Review
Mammographic density
Potential mechanisms of breast cancer risk associated with mammographic density: hypotheses based on epidemiological evidence

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Abstract

There is now extensive evidence that mammographic density is an independent risk factor for breast cancer that is associated with large relative and attributable risks for the disease. The epidemiology of mammographic density, including the influences of age, parity and menopause, is consistent with it being a marker of susceptibility to breast cancer, in a manner similar to the concept of 'breast tissue age' described by the Pike model. Mammographic density reflects variations in the tissue composition of the breast. It is associated positively with collagen and epithelial and non-epithelial cells, and negatively with fat. Mammographic density is influenced by some hormones and growth factors as well as by several hormonal interventions. It is also associated with urinary levels of a mutagen. Twin studies have shown that most of the variation in mammographic density is accounted for by genetic factors. The hypothesis that we have developed from these observations postulates that the combined effects of cell proliferation (mitogenesis) and genetic damage to proliferating cells by mutagens (mutagenesis) may underlie the increased risk for breast cancer associated with extensive mammographic density. There is clearly a need for improved understanding of the specific factors that are involved in these processes and of the role played by the several breast tissue components that contribute to density. In particular, identification of the genes that are responsible for most of the variance in percentage density (and of their biological functions) is likely to provide insights into the biology of the breast, and may identify potential targets for preventative strategies in breast cancer.

Introduction

Following Wolfe's original studies [1,2], the proportion of the breast area in the mammogram that is occupied by radiologically dense breast tissue (mammographic density) is now recognized to be a strong risk factor for breast cancer that may account for a large fraction of the disease [3,4] (see the review by Vachon and coworkers in this series [5]). In the present paper we review what is known of the aetiology of mammographic density and outline hypotheses for its association with risk for breast cancer.

We describe below the evidence that mammographic density is a marker of susceptibility to breast cancer, and we review what is known of the histology of radiologically dense breast tissue, and the influence of other risk factors for breast cancer. We describe associations of hormones, growth factors and a mutagen with mammographic density, and the evidence that mammographic density is influenced by genetic variants.

We propose that cumulative exposure to mammographic density may be an important determinant of breast cancer incidence, and that the risk for breast cancer associated with mammographic density may be explained by the combined effects of mitogens, which influence cell proliferation and the size of the cell population in the breast, and mutagens, which influence the likelihood of genetic damage to those cells. Figure 1 panels a and b, respectively, provide a schematic overview and a more detailed description of aspects of these hypotheses that are examined in the sections that follow. The available evidence is incomplete in many of these areas, however. In addition, all studies of the aetiology of mammographic density are constrained by the limitations of current methods of measuring density (see the review by Yaffe and coworkers in this series [5]).

Ultimately, the risk for breast cancer associated with mammographic density will be elucidated by an improved understanding of the biology of the breast (see the review by Tisty and coworkers in this series [5]). However, just as epidemiological methods have identified mammographic density as an important risk factor for breast cancer, whose

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IGF = insulin-like growth factor; IGFBP = insulin-like growth factor binding protein; ROS = reactive oxygen species; MDA = malondialdehyde.

Figure 1
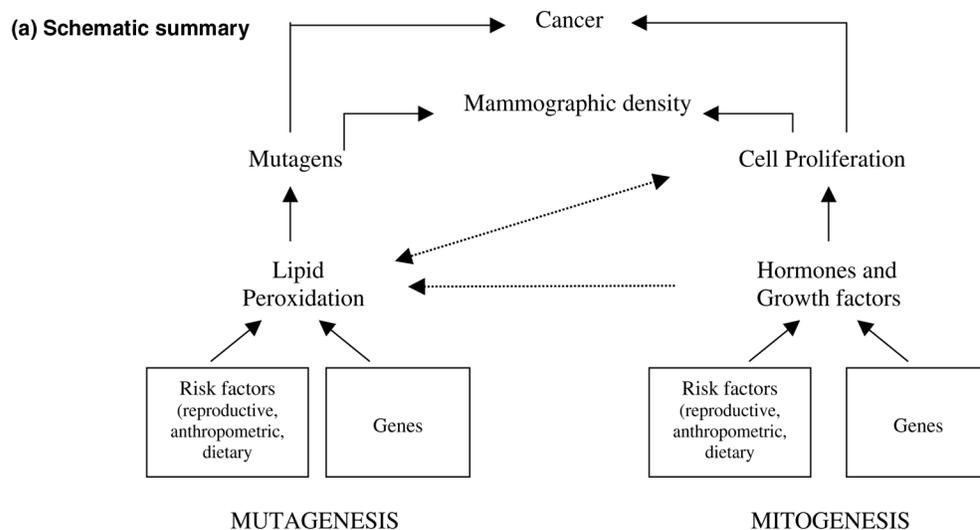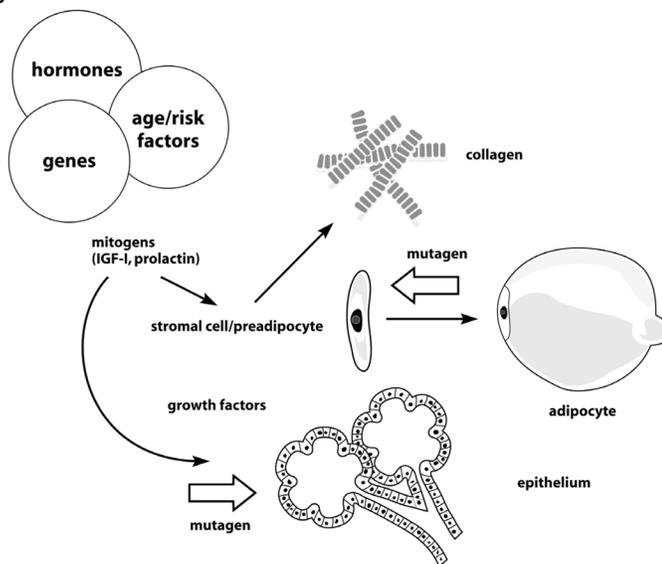
Hypotheses. **(a)** Schematic summary. We postulate that the combined effects of cell proliferation (mitogenesis) and genetic damage to proliferating cells caused by mutagens (mutagenesis) may underlie the increased risk for breast cancer associated with extensive mammographic density. Mitogenesis and mutagenesis are related processes. Increased cell proliferation increases susceptibility to mutations but also increases lipid peroxidation, which can in turn increase cell proliferation (see text). **(b)** Biological hypothesis. The tissue components (epithelial cells, stromal cells, collagen and fat) that are responsible for variations in mammographic density are related to each other in several ways. Stromal fibroblasts produce collagen, and some are pre-adipocytes that differentiate into adipocytes. Stromal and epithelial cells influence each other through paracrine growth factors, and both cell types are influenced by endocrine stimuli to cell proliferation (mitogenesis). Genetic damage to either stromal or epithelial cells caused by mutagens (mutagenesis) could initiate carcinogenesis (see text).

biology is likely to play an important role in the aetiology of the disease, epidemiological approaches may be able to suggest potential pathways and mechanisms that are responsible for risk.

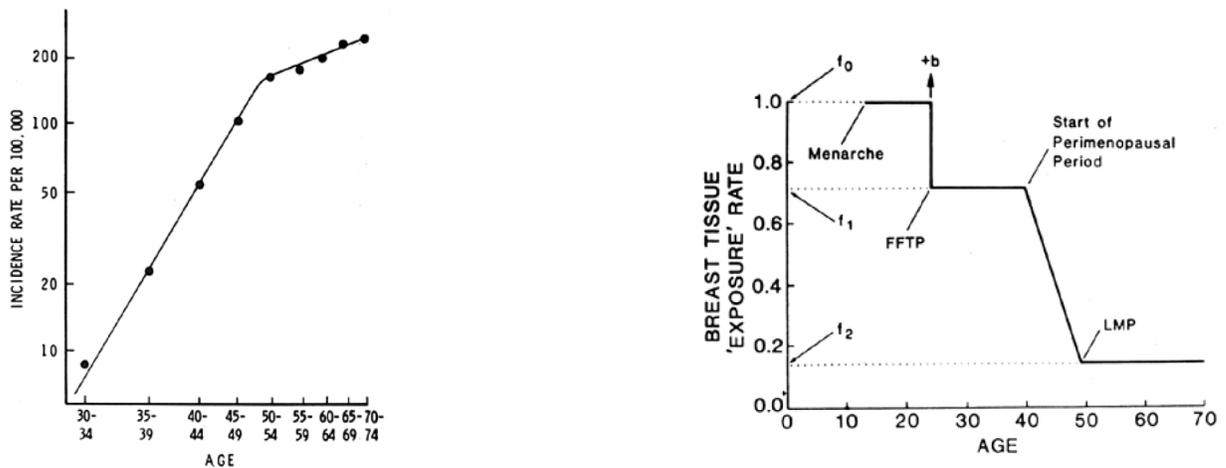## Cumulative exposure to mammographic density and breast cancer incidence

The average percentage mammographic density declines with increasing age (Figure 2a), whereas breast cancer

Figure 2

(a) Percent mammographic density according to age
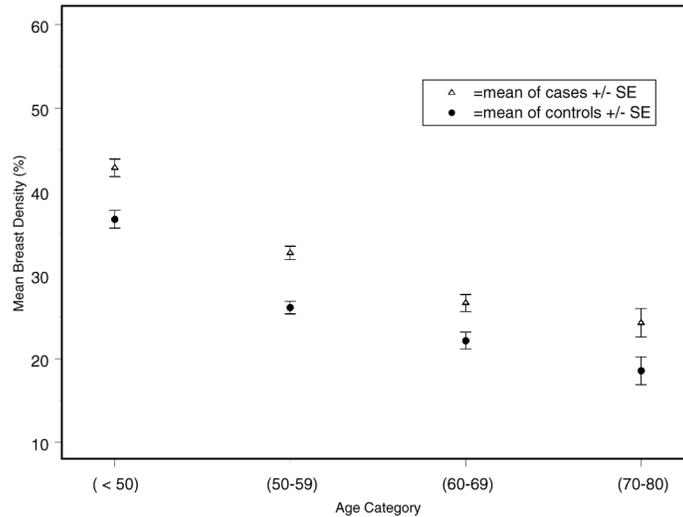
(b) Incidence of breast cancer and breast tissue age according to the Pike Model

Age, mammographic density and the incidence of breast cancer. **(a)** Baseline percentage mammographic density in women from three mammographic screening programmes according to those who developed breast cancer 1 to 8 years later (cases) or remained free from breast cancer (control individuals). Average percentage density in the baseline mammogram declined with increasing age at enrolment, both in women who eventually developed breast cancer and in those who remained free from disease. At all ages, percentage density was greater in those who developed breast cancer. Data from Boyd and coworkers [4]. **(b)** On the left is shown a log-log plot of the age-specific incidence of breast cancer. Adapted from Pike and coworkers [6]. To the right is shown the Pike model of breast tissue ageing. 'b' represents a one time increase in risk associated with first full-term pregnancy (FFTP). See Pike and coworkers [6]. LMP, last menstrual period.

incidence increases with age (Figure 2b [left]). This apparent paradox may be resolved, however, by reference to a model of breast cancer incidence proposed by Pike and coworkers [6]. This model is based on the concept that it is the rate of breast tissue 'ageing' or 'exposure', rather than chronological age, that is the relevant measure for describing the age-specific incidence of breast cancer (Figure 2b [right]). Breast tissue ageing is thought to be closely related to the mitotic activity of breast epithelial or stem cells and their susceptibility to genetic damage. According to the model, shown in Figure 2b (right), the rate of breast tissue ageing is most rapid at the time of menarche, slows with pregnancy,

slows further during the peri-menopausal period, and is least after the menopause. After fitting numeric values for these parameters, Pike and coworkers [6] showed that cumulative exposure to breast tissue ageing, given by the area under the curve in Figure 2b (right), described the age-incidence curve for breast cancer in the USA, also shown in Figure 2b (left). Thus, cumulative exposure to breast tissue ageing and the age-specific breast cancer incidence both increase with age, but the rate of increase slows with age, particularly after menopause.

Mammographic density shares many of the features of 'breast tissue age' and is influenced by similar factors. Detailed descriptions of the associations of risk factors with mammographic density can be found elsewhere [7-9]. Body size in particular is strongly and inversely associated with mammographic density, and is a risk factor for breast cancer independent of mammographic density [10]. We focus here on the associations of mammographic density with age, parity and menopause, variables in the Pike model that are also associated with variations in one or more of the histological features of the breast [11].

In addition to the effects of age referred to above, mammographic density is less extensive in women who are parous and in those with a larger number of live births (Figure 3). In these data each pregnancy was associated with an average 2% difference in percentage density [4]. Post-menopausal women have consistently been found to have less extensive mammographic density than premenopausal women, and a longitudinal study of the effects of the menopause on mammographic density [12] showed that percentage density was reduced by about 8% on average over the menopause.

All risk factors for breast cancer must ultimately exert their influence by an effect on the breast. These findings suggest that, for at least some risk factors, this influence includes an effect on the number of cells and the quantity of collagen in the breast, which is reflected in differences in mammographic density and which may mediate the effect of the factor on breast cancer risk (see 'Breast histology and radiological features', below). The concept of breast tissue age in the Pike model is related to the effects of hormones on the kinetics of breast cells and the accumulation of genetic damage. As we discuss below, mammographic density may reflect cumulative exposure to stimuli to division of breast cells that predisposes them to genetic damage by mutagens.

In addition to the cross-sectional data shown in Figure 2a, longitudinal studies [13,14] have found that percentage mammographic density in women who develop breast cancer was greater than in those who remained free from disease, but neither study showed that the rate of change over time was related to breast cancer risk. Both studies were based primarily on postmenopausal women, and it remains possible

Figure 3
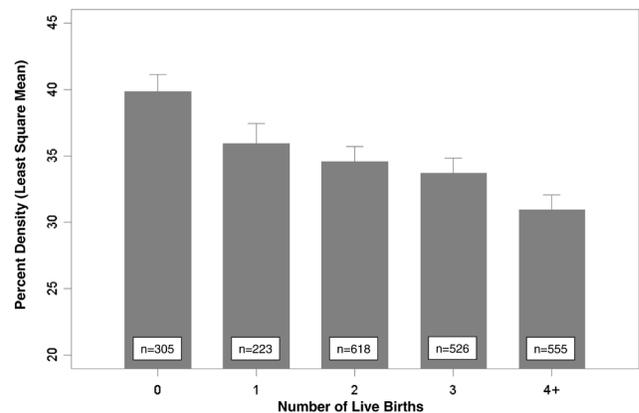
Parity and mammographic density. Least square means of percentage mammographic density according to number of live births, adjusted for age, body mass index, age at menarche, age at first birth, menopausal status, age at menopause, previous use of menopausal hormone therapy (ever/never) and breast cancer in first degree relatives (0, 1, 2+). The height of the bar is the least square mean of percentage density, and half width of the error bar represents the standard error. Data from Boyd and coworkers [4].

that differences in rate of change in mammographic density earlier in life may be related to later risk for breast cancer.

Breast histology and radiological features
Breast histology and mammographic density
Studies of the relationship between breast tissue histology and the radiological appearance of the breast (described in detail by Boyd and coworkers [9]), using surgical biopsies or mastectomy specimens, have found greater amounts of epithelium and/or stroma to be associated with mammographic density.

Li and coworkers used quantitative microscopy to examine histological features of randomly selected tissue blocks from breast tissue obtained at forensic autopsy [15,16] and determined the proportions of the biopsy occupied by cells (estimated by nuclear areas), glandular structures and collagen [11]. Figure 4 from that study [11] shows the inverse association of percentage density (in the image of the breast tissue slice from which the biopsy was taken) with age, and associations of percentage density with measured components of breast tissue, expressed as a percentage of the total area of the section. Greater percentage mammographic density was associated with a significantly greater total nuclear area, a greater nuclear area of both epithelial and nonepithelial cells, a greater proportion of collagen, and a greater area of glandular structures. Of the tissue components measured, collagen was present in the greatest quantity, was most strongly associated with percentage density, and explained 29% of the variance in percentage density. Nuclear area and glandular area accounted for between 4% and 7% of the variance in percentage density.

Figure 4
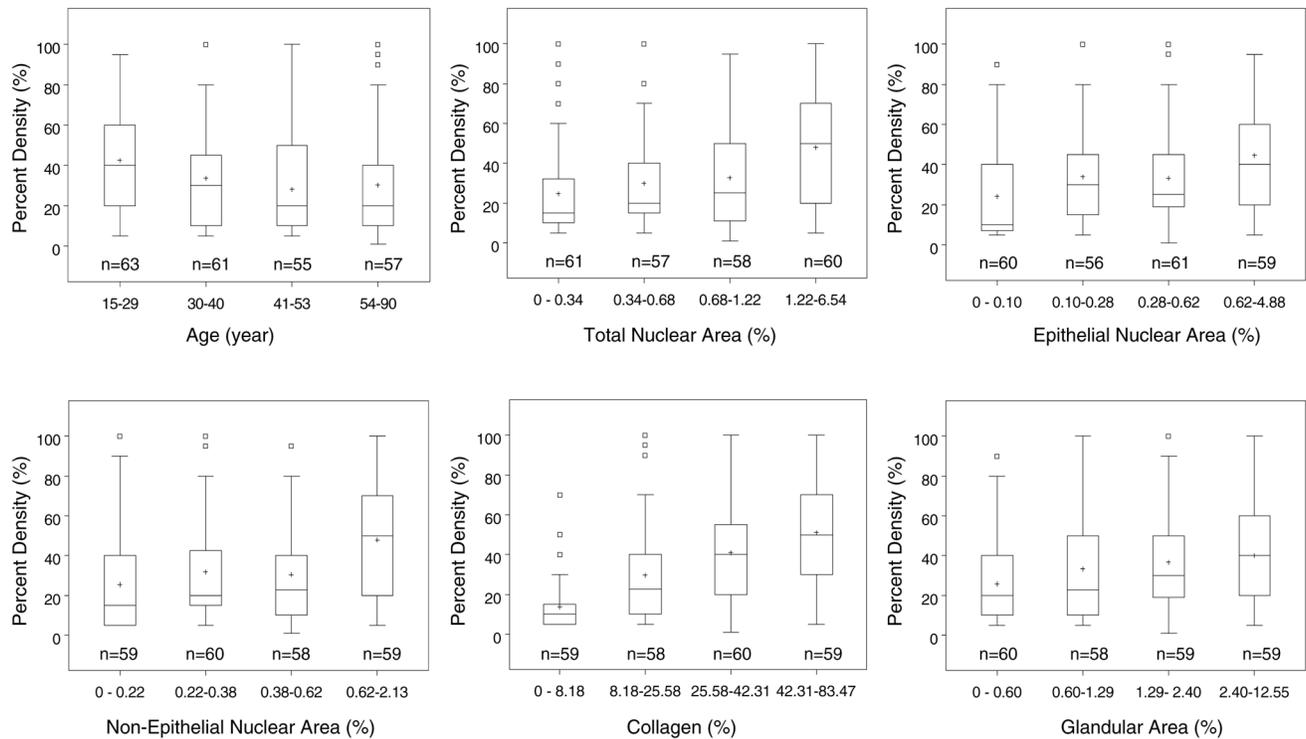
Percentage mammographic density, age, and histological measures. Boxplots showing the associations of percentage density with age and histological measures. Median is shown by a horizontal line, mean by the '+' symbol, interquartile range by the columns, 1.5× the interquartile range by the whiskers, and outliers are shown separately. P values from linear regression, using continuous variables adjusted for age, were as follows: age, $P = 0.04$; total nuclear area, $P < 0.001$; epithelial nuclear area, $P \leq 0.001$; nonepithelial nuclear area, $P < 0.001$; collagen, $P < 0.001$; glandular area, $P < 0.001$. Data from Li and coworkers [11].

Greater body weight, parity and greater number of births, and postmenopausal status were associated with quantitative differences in one or more of the tissue features measured in the autopsy samples described above (see Li and coworkers [11] for details). For example, greater body weight and postmenopausal status were inversely associated with all of the histological measures, and the percentage of collagen in the biopsy declined with parity and increasing number of live births. Each live birth was associated with an average reduction by 2% [11]. These factors were all associated with variations in mammographic density in this [11] and other studies [8,9], and with risk for breast cancer [17].

Breast histology and risk of breast cancer

Extensive mammographic density is associated with an increased risk for atypical hyperplasia and *in situ* breast cancer [18], which are associated with an increased risk for subsequent invasive breast cancer [19,20]. The reductions in collagen and glandular tissue, and the increase in fat that occur in the breast with increasing age have long been recognized by pathologists as 'involution', and Milanese and coworkers [21] demonstrated, using a definition that focused the degree of involution in the terminal duct lobular unit, that

greater involution was associated with a reduced risk for breast cancer. The reduction in mammographic density with age is associated with smaller proportions of collagen and glandular tissue in the breast [11] and may be related to involution of the terminal duct lobular unit.

Relationships among histological structures that are responsible for density

Epithelial and stromal cells, collagen and fat - the tissue components that contribute to mammographic density - are related to each other in several ways that are illustrated in Figure 1b. Epithelial and stromal cells communicate by means of paracrine growth factors (see the review by Tisty and coworkers in this series [5]). Collagen is a product of stromal fibroblasts, and adipocytes develop from stromal pre-adipocytes [22]. Factors that affect one of these components may therefore affect the others, directly or indirectly, and each component has properties that may influence risk for and progression of breast cancer.

Breast cancer arises from epithelial cells, and the number and proliferative state of these cells may influence both the radiological density of the breast and the probability of

genetic damage that may give rise to cancer. In addition, collagen and stromal matrix are products of stromal cells that may, through their mechanical properties, facilitate tumour invasion [23]. Metalloproteinases that regulate stromal matrix can also regulate the activation of growth factors and influence susceptibility to breast cancer [24,25].

Mitogenesis
Mitogens as potential mediators of effects: hormones and growth factors.
As shown in Figure 1a,b, the effects of age and other factors on breast tissue composition that are described above (and further below) are likely to be mediated at least in part by one or more of the several endocrine, paracrine and autocrine mechanisms that regulate the growth and development of breast stroma and epithelium. Variations in exposure or response to one or more of these mechanisms may explain the effects that genetic and environmental factors have on differences in breast tissue composition. Most studies to date have focused on endocrine influences.

Blood levels of hormones and growth factors
The results of cross-sectional studies that have examined blood levels of steroid sex hormones and growth factors in relation to mammographic density are summarized in Tables 1 and 2. The studies vary in design, size, method of sampling patients, methods of measuring density, and methods of analysis. All have assessed the association between the blood and mammographic measures after adjustment for other factors that are known to influence density.

Most studies of blood oestrogen levels and percentage mammographic density have found either no association or an inverse association with estrone levels (five out of seven studies) [26-30] or total or free estradiol (seven out of eight studies) [26-32] in premenopausal or postmenopausal women. An exception is the study carried out in the Postmenopausal Estrogen/Progestin Intervention Trial [33], which identified a positive association between percentage density and estrone, estradiol and free estradiol levels in postmenopausal women. Bremnes and coworkers [32] found a positive association of mammographic density with estrone levels (which was statistically significant only in women with insulin-like growth factor [IGF]-I levels below the median) but not with estradiol or free estradiol levels. Progesterone levels have not been shown to be associated with mammographic density in premenopausal or postmenopausal women. Sex hormone binding globulin has been found to have a significant positive association with mammographic density in two studies after adjustment for other variables [31,32], and in four other studies before adjustment [26,27,30,33]. Testosterone and androstenedione have not been shown to be associated with mammographic density in post-menopausal women and have not yet been studied in premenopausal women.

Blood levels of growth hormone have been found to be positively associated with mammographic density in pre-menopausal women, but this association became non-significant after adjustment for body size [31]. Because growth hormone is one of the factors that influences body size, this may be over-adjustment. Prolactin levels were found to be positively associated with the area of dense tissue in premenopausal women in one study [31], with percentage mammographic density in postmenopausal women in two studies [31,34], and in a further study statistical significance was lost after adjustment for other variables [27]. Mammo-graphic density was found to be positively associated with serum IGF-I levels in premenopausal women in three [31,35,36] out of five studies [37,38], and one study found an association in postmenopausal women [39]. Results with IGF-binding protein (IGFBP)-3 and the ratio of IGF-I to IGFBP-3 have been inconsistent. In a longitudinal study, women with higher levels of serum IGF-I during the pre-menopausal period experienced a smaller increase nondense area and a slightly smaller decrease in dense area during menopause [40].

Growth factors in breast tissue
To date, few studies have examined growth factors or stromal matrix proteins in breast tissue in relation to mammographic density. One study [41] was conducted in formalin-fixed paraffin blocks of breast tissue ($n = 92$) surrounding benign lesions, half from breasts with little or no radiological density and half from breasts with extensive density, and included groups matched for age at the time of biopsy. Similar to the results of the study conducted by Li and coworkers [11] described above, breast tissue from women with extensive densities had a greater nuclear area and a larger stained area of collagen. In addition, stained areas of immunohisto-chemistry for tissue inhibitor of matrix metalloproteinase-3 and IGF-I were greater in women with extensive density than in those with little breast density [41]. Stromal proteoglycans that are expressed in association with breast cancer have also been found to be associated with mammographic density [42].

Breast tissue response to hormones
Combined estrogen-progesterone menopausal hormone therapy, but not estrogen therapy alone, is associated with a small increase in risk for breast cancer [43], and increases mammographic density [44-46]. Percentage density is reduced by tamoxifen [47], and by a gonadotrophin-releasing hormone agonist [48] that reduces exposure to estrogen and progesterone in premenopausal women. The average reduc-tions in percentage density associated with these hormonal interventions are modest, in general less than 10%.

Hormones and growth factors: risk factors and risk for breast cancer
Estradiol and testosterone blood levels have been shown to be related to risk for breast cancer in premenopausal and

Table 1

Studies of steroid sex hormones, SHBG, and mammographic density

Hormone	Number of studies[a]	Menopausal status	Direction of association [ref.]		
			Positive	None	Inverse
Estrone	7	Premenopausal		Noh et al. [26]	
		Postmenopausal	Bremnes et al. [32], Greendale et al. [33]	Tamimi et al. [27][2], Aiello et al. [28][b], Warren et al. [29], Verheus et al. [30][2]	Aiello et al. [28][c]
Estradiol	8	Premenopausal		Noh et al. [26][1], Boyd et al. [31]	
		Postmenopausal	Greendale et al. [33]	Tamimi et al. [27][2], Aiello et al. [28][b], Warren et al. [29], Verheus et al. [30][2], Boyd et al. [31], Bremnes et al. [32]	Aiello et al. [28][c]
Free estradiol	8	Premenopausal		Noh et al. [26], Boyd et al. [31]	
		Postmenopausal	Greendale et al. [33]	Tamimi et al. [27][2], Aiello et al. [28][b], Warren et al. [29], Verheus et al. [30][2], Bremnes et al. [32]	Aiello et al. [28][c], Boyd et al. [31]
Progesterone	5	Premenopausal		Noh et al. [26][1], Boyd et al. [31][1]	
		Postmenopausal		Tamimi et al. [27], Warren et al. [29], Boyd et al. [31], Greendale et al. [33]	
SHBG	8	Premenopausal		Noh et al. [26][1], Boyd et al. [31][1]	
		Postmenopausal	Boyd et al. [31], Bremnes et al. [32]	Tamimi et al. [27][1], Aiello et al. [28][b,c], Warren et al. [29], Verheus et al. [30][1], Greendale et al. [33][1]	
Testosterone	6	Premenopausal		NA	
		Postmenopausal		Tamimi et al. [27], Aiello et al. [28][b], Warren et al. [29], Verheus et al. [30][2], Bremnes et al. [32], Greendale et al. [33]	Aiello et al. [28][c]
Androstenedione	5	Premenopausal		NA	
		Postmenopausal		Tamimi et al. [27], Aiello et al. [28][b], Warren et al. [29], Verheus et al. [30], Bremnes et al. [32]	Aiello et al. [37][c]

Associations shown are for percentage density. Associations were classified as positive, none, or inverse according to the direction of effect and the statistical significance of the values after adjustment for other factors, using a criterion of $P < 0.05$. Several associations were statistically significant before adjustment for other factors and these are indicated as follows: [1]positive association before adjustment and [2]inverse association before adjustment. [a]Some studies included both premenopausal and postmenopausal women. [b]Results for never users of hormones. [c]Results for users of hormones. SHBG, sex hormone binding globulin; NA, not assessed.

postmenopausal women [49,50] but, as discussed above, have not consistently been shown to be associated with mammographic density, suggesting that they may influence risk through pathways that are unrelated to density. In support of this idea, Tamimi and coworkers [51] recently reported that circulating sex steroid levels and mammographic density are independently associated with breast cancer risk in postmenopausal women. However, it remains possible that other forms of estrogen not measured in these studies, including estrogen metabolites, may influence mammographic density [52] and the associated risk for breast cancer [53]. Estrogens can induce lipid peroxidation (see below) [54], and catechol estrogens (metabolites of estrone and estradiol) can react with DNA to form adducts [55] that may initiate cancer.

Blood levels of IGF-I and prolactin have also been found to be associated with risk for breast cancer, IGF-I predominantly in premenopausal women, and prolactin in both premenopausal and postmenopausal women [56,57]. IGF-I is a known mitogen for breast epithelium that is produced in the breast stroma, as well as by the liver in response to growth hormone [58], and administration of growth hormone to ageing primates has been shown to induce epithelial proliferation [59]. Prolactin increases cell proliferation and decreases apoptosis in the breast, and higher blood levels have been found to be associated with an increased risk for breast cancer in both premenopausal and postmenopausal women [60]. Prolactin plays an important role in the development and progression of mammary tumours in rodents [61]. Mammographic density, IGF-I and prolactin levels are all

Table 2

Studies of pituitary hormones, related growth factors, and mammographic density

Hormone	Number of studies[a]	Menopausal status	Direction of association [ref.]		
			Positive	None	Inverse
Growth hormone	1	Premenopausal		Boyd et al. [31][1]	
		Postmenopausal		Boyd et al. [31][1]	
Prolactin	4	Premenopausal	Boyd et al. [31] (dense area only)		
		Postmenopausal	Boyd et al. [31], Greendale et al. [34]	Tamimi et al. [27][1], Bremnes et al. [39]	
IGF-I	7	Premenopausal	Boyd et al. [31], Byrne et al. [35], Diorio et al. [36]	Maskarinec et al. [37], dos Santo Silva et al. [38]	
		Postmenopausal	Bremnes et al. [39]	Aiello et al. [28][b,c], Boyd et al. [31], Byrne et al. [35], Diorio et al. [36][1], dos Santo Silva et al. [38]	
IGFBP-3	7	Premenopausal		Boyd et al. [31], Byrne et al. [35], Maskarinec et al. [37], dos Santos Silva et al. [38]	Diorio et al. [36]
		Postmenopausal		Aiello et al. [28][b,c], Boyd et al. [31], Byrne et al. [35], dos Santos Silva et al. [38][2], Bremnes et al. [39]	
IGF-I/IGFBP-3 ratio	7	Premenopausal	Boyd et al. [31], Byrne et al. [35], Maskarinec et al. [37]	Diorio et al. [36][1], dos Santos Silva et al. [38]	
		Postmenopausal	Bremnes et al. [39]	Aiello et al. [28][b], Boyd et al. [31], Byrne et al. [35], Diorio et al. [36][1], dos Santos Silva et al. [38]	Aiello et al. [28][c]

Associations shown are for percentage density, unless otherwise indicated. Associations were classified as positive, none, or inverse according to the direction of effect and the statistical significance of the values after adjustment for other factors, using a criterion of $P < 0.05$. Several associations were statistically significant before adjustment for other factors and these are indicated as follows: [1]positive association before adjustment and [2]inverse association before adjustment. [a]Some studies included both premenopausal and postmenopausal women. [b]Results for never users of hormones. [c]Results for users of hormones. IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein.

influenced by age, parity and number of births in a similar manner [62,63].

Mutagenesis
Mutagens as potential mediators of effects: oxidative stress
Oxidative stress occurs when an excess of reactive oxygen species (ROS) is produced in relation to antioxidant defences and can cause oxidative damage to DNA, protein and lipid molecules. DNA damage can lead to mutagenesis and increased risk for cancer [64]. Inflammation is also associated with increased ROS and may be an additional pathway that relates oxidative stress to cancer risk [65].

A variety of biomarkers for measurement of oxidative stress *in vivo* have been proposed, including markers of oxidative damage to DNA, protein and lipids [66], but a recent validation study in rats indicated that blood or urinary isoprostanes and urinary malondialdehyde (MDA) are the best

indicators of *in vivo* oxidative stress [67]. These compounds are products of lipid peroxidation produced from the free radical mediated oxidation of arachidonic acid. Isoprostane is a prostaglandin-like compound [68] and MDA is a known mutagen [69,70].

Urinary malondialdehyde and mammographic density
A positive association between mammographic density and 24-hour urinary MDA excretion was observed in three independent studies [71-73]. In premenopausal and postmenopausal women, representing a wide range of mammographic density [72,73], urinary MDA excretion was 23% to 30% higher in the highest quintile of mammographic density as compared with the lowest, after adjustment for age and body mass index or waist circumference (Table 3). Measures of body size, which are negatively associated with mammographic density and positively associated with oxidative stress, are important potential confounders of the relationship between urinary MDA and percentage mammo-

Table 3

Urinary excretion of MDA by quintile of percentage mammographic density

| | Quintiles of percentage density | | | | | |
	1	2	3	4	5	P value for trend
Premenopausal ($n = 160$)	2.76[a]	2.29	2.86	2.75	3.62	0.02
Postmenopausal ($n = 175$)	3.19	3.23	3.67	3.46	3.88	0.13
All women ($n = 335$)	3.02	2.76	3.10	3.38	3.68	0.01

Values for malondialdehyde (MDA) are expressed as mmol/day. For 'All women', values are also adjusted for menopausal status. [a]Least square mean of MDA adjusted for age and waist circumference (negative inverse). Data are from Hong and coworkers [73].

graphic density. This relationship becomes stronger [71], or is only evident [72,73], after adjustment for body size. Serum levels of MDA and MDA DNA adducts were not associated with mammographic density [73].

It is unknown whether systemic levels of lipid peroxidation markers, such as urinary MDA, reflect breast tissue levels. However, women with breast cancer who received radiation to the breast (which induces oxidative stress and inflammation) exhibited significantly increased urinary excretion of isoprostanes as compared with those women with breast cancer who did not receive such treatment [74].

Oxidative stress: risk factors and risk of breast cancer

The protective effects of higher fruit and vegetable intake and serum antioxidant levels on breast cancer risk seen in some studies, and studies showing that genetic polymorphisms in some antioxidant enzymes are associated with breast cancer risk provide indirect evidence for a role of oxidative stress in the development of breast cancer [75,76]. Direct evidence of an association of oxidative stress with breast cancer risk arises from case control studies of patients with and without breast cancer. Plasma MDA was elevated in breast cancer patients relative to levels in healthy control individuals [77-79]. Levels of MDA DNA adducts and 8-hydroxy-2-deoxy-guannosine (markers of DNA damage) were significantly higher in normal breast tissue of cancer patients than in the breast tissue of control individuals without cancer [80-82]. Recently, a large case control study identified a significant trend toward increasing breast cancer risk with increasing urinary excretion of isoprostanes [74]. A limitation of the studies cited above is that the markers of oxidative stress were measured in biological samples collected after breast cancer diagnosis, and therefore the higher levels of oxidative stress in cases could be due to the presence of cancer or its treatment.

Several lines of evidence suggest that there is an association between oxidative stress and some factors that are known or suspected to influence risk for breast cancer. Chinese women living in China have lower levels of urinary MDA excretion [83] and lower breast cancer risk than do Chinese

women living the USA, and Chinese American women have lower urinary isoprostane excretion than Caucasian American women [84]. The lower risk and oxidative stress observed in Asian women may be related to their lower body weight and dietary fat intake compared with Caucasian women. Lower body weight is associated with lower breast cancer risk [85] and lower levels of isoprostane [86,87]. Lower dietary fat intake may be associated with reduced breast cancer risk [88] and with reduced oxidative stress [84,89]. Chronic moderate levels of activity increase antioxidant activity [90,91] and are associated with reduced breast cancer risk [92]. However, the role that these factors play in risk for breast cancer associated with mammographic density is not yet clear. For example, Asian women tend to have greater percentage density than do Caucasian women (probably as a result of smaller breast size) [93], the effect of body weight on breast cancer is probably independent of mammographic density [10], and physical activity does not appear to be associated with mammographic density [94,95].

In terms of reproductive risk factors known to be associated with mammographic density, markers of oxidative stress are higher in postmenopausal than in premenopausal women [73,96] and may be reduced by menopausal hormone therapy [97] and tamoxifen [98]. However, estrogen and its metabolites have both anti-oxidant and pro-oxidant effects [99], and urinary isoprostane excretion was not associated with blood estrogen levels [100]. Higher alcohol intake is associated with higher breast cancer risk [101], plasma isoprostane levels [102], and mammographic density [72,103].

Relationship of mitogenesis and mutagenesis

Increased cell proliferation can cause an increase in production of ROS and lipid peroxidation, and the products of lipid peroxidation themselves can promote cell proliferation via cell signalling [104] (Figure 1a). Interestingly, MDA and isoprostanes (products of lipid peroxidation) have been reported to be mediators of the increased cell proliferation and collagen production seen in hepatic fibrosis [105]. Fibrosis, a response to tissue injury and inflammation (which increase oxidative stress), involves the proliferation and

activation of fibroblasts and results in accumulation of extra-cellular matrix and collagen [106]. It is unknown whether the process of fibrosis is related to mammographic density and increased risk for breast cancer. However, chronic inflam-mation and/or the wound healing response may be involved in the initiation or promotion of cancer [24,107], and the presence of breast cancer is associated with reactive stroma, a process that resembles fibrosis [108] that is thought to promote tumour progression and invasion. Thus, the associa-tion of increased MDA with higher mammographic density may be either a cause or an effect of increased cell proliferation and collagen production, and the risk for breast cancer may be increased by these processes as well as by mutagenesis. As shown in the Figure 1b both stromal and epithelial cells are potential sites of mutagenesis, either of which might initiate processes that ultimately give rise to breast cancer.

Heritability of mammographic density

Parity, menopause and other risk factors explain only 20% to 30% of the variance in mammographic density [8,109]. Early studies of mother-daughter sets [110,111] and small twin studies [111,112] suggested that genetic factors might explain a proportion of the variation (the heritability) of breast tissue patterns within a given population. A segregation analysis of nuclear family data conducted Pankow and co-workers [113] yielded findings consistent with a single mode of inheritance of one or more major genes, but it could not distinguish between dominant, recessive, or co-dominant models.

Twin studies conducted in Australia and North America identified correlations between twin pairs in percentage mammographic density that were, respectively, 0.61 and 0.67 for monozygotic twin pairs, and 0.25 and 0.27 for dizygotic twin pairs [114]. After adjustment for the other risk factors associated with differences in mammographic density, the proportion of the residual variation accounted for by additive genetic factors (heritability) was 63% (95% confidence interval 59% to 67%) in the studies combined, and was similar in each of the two studies. These two twin studies thus replicate each other in providing compelling evidence that the wide variation in percentage mammographic density among women is strongly influenced by genetic factors.

The search for genes associated with mammographic density is in its infancy and few have been found to date. Several large-scale genome-wide linkage and association studies are in progress and can be expected to report their findings within the next few years. The preliminary results from one genome-wide sib-pair linkage study [115] provide evidence for linkage at a region on chromosome 6. Vachon and coworkers [116] recently reported results of a genome-wide linkage scan that showed that a putative locus on chromosome 5p may account for a large proportion of the

variance in mammographic density. Among association studies conducted to date, variations in genes concerned with estrogen metabolism [117-120], the estrogen [121] and androgen [122] receptors, IGFBP-3 [123], IGF [124] and growth hormone [125] have been shown to be associated with mammographic density. To date, few of these findings have been replicated, and some that have been replicated [117,118] have also been contradicted [119,120].

Among potential genetic influences suggested by our hypothesis shown in Figure 1a,b are effects on the produc-tion and metabolism of breast mitogens [126], effects on the change in mitogens that occurs with ageing [127], the response of stromal and epithelial breast tissue to stimulation by mitogens [128], and tissue modelling in the breast [129]. The production and metabolism of mutagens may also be under genetic control [130], as is the repair of DNA damage caused by mutagens [131]. Some factors already found to be associated with mammographic density are also involved in processes that generate mutagens or modify their effects. These include catechol-O-methyltransferase, which is involved in the metabolism of catechol estrogens with pro-oxidant and anti-oxidant activities [117], and cytochrome P450 1A2 [73], which has been found to be associated with serum and urinary MDA levels. These associations require confirmation, however.

Summary

There is now extensive 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. The hypotheses that we have developed from the observations described above are summarized here and illustrated in Figure 1a,b.

Cumulative exposure to mammographic density and breast cancer risk

Mammographic density reflects variations in the tissue composition of the breast, and is associated positively with collagen and epithelial and nonepithelial cells, and negatively with fat. Increasing age, parity, and menopause are all associated with reductions in the epithelial and stromal tissues in the breast, and with an increase in fat. These histological changes are reflected in the radiological appearance of the breast, and are consistent with mammo-graphic density being a marker of susceptibility to breast cancer, in a manner similar to the concept of 'breast tissue age' described in the Pike model [6,132]. Like breast tissue age, variations in mammographic density may reflect the mitotic activity of breast cells and differences in susceptibility to genetic damage, and cumulative exposure to density may have an important influence on breast cancer incidence.

Mitogens, mutagens and mammographic density

Mammographic density is influenced by some hormones and growth factors, as well as by several hormonal interventions,

and is associated with urinary levels of a mutagen. We postulate that the combined effects of cell proliferation (mitogenesis) and genetic damage to proliferating cells by mutagens (mutagenesis) may underlie the increased risk for breast cancer associated with extensive mammographic density. As described above under 'Relationship of mitogenesis and mutagenesis', mitogenesis and mutagenesis are not independent processes. Increased cell proliferation can increase lipid peroxidation, and the products of lipid peroxidation can increase cell proliferation.

Blood levels of IGF-I and prolactin are among the endocrine stimuli to cell proliferation that have been found to be positively associated with both mammographic density and breast cancer risk, respectively, in premenopausal and post-menopausal women [56,57].

Autocrine and paracrine stimuli to the proliferation of epithelial and stromal cells, which regulate the growth, development and involution of the breast [25,133], have to date received little attention in relation to mammographic density, but one study [41] has identified an association of density with IGF-I in breast tissue. Animal models have shown that growth factors from fibroblasts can stimulate or inhibit epithelial proliferation, and that the genetic modification of fibroblasts can induce cancer [24,25,133].

The proliferation of cells that results from stimulation by endocrine, autocrine and paracrine growth factors increases risk for mutation [134]. To date the association of only one mutagen has been examined in relation to mammographic density, but greater levels of urinary excretion of MDA (a mutagenic product of lipid peroxidation) was found in three independent studies to be associated with more extensive mammographic density.

Potential areas for genetic influence include variation in the regulation of the hormones and growth factors that act on the breast, the response and modelling of breast tissue to these stimuli, and the processes that are involved in oxidative stress and the generation of mutagens.

Conclusion

Although there is evidence that both mitogenic and mutagenic processes are involved in determining the risk for breast cancer associated with mammographic density, there is clearly a need for an improved understanding of the

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edited by Norman Boyd.

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specific factors involved and of the role played by the several breast tissue components that contribute to density. In particular, the identification of the genes that are responsible for most of the variance in percentage density (and of their biological functions) is likely to provide insights into the biology of the breast and may identify potential targets for preventative strategies for breast cancer.

Competing interests
The authors declare that they have no competing interests.

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