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Association analysis between breast cancer genetic variants and mammographic density in a large population-based study (Determinants of Density in Mammographies in Spain) identifies susceptibility loci in *TOX3* gene

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Abstract ***Background:*** Mammographic density (MD) is regarded as an intermediate phenotype in breast cancer development. This association study investigated the influence of 14 breast cancer susceptibility loci identified through previous genome-wide association studies on MD among the participants in the "Determinants of Density in Mammographies in Spain" (DDM-Spain) study.

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Methods: Our study covered a total of 3348 Caucasian women aged 45–68 years, recruited from seven Spanish breast cancer screening centres having DNA available. Mammographic density was blindly assessed by a single reader using a semiquantitative scale. Ordinal logistic models, adjusted for age, body mass index and menopausal status, were used to estimate the association between each genotype and MD.

Results: Evidence of association with MD was found for variant rs3803662 (*TOX3*) (Odds Ratio (OR) = 1.13, 95% Confidence Interval (CI) = 1.03–1.25), and marginal evidence of association for susceptibility loci rs3817198 (*LSP1*) (OR = 1.09, 95% CI = 1.00–1.20) and rs2981582 (*FGFR2*) (OR = 0.92, 95% CI = 0.84–1.01). Two other loci were associated with MD solely among pre-menopausal women, namely, rs4973768 (*SLC4A7*) (OR = 0.83, 95% CI = 0.70–1.00) and rs4415084 (*MEPS30*) (OR = 1.22, 95% CI = 1.00–1.49).

Conclusions: Our findings lend some support to the hypothesis which links these susceptibility loci to MD.

1. Introduction

Genome-wide association studies (GWAS) have proved successful in identifying new genetic variants associated with breast cancer.[1–5] These studies and candidate-gene association analyses have confirmed the contribution of common polymorphisms to the pathogenesis of breast tumours.[6,7]

Measurement of mammographic breast density has been proposed as an intermediate phenotype for breast tumours,[8–10] though the biology underlying the association between mammographic density (MD) and breast cancer is still unknown. Mammographic density reflects variations in the amounts of fat, and stromal and epithelial tissue in the breast gland. Twin and family studies have shown that MD is an inherited trait, and genetic variation accounts for at least a 60% of its variability.[8,11,12]

At least five association analyses of mammographic density (MD) and breast cancer susceptibility loci have been published.[13–17] Two of these found a significant association with rs3817198 SNP (*LSP1*),[13,16] two also reported associations with susceptibility variants located in the *TOX* gene,[13,14] and another reported several associations, all consistent with the effect of these single-nucleotide polymorphisms (SNPs) on breast cancer, including rs4666451 (chromosome 2), rs10941679 (chromosome 5), rs13281615 (8q), rs10995190 (MRPS30/HCNI) and rs2046210 (ESRI).[16] Only one study failed to observe any relationship between MD and breast cancer susceptibility variants.[15] On the other hand, a recent meta-analysis of five GWAS reported a new loci, rs10995190 in *ZNF365*, associated with both percentage mammographic density and breast cancer.[17]

In general, the above-mentioned studies had limited statistical power and showed marginally significant associations. Some focused on subgroups of women (pre-menopausal women, users of post-menopausal hormones or steroid receptor-positive cases). Accordingly, we decided to investigate the possible association between breast cancer susceptibility variants identified through previous GWAS[1–4] and mammographic density in the multicentre "Determinants of Density in Mammographies in Spain" (DDM-Spain) study, a cross-sectional study including 3574 pre- and post-menopausal women recruited at seven specific screening centres within the Spanish breast cancer screening program network.[18–20] We also assessed whether the association between mammographic density and these genetic variants was similar in pre- and post-menopausal women.

2. Materials and methods

2.1. Subjects and mammograms

The DDM-Spain study is a cross-sectional study, which aimed to identify genetic, reproductive and lifestyle characteristics associated with mammographic patterns/densities that might enhance the risk of developing breast cancer. Briefly, women aged 45 years and over who attended the regional Breast Cancer Screening Programmes at the recruiting centres established in Barcelona, Burgos, Corunna (*Coruña*), Palma de Mallorca, Pamplona, Valencia and Zaragoza from September 2006 to June 2007 were invited to participate in the study. Exclusion criteria included evidence of previous breast or ovarian cancer, inability to answer the questionnaire, physical impairment to perform the mammogram and previous breast implants.

The study was reviewed and approved by the Bioethics Committee of the Carlos III Institute of Health (*Instituto de Salud Carlos III*) (Madrid) and all subjects gave their written consent. The intended sample size was 500 women per centre, implying a total of 3500 women. The final sample consisted of 3574 women (range 497–536 per centre). Of the total sample, 3348 Caucasian women were genotyped and included in this study.

Menopausal status was self-reported and defined as absence of menstruation in the preceding 12 months. Inconsistencies between the three questionnaire items inquiring about menopausal status (number of periods in the preceding 12 months, year and cause of