Published in final edited form as:
Cancer Epidemiol Biomarkers Prev. 2013 January ; 22(1): 127–134. doi: 10.1158/1055-9965.EPI-12-0769.

Genetic susceptibility loci for subtypes of breast cancer in an African American population

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Abstract

Background—Most genome-wide association scans (GWAS) have been carried out in European ancestry populations; no risk variants for breast cancer have been identified solely from African ancestry GWAS data. Few GWAS hits have replicated in African ancestry populations.

Methods—In a nested case-control study of breast cancer in the Black Women's Health Study (1,199 cases/1,948 controls), we evaluated index SNPs in 21 loci from GWAS of European or Asian ancestry populations, overall, in subtypes defined by estrogen (ER) and progesterone (PR) receptor status (ER+/PR+, n=336; ER−/PR−, n=229), and in triple-negative breast cancer (TNBC, N=81). To evaluate the contribution of genetic factors to population differences in breast cancer subtype, we also examined global percent African ancestry.

Results—Index SNPs in five loci were replicated, including three associated with ER−/PR− breast cancer (*TERT* rs10069690 in 5p15.33, rs704010 in 10q22.3, and rs8170 in 19p13.11): per allele odds ratios were 1.29 (95% confidence interval (CI) 1.04–1.59), p=0.02, 1.52 (95% CI 1.12–2.08), p=0.01, and 1.30 (95% CI 1.01–1.68), p=0.04, respectively. Stronger associations were observed for TNBC. Furthermore, cases in the highest quintile of percent African ancestry were three times more likely to have TNBC than ER+/PR+ cancer.

Conclusions—These findings provide the first confirmation of the TNBC SNP rs8170 in an African ancestry population, and independent confirmation of the *TERT* ER− SNP. Further, the risk of developing ER− breast cancer, particularly TNBC, increased with increasing proportion of global African ancestry.

Impact—The findings demonstrate the importance of genetic factors in the disproportionately high occurrence of TNBC in African American women.

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Disclosure of Potential Conflict of Interest:
The authors certify that they have no conflicts of interest.

Disclaimer:
The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health. Data on breast cancer pathology were obtained from several state cancer registries (AZ, CA, CO, CT, DE, DC, FL, GA, IL, IN, KY, LA, MD, MA, MI, NJ, NY, NC, OK, PA, SC, TN, TX, VA) and results reported do not necessarily represent their views.

Keywords

breast carcinoma; triple-negative; genetic susceptibility; GWAS replication; African-American; SNPs

Introduction

Genome-wide association scans (GWAS) of European and Asian ancestry populations have identified over 25 loci, including more than 30 single nucleotide polymorphisms (SNPs), associated with breast cancer risk (1–16). Most breast cancer SNPs appear to be more strongly associated with estrogen-receptor (ER) positive breast cancer, the molecular subtype that predominates in European and Asian ancestry populations (2, 4, 17–19). To date, no new loci have been identified solely from GWAS data in African ancestry women, but a combined analysis of data on estrogen-receptor negative (ER−) breast cancer from the African American Breast Cancer (AABC) GWAS and the European ancestry Triple-Negative GWAS identified a variant in the *TERT* gene at 5p15 that was associated with ER − breast cancer at genome-wide significance (5). In addition, a GWAS of European ancestry BRCA1 mutation carriers identified a SNP at 19p13 associated with ER− breast cancer (12). Further work, also in European ancestry populations, indicated that this SNP (rs8170) is specific to triple-negative breast cancer (20). In the U.S., ER− breast cancer is twice as common among women of African ancestry compared with women from other ancestral groups, and in Africa, ER− breast cancer is the leading type of breast cancer (21–23). Triple-negative breast cancer is also markedly more common in African ancestry populations (21, 24). Women with ER− breast cancer, and especially those with triple-negative breast cancer, have poorer outcomes (24, 25). Thus, determining whether genetic loci from European and Asian ancestry GWAS are also important for breast cancer, particularly ER− and triple-negative breast cancer, in African ancestry populations is of great interest.

We carried out replication genotyping of 24 SNPs, representing 21 distinct loci, in the Black Women's Health Study, a large prospective cohort of African American women. We assessed associations with breast cancer overall as well as within specific molecular subtypes of breast cancer. To assess whether genetic factors contribute to the higher incidence of ER− and triple-negative breast cancer in African American women, we also examined the relation of global ancestry (African vs. European) to subtype of breast cancer.

Design and Methods

Study population

At baseline in 1995, approximately 59,000 African American women from all regions of the U.S. enrolled in the Black Women's Health Study (BWHS) by completing mail questionnaires that included comprehensive questions on medical history, use of medications, demographic factors, body size, reproductive history, family history of breast cancer, and behaviors. Participants have been followed by mail questionnaires every two years since then, with complete follow-up on approximately 80% of the baseline cohort in each cycle of follow-up. Participants are asked about new diagnoses of cancer on each questionnaire and pathology reports and/or state cancer registry data are obtained to confirm self-reports of breast cancer and to obtain data on tumor characteristics. Searches of the National Death Index and state cancer registries are carried out each year to learn about cancer in women who have become lost to follow-up or have died before having a chance to report a new diagnosis of cancer.

DNA samples were obtained from BWHS participants by the mouthwash-swish method with all samples stored in freezers at −80°C (26). Saliva samples were provided by approximately 50% of BWHS participants (26,800 women). Women who provided a sample were slightly older than women who did not, but the two groups were similar with regard to geographic region of residence, educational level, body mass index, and family history of breast cancer.

Cases for the present study were all participants who had been diagnosed with breast cancer as of 2010 and had provided a DNA sample. There were 1199 breast cancer cases in total; 180 of the cases were classified as ductal carcinoma in situ without an invasive component. Data on ER and PR status were available for only 52% of cases since many cases had been diagnosed before these assays were routinely carried out. HER2 status, which has been routinely assessed even more recently, was available from medical record data for 34% of cases. The analyses included 336 cases classified as ER+/PR+, 229 classified as ER−/PR−, and 81 classified as triple-negative (ER−, PR−, HER2−). We have previously shown that BWHS breast cancer cases without information on ER/PR status are similar to BWHS cases with known ER/PR status with regard to known and suspected risk factors for breast cancer (27). Controls were selected from among BWHS participants with DNA samples who were free of breast cancer through 2010. Approximately two controls were selected for each case, matched on year of birth (±1 year) and geographic region of residence (Northeast, South, Midwest, and West). The study protocol was approved by the Institutional Review Board of Boston University.

SNP selection

We genotyped 24 SNPs representing 21 different regions. These index SNPs had reached genome-wide significance in one or more GWAS of European or Asian ancestry populations. We assessed as the “coded” allele whichever allele was tested in the original publication; in some cases it was the minor allele and in others it was not. This allows for direct comparison of the direction of the association in our study with the direction observed in the discovery study.

We also selected 30 ancestry informative markers (AIMs) to estimate and control for population stratification due to European admixture. The 30 AIMs were selected from a list of validated SNPs in which the top 30 AIMs had allele frequency differences between Africans and Europeans of at least 0.75 (28). We used a Bayesian approach, as implemented in Admixmap software to estimate individual admixture proportions (29, 30). We have previously shown that estimation of % European vs. African ancestry with 30 AIMs correlates well with the ancestry proportion estimate obtained with a full admixture panel of approximately 1,500 AIMs (31).

Genotyping and quality control

DNA was isolated from the mouthwash samples at the Boston University Molecular Core Genetics Laboratory using the QIAAMP DNA Mini Kit (Qiagen). Whole genome amplification was done with the Qiagen RePLI-g Kits using the method of multiple displacement amplification with input of 50 nanograms of genomic DNA per reaction. Amplified samples underwent purification and PicoGreen quantification at the Broad Institute Biological Samples Platform (Cambridge, MA) before being plated for genotyping. Genotyping was carried out at the Broad Institute Genetic Analysis Platform, using the Sequenom MassArray iPLEX technology. Two percent of samples were blinded duplicates included to assess reproducibility of genotypes. An average reproducibility of 99% was obtained. All SNPs with a call rate of <90% or a deviation from Hardy-Weinberg equilibrium of $P<0.001$ in the control sample were excluded. We also excluded samples with

call rates of <80%. Genotyping was carried out in several different batches. Mean call rate in the final data set was 99% for SNPs and 99% for samples.

Data analysis

Summary statistics for the genotype data were computed with PLINK software version 1.06 (32). We tested for association with breast cancer using the Cochran-Armitage trend test of an additive genetic model. We used PROC LOGISTIC from SAS statistical software version 9.1.3 (SAS Institute, Inc.) to estimate odds ratios (OR) and 95% confidence intervals (95% CI). For loci with more than one index SNP, we carried out haplotype analyses using the conditional haplotype method to determine whether the SNPs were independent signals in our data or were tagging the same causal variant (33, 34). We adjusted the ORs for age, geographic region of residence (Northeast, South, Midwest, West), place of birth (U.S., other country), and percent African versus European ancestry (continuous variable). We carried out analyses for all breast cancers combined and separately for the ER+/PR+ and ER −/PR− subtypes, each group compared with all controls. For SNPs that had been previously identified as ER− or triple-negative variants, we carried out an analysis restricted to triple-negative breast cancer, that is, cases that were negative for ER, PR, and HER2. We also carried out sub-analyses among invasive cases only and among early-onset cases (age of diagnosis <45).

We assessed the relation of global ancestry (African vs. European) to breast cancer subtype by calculating ORs and 95% CIs for quintiles of percent average African ancestry relative to the lowest quintile; in these analyses we compared ER−/PR− and triple-negative subtypes to the ER+/PR+ subtypes. Analyses were adjusted for age, geographic region of residence, and country of birth. We additionally controlled for number of full-term births, age at first full-term birth, age at menarche, menopausal status, use of menopausal hormones, and body mass index.

Results

The analyses included 1,199 breast cancer cases and 1,948 controls. There were 336 breast cancer cases classified as ER+/PR+ and 229 as ER−/PR−. A subset of the ER−/PR− cases could also be classified according to HER2 expression and of them, 81 were classified as triple-negative. As shown in Table 1, cases and controls were similar with respect to year of birth, geographic region, and percent African ancestry; as expected, a greater proportion of cases than controls reported a first degree family history of breast cancer.

Overall breast cancer

Results for SNPs in 21 loci previously associated with breast cancer risk in European or Asian ancestry populations are given in Table 2. Index SNPs at two loci were replicated for overall breast cancer in the BWHS: 10q22 rs704010, OR = 1.24, 95% CI 1.04–1.47, p=0.01 and 11p15 rs3817198, OR=1.21, 95% CI 1.01–1.46, p=0.04. At a third locus, 9q31.2, the OR for the index SNP, rs865686, was 1.11, 95% CI 0.99–1.24, p=0.07, in the opposite direction of the association identified in the European ancestry discovery population (10).

Results were similar when analyses were restricted to invasive cases only (N cases = 1027) and to cases diagnosed before age 45 (N cases = 337).

ER+/PR+ breast cancer

Several SNPs in the *FGFR2* gene (10q26) have been associated with breast cancer in multiple studies, and the observed associations appear to driven by the association with ER+ breast cancer (6, 11, 17, 35). One of the three *FGFR2* index SNPs we tested was associated

with ER+/PR+ breast cancer (rs2981579, OR= 1.28, 95% CI 1.04–1.59), but not with ER−/PR− cancer or all subtypes combined. Haplotypes of the three index SNPs are shown in Table 3. The results indicate that rs2981579 may be most important in our sample, as haplotypes carrying the rs2981579-T allele tended to show increased risk, but all three SNPs may be tagging the same signal even in this African ancestry population. As shown in Table 3, the G allele of rs1219648 and the A allele of rs2981582 appear most often in the presence of the T allele of rs2981579. In fact, the observed frequency of the TGA haplotype (35%) is almost three times its expected frequency (12%) under linkage equilibrium of the three SNPs.

Consistent with our report from the first 886 cases in our study (36), there was a suggestive association of the 5p12 index SNP rs4415084 with ER+/PR+ breast cancer, OR = 1.19, 95% CI 0.98–1.43, p=0.07.

ER−/PR− breast cancer

Two SNPs, rs10069690 in 5p13.33 (*TERT* gene) and rs8170 in 19p13.11, were previously associated with ER− and/or triple-negative breast cancer at a genome-wide level of significance (5, 12). Both were replicated in our analyses of ER−/PR− breast cancer: the OR for rs10069690 was 1.29, 95% CI 1.04–1.59, p=0.02 and the OR for rs8170 was 1.30, 95% CI 1.01–1.68, p=0.04.

The 10q22 index SNP, associated with overall breast cancer risk in our data, was also strongly associated with ER−/PR− breast cancer (OR=1.52, 95% CI 1.12–2.08, p=0.01).

Triple-negative breast cancer

We further assessed the association of the two ER−/PR− SNPs in relation to risk of triple-negative breast cancer. Both SNPs were significantly associated with triple-negative breast cancer, with higher ORs than were observed for ER−/PR− cases. The OR for rs10069690 was 1.42, 95% CI 1.02–1.99, p=0.037 and the OR for rs8170 was 1.48, 95% CI 1.03–2.15, p=0.035.

Because the MAF of rs10069690 was markedly higher in our study population (57%) relative to the CEU population (27%), we estimated the attributable risk ratio (*ARR*) of TNBC for the association with rs10069690. We calculated $ARR = \frac{\sum_i f_A(i) OR^i}{\sum_i f_E(i) OR^i}$ where $f_A(i)$ and $f_E(i)$ are the probabilities of having 0, 1, or 2 copies of the high-risk allele in African ancestry and European ancestry women, respectively. We calculated the *ARR* under two different scenarios, assuming Hardy-Weinberg proportions and an additive effect on the logarithmic scale. First, we assumed the same effect size of the high-risk allele in African ancestry and European ancestry women. We calculated a common OR (1.26) by doing a meta-analysis of our results (OR = 1.42) with previously reported findings (OR = 1.25) (5). In this case, the estimated ARR was 15% (95% CI 9%–25%). Second, we assumed a true difference in effect size between African and European ancestry women based on data from our study and the previous study (5). In a meta-analysis of our results (OR=1.42) and results from the African American women from the previous study (OR=1.33), the OR in an African ancestry population was estimated to be 1.34, whereas the OR in European ancestry women from that study was 1.23. Under these assumptions, the estimated ARR was 26% (95% CI 12%–44%).

We assessed the potential interaction between SNPs rs10069690, located in the telomerase gene (*TERT*), and rs8170, located in the gene encodingBABAM1, which interacts with

BRCA1 in the DNA repair process and down-regulates telomerase activity (37). We estimated ORs for the T-allele of rs10069690 separately among GG homozygous and among carriers of the high-risk A-allele (GA + AA) of rs8170. For ER−/PR− breast cancer, the OR for the T-allele of rs10069690 was higher among carriers of the A-allele of rs8170 (OR = 1.49, 95% CI 1.06–2.09) than among GG homozygous of rs8170 (OR = 1.15, 95% CI 0.88–1.51) (p for interaction = 0.24). A similar pattern was observed for triple-negative breast cancer. Because there were only 81 triple-negative cases, we used a recessive model for the high-risk T-allele of rs10069690 (i.e. TT vs. CC+CT reference group). Among carriers of the high-risk A-allele (GA+AA) of rs8170, the OR for TT-homozygous in rs10069690 was 1.60, 95% CI 0.82–3.11 compared to OR of 1.06, 95% CI 0.56–2.01 among GG-homozygous for rs8170 (p for interaction = 0.38).

Global ancestry in relation to subtype

Mean percent African ancestry was significantly higher in ER−/PR− and triple-negative breast cancer cases (81.6%, p=0.05 and 83.0%, p=0.008, respectively) relative to ER+/PR+ cases (79.9%) (Table 4). Women in the highest quintile of African ancestry had two times the odds of having the ER−/PR− subtype versus ER+/PR+ subtype relative to women in the lowest quintile, OR=2.08 (95% CI 1.16–3.71), and three times the odds of triple-negative breast cancer (OR=3.04, 95% CI 1.41–6.58), controlling for age, geographic region of residence, and country of birth. Results were similar in models that additionally controlled for reproductive and anthropometric risk factors for breast cancer: OR = 1.98 (95% CI 1.09–3.58) for ER−/PR− versus ER+/PR+ and OR = 2.70 (95% CI 1.22–5.96) for triple-negative versus ER+/PR+ (data not shown).

Discussion

In this nested case-control study of approximately 1,200 African American breast cancer cases and 1,900 matched controls from an ongoing prospective cohort study, we found that 5 out of 24 index SNPs identified in GWAS of European ancestry or Asian ancestry populations transferred to African Americans. Importantly, both of the SNPs previously reported to be associated with ER− breast cancer were significantly associated with ER− breast cancer in our sample. Rs10069690 in the *TERT* gene was identified in a meta-analysis of GWAS data from African and European ancestry populations (5); our study is the first confirmation of the association in an independent sample of African Americans. Of note, the risk allele frequency in our study was 0.57, as compared with only 0.27 in the 1000 Genomes European population. Thus, to the extent that this SNP is tagging a functional allele, the observed association likely explains part of the excess incidence of ER− breast cancer in African American women. If the true effect size is about the same for European and African ancestry populations, then this locus may account for a 15% (95% CI 9%–25%) higher incidence of triple-negative breast cancer in African American women. However, it is possible that the true effect size may be higher in African ancestry women: pER−allele ORs were 1.42 in our study and 1.33 among African Americans in the previous study (5), whereas the OR among European ancestry women in that study was 1.23. If there is indeed a difference in effect size, the locus may account for 26% (95% CI 12%–44%) of the excess incidence of triple-negative breast cancer in African American women. The other ER− SNP, rs8170, identified in a European ancestry population and, to date, replicated only in European ancestry populations (12, 20), appears to be specific to triple-negative breast cancer. Our study provides the first evidence that this SNP is also associated with triple-negative breast cancer in an African ancestry population. This is a common variant, with a moderately strong effect. In our data, the risk allele frequency was 0.25 in triple-negative cases and each allele was associated with a 48% increase in risk; thus the causal variant tagged by this allele may have an appreciable effect on risk of triple-negative breast cancer.

There is no evidence, however, that it would explain population differences in incidence of subtypes, since both the risk allele frequency and per allele odds ratios were similar in our study of African American women and the previous study of European ancestry women.

It is not clear how these variants (or more specifically the causal variants they are tagging) may act to affect risk of ER− (and triple negative) breast cancer. SNP rs10069690 in chromosome 5p15 is located in the *TERT* gene, which encodes the reverse transcriptase of the telomerase holoenzyme that controls telomere elongation. De-regulation of telomerase activity has been linked with the transformation process in a variety of cancer types. SNP rs8170 in chromosome 19p13 is located in the *BABAM1* gene (also known as *MERIT40* or *C19orf62*) whose protein product is a component of the BRCA1-A complex that plays a major role in the repair of DNA damage. Recent evidence has shown that BRCA1 is also present at the telomere (38) and is able to down-regulate telomerase activity(39), possibly in part through its presence in the telomere (38). It is noteworthy that most of the BRCA1-associated breast cancers are triple-negative and basal-like (40), and BRCA1-related pathways seem to be de-regulated in basal-like breast cancers (41). These observations suggest a potential synergistic action of the 5p15 and 19p13 SNPs. Mutations in the *BABAM1* gene might result in de-regulation of the BRCA1-A complex activity. A particular effect would be the failure of the BRCA1-A complex to locate at the telomere and down-regulate telomerase activity. Because telomerase activity is regulated by a variety of mechanisms, mutations in the *BABAM1* may not increase risk of breast cancer per se; rather they may make the carriers of such mutations more susceptible to further de-regulation of telomerase activity, for example, through mutations in the *TERT* gene. To test this hypothesis, we assessed possible interaction of SNPs rs10069690 and rs8170 in relation to ER−/PR− and triple-negative breast cancer. Our results provided some evidence of a synergistic effect between the two SNPs, but the interaction terms were not statistically significant (p=0.24 for ER−/PR− breast cancer and p=0.37 for triple-negative breast cancer).

We have published previously on two specific loci (36, 42). These BWHS reports were based on earlier genotyping runs that did not include breast cancer cases from 2009 through 2011. In analyses of 906 cases and 1,111 controls, we did not replicate the index SNP in the chromosome 16q12 locus, but in fine-mapping we identified an association with breast cancer risk for four SNPs located in a small LD block in the *LOC643714* gene loci (42). In similar analyses of the 5p12 locus in 886 BWHS cases and 1,089 controls, the p-value was 0.06 for an association of the index SNP rs4415084 with overall breast cancer risk and 0.03 for ER+ breast cancer (36). The association was weaker in the present larger analysis of 1199 cases and 1949 controls, with a p-value of 0.10 for the association with overall breast cancer and 0.07 for ER+/PR+ breast cancer.

There have been several other previous reports on transferability of European/Asian GWAS index SNPs in African ancestry populations. In a combined analysis of two studies conducted by investigators at Vanderbilt University, two index SNPs were replicated, one in 10q26 and one in chr2q35 (43). In a GWAS of African American cases and controls from a collaboration of nine case-control and cohort studies (AABC), four index SNPs (at 2q35, 9q31, 10q26, 19p13) were replicated (44). The 19p13 SNP replicated in AABC (rs2363956), failed genotyping in our study, based on Hardy-Weinberg equilibrium. The AABC did not report results for rs8170 in 19p13. In GWAS data from African American women in the Women's Health Initiative, SNPs at 5p12 and 16q12.2 were replicated (45). Finally, in data from a collaborative study that included African ancestry cases and controls from Africa, Barbados, and the U.S. (46), the only index SNP replicated was a SNP at 10q26. This study also replicated a SNP in 6q25 that had been identified in multi-ethnic fine-mapping of the region(47), and a SNP in 16q12 that had been identified in previously reported fine-mapping in our study (42).

Among the previous replication studies, only the AABC reported results according to ER and PR status (44). The SNP at 2q35 was associated with ER+ cancer only; SNPs at 9q31 and 10q26 were associated with ER− cancer only; and the SNP at 19p13 was associated with both types. We observed an association with the 10q26 locus only for ER+/PR+ breast cancer and with the 19p13 locus only for ER−/PR− cancer. In addition, whereas the AABC study replicated the index SNP in the 9q31 locus reported by Fletcher et al. (10), an opposite association was observed in our study; our results may be due to chance and, indeed, the p-values for per allele ORs were 0.40 for ER−/PR− cancer and 0.07 for overall breast cancer.

Previous research has demonstrated that African American women have a markedly higher incidence of both ER− breast cancer and triple-negative breast cancer (21, 24). Here we show that global percent African vs. European ancestry in African American women is associated with subtype: relative to women with ER+/PR+ breast cancer, women with ER−/PR− cancer were twice as likely to be in the highest quintile of African ancestry and women with triple negative breast cancer were three times as likely to be in that quintile. Fejerman et al similarly found a significant association of global ancestry with ER−/PR− relative to ER+/PR+ cancer, but did not assess triple-negative cancer (48). These findings suggest that there may be African ancestry specific variants that increase susceptibility to specific subtypes of cancer. Therefore, research that extends beyond replication of variants identified in populations with less genetic diversity is warranted. Such studies are likely to provide novel insight into the pathogenesis of breast cancer especially breast cancer in African ancestry populations.

A strength of our study is that all cases and controls arose from the same population – the 59,000 African American women who enrolled in Black Women's Health Study cohort study in 1995. This is likely to have minimized confounding due to population stratification. In addition, all analyses were adjusted for percent African vs. European ancestry based on a set of previously validated AIMs and were also adjusted for geographic region of residence. Results from immunohistochemistry assays conducted at the time of breast cancer diagnosis were available for 52% of cases, thus permitting separate analyses according to subtypes defined by ER and PR status. A limitation is that HER2 assays have been routinely performed only in recent years and therefore HER2 data were available for 34% percent of cases, limiting power to assess the triple-negative subtype separately.

Another limitation is the overall sample size of 1199 cases and 1948 controls. Although this represents the largest genetic analysis of breast cancer in African American women from a single study, we had only 20% power to detect an OR of 1.10 for a risk allele with a frequency of 0.10. Insufficient power may explain our failure to replicate index SNPs in some of the loci assessed. An important next step is to use the weaker LD in African ancestry populations to fine-map the regions identified by the index SNPs, as we have previously demonstrated for two loci (36, 42).

In summary, there are several important findings from this study. We have demonstrated that a SNP in 19p13.11, previously shown to be associated with triple-negative breast cancer in European ancestry populations, is also associated with triple-negative breast cancer in an African ancestry population. The risk allele of this SNP is common, appearing in 25% of triple negative cases in our study. A second SNP, rs10069690 in the *TERT* gene, was also replicated for triple-negative breast cancer and this SNP has a markedly higher risk allele frequency in African ancestry women than in European ancestry women. Three other GWAS SNPs were replicated, either for all subtypes combined or for ER+/PR+ breast cancer. The five loci replicated in this African American population may hold particular promise for uncovering trans-ethnic functional SNPs that will inform the pathophysiology of breast cancer. In addition, we have shown, in an African American population, that percent

African ancestry is strongly associated with triple-negative breast cancer. This finding provides further evidence that genetic factors play a role in the disproportionately high prevalence of this breast cancer subtype among African American women and highlights the need for further discovery of variants that contribute to this disparity.

Acknowledgments

The authors thank the Black Women's Health Study participants and staff.

Grant Support:

This work was supported by grants R01 CA058420 and R01 CA098663-06A2 from the National Institutes of Health, National Cancer Institute, Division of Cancer Control and Population Sciences, and a grant from the Susan G. Komen for the Cure Foundation.

REFERENCES

1. Thomas G, Jacobs KB, Yeager M, Kraft P, Wacholder S, Orr N, et al. Multiple loci identified in a genome-wide association study of prostate cancer. Nat Genet. 2008; 40:310–315. [PubMed: 18264096]
2. Stacey SN, Manolescu A, Sulem P, Rafnar T, Gudmundsson J, Gudjonsson SA, et al. Common variants on chromosomes 2q35 and 16q12 confer susceptibility to estrogen receptor-positive breast cancer. Nat Genet. 2007; 39:865–869. [PubMed: 17529974]
3. Ahmed S, Thomas G, Ghoussaini M, Healey CS, Humphreys MK, Platte R, et al. Newly discovered breast cancer susceptibility loci on 3p24 and 17q23.2. Nat Genet. 2009; 41:585–590. [PubMed: 19330027]
4. Stacey SN, Manolescu A, Sulem P, Thorlacius S, Gudjonsson SA, Jonsson GF, et al. Common variants on chromosome 5p12 confer susceptibility to estrogen receptor-positive breast cancer. Nat Genet. 2008; 40:703–706. [PubMed: 18438407]
5. Haiman CA, Chen GK, Vachon CM, Canzian F, Dunning A, Millikan RC, et al. A common variant at the TERT-CLPTM1L locus is associated with estrogen receptor-negative breast cancer. Nat Genet. 2011; 43:1210–1214. [PubMed: 22037553]
6. Easton DF, Pooley KA, Dunning AM, Pharoah PD, Thompson D, Ballinger DG, et al. Genome-wide association study identifies novel breast cancer susceptibility loci. Nature. 2007; 447:1087–1093. [PubMed: 17529967]
7. Gold B, Kirchhoff T, Stefanov S, Lautenberger J, Viale A, Garber J, et al. Genome-wide association study provides evidence for a breast cancer risk locus at 6q22.33. Proc Natl Acad Sci U S A. 2008; 105:4340–4345. [PubMed: 18326623]
8. Zheng W, Long J, Gao YT, Li C, Zheng Y, Xiang YB, et al. Genome-wide association study identifies a new breast cancer susceptibility locus at 6q25.1. Nat Genet. 2009; 41:324–328. [PubMed: 19219042]
9. Turnbull C, Ahmed S, Morrison J, Pernet D, Renwick A, Maranian M, et al. Genome-wide association study identifies five new breast cancer susceptibility loci. Nat Genet. 2010; 42:504–507. [PubMed: 20453838]
10. Fletcher O, Johnson N, Orr N, Hosking FJ, Gibson LJ, Walker K, et al. Novel breast cancer susceptibility locus at 9q31.2: results of a genome-wide association study. J Natl Cancer Inst. 2011; 103:425–435. [PubMed: 21263130]
11. Hunter DJ, Kraft P, Jacobs KB, Cox DG, Yeager M, Hankinson SE, et al. A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. Nat Genet. 2007; 39:870–874. [PubMed: 17529973]
12. Antoniou AC, Wang X, Fredericksen ZS, McGuffog L, Tarrell R, Sinilnikova OM, et al. A locus on 19p13 modifies risk of breast cancer in BRCA1 mutation carriers and is associated with hormone receptor-negative breast cancer in the general population. Nat Genet. 2010; 42:885–892. [PubMed: 20852631]

13. Long J, Cai Q, Shu XO, Qu S, Li C, Zheng Y, et al. Identification of a functional genetic variant at 16q12.1 for breast cancer risk: results from the Asia Breast Cancer Consortium. PLoS Genet. 2010; 6:e1001002. [PubMed: 20585626]
14. Cai Q, Long J, Lu W, Qu S, Wen W, Kang D, et al. Genome-wide association study identifies breast cancer risk variant at 10q21.2: results from the Asia Breast Cancer Consortium. Hum Mol Genet. 2011; 20:4991–4999. [PubMed: 21908515]
15. Ghoussaini M, Fletcher O, Michailidou K, Turnbull C, Schmidt MK, Dicks E, et al. Genome-wide association analysis identifies three new breast cancer susceptibility loci. Nat Genet. 2012; 44:312–318. [PubMed: 22267197]
16. Long J, Cai Q, Sung H, Shi J, Zhang B, Choi JY, et al. Genome-wide association study in east Asians identifies novel susceptibility loci for breast cancer. PLoS Genet. 2012; 8:e1002532. [PubMed: 22383897]
17. Garcia-Closas M, Hall P, Nevanlinna H, Pooley K, Morrison J, Richesson DA, et al. Heterogeneity of breast cancer associations with five susceptibility loci by clinical and pathological characteristics. PLoS Genet. 2008; 4:e1000054. [PubMed: 18437204]
18. Reeves GK, Travis RC, Green J, Bull D, Tipper S, Baker K, et al. Incidence of breast cancer and its subtypes in relation to individual and multiple low-penetrance genetic susceptibility loci. Jama. 2010; 304:426–434. [PubMed: 20664043]
19. Broeks A, Schmidt MK, Sherman ME, Couch FJ, Hopper JL, Dite GS, et al. Low penetrance breast cancer susceptibility loci are associated with specific breast tumor subtypes: findings from the Breast Cancer Association Consortium. Hum Mol Genet. 2011; 20:3289–3303. [PubMed: 21596841]
20. Stevens KN, Fredericksen Z, Vachon CM, Wang X, Margolin S, Lindblom A, et al. 19p13.1 is a triple-negative-specific breast cancer susceptibility locus. Cancer Res. 2012; 72:1795–1803. [PubMed: 22331459]
21. Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California cancer Registry. Cancer. 2007; 109:1721–1728. [PubMed: 17387718]
22. Stark A, Kleer CG, Martin I, Awuah B, Nsiah-Asare A, Takyi V, et al. African ancestry and higher prevalence of triple-negative breast cancer: findings from an international study. Cancer. 2010; 116:4926–4932. [PubMed: 20629078]
23. Huo D, Ikpatt F, Khramtsov A, Dangou JM, Nanda R, Dignam J, et al. Population differences in breast cancer: survey in indigenous African women reveals over-representation of triple-negative breast cancer. J Clin Oncol. 2009; 27:4515–4521. [PubMed: 19704069]
24. Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. Jama. 2006; 295:2492–2502. [PubMed: 16757721]
25. Chlebowski RT, Chen Z, Anderson GL, Rohan T, Aragaki A, Lane D, et al. Ethnicity and breast cancer: factors influencing differences in incidence and outcome. J Natl Cancer Inst. 2005; 97:439–448. [PubMed: 15770008]
26. Cozier YC, Palmer JR, Rosenberg L. Comparison of methods for collection of DNA samples by mail in the Black Women's Health Study. Ann Epidemiol. 2004; 14:117–122. [PubMed: 15018884]
27. Rosenberg L, Boggs DA, Wise LA, Adams-Campbell LL, Palmer JR. Oral contraceptive use and estrogen/progesterone receptor-negative breast cancer among African American women. Cancer Epidemiol Biomarkers Prev. 2010; 19:2073–2079. [PubMed: 20647407]
28. Smith MW, Patterson N, Lautenberger JA, Truelove AL, McDonald GJ, Waliszewska A, et al. A high-density admixture map for disease gene discovery in african americans. Am J Hum Genet. 2004; 74:1001–1013. [PubMed: 15088270]
29. McKeigue PM, Carpenter JR, Parra EJ, Shriver MD. Estimation of admixture and detection of linkage in admixed populations by a Bayesian approach: application to African-American populations. Ann Hum Genet. 2000; 64:171–186. [PubMed: 11246470]

30. Hoggart CJ, Parra EJ, Shriver MD, Bonilla C, Kittles RA, Clayton DG, et al. Control of confounding of genetic associations in stratified populations. Am J Hum Genet. 2003; 72:1492–1504. [PubMed: 12817591]
31. Ruiz-Narvaez EA, Rosenberg L, Wise LA, Reich D, Palmer JR. Validation of a small set of ancestral informative markers for control of population admixture in African Americans. Am J Epidemiol. 2011; 173:587–592. [PubMed: 21262910]
32. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81:559–575. [PubMed: 17701901]
33. Valdes AM, Thomson G. Detecting disease-predisposing variants: the haplotype method. Am J Hum Genet. 1997; 60:703–716. [PubMed: 9042931]
34. Valdes AM, McWeeney S, Thomson G. HLA class II DR-DQ amino acids and insulin-dependent diabetes mellitus: application of the haplotype method. Am J Hum Genet. 1997; 60:717–728. [PubMed: 9042932]
35. Nordgard SH, Johansen FE, Alnaes GI, Naume B, Borresen-Dale AL, Kristensen VN. Genes harbouring susceptibility SNPs are differentially expressed in the breast cancer subtypes. Breast Cancer Res. 2007; 9:113. [PubMed: 18036273]
36. Ruiz-Narvaez EA, Rosenberg L, Rotimi CN, Cupples LA, Boggs DA, Adeyemo A, et al. Genetic variants on chromosome 5p12 are associated with risk of breast cancer in African American women: the Black Women's Health Study. Breast Cancer Res Treat. 2010; 123:525–530. [PubMed: 20140701]
37. Kraft P, Haiman CA. GWAS identifies a common breast cancer risk allele among BRCA1 carriers. Nat Genet. 2010; 42:819–820. [PubMed: 20877320]
38. Ballal RD, Saha T, Fan S, Haddad BR, Rosen EM. BRCA1 localization to the telomere and its loss from the telomere in response to DNA damage. J Biol Chem. 2009; 284:36083–36098. [PubMed: 19797051]
39. Xiong J, Fan S, Meng Q, Schramm L, Wang C, Bouzahza B, et al. BRCA1 inhibition of telomerase activity in cultured cells. Mol Cell Biol. 2003; 23:8668–8690. [PubMed: 14612409]
40. Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A. 2003; 100:8418–8423. [PubMed: 12829800]
41. Turner NC, Reis-Filho JS, Russell AM, Springall RJ, Ryder K, Steele D, et al. BRCA1 dysfunction in sporadic basal-like breast cancer. Oncogene. 2007; 26:2126–2132. [PubMed: 17016441]
42. Ruiz-Narvaez EA, Rosenberg L, Cozier YC, Cupples LA, Adams-Campbell LL, Palmer JR. Polymorphisms in the TOX3/LOC643714 locus and risk of breast cancer in African-American women. Cancer Epidemiol Biomarkers Prev. 2010; 19:1320–1327. [PubMed: 20406955]
43. Zheng W, Cai Q, Signorello LB, Long J, Hargreaves MK, Deming SL, et al. Evaluation of 11 breast cancer susceptibility loci in African-American women. Cancer Epidemiol Biomarkers Prev. 2009; 18:2761–2764. [PubMed: 19789366]
44. Chen F, Chen GK, Millikan RC, John EM, Ambrosone CB, Bernstein L, et al. Fine-mapping of breast cancer susceptibility loci characterizes genetic risk in African Americans. Hum Mol Genet. 2011; 20:4491–4503. [PubMed: 21852243]
45. Hutter CM, Young AM, Ochs-Balcom HM, Carty CL, Wang T, Chen CT, et al. Replication of breast cancer GWAS susceptibility loci in the Women's Health Initiative African American SHARe Study. Cancer Epidemiol Biomarkers Prev. 2011; 20:1950–1959. [PubMed: 21795501]
46. Huo D, Zheng Y, Ogundiran TO, Adebamowo C, Nathanson KL, Domchek SM, et al. Evaluation of 19 susceptibility loci of breast cancer in women of African ancestry. Carcinogenesis. 2012; 33:835–840. [PubMed: 22357627]
47. Stacey SN, Sulem P, Zanon C, Gudjonsson SA, Thorleifsson G, Helgason A, et al. Ancestry-shift refinement mapping of the C6orf97-ESR1 breast cancer susceptibility locus. PLoS Genet. 2010; 6:e1001029. [PubMed: 20661439]
48. Fejerman L, Haiman CA, Reich D, Tandon A, Deo RC, John EM, et al. An admixture scan in 1,484 African American women with breast cancer. Cancer Epidemiol Biomarkers Prev. 2009; 18:3110–3117. [PubMed: 19843668]

Table 1

Characteristics of breast cancer cases and controls in the Black Women's Health Study

Characteristic	Cases (n=1199)	Controls (n=1948)
Mean age (years)	47	47
Region of residence (%)		
Northeast	27	25
South	31	31
Midwest	25	25
West	17	18
US born (%)	97.7	97.5
First-degree family history of breast cancer (%)	13.4	8.2
Global percent African ancestry (%)	80.6	80.5

Table 2

Breast cancer index SNPs: original GWAS results and results from the Black Women's Health Study (BWHS)

Chromosome	SNP	GWAS OR	Coded allele/ reference allele	Coded allele frequency	BWHS results: OR (95% CI), p value		
					All cases	ER+/PR+	ER−/PR−
1p11.2	rs11249433[1]	1.16	C/T	0.13	0.97 (0.80–1.18), 0.76	1.15 (0.86–1.56), 0.35	0.76 (0.52–1.13), 0.17
2q35	rs13387042[2]	1.20	A/G	0.73	1.06 (0.93–1.20), 0.38	1.05 (0.85–1.30), 0.65	1.06 (0.83–1.35), 0.63
3p24.1	rs4973768[3]	1.11	T/C	0.37	0.92 (0.82–1.03), 0.16	0.98 (0.81–1.18), 0.82	0.89 (0.71–1.11), 0.31
5p12	rs4415084[4]	1.16	T/C	0.61	1.10 (0.98–1.22), 0.10	1.19 (0.98–1.43), 0.07	0.94 (0.76–1.16), 0.58
5p15.33 (ER−)	rs10069690[5]	1.18	T/C	0.57	1.05 (0.94–1.17), 0.34	1.02 (0.85–1.21), 0.86	**1.29 (1.04–1.59), 0.02**
5q11.2[a]	rs16886165[1]	1.23	G/T	0.29	1.02 (0.89–1.17), 0.79	1.20 (0.96–1.50), 0.10	0.94 (0.73–1.21), 0.63
5q11.2	rs889312[6]	1.13	C/A	0.32	1.03 (0.92–1.16), 0.61	1.08 (0.89–1.30), 0.43	0.94 (0.75–1.18), 0.61
6q22.33	rs2180341[7]	1.41	G/A	0.32	1.03 (0.90–1.18), 0.64	1.15 (0.93–1.42), 0.21	0.91 (0.71–1.17), 0.47
6q25.1	rs2046210[8]	1.29	A/G	0.61	1.10 (0.97–1.24), 0.13	1.16 (0.94–1.43), 0.17	1.12 (0.87–1.42), 0.38
8q24.21	rs13281615[6]	1.08	C/T	0.43	0.94 (0.85–1.05), 0.30	0.93 (0.77–1.11), 0.41	0.91 (0.74–1.13), 0.40
9p21.3	rs1011970[9]	1.09	T/G	0.34	1.01 (0.90–1.14), 0.81	0.96 (0.80–1.16), 0.67	0.90 (0.72–1.12), 0.35
9q31.2	rs865686[10]	0.89	G/T	0.47	1.11 (0.99–1.24), 0.07	1.11 (0.92–1.34), 0.29	1.10 (0.89–1.36), 0.40
10p15.1	rs2380205[9]	0.94	T/C	0.60	1.03 (0.91–1.15), 0.65	1.13 (0.92–1.38), 0.23	1.08 (0.86–1.36), 0.50
10q21.2[a]	rs10995190[9]	0.86	A/G	0.16	0.98 (0.85–1.13), 0.81	0.91 (0.72–1.15), 0.44	1.01 (0.77–1.32), 0.96
10q22.3	rs704010[9]	1.07	A/G	0.10	**1.24 (1.04–1.47), 0.01**	1.27 (0.96–1.66), 0.09	**1.52 (1.12–2.08), 0.01**
10q26.13	rs2981579[1]	1.17	T/C	0.60	1.04 (0.92–1.18), 0.48	**1.28 (1.04–1.59), 0.02**	1.08 (0.85–1.36), 0.56
10q26.13	rs1219648[11]	1.32	G/A	0.42	1.01 (0.91–1.12), 0.82	1.07 (0.90–1.27), 0.44	0.99 (0.80–1.21), 0.89
10q26.13	rs2981582[6]	1.26	A/G	0.49	1.05 (0.92–1.19), 0.46	1.14 (0.90–1.45), 0.28	1.03 (0.80–1.31), 0.84
11p15.5	rs3817198[6]	1.07	C/T	0.11	**1.21 (1.01–1.46), 0.04**	1.22 (0.90–1.65), 0.21	1.18 (0.82–1.70), 0.37
11q13.3	rs614367[9]	1.15	T/C	0.14	1.04 (0.88–1.21), 0.66	0.92 (0.70–1.22), 0.58	0.97 (0.71–1.33), 0.85
14q24.1	rs999737[1]	1.06	C/T	0.95	0.88 (0.68–1.13), 0.32	0.71 (0.48–1.06), 0.10	1.18 (0.68–2.06), 0.56
16q12.1	rs3803662[6]	1.20	T/C	0.51	0.96 (0.83–1.10), 0.54	0.99 (0.78–1.26), 0.94	0.95 (0.74–1.22), 0.69
17q23.2	rs6504950[3]	0.95	A/G	0.35	0.95 (0.85–1.07), 0.42	1.10 (0.92–1.33), 0.29	0.99 (0.79–1.23), 0.90
19p13.11 (ER−)	rs8170[12]	1.26	A/G	0.19	1.01 (0.88–1.16), 0.87	1.00 (0.80–1.25), 0.99	**1.30 (1.01–1.68), 0.04**

*Odds ratios adjusted for age, geographic region of residence, and country of birth

[a] Proxies: GWAS: rs16886165; Proxy: rs16886164 (r2=1.0 in HapMap YRI)
GWAS: rs10995190; Proxy: rs10995189 (r2=1.0 in HapMap YRI)

Table 3

FGFR2 haplotypes in data from the Black Women's Health Study, 1199 breast cancer cases and 1948 controls

Region				Haplotype frequencies				OR (95% CI)		
				All cases	ER+/PR+	ER−/PR−	Controls	All cases	ER+/PR+	ER−/PR−
10q26	Index 1	Index 2	Index 3							
	rs2981579	rs1219648	rs2981582							
	C	A	G	0.36	0.33	0.35	0.37	1.00 (ref)	1.00 (ref)	1.00 (ref)
	C	**G**	G	0.02	0.01	0.02	0.02	0.81 (0.50–1.33)	0.38 (0.11–1.31)	1.09 (0.45–2.66)
	T	A	G	0.08	0.09	0.07	0.08	0.91 (0.72–1.16)	1.17 (0.79–1.74)	0.87 (0.53–1.42)
	T	A	**A**	0.13	0.13	0.12	0.12	1.02 (0.84–1.25)	1.19 (0.84–1.68)	1.07 (0.72–1.58)
	T	**G**	G	0.04	0.05	0.05	0.05	1.02 (0.74–1.40)	1.27 (0.76–2.15)	1.08 (0.59–1.98)
	T	**G**	**A**	0.37	0.39	0.38	0.35	1.05 (0.91–1.20)	1.25 (0.99–1.59)	1.15 (0.88–1.50)

*Odds ratios adjusted for age, geographic region of residence, and country of birth.

Alleles in bold indicate risk allele for that SNP

Pairwise r2 : rs2981579/rs1219648 0.34; rs2981579/rs2981582 0.54; rs1219648/rs2981582 0.37;

Table 4

Association of global percent African ancestry with breast cancer subtypes in the Black Women's Health Study

	ER+/PR+ cases	ER−/PR− cases		Triple negative cases	
Mean % African ancestry	**79.9**	**81.6**	**P = 0.05**	**83.0**	**P = 0.008**
Quintile of % African ancestry (median in ER+/PR+)	N	N	Odds ratio * (95% CI)		Odds ratio * (95% CI)
1 (64.8)	73	37	1.00 (ref)	12	1.00 (ref)
2 (76.6)	61	45	1.47 (0.84–2.60)	9	0.92 (0.37–2.25)
3 (82.3)	78	55	1.41 (0.82–2.43)	19	1.57 (0.73–3.37)
4 (87.5)	73	40	1.09 (0.62–1.91)	16	1.20 (0.53–2.68)
5 (92.4)	51	52	2.08 (1.16–3.71)	25	3.04 (1.41–6.58)

* Odds ratios adjusted for age, geographic region of residence, and country of birth