A functioningally significant SNP in TP53 and breast cancer risk in African-American women

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A coding region polymorphism exists in the TP53 gene (Pro47Ser; rs1800371) in individuals of African descent, which reduces p53 tumor suppressor function in a mouse model. It has been unclear whether this functionally significant polymorphism alters cancer risk in humans. This analysis included 6907 women with breast cancer and 7644 controls from the AMBER, ROOT, and AABC consortia. We used multivariable logistic regression to estimate associations between the TP53 Pro47Ser allele and overall breast cancer risk. Because polymorphisms in TP53 tend to be associated with cancer risk in pre-menopausal women, we also limited our analyses to this population in the AMBER and ROOT consortia, where menopausal status was known, and conducted a fixed effects meta-analysis. In an analysis of all women in the AMBER, ROOT, and AABC consortia, we found no evidence for association of the Pro47Ser variant with breast cancer risk. However, when we restricted our analysis to only pre-menopausal women from the AMBER and ROOT consortia, there was a per allele odds ratio of 1.72 (95% confidence interval 1.08–2.76; p-value = 0.023). Although the Pro47Ser variant was not associated with overall breast cancer risk, it may increase risk among pre-menopausal women of African ancestry. Following up on more studies in human populations may better elucidate the role of this variant in breast cancer etiology.

INTRODUCTION

The p53 tumor suppressor (TP53) holds distinction as the most frequently mutated gene in human cancer. In addition to inactivating mutations in human cancer, there are a number of single-nucleotide polymorphisms (SNPs) in TP53, and in genes whose protein products regulate p53, MDM2, and MDM4; these SNPs modify TP53 (hereafter p53) pathway signaling, and can confer increased risk for cancer. Of note, the proline 72 allele in TP53, and the G allele of SNP309 in MDM2, have previously been associated with increased breast cancer risk in pre-menopausal women. More than two decades ago, an inherited polymorphism in TP53 that converts the proline residue at amino acid 47 to a serine was reported in African Americans (Pro47Ser, rs1800371, G/A). We showed that the serine 47 (hereafter S47) variant has significantly impaired ability to induce programmed cell death in multiple human cell lines engineered to contain inducible human forms of WT p53 and S47 (ref. 7). More recently, we created a mouse model for this variant, and showed that it confers significantly increased spontaneous cancer risk. The Pro47Ser polymorphism appears to be restricted to African-descent populations at a minor allele frequency (MAF) of 1.6%, and is
As noted above, previous studies of SNPs in p53 and MDM2 are extremely rare in populations of non-Finnish Europeans (3/126766), and non-existent in populations of Finnish Europeans (0/26082) or East Asian descent (0/18924), according to the recently released GnomAD data (http://gnomad.broadinstitute.org/variant/17-7579548-G-A). Because of the functional significance of this variant in cell lines and mouse models, we sought to test the hypothesis that the S47 variant might be associated with breast cancer risk in African American women, particularly among pre-menopausal women. To do this, we observed a per-allele OR of 1.72 (95% CI 1.08–2.76), respectively. Because the SNP was imputed from genotype data in AMBER, we repeated this analysis limited to the >98% of pre-menopausal samples for which the maximum posterior genotype probability was >90%. The comparable OR was 2.07 (95% CI 1.18–3.65; p = 0.01).

In a fixed-effects meta-analysis of data from the ROOT and AMBER consortia where data of menopausal status were available, we observed a per-allele OR of 1.72, 95% CI, 1.08–2.76, p = 0.023 as shown in Table 1 and in a forest plot in Fig. 1.

**RESULTS**

In the AMBER consortium, imputed data on the Pro47Ser polymorphism (rs1800371) were available for 3310 cases and 3698 controls. As shown in Table 1, the Pro47Ser SNP is rare, with a MAF of 1.4% in controls. The MAF of rs1800371 in all participating studies of the three consortia are shown in Table 1, ranging from 0.9 to 1.8%. There was no association between rs1800371 and risk of overall breast cancer (odds ratio (OR) 1.17, 95% confidence interval (CI) 0.84–1.62, p = 0.34) (Table 1). Similarly, there were no significant associations observed in ROOT or in African American Breast Cancer Consortium (AABC).

As noted above, previous studies of SNPs in p53 and MDM2 observed associations with breast cancer only among premenopausal women. Thus, we performed analysis among that group only. In both AMBER and ROOT, there were suggestions of increased risk of breast cancer with per allele ORs of 1.79 (95% CI, 1.07–2.99) and 1.39 (95% CI, 0.51–3.78), respectively. Because the SNP was imputed from genotype data in AMBER, we repeated this analysis limited to the >98% of pre-menopausal samples for which the maximum posterior genotype probability was >90%. The comparable OR was 2.07 (95% CI 1.18–3.65; p = 0.01).

In a fixed-effects meta-analysis of data from the ROOT and AMBER consortia where data of menopausal status were available, we observed a per-allele OR of 1.72, 95% CI, 1.08–2.76, p = 0.023 as shown in Table 1 and in a forest plot in Fig. 1.

**DISCUSSION**

We previously showed that, in human cell lines and a mouse model, the S47 variant exhibits a modest decrease in apoptosis in response to most genotoxic stresses compared with wild-type p53, but exhibits a significant defect in cell death induced by cisplatin. The variant shows impaired ability to transactivate a subset of p53 target genes, including two involved in metabolism: Gls2 (glutaminase 2) and Sco2. We showed that mice expressing S47 in homozygous or heterozygous form are susceptible to spontaneous cancers of diverse histological types. Because of the strong functional significance of this variant, and it’s specificity to populations of African ancestry, it could play a role in the earlier
driven by AMBER studies, which has a higher MAF than ROOT studies (1.5% vs. 1.1%). If admixture with European ancestry dilutes associations with this African-specific variant, which appeared to be more apparent in ROOT than in AMBER, that could explain the lack of significance in ROOT. It may thus need much larger studies to be able to observe a strong association of this rare SNP in African American populations, especially for testing heterogeneity by tumor estrogen receptor (ER) status. It is also possible that there truly is no effect of the S47 variant on human breast cancer. Even though the tumor suppressive function of the Pro47Ser protein is compromised in mice, the effect may be tissue specific, and not relevant in the mammary gland; similar findings have been found for other p53 variants. Nonetheless, studies such as these, taking findings from cell lines and mouse models to human populations, are needed to understand the basis of cancer etiology.

**METHODS**

Based upon our laboratory findings, we collaborated with investigators from the AMBER Consortium, which includes data and samples from the Black Women’s Health Study (BWHS), the Carolina Breast Cancer Study, and the Women’s Circle of Health Study (WCHS). All studies were approved by the affiliated institutional review boards. BWHS is a prospective cohort study with participants across the US enrolled by mailed questionnaires and followed with biennial and 5-year interval follow-up questionnaires. CBCS and WCHS are both case–control studies, with CBCS 1 and 2 conducted with population-based sampling and in-person interviews from 1993–2001 in 24 counties in North Carolina. WCHS, initiated in 2002 in metropolitan New York City and several counties in eastern New Jersey, is still ongoing in NJ. For these analyses, we included samples from women with invasive cancer or ductal carcinoma in situ (n = 3130), confirmed by pathology reports or registry records from which we also obtained data on ER status, and 3698 controls. Genotyping was performed at the Center for Inherited Disease Research as part of a larger project, using the Illumina HumanExome Beadchip Plus v1.1 plus 200,000 custom beadtypes. Imputation based on 1000 Genomes data was carried out at the University of Washington. The observed expected variance ratio, a measure of squared correlation between the imputed genotypes and the true genotypes for the imputed SNP was 0.91. We examined associations between the imputed SNP rs1800371 in TP53 and breast cancer overall, ER positive cancer, and ER-negative cancer.

Collaborating with three large consortia of breast cancer in women of African ancestry, our findings suggest that the S47 variant may contribute to increased risk of pre-menopausal breast cancer in individuals of African descent. However, because of the relatively low prevalence of the variant, despite the fact that the studies are the largest available for breast cancer in women of African descent, associations were of borderline significance in AMBER and non-significant in ROOT. The combined meta-analysis, including 1942 pre-menopausal cases and 2388 controls, was also of borderline significance.

Although the suggestions of increased risk could be interpreted as null because of the borderline significance of the associations, the high frequency of sporadic cancer in the S47 mouse strain strengthens our hypothesis that this rare variant may increase risk among women of African ancestry, but the sample sizes do not allow for clear associations. The observed increased risk among premenopausal women from the meta-analysis might be onset and more aggressive nature of breast cancer among women of African descent.

Fig. 1 Forest plot of meta-analysis of TP53 rs1800371 with breast cancer risk among pre-menopausal women in the AMBER and ROOT consortia. Study-specific odds ratios (OR) from each of BWHS, WCHS, CBCS, and ROOT is indicated as a shaded square, with 95% confidence interval (CI) shown as a horizontal line. The meta-OR is indicated as a diamond, with the width corresponding to the 95% CI. The analyses were performed for all cases, ER-positive cancer, and ER-negative cancer.
described above. After stratification by menopausal status and ER status, the study-specific estimates for this rare SNP were not stable. Therefore, in the meta-analysis we used risk estimates from ROOT as one study but study-specific risk estimates from BWHS, WHS, and CBS, where adequate sample size and reliable estimates were available from each study. AABC was excluded from these analyses because menopausal status was not known for their participants.

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AUTHOR CONTRIBUTIONS
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COMPETING INTERESTS
The authors have no competing interests.

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