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Research Article

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Pectoral Muscle Attenuation as a Marker for Breast Cancer Risk in Full-Field Digital Mammography

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Abstract

Background: Mammographic percent density is an established marker of breast cancer risk. In a study of screen film mammograms, we recently reported a novel feature from the pectoral muscle region to be associated with breast cancer risk independently of area percent density (APD). We now investigate whether our novel feature is associated with risk in a study based on full-field digital mammography (FFDM).

Methods: We carried out a breast cancer risk analysis using a data set of 3,552 healthy controls and 278 cases. We included three image-based measures in our analyses: volumetric percent density (VPD), APD, and the mean intensity of the pectoral muscle (MIP). The datasets comprised different machine vendors and models. In addition, the controls dataset was used to test for the association of our measures against *rs10995190*, in the *ZNF365* gene, a genetic variant known to be associated with mammography density and breast cancer risk.

Results: MIP was associated with breast cancer risk [per SD OR, 0.811; 95% confidence interval (CI), 0.707–0.930; $P = 0.0028$] after adjusting for conventional covariates and VPD. It was also associated with the genetic variant *rs10995190* after adjusting for VPD and other covariates (per allele effect = 0.111; 95% CI, 0.053–0.170; $P = 1.8 \times 10^{-4}$). Results were similar when adjusting for APD instead of VPD.

Conclusion: MIP is a novel mammographic marker, which is associated with breast cancer risk and the genetic variant *rs10995190* independently of PD measures.

Impact: Inclusion of MIP in risk models should be considered for studies using PD from FFDM. *Cancer Epidemiol Biomarkers Prev; 1–7.*

Introduction

There is large body of evidence that mammographic density (which reflects the fibro-glandular tissue in a woman's breast) is a strong marker for breast cancer risk (1, 2). The ratio of the proportion of fibro-glandular/dense tissue (the part of the breast which appears white on a mammogram) to the total breast area is often taken as a quantitative measure and is termed percent mammographic density. There are a number of approaches, mostly area-based, which quantitatively measure mammographic density in screen film mammography; some are automatic, others are semiautomatic (require user intervention).

A number of algorithms have been proposed to infer mammographic density from two dimensional full-field digital mammography (FFDM) images. As well as using area-based, semiautomated thresholding algorithms (3), it is also possible to use automated volumetric approaches such as Volpara (4) and Quantra (5), which calibrate the imaging system and then, based on physics models and image acquisition parameters, determine the amount of tissue density in the compressed breast.

There is, however, still a scarcity of large-scale research studies assessing mammographic density from FFDM images in conjunction with breast cancer risk, although prior studies (4, 6–9), have demonstrated that Volpara can be an effective volumetric measurement method.

We recently introduced a novel metric, the mean intensity of pectoral muscle (MIP), and demonstrated its association with breast cancer risk independently of area-based mammographic density, measured by Cumulus (3), in screen film mammography (10). Although we adjusted for geographical region and date of mammogram, in theory it was still possible that the observed association could have been due to unknown inherited system differences between cases and controls. We therefore tested also for association with the common genetic variant for mammographic density and breast cancer risk, *rs10995190*, in the gene *ZNF365*, first using the same dataset, and then in an independent cohort, also with screen film mammograms. Genotypes are not likely to be associated with mammographic machine/system type, hence any association of MIP with genotype was expected to be causal. MIP was found to be strongly associated with *rs10995190* after adjusting for our measurement of area percent density (APD). Our interest in the pectoral muscle region was originally triggered by an observed illuminance fluctuation in screen film mammograms (10). We hypothesized that the image illuminance is governed by volumetric mammographic density. We used the mean intensity of pixels in the pectoral muscle as an independent monitor of exposure conditions related to volumetric density. It is important to establish whether the associations we observed in film mammograms (with both breast cancer risk and *rs10995190*) extend to FFDM.

This article has two main purposes. The first is to explore whether MIP is associated with breast cancer risk (after

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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Table 1. Mammography machine types used in this study

Manufacturer	Model	Station name	Code	Cases ($n = 278$)	Controls[a] ($n = 3,552$)
GE Medical Systems	Senographe Essential Version ADS_53.40	HBGMG03	1	59	1201
GE Medical Systems	Senographe Essential Version ADS_53.40	LKAMG01	2	34	63
Sectra Imtec AB	L30	BDCHK1	3	3	56
Sectra Imtec AB	L30	SECTRA_MDM_1	4	41	350
Sectra Imtec AB	MDM 1.5	BDCHK2	5	28	97
Sectra Imtec AB	MDM 1.5	BDCHK3	6	27	91
Philips Digital Mammography Sweden AB	L30	BDCHK1	7	21	353
Philips Digital Mammography Sweden AB	L30	BDCHK2	8	25	449
Philips Digital Mammography Sweden AB	L30	BDCHK3	9	29	495
Philips Digital Mammography Sweden AB	L30	BDCHK4	10	11	397

[a]Used in the genetic association study ($n = 3,552$).

adjusting/accounting for percent density, measured using both a volumetric and area approaches) based on FFDM images. Unlike in our study of screen film mammograms, we have information on the machine used for each mammogram. Although we can adjust for machine type in our case–control analysis, we cannot be completely sure that we account for all machine differences between cases and controls. We therefore also explore association with the genetic marker *rs10995190*, as we did in our study with screen film mammograms (genotype is not likely to be associated with machine type). The second purpose of this article is to better understand what our novel metric, MIP, represents by studying its association with image acquisition parameters (which are not available in analog images).

Materials and Methods

Study population

We extracted data from the Karolinska Mammography Project for Risk Prediction of Breast Cancer (KARMA) study (http://karmastudy.org/), a prospective cohort study started in 2011, comprising 70,877 women attending mammography screening or clinical mammography at four hospitals in Sweden. Blood samples were obtained from participants at study entry. Participants were also prompted to fill out a detailed web-based questionnaire and permission was requested to store digitally both raw and processed FFDM images, and to link information from Swedish national registers on inpatient care and cancer. The case–control study analysis presented in this article is based on 278 women diagnosed with incident breast cancer. For these women, we have both raw and processed prediagnostic digital (MLO) images (up to 30 months before diagnosis) together with complete data on the covariates used in the statistical analyses described below. We needed both raw and processed images because Volpara requires raw images and for reasons we describe later, MIP and our measure of area PD are calculated from processed images (the images that are routinely used for screening and stored). Mammograms, for the 278 women with breast cancer included in this study, have been taken on 10 different mammography machine systems (sub-models of GE, Sectra, and Philips vendors). As our control dataset, we used a subset of healthy KARMA women that have been included in a genetic association study based on *iCOGS*, a custom Illumina iSelect genotyping array (http://www.nature.com/icogs/). These women were included as controls in our case–control study if their mammograms had been taken on one of the 10 mammography machines used for the cases (and raw and processed MLO images and complete covariate data were available). We selected 3,552 women in this way. These women also contributed to the genetic association analyses. In total, we selected 3,830 women with raw and processed MLO images and complete covariate data. See Table 1 for a summary of the datasets. We ensured that all images included in our analyses had Volpara measurements, had both raw and processed images and did not contain implants or Volpara software warnings.

The average value of the LMLO and RMLO views was used for MIP and our measure of APD (see below) and volumetric percent density (VPD; see below) in the genetic association analysis (of healthy women). For the case–control study analysis, only one view was used; the contralateral view was chosen for the cases and view (left/right) was randomly chosen for the controls.

Volumetric mammographic density measurement

Volumetric density was measured using the commercial software, Volpara (version 1.4.5 | 5212 |). Its volume percent density measure has recently been demonstrated to correlate well with density measured from MRI images (4, 6, 7), the Breast Imaging-Reporting And Data System (BIRADS; refs. 7, 8), and a semiautomated area-based approach, Cumulus (9). Volpara requires raw mammograms. We refer to Volpara's measure of VPD.

Mean intensity of the pectoral muscle

We extracted MIP from processed FFDM images for the following reasons. First, raw images are typically not considered in the clinical evaluation or stored in the PACS (picture archiving and communication system); see Fowler and colleagues (11) and van Engeland and colleagues (12). Second, they are closer in appearance to digitized screen film images than raw images are. Third, it is easier to accurately segment the pectoral muscle in processed FFDM because breast anatomy in raw images is not visually distinguishable without preprocessing contrast enhancement.

We first used a fully automated algorithm for mammography segmentation, which we have described in detail elsewhere (13). The segmentation method identifies the pectoral muscle region on a given FFDM image from which the arithmetic

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mean, of the pixel intensity values, is derived. Intensity within each mammographic image is first block-wise equalized using contrast-limited adaptive histogram equalization (14). This algorithm enhances the contrast within each block in the image so that the histogram of the output block approximately matches a uniform distribution. The resulting image is then converted to double precision (floating-point format) and normalized to the interval (0–1). MIP values, therefore, also fall within this range.

Area mammographic density measurement

From the processed FFDM images, we automatically generated the APD using our in-house built algorithm reported in detail in ref. (13). The algorithm uses compound preprocessing steps to automatically segment mammographic images into three main fragments: The pectoral muscle, the breast area, and the dense area.

Genetic data

Genome-wide association studies have identified a handful of SNPs associated with mammographic density (15). Of these variants, SNP *rs10995190*, in the gene *ZNF365*, has the strongest association and has been confirmed to be associated with both mammographic density ($P = 9.6 \times 10^{-10}$; ref. 15) and breast cancer risk ($P = 1 \times 10^{-36}$; ref. 16). For the 3,552 healthy women included in this study, genotype data on the SNP *rs10995190* were extracted from iCOGs. Genotypes were coded as 0, 1, or 2, corresponding to the number of copies of the rare allele.

Questionnaire data

Information on age, body mass index (BMI), hormone replacement therapy (HRT) status, reproductive history, and other breast cancer risk factors was collected via a web-based questionnaire at study entry. Menopausal status was defined according to information on last year menstruation status, previous oophorectomy and age at study entry.

Ethical statement

The Karma study has an ethical committee approval by the Ethical Committee at Karolinska Institutet (Dnr 2010/958-31/1) and all participants provided written informed consent.

Statistical analysis

Because our genetic association analyses assumes that outcome variables are normally distributed, we transformed MIP, APD, and VPD before analyses. The distributions of MIP, APD, and the acquisition parameters were notably different for GE machine types than for Sectra/Philips machine types (Supplementary Figs. S1 and S2). The distributions of VPD were more similar across machine types (Supplementary Fig. S3). For MIP and APD, we applied two different Box-Cox transformations (using the *R* package MASS; ref. 17), one for GE machine types and one for Sectra/Philips machine types. To account for any further possible influence of machine on MIP and APD (unequal variances across machines), we first fitted linear regression models treating MIP/APD as an outcome variable, for each individual machine type, adjusting for age and BMI as covariates. Standardized residuals were then used to represent MIP and APD in subsequent analyses. VPD measurements were transformed by taking the logarithm, as in Ellison-Loschmann and colleagues (18) and Cheddad and colleagues (13).

Case–control analysis. We examined the association between the MIP, APD, and VPD measures and breast cancer status, based on the dataset of 278 cases and 3,552 controls, using unconditional logistic regression (case–control status as dependent variable and each of the measures as the independent variable), adjusting for age, BMI, menopausal status, HRT, parity, age at first birth, and machine type. Effect estimates are presented as ORs. We first included MIP and VPD, one at a time, and subsequently included both measurements as covariates, to study independence of their associations. We repeated the above analyses, additionally adjusting for a selection of acquisition parameters (*kVp*, Exposure Time, X-ray Tube Current, Exposure, Exposure in *uAs*, Body Part Thickness, Compression Force, Relative X-ray Exposure and Organ Dose). We did this to protect against unknown machine differences (in case the adjustment by machine, model, and station name did not completely account for machine differences between cases and controls). We then performed the same analyses but using MIP and APD instead of MIP and VPD.

Genetic association. We fitted linear regression models treating MIP and VPD measures one at a time as outcome variables and included *rs10995190* (treated as a continuous variable) along with age, BMI, menopausal status, HRT, parity, age at first birth, and machine type as covariates. We carried out Wald tests to evaluate the association between *rs10995190* and each of the outcome variables. We then carried out similar tests of association for each of the outcome variables (MIP and VPD), additionally adjusting for the other measures. For example, for MIP as outcome variable, we additionally included VPD measurement as a covariate when testing for association between MIP and *rs10995190*. We then performed the same analyses but using MIP and APD instead of MIP and VPD.

Regression analysis to understand MIP. We examined the association of the nine acquisition parameters (listed earlier) with MIP. Correlations between acquisition parameters vary across machines, so machine-specific analyses were carried out. We fitted a series of linear regression models with MIP as an outcome variable and acquisition parameters as covariates, adjusting for VPD. To compare nested models, we carried out likelihood ratio tests by comparing residual deviances (−2 times log likelihood differences from a model that is a perfect fit to the data). By comparing the fit of different models (with different subsets of acquisition parameters included as covariates), we can learn about which acquisition parameters drive the value of MIP. We also fitted linear regression models (MIP as outcome) using age, BMI, and VPD measurements as covariates to understand MIP's relationship with these key variables.

R (version 3.1.1) was used for data management, statistical analyses, and graphics (19). MATLAB (version 8.3) was used for image processing and analysis (20). All reported tests are two-sided. *P* values <0.05 were considered to be statistically significant.

Results

Characteristics of women included in this study are summarized in Table 2. In this table, we also summarize tests of association between key characteristics and those measures taken from the mammographic images.

Despite the relatively small number of cases in the case–control study, we observed that VPD, APD, and our MIP measure were

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Table 2. Key characteristics of individuals included in this study [mean (SD) or *n* (%)]

	Cases used for case–control study ($n = 278$)	Controls used for case–control study ($n = 3{,}552$)	P^a (cases controls comparison)	Association with MIP *P* value (effect direction)	Association with VPD *P* value (effect direction)	Association with APD *P* value (effect direction)
Age[b]	58 (9.341)	53 (9.189)	1.3×10^{-18}	1.8×10^{-6} (−)	5.8×10^{-95} (−)	1.2×10^{-91} (−)
BMI	26.231 (4.530)	25.337 (4.282)	0.0013	3.3×10^{-19} (+)	8.2×10^{-232} (−)	1.0×10^{-218} (−)
Postmenopausal			0.2074	0.0283	1.2×10^{-15}	7.8×10^{-16}
No	90(32)	1,770 (50)				
Yes	188 (68)	1,782 (50)				
HRT use			0.0154	0.9990	0.8179	0.9598
Never	192(69)	2,575 (73)				
Past	67(24)	794 (22)				
Current	19(7)	183 (5)				
Parity and age at first birth			0.2660	0.6540	2.2×10^{-4}	0.9362
Nulliparous	31(11)	481 (14)				
Parity ≤2 and age at first birth ≤25	67 (24)	778 (22)				
Parity ≤2 and age at first birth >25	117 (42)	1,413 (40)				
Parity >2 and age at first birth ≤25	44 (16)	511 (14)				
Parity >2 and age at first birth >25	19 (7)	369 (10)				
MIP (processed)	3.088 (0.969)	3.414 (0.998)	9.3×10^{-6}	–	1.1×10^{-100} (−)	1.9×10^{-85} (−)
VPD (raw)	2.025(0.507)	2.038 (0.549)	0.0046	1.1×10^{-100} (−)	–	$<1.2 \times 10^{-300}$ (+)
APD (processed)	4.386 (0.963)	4.424 (1.003)	0.0003	1.9×10^{-85} (−)	$<1.2 \times 10^{-300}$ (+)	–

[a]*P* values are those of the Wald test (logistic regression, unadjusted) or of LR tests for menopausal status, HRT use, and parity.
[b]For age and BMI rows, the tests' *P* values were obtained after adjusting for machine type, else the *P* values were obtained after adjusting for age, BMI, and machine type.

significantly associated with cancer status (3.7×10^{-4}, 1.1×10^{-5}, and 4.6×10^{-5}, respectively); see Table 3. This association remained when we accounted for the acquisition parameters in the model; see Table 3. Even with both VPD and MIP included as covariates, each of the measures remained (independently) associated with case–control status both without adjustment for acquisition parameters ($P = 0.0259$ and $P = 0.0028$ for VPD and MIP, respectively) and with adjustment for acquisition parameters ($P = 0.0016$ and $P = 0.0012$ for VPD and MIP, respectively). We observed a similar result when APD and MIP were studied together; each of the measures remained (independently) associated with case–control status both without adjustment for acquisition parameters ($P = 6.1 \times 10^{-4}$ and $P = 0.0027$ for APD and MIP, respectively) and with adjustment for acquisition parameters ($P = 1.1 \times 10^{-5}$ and $P = 6.6 \times 10^{-4}$ for APD and MIP, respectively).

The results of our genetic association analysis (with *rs10995190*) are presented in Table 4. We found strong evidence of association for VPD, APD, and MIP ($P = 4.1 \times 10^{-6}$, $P = 3.2 \times 10^{-9}$ and $P = 8.5 \times 10^{-8}$ for VPD, APD and MIP independently). We found evidence of association between MIP and *rs10995190* even after adjusting for VPD ($P = 1.8 \times 10^{-4}$), or adjusting for APD ($P = 5.8 \times 10^{-4}$). VPD also contained information independent of MIP in terms of association with *rs10995190* ($P = 0.01028$); similarly for APD the *P* value for the association was $P = 2.0 \times 10^{-5}$. Additional adjustment for the acquisition parameters had little effect on these results. The direction of the association for the SNP with VPD/APD was negative and was positive for MIP, which is consistent with what we have previously reported (10).

As we noted earlier, the distribution of MIP (and the acquisition parameters) differed between GE and Sectra/Philips machine types. Because of these machine differences, we considered it relevant to assess the performance of MIP for GE machine types (1 and 2) and for Sectra/Philips machine types (3–10), separately. For both machine types MIP were associated with case–control status ($P = 7.0 \times 10^{-4}$ and $P = 0.008$ for GE machines and Sectra/Philips machines, respectively) and the genetic variant *rs10995190* ($P = 5.1 \times 10^{-4}$ and $P = 3.8 \times 10^{-5}$ for GE machines and Sectra/Philips machines, respectively). We note that none of the acquisition parameters were significantly associated with case–control status or *rs10995190* when considering all machine types together or stratifying by the two groups of machines.

Table 3. Effect estimates for automated measures of mammographic density on case–control status, $n = 3{,}830$ (278 cases and 3,552 controls)

	MIP, VPD, and PD one at a time in the model		MIP and VPD included together in the model		MIP and APD included together in the model	
Outcome	Estimate (95% CI)	*P*	Estimate (95% CI)	*P*	Estimate (95% CI)	*P*
(A)						
VPD (raw)	1.776 (1.295–2.438)	3.7×10^{-4}	1.470 (1.047–2.063)	0.0259	—	—
APD (processed)	1.340 (1.176–1.527)	1.1×10^{-5}	—	—	1.265 (1.107–1.448)	6.1×10^{-4}
MIP (processed)	0.767 (0.674–0.871)	4.6×10^{-5}	0.811 (0.707–0.930)	0.0028	0.816 (0.714–0.932)	0.0027
(B)						
VPD (raw)	2.217 (1.476–3.336)	1.3×10^{-4}	1.959 (1.290–2.977)	0.0016	—	—
APD (processed)	1.474 (1.259–1.730)	1.7×10^{-6}	—	—	1.428 (1.219–1.675)	1.1×10^{-5}
MIP (processed)	0.759 (0.660–0.871)	9.4×10^{-5}	0.791 (0.686–0.911)	0.0012	0.784 (0.682–0.902)	6.6×10^{-4}

NOTE: Estimates (point estimates and 95% CIs) are presented as ORs. A, with full adjustment (age, BMI, menopausal status, HRT use, parity, age at first birth, and machine type) and B, full adjustment plus additionally adjusting for acquisition parameters. OR estimates, CI estimates, and *P* values (Wald tests) are based on estimated coefficients for VPD, APD, and MIP in logistic regression models with case–control status as outcome.

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Table 4. Effect estimates for mammographic density SNP, *rs10995190*, on automated measures of mammographic density ($n = 3{,}552$, the controls dataset)

	Without adjustment for the other outcome		With adjustment for the other outcome	
Outcome	**Estimate (95% CI)**	***P***	**Estimate (95% CI)**	***P***
A				
Adjustment (i)				
VPD (raw)	−0.061(−0.087 to −0.035)	4.1×10^{-6}	−0.031(−0.054 to −0.007)	0.0103
MIP (processed)	0.176 (0.111–0.240)	8.5×10^{-8}	0.111 (0.053–0.170)	1.8×10^{-4}
Adjustment (ii)				
VPD (raw)	−0.044 (−0.063 to −0.025)	8.7×10^{-6}	−0.032 (−0.051 to −0.014)	7.8×10^{-4}
MIP (processed)	0.148 (0.090–0.206)	6.3×10^{-7}	0.117 (0.060–0.173)	5.4×10^{-5}
B				
Adjustment (i)				
APD (processed)	−0.193 (−0.256 to −0.129)	3.2×10^{-9}	−0.130 (−0.190 to −0.070)	2.0×10^{-5}
MIP (processed)	0.176 (0.111–0.240)	8.5×10^{-8}	0.106 (0.046–0.166)	5.8×10^{-4}
Adjustment (ii)				
APD (processed)	−0.151 (−0.200 to −0.102)	1.4×10^{-9}	−0.131 (−0.179 to −0.082)	1.2×10^{-7}
MIP (processed)	0.148 (0.090–0.206)	6.3×10^{-7}	0.118 (0.061–0.176)	5.7×10^{-5}

NOTE: Adjustment (i), adjusted for age, BMI, menopausal status, HRT use, parity, age at first birth, and machine types. Adjustment (ii), adjusted for age, BMI, menopausal status, HRT use, parity, age at first birth, machine types, and acquisition parameters. Point estimates, interval estimates, and *P* values (Wald tests) are based on estimated coefficients for the SNP in linear regression models with A, MIP and VPD as outcomes; and B, MIP and APD as outcomes.

We next carried out a detailed analysis of association between image acquisition parameters and MIP. Although the acquisition parameters that best accounted for the associations differed to some extent across machines, MIP was most strongly associated with Body Part Thickness and/or exposure parameters; see Supplementary Methods and Materials and the Supplementary Table S1 therein.

Using the controls dataset, we also studied the relationships between MIP, mammographic volumetric density, age, and BMI from images from a subset of machines with large numbers of images. MIP was observed to be negatively associated with VPD for the GE machine type 1 ($r = -0.402$, $P = 8.5 \times 10^{-48}$), for the Sectra machine type 4 ($r = -0.406$, $P = 2.7 \times 10^{-15}$), and for the Philips machine type 9 ($r = -0.390$, $P = 2.0 \times 10^{-19}$). These associations remained strongly significant after adjusting for age and BMI ($P = 2.6 \times 10^{-30}$, $P = 1.5 \times 10^{-16}$, and $P = 2.8 \times 10^{-20}$, for the GE type 1, Sectra type 4, and Philips type 9 machines, respectively). Studying images from one machine at a time, we found that the correlation coefficient between MIP and volumetric percent mammographic density (VPD) was always negative (values ranged from −0.22 to −0.50).

Discussion

In this study, we have investigated the association between a novel image-based marker from FFDM images, the MIP, and cancer status in a case–control study of breast cancer. We have also studied its association with a genomic marker known to be associated with density and breast cancer risk. In both cases, we found strong evidence of association and our results were completely in line with our previous findings based on screen film mammograms.

Our analysis suggests that MIP helps recalibrate volumetric measurements. In other words, MIP picks up imperfection in density measurements as we also argued in our previous work on screen film images (10).

It could be that the intrinsic calibration model used by Volpara is not optimal, and therefore MIP reflects an external biologic reference for dense tissue thickness correction analogous to the physical calibration phantoms (21–24). Researchers in refs. (21–24) deduce dense tissue thickness at each pixel in a given image by calibrating the imaging system via an external physical phantom (plastic calibration device or a step wedge) affixed to the top of the compression paddle, which is X-rayed together with the breast. Although such algorithms are able to be used on both FFDM and screen film imaging systems, they are not able to calibrate already acquired mammographic images. The advantage of MIP over these phantoms is that it can be implemented in retrospective studies and is readily available. Therefore, MIP, as an image-based marker, appears to hold some important information that has a pronounced significant association with breast cancer status and the density genetic marker.

The fact that MIP is so strongly associated with *rs10995190* lends credibility to our hypothesis that MIP reflects an external biologic reference for dense tissue thickness correction, and points away from other biologic explanations for why MIP is associated with breast cancer risk. We may otherwise, for example, have believed that MIP could reflect ageing (biologic age as opposed to our chronologic age) because one of the most noticeable aging effects is the reduction in muscle size (25–27) and the number of muscle fibers in the human minor pectoral muscle (28).

It is possible that breast compression, if not prudently handled, may result in part of the breast overlaying the pectoral muscle on X-ray mammograms so that fragments of dense tissue missed by volumetric or area-based measures may appear in the pectoral muscle region. This phenomenon, though, does not occur often because the compression pads are designed in a way to prevent superimposition of breast tissue shadow (29). Moreover, such missed density forms only a density residue that is unlikely to trigger the significant associations that we observed with MIP.

In digitized screen film mammography, automatic exposure control (AEC) is regulated by placing an X-ray sensor under the film and in the middle of the breast tissue (the area with the lowest transmission) to terminate the exposure once a predetermined level of radiation is reached. That is, the AEC jointly optimizes image acquisition and display. On the other hand, in FFDM systems, the AEC is automatically estimated and controlled to maximize signal-to-noise ratio in relation to patient radiation dose, whereas the optimization of display (brightness and contrast) is done at the post-acquisition phase (30, 31) and is vendor's trade secret. In both technologies, the exposure is affected by breast thickness and estimated breast composition. Hence, the image pixels in the pectoral muscle may be an independent

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monitor of exposure parameters conditions related to the volumetric density. In other words, MIP could be capturing meaningful information, which is related to the acquisition parameters (machine–organ interaction). The relationship between MIP and the acquisition parameters is, however, complex and differs across machines. Our analysis of association between MIP and the acquisition parameters does though provide a further hint that MIP is responding to volumetric density because, in the machines we investigated, MIP is strongly related to Body Part Thickness and/or exposure parameters, parameters that are highly driven by amount of density and breast composition.

We found no association of the acquisition parameters with breast cancer status or the SNP *rs10995190*. This result is not inconsistent with our observation that MIP is associated with acquisition parameters. The acquisition parameters are not able to explain MIP values to a very large extent and relationships between MIP and acquisition parameters vary by machine. It appears that MIP is able to provide a relevant, machine-independent, measure of mammographic density (as reported in the results, MIP was associated with both case–control status and *rs10995190* when dividing the data broadly by machine type; GE vs. Sectra/Philips). Olson and colleagues (32) also reported a lack of association between acquisition parameters and breast cancer status, after manually extracting a selection of image acquisition parameters from film images.

Other researchers have investigated the importance of intensity-based and textural-based mammographic features. Nielsen and colleagues (33) derived a mammographic texture resemblance (MTR) marker for breast cancer risk, which they found to be associated with risk independently of density and which they validated in a separate cohort (34). The risk estimate obtained in (OR, 0.82; ref. 10), is similar to the one reported here (OR, 0.81). The reported (per SD) ORs for the MTR marker are 1.3 and 1.36 (in refs. 33, 34, respectively). Unlike in ref. (33, 34), our associations are validated in a genetic variant that guards against associations being due to machine artefacts—cases–controls may use different machines, but machines are not likely to be associated with genotypes.

VPD was inversely associated with age and BMI. MIP, which is inversely associated with VPD, is positively associated with BMI, but inversely associated with age. The directions of association with MIP are in line with our previous findings in digitized film mammograms (10). In our analyses (risk analysis and genetic association), we observed that, in all cases, the *P* values from tests of association with MIP were smaller than those from tests of association with VPD.

It should be emphasized that the use of raw FFDM images in this study was solely because Volpara, as an established volumetric measurement tool, works only on raw images. However, MIP is likely to complement mammographic density measurements generated either by area-based tools or volumetric-based ones from either raw or processed images.

The uniqueness of our technique lies in the simplicity and practicability of attaining additional information out of the pectoral muscle, which has not been studied before, from readily available mammographic images. Moreover, this work is the first that attempts to study image-based markers in combination with the acquisition parameters on FFDM images. To date, this is also the first study to combine multiple FFDM machine types for breast cancer risk and genetic association analyses.

Taken together, the results reported here, along with our previous work (10), suggest that our MIP metric is a viable density calibration measure that is consistent across both digitized film mammography and FFDM systems. Therefore, it is possible that MIP could be used to either add to breast cancer risk prediction models or to help optimize the performance of current mammographic density measurements (area or volumetric based). Further research is warranted to validate our findings on independent datasets and to find out how MIP can be best combined with existing mammographic density measures.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: A. Cheddad, K. Czene, K. Humphreys
Development of methodology: A. Cheddad, K. Humphreys
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K. Czene, P. Hall
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Cheddad, K. Humphreys
Writing, review, and/or revision of the manuscript: A. Cheddad, K. Czene, K. Humphreys
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): P. Hall
Study supervision: P. Hall, K. Humphreys

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References

1. Boyd NF, Guo H, Martin LJ, Sun L, Stone J, Fishell E, et al. Mammographic density and the risk and detection of breast cancer. N Engl J Med 2007;356:227–36.
2. Schreer I. Dense breast tissue as an important risk factor for breast cancer and implications for early detection. Breast Care 2009;4: 89–92.
3. Byng JW, Boyd NF, Fishell E, Jong RA, Yaffe MJ. The quantitative analysis of mammographic densities. Phys Med Biol 1994;39:1629–38.
4. Highnam R, Brady S, Yaffe M, Karssemeijer N, Harvey J. Robust breast composition measurement—VolparaTM. In:Martí J, Oliver A, Freixenet J, Martí R, editors. Digital mammography: Springer Berlin Heidelberg; 2010. p. 342–9.
5. Hologic. Inc [Internet]. USA: Quantra. [cited 2015 Mar 10]. Available from: http://www.hologic.com/.
6. Gubern-Mérida A, Kallenberg M, Platel B, Mann RM, Martí R, Karssemeijer N. Volumetric breast density estimation from full-field digital mammograms: a validation study. PLoS ONE 2014;9:e85952.
7. Seo JM, Ko ES, Han BK, Ko EY, Shin JH, Hahn SY. Automated volumetric breast density estimation: a comparison with visual assessment. Clin Radiol 2013;68:690–5.

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8. McEntee MF, Damases CN. Mammographic density measurement: a comparison of automated volumetric density measurement to BIRADS. Proc SPIE 9037 Medical Imaging; 2014: SPIE; 2014. p. 90370T-T-8.
9. Jeffreys M, Harvey J, Highnam R. Comparing a new volumetric breast density method (VolparaTM) to Cumulus. In:Martí J, Oliver A, Freixenet J, Martí R, editors. Digital mammography: Springer Berlin Heidelberg; 2010. p. 408–13.
10. Cheddad A, Czene K, Shepherd JA, Li J, Hall P, Humphreys K. Enhancement of mammographic density measures in breast cancer risk prediction. Cancer Epidemiol Biomarkers Prev 2014;23:1314–23.
11. Fowler EE, Vachon CM, Scott CG, Sellers TA, Heine JJ. Automated percentage of breast density measurements for full-field digital mammography applications. Acad Radiol 2014;21:958–70.
12. van Engeland S, Snoeren PR, Huisman H, Boetes C, Karssemeijer N. Volumetric breast density estimation from full-field digital mammograms. IEEE Trans Med Imaging 2006;25:273–82.
13. Cheddad A, Czene K, Eriksson M, Li J, Easton D, Hall P, et al. Area and volumetric density estimation in processed full-field digital mammograms for risk assessment of breast cancer. PLoS ONE 2014;9:e110690.
14. Zuiderveld K. Contrast limited adaptive histogram equalization. San Diego, CA: Academic Press Professional, Inc; 1994.
15. Lindström S, Vachon CM, Li J, Varghese J, Thompson D, Warren R, et al. Common variants in ZNF365 are associated with both mammographic density and breast cancer risk. Nat Genet 2011;43:185–7.
16. Michailidou K, Hall P, Gonzalez-Neira A, Ghoussaini M, Dennis J, Milne RL, et al. Large-scale genotyping identifies 41 new loci associated with breast cancer risk. Nat Genet 2013;45:353–61, 61e1–2.
17. Venables B, Ripley B. Modern applied statistics with S. 4th ed. New York: Springer; 2002.
18. Ellison-Loschmann L, McKenzie F, Highnam R, Cave A, Walker J, Jeffreys M. Age and ethnic differences in volumetric breast density in new zealand women: a cross-sectional study. PLoS ONE 2013;8:e70217.
19. The R Foundation. org [Internet]. Austria: the R project for statistical computing; [cited 2015 Mar 10]. Available from: http://www.r-project.org/.
20. The MathWorks, Inc. [Internet]. USA: MATLAB. [cited 2015 Mar 10]. Available from: http://www.mathworks.se/.
21. Shepherd JA, Kerlikowske K, Ma L, Duewer F, Fan B, Wang J, et al. Volume of mammographic density and risk of breast cancer. Cancer Epidemiol Biomarkers Prev 2011;20:1473–82.
22. Malkov S, Wang J, Kerlikowske K, Cummings SR, Shepherd JA. Single x-ray absorptiometry method for the quantitative mammographic measure of fibroglandular tissue volume. Med Phys 2009;36:5525–36.
23. Pawluczyk O, Augustine BJ, Yaffe MJ, Rico D, Yang J, Mawdsley GE, et al. A volumetric method for estimation of breast density on digitized screen-film mammograms. Med Phys 2003;30:352–64.
24. Boyd N, Martin L, Gunasekara A, Melnichouk O, Maudsley G, Peressotti C, et al. Mammographic density and breast cancer risk: evaluation of a novel method of measuring breast tissue volumes. Cancer Epidemiol Biomarkers Prev 2009;18:1754–62.
25. Porter MM, Vandervoort AA, Lexell J. Aging of human muscle: structure, function and adaptability. Scand J Med Sci Sports 1995;5:129–42.
26. Lexell J. Human aging, muscle mass, and fiber type composition. J Gerontol A Biol Sci Med Sci 1995;50 Spec No:11–6.
27. Deschenes MR. Effects of aging on muscle fibre type and size. Sports Med 2004;34:809–24.
28. Sato T, Akatsuka H, Kito K, Tokoro Y, Tauchi H, Kato K. Age changes in size and number of muscle fibers in human minor pectoral muscle. Mech Ageing Dev 1984;28:99–109.
29. Andolina V, Lillé S. Mammographic imaging: a practical guide. Philadelphia: Lippincott Williams & Wilkins; 2010.
30. Pisano ED, Yaffe MJ. Digital mammography. Radiology 2005;234:353–62.
31. Williams MB, Raghunathan P, More MJ, Seibert JA, Kwan A, Lo JY, et al. Optimization of exposure parameters in full field digital mammography. Med Phys 2008;35:2414–23.
32. Olson JE, Sellers TA, Scott CG, Schueler BA, Brandt KR, Serie DJ, et al. The influence of mammogram acquisition on the mammographic density and breast cancer association in the Mayo Mammography Health Study cohort. Breast Cancer Res 2012;14:R147.
33. Nielsen M, Karemore G, Loog M, Raundahl J, Karssemeijer N, Otten JD, et al. A novel and automatic mammographic texture resemblance marker is an independent risk factor for breast cancer. Cancer Epidemiol 2011;35:381–7.
34. Nielsen M, Vachon CM, Scott CG, Chernoff K, Karemore G, Karssemeijer N, et al. Mammographic texture resemblance generalizes as an independent risk factor for breast cancer. Breast Cancer Res 2014;16:R37.

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