A genome-wide association study of mammographic texture variation

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Abstract

Background: Breast parenchymal texture features, including grayscale variation (V), capture the patterns of texture variation on a mammogram and are associated with breast cancer risk, independent of mammographic density (MD). However, our knowledge on the genetic basis of these texture features is limited.

Methods: We conducted a genome-wide association study of V in 7040 European-ancestry women. V assessments were generated from digitized film mammograms. We used linear regression to test the single-nucleotide polymorphism (SNP)-phenotype associations adjusting for age, body mass index (BMI), MD phenotypes, and the top four genetic principal components. We further calculated genetic correlations and performed SNP-set tests of V with MD, breast cancer risk, and other breast cancer risk factors.

Results: We identified three genome-wide significant loci associated with V: rs138141444 (6q24.1) in ECT2L, rs79670367 (8q24.22) in LINC01591, and rs113174754 (12q22) near PGAM1P5. 6q24.1 and 8q24.22 have not previously been associated with MD phenotypes or breast cancer risk, while 12q22 is a known locus for both MD and breast cancer risk. Among known MD and breast cancer risk SNPs, we identified four variants that were associated with V at the Bonferroni-corrected thresholds accounting for the number of SNPs tested: rs335189 (5q23.2) in PRDM6, rs13256025 (8p21.2) in EBF2, rs11836164 (12p12.1) near SSPN, and rs17817449 (16q12.2) in FTO. We observed significant genetic correlations between V and mammographic dense area (r_g = 0.79, P = 5.91 × 10^{-5}), percent density (r_g = 0.73, P = 1.00 × 10^{-4}), and adult BMI (r_g = -0.36, P = 3.88 × 10^{-7}). Additional significant relationships were observed for non-dense area (z = -4.14, P = 3.42 × 10^{-5}), estrogen receptor-positive breast cancer (z = 3.41, P = 6.41 × 10^{-4}), and childhood body fatness (z = -4.91, P = 9.05 × 10^{-7}) from the SNP-set tests.

Conclusions: These findings provide new insights into the genetic basis of mammographic texture variation and their associations with MD, breast cancer risk, and other breast cancer risk factors.

Keywords: Breast cancer, Breast parenchymal texture feature, Texture variation, V measure, Mammographic density, GWAS, Genetic correlation
tissue in the breast, 18 loci for non-dense area (NDA), which reflects the amount of fatty tissue in the breast, and 24 loci for percent density (PD), defined as the percentage of area on a mammogram that is occupied by dense tissue [9].

Yet, MD is a global metric that ignores local patterns of variability in breast density [10]. Women with the same level of PD may have substantial heterogeneity in the structural patterns of breast parenchyma, which are assessed as texture features. Compared to MD phenotypes, breast parenchymal texture features are more refined and localized and are fully automated measures of the variation in parenchymal patterns on a mammogram [11]. Growing evidence suggests that texture features are independent breast cancer risk factors [12–16]. Heine et al. developed a summary measure of texture features called V, which captures the grayscale variation on a mammogram [12]. Recent studies have shown that a higher value of V, reflecting greater texture variation, is associated with an increased risk of breast cancer, independent of MD [12, 16]. Understanding the mechanisms underlying texture variation and breast cancer risk, especially the role of genetic variants, would provide additional insights into the development of breast cancer. However, to date, no GWAS has been conducted on breast parenchymal texture features.

In the present study, we performed a GWAS of mammographic texture variation within the Nurses’ Health Studies and Mayo Mammography Health Study cohorts. We also leveraged summary statistics of breast cancer risk and MD phenotypes from previous GWAS to identify shared susceptibility loci for V, MD, and breast cancer risk. We further assessed the genetic relationships of V with MD phenotypes, breast cancer risk, and other breast cancer risk factors by estimating genetic correlations and performing single-nucleotide polymorphism (SNP)-set tests.

**Methods**

**Study population**

The Nurses’ Health Study (NHS) is a prospective cohort study established in 1976. A total of 121,700 female registered nurses aged 30 to 55 residing in 11 states within the USA completed an initial questionnaire at that time. NHSII was established in 1989 when 116,671 female registered nurses aged 25 to 42 residing in 14 states within the USA completed an initial questionnaire at that time. Blood samples were collected from 32,826 women in NHS cohort from 1989 to 1990 and 29,611 women in NHSII cohort from 1996 to 1999, which form the blood subcohorts. Women in each cohort have been followed by self-administered questionnaires to collect updated exposure and newly diagnosed disease information every two years.

The Mayo Mammography Health Study (MMHS) is a prospective cohort study of 19,924 women who had a screening mammogram from 2003 to 2006 at the Mayo Clinic in Rochester, MN and agreed to participate in the study. Participants were at least 35 years old, residents of Minnesota, Iowa, or Wisconsin, and had no personal history of breast cancer. Participants completed a baseline questionnaire and provided consent to access any residual blood samples from clinical tests over the time period. Breast cancer diagnostic information was obtained through linkage to state-wide cancer registry data and mailed questionnaires.

**Mammogram collection and processing**

The mammogram collection and processing procedure in NHS and NHSII is described elsewhere [16, 17] and is briefly summarized here. Pre-diagnostic screening mammograms conducted as close as possible to the blood draw date were collected within NHS and NHSII breast cancer case–control studies nested in the blood subcohorts [18]. A total of 6258 film mammograms were initially collected; 437 mammograms with missing V or BMI were further excluded. The study protocol was approved by the institutional review boards of the Brigham and Women’s Hospital and Harvard T.H. Chan School of Public Health. Film mammogram craniocaudal views of both breasts were digitized using a Lumysis 85 laser film scanner or a VIDAR CAD PRO Advantage scanner (VIDAR Systems Corporation, Herndon, VA, USA). Digi-
tized images were grouped based on resolution (mean resolution = 171 μm, 232 μm, 300 μm, and images with isolated resolutions). Here, we evaluated the groups of images with average resolutions of 171 μm (high resolution) and 300 μm (low resolution). Images with isolated resolutions were down-sampled to 300 μm and added to the low-resolution group. All 171 μm images were further adjusted to 300 μm to form a larger dataset of low-resolution images.

Details of mammogram acquisition, retrieval, and digitization for MMHS are described elsewhere [12, 19]. Briefly, women in MMHS who agreed to participate provided written informed consent to access their mammograms. A total of 19,924 women were followed up for incident cancer events. We used a case-cohort design with a random sample of 2300 women from the entire MMHS cohort as the subcohort. We collected film mammograms from 1194 breast cancer cases identified through August 2019 and 2167 controls in the subcohort, excluding women who were diagnosed within 60 days of the enrollment mammogram and women with a digital mammogram. The study protocol was approved by the Mayo Clinic institutional review board. Film mammograms of both craniocaudal views were digitized on the
Array 2905 laser digitizer (Array Corporation, Roden, The Netherlands) with 50 μm (limiting) pixel spacing and further down-sampled to 200 μm. Both the original 50 μm images (high resolution) and the down-sampled 200 μm images (low resolution) were used for calculation of V.

**Assessment of V**

V is an automated measure of the grayscale variation on a mammogram. The algorithm for generating V has been described previously by Heine et al. [12, 20, 21]. Briefly, there are three main steps: segmentation, erosion, and calculation of variation. First, the breast is segmented from the background. Then, the segmented breast area is eroded by 25% or 35% along a radial direction to retain the regions where the breast was in contact with the compression paddle. Finally, the V is calculated as the standard deviation of the pixel values within the eroded breast region. Normalization processes, including spatial normalization, feature distribution normalization, and resolution estimation, were applied to the images before calculation of V to account for resolution and intensity scale differences [17].

We generated four assessments of V with different proportions of erosion and image resolutions: V with 35% erosion and low resolution (V65L), V with 25% erosion and low resolution (V75L), V with 35% erosion and high resolution (V65H), and V with 25% erosion and high resolution (V75H). These four V assessments were highly correlated with each other (Additional file 1: Fig. S1). We used V65L as our primary univariate outcome, as it had the largest sample size.

**MD phenotypes and other covariates**

MD phenotypes were assessed from digitized film mammograms using Cumulus [22], a semi-automated software, by a single trained reader [12, 23]. DA and NDA were generated for each mammogram; PD was calculated as DA divided by the total breast area. DA, NDA, and PD measures in the left and right breasts were averaged. Figure 1 shows the scatter plots and correlations of V65L and the three MD phenotypes. Body mass index (BMI) was measured at mammogram collection for all participants. Women were considered as breast cancer cases if they were diagnosed with breast cancer after blood or mammogram collection but before June 1, 2004 (NHS), June 1, 2007 (NHSII), or August 2019 (MMHS). Age at mammogram collection was also retrieved.

**Genotyping, quality control, and imputation**

The full genotyping and quality control pipeline for NHS and NHSII is described elsewhere [24]. In the present study, we used genotype data from four platforms: Affymetrix 6.0, Illumina HumanHap, Illumina Omni-Express, and Illumina OncoArray. Variants with call rate < 95% or Hardy–Weinberg equilibrium \( P < 1 \times 10^{-6} \) were excluded. European ancestry principal component (PC) outliers or samples with call rate < 90%, gender discordance, or extreme heterozygosity were excluded.

The full genotyping and quality control pipeline for MMHS is also described elsewhere [25]. Here, we used genotype data from iCOGS and OncoArray platforms. Variants with a call rate < 95% or not in Hardy–Weinberg equilibrium were excluded. Samples with a call rate < 95%, extreme heterozygosity, or of non-European ancestry based on genetic PCs were further excluded.

All genotype data were imputed to the 1000 Genomes Phase 3 version 5 reference panel separately by study and platform [26]. The number of individuals included in our GWAS by study and platform can be found in Additional file 2: Table S1.

![Fig. 1](image-url) Scatter plots of V65L by three MD phenotypes. Spearman correlation between the two measures is shown on each plot. MD mammographic density, DA dense area, NDA non-dense area, PD percent density
Association test
V assessments were standardized to have mean zero and unit standard deviation before analysis. SNP association analyses were performed within each study by platform using linear regression assuming an additive dosage effect. RVtests [27] was used for NHS/NHSII cohorts (Affymetrix 6.0, Illumina HumanHap, Illumina Omni-Express, and Illumina OncoArray), and variants were removed from individual platform results if the expected minor allele counts were below 10. PLINK 2.0 [28] was used for MMHS cohorts (iCOGS and OncoArray). We ran six models adjusting for different covariates, including age, the top four genetic PCs, BMI, PD, DA, and NDA (Table 1). Fixed effect meta-analyses across studies and platforms were conducted for each model using METAL [29]. Cochran's Q statistic was used to check for heterogeneity of the SNP associations across studies and platforms. Quantile–quantile plots and genomic inflation factors were used to assess systematic inflation in test statistics due to population substructure. Manhattan plots were generated to visualize the overall GWAS results. LocusZoom plots [30] of the 1 Mb region centered around the identified lead SNPs were generated to visualize the regional association results and nearby genes.

Given that the four V assessments were highly correlated with each other and might be proxies for an underlying latent phenotype, we performed multivariate phenotype association tests to pool association evidence across the four V assessments and get a single summary test statistic for each variant. We used R package MPAT [31] to obtain the summary test statistic for each variant. We used R package MPAT across the four V assessments and get a single summary phenotype association tests to pool association evidence underlying latent phenotype, we performed multivariate related with each other and might be proxies for an genes.

To visualize the regional association results and nearby clumping to remove any SNPs in LD (r² < 0.1) with SNPs of smaller P value (see Additional file 1: Table S3). The test statistic for V and each trait was

\[ Z = \frac{\sum_n \text{sgn}(Z_V \cdot Z_{\text{Trait}}) \cdot |Z_V|}{\sqrt{n}} \]

where \( Z_V \) is the Z score from the SNP-specific association with V and \( Z_{\text{Trait}} \) is the Z score from the SNP-specific association with the trait of interest, and n is the total number of tested genome-wide significant SNPs for that trait.

Sensitivity analysis
Our study population contains both women who developed breast cancer and women who did not develop breast cancer during the follow-up period after mammogram collection. We therefore further adjusted for

| Table 1 Covariates adjusted in the six GWAS models |
|-----------------|-----------------|
| Model | Covariates |
| 0 | Age, genetic PCs |
| 1 | Age, genetic PCs, BMI |
| 2 | Age, genetic PCs, BMI, PD |
| 3 | Age, genetic PCs, BMI, DA |
| 4 | Age, genetic PCs, BMI, NDA |
| 5 | Age, genetic PCs, BMI, PD, DA, NDA |

GWAS genome-wide association study, PCs principal components, BMI body mass index, PD percent density, DA dense area, NDA non-dense area

V, MD phenotypes, and breast cancer susceptibility variants
We evaluated whether the identified V loci were also associated with MD phenotypes or breast cancer risk using GWAS results from Breast Cancer Association Consortium (BCAC) [8, 25, 32]. To further identify shared susceptibility SNPs between V, MD phenotypes, and breast cancer risk, we conducted in silico lookups of 72 genome-wide significant MD phenotype SNPs identified by Sieh et al. [6] and Chen et al. [8], and 195 genome-wide significant breast cancer risk SNPs identified by Michailidou et al. [25] and Zhang et al. [32] in our GWAS of V. These candidate SNPs were considered significant for V if they passed the Bonferroni-corrected thresholds accounting for the number of MD (\( P < 0.05/72 \)) or breast cancer (\( P < 0.05/195 \)) SNPs tested in Model 0 for any V assessment.

Genetic correlation and SNP-set test
Genetic correlations of V with MD phenotypes, breast cancer risk, overall and stratified by estrogen receptor (ER) status, adult BMI, childhood body fatness, age at menarche, and age at natural menopause were estimated using linkage disequilibrium (LD) score regression [33, 34]. Sources of summary statistics of these traits for estimating genetic correlations are summarized in Additional file 1: Table S2.

While genetic correlation quantifies the shared genetic contribution to two traits on genome-wide scale, it may also capture the contribution of other traits due to pleiotropy (e.g., the effect of BMI on the correlation between V and PD). Therefore, we further performed SNP-set tests to assess the genetic relationship between V and the above-mentioned traits using only reported genome-wide significant SNPs for those traits. SNPs for each trait were collected from published GWAS followed by LD clumping to remove any SNPs in LD (\( r^2 > 0.1 \)) with SNPs of smaller P value (see Additional file 1: Table S3). The test statistic for V and each trait was
breast cancer case–control status in Model 5 to assess its impact on the genetic associations. We performed a multicollinearity check for the identified genome-wide significant SNPs for Model 5, where we adjusted for all three MD phenotypes, by calculating the variance inflation factor (VIF). To assess the potential impact of outliers on the association results at the identified GWAS loci, we calculated the studentized residuals for all samples for each genome-wide significant SNP. Samples with absolute studentized residual greater than 3 were considered as outliers.

**Results**

Our GWAS meta-analysis of V comprised 7040 women of European ancestry within the NHS, NHSII, and MMHS cohorts (Table 2). Women in MMHS were older and had higher BMI and lower MD compared to women in NHS and NHSII. Quantile–quantile plots and genomic inflation factors indicate there was no evidence of systematic inflation of the GWAS test statistics (Additional file 1: Fig. S2). Manhattan plots showing the -log10(P) for all tested SNPs across chromosomes are presented in Additional file 1: Fig. S3. Quantile–quantile plots of the heterogeneity P value indicate there was limited evidence of systematic heterogeneity in the test results across studies and platforms (Additional file 1: Fig. S4).

In total, we identified three independent loci that reached the genome-wide significant threshold of \( P < 5 \times 10^{-8} \) in any model for any V assessment: 6q24.1 (ECT2L), 8q24.22 (LINC01591), and 12q22 (PGAM1P5) (Table 3). 6q24.1 (Lead SNP: rs138141444, \( P = 1.24 \times 10^{-8} \) for V75H, Model 0) is a novel locus that has not previously been associated with MD phenotypes or breast cancer risk. Figure 2a shows the regional association results for 6q24.1 from Model 0 for V75H where the association was genome-wide significant. The association results for 6q24.1 were consistent across models with the same direction and similar effect sizes as well as \( P \) values. 8q24.22 (Lead SNP: rs79670367, \( P = 2.38 \times 10^{-8} \) for VSUM, Model 5) is neither a MD nor breast cancer risk locus. Figure 2b shows the regional association results for 8q24.22 from Model 5 for VSUM. The association between V and rs79670367 was more significant when we adjusted for PD (Model 2), DA (Model 3), or both (Model 5) and became less significant without adjustment for any MD phenotypes (Model 0 and 1) or adjusting for NDA only (Model 4). The direction of association was consistent across models. 12q22 (Lead SNP: rs113174754, \( P = 4.42 \times 10^{-8} \) for VSUM, Model 3) has previously been associated with NDA (rs11836367, \( P = 8.40 \times 10^{-9} \), \( r^2 = 0.59 \) with rs113174754) [6], overall breast cancer risk (rs113174754, \( P = 1.08 \times 10^{-5} \)), and ER+ breast cancer risk (rs113174754, \( P = 1.37 \times 10^{-5} \)) [25]. This locus is also significantly associated with breast size (rs17356907, \( P = 1.30 \times 10^{-3} \), \( r^2 = 0.47 \) with rs113174754) [35]. Figure 2c shows the regional association results for 12q22 from Model 3 for VSUM. The association between V and rs113174754 became non-significant when we adjusted for NDA. The direction of association with V was consistent across models and consistent with the association with NDA (opposite direction) and breast cancer risk (same direction).

We identified four additional loci that had previously been associated with MD phenotypes or breast cancer risk and reached the Bonferroni-corrected thresholds accounting for the number of MD or breast cancer SNPs tested \( (P<0.05/72 = 6.94 \times 10^{-4}) \) for MD, \( (P<0.05/195 = 2.56 \times 10^{-4}) \) for breast cancer risk) in Model 0: 5q23.2 (PRDM6), 8p21.2 (EBF2), 12p12.1 (STIP1), and 16q22.2 (FTO) (Table 3). 5q23.2 (Lead SNP: rs335189, \( P = 7.30 \times 10^{-5} \) for VSUM, Model 0) is a known locus for DA (\( P = 2.84 \times 10^{-11} \)) and PD (\( P = 5.78 \times 10^{-10} \)) [8]. The associations with V were significant in Model 0, Model 1, and Model 4 but not with adjustment for DA, PD, or both (Model 2, 3, and 5). 8p21.2 (Lead SNP: rs13256025, \( P = 5.74 \times 10^{-5} \) for VSUM, Model 0) has previously been associated with breast cancer risk (\( P = 1.40 \times 10^{-10} \)) [32]. The associations with V were significant in Models 0 and 1 and became non-significant when we adjusted for DA, NDA, or PD (Models 2, 3, 4, and 5). Although this locus has not been reported as a MD locus, the \( P \) value of the association between the lead SNP and PD was close to the genome-wide significant threshold (\( P = 4.46 \times 10^{-7} \)) [8]. 12p12.1 (Lead SNP: rs11836164, \( P = 6.69 \times 10^{-5} \) for VSUM, Model 0) is a known locus for DA (\( P = 1.66 \times 10^{-9} \)) [8]. The associations with V were significant in Models 0, 1, and 4 and became non-significant when we adjusted for DA, PD, or both (Models 2, 3, and 5). 16q22.2 (Lead SNP: rs17817449, \( P = 1.12 \times 10^{-6} \) for VSUM, Model 0) is a known locus for PD (\( P = 5.06 \times 10^{-5} \)) [8], overall (\( P = 2.52 \times 10^{-21} \)), ER+ (\( P = 5.59 \times 10^{-14} \)), and ER−breast cancer risk

| Table 2 Characteristics of NHS/NHSII and MMHS study population |
|---------------------------------------------------------------|
| **NHS/NHSII (n = 4831)** | **MMHS (n = 2209)** |
| Mean (SD) | Mean (SD) |
| Age (years) | 53.8 (9.2) | 58.9 (11.9) |
| BMI (kg/m²) | 25.9 (5.3) | 28.0 (6.2) |
| Dense area | 43.4 (29.3) | 23.8 (17.0) |
| Non-dense area | 109.3 (73.7) | 130 (67.1) |
| Percent density | 32.8 (19.7) | 17.9 (12.9) |

*NHS Nurses’ Health Study, MMHS Mayo Mammography Health Study, SD standard deviation, BMI body mass index*
Table 3 Novel V loci and their associations with MD phenotypes and breast cancer risk, overall and stratified by ER status

| Region | Lead SNP | Position | Gene | EA/OA | EAF | V65L | VSUM | MD phenotypes<sup>b</sup> | Breast cancer risk<sup>b</sup> |
|--------|----------|----------|------|-------|-----|------|------|---------------------------|-------------------------------|
|        |          |          |      |       |     |      |      | Model β (SE) | P value | Model Z score | Z score | P value | Model β (SE) | P value |
| Genome-wide significant loci<sup>c</sup> | | | | | | | | | | | | | | |
| 6q24.1<sup>d</sup> | rs138141444 | 139157426 | ECT2L | CT/C | 0.58 | 0 | −0.08 (0.02) | 1.02E−05 | 0 | −5.32 | 1.04E−07 | DA | −0.31 | 7.55E−01 | Overall | 0.00 (0.01) | 8.09E−01 |
| 1 | −0.07 (0.02) | 3.06E−05 | 1 | −0.06 (0.01) | 6.43E−05 | 2 | −0.06 (0.01) | 2.28E−04 | 3 | −0.06 (0.02) | 1.14E−04 | 4 | −0.05 (0.01) | 1.95E−04 | 5 | −0.08 (0.01) | 1.25E−02 | |
| 2 | −0.06 (0.01) | 6.00E−07 | PD | −0.01 9.90E−01 ER− | 8.09E−01 |
| 3 | −0.01 9.90E−01 ER− | 2.32E−01 |
| 4 | −0.01 9.90E−01 ER− | 2.32E−01 |
| 5 | −0.01 9.90E−01 ER− | 2.32E−01 |
| 8q24.22 | rs79670367 | 136280666 | LINC01591 | A/C | 0.02 | 0 | −0.34 (0.14) | 1.71E−02 | 0 | −2.55 | 1.09E−02 | DA | 0.13 | 8.99E−01 | Overall | −0.08 (0.03) | 1.92E−02 |
| 1 | −0.41 (0.14) | 2.75E−03 | 1 | −0.57 (0.11) | 4.63E−07 | 2 | −0.55 (0.12) | 2.41E−06 | 3 | −0.55 (0.13) | 2.23E−03 | 4 | −0.56 (0.11) | 3.61E−07 | 5* | −0.56 (0.11) | 2.38E−08 | |
| 2 | −0.57 | 9.32E−08 | PD | −0.64 | 5.25E−01 | ER− | −0.10 (0.06) | 1.25E−01 |
| 3 | −0.10 (0.06) | 1.25E−01 |
| 4 | −0.10 (0.06) | 1.25E−01 |
| 5 | −0.10 (0.06) | 1.25E−01 |
| 12q22 | rs113174754 | 96021847 | PGAM1P5 | A/ACC | 0.05 | 0 | 0.07 (0.02) | 1.25E−04 | 0 | 4.53 | 5.91E−06 | DA | −2.34 | 1.95E−02 | Overall* | 0.07 (0.01) | 1.08E−24 |
| 1 | 0.07 (0.02) | 8.74E−05 | 1 | 4.61 | 4.05E−06 | NDA | −3.97 | 7.26E−05 | ER+* | 0.07 (0.01) | 1.37E−18 |
| 2 | 3.39E−05 | 2 | 4.62 | 3.82E−06 | PD | 0.81 | 4.17E−01 | ER− | 0.06 (0.01) | 3.44E−06 |
| 3 | 3.40E−07 | 3* | 5.47 | 4.42E−08 | |
| 4 | 4.05E−06 | 2.65E−04 |
| 5 | 4.05E−06 | 2.65E−04 |
| Region       | Lead SNP  | Position     | Gene\(^a\) | EA/OA | EAF | V6SL | VSUM       | MD phenotypes\(^b\)       | Breast cancer risk\(^b\) |
|--------------|-----------|--------------|------------|-------|-----|------|------------|---------------------------|--------------------------|
|              |           |              |            |       |     |      |            | Model β (SE) P value Z score | Model Z score P value Phenotype Z score P value Phenotype β (SE) P value |
| 5q23.2       | rs335189  | 122446856    | PRDM6      | C/G   | 0.70|      |            | 2.15E−04 0 2.15E−04 0 | −3.97 7.30E−05 DA* −6.66 2.84E−11 Overall | −0.03 (0.01) 9.55E−05 |
|              |           |              |            |       |     |      |            | 1 −0.06 (0.02) −0.06 (0.02) | −3.84 1.24E−04 NDA 260 9.39E−03 ER+ | −0.03 (0.01) 1.54E−03 |
|              |           |              |            |       |     |      |            | 2 −0.03 (0.01) −0.03 (0.01) | −2.26 2.35E−02 PD* −6.20 5.78E−10 ER− | −0.01 (0.01) 3.25E−01 |
|              |           |              |            |       |     |      |            | 3 −0.04 (0.02) −0.04 (0.02) | −3.13 1.73E−03 Overall | 0.03 (0.01) 6.20 9.39E−03 |
|              |           |              |            |       |     |      |            | 4 −0.06 (0.02) −0.06 (0.02) | −3.51 4.50E−04 | 0.04 (0.01) 2.61E−02 5 9.85E−03 |
|              |           |              |            |       |     |      |            | 5 −0.03 (0.01) −0.03 (0.01) | −2.58 9.85E−03 | 0.04 (0.01) 6.95E−02 0 |
| 8p21.2       | rs13256025| 25831778     | EBF2       | T/C   | 0.20|      |            | 6.23E−05 0 6.23E−05 0 | 4.02 5.74E−05 DA 3.32 9.17E−04 Overall | 0.04 (0.01) 2.12E−07 |
|              |           |              |            |       |     |      |            | 1 0.08 (0.02) 0.08 (0.02) | 3.94 8.01E−05 NDA −4.26 2.01E−05 ER+ | 0.03 (0.01) 8.77E−04 |
|              |           |              |            |       |     |      |            | 2 0.04 (0.02) 0.04 (0.02) | 2.54 1.11E−02 PD 5.05 4.46E−07 ER− | 0.06 (0.01) 1.78E−04 |
|              |           |              |            |       |     |      |            | 3 0.05 (0.03) 0.05 (0.03) | 2.92 3.47E−03 Overall | 0.01 (0.01) 6.93E−03 2.37 1.79E−02 |
|              |           |              |            |       |     |      |            | 4 0.06 (0.02) 0.06 (0.02) | 3.23 1.22E−03 | 0.04 (0.02) 3.31E−02 5 1.79E−02 |
|              |           |              |            |       |     |      |            | 5 0.04 (0.02) 0.04 (0.02) | 2.37 1.79E−02 | 0.01 (0.01) 6.95E−02 0 |
| 12p12.1      | rs11836164| 26446625     | SSPN       | T/C   | 0.76|      |            | 8.22E−06 0 8.22E−06 0 | −3.99 6.69E−05 DA* −6.03 1.66E−09 Overall | 0.01 (0.01) 6.95E−02 |
|              |           |              |            |       |     |      |            | 1 −0.08 (0.02) −0.08 (0.02) | −4.05 5.19E−05 NDA 0.73 4.67E−01 ER+ | 0.00 (0.01) 7.35E−01 |
|              |           |              |            |       |     |      |            | 2 −0.04 (0.02) −0.04 (0.02) | −2.37 1.77E−02 PD −5.43 5.55E−08 ER− | 0.03 (0.01) 5.72E−02 |
|              |           |              |            |       |     |      |            | 3 −0.05 (0.02) −0.05 (0.02) | −2.55 1.09E−02 | 0.01 (0.01) 3.18E−03 3 1.78E−03 |
|              |           |              |            |       |     |      |            | 4 −0.08 (0.02) −0.08 (0.02) | −4.17 3.03E−05 | 0.04 (0.02) 6.93E−03 5 1.34E−02 |
|              |           |              |            |       |     |      |            | 5 −0.04 (0.02) −0.04 (0.02) | −2.47 1.34E−02 | 0.01 (0.01) 6.95E−02 0 |
Table 3 (continued)

| Region | Lead SNP   | Position | Gene<sup>a</sup> | EA/OA | EAF     | V65L | VSUM | MD phenotypes<sup>b</sup> | Breast cancer risk<sup>b</sup> |
|--------|------------|----------|------------------|-------|---------|------|------|-------------------------|-------------------------------|
|        |            |          |                  |       |         |      |      |                         |                               |
|        | 16q12.2    | rs17817449 | 53813367         | FTO   | T/G     | 0.61 | 0    | 1.63E-07               | 0.006                     |
|        |            |           |                  |       |         |      |      | 4.87                    | 2.52E-21                   |
|        |            |           |                  |       |         |      |      | 1.12E-06               |                               |
|        |            |           |                  |       |         |      |      | DA                      | 464                         |
|        |            |           |                  |       |         |      |      | 3.49E-06               | 0.107                     |
|        |            |           |                  |       |         |      |      | Model Z score           | Model Z score              |
|        |            |           |                  |       |         |      |      | 4.87                    | 2.46E-04                  |
|        |            |           |                  |       |         |      |      | 3.32                    | 8.93E-04                  |
|        |            |           |                  |       |         |      |      | 4.36E-03               | 5.85                      |
|        |            |           |                  |       |         |      |      | PD*                     | 5.06E-09                  |
|        |            |           |                  |       |         |      |      | ER-*                    | 1.80E-10                  |
|        |            |           |                  |       |         |      |      | Overall*                | 2.52E-21                  |
|        |            |           |                  |       |         |      |      | 0.06                    | 0.06                      |
|        |            |           |                  |       |         |      |      | 0.006                   | 5.59E-14                  |
|        |            |           |                  |       |         |      |      | 0.107                   | 5.85                      |
|        |            |           |                  |       |         |      |      | 0.06                    | 1.80E-10                  |
|        |            |           |                  |       |         |      |      | 0.006                   | 5.59E-14                  |
|        |            |           |                  |       |         |      |      | 0.107                   | 5.85                      |
|        |            |           |                  |       |         |      |      | 0.06                    | 1.80E-10                  |

<sup>a</sup> Nearest gene within 500 kb of the lead SNP

<sup>b</sup> Summary statistics were from Breast Cancer Association Consortium [8, 25]

SNP single-nucleotide polymorphism, EA effect allele, OA other allele, EAF effect allele frequency, MD mammographic density, DA dense area, NDA non-dense area, PD percent density, ER estrogen receptor

Genome-wide significant loci (P < 5 x 10^-8) in any model for any V assessment

6q24.1 (rs13814444) is genome-wide significant for V75L (Additional file 2: Table S4)

Significant V loci at the Bonferroni-corrected threshold of P < 6.94 x 10^-4 accounting for the 72 MD phenotype SNPs tested or P < 2.56 x 10^-4 accounting for the 195 breast cancer SNPs tested based on Model 0

Genome-wide significant associations (P < 5 x 10^-8)
This locus is also significantly associated with BMI (rs17817449, P = 5.10 × 10^{-19}) and breast size (rs62033406, P = 3.70 × 10^{-7}, r^2 = 0.89 with rs17817449) [35].

The associations with V were significant in Model 0 and became non-significant when we adjusted for BMI or any MD phenotype. The directions of association with V were consistent with those significant associations with MD (same direction for PD and DA, opposite direction for NDA) or breast cancer (same direction) for all four loci. Association results of all identified V loci for all models and V assessments can be found in Additional file 2: Table S4.

We observed significant positive genetic correlations between V and dense area (rg = 0.79, P = 5.91 × 10^{-5} for VSUM, Model 0) and percent density (rg = 0.73, P = 1 × 10^{-4} for VSUM, Model 0) (Fig. 3a). The correlations became non-significant using GWAS results from Model 2. Positive correlations were also observed with overall (rg = 0.20, P = 6.90 × 10^{-3} for VSUM, Model 0) and ER+ (rg = 0.22, P = 4.60 × 10^{-3} for VSUM, Model 0) breast cancer and became non-significant when adjusting for PD. We also observed a significant negative association with adult BMI (rg = −0.36, P = 3.88 × 10^{-7} for VSUM, Model 0), which became non-significant when adjusting for BMI. A strong negative correlation was observed for NDA (rg = −0.60, P = 5.20 × 10^{-3} for VSUM, Model 0) before adjusting for PD. Genetic correlation results were similar across V assessments; the full results are summarized in Additional file 2: Table S7.

In addition to the genetic relationships of V with DA, NDA, PD, and breast cancer risk identified by genetic correlations, we further identified a significant positive association between V and ER+ breast cancer (z = 3.41, P = 6.41 × 10^{-4} for VSUM, Model 0) and a significant negative association between V and childhood body fatness from the SNP-set test using genome-wide significant SNPs for childhood body fatness (z = −4.91, P = 9.05 × 10^{-7} for VSUM, Model 0) (Fig. 3b). The overall pattern of the associations was similar for genetic correlation and SNP-set test. It is worth noting that for MD phenotypes and childhood body fatness, the associations with V remained nominally significant (P < 0.05) if we further adjust for BMI and PD in the SNP-set test. Plots showing Z scores from GWAS of V and GWAS of MD phenotypes [8] and breast cancer [25] for the SNPs included in the SNP-set tests are presented in Fig. 4. SNP-set test results across all models and V assessments can be found in Additional file 2: Table S8.

No substantial change on the top findings was observed after including breast cancer case–control status as a covariate (Additional file 2: Table S4). There was no multicollinearity issue for the effect estimates of the genome-wide significant SNPs in Model 5 (VIFs all close to 1). There were 10 outliers with absolute studentized
residual greater than 3 for rs79670367 at 8q24.22 from Model 5 for V65L. The effect estimates for the effect allele increased by 24% after removing those outliers. No substantial impact of outliers was found for other identified V SNPs.

Discussion

While MD continues to be one of the most well-established and widely used mammographic risk factors for breast cancer, there are gaps in our knowledge of mammographic features themselves and their relationship with breast cancer risk. Current MD measures do not capture the heterogeneity in the distribution of dense breast tissue on a mammogram, known as texture variation. Increasing evidence has shown that the performance of texture variation on discriminating breast cancer outcomes is either comparable or even higher than the performance of MD measures [12, 16, 37, 38]. Understanding the contributing mechanisms of texture variation on breast cancer risk, especially the involved genetic components, would expand our knowledge on breast cancer development. In this study, we performed the first GWAS meta-analysis of mammographic texture variation, focusing on a summary measure of grayscale variation on mammograms (V). We identified three genome-wide significant V loci: 6q24.1 (ECT2L), 8q24.22 (LINC01591), and 12q22 (PGAM1P5), the first two of which have not previously been associated with MD or breast cancer risk. Four additional loci for MD or breast cancer risk, 5q23.2 (PRDM6), 8p21.2 (EBF2), 12p12.1 (SSPN), and 16q12.2 (FTO), were also found associated with V.

Different models of the SNP-V association were fit to capture different effects (Table 1). Model 0 with only age and genetic PCs as covariates can capture both the effect of genetic variants on V and the effect that was mediated by BMI or MD phenotypes. We also fit Model 5 adjusting for all MD phenotypes together to assess the variant effect that was independent of all adjusted covariates. Although PD can be calculated from DA and NDA, previous GWAS of MD still identified different loci and genetic effects for different MD measures. We therefore
fit the fully adjusted Model 5 to minimize the effect of MD phenotypes on the V associations. Collinearity issue in Model 5 did not have an impact on the effect estimates of the variants. Comparing the results from different models may also provide evidence for the underlying relationships between the genetic variants, V, and other adjusted covariates as well as boost power to detect V SNPs. For example, if we observed a SNP-V association in models with and without adjustment for MD, then it is likely that the SNP influences V through other pathways that are independent of density; if the SNP-V association was only observed in model without adjusting for density, then it indicates that the SNP effect on V might be largely mediated by density. Downstream analyses need to be performed to confirm the relationships. Both V65L and the calculated summary statistics of the four V assessments, VSUM, were used as our primary outcomes. We have a larger sample size thus a greater power for low-resolution V assessments compared to high-resolution assessments (sample size for V65L and V75L = 7040; sample size for V65H and V75H = 4763). Although a previous study looking at the relationship between V and breast cancer risk in NHS/NHSII used a different assessment, V75L, as the outcome [16], these two low-resolution V assessments were highly correlated with each other (ρ = 0.98, Additional file 1: Fig. S1) and there was no substantial difference in the GWAS results of these two assessments (Additional file 2: Table S4). Using VSUM also has the advantage of boosting power given that the SNP associations were similar across different V assessments.

Among the three genome-wide significant V loci, 12q22 is also associated with NDA and breast cancer risk in consistent direction, suggesting that at least part of its genetic effect on V is mediated by NDA or the genetic effect on NDA is mediated by V, and there are potential...
shared biological pathways between these three traits. These hypotheses are further supported by the fact that 12q22 is also associated with total breast size and its association with V was most significant when adjusting for PD and became non-significant when adjusting for NDA. The lead variant rs113174754 at 12q22 is an indel near pseudogene PGAM1P5 and is 30 kb upstream of protein coding gene NTN4 (see Fig. 2c). NTN4 encodes a member of the netrin family of proteins, which involved in axon guidance, tumorigenesis, and angiogenesis. NDA SNP at 12q22 (rs11836367–C, correlated with the effect allele of rs113174754) has been found to downregulate NTN4 in mammary tissue [6]. NTN4 has also been identified as a candidate breast cancer risk gene by colocalization analysis, where the C allele of SNP rs61938093 ($r^2 = 0.48$ with the effect allele of rs113174754) at this region reduced NTN4 promoter activity and knockdown of NTN4 promoted breast cell proliferation and tumor growth [39]. These findings suggest a shared genetic basis and potential biological mechanisms for mammographic risk factors, especially breast adipose tissue (represented by NDA), and breast cancer risk at this locus, and may also explain the observed association between V and breast cancer risk. 6q24.1 and 8q24.22 are V loci that have not been seen associated with MD phenotypes or breast cancer risk. The lead variant rs138141444 at 6q24.1 is an intronic indel in ECT2L. The lead variant rs79670367 at 8q24.22 is an intronic SNP in LINCO1591. Neither these two genes nor nearby genes have been associated with breast cancer risk. The genetic effects of these two loci on V are therefore likely through mechanisms not mediated by MD. It should also be noted that the effect allele frequency for rs79670367 is less than 5% and the outlier analysis indicated that the association results might be influenced by influential outliers. Moreover, only about half of the samples have genotype data on this variant (available in NHS/NHSII Illumina HumanHap and MMHS OncoArray). Further studies are needed to confirm the findings at these two loci.

Four additional V loci have previously been associated with breast cancer risk or MD phenotypes. The lead variant rs13256025 at 8p21.2 is an intronic SNP in protein coding gene EBF2. EBF2 encodes well-conserved DNA-binding helix–loop–helix transcription factors, which involved in differentiation of osteoblasts. Although little is known about the role of EBF2 in breast cancer development, studies have shown that inactivation of EBF genes can lead to tumorigenesis via accumulation and expansion of undifferentiated progenitor cells [40]. 16q12.2 is associated with both PD and breast cancer risk in the same direction with its lead SNP rs17817449 located in FTO. FTO is a well-established susceptibility gene for obesity [41]. In our analysis, the association was only significant in the base model and became non-significant when adjusting for BMI, suggesting that its genetic effect on V might be mediated by BMI. FTO is overexpressed in breast cancer cells, which affects the energy metabolism of the cells [42]. 5q23.2 is a known locus for DA and PD. The lead variant rs335189 is an intronic SNP in PRDM6. PRDM6 encodes a transcriptional repressor involved in the regulation of endothelial cell proliferation, survival, and differentiation and may play a role in breast cancer tumorigenesis [7, 43]. The lead variant rs11836164 at 12p12.1 is an intronic SNP near SSPN and is only associated with DA. Functional analysis needs to be performed to further investigate the role of identified V SNPs in mammary development and breast cancer etiology.

Consistent with the phenotypic relationships we observed for V and MD measures, there were strong positive genetic correlations of V with DA and PD, and negative genetic correlations with NDA. The positive genetic correlations between V and breast cancer risk (overall and ER+ specific) were also nominally significant, further supporting that the observed phenotypic association between V and breast cancer risk can at least be partially explained by shared genetic components. The magnitude of these genetic correlations is comparable to those between MD and breast cancer risk [6]. A genetic variant can be associated with multiple traits, which is known as pleiotropy. Studies have shown that jointly analyzing GWAS data of multiple traits can boost power to detect genetic associations for each trait and improve the prediction performance [44, 45]. In our analysis, we observed significant genetic correlations of V with MD phenotypes and BMI using genome-wide association results. It is therefore very likely that a substantial number of variants are associated with both MD phenotypes, especially NDA, and BMI, which would dilute the correlations we observed for any pair of the traits. SNP-set tests may provide more evidence for the shared mechanism underlying two traits using only susceptibility variants. Here, we found that even if we adjust for PD in the model, there were still significant correlations between V and PD based on genome-wide significant SNPs for PD, indicating that the genetic contribution of V cannot be fully explained by PD and PD is either a mediator or collider of the association between the genetic variants and V (Fig. 4a). Correlations of V with breast cancer and childhood body fatness were also stronger at the susceptibility variants. There were still correlations, though not significant, after adjusting for PD, providing evidence for the genetic relationship between V and these traits that were not mediated by MD (Fig. 4b).

Our study focuses on a summary texture measure, V, but there are also many other texture features that may capture more of the parenchymal complexity. For
example, Manduca et al. systematically evaluated 1443 textural features and identified six independently validated strongest features [13]. Malkov et al. identified 15 texture features that were significantly associated with breast cancer risk, several of which were only weakly correlated with PD [46]. V is a summary measure of the grayscale variation in the entire eroded breast region thus may not be optimal for assessing local patterns in specific regions of interest, which can be quantified by other texture analyzing methods [11]. In addition, estimation of V depends on parameters such as proportion of breast erosion and digital resolution, which may also not be optimal. Studying the genetics of other features or their combinations may provide additional information for the genetic architecture of breast parenchymal texture variation. Our study included breast cancer cases, which might be concerning since V has been associated with breast cancer risk. However, both theoretical [47] and empirical [48] evidence suggests that including cases of a rare outcome does not bias the association estimates in GWAS of a secondary outcome, except when both the genetic variant being analyzed and the secondary outcome are very strong risk factors—stronger than those exhibited by breast cancer risk SNPs, V, or BMI. Indeed, we did not observe any substantial changes on the top findings after further adjusting for breast cancer case-control status in the model. Moreover, the direction of the associations we observed—e.g., a breast cancer risk allele was positively associated with V—is opposite of those expected if the SNP-V association is solely an artifact due to collider bias. Multiple testing issue caused by studying four V assessments may also be a concern, and we therefore estimated a single summary test statistic, VSUM, to minimize the impact of multiple testing and to boost power. Studying the computerized automated texture feature can also reduce the potential bias caused by measurement error that studies on semi-automated MD measures are usually susceptible to.

Conclusions
In conclusion, we performed a GWAS of breast parenchymal texture variation, V, and identified three independent loci at genome-wide significance, including 12q22 (PGAM1LPS) that are associated with MD phenotypes and breast cancer risk and 6q24.1 (ECT2L) and 8q24.22 (LINC01591) that are novel V susceptibility loci. Four additional V loci were identified from looking up MD and breast cancer susceptibility SNPs in GWAS of V, including 5q23.2 (PRDM6), 8p21.2 (EBF2), 12p12.1 (SSPN), and 16q12.2 (FTO). These findings provide the first evidence of the genetic basis of V and shared genetic components between V, MD, and breast cancer risk. Future studies are needed to confirm our findings and further improve our understanding of the mechanisms underlying the relationship between texture features, MD, and breast cancer development.

Abbreviations
BCAC: Breast Cancer Association Consortium; BMI: Body mass index; DA: Dense area; ER: Estrogen receptor; GWAS: Genome-wide association study; LD: Linkage disequilibrium; MD: Mammographic density; MMHS: Mayo Mammography Health Study; NDA: Non-dense area; NSHS: Nurse’s Health Study; PC: Principal component; PD: Percent density; SNP: Single-nucleotide polymorphism; VIF: Variance inflation factor; V75H: V with 25% erosion and high resolution; V75L: V with 25% erosion and low resolution; V65H: V with 35% erosion and high resolution; V65L: V with 35% erosion and low resolution.

Supplementary Information
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Author contributions XI, PK, RMT, and CVM conceived and designed the study. RMT and ETW prepared the mammographic texture variation data for NHS/NHSII. CMV and JH prepared the mammographic texture variation data for MMHS. CT prepared the genotype data for NHS/NHSII. SW prepared the genotype data for MMHS. HC and SL prepared the mammographic density GWAS data. YL analyzed and interpreted the data and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials NHS/NHSII: The data that support the findings of this study are available from the Nurses’ Health Studies; however, they are not publicly available. Investigators interested in using the data can request access, and feasibility will be discussed at an investigators’ meeting. Limits are not placed on scientific questions or methods, and there is no requirement for co-authorship. Additional data sharing information and policy details can be accessed at http://www.nurseshealthstudy.org/researchers. MMHS: The summary statistics generated from the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate The study protocol was approved by the institutional review boards of the Brigham and Women’s Hospital, Harvard T.H. Chan School of Public Health, and the Mayo Clinic and was in accordance with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. All participants provided written informed consent.

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

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