Genetic variation in *IGFBP2* and *IGFBP5* is associated with breast cancer in populations of African descent

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Abstract

The insulin-like growth factor (IGF) signaling pathway is thought to play a major role in the etiology of breast cancer. Although incidence rates of breast cancer overall are lower in African Americans than in Caucasians, African-American women have a higher incidence under age 40 years, are diagnosed with more advanced disease, and have poorer prognosis. We investigated the association of breast cancer and genetic variants in genes in the IGF signaling pathway in a

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population-based case–control study of African-American women. We found significant associations at a locus encompassing parts of the *IGFBP2* and *IGFBP5* genes on chromosome 2q35, which we then replicated in a case–control study of Nigerian women. Based on those initial findings, we genotyped a total of 34 single nucleotide polymorphisms (SNPs) across the region in both study populations. Statistically significant associations with breast cancer were observed across approximately 50 kb of DNA sequence encompassing three exons in the 3′ end of *IGFBP2* and three exons in the 3′ end of *IGFBP5*. SNPs were associated with breast cancer risk with *P* values as low as *P* = 0.0038 and *P* = 0.01 in African-Americans and Nigerians, respectively. This study is the first to report associations between genetic variants in *IGFBP2* and *IGFBP5* and breast cancer risk.

Introduction

Breast cancer is the leading female cancer in the United States, with one in eight women predicted to be diagnosed during their lifetime. Overall, African-American women have a lower incidence of breast cancer than Caucasians, but they have a higher incidence before age 40 years, are diagnosed with more aggressive disease, and have poorer 5-year survival (Aziz et al. 1999; English et al. 2002; Marbella and Layde 2001; Ries et al. 2004; Swanson and Lin 1994; Trock 1996). The reasons for these racial differences are not known.

Extensive evidence from in vivo and in vitro model systems and human studies [reviewed in Jerome et al. (2003), Pollak et al. (2004), Sachdev and Yee (2001)] supports a major role for the insulin-like growth factor-I (IGF1) signaling pathway in breast cancer pathogenesis. Moreover, for pre-menopausal breast cancer, significant positive associations have been reported with serum levels of IGF1 (Bohlke et al. 1998; Bruning et al. 1995; Hankinson et al. 1998; Toniolo et al. 2000; Yu et al. 2002). Mammographic density, a strong risk factor for breast cancer, has also been positively associated with the ratio of IGF-1 to IGFBP-3 (insulin-like growth factor binding protein 3) in pre-menopausal women, but not in post-menopausal women (Byrne et al. 2000). Interestingly, in several studies, higher serum IGF-I levels have been reported in healthy African-Americans as compared to healthy Caucasians, among both males and females (Jernstrom et al. 2001; Wong et al. 1999; Yanovski et al. 2000). Thus we hypothesize that genetic variation in the IGF signaling pathway may contribute to racial/ethnic disparities in breast cancer incidence and disease progression. We focused on African-Americans, because they are more likely to be diagnosed with aggressive breast cancer, yet are under-represented in research investigating genetic risk factors for breast cancer.

As part of an ongoing study, we assessed genetic variants in the IGF1 signaling pathway, specifically in the *IGF1*, *IGFBP1*, *IGFBP2*, *IGFBP3*, and *IGFBP5* genes, and their association with breast cancer in a case–control study of African-American women. We found significant associations with SNPs in a region that spanned the 3′ ends of *IGFBP2* and *IGFBP5*. We then confirmed the results in a Nigerian case-control study. Based on these findings, we genotyped a total of 34 SNPS, including one candidate functional SNP, in both study populations to further investigate the association with breast cancer. The results of the analysis of the 34 SNPs are reported here.

Materials and methods

Study populations

All study participants provided written informed consent and the study was approved by the Institutional Review Boards of the participating institutions.

African-Americans

The African-American study population includes females aged 35–79 years who participated in a multiethnic population-based case-control study of breast cancer (John et al. 2003; Wang et al. 2005), and females aged <65 years who participated in the Northern California component of the Breast Cancer Family Registry (John et al. 2004). Both studies identified newly diagnosed, invasive breast cancer cases through the population-based Greater San Francisco Bay Area Cancer Registry, and unrelated population controls through random-digit dialing (RDD) that were frequency matched to cases on race/ethnicity and expected 5-year age distribution. For the Breast Cancer Family Registry, given limited funding for this activity, a 1:1 match of cases:controls was not the objective. However, the same proportion of controls and cases participated in the study, so that there was no apparent selection bias in controls. This analysis is based on cases diagnosed between May 1997 and April 1999 and matched controls from the multi-ethnic case–control study and cases diagnosed between January 1995 and September 1998 and matched controls enrolled in the family registry. Both studies administered a brief telephone-screening interview to assess study eligibility, family and personal history of breast cancer, and self-identified race/ ethnicity. Eligible cases and controls were invited to participate in an in-person interview and collection of a blood sample. This analysis is based on 460 African-American cases and 279 African-American controls with available DNA samples.

Nigerians

The Nigerian study population includes women aged 24–90 years newly diagnosed with histologically confirmed, invasive breast cancer, identified at the Surgical Oncology and Radiotherapy Units of the University College Hospital (UCH) in Ibadan, Nigeria (Adebamowo et al. 2003). All consecutive cases diagnosed between March 1998 and December 2003 were eligible, and 94% participated. Unrelated controls aged 18–80 years were identified through population-based recruitment. A community adjoining the hospital was randomly selected from a list of all the communities in the area. This community is stable, socio-economically diverse and represents the diversity of patients seen at the UCH. Samples from this community were examined as part of the HapMap study for internal control, and there was no evidence of relatedness (unpublished data). Controls were randomly selected from the community population register and were eligible if a study nurse found no evidence of breast cancer by clinical breast examination. The primary objective of the collection was breast cancer cases and not 1:1 matching of cases and controls, so that far fewer controls were recruited than cases. Cases and controls completed an in-person interview and provided a blood sample. Ethnicity was based on self-identification and 77% of the participants were Yoruba, 14% Hausa, and 9% Ibo. This analysis is based on 461 cases and 163 controls with available DNA samples.

SNP selection

In total, 34 SNPs were genotyped in the African-American and Nigerian study populations. These included seven SNPs in *IGFBP2* and 10 SNPs in *IGFBP5*, selected from the NIEHS Panel 1 (http://egp.gs.washington.edu/welcome.html), a panel of 90 individuals representing the ethnic diversity in the United States, including Caucasians, Hispanics, Asian Americans, and African-Americans. These 12 SNPs were selected with an r^2 threshold of 0.80 using the program LDSelect (Carlson et al. 2004), and having minor allele frequencies greater than 0.05. In addition, 16 SNPs in the interval between rs9341177 and rs11575195 were subsequently selected and genotyped to increase the density of markers in the region showing the strongest evidence for association. These 16 SNPs included all SNPs with minor allele frequencies (MAF) >0.10 in the Yoruban population in the HapMap database (public release #20), for which we could design assays. An additional putative functional SNP in *IGFBP5* was included.

SNP genotyping

Genotyping was performed by the MGB Taqman probe assay from Applied Biosystems Inc. (ABI) or MGB Eclipse™ probe assay from Nanogen Inc. Primer and probe sequences are available on request. Duplicates of 46 DNA samples and water controls were genotyped for quality control. The laboratory technician was blinded as to whether samples were duplicates, cases, or controls. The order of the DNA samples on 384-well plates was randomized in order to ensure the same study conditions for samples from cases and controls. Genotyping call rates ranged from 95 to 99% and duplicate concordance rates were higher than 99%.

For the MGB Taqman probe assays, reactions were carried out using 10 ng genomic DNA according to manufacturer's protocols. The fluorescence profile was read on an ABI PRISM 7900 HT instrument and the results analyzed with Sequence Detection Software (ABI). For the MGB Eclipse™ probe assays, reactions were carried out using 10 ng genomic DNA according to manufacturer's protocols. Endpoint dissociation melting curves were generated on the ABI PRISM 7900 HT instrument by monitoring fluorescence while heating the reactions from 30 to 80°C at a 10% rate. Genotypes were assigned using the Eclipse-MeltMacro_v2.328 program.

Statistical analysis

Hardy–Weinberg equilibrium (HWE) was assessed in cases and controls separately and the maximum likelihood estimate of the inbreeding coefficient (*f*) was reported if a statistically significant ($P < 0.05$) deviation from HWE was observed. The inbreeding coefficient ranges from −1 to 1, with −1 indicating complete heterozygosity (no homozygotes observed), 0 indicating genotypes in perfect HWE proportions, and 1 indicating complete homozygosity.

Each SNP was individually tested for the association with breast cancer using a logistic regression model with the genotype modeled as an additive variable, with levels 0, 1 and 2 representing the number of copies of the minor allele. This model is equivalent to the Armitage trend test (Armitage 1955) and has been shown to be robust to deviations from Hardy–Weinberg equilibrium and most powerful in the absence of dominance (Sasieni

1997). Deviation from additivity was tested using a second logistic regression model that included an additional parameter for deviation from additivity. If a SNP showed significant evidence for non-additivity, dominance and recessive models were also fit. Odds ratio estimates and 95% confidence intervals (CI) were obtained from the most statistically significant genetic model. This multiple model approach ensured that each SNP was tested with a model that provided maximum power (Chapman and Clayton 2007). All logistic regression models included age at diagnosis (cases) or age at enrollment (controls) as a covariate. Odds ratio estimates and 95% confidence intervals (CI) were obtained from the most statistically significant genetic model. A variable for self-identified ethnicity was included in the analysis of the Nigerian data for four SNPs that showed significant (*P* value <0.05) ethnic differences in allele frequencies. No adjustment for multiple testing was done. Given the correlation between SNPs due to linkage disequilibrium, and the increased prior probability of observing association at a candidate gene, a Bonferroni type of adjustment for multiple testing is not appropriate.

A simulation study was carried out to assess the statistical significance of the association findings. Data sets were generated under the null hypothesis of no association between SNP genotypes and breast cancer status by randomly assigning individuals to either case or control status, while maintaining the individuals' genotypes and the study sample sizes. This simulation approach maintains the LD structure among the SNPs. Results were based on 10,000 simulated datasets.

Results

IGFBP2 and *IGFBP5* are in a tail to tail configuration on chromosome 2q. Thirty-four SNPs were genotyped spanning from intron 1 of *IGFBP2* to intron 1 of *IGFBP5*. The non-synonomous SNP, rs11575194 in the third exon of *IGFBP5*, was not included in the analysis because of the rarity of the minor allele (observed three times in African-Americans and not at all among Nigerians). Thus, 33 SNPs were analyzed for association with breast cancer in the two population samples. Deviations from Hardy–Weinberg equilibrium (*P* value < 0.01) only were observed for three SNPs in African-Americans (rs9341151, rs9341163 and rs2241193). None of the three SNPs were associated with breast cancer. Minor allele frequencies less than 0.05 were observed for one SNP in the African-American sample and for two SNPs in the Nigerian sample. These SNPs were included in the analysis, even though this study had very modest power to detect an association with an allele of such low frequency (Tables 1, 2).

Associations with breast cancer risk in African-American women are shown in Table 1. Eleven of the 34 SNPs were associated at a *P* value less than 0.05, three of which showed *P* values less than 0.01. The strongest association was found for SNP rs9341223 (*P* value = 0.0038). SNPs rs9341218 (*P* value = 0.0067) and rs9288522 (*P* value = 0.015) were in linkage disequilibrium with rs9341223, showing r^2 values of 0.63 and 0.58 between each respective pair. Seven SNPs showed significant non-additive effects, all of which showed the highest statistical significance under the recessive inheritance model. For all SNPs showing evidence for association, with the exception of rs9288521 and rs7426116, odds ratio estimates indicate that the minor allele confers increased risk. The significant

associations observed at SNPs rs9341134, rs9341223, rs11575213, rs6746360 and rs11575161 in *IGFBP2* and *IGFBP5* led to the subsequent replication in the Nigerian study.

Associations with breast cancer risk in the Nigerian case–control study are shown in Table 1. Nine of the 33 SNPs analyzed showed evidence for association with *P* values less than 0.05, including three of the 11 SNPs associated with risk in African-American women. Notably, the SNP most strongly associated with risk in African-Americans, rs9341223, was also significantly associated with risk in Nigerians (*P* value = 0.035). For this SNP and for SNP rs11575213, different genetic models were significant for the two populations (recessive in African-Americans and additive in Nigerians).

In Fig. 1, the locations and associations for the 33 SNPs tested across the *IGFBP2* and *IGFBP5* region in both populations are shown. The $-1 \times \log 10$ (*P* value) for the genetic model (additive, dominant or recessive) providing the strongest evidence for association is shown for each SNP. The locations of *IGFBP2* and *IGFBP5* are shown along the top of the figure, with bars indicating locations of exons. The two genes are oriented tail to tail with their 3′ untranslated regions (UTRs) separated by approximately 7,670 bp. The SNPs showing statistical significance are predominately clustered around the three 3′ exons and UTR of *IGFBP2*, the interval between the two genes, and the three 3′ exons and UTR of *IGFBP5*. The total region investigated spans 48,876 bp between SNPs rs7603372 and rs2241193. In the African-American women, all five of the SNPs showing evidence for association under the recessive model between SNPs rs9288522 and rs6746360 had significant r^2 values between 0.58 and 0.63. The multiple significant associations observed at SNPs under the additive model or outside this region in the African-Americans cannot be explained by linkage disequilibrium. In the Nigerian women, all six SNPs showing evidence for association between SNPs rs9341178 and rs11575213 had significant r^2 values between 0.28 and 0.64. All other SNPs showing evidence for association were not significantly correlated through linkage disequilibrium. Linkage disequilibrium patterns across the region with added marker density (between rs9341177 and rs11575195) were complex in both population samples and clear block patterns were not discernable. Haplotypes within two small regions of high linkage disequilibrium were no more informative than the individual SNPs constituting the haplotypes.

Tables 2 and 3 show the genotype counts for those SNPs showing evidence for association at *P* value < 0.05, in the African-American and Nigerian samples, respectively. HWE testing of SNPs using the combined case and control samples showed one SNP (rs4674107) with evidence for a deviation from equilibrium (*P* = 0.02). An excess of homozygous genotypes among cases and a deficiency of homozygous genotypes among controls could result from the disease association of a SNP that is acting under a recessive model of inheritance. It should be noted that the HWE test is highly sensitive to the number of homozygous genotype counts for rare alleles.

Using simulation, we assessed the probability of observing a *P* value less than that which was observed at each specific SNP. The last columns of Table 2 show the proportion of replicates showing *P* values less than observed at the individual SNP. The high correlation between the *P* values calculated from the real data and from the simulated data indicates that

the individual results are robust. One possible exception is SNP rs9288521 that showed evidence for association under a recessive model but with very few genotypes homozygous for the minor allele.

We next analyzed the replicate datasets in order to assess the significance of our findings in the context of the 33 SNPs that were tested within each population. This analysis provides an estimate of the probability of observing results similar to those reported while taking into account the number of SNPs tested, the LD between the SNPs and the genetic models tested. There were three SNPs showing P values less than 0.01 in the African-American sample, as well as in the Nigerian sample. From the simulated data-sets, we assessed the probability of observing any three SNPs with P values less than 0.01 in each dataset. The simulated African-American data showed 152/10,000 replicates in which any three or more SNPs showed P values less than 0.01, providing a rough estimate of the probability of this observation as 0.015. Similarly, there was 149/10,000 simulated Nigerian datasets in which three or more SNPs showed P values less than 0.01. The African-American and Nigerian datasets both showed three or more SNPs with P values less than 0.01 for the same iteration of the datasets in only 3/10,000 replicates, providing a rough estimate of 0.0003 as the probability of such a replication occurring by chance.

Discussion

We initially found an association of breast cancer risk in African-American women with a locus on chromosome 2q35, encompassing parts of *IGFBP2* and *IGFBP5*, and subsequently replicated this association in Nigerian women. The association was statistically significant in both populations. A total of 34 SNPs were genotyped in this region. Significant associations with individual SNPs at a common locus were observed in the two independent populations of African descent. Given the complex etiology of breast cancer, the genetic diversity within and between the two populations studied, and the relatively small sample size in both studies, the inconsistent nature of the results is not unexpected. It is unlikely that any of the genetic variants tested in this study are causal; the observed associations likely depend on the allele frequencies in the two populations, as well as the linkage disequilibrium patterns across the region. It is also possible that the associations are due to different etiological variants, or sets of variants, in the two populations, and that the effects are dependent on different genetic backgrounds and/or interactions with different environmental factors. Ideally, future association studies of this candidate region should be based on additional SNPs and larger sample sizes, accounting for population substructure, to reduce any random effects of sampling. Functional studies will need to be done to confirm the actual causal variant.

The IGF pathway is a plausible candidate pathway to investigate, because it is a key factor in the development and progression of breast cancer, based on evidence from more than 1,100 published papers, ranging from in vivo and in vitro studies in humans and mice to epidemiologic studies [reviewed in Jerome et al. (2003), Pollak et al. (2004) and Sachdev and Yee (2001)]. There have been a limited number of epidemiologic studies of the association of breast cancer risk and genetic variation in genes in the IGF pathway, focused on *IGF1*, *IGFBP1*, *IGFBP3*, and *IGFALS*. For *IGF1*, two reports showed significant

associations (Canzian et al. 2006; Cleveland et al. 2006), whereas others found no association (Al-Zahrani et al. 2006; Gonzalez-Zuloeta Ladd et al. 2007; Missmer et al. 2002; Setiawan et al. 2006; Wagner et al. 2005). For *IGFBP3*, one study in Chinese women found a significantly elevated breast cancer risk associated with genetic variants in *IGFBP3* (Ren et al. 2004), as did a study in German women (Wagner et al. 2005), whereas other studies found no associations (Al-Zahrani et al. 2006; Canzian et al. 2006; Cheng et al. 2006; Schernhammer et al. 2003). The inconsistent results may be due to many factors such as differences in genetic variants examined in the genes and/or in study design (e.g., restriction to post-menopausal or pre-menopausal breast cancers). No positive associations have been reported for *IGFBP1* (Canzian et al. 2006; Cheng et al. 2006) and *IGFALS* (Canzian et al. 2006). A study investigating breast cancer survival among Chinese women found significant associations with two SNPs in *IGFBP3* and one SNP in *IGF1R*, and no associations with SNPs in *IGF1* or *IGFALS* (Deming et al. 2007). To our knowledge, no studies have been published investigating the association of genetic variation in *IGFBP2* and *IGFBP5* and breast cancer risk.

The insulin-like growth factor binding proteins (IGFBPs) serve as growth modulators, both independently and as regulators of IGFs (Mohan and Baylink 2002; Rosenzweig 2004). The different binding proteins may modulate IGF action differently, and the same binding protein may play a different role under different cellular conditions (Jones and Clemmons 1995). IGFBP-2 is expressed in invasive and in situ breast cancers, but not in normal tissues (Busund et al. 2005). It is involved in apoptosis by IGF signaling through the phosphoinositol-3-kinase (PI3K) pathway (Martin and Baxter 2007), and also independently of IGF (Frommer et al. 2006). IGFBP-2 also interacts with integrin to regulate tumor growth and migration (Pereira et al. 2004; Schutt et al. 2004). IGFBP-5 mRNA and protein are found in breast cancer tissues and in primary breast cancer cell cultures (Beattie et al. 2006), and are involved in apoptosis of the mammary gland, in both in vivo and in vitro mouse studies, through inhibition of IGF-signaling and independently of IGF through interactions with molecules in the extracellular matrix (Flint et al. 2003; Marshman et al. 2003). This same effect has been observed in human breast cancer cells (Butt et al. 2005, 2003). IGFBP-2 and IGFBP-5 are also associated with tamoxifen resistance (Becker et al. 2005; Maxwell and van den Berg 1999). IGFBP-2 and IGFBP-5 thus appear to have multiple roles in breast cancer, and variants in these genes could contribute to risk.

The causative genetic variants that underlay the observed associations across *IGFBP2* and *IGFBP5* must still be identified before it can be determined how these genes alter breast cancer risk and whether one or both genes are involved. Currently, one can only hypothesize that the genetic variant alters the expression of the IGFBP in the cell, the binding of IGF-1 to the IGFBP, the proteolytic action of the proteases, or the localization within the cell. Any alterations in the IGFBP protein could result in changes in amounts of IGF-1 to bind to the IGF receptor to activate the downstream phosphoinositol-3-kinase (PI3K) and the Rasmitogen-activated protein kinase (MAPK) pathways, or alter the IGFBP independent effects associated with apoptosis, cell adhesion, or cell migration.

In conclusion, the finding of a significant association in two independent populations of African descent, the consistent localization in the two populations, and the biological

plausibility of the association between the candidate genes and the phenotype provide strong evidence for association with this locus. Further replication in larger populations of African descent, as well as in non-African populations, are required to increase confidence in the reported association and to localize the etiological component of the association.

Acknowledgments

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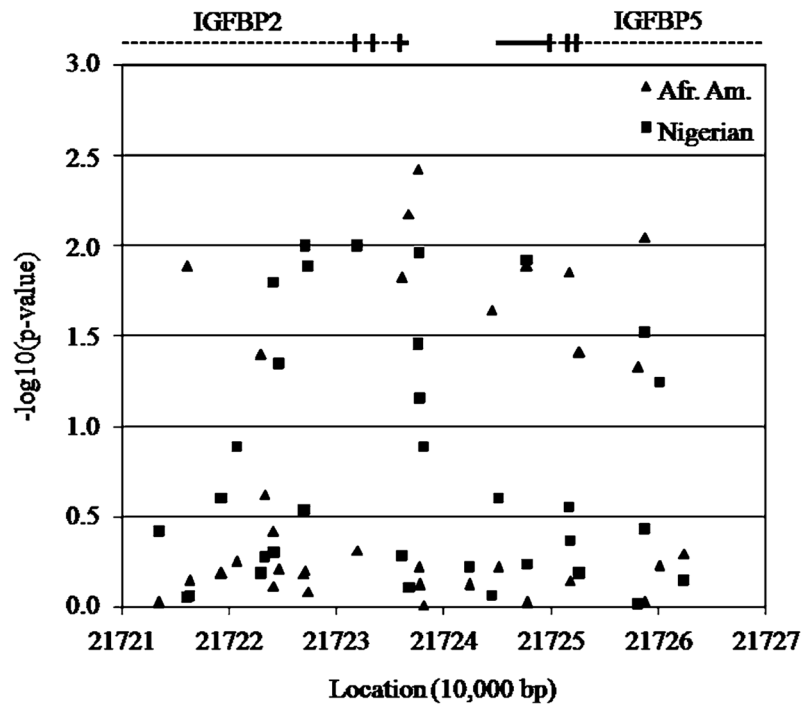
Fig. 1.
Associations of individual SNPs in the *IGFBP2* and *IGFBP5* region and breast cancer risk in African-American and Nigerian women

Table 1

Associations of genetic variants across *IGFBP2* and *IGFBP5* and breast cancer risk in African–American and Nigerian women

SNP	African-Americans							Nigerians						
	MAF[a]		Association tests		Odds ratio	95% CI		MAF+		Association tests		Odds ratio	95% CI	
	Case	Control	Model	P value		Lower	Upper	Case	Control	Model*	P value		Lower	Upper
rs7603372	0.28	0.28	Add	0.94	0.99	0.78	1.25	0.18	0.19	Add	0.38	0.86	0.61	1.21
rs9341134	0.15	0.10	Add	0.013	1.54	1.10	2.15	0.16	0.16	Add	0.88	1.03	0.70	1.51
rs7600691	0.24	0.23	Add	0.71	1.90	0.92	3.95	0.33	0.32	Add	0.87	1.02	0.78	1.34
rs9341145	0.04	0.03	Add	0.65	1.14	0.65	2.00	0.02	0.01	Add	0.25	2.15	0.58	8.00
rs9341151	0.14	0.16	Add	0.56	0.92	0.69	1.22	0.26	0.21	Add	0.13	1.28	0.93	1.75
rs9288521	0.11	0.10	Rec	0.04	0.24	0.06	0.93	0.10	0.10	Add	0.65	1.11	0.71	1.74
rs9341157	0.12	0.11	Add	0.24	1.23	0.87	1.74	0.18	0.15	Add	0.53	1.13	0.78	1.64
rs9341163	0.20	0.21	Add	0.77	0.96	0.75	1.24	0.27	0.31	Rec	0.016	0.47	0.26	0.87
rs9341164	0.27	0.25	Add	0.38	1.12	0.87	1.42	0.33	0.31	Add	0.50	1.11	0.83	1.48
rs9341165	0.08	0.07	Add	0.62	1.10	0.74	1.64	0.11	0.11	Rec	0.045	0.30	0.09	0.97
rs9341177	0.15	0.16	Add	0.66	0.93	0.69	1.26	0.22	0.20	Add	0.29	1.19	0.86	1.65
rs9341178	0.25	0.24	Add	0.63	1.06	0.83	1.36	0.34	0.30	Rec	0.01	2.62	1.26	5.47
rs9341182	0.07	0.08	Add	0.83	0.96	0.64	1.44	0.13	0.08	Dom	0.013	1.95	1.15	3.31
rs7567238	0.15	0.14	Add	0.49	1.11	0.82	1.50	0.20	0.15	Dom	0.01	1.76	1.14	2.70
rs9288522	0.35	0.33	Rec	0.015	1.85	1.13	3.03	0.44	0.43	Add	0.52	1.09	0.83	1.44
rs9341218	0.31	0.26	Rec	0.0067	2.34	1.27	4.33	0.39	0.39	Add	0.78	1.04	0.79	1.37
rs9341223	0.26	0.23	Rec	0.0038	2.98	1.42	6.26	0.31	0.25	Add	0.035	1.38	1.02	1.87
rs9341226	0.12	0.11	Add	0.60	1.09	0.79	1.51	0.17	0.10	Add	0.01	1.76	1.14	2.73
rs9341227	0.05	0.06	Add	0.75	0.93	0.58	1.48	0.06	0.09	Add	0.07	0.62	0.37	1.04
rs9288523	0.07	0.07	Add	0.98	1.00	0.68	1.50	0.09	0.07	Add	0.13	1.48	0.89	2.49
rs2067041	0.42	0.41	Add	0.75	1.04	0.83	1.30	0.48	0.50	Add	0.60	0.93	0.72	1.22
rs4674107	0.05	0.08	Add	0.023	0.62	0.41	0.94	0.01	0.01	Add	0.86	0.81	0.08	8.14
rs3276	0.10	0.11	Add	0.60	0.91	0.65	1.28	0.11	0.08	Add	0.25	1.32	0.83	2.10
rs11575213	0.29	0.28	Rec	0.013	2.24	1.18	4.25	0.36	0.28	Add	0.012	1.44	1.08	1.90
rs11575212	0.14	0.14	Add	0.94	1.01	0.74	1.38	0.16	0.16	Add	0.58	1.11	0.77	1.60
rs6746360	0.28	0.27	Rec	0.014	2.23	1.18	4.23	0.35	0.32	Add	0.28	1.16	0.88	1.53

| SNP | African-Americans | | | | | | | Nigerians | | | | | | |
| | MAF[a] | | Association tests | | Odds ratio | 95% CI | | MAF+ | | Association tests | | Odds ratio | 95% CI | |
	Case	Control	Model	P value		Lower	Upper	Case	Control	Model*	P value		Lower	Upper
rs11575195	0.10	0.10	Add	0.72	1.07	0.75	1.51	0.10	0.10	Add	0.43	1.20	0.76	1.89
rs7585038	0.10	0.07	Add	0.039	1.51	1.02	2.23	0.14	0.13	Add	0.65	1.10	0.74	1.63
rs11575161	0.17	0.17	Rec	0.047	2.71	1.01	7.25	0.07	0.07	Add	0.96	0.99	0.59	1.65
rs7420849	0.27	0.27	Add	0.93	1.01	0.80	1.27	0.21	0.19	Add	0.37	1.17	0.84	1.63
rs7426116	0.20	0.27	Add	0.009	0.72	0.57	0.92	0.18	0.23	Add	0.030	0.70	0.51	0.97
rs741384	0.46	0.44	Add	0.59	1.06	0.86	1.31	0.44	0.38	Add	0.057	1.30	0.99	1.69
rs2241193	0.21	0.22	Add	0.51	0.92	0.72	1.18	0.18	0.19	Add	0.71	0.94	0.66	1.33

[a]Minor allele frequency

Add additive, Dom dominant, Rec recessive

Table 2

SNPs showing evidence for association with breast cancer in African-Americans

SNP	Cases				f	Controls				f	Model	Obs. P value	Simul. P value
	1/1	1/2	2/2	HWE		1/1	1/2	2/2	HWE				
rs9341134	332	118	9	0.69	NS	216	47	4	0.46	NS	Add	0.013	0.011
rs9288521	346	95	3	0.16	NS	226	40	7	0.01	0.18	Rec	0.040	0.008
rs9288522	204	180	70	0.005	0.13	112	129	24	0.12	NS	Rec	0.015	0.013
rs9341218	231	181	51	0.09	NS	144	116	14	0.12	NS	Rec	0.0067	0.0037
rs9341223	256	157	41	0.02	0.11	156	110	9	0.04	−0.12	Rec	0.0038	0.0021
rs4674107	420	37	5	0.004	0.17	232	39	2	0.80	NS	Add	0.023	0.021
rs11575213	243	174	45	0.10	NS	138	128	13	0.01	−0.15	Rec	0.013	0.011
rs6746360	245	174	45	0.09	NS	143	123	13	0.03	−0.13	Rec	0.014	0.011
rs7585038	373	84	6	0.62	NS	238	34	2	0.55	NS	Add	0.039	0.038
rs11575161	324	114	23	0.005	0.26	185	84	5	0.17	NS	Rec	0.047	0.042
rs7426116	297	144	22	0.41	NS	153	98	24	0.16	NS	Add	0.009	0.008

Table 3

SNPs showing evidence for association with breast cancer in Nigerians

| SNP | Cases | | | | f | Controls | | | | f | Model | Obs. P value | Simul. P value |
	1/1	1/2	2/2	HWE		1/1	1/2	2/2	HWE				
rs9341163	271	205	35	0.65	NS	77	51	20	0.02	0.19	Rec	0.016	0.012
rs9341165	405	101	8	0.57	NS	121	21	6	0.003	0.28	Rec	0.045	0.053
rs9341178	231	214	65	0.17	NS	69	67	10	0.23	NS	Rec	0.010	0.009
rs9341182	390	116	6	0.40	NS	128	17	3	0.05	NS	Dom	0.013	0.014
rs7567238	323	170	20	0.68	NS	111	30	6	0.06	NS	Dom	0.010	0.007
rs9341223	248	239	48	0.37	NS	86	60	9	0.73	NS	Add	0.035	0.031
rs9341226	355	142	17	0.55	NS	114	25	2	0.66	NS	Add	0.010	0.009
rs11575213	223	240	71	0.61	NS	84	65	13	0.93	NS	Add	0.012	0.011
rs7426116	351	141	21	0.17	NS	90	48	11	0.22	NS	Add	0.030	0.033