Original Report

Title: 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. We now investigate whether our novel feature is associated with risk in a study based on full field digital mammograms (FFDM).

Methods: We carried out a breast cancer risk analysis using a data set of 3552 healthy controls and 278 cases. We included three image-based measures in our analyses: Volumetric percent density (VPD), area percent density (APD) and the mean intensity of the pectoral muscle (MIP). The data sets comprised different machine vendors and models. Additionally, the controls data set 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 s.d 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= 1.8×10^{-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.

250 words

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 semi-automatic (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, semi-automated 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 data set, 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. Our interest in the pectoral muscle region was originally triggered by an observed illuminance fluctuation in screen film mammograms (10). We hypothesised 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 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 ***Kar****olinska* ***Ma****mmography 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 pre-diagnostic digital (MLO) images (up to 30 months prior to diagnosis) together with complete data on the covariates used in the statistical analyses described below. We needed both raw and processed images since 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 data set, we used a subset of healthy KARMA women which 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 3552 women in this way. These women also contributed to the genetic association analyses. In total, we selected 3830 women with raw and processed MLO images and complete covariate data. See Table 1 for a summary of the data sets. 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 area percent density (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 (VPD)

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 magnetic resonance imaging (MRI) images (4, 6, 7), the Breast Imaging-Reporting And Data System (BIRADS) (7, 8) and a semi-automated area based approach, Cumulus (9). Volpara requires raw mammograms. We refer to Volpara's measure of volumetric percent density as VPD.

Mean Intensity of the Pectoral muscle (MIP)

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 et al. (11) and van Engeland et al. (12). Second, they are closer in appearance to digitised screen film images than raw images are. Third, it is easier to accurately segment the pectoral muscle in processed FFDM since breast anatomy in raw images is not visually distinguishable without pre-processing 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 mean, of the pixel intensity values, is derived. Intensity within each mammographic image is first block-wise equalised using contrast limited adaptive histogram equalisation (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 normalised to the interval (0 - 1). MIP values therefore also fall within this range.

Area mammographic density measurement (APD)

From the processed FFDM images we automatically generated the APD using our in-house built algorithm reported in detail in (13). The algorithm uses compound pre-processing 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 (GWAS) have identified a handful of single nucleotide polymorphisms (SNP) 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 ($p=9.6\times10^{-10}$) (15) and breast cancer risk ($p=1\times10^{-36}$) (16). For the 3552 healthy women included in this study, genotype data on the SNP *rs10995190* was 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, 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 Methods

Because our genetic association analyses assumes that outcome variables are normally distributed we transformed MIP, APD and VPD prior to analyses. The distributions of MIP, APD and the acquisition

parameters were notably different for GE machine types than for Sectra/Philips machine types (Supplementary Fig. S1 and Supplementary Fig. 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; (17)), one for GE machine types and one for Sectra/Philips machine types. In order 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. Standardised residuals were then used to represent MIP and APD in subsequent analyses. VPD measurements were transformed by taking the logarithm, as in Ellison-Loschmann et al. (18) and Cheddad et al. (13).

A) Case-control analysis: We examined the association between the MIP, APD and VPD measures and breast cancer status, based on the data set of 278 cases and 3552 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 odds ratios. 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.

B) 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.

C) 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 in order 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 summarised in Table 2. In this table we also summarise 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 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 ($p=6.1\times10^{-4}$ and p=0.0027 for APD and MIP, respectively) and with adjustment for acquisition parameters ($p=1.1\times10^{-5}$ and $p=6.6\times10^{-4}$ 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 ($p=4.1\times10^{-6}$, $p=3.2\times10^{-9}$ and $p=8.5\times10^{-8}$ for VPD, APD and MIP independently). We found evidence of association between MIP and *rs10995190* even after adjusting for VPD ($p=1.8\times10^{-4}$), or adjusting for APD ($p=5.8\times10^{-4}$). 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 $p=2.0\times10^{-5}$. 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&2) and for Sectra/Philips machine types (3-10), separately. For both machine types MIP was associated with case-control status ($p=7.0\times10^{-4}$and p=0.008 for GE machines and Sectra/Philips machines, respectively) and the genetic variant *rs10995190* ($p=5.1\times10^{-4}$and and $p=3.8\times10^{-5}$ 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.

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 Table S1 therein.

Using the controls data set, 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 ($r = -0.402$, $p = 8.5\times10^{-48}$), for the Sectra machine type 4 ($r = -0.406$, $p = 2.7\times10^{-15}$) and for the Philips machine type 9 ($r = -0.390$, $p = 2.0\times10^{-19}$). These associations remained strongly significant after adjusting for age and BMI ($p = 2.6\times10^{-30}$, $p = 1.5\times10^{-16}$ and $p = 2.8\times10^{-20}$, 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 mean intensity of the pectoral muscle, 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 biological reference for dense tissue thickness correction analogous to the physical calibration phantoms (21-24). Researchers in (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 which 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 biological reference for dense tissue thickness correction, and points away from other biological explanations for why MIP is associated with breast cancer risk. We may otherwise, for example, have believed that MIP could reflect ageing (biological age as opposed to our chronological age) since one of the most noticeable aging effects is the reduction in muscle size (25-27) and the number of muscle fibres 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 which is unlikely to trigger the significant associations that we observed with MIP.

In digitised 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 optimises image acquisition and display. On the other hand, in FFDM systems, the AEC is automatically estimated and controlled to maximise signal-to-noise ratio in relation to patient radiation dose, while the optimisation 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 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 since, in the machines we investigated, MIP is strongly related to *Body Part Thickness* and/or exposure parameters, parameters which 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 versus Sectra/Philips)). Olson et al. (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 et al. (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 (10), (OR= 0.82), is similar to the one reported here, (OR=0.81). The reported (per s.d.) odds ratios for the MTR marker are 1.3 and 1.36 (in (33, 34), respectively). Unlike in (33, 34), our associations are validated a genetic variant which 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 digitised 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 emphasised 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 digitised film mammography and FFDM systems. Therefore, it is possible that MIP could be utilised to either add to breast cancer risk prediction models or to help optimise the performance of current mammographic density measurements

(area or volumetric based). Further research is warranted to validate our findings on independent data sets and to find out how MIP can be best combined with existing mammographic density measures.

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Table 1. Mammography machine types used in this study.

Manufacturer	Model	Station Name	Code	Cases (n= 278)	Controls* (n= 3552)
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

* Used in the genetic association study (n= 3552).

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Table 2: Key characteristics of individuals included in this study (mean (s.d) or n (%)).

	Cases used for case-control study (n= 278)	Controls used for case-control study (n= 3552)	P-value (*) (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(€)	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)	1770 (50)				
Yes	188 (68)	1782 (50)				
HRT use			0.0154	0.9990	0.8179	0.9598
Never	192(69)	2575 (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)	1413 (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} (-)	-	$<1.2\times10^{-300}$ (+)
APD (Processed)	4.386 (0.963)	4.424 (1.003)	0.0003	1.9×10^{-85} (-)	$<1.2\times10^{-300}$ (+)	-

(*) P-values are those of Wald test (logistic regression, unadjusted) or of LR tests for menopausal status, HRT use and parity. (€) 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.

Table 3: Effect estimates for automated measures of mammographic density on case-control status, n=3830 (278 Cases, 3552 Controls). Odds ratio estimates, confidence interval 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.

	MIP, VPD & PD one at a time in the model		MIP& VPD included together in the model		MIP & APD included together in the model	
Outcome	**Estimate (95%CI)**	**p-value**	**Estimate (95%CI)**	**p-value**	**Estimate (95%CI)**	**p-value**
(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}

* Estimates (point estimates and 95% CIs) are presented as odds ratios. (a) with full adjustment (age, BMI, menopausal status, HRT use, parity, age at first birth and machine type), (b) full adjustment plus additionally adjusting for acquisition parameters.

Table 4. Effect estimates for mammographic density SNP, *rs10995190*, on automated measures of mammographic density (n=3552, the controls data set). 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.

(a)				
Outcome	**Without adjustment for the other outcome**		**With adjustment for the other outcome**	
	Estimate (95%CI)	**p-value**	**Estimate (95%CI)**	**p-value**
Adjustment (i)				
VPD (raw)	-0.061(-0.087, -0.035)	4.1×10^{-6}	-0.031(-0.054, -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, -0.025)	8.7×10^{-6}	-0.032 (-0.051, -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)				
	Without adjustment for the other outcome		**With adjustment for the other outcome**	
	Estimate (95%CI)	**p-value**	**Estimate (95%CI)**	**p-value**
Adjustment (i)				
APD (Processed)	-0.193 (-0.256, -0.129)	3.2×10^{-9}	-0.130 (-0.190, -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, -0.102)	1.4×10^{-9}	-0.131 (-0.179, -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}

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.

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