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Common variants in *ZNF365* are associated with both mammographic density and breast cancer risk

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S.L., C.M.V., J.L.H., P.K., P.H., D.F.E., N.F.B. and R.M.T., designed and executed the study. S.L., J.Li., J.V., A.D.P., D.W., C.G.S., V.S.P., J.S, K.C. and P.K., led the statistical analysis. C.M.V., D.T., R.W., J.B., J.Le., T.A., N.J.W., R.J.F.L., A.D.P., L.J.M, S.E.H., A.H., D.J.H, J.L.H, M.C.S.,S.J.C., I.d.S.S., J.Liu, L.E., F.J.C.,C.A., K.C., P.H., D.F.E., N.F.B., and R.M.T., collected and provided data to the initial GWAS analysis and replication studies. S.L., C.M.V., J.Li., D.T., R.J.F.L., J.S., D.F.E. and R.M.T. wrote the manuscript, with contributions from all the authors.

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Abstract

High percent mammographic density adjusted for age and body mass index (BMI) is one of the strongest risk factors for breast cancer. We conducted a meta-analysis of five genome-wide association studies of percent mammographic density and report an association with rs10995190 in *ZNF365* (combined $P=9\times6\cdot10^{-10}$). This finding might partly explain the underlying biology of the recently discovered association between common variants in *ZNF365* and breast cancer risk.

Percent mammographic density reflects the proportion of stromal and epithelial tissues in relation to fat tissue in the breast. Women with more than 75% dense tissue in the breast are at a four- to five-fold greater risk of breast cancer than women of the same age and BMI with little or no dense tissue 1-3. Percent mammographic density has thus been considered an intermediate phenotype of breast cancer 4-7 and identifying its determinants may provide novel insights into the etiology of breast cancer.

Lifestyle factors including age, parity, BMI and exogenous hormone levels explain only 20-30% of the between-women variation in percent mammographic density 8. It has been estimated that 61-67% of the residual variation could be attributable to genetic factors 9 but linkage and candidate gene association studies have been largely unsuccessful in reproducibly identifying loci related to mammographic density.

To this end, we conducted a meta-analysis of five genome-wide association studies (GWAS) of percent mammographic density adjusted for age and BMI within the Marker Of DEnsity (MODE) consortium: the Nurses' Health Study (NHS) (n=1,590), the Singapore and Swedish Breast Cancer Study (SASBAC) study (n=1,258), the European Prospective Investigation Into Cancer and Nutrition - (EPIC-Norfolk) (n=1,142), the Minnesota Breast Cancer Family study (MBCFS) (n=571) and the TORONTO/MELBOURNE study (n=316). The total sample size was 4,877 women. All women were of self-described European descent and the majority (89%) was postmenopausal at the time of mammogram.

Study design, population characteristics and genotyping platforms varied across studies (Supplementary Tables 1-3). For all studies, percent mammographic density was measured using the CUMULUS software 10. Genotypes for more than 2 million SNPs were imputed within each study using Phase II data from HapMap CEU individuals. All studies except TORONTO/MELBOURNE used linear regression treating percent mammographic density as a quantitative trait. TORONTO/MELBOURNE selected women in the top and bottom 10% of percent mammographic density and treated women with high density as “cases” and women with low density as “controls” in a logistic regression model. The differences in study design (extreme sampling vs. continuous trait) did not allow us to perform meta-analysis based on the estimated effect size in each study as units of density measurement were not comparable across studies 11. Instead, a combined test for each SNP was derived by combining p-values and the direction of association for each study, weighted by the

square-root of the sample size and the study-specific inflation factor. We calculated an effective sample size for the TORONTO/MELBOURNE study (n=1,109) to account for their sampling of women in the tails of the distribution (Supplementary Information).

The quantile-quantile plot and Manhattan plots are depicted in Supplementary Figures 1 and 2. The overall genetic inflation factor was λ=1.033. Although no SNP met the commonly-used genome-wide significance criterion of $P<5\times10^{-8}$, six SNPs within the same linkage disequilibrium (LD) block in intron 4 of *ZNF365* had p-values $<10^{-6}$, with the smallest p-value being observed for rs10995195 for which the 'C' allele was associated with lower mammographic density ($P=4.0\times10^{-7}$, Supplementary Table 4).

A recent GWAS by Turnbull and colleagues, including 3,659 breast cancer cases and 4,897 controls in the first stage and 12,576 cases and 12,223 controls in the second stage, found that the rs10995190 'A' allele in *ZNF365* was associated with decreased breast cancer risk (OR: 0.86, 95% CI: 0.82-0.91, $P=5.1\times10^{-15}$) 12. The rs10995190 'A' allele is in high LD with the rs10995195 'C' allele (pair-wise r^2 =0.94 in HapMap CEU) and was ranked third in our meta-analysis of percent mammographic density ($P=5.7\times10^{-7}$; Figure 1).

We attempted to replicate the association between rs10995190 and percent mammographic density in 1,690 women from the Mayo Clinic Breast Cancer Study (MCBCS) genotyped as a part of the replication in the breast cancer case-control GWAS by Turnbull colleagues, and in additional 1,145 women without breast cancer from the Sisters in Breast Screening Study (SIBS) through *in silico* replication (Supplementary Information). We found that the 'A' allele of rs10995190 was associated with lower percent mammographic density in our replication studies (P=0.0004), resulting in a combined P-value of 9.6×10^{-10} (Table 1). Adjusting for breast cancer case-control status in NHS and MCBCS ($P=6.4\times10^{-9}$) or excluding breast cancer cases ($P=1.1\times10^{-7}$) did not change the statistical significance of this association. For two of the three case-control studies (NHS and MCBCS), there was a significant association between rs10995190 and mammographic density among the controls (Table 1). Therefore, we find it unlikely that the association between rs10995190 and mammographic density is driven by confounding due to inclusion of breast cancer cases. Across studies with genotype data for rs10995190 (not considering studies with imputed data), the mean change in percent mammographic density per minor allele was −2.01. Based on this estimate, rs10995190 would explain ~0.5% of the variance in percent mammographic density.

To assess the extent to which the observed association between rs10995190 and breast cancer risk might be mediated through mammographic density, we estimated the association between rs10995190 and breast cancer risk before and after adjustment for mammographic density using case-control data from NHS, SASBAC and MCBCS (Supplementary Table 5). From the pooled analysis, including 2,107 breast cancer cases and 2,433 controls, we observed a significant association between rs10995190 and breast cancer risk, with an effect size similar to that previously reported (OR: 0.85, 95% CI: 0.76-0.96, P=0.008) 12. Adjusting for mammographic density slightly attenuated the association (OR: 0.90, 95% CI: 0.80–1.01, P=0.09). These results demonstrate that genetic variation in *ZNF365* could influence breast cancer risk by influencing the proportion of dense tissue in the breast, although it remains possible that the same locus influences both phenotypes independently.

In addition, we examined if any other known breast cancer SNPs were associated with mammographic density in our study (Supplementary Table 6). Out of 22 SNPs tested (excluding rs10995190), two SNPs showed an association with mammographic density; rs2046210 (*ESRI*, P=0.005) and rs3817198 (*LSP1*, P=0.04). Both associations were in the expected direction as determined by corresponding breast cancer associations. We also

examined these associations stratified by case-control status, recognizing the lower statistical power due to the smaller sample size (Supplementary Table 6).

A potential limitation in this study is the inherent measurement error in mammographic density. In all seven studies, mammographic density was read using the same computer-assisted thresholding method which has been shown to be highly reproducible with intra- and inter-reader reproducibility within sites generally greater than 0.9 10. In addition, the European studies used the medio-lateral oblique (MLO) view, while other studies used the cranio-caudal (CC) view. Although the percent density measurements from the MLO view have been shown to be lower than those from the CC view 13,14, both measures are strong predictors of breast cancer risk. By conducting study-specific GWAS before pooling summary statistics in a meta-analysis, we minimized the impact of differences in density measurements across studies.

Mammographic density attenuated the association with breast cancer risk suggesting that the influence of *ZNF365* on breast cancer risk may be mediated in part by mammographic density. Given that there is measurement error in our phenotype, our ability to demonstrate mediation through mammographic density is reduced. Nonetheless, these results demonstrate how an intermediate phenotype can help shed light on the mechanisms underlying observed SNP-disease associations. The association with rs10995190, while highly statistically significant, explains only 0.5% of the variance in percent mammographic density. Further GWAS analyses in larger sample sizes will most likely result in identification of additional variants.

In summary, we report a novel association between common genetic variation in *ZNF365* and percent mammographic density adjusted for age and BMI. The same genetic variant was recently identified as a breast cancer susceptibility locus suggesting that one or more variants in the *ZNF365* locus acts on breast cancer risk by influencing the proportion of dense tissue in the breast.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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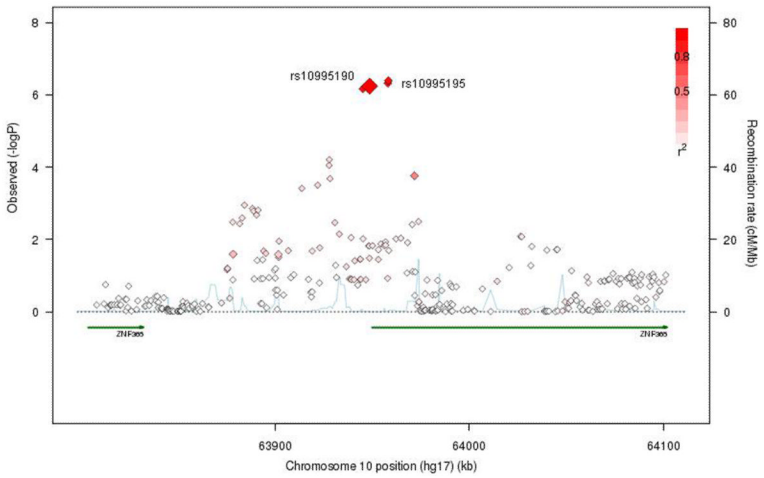

Figure 1.

Regional association plot for ZNF365 across a 300kb window. Association of individual SNPs is plotted as $-\log_{10}(P)$ against chromosomal base-pair position. Results of both genotyped and imputed SNPs are provided. Colors indicate the LD relationship between rs10995190 and the other markers (red, $r^2>0.8$). The right-hand Y axis shows the recombination rate estimated from the HapMap CEU population. All p-values are from the discovery phase.

Table 1

Association between rs10995190 and percent mammographic density.

	Cohort	*N*	*MAF*[1]	*Effect allele*	*Genotyped/Imputed*[2]	*Effect*[3]	95% CI	*P*[4]	P(Het)
Discovery Phase	EPIC Norfolk	1,142	0.15	A	Imputed (0.99)	−0.12	−0.23 to 0.03	0.12	
	SASBAC[5](1)	518	0.13	A	Genotyped	−0.26	−0.54 to 0.02	0.07	
	SASBAC[5](2)	740	0.15	A	Genotyped	0.01	−0.21 to 0.23	0.95	
	NHS	1,590	0.15	A	Genotyped	−0.29	−0.45 to −0.13	0.0005 (0.001)	
	NHS[5](1)	806	0.14	A	Genotyped	−0.20	−0.43 to 0.03	0.08	
	NHS[5](2)	784	0.16	A	Genotyped	−0.35	−0.58 to −0.12	0.003	
	TORONTO/MELBOURNE	316	0.15	A	Genotyped	−0.56[6]	−1.02 to −0.10	0.02	
	MBCFS	571	0.14	A	Genotyped	−0.26	−0.48 to −0.05	0.02	
	COMBINED	**4,877**		**A**		**−0.18**	**−0.29 to −0.08**	**5.70×10^{-7}**	**0.39**
Replication Phase	MCBCS	1,690	0.15	A	Genotyped	−0.23	−0.36 to −0.10	0.0006 (0.003)	
	MCBCS[5](1)	783	0.13	A	Genotyped	−0.04	−0.22 to −0.14	0.63	
	MCBCS[5](2)	907	0.16	A	Genotyped	−0.29	−0.46 to −0.12	0.001	
	SIBS	1,145	0.14	A	Imputed (0.99)	−0.11	−0.28 to 0.06	0.20	
	COMBINED	**2,835**		**A**		**−0.18**	**−0.30 to −0.07**	**0.0004**	**0.27**
Combined		**7,712**		**A**		**−0.18**[7]	**−0.25 to −0.12**	**9.63×10^{-10}**	**0.29**

[1] MAF=Minor allele frequency

[2] If imputed the imputation quality score is indicated in parenthesis

[3] The effect estimate measures change in square-root transformed mammographic density adjusted for age, BMI and other covariates (see supplementary information) per minor allele for all studies except TORONTO/MELBOURNE which performed a logistic regression based on extreme sampling as described in the supplementary information.

[4] P-values in parenthesis are based on linear regression taking breast cancer case-control status into account.

[5] (1) – Breast Cancer Cases, (2) - Controls

[6]The effect estimate for the TORONTO/MELBOURNE study is based on a logistic regression model as explained in the supplementary information section. Therefore, their effect estimate has a different interpretation compared to the cross-sectional studies.

[7]The combined effect estimate does not include the TORONTO/MELBOURNE study.