Breast Density and Breast Cancer Risk: Understanding of Biology and Risk

Andreas Pettersson · Rulla M. Tamimi

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Abstract Mammographic density is a strong risk factor for breast cancer, independent of other known risk factors. While a number of hypotheses have been proposed, the biological mechanisms underlying the association between mammographic density and breast cancer remain, however, largely unknown. Understanding these mechanisms may help identify opportunities to reduce mammographic density and ultimately breast cancer risk. We review three major areas of research that may help us better understand the biologic underpinnings of mammographic density: heritability and genetics, breast tissue histology and (molecular) pathology, and experimental studies. We summarize the main consistent findings in the literature and identify key areas for future research.

Keywords Mammographic density · Breast density · Breast cancer · Genetics · Gene expression · Molecular markers

Introduction

On a mammogram, epithelial cells and stroma (excluding the adipose tissue component of the stroma) appear dense (white/light) whereas adipose tissue appears nondense (dark). In the

research setting, mammographic density is often measured using a computer-assisted thresholding technique and quantified as absolute or percentage dense area on a mammogram. Mammographic density is a strong risk factor for breast cancer independent of other established risk factors. For example, women with ≥ 75 % mammographic density have a 4.6-fold increased risk of breast cancer relative to women with < 5 % according to one meta-analysis [1].

The biological mechanisms underlying the association between mammographic density and breast cancer risk are largely unknown. Understanding these mechanisms may help identify opportunities to reduce mammographic density and ultimately breast cancer risk. Several non-mutually exclusive hypotheses explaining the association between mammographic density and breast cancer risk have been proposed, including (but not limited to) the following.

1. Mammographic density reflects the number of mammary stem cells ‘at risk’ of developing breast cancer [2].
2. Mammographic density reflects the combined effects of cell proliferation (mitogenesis) and genetic damage to proliferating cells by mutagens (mutagenesis) [3].
3. Mammographic density reflects local estrogen production in the breasts [4].
4. Mammographic density reflects the degree of lobular involution of the breasts [5].
5. Mammographic density reflects mechanical properties (e.g., extracellular matrix [ECM] and tissue stiffness, or quantitative or structural changes of the stromal collagen such as cross-linking) of the breast tissue [6, 7].

In addition to these hypotheses, it has been suggested that the absolute nondense area (i.e., the amount of breast adipose tissue) may also influence breast cancer risk. Higher absolute nondense area (i.e., higher amounts of breast adipose tissue) has been linked with both increased [8] and decreased [9]

A. Pettersson (✉) · R. M. Tamimi (✉)
Department of Epidemiology, Harvard School of Public Health, 677
Huntington Avenue, Boston, MA 02115, USA
e-mail: apetters@hsph.harvard.edu
e-mail: rulla.tamimi@channing.harvard.edu

A. Pettersson
Clinical Epidemiology Unit, Department of Medicine, Karolinska
Institutet, 171 77 Stockholm, Sweden

R. M. Tamimi
Channing Division of Network Medicine, Brigham and Women’s
Hospital, 181 Longwood Avenue, Boston, MA 02115, USA

breast cancer risk. In a recent meta-analysis including data from 13 case-control studies, absolute nondense area was inversely associated with breast cancer risk (odds ratio [OR] for a one standard deviation increment in absolute nondense area among postmenopausal women=0.85, 95 % CI 0.75–0.96) [10]; however, because of between-study heterogeneity, it remains unclear if the inverse association between the absolute nondense area and breast cancer risk is independent of the absolute dense area.

In this review, we focus on studies that add to our understanding of the biologic mechanisms by which mammographic density may increase breast cancer risk, with emphasis on the heritability and genetics of mammographic density, mammographic density and breast tissue histology and (molecular) pathology, and experimental studies.

Heritability and Genetics of Mammographic Density

Twin Studies and Estimates of Heritability of Mammographic Density

Evidence from twin studies provides convincing and quantitative estimates of the heritability of mammographic density [11–13]. Boyd et al. examined the heritability of mammographic density by comparing percentage mammographic density in 353 pairs of monozygotic twins and 246 pairs of dizygotic twins from Australia and North America [11]. It was estimated from these twin studies that genetics accounts for 60–67 % of the variation in percentage mammographic density. These estimates of heritability were adjusted for several life-style factors known to be associated with mammographic density, including age, body mass index (BMI), age at menarche, menopausal status, parity, and age at first birth. The correlation between percentage mammographic density in monozygotic twins was twice as high as that in dizygotic twins. Additional analyses from these studies also revealed that the heritability of mammographic density among women over 50 years was similar to that among women 50 or younger [14], and that genetic factors explain a large proportion of the variation in both absolute dense area and absolute nondense area [15].

Genome-wide Association Studies (GWAS) of Mammographic Density

Given the strong heritability of mammographic density, several investigators have examined genetic predictors of breast density as well as conducted genome-wide association studies (GWAS). The Markers of Density (MODE) consortium conducted a meta-analysis of five GWAS of percentage mammographic density adjusted for age and BMI [16]. In total, 4,877 women of European descent, who were primarily

postmenopausal at the time of mammogram (89 %), contributed to these analyses. In silico replication of the top hits from the meta-analysis revealed a genome-wide significant hit in the pooled analysis for rs10995190 ($P=9.6 \times 10^{-10}$) in the gene *ZNF365*. This single nucleotide polymorphism (SNP) explains <1 % of the variance in percentage mammographic density. A large GWAS of breast cancer had also identified this region as a breast cancer risk SNP [17]. To assess the extent to which the observed association between rs10995190 and breast cancer risk might be mediated through mammographic density, the authors estimated the association both before and after adjustment for mammographic density for studies with data on both breast cancer cases and controls. From the pooled analysis, the association between rs10995190 and breast cancer risk was (OR: 0.85, 95 % CI: 0.76–0.96, $p=0.008$). Adjusting for mammographic density slightly attenuated the association (OR: 0.90, 95 % CI: 0.80–1.01, $p=0.09$). These results suggest that genetic variation in *ZNF365* could influence breast cancer risk by influencing the proportion of dense tissue in the breast, although it remains possible that the same locus influences both phenotypes independently.

To further explore the shared genetic basis of mammographic density and breast cancer risk, Varghese et al. [18] created genetic scores based on the top 1–10 % of the MODE GWAS mammographic density SNPs and examined the scores in relation to breast cancer risk in an independent study population (U2K GWAS: 3,628 cases and 5,190 controls). Women in the top 10 % of the mammographic density genetic score distribution were at a 31 % (OR: 1.31, 95 % CI: 1.08–1.59) increased risk of developing breast cancer compared with women in the lowest 10 % of the score. These findings support the hypothesis that breast density and breast cancer have a shared genetic basis.

Breast Cancer Risk Loci in Relation to Mammographic Density

An international consortium, DENSEP, pooled together 19 studies (16,895 Caucasian women) with data on established breast cancer susceptibility variants and quantitative mammographic density measures [19]. The initial publication pooled cross-sectional analyses of common breast cancer susceptibility variants in 14 independent loci and mammographic density measures. The strongest associations were seen with rs3817198 (*LSP1*) and absolute dense area ($p=0.00005$) and percentage dense area ($p=0.001$). There was also evidence of an inverse association between rs10483813 (*RAD51L1*) and percentage dense area ($p=0.003$), but not with absolute dense area ($p=0.07$). There were nominal associations of multivariate adjusted percentage dense area and absolute dense area with rs2046210 (*ESR1*), rs1045485/rs17468277 (*CASP8*), rs4973768 (*SLC4A7/NEK10*), and rs3803662 (*TOX3*), which

were in the direction of the published corresponding breast cancer associations but not statistically significant after taking into account multiple testing. None of the investigated SNPs were associated with absolute nondense area. The two common variants in *LSP1* and *RAD51L1* explained 0.2 % (combined, 0.1 % for each) of the variance in adjusted percentage dense area and absolute dense area.

The MODE consortium also examined if any other known breast cancer SNPs were associated with mammographic density. Out of 22 SNPs tested (excluding rs10995190), two SNPs showed an association with percentage dense area: rs2046210 (*ESRI*, $p=0.005$) and rs3817198 (*LSP1*, $p=0.04$). Both associations were in the expected direction as determined by the corresponding breast cancer associations.

Together, these data are consistent with the hypothesis that mammographic density is a polygenic trait, influenced by many common low-penetrance variants, and/or rarer variants with larger effects, which cannot be identified through current GWAS. The SNPs identified have demonstrated a consistent relation with mammographic density and breast cancer risk. There is thus some evidence supporting the hypothesis that some of the effect of breast cancer risk loci on breast cancer risk is mediated through mammographic density.

Mammographic Density and Breast Tissue Histology and (Molecular) Pathology

Mammographic Density and Breast Tissue Components

Histologically, the majority of the breast tissue is occupied by stroma and adipose tissue. For example, in breast tissue obtained from biopsies of non-invasive breast lesions, epithelia has been shown to comprise on average ~5 % of the tissue, with adipose tissue and collagen comprising the remaining ~95 % [20, 21]. Likewise, among 300 women in the Nurses' Health Study undergoing biopsy for benign breast disease, the mean epithelial proportion was 5.3 % and the mean stromal proportion was 58.7 % [22]. That the major dense breast tissue component is stroma rather than epithelium has led to the notion that the differences in mammographic density, and the association between mammographic density and breast cancer risk, may largely be explained by differences in the stroma rather than the epithelium.

Numerous studies have investigated the relationship (measured within or between study subjects) between mammographic density and the amount or proportion of epithelial cells, adipose tissue, and/or stroma in breast tissue samples [4, 20, 23–29]. Consistently, these studies report a positive association between mammographic density and the amount or proportion of stroma and/or collagen. Likewise, the amount or proportion of adipose tissue has been consistently inversely

associated with mammographic density [4, 20, 25, 28, 29]. The studies examining the association between mammographic density and the amount or proportion of epithelial cells (in some studies measured as ducts or lobules or glandular area rather than epithelial cells [24, 26, 28], or total nuclear area rather than epithelial nuclear area [23]) are somewhat mixed, although all studies report a positive association [4, 20, 23–25, 27] or no association [26, 28, 29].

In summary, existing data suggest that epithelial cells comprise a small proportion (~5 %) of the breast tissue, whereas stroma and adipose tissue comprise a large proportion (~95 %). Moreover, mammographic density appears to be positively associated with the amount or proportion of both epithelia and stroma (perhaps more strongly with stroma than epithelia), and inversely associated with the amount or proportion of adipose tissue.

Mammographic Density and Histological Breast Cancer Risk Factors

Many studies have assessed the association between mammographic density and histological or cytomorphological breast cancer risk factors, such as carcinoma in situ, atypical hyperplasia, and hyperplasia without atypia [20, 26, 30–40]. Although results from these studies have been mixed, several studies have reported a positive association between mammographic density and these and other proliferative lesions (reviewed in, for example, Boyd et al. [21]). These findings have contributed to the hypothesis that mammographic density reflects proliferation of breast epithelium and stroma.

Mammographic Density and Lobular Involution

It has been hypothesized that mammographic density reflects the degree of lobular involution, a process during which epithelial cells undergo apoptosis and are replaced by adipose tissue. In support of this hypothesis, Ghosh et al. reported an inverse association between mammographic density (defined by Wolfe's parenchymal patterns) and lobular involution among 2,667 women in the Mayo benign breast disease (BBD) cohort [5]. Compared with those with complete involution, women with no involution had an OR of 1.7 (95 % CI 1.2–2.3) of high density (DY pattern). The corresponding OR among those with partial involution was 1.3 (95 % CI 1.0–1.6). In the same paper, the authors confirmed these findings among 317 women with benign breast disease in the Mayo Mammography Health Study (MMHS), among whom involution was found to be inversely associated with percentage mammographic density (as measured by Cumulus, a computer-assisted thresholding method). Involution was not, however, associated with the absolute dense area, but rather strongly positively associated with the absolute nondense area. Intriguingly, in a follow-up study based on the Mayo

BBD cohort, Ghosh et al. found that mammographic density and lobular involution are independent risk factors for breast cancer [5], arguing against the hypothesis that the association between mammographic density and breast cancer risk is explained to any large degree by involution. Ghosh et al. also found that women with dense breasts and no lobular involution had the highest breast cancer risk and speculated that the stromal-rich environment in dense breasts results in a preponderance of growth factors that stimulate the epithelium in a non-involuting breast, which could lead to malignant transformation.

Mammographic Density and Tissue Markers

To date, at least ten studies have assessed the association between mammographic density and different immunohistochemical (IHC) markers (see Table 1 for details).

Five studies assessed the association between mammographic density and estrogen receptors (ER α and/or ER β) and/or progesterone receptor (PR) expression [24, 25, 41–43]. With the exception of the study by Lundström et al. [41], results were null, suggesting that mammographic density is not associated with ER or PR expression in non-cancerous breast tissue.

Five studies assessed the association between mammographic density and Ki67, a proliferation marker [24, 25, 40, 42, 43]. All but one study [24] reported no association. Lack of association between mammographic density and cellular proliferation has also been reported by Stomper et al., who measured DNA S-phase percentages in dense ($n=34$) and fatty ($n=66$) breast tissue ≥ 2 cm away from the mammographic lesion [38]. Moreover, Hawes et al. found lower proliferation rates in high and medium density compared with low density of connective tissue in tissue blocks from 12 women undergoing reduction mammoplasty [44]. Taken together, these studies do not support the hypothesis that mammographically dense tissue reflects proliferation of breast epithelium and/or stroma.

Vachon et al. reported higher aromatase expression in mammographically dense versus nondense regions of 49 healthy volunteers undergoing ultrasound-guided core needle biopsies, supporting the hypothesis that local estrogen production in the breasts influences breast density [4]. This interesting finding, which may also potentially be explained by high breast density causing higher aromatase expression due to, for example, ‘mechanical’ properties (e.g., cell density) of dense breast tissue [45], has not been replicated to date.

Guo et al., in biopsy material surrounding non-invasive lesions from 46 women with mammographically dense breasts (>50 % density) and 46 women with mammographically nondense breasts (<25 % density), found higher expression of insulin-like growth factor 1

(IGF-I) and TIMP-3, a tissue inhibitor of matrix-degrading matrix metalloproteinases (MMPs), in dense compared with nondense breasts [23]. This finding is in line with the hypothesis that growth factors within the stroma affect stromal and epithelial proliferation, mammographic density, and breast cancer risk. However, Steude et al., under the hypothesis that MMPs and their tissue inhibitors affect breast density and breast cancer risk through degradation of the ECM and activation of growth factors, found no association between mammographic density (as measured by Cumulus) and MMP-1, MMP-12 or TIMP-3 in benign and/or malignant tissue from 277 women undergoing breast cancer surgery [46]. In fact, although not statistically significant, Steude et al. found lower TIMP-3 expression in women with higher mammographic density.

Additionally, there are single studies that have evaluated other markers. These studies reported no association between mammographic density and transforming growth factor- α (TGF α) [23], proliferating cell nuclear antigen (PCNA) [42] and human epidermal growth factor receptor 2 (HER2) [42] expression, but a positive association with collagen-related markers (lumican, decorin, and syndecan-1) [26] and with stromal COX2 [43] expression.

Mammographic Density and Breast Tissue Gene Expression

Four studies have investigated the association between mammographic density and breast tissue gene expression.

Yang et al. conducted gene expression profiling in excisional biopsies, taken >5 cm away from the tumors, from mastectomy samples in women undergoing surgery for breast cancer [43]. BIRADS (Breast Imaging Reporting and Data System) breast density scores from pre-operative mammograms were used to quantify mammographic density. Participants were divided into those with dense ($n=28$; BIRADS 3–4) and nondense ($n=38$; BIRADS 1–2) breasts. Out of approximately 34,000 genes, 26 genes showed higher expression (≥ 1.5 -fold higher at $p \leq 0.001$), and 47 genes showed lower expression, in dense versus nondense tissue. Canonical pathway analysis suggested decreased TGF β signaling in dense versus nondense tissue. TGF β is a growth factor with both tumor suppressing and tumor promoting properties [47]. Interestingly, Yang et al. also found higher levels of stromal COX2 expression (as assessed by IHC; see above); COX2 can inhibit TGF β , but can also stimulate aromatase expression.

Haakensen et al. conducted gene expression profiling in biopsy material from 79 women without breast cancer and 64 women with breast cancer [48]. Among women without breast cancer, the biopsy was taken from an area with ‘no visible pathology’ but with some breast density.

Table 1 Summary of studies evaluating the association between mammographic density and immunohistochemical (IHC) tissue markers

Study (Reference)	Year	Country	Study population	Material	N	Mammographic density	IHC markers	Main results	Adjusted for
Guo et al. [23]	2001	Canada	Women undergoing fine needle biopsies of the breast; nonmalignant findings	Tissue surrounding but remote from benign lesions	92	Percentage density (semiquantitative scale) of the entire breast on mammograms taken at the time of biopsy. Divided into low density ($n=46$; $<25\%$ density) and high density ($n=46$; $>50\%$ density). Density at the site of biopsy also investigated; highly correlated with density of the whole breast	TGF α IGF-1 TIMP-3	Density positively associated with IGF-1 and TIMP-3 expression. No association between mammographic density and TGF α expression	Age at biopsy
Alowami et al. [26]	2003	Canada	Women undergoing lumpectomy or mastectomy because of benign or pre-invasive breast disease identified on mammograms	Tissue distant from the lesion	62	Percentage density (semiquantitative scale) of the surrounding tissue distant from the lesion on diagnostic mammograms. Divided into low density ($n=35$; $\leq 25\%$ density) and high density ($n=27$; $\geq 50\%$ density)	Lumican Decorin	Density positively associated with lumican and decorin expression	None
Lundström et al. [41]	2006	Sweden	Women undergoing surgery for breast cancer (all women were postmenopausal)	Radiologically benign tissue ≥ 2 cm from the tumor from both low- and high-dense areas	?	X-ray of tissue specimen. Divided into low and high density. Note: all analyses performed within each participant	Syndecan-1 ER α ER β	Higher Syndecan-1 expression in epithelium and stroma from dense versus nondense tissue. Higher ER α in epithelium from dense versus nondense tissue. No association between ER β epithelial expression and density	None, all analyses performed within each participant
Khan et al. [40]	2007	USA	Women at high risk of breast cancer undergoing RPFNA	Material from breast aspirations	344	Percentage density assessed by Cumulus on mammograms within 6 months of RPFNA	Ki67	No association between density and Ki67 in breast epithelial cells	Age, menopausal status, 5-year Gail risk, HRT status, BMI, height and weight
Harvey et al. [24]	2008	USA	Women undergoing mastectomy for breast cancer (all women were postmenopausal, half ($n=28$) using HRT and half not using HRT)	Mastectomy tissue from each quadrant not showing evidence of cancer	56	Percentage density (computer-assisted) of the contralateral mammogram	ER PR Ki67	Density positively associated with Ki67 expression in ducts and lobule type 1. No association between mammographic density and ER or PR expression	Age at mastectomy, HRT use
Verheus et al. [42]	2009	USA	Women in the Multiethnic Cohort (MEC) undergoing surgery for breast cancer	Up to 4 cores from areas of malignant and benign tissue	159	Mammographic density (Cumulus) on pre-diagnostic mammograms	ER α ER β PR HER2	No association between density and the markers	Age at mammogram, BMI, ethnicity, parity, age at first live birth, age at menarche,

Table 1 (continued)

Study (Reference)	Year	Country	Study population	Material	N	Mammographic density	IHC markers	Main results	Adjusted for
Steude et al. [46]	2010	USA	Women in the MEC cohort undergoing surgery for breast cancer	Up to 4 cores from areas of malignant and benign tissue from tissue block put on TMAs	277	Mammographic density (Cumulus) on pre-diagnostic mammograms	PCNA Ki67 MMP-1 MMP-12 TIMP-3	No association between density and the markers	menopausal status, HRT use and family history of breast cancer Age at mammogram, BMI, ethnicity, parity, age at first live birth, age at menarche, menopausal status, HRT use, family history of breast cancer and tumor stage
Yang et al. [43]	2010	USA	Women undergoing mastectomy for breast cancer	Excision biopsies from mastectomy specimens >5 cm from the tumor	66	BIRADS on pre-operative mammograms. Low density ($n=28$; BIRADS 1–2) and high density ($n=38$; BIRAD 3–4)	ER PR Ki67 COX2	Higher stromal COX2 expression in dense versus nondense tissue. No association with ER, PR or Ki67 in epithelium or stroma	ER, PR, Ki67 and COX2
Vachon et al. [4]	2011	USA	Healthy volunteers with normal screening mammograms undergoing ultrasound-guided core-needle biopsies	Biopsy tissue from both dense and nondense regions of the breasts	49	Mammographically dense and nondense tissue. Note: all analyses performed within each participant	Aromatase	Higher aromatase expression in dense versus nondense tissue. Higher aromatase expression in epithelial cells and stromal cells from dense versus nondense tissue, but lower aromatase expression in adipocytes from dense versus nondense tissue	None, all analyses performed within each participant
Ghosh et al. [25]	2012	USA	Healthy volunteers with normal screening mammograms undergoing ultrasound-guided core-needle biopsies	Biopsy tissue from both dense and nondense regions of the breasts	59	Mammographically dense and nondense tissue. Note: all analyses performed within each participant	Er α PR Ki67	No association between density and the markers	None, all analyses performed within each participant

BIRADS Breast Imaging Reporting and Data System, *ER* estrogen receptor, *HER2* human epidermal growth factor receptor 2, *HRT* hormone replacement therapy, *IGF-1* insulin-like growth factor 1, *MMP* matrix metalloproteinase, *PCNA* proliferating cell nuclear antigen, *PR* progesterone receptor, *RPFNA* random perianelolar fine needle aspiration, *TGF α* transforming growth factor- α , *TIMP* tissue inhibitor of matrix-degrading MMPs, *TMAs* tissue microarrays

Among women with breast cancer, the tissue came from the tumor. Mammographic density was assessed using the computer-assisted thresholding technique, Madena, and was treated as a continuous variable in the analyses. Out of 9,767 probes, 25 probes (covering 24 genes) were significantly differentially expressed by breast density. Pathway analysis revealed no significant pathway, but among the 24 differentially expressed genes were *ESR1*, encoding for the ER α , and three genes in the uridine 5'-diphospho-glucuronosyltransferase (UGT) family, gene products which inactivate estrogen metabolites. The expression of these genes was lower in high-density tissue as compared with low-density tissue and in tissue from breast cancers compared with low-density tissue.

Sun et al. conducted gene expression profiling in non-malignant tissue adjacent to tumor (<2 cm from the tumor margin) among 118 women undergoing breast cancer surgery [29]. Mammographic density was measured with Cumulus in the pre-treatment mammogram from the contralateral breast. Using unsupervised hierarchical clustering, the tissue was classified into extra-tumoral subtypes 'Active' or 'Inactive'; the Active subtype had previously been shown to be associated with high expression of genes involved in activation of fibrosis, cellular movement, increased TWIST expression and positive expression of TGF β signatures, whereas the Inactive subtype had previously been shown to be associated with higher expression of cell adhesion and cell–cell contact genes. Mean percentage density was higher in the Inactive (32 %) compared with the Active (23 %) subgroup. Moreover, both the Inactive subtype and percentage density were positively associated with gene expression signatures of 'young age' and 'estrogen response,' and inversely associated with 'obesity' and 'TGF- β ' signatures. These findings are in line with the results from Yang et al. [43] that TGF β signaling is inversely associated with mammographic density.

Lisanti et al. [49] isolated fibroblasts from women with high breast density ($n=7$) and women with low breast density ($n=6$). They conducted gene-set enrichment analyses and identified gene sets related to high density including stress response, inflammation, stemness, signal transduction, and cancer. In comparing gene signatures from high breast density with that of tumor stroma, five shared biological processes emerged (stress signaling, stemness, angiogenesis, inflammation, and fibrosis), many of which are associated with wound healing.

Several of the gene expression studies, as well as the IHC marker studies described above, have been conducted with little acknowledgment to the tissue heterogeneity. There is heterogeneity with respect to the tissue sources (e.g., benign biopsy, tissue adjacent to tumors, reduction mammoplasties), and there is cellular heterogeneity (e.g., epithelial, stromal, adipocytes). Thus, although several promising findings have

Table 2 Summary of evidence from the literature

1. Twin studies suggest that mammographic density is largely determined by genetic factors (heritability ~60 %), with the remaining variation being explained by environmental factors, especially BMI. GWAS studies have identified SNPs associated with breast density and breast cancer, supporting a shared heritability of both. However, to date the SNPs identified from GWAS of mammographic density explain only a small proportion of the variability
2. The majority of the breast tissue is stroma and adipose tissue (~95 %), with epithelial cells comprising the remaining ~5 %
3. Mammographic density is positively associated with the amount or proportion of stroma, inversely associated with the amount or proportion of adipose tissue, and likely positively associated with the amount or proportion of epithelial tissue
4. The extent to which the association between mammographic density and breast cancer risk is explained by the numbers of cells 'at risk' of cancer formation remains unclear
5. There appears to be no association between mammographic density and the proliferative activity of the breast tissue, as determined by, for example, Ki67 expression
6. A growing body of evidence suggest that crosstalk between the epithelial cells, stroma, and adipose tissue is important for determining mammographic density and potentially breast cancer risk. Data from both epidemiological and experimental studies suggest several plausible mechanisms, including increased local estrogen production, altered levels of growth factors (e.g., increased IGF-1 expression and decreased TGF β expression), decreased CD36 expression and changes in mechanical properties
7. Although mammographic density is associated with the degree of lobular involution, they appear to be independent risk factors for breast cancer

GWAS genome-wide association studies, SNPs single nucleotide polymorphisms

emerged from these studies, it is possible that some of the lack of consistency in findings can be explained by tissue heterogeneity.

Experimental Studies

As described above, radiologically dense areas of the breast are characterized by low adipose content and high stromal/ECM content. This observation, paired with a similar characterization observed in stromal tissue adjacent to tumors, led DeFilippis et al. [50] to examine and compare molecular characteristics of fibroblasts from breast tissue of high and low mammographic density. Gene expression profiling of fibroblasts from high and low density revealed the greatest difference in expression for CD36; CD36 was down-regulated approximately 4-fold in high-density-associated fibroblasts relative to those from the low-density fibroblasts. CD36, also known as fatty acid translocase, is an integral membrane protein. It is a marker of terminal differentiation, and involved in adipocyte differentiation, immune signaling, TGF β activation, and cell–ECM interactions [51, 52]. CD36 knockout mouse models demonstrated decreased fat accumulation and

increased matrix accumulation [50]. Interestingly, there are in vitro data to suggest that CD36 expression can be increased by common drugs such as aspirin [53] and statins [54].

A few groups have proposed that extracellular matrix and tissue stiffness, or quantitative or structural changes of the stromal collagen such as cross-linking, may partly explain the association between mammographic density and breast cancer risk [6, 7]. The mechanisms by which mechanical or structural properties of the breast tissue may be related to breast cancer risk have been discussed and reviewed in detail previously [55–57].

Chew et al. developed a xenograft model, in which human reduction mammoplasty samples selected from areas of high and low mammographic density are propagated in biochambers implanted in SCID (severe combined immunodeficiency) mice [58]. This recent study evaluated the effects of parity and lactation on breast tissue using these models. During pregnancy, there were higher levels of Ki67 in the high-density breast tissue compared with the lower density breast tissue. However, there were no differences in Ki67 levels in tissue sampled during postpartum involution. Additionally, the high-density samples showed increases in adipose and decreases in stromal content after postpartum involution, which was not seen from tissues of low density. These data demonstrate that in these model systems there are dynamic changes in molecular markers and cellular tissue composition that vary by breast density. The short time over which these changes in both molecular markers and breast tissue composition occur may suggest that evaluating biomarkers and breast density at one point in time is inadequate.

Conclusion

We have summarized the main consistent findings from the literature in Table 2. Based on these findings, we identified key gaps in knowledge and propose the following for future research:

1. *Genetics* Continued research to (a) identify genetic predictors of mammographic density (i.e., missing heritability) and (b) better understand the extent of shared heritability of mammographic density and breast cancer risk.
2. *Cells at risk* To prioritize research addressing the fundamental question of to what extent the association between mammographic density and breast cancer risk is explained by the ‘number of cells at risk’ hypothesis.
3. *Role of breast adipose tissue* The results from studies examining different mammographic density phenotypes indicate that the ratio of dense to nondense tissue may reflect some biological mechanisms important in breast cancer development, and/or that higher absolute nondense tissue (more breast adipose tissue) may be protective for

breast cancer. Additional work is necessary to understand how the dense and non-dense tissue influences the risk of breast cancer.

4. *Dynamic nature of breast density and timing of mammographic density assessment* Most studies have assessed only a single measure of mammographic density, often postmenopausal. Mammographic density is highest at younger ages, and tends to decline with age and after menopause. Additional studies assessing mammographic density earlier in life will be important for identifying and understanding factors that influence the formation of dense breast tissue. Understanding which factors are involved and how they influence changes in mammographic density (and how those changes influence breast cancer risk) will be important for identifying potential breast cancer prevention strategies.
5. *Tissue heterogeneity* Future studies including breast tissue samples (e.g., IHC marker studies and gene expression studies) should strive to better acknowledge and characterize tissue heterogeneity (e.g., cellular heterogeneity) as well as minimize, to the extent possible, heterogeneity that may influence our interpretation of the findings.
6. *Experimental studies* Future studies should follow up on promising pathways that have already been published (see Table 2). Additionally, it will be difficult for human studies to determine temporal relationships of cause and effect. Experimental studies may help to elucidate the temporal relationship of associations that are published from cross-sectional epidemiological studies.
7. *Integrative studies* Additional studies that are able to study mammographic density, breast tissue specimens, and long-term outcomes of breast cancer are needed to identify mechanisms that ultimately influence breast cancer risk.

Compliance with Ethics Guidelines

Conflict of Interest A. Pettersson and R.M. Tamimi both declare no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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