8) assessed whether tumors originated within the quadrant with the highest prediagnostic

Table 2. Odds of a square becoming the location of a subsequent breast tumor in relation to its percent MD 5 years prior to diagnosis

Grid length (cm)	Within breast quartiles of square-specific percent MD	No. of squares in which tumor arises[a]	OR (95% CI)[b]
4	1 (lowest)	26	1
	2	48	1.5 (0.9, 2.5)
	3	66	2.5 (1.6, 4.1)
	4 (highest)	74	2.6 (1.6, 4.2)
			P for linear trend <0.001
3	1 (lowest)	19	1
	2	44	2.3 (1.3, 4.0)
	3	69	3.9 (2.3, 6.4)
	4 (highest)	91	4.6 (2.8, 7.6)
			P for linear trend <0.001
2	1 (lowest)	15	1
	2	32	2.1 (1.1, 3.8)
	3	71	4.9 (2.8, 8.6)
	4 (highest)	111	6.4 (3.7, 11.1)
			P for linear trend <0.001
1	1 (lowest)	3	1
	2	32	6.1 (1.9, 20.1)
	3	63	16.6 (5.2, 53.2)
	4 (highest)	130	25.5 (8.1, 80.3)
			P for linear trend <0.001

[a]The total number is less than 231 because tumor squares for which no control squares of similar size (i.e., within 10%) could be identified were excluded (see Methods section).

[b]OR and 95% CI estimated by using a conditional logistic regression model where the matching set is a woman's prediagnostic breast consisting of a square where the tumor will subsequently originate (tumor square) and several tumor-free squares (control squares; see Methods section).

MD. Applying this approach to our data yielded a rather weak association ($P = 0.07$) because 75% of our tumor squares (regardless of grid length) had a higher density than the highest density quadrant. The use of a validated registration technique (10) to accurately align serial digitized mammograms is a major strength of our study because it allowed the measurement of localized MD at a much smaller level. The accuracy of the affine registration is somewhat lower for images with little density (10), but it is unlikely that this affected substantially our findings because practically all prediagnostic images had some density (fifth percentile of prediagnostic whole-breast MD, 16.8%), and the magnitude of the association between square-specific MD and tumor location did not vary according to prediagnostic whole-breast MD level.

Radiologists were unable to identify the tumor on the diagnostic films for 17% of cases, mainly because their breasts were dense. This, coupled with the fact that these cases had higher prediagnostic whole-breast MD than those included in the study (mean difference 7.8%, 95% CI 2.6%–13.0%), provides further indirect support to the hypothesis that tumors arise predominantly within prediagnostic dense tissue. As density, and its spatial distribution within the breast, change with age, it would be informative to assess images taken further back in time. The participants were mainly premenopausal at diagnosis. Replication of the findings in postmenopausal women of screening ages is particularly relevant as overall MD is lower, hence, small amounts of radiodense tissue would be identified as at risk.

The density measurements had a high reliability, and were carried out blindly to subsequent tumor location, but the interpretation of the findings is constrained by the 2-dimensional nature of the interactive threshold method. Three-dimensional imaging methods (e.g., MRI and breast tomosynthesis; refs. 13, 14) will provide a better assessment of the value of localized density as a predictor of subsequent tumor location, but they will need to be modified to incorporate quantification of whole breast and localized amounts of fibroglandular tissue.

Relevance of the findings

The significance of the findings is 2-fold. First, they suggest that MD is directly involved in the pathogenesis of breast cancer. Second, the findings provide new avenues for the clinical management of women with high MD.

The biological basis for the MD-breast cancer association is not well characterized. Our findings suggest that high MD is likely to be causally related to breast cancer rather than a simple correlate of its determinants. Breast cancer arises from epithelial cells lining the ducts or lobules of the breast. The greater the number of these cells, the higher the amount of radiodense tissue and the likelihood that they may undergo a malignant transformation, consistent with the observation that high MD is associated with increased risk of both invasive cancer and proliferative lesions thought to be precursors of breast cancer (e.g., DCIS, hyperplasia with or without atypia, and columnar cell lesions; refs. 15, 16). Thus, MD may reflect higher rates of epithelial proliferation, which are likely to increase the risk of somatic mutations, epigenetic alterations, and carcinogenesis, and/or slower rates of involution (17–19). Fifteen percent of tumors arose within areas of the breast that were in the lower half of that breast's square-density distribution. This is consistent with the observation that breast cancer risk is lower, but not nonexistent, in women with entirely fatty breast (1). These tumors may have arisen within the dense tissue of tumor squares that were not entirely fatty or through MD-unrelated pathways.

Current breast cancer risk assessment models do not incorporate MD (20), although the addition of this variable may increase slightly their discriminatory power (21, 22), MD is more strongly associated with breast cancer risk than any of the variables included in the Gail's model (23), the most widely used risk prediction model, and unlike most of these variables MD may be modified by hormone interventions (e.g., tamoxifen; ref. 24). If MD is causally related to breast cancer, as strongly implied by our findings, factors that reduce within-woman MD are likely to lower risk and improve detection of early lesions by increasing the sensitivity of mammography.

Whole-breast MD, along with other independent risk factors, allows identification of women at high-breast cancer risk. Once a woman is considered as high risk, our findings imply that examination of localized MD may provide information on the likely location of a subsequent tumor within a breast. The ability to identify, within a specific breast, the tissue that is at increased risk may open up the possibility of more localized approaches for the management of high-risk women—for example, localized dense tissue excision, localized preventive chemotherapy, targeted radiotherapy could be explored in future studies, as in the present case-only study specificity could not be assessed. Cancer detection (including computer-assisted systems) could focus more on localized patterns of density and their between-screen changes. Under current U.K. guidelines (25), a woman with a predicted risk more than 8% from age 40 to 49 years or a lifetime risk more than 30% should be offered tertiary care, including additional MRI screening and consideration of risk reduction surgery (i.e., prophylactic mastectomy and/or oophorectomy). Prophylactic mastectomy reduces breast cancer risk by 90 to 100% (26, 27), but is the least acceptable option (27). Intensive screening yields frequent false positives, overdiagnosis, and overtreatment (28). No randomized trials have compared the benefits from these various strategies, and it is unlikely that such trials will ever be possible as patients, and their clinicians, are unlikely to accept random assignment between prophylactic surgery and intensive screening. Our findings among breast cancer cases need to be replicated in follow-up studies of high-risk women undergoing repeat-screen mammography, but if confirmed, they could lead to the development and evaluation of new localized approaches as alternatives to prophylactic mastectomy or intensive screening.

Conclusions

Whole-breast density is a well-established risk factor for breast cancer. This study goes a step further by clearly showing that tumors arise predominantly within localized (1-cm^2 squares) areas that were radiodense several years prior to diagnosis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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