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A Collaborative Study of the Etiology of Breast Cancer Subtypes in African American Women: the AMBER Consortium

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Abstract

Purpose—Breast cancer is a heterogeneous disease, with at least five intrinsic subtypes defined by molecular characteristics. Tumors that express the estrogen receptor (ER+) have better outcomes than ER− tumors, due in part to the success of hormonal therapies that target ER+ tumors. The incidence of ER− breast cancer, and the subset of ER− cancers that are basal-like, is about twice as high among African American (AA) women as among U.S. women of European descent (EA). This disparity appears to explain, in part, the disproportionately high mortality from breast cancer that occurs in AA women. Epidemiologic research on breast cancer in AA women lags behind research in EA women. Here, we review differences in the etiology of breast cancer subtypes among AA women and describe a new consortium of ongoing studies of breast cancer in AA women.

Methods—We combined samples and data from four large epidemiologic studies of breast cancer in AA women, two cohort and two case-control, creating the AMBER consortium. Tumor tissue is obtained and stored in tissue microarrays, with assays of molecular markers carried out at a pathology core. Genotyping, carried out centrally, includes a whole exome SNP array and over 180,000 custom SNPs for fine-mapping of GWAS loci and candidate pathways.

Results—To date, questionnaire data from 5,739 breast cancer cases and 14,273 controls have been harmonized. Genotyping of the first 3,200 cases and 3,700 controls is underway, with a total of 6,000 each expected by the end of the study period.

Conclusions—The new consortium will likely have sufficient statistical power to assess potential risk factors, both genetic and non-genetic in relation to specific subtypes of breast cancer in AA women.

Keywords

breast cancer; molecular subtypes; epidemiology; African American

INTRODUCTION

Breast cancer is a heterogeneous disease.[1, 2] Assessing etiologic factors for all breast cancers combined may result in weak or null findings that obscure important risk relationships for specific disease subgroups. The earliest and most frequently used molecular

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classification of breast cancer is by hormone receptor status; women with tumors that express the estrogen receptor (ER+) have better outcomes than those with ER− tumors, due in part to the success of hormonal therapies that target ER+ tumors.[3–5] Presence or absence of progesterone receptors is also a prognostic factor.[6]

The incidence of ER/PR positive and negative tumors varies by race in the United States.[7–11] Non-Hispanic white women have the highest rates of ER+ tumors, while non-Hispanic black women have the highest rates of ER− tumors. Findings from studies of all breast cancers have been useful for understanding risk of breast cancer in European ancestry (EA) women because about 80–85% of cancers in EA women are ER+. By contrast, only 60–65% of breast cancers in women of African ancestry (AA) are ER+.[11] Breast cancer mortality is higher in AA women than in EA women at every age, and the mortality disparity is not fully explained by factors related to access to optimal and timely screening and care.[12–14] The higher incidence of ER− tumors likely plays a role in these survival disparities. A better understanding of the etiology of ER− breast cancer is essential for reducing breast cancer incidence and mortality in AA women.

While the most common classification of breast tumors for etiologic research has been by ER and PR status, additional markers have been identified as having prognostic value and are increasingly being assayed at the time of diagnosis. Five “intrinsic” subtypes of breast cancer that exhibit different clinical outcomes and express different therapeutic targets have been identified and validated across multiple datasets and study populations.[1, 2, 15–19] A validated panel of immunohistochemical (IHC) markers has been developed to identify the intrinsic subtypes: Luminal A (ER+ and/or PR+ and HER2−), Luminal B (ER+ and/or PR+ and either HER2+ or Ki-67+), HER2+/ER− (ER− PR− HER2+), Basal-like (ER− PR− HER2−, CK5/6+ and/or HER1+) and Unclassified (ER− PR− HER2− CK5/6− HER1−).[20–22] Notably, the intrinsic subtypes distinguish between two subtypes of triple negative breast cancer: the poor prognosis “basal-like” subtype that exhibits high proliferation rates and expression of cytokeratins 5/6, HER1/EGFR and other potential therapeutic targets, and the “Unclassified” group that shows more favorable prognosis and lacks expression of the basal-like markers.[22] As demonstrated in data from the Carolina Breast Cancer Study (CBCS), the intrinsic subtypes exhibit differing clinical profiles that justify consideration as separate disease entities.[20] Reports from CBCS[23] and others[24] suggest that the intrinsic subtypes may also have different etiologic patterns, but the data are not definitive.

We hypothesize that differential distributions of reproductive and other lifestyle factors may play a role in the higher prevalence of ER− tumors in AA women. Rare and common genetic variants that infer susceptibility may also be important, either alone or in concert with exposures. Here we review differences in the etiology of breast cancer subtypes among AA women and describe a new consortium designed to investigate risk factors for distinct breast cancer subtypes among AA women.

FACTORS THAT MAY EXPLAIN DIFFERENCES IN SUBTYPES

Reproductive factors

Several studies[23, 25–28] have investigated risk factors for breast cancer in AA women, but each had limited statistical power for examining risk factors for specific subtypes. The CBCS,[23] the Black Women’s Health Study (BWHS),[29] and a growing number of other studies have provided intriguing data showing that parity is associated with a reduced risk of ER+ breast cancer but increases risk of ER− and basal-like breast cancer.[30–32] Importantly, breastfeeding appears to ameliorate the increased risk or ER− and basal-like breast cancer associated with parity[23, 29] New research on pregnancy-associated breast cancer suggests that immune/inflammation pathways may mediate the relationship between

parity, lactation, and breast cancer.[33] There is also evidence that oral contraceptive use is a stronger risk factor for ER− than ER+ breast cancer.[34, 35]

Reproductive factors differ in prevalence between AA and EA women. AA women have an earlier age at menarche, are more likely to have their babies at a young age, and are less likely to breastfeed their babies.[36] Taken together, these findings raise the provocative hypothesis that the higher incidence of ER−, triple negative, and basal-like breast cancer in AA women may be explained, in part, by childbearing and lactation patterns. Confirmation of these findings in a statistically powerful study of breast cancer subtypes could have important public health implications. The results might provide the impetus for intensified efforts to encourage breastfeeding and facilitate it in the workplace, not only for the health of babies, but also to reduce risk of the more lethal forms of breast cancer.

Obesity and physical activity

An epidemic of obesity has broadly affected the American population, particularly AA women,[37, 38] of whom more than half are obese (body mass index (BMI) ≥30kg/m^2). Abdominal obesity, often measured by waist circumference or waist to hip ratio (WHR),[39] has also increased greatly; more than 70% of AA women ages 20–79 had a waist circumference of 88 cm or greater in 1999–2000,[40] a level of abdominal obesity associated with severe adverse health consequences.[41] While overall obesity is associated with many adverse health outcomes,[42, 43] the health effects of abdominal obesity may be even worse, reflecting metabolic derangements (e.g., dyslipidemia, glucose intolerance) associated with abdominal fat.[39, 42–49] In the majority of studies conducted in EA women, high BMI is associated with reduced risk of premenopausal breast cancer and increased risk of postmenopausal breast cancer.[25, 50–52] Abdominal adiposity is associated with increased risk of both premenopausal and postmenopausal breast cancer.[39, 53] The association of overall obesity with increased risk of postmenopausal breast cancer has been most consistently noted for ER+/PR+ tumors.[54–56] Results on other subtypes are variable. In the CBCS, high BMI was not associated with increased risk of luminal A tumors in postmenopausal women.[23] However, among both pre- and postmenopausal women, higher WHR was strongly associated with increased risk of basal-like tumors, independent of BMI, with a weaker association for luminal A tumors.

Evidence for a BMI association in AA women is sparse, with inconsistent results from early case-control studies.[27, 50, 57–59] The Women's CARE study reported an inverse association of BMI at age 18 with premenopausal ER−/PR− breast cancer and a positive association of recent BMI with postmenopausal ER+/PR+ breast cancer.[60] In the BWHS, high BMI at age 18 was associated with reduced risk of both pre- and postmenopausal breast cancer, and current BMI was inversely associated with premenopausal cancer.[25] There was suggestive evidence of a positive association of high BMI with ER+/PR+ tumors. The CBCS found no association of BMI with postmenopausal breast cancer and positive associations of high WHR with risk of both pre- and postmenopausal breast cancer.[50] CBCS results on intrinsic subtypes (described above) were not presented by race. Recently, the Women's Circle of Health Study (WCHS) observed significant inverse associations of high BMI with ER−/PR− breast cancer among postmenopausal women. Similar to the CBCS, higher waist circumference was associated with increased risk of premenopausal breast cancer in the WCHS after adjustment for BMI.

Obesity may affect breast cancer risk through several different and overlapping pathways involving changes in circulating cytokine and adipokine levels, insulin resistance, circulating insulin levels, sex hormones, and growth factors.[61, 62] Because AA women are more likely to be obese and to have high WHR, one would predict that there would be strong positive associations between obesity and the aggressive breast tumors seen more

commonly in AA women. This does not appear to be the case, however, and there is clearly a need for further epidemiologic and mechanistic research.

Based on the results of numerous epidemiologic investigations of overall breast cancer, the World Cancer Research Fund/American Institute for Cancer Research has judged it probable that exercise reduces risk of postmenopausal breast cancer and considers evidence for premenopausal cancer to be limited.[63] Some studies suggest that an inverse association of exercise with breast cancer risk may be stronger among leaner women;[64–67] if so, this would be particularly relevant to AA women because of their higher prevalence of obesity. Data on exercise and subtypes of breast cancer are limited: a few studies found a stronger inverse association with ER− cancer than with ER+ cancer[66, 68, 69] while others found no difference.[67, 70, 71] To date, there are no published results on subtypes specifically from AA women.

Evolutionary factors - Inflammation and vitamin D

Higher proportion of African ancestry, as estimated through the use of Ancestry-Informative Markers (AIMs), a set of polymorphic markers that vary between populations with different geographic origins, has been shown to be associated with ER− and triple negative breast cancer.[72, 73] The reasons for this association are unclear. It may be that certain genetic variants, more prevalent among Africans, confer susceptibility to ER −, triple negative, basal-like breast cancers, as discussed below. It is also possible that other factors associated with evolution over millennia in Africa could play an etiologic role. For example, higher melanin content and dark skin pigmentation are considered to be the ancestral skin color, with migration to northern hemispheres resulting in lighter skin, perhaps for better absorption of ultraviolet light for the synthesis of vitamin D. In North America, vitamin D deficiency (≤15 ng/ml) is almost 10 times higher in AA than in EA women,[74] with the prevalence of severe vitamin D deficiency among AAs at 29% in 2001–2004.[75] In the WCHS, severe vitamin D deficiency (< 10 ng/ml) was present in 34% of AAs, but only 6% of EAs.[76] Furthermore, higher proportion of African ancestry, measured by AIMS, was associated with lowest levels of serum 25(OH)D.[76] Thus, it is possible that high melanin content allows for adequate sun absorption and vitamin D synthesis in a rural environment in sub-Saharan African but results in low serum 25(OH)D in northern regions and in societies where most time is spent indoors.

In regard to the relationship between levels of vitamin D and breast cancer risk, a recent meta-analysis of data from nine prospective studies concluded that higher levels of 25(OH)D were associated with lower risk of breast cancer risk in postmenopausal, but not premenopausal women.[77] However, as is the case for reproductive risk factors, associations may vary according to breast cancer subtypes. In a study of premenopausal EA women, we found that levels of 25(OH)D were highest among controls, and lowest among women with triple negative breast cancer, particularly in comparison to those with luminal A subtype.[78] Lower levels of 25(OH)D were observed in women with triple negative breast cancer in two other studies,[79, 80] whereas some studies that examined 25(OH)D levels according to ER and PR status without considering HER2 status did not find such associations.[81–83] These results, taken together, suggest that low serum 25(OH)D in AAs could be associated with more aggressive breast cancers.

There may have also been evolutionary selection on the innate and adaptive immune systems for more robust inflammatory responses to withstand endemic infectious disease. A less restrained immune response, while beneficial for resisting and surviving infectious diseases, may play a role in malignant transformation and cancer risk in later life. Indeed, biomarkers of low grade chronic inflammation have been associated with greater risk of cancer, higher tumor grade, and poorer cancer survival,[84–87] and SNPs and levels of

several pro-inflammatory cytokines related to innate immunity have been associated with breast cancer risk,[88] stage, and progression.[89] SNPs associated with increased levels of pro-inflammatory cytokines, such as IL1, IL2, IL6, and IL18, are more common in AA than in EA women.[90] In addition to differences by ancestry in non-specific innate immune system pathways, there is natural selection in sub-Saharan Africa for genotypes by specific families of infectious agents such as protozoa and helminths, with a shift to TH2 immunity for exposure to helminths. Endemic malarial parasites have dominated the infectious landscape in sub-Saharan Africa for millennia, with a profound influence on the selection of genetic variation.[91] Thus, specific genetic profiles that confer resistance in Africans have evolved, such as the β-globin locus and other hemoglobin genes, G6PD, the Duffy antigen gene,[92] and loss of T cell function.[93] Although data are lacking from large prospective epidemiological studies on immune factors and breast cancer risk, in the WCHS, SNPs in both innate and adaptive immune response pathways were differentially distributed by race, with associations noted between several SNPs in these immune pathways and breast cancer risk.[94, 95] In mice, specific malaria-driven genetic changes such as loss of the Duffy antigen receptor result in higher serum chemokine levels and increased inflammatory milieu, predisposing to the development of more aggressive prostate tumors.[96] A similar mechanism could exist for human breast cancer; recently, using serum obtained prior to surgery from women with incident breast cancer, Hong and colleagues showed that levels of IL-5, a Th2 cytokine, were higher in women with ER− than in ER+ tumors and that women with highest ratios of TH1 cytokines to IL-5 were least likely to have ER− or triple negative breast cancer.[97]

Genomics and breast cancer subtypes in AA women

To date, there have been only two genome wide association studies (GWAS) of breast cancer in women of African ancestry, one with approximately 3,000 cases (AABC)[98, 99] and the other with almost 2,000 cases (ROOT).[100] No new loci have been identified as associated with breast cancer risk at a genome-wide significance level in either GWAS or in a meta-analysis of both scans. By comparison, over 70 breast cancer loci have been identified in GWAS data from EA populations.[101, 102] There have been several reports of transferability of European/Asian GWAS index SNPs to African ancestry populations, with replication of only a few of the index SNPs.[72, 98, 103–105]

Only the AABC and BWHS have reported genetic results according to ER and PR status.[72, 98] A meta-analysis of AABC data with data from a GWAS of triple-negative breast cancer in EA women identified a new locus on the *TERT* gene associated with ER− breast cancer.[106] A SNP in the 19p13 region that was associated with ER− and triple-negative breast cancer in EA was replicated in the BWHS for both subtypes.[72] Global percent African vs. European genetic ancestry in AA women was associated with subtype in the BWHS.[72] 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. A similar association of global ancestry with ER−/PR− relative to ER+/PR+ cancer was observed in an admixture scan of AA breast cancer that included cases and controls from CBCS, WCHS, Multiethnic Cohort (MEC) and other studies.[73] These findings suggest that there may be African ancestry specific variants that increase susceptibility to specific subtypes of cancer. However, studies to date have been underpowered to detect even common variants that may be African ancestry specific.

THE AMBER CONSORTIUM

Because of the critical gaps in knowledge discussed above, it is essential that more research be directed toward understanding the causes of ER− and basal-like breast cancer in AA

women. It is clear that such research will be effective only if studies with appreciable numbers of AA women combine their data for increased statistical power. To this end, the authors initiated collaborations among four of the largest ongoing studies of breast cancer in AA women: two case-control studies (CBCS and WCHS) and two prospective cohort studies (BWHS and MEC). The collaboration, African American Breast Cancer Epidemiology and Risk (AMBER), is designed to pool existing data, continue accrual of new cases with periodic additions to the pooled data set, and carry out subtyping assays of tumor tissue samples, genotyping assays of DNA samples, and statistical analyses of questionnaire data within dedicated cores so that the same methods are applied across studies. We expect that by study end, AMBER will include more than 6,000 AA women with breast cancer and more than 6,000 AA controls for evaluation of breast cancer risk factors by subtype. The contributing studies are described briefly below.

The Carolina Breast Cancer Study (CBCS) is a North Carolina population-based case control study of breast cancer, conducted in three phases.[107, 108] The current study phase, phase 3 (years 2008–2014), includes women resident in 44 counties. CBCS phases 1 and 2 were conducted in 24 counties. Breast cancer cases are identified using Rapid Case Ascertainment in cooperation with the NC Central Cancer Registry. Controls were identified for phases 1 and 2 only (1993–1996 and 1996–2001), using Division of Motor Vehicles lists for women under age 65 and Health Care Financing Administration lists for women 65 and older. Randomized recruitment was used to oversample AA women and women under age 50. The age range of study participants is 20 to 74. Procedures for recruiting and enrolling study participants were approved by the Institutional Review Board of the UNC School of Medicine and informed consent was obtained for each participant. Cases of invasive breast cancer were enrolled in all three phases and cases of *in-situ* breast cancer were enrolled during Phase 2 only; all cases of *in-situ* breast cancer were eligible, with no over-sampling according to age or race. Controls enrolled in phases 1 and 2 were frequency matched to cases based upon age (+/− 5 years) and race. Rapid Case Ascertainment has resulted in an average time interval of six months between date of diagnosis and date of interview. In-depth interviews are conducted by study nurses in participants' homes to obtain information on family history and other risk factors for breast cancer, including reproductive history, physical activity, breast cancer screening and access to health care and other lifestyle factors. The interviewers measure height, weight, and waist circumference, and obtain blood or mouth rinse samples. During Phases 1 and 2, 787 AA invasive cases and 718 AA controls and 107 AA *in situ* cases and 70 AA controls were enrolled. Overall response rates (product of contact and cooperation rates) were 74% for AA cases and 54% for AA controls. To date, the response rate for Phase 3 for AA cases is 70.5%.

Blood samples were collected into ACD-anticoagulated tubes. During phases 1 and 2, 86% of AA cases and 86% of AA controls provided blood samples that yielded sufficient DNA and plasma for laboratory analyses. There were no statistically significant differences between persons with and without DNA according to age, menopausal status, family history of breast cancer, smoking, hormone use, age at menarche, parity or other breast cancer risk factors. Stage at diagnosis did not differ significantly between cases who gave DNA and those who did not. In phase 3, blood samples have been obtained from 58% of cases; 39% of cases have provided mouth rinse or saliva samples. Thus, viable DNA samples have been obtained from 97% of enrolled participants in phase 3.

The Women's Circle of Health Study (WCHS) is a multi-site case–control study in New York City (NYC) and New Jersey (NJ) aimed at evaluating risk factors for early and aggressive breast cancer in women of AA and EA ancestry.[109, 110] Cases are women with primary, histologically confirmed invasive breast cancer or ductal carcinoma in situ,

ages 20–75 years. Controls are frequency matched on age and race. Both cases and controls must have no previous history of cancer other than non-melanoma skin cancer.

Recruitment in NYC took place between January 2002 and December 2008 and involved hospital-based ascertainment of cases, while controls were identified through random digit dialling (RDD), frequency matching to telephone prefixes of cases. The sampling frame was designed so that cells categorized by age were filled in similar proportions to those of the cases. Recruitment at the NJ site started in March 2006 and is ongoing. Phase I of the study ended in April 2012 and covered seven counties in NJ (Bergen, Essex, Hudson, Mercer, Middlesex, Passaic, and Union). WCHS2 includes two additional counties for a total of nine counties. Cases in NJ were identified from 2006 to 2012 by the NJ State Cancer Registry using rapid case ascertainment. Controls were initially recruited though RDD (2006 to 2010) and later through community-based efforts (2009–2012). In-person interviews ascertained data on established and suspected risk factors for breast cancer, including family history, reproductive and menstrual history, hormone use, alcohol intake and smoking, occupational history, physical activity, and dietary intake. Women were also asked to report their weight and height one year before diagnosis (for cases) or reference date (for controls), and at several times during their life. Anthropometric measurements were taken at the end of the visit using a standardized protocol and measuring instruments. Body composition (lean and fat mass, percent body fat) was measured by bioelectrical impedance analysis using a Tanita® TBF-300A scale. Initially blood sample were obtained from participants, but in 2007, to reduce costs and increase participation, sample collection was restricted to saliva samples obtained Oragene Kits (DNA Genotek, Inc, Ottawa, ON, Canada).

Among eligible AA women, 75% in NY and 54% in NJ completed an interview and provided a biologic specimen. As of this writing, 979 AA cases and 958 AA controls have been enrolled in WCHS phases 1 and 2. We estimate that we will enroll at least 250 additional AA cases each year for the next four years. The study was approved by the Institutional Review Boards at the University of Medicine and Dentistry of New Jersey, Mount Sinai School of Medicine, and Roswell Park Cancer Institute and all participants provide written informed consent before participating in the study.

Black Women's Health Study (BWHS)

The BWHS is an ongoing prospective follow-up study of health and illness among U.S. black women, with a focus on cancer.[111] The study began in 1995 when 59,000 AA women 21–69 years of age from across the United States completed a 14-page postal health questionnaire. The median age at entry was 38, and participants were residents of 17 states in mainland U.S.: Northeast, 28%; South, 30%; Midwest, 23%; West, 19%. The baseline questionnaire elicited information on a wide range of variables, including demographic factors, use of medical care, family history of breast cancer, reproductive and medical history, current and past cigarette and alcohol use, current weight and weight at age 18, height, waist and hip circumference, use of vitamin supplements and medications, diet, and participation in exercise. Biennial follow-up questionnaires ascertain new cases of breast cancer and other illnesses and update covariate information. Deaths are identified through linkage to the National Death Index and by reports from family and friends of participants. Follow-up is complete for 80% of the baseline cohort after seven follow-up cycles. Medical record and cancer registry data are sought for all participants who report a diagnosis of breast cancer. Data are abstracted on year of diagnosis, histology, grade, tumor size, lymph node involvement, metastases, estrogen receptor status, progesterone receptor status, and HER-2 neu expression. To date, either medical records or cancer registry data have been obtained for 1,144 women who reported incident breast cancers; 99% were confirmed as breast cancer. Eighty-three percent had invasive cancers and 17% carcinoma *in situ.*

From 1999–2007, all BWHS participants with known addresses were invited to provide saliva sample by the mouthwash-swish method.[112] Samples were provided by 26,814 women, for a response rate of 50%. Respondents were somewhat older than non-respondents, but were similar with regard to geographic region, educational level, BMI, age at menarche, parity, oral contraceptive use, menopausal hormone use, and family history of breast cancer. These comparisons indicate that participants with stored samples are highly representative of all BWHS participants.

The Multiethnic Cohort (MEC) is a prospective cohort study that was designed to provide prospective data on cancer and other chronic diseases.[113] To maximize the diversity of exposures, the MEC targeted a range of ethnic groups spanning all socioeconomic levels. The MEC includes men and women aged 45–75 at recruitment, primarily from five different racial-ethnic groups, including 16,594 AA women. The cohort was assembled in 1993–1996 by mailing a self-administered, 26-page questionnaire to persons identified primarily through the driver's license files for the state of Hawaii and the county of Los Angeles in California, supplemented with other sources. The baseline questionnaire obtained information on demographics, medical and reproductive histories, medication use, family history of various cancers, physical activity and an extensive quantitative food frequency questionnaire. Follow-up questionnaires were sent in 1999, 2003, and 2010.

Identification of incident breast cancer in study participants is by regular linkage with the Los Angeles County Cancer Surveillance Program and the State of California Cancer Registry, both of which are NCI-funded Surveillance, Epidemiology, and End Results (SEER) registries. Deaths in the cohort are identified by linkage to the state death-certificate files in CA and with the National Death Index for deaths occurring in other states. Out-migration in the MEC is low. Based on the extensive tracking of a random sample of the cohort, the out-migration rate was 3.7% after 7 years of follow-up.

Biospecimen collection in the MEC began in 1996 with the collection of blood and urine specimens from incident cases of breast, prostate and colorectal cancer, together with a cross-section of the cohort (N~6,000), for nested case-control studies of genetic susceptibility and cancer. In the current cycle of the MEC, the effort was expanded and included the prospective collection of biospecimens (fasting blood, urine, and in a small subset, buccal cells) from all eligible MEC participants in Hawaii and Los Angeles, respectively.

METHODS

The AMBER consortium is an NCI-funded Program Project consisting of four cores and four scientific projects. Core A coordinates administration. Core B includes data collection in the case-control studies and collection of tumor blocks in CBCS, WCHS, and BWHS. Core C, the Biospecimen Core, has both a genomics and a pathology component. The genomics component is responsible for obtaining DNA samples from all studies, preparing the samples for genotyping, overseeing genotyping of all samples in a single laboratory, and participating in statistical analyses of the genotype data. In the first year of AMBER, aliquots of DNA from approximately 3200 breast cancer cases and 3700 controls from among BWHS, CBCS, and WCHS participants were assembled at Core C, quantified by pico green and shipped to the Center for Inherited Disease Research (CIDR) laboratory for genotyping, which began in June 2013. The pathology component of Core C is responsible for all laboratory activities pertaining to subtyping of breast cancers. TMAs are constructed at two locations, with CBCS TMAs created at a UNC lab and WCHS and BWHS TMAs constructed at a Roswell Park Cancer Institute lab. A number of validation studies were conducted to test the validity and reliability of the immunohistochemistry assays for each

molecular marker and the "calling" of IHC results. Based on those results, a protocol was developed such that staining of all samples will be carried out in a single lab at UNC and scoring of results will be done using the Aperio imaging system. MEC tumor tissue will be collected and evaluated under separate funding and protocols, with data on subtypes contributed to AMBER for pooled classifications and analyses.

The Biostatistics and Data Management core, Core D, has responsibility for establishing and maintaining a database that includes individual-level pathology, genomic, serum biomarker, and questionnaire data from all four studies. It is also responsible for preparing and distributing analytic data files and providing biostatistical support for all analyses. Working closely with the other Cores and scientific investigators, Core D conducts quality control and cleaning of both genotyping data and questionnaire data.

Variables to be used as main exposures or important covariates were harmonized by Core D in a two-step process. First, based on a review of all questionnaires, a list of desired variables was created, with specification of whether a given variable should be provided as continuous or categorical, and if categorical, specification of the categories. The intent was to allow investigators from each individual study to carry out cleaning and recoding of their own data insofar as possible. After receipt of the data, Core D investigators conducted quality checks and multiple harmonizing recoding in an iterative process with study investigators to derive common variables that retained as detailed information as possible.

Statistical analyses of questionnaire data

The cohort studies, BWHS and MEC, provided nested case-control data, with up to four controls randomly selected from among women without breast cancer, matched on year of birth and on having completed the same questionnaire as the last questionnaire completed by the case before her diagnosis of breast cancer. The case year of diagnosis was considered to be the index date for all controls in the same risk set. For time-varying exposure measures, exposure values were taken from the most recent questionnaire completed before the index date. The nested case-control data from BWHS and MEC were then pooled with case-control data from CBCS and WCHS to create an AMBER database for all future analyses.

Associations between exposure variables of interest and specific breast cancer subtypes will be assessed in the pooled data by calculating odds ratios and 95% confidence intervals using polytomous logistic regression adjusted for study site, five-year age group, calendar year of interview , geographic region, years of education, and breast cancer risk factors. In addition, a random-effects model will be used to estimate combined odds ratios and between-study heterogeneity with be assessed with *Q* test statistics.

Case-case analyses will also be carried out. In initial analyses, ER− cases will be compared with ER+ cases with respect to exposure. After subtyping by the Pathology Core is complete, each of the other subtypes will be compared with the most common subtype, luminal A. Case-case analyses will provide additional statistical power because it will be possible to include cases from CBCS3, for which no controls are available. The validity of the case-case analyses will be demonstrated by comparing case-control results with case-case results for the same sets of cases.

Current Status

As shown in Table 1, for the first round of analyses of questionnaire data in the AMBER collaboration, there are 2,867 ER+ breast cancer cases, 1,480 ER− breast cancer cases, and, from among the ER− cases, 619 triple-negative cases. A total of 530 breast cancer cases diagnosed before age 40 are included.

DNA samples from approximately 3,200 breast cancer cases and 3,700 controls from CBCS, WCHS, and BWHS are being genotyped in the first round of genotyping; by the time genotyping is complete and the data are cleaned, pathology data sufficient to classify the cases by ER and PR status will be available for most of those cases.

PROJECTS AND GOALS

AMBER will integrate data from all levels (molecular to behavioral) to understand breast cancer risk, with the goal of improvement in health. The first AMBER scientific projects will focus on the topics addressed in the introduction: associations between breast cancer subtypes and reproductive risk factors, body size, physical activity, vitamin D and immune/inflammatory pathways, and genomics. Each of the projects will provide important information regarding risk factors for breast cancer subgroups. However, the greatest impact will result from interactions among the projects. The study design for the AMBER consortium is motivated in large part by the desire to integrate analyses of genome-wide genetic variation and modifiable epidemiologic risk factors to study gene-environment interactions.[114] AMBER will forge a link between GWAS results and population-based epidemiology by integrating findings from the genomics project into the projects that address modifiable risk factors.

AMBER is the most powerful study, to date, of breast cancer in AA women. With information on pathologic tumor characteristics in a large set of cases, there will be, for the first time, adequate statistical power to classify this heterogeneous disease into more homogeneous subgroups. Using harmonized epidemiological data and biospecimens, it should be possible to identify modifiable and genetic risk factors for molecular subtypes, including the most lethal. Our hope is that AMBER will be able to provide definitive answers to the preponderance of aggressive breast cancer in AA women, paving the way for prevention initiatives.

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References

1. Parker JS, Mullins M, Cheang MC, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol. 2009; 27(8):1160–7. [PubMed: 19204204]
2. Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. Nature. 2000; 406(6797):747–52. [PubMed: 10963602]
3. Goss PE, Ingle JN, Martino S, et al. Efficacy of letrozole extended adjuvant therapy according to estrogen receptor and progesterone receptor status of the primary tumor: National Cancer Institute of Canada Clinical Trials Group MA. 17. J Clin Oncol. 2007; 25(15):2006–11. [PubMed: 17452676]
4. Berry DA, Cirrincione C, Henderson IC, et al. Estrogen-receptor status and outcomes of modern chemotherapy for patients with node-positive breast cancer. Jama. 2006; 295(14):1658–67. [PubMed: 16609087]

5. Burstein HJ, Prestrud AA, Seidenfeld J, et al. American Society of Clinical Oncology clinical practice guideline: update on adjuvant endocrine therapy for women with hormone receptor-positive breast cancer. J Clin Oncol. 2010; 28(23):3784–96. [PubMed: 20625130]
6. Bardou VJ, Arpino G, Elledge RM, et al. Progesterone receptor status significantly improves outcome prediction over estrogen receptor status alone for adjuvant endocrine therapy in two large breast cancer databases. J Clin Oncol. 2003; 21(10):1973–9. [PubMed: 12743151]
7. Jemal A, Ward E, Thun MJ. Recent trends in breast cancer incidence rates by age and tumor characteristics among U.S. women. Breast Cancer Res. 2007; 9(3):R28. [PubMed: 17477859]
8. Anderson WF, Chatterjee N, Ershler WB, et al. Estrogen receptor breast cancer phenotypes in the Surveillance, Epidemiology, and End Results database. Breast Cancer Res Treat. 2002; 76(1):27–36. [PubMed: 12408373]
9. Chu KC, Anderson WF. Rates for breast cancer characteristics by estrogen and progesterone receptor status in the major racial/ethnic groups. Breast Cancer Res Treat. 2002; 74(3):199–211. [PubMed: 12206512]
10. Furberg H, Millikan R, Dressler L, et al. Tumor characteristics in African American and white women. Breast Cancer Res Treat. 2001; 68(1):33–43. [PubMed: 11678307]
11. Gapstur SM, Dupuis J, Gann P, et al. Hormone receptor status of breast tumors in black, Hispanic, and non-Hispanic white women. An analysis of 13,239 cases. Cancer. 1996; 77(8):1465–71. [PubMed: 8608530]
12. Joslyn SA. Racial differences in treatment and survival from early-stage breast carcinoma. Cancer. 2002; 95(8):1759–66. [PubMed: 12365025]
13. Amend K, Hicks D, Ambrosone CB. Breast cancer in African-American women: differences in tumor biology from European-American women. Cancer Res. 2006; 66(17):8327–30. [PubMed: 16951137]
14. Chlebowski RT, Chen Z, Anderson GL, et al. Ethnicity and breast cancer: factors influencing differences in incidence and outcome. J Natl Cancer Inst. 2005; 97(6):439–48. [PubMed: 15770008]
15. Yang XR, Sherman ME, Rimm DL, et al. Differences in risk factors for breast cancer molecular subtypes in a population-based study. Cancer Epidemiol Biomarkers Prev. 2007; 16(3):439–43. [PubMed: 17372238]
16. Kim MJ, Ro JY, Ahn SH, et al. Clinicopathologic significance of the basal-like subtype of breast cancer: a comparison with hormone receptor and Her2/neu-overexpressing phenotypes. Hum Pathol. 2006; 37(9):1217–26. [PubMed: 16938528]
17. Kurebayashi J, Moriya T, Ishida T, et al. The prevalence of intrinsic subtypes and prognosis in breast cancer patients of different races. Breast. 2007; 16 (Suppl 2):S72–7. [PubMed: 17714947]
18. Lin CH, Liau JY, Lu YS, et al. Molecular subtypes of breast cancer emerging in young women in Taiwan: evidence for more than just westernization as a reason for the disease in Asia. Cancer Epidemiol Biomarkers Prev. 2009; 18(6):1807–14. [PubMed: 19505913]
19. Bauer KR, Brown M, Cress RD, et al. 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(9):1721–8. [PubMed: 17387718]
20. Carey LA, Perou CM, Livasy CA, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. Jama. 2006; 295(21):2492–502. [PubMed: 16757721]
21. Cheang MC, Chia SK, Voduc D, et al. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Natl Cancer Inst. 2009; 101(10):736–50. [PubMed: 19436038]
22. Cheang MC, Voduc D, Bajdik C, et al. Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. Clin Cancer Res. 2008; 14(5):1368–76. [PubMed: 18316557]
23. Millikan RC, Newman B, Tse CK, et al. Epidemiology of basal-like breast cancer. Breast Cancer Res Treat. 2008; 109(1):123–39. [PubMed: 17578664]
24. Yang XR, Chang-Claude J, Goode EL, et al. Associations of breast cancer risk factors with tumor subtypes: a pooled analysis from the Breast Cancer Association Consortium studies. J Natl Cancer Inst. 2011; 103(3):250–63. [PubMed: 21191117]

25. Palmer JR, Adams-Campbell LL, Boggs DA, et al. A prospective study of body size and breast cancer in black women. Cancer Epidemiol Biomarkers Prev. 2007; 16(9):1795–802. [PubMed: 17855697]
26. Trivers KF, Lund MJ, Porter PL, et al. The epidemiology of triple-negative breast cancer, including race. Cancer Causes Control. 2009; 20(7):1071–82. [PubMed: 19343511]
27. Schatzkin A, Palmer JR, Rosenberg L, et al. Risk factors for breast cancer in black women. J Natl Cancer Inst. 1987; 78(2):213–7. [PubMed: 3468283]
28. Ogundiran TO, Huo D, Adenipekun A, et al. Case-control study of body size and breast cancer risk in Nigerian women. Am J Epidemiol. 2010; 172(6):682–90. [PubMed: 20716701]
29. Palmer JR, Boggs DA, Wise LA, et al. Parity and lactation in relation to estrogen receptor negative breast cancer in African American women. Cancer Epidemiol Biomarkers Prev. 2011; 20(9): 1883–91. [PubMed: 21846820]
30. Phipps AI, Chlebowski RT, Prentice R, et al. Reproductive history and oral contraceptive use in relation to risk of triple-negative breast cancer. J Natl Cancer Inst. 2011; 103(6):470–7. [PubMed: 21346227]
31. Kwan ML, Kushi LH, Weltzien E, et al. Epidemiology of breast cancer subtypes in two prospective cohort studies of breast cancer survivors. Breast Cancer Res. 2009; 11(3):R31. [PubMed: 19463150]
32. Shinde SS, Forman MR, Kuerer HM, et al. Higher parity and shorter breastfeeding duration: association with triple-negative phenotype of breast cancer. Cancer. 2010; 116(21):4933–43. [PubMed: 20665494]
33. Schedin P. Pregnancy-associated breast cancer and metastasis. Nat Rev Cancer. 2006; 6(4):281–91. [PubMed: 16557280]
34. Ma H, Bernstein L, Ross RK, et al. Hormone-related risk factors for breast cancer in women under age 50 years by estrogen and progesterone receptor status: results from a case-control and a case-case comparison. Breast Cancer Res. 2006; 8(4):R39. [PubMed: 16846528]
35. Rosenberg L, Boggs DA, Wise LA, et al. Oral contraceptive use and estrogen/progesterone receptor-negative breast cancer among African American women. Cancer Epidemiol Biomarkers Prev. 2010; 19(8):2073–9. [PubMed: 20647407]
36. Bernstein L, Teal CR, Joslyn S, et al. Ethnicity-related variation in breast cancer risk factors. Cancer. 2003; 97(1 Suppl):222–9. [PubMed: 12491485]
37. Kim SY, Dietz PM, England L, et al. Trends in pre-pregnancy obesity in nine states, 1993–2003. Obesity (Silver Spring). 2007; 15(4):986–93. [PubMed: 17426334]
38. Ogden CL, Carroll MD, Curtin LR, et al. Prevalence of overweight and obesity in the United States, 1999–2004. Jama. 2006; 295(13):1549–55. [PubMed: 16595758]
39. Harvie M, Hooper L, Howell AH. Central obesity and breast cancer risk: a systematic review. Obes Rev. 2003; 4(3):157–73. [PubMed: 12916817]
40. Okosun IS, Chandra KM, Boev A, et al. Abdominal adiposity in U.S. adults: prevalence and trends, 1960–2000. Prev Med. 2004; 39(1):197–206. [PubMed: 15208003]
41. National Institutes of Health/National Heart Lung and Blood Clinical Guidelines on identification, evaluation, and treatment of overweight and obesity in adults. The evidence report. Obes Res. 1998; 6:51S–209S. [PubMed: 9813653]
42. National Task Force on the Prevention and Treatment of Obesity. Overweight obesity, and health risk. Arch Intern Med. 2000; 160(7):898–904. [PubMed: 10761953]
43. Flegal KM, Graubard BI, Williamson DF, et al. Excess deaths associated with underweight, overweight, and obesity. Jama. 2005; 293(15):1861–7. [PubMed: 15840860]
44. Ballard-Barbash R. Anthropometry and breast cancer. Body size--a moving target Cancer. 1994; 74(3 Suppl):1090–100.
45. Despres JP. Abdominal obesity as important component of insulin-resistance syndrome. Nutrition. 1993; 9(5):452–9. [PubMed: 8286886]
46. Kirschner MA, Samojlik E, Drejka M, et al. Androgen-estrogen metabolism in women with upper body versus lower body obesity. J Clin Endocrinol Metab. 1990; 70(2):473–9. [PubMed: 2298859]

47. Kissebah AH, Krakower GR. Regional adiposity and morbidity. Physiol Rev. 1994; 74(4):761–811. [PubMed: 7938225]
48. Okosun IS, Boltri JM, Anochie LK, et al. Racial/ethnic differences in prehypertension in American adults: population and relative attributable risks of abdominal obesity. J Hum Hypertens. 2004; 18(12):849–55. [PubMed: 15361887]
49. Stevens J. Obesity, fat patterning and cardiovascular risk. Adv Exp Med Biol. 1995; 369:21–7. [PubMed: 7598009]
50. Hall IJ, Newman B, Millikan RC, et al. Body size and breast cancer risk in black women and white women: the Carolina Breast Cancer Study. Am J Epidemiol. 2000; 151(8):754–64. [PubMed: 10965972]
51. Lahmann PH, Hoffmann K, Allen N, et al. Body size and breast cancer risk: findings from the European Prospective Investigation into Cancer And Nutrition (EPIC). Int J Cancer. 2004; 111(5): 762–71. [PubMed: 15252848]
52. van den Brandt PA, Spiegelman D, Yaun SS, et al. Pooled analysis of prospective cohort studies on height, weight, and breast cancer risk. Am J Epidemiol. 2000; 152(6):514–27. [PubMed: 10997541]
53. Connolly BS, Barnett C, Vogt KN, et al. A meta-analysis of published literature on waist-to-hip ratio and risk of breast cancer. Nutr Cancer. 2002; 44(2):127–38. [PubMed: 12734058]
54. Althuis MD, Fergenbaum JH, Garcia-Closas M, et al. Etiology of hormone receptor-defined breast cancer: a systematic review of the literature. Cancer Epidemiol Biomarkers Prev. 2004; 13(10): 1558–68. [PubMed: 15466970]
55. Colditz GA, Rosner BA, Chen WY, et al. Risk factors for breast cancer according to estrogen and progesterone receptor status. J Natl Cancer Inst. 2004; 96(3):218–28. [PubMed: 14759989]
56. Setiawan VW, Monroe KR, Wilkens LR, et al. Breast cancer risk factors defined by estrogen and progesterone receptor status: the multiethnic cohort study. Am J Epidemiol. 2009; 169(10):1251–9. [PubMed: 19318616]
57. Adams-Campbell LL, Kim KS, Dunston G, et al. The relationship of body mass index to reproductive factors in pre- and postmenopausal African-American women with and without breast cancer. Obes Res. 1996; 4(5):451–6. [PubMed: 8885209]
58. Austin H, Cole P, Wynder E. Breast cancer in black American women. Int J Cancer. 1979; 24(5): 541–4. [PubMed: 575111]
59. Zhu K, Caulfield J, Hunter S, et al. Body mass index and breast cancer risk in African American women. Ann Epidemiol. 2005; 15(2):123–8. [PubMed: 15652717]
60. Berstad P, Coates RJ, Bernstein L, et al. A case-control study of body mass index and breast cancer risk in white and African-American women. Cancer Epidemiol Biomarkers Prev. 2010; 19(6): 1532–44. [PubMed: 20501755]
61. Sachdev D, Yee D. The IGF system and breast cancer. Endocr Relat Cancer. 2001; 8(3):197–209. [PubMed: 11566611]
62. Hankinson SE, Schernhammer ES. Insulin-like growth factor and breast cancer risk: evidence from observational studies. Breast Dis. 2003; 17:27–40. [PubMed: 15687675]
63. Wiseman M. The second World Cancer Research Fund/American Institute for Cancer Research expert report. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. Proc Nutr Soc. 2008; 67(3):253–6. [PubMed: 18452640]
64. Friedenreich CM, Cust AE. Physical activity and breast cancer risk: impact of timing, type and dose of activity and population subgroup effects. Br J Sports Med. 2008; 42(8):636–47. [PubMed: 18487249]
65. McTiernan A, Kooperberg C, White E, et al. Recreational physical activity and the risk of breast cancer in postmenopausal women: the Women's Health Initiative Cohort Study. Jama. 2003; 290(10):1331–6. [PubMed: 12966124]
66. Dallal CM, Sullivan-Halley J, Ross RK, et al. Long-term recreational physical activity and risk of invasive and in situ breast cancer: the California teachers study. Arch Intern Med. 2007; 167(4): 408–15. [PubMed: 17325304]

67. Bernstein L, Patel AV, Ursin G, et al. Lifetime recreational exercise activity and breast cancer risk among black women and white women. J Natl Cancer Inst. 2005; 97(22):1671–9. [PubMed: 16288120]
68. Peters TM, Schatzkin A, Gierach GL, et al. Physical activity and postmenopausal breast cancer risk in the NIH-AARP diet and health study. Cancer Epidemiol Biomarkers Prev. 2009; 18(1):289–96. [PubMed: 19124511]
69. Adams SA, Matthews CE, Hebert JR, et al. Association of physical activity with hormone receptor status: the Shanghai Breast Cancer Study. Cancer Epidemiol Biomarkers Prev. 2006; 15(6):1170–8. [PubMed: 16775177]
70. Bardia A, Hartmann LC, Vachon CM, et al. Recreational physical activity and risk of postmenopausal breast cancer based on hormone receptor status. Arch Intern Med. 2006; 166(22): 2478–83. [PubMed: 17159013]
71. Enger SM, Ross RK, Paganini-Hill A, et al. Body size, physical activity, and breast cancer hormone receptor status: results from two case-control studies. Cancer Epidemiol Biomarkers Prev. 2000; 9(7):681–7. [PubMed: 10919738]
72. Palmer JR, Ruiz-Narvaez EA, Rotimi CN, et al. Genetic susceptibility loci for subtypes of breast cancer in an African American population. Cancer Epidemiol Biomarkers Prev. 2013; 22(1):127–34. [PubMed: 23136140]
73. Fejerman L, Haiman CA, Reich D, et al. An admixture scan in 1,484 African American women with breast cancer. Cancer Epidemiol Biomarkers Prev. 2009; 18(11):3110–7. [PubMed: 19843668]
74. Nesby-O'Dell S, Scanlon KS, Cogswell ME, et al. Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988–1994. Am J Clin Nutr. 2002; 76(1):187–92. [PubMed: 12081833]
75. Ginde AA, Liu MC, Camargo CA Jr. Demographic differences and trends of vitamin D insufficiency in the US population, 1988–2004. Arch Intern Med. 2009; 169(6):626–32. [PubMed: 19307527]
76. Yao S, Zirpoli G, Bovbjerg DH, et al. Variants in the vitamin D pathway, serum levels of vitamin D, and estrogen receptor negative breast cancer among African-American women: a case-control study. Breast Cancer Res. 2012; 14(2):R58. [PubMed: 22480149]
77. Bauer SR, Hankinson SE, Bertone-Johnson ER, et al. Plasma vitamin D levels, menopause, and risk of breast cancer: dose-response meta-analysis of prospective studies. Medicine (Baltimore). 2013; 92(3):123–31. [PubMed: 23625163]
78. Yao S, Sucheston LE, Millen AE, et al. Pretreatment serum concentrations of 25-hydroxyvitamin D and breast cancer prognostic characteristics: a case-control and a case-series study. PLoS One. 2011; 6(2):e17251. [PubMed: 21386992]
79. Peppone LJ, Rickles AS, Janelsins MC, et al. The association between breast cancer prognostic indicators and serum 25-OH vitamin D levels. Ann Surg Oncol. 2012; 19(8):2590–9. [PubMed: 22446898]
80. Rainville C, Khan Y, Tisman G. Triple negative breast cancer patients presenting with low serum vitamin D levels: a case series. Cases J. 2009; 2:8390. [PubMed: 19830074]
81. Kawase T, Matsuo K, Suzuki T, et al. Association between vitamin D and calcium intake and breast cancer risk according to menopausal status and receptor status in Japan. Cancer Sci. 2010; 101(5):1234–40. [PubMed: 20151981]
82. Eliassen AH, Spiegelman D, Hollis BW, et al. Plasma 25-hydroxyvitamin D and risk of breast cancer in the Nurses' Health Study II. Breast Cancer Res. 2011; 13(3):R50. [PubMed: 21569367]
83. Blackmore KM, Lesosky M, Barnett H, et al. Vitamin D from dietary intake and sunlight exposure and the risk of hormone-receptor-defined breast cancer. Am J Epidemiol. 2008; 168(8):915–24. [PubMed: 18756015]
84. Heikkila K, Ebrahim S, Lawlor DA. A systematic review of the association between circulating concentrations of C reactive protein and cancer. J Epidemiol Community Health. 2007; 61(9):824–33. [PubMed: 17699539]

85. Gong Z, Quan L, Yao S, et al. Innate immunity pathways and breast cancer risk in African American and European-American women in the Women's Circle of Health Study (WCHS). PLoS One. 2013 (In Press).
86. Quan L, Gong Z, Yao S, et al. Cytokine and cytokine receptor genes of adaptive immune response are differentially associated with breast cancer risk in women of African ancestry and European ancestry. Int J Cancer. 2013 (In Press).
87. Pierce BL, Ballard-Barbash R, Bernstein L, et al. Elevated biomarkers of inflammation are associated with reduced survival among breast cancer patients. J Clin Oncol. 2009; 27(21):3437–44. [PubMed: 19470939]
88. Lyon DE, McCain NL, Walter J, et al. Cytokine comparisons between women with breast cancer and women with a negative breast biopsy. Nurs Res. 2008; 57(1):51–8. [PubMed: 18091292]
89. Rao VS, Dyer CE, Jameel JK, et al. Potential prognostic and therapeutic roles for cytokines in breast cancer (Review). Oncol Rep. 2006; 15(1):179–85. [PubMed: 16328053]
90. Ness RB, Haggerty CL, Harger G, et al. Differential distribution of allelic variants in cytokine genes among African Americans and White Americans. Am J Epidemiol. 2004; 160(11):1033–8. [PubMed: 15561982]
91. Zabaleta J, Schneider BG, Ryckman K, et al. Ethnic differences in cytokine gene polymorphisms: potential implications for cancer development. Cancer Immunol Immunother. 2008; 57(1):107–14. [PubMed: 17618436]
92. Kwiatkowski DP. How malaria has affected the human genome and what human genetics can teach us about malaria. Am J Hum Genet. 2005; 77(2):171–92. [PubMed: 16001361]
93. Campbell MC, Tishkoff SA. African genetic diversity: implications for human demographic history, modern human origins, and complex disease mapping. Annu Rev Genomics Hum Genet. 2008; 9:403–33. [PubMed: 18593304]
94. Quan L, Gong Z, Yao S, et al. Cytokine and cytokine receptor genes of adaptive immune response are differentially associated with breast cancer risk in American women of African and European ancestry. Int J Cancer. 2013
95. Gong Z, Quan L, Yao S, et al. Innate immunity pathways and breast cancer Risk in African American and European-American women in the Women's Circle of Health Study (WCHS). PLoS One. 2013; 8(8):e72619. [PubMed: 23991131]
96. Shen H, Schuster R, Stringer KF, et al. The Duffy antigen/receptor for chemokines (DARC) regulates prostate tumor growth. FASEB J. 2006; 20(1):59–64. [PubMed: 16394268]
97. Hong CC, Yao S, McCann SE, et al. Pretreatment levels of circulating Th1 and Th2 cytokines, and their ratios, are associated with ER-negative and triple negative breast cancers. Breast Cancer Res Treat. 2013; 139(2):477–88. [PubMed: 23624818]
98. Chen F, Chen GK, Millikan RC, et al. Fine-mapping of breast cancer susceptibility loci characterizes genetic risk in African Americans. Hum Mol Genet. 2011; 20(22):4491–503. [PubMed: 21852243]
99. Chen F, Chen GK, Stram DO, et al. A genome-wide association study of breast cancer in women of African ancestry. Hum Genet. 2013; 132(1):39–48. [PubMed: 22923054]
100. Huo D, Zheng Y, Ogundiran TO, et al. Evaluation of 19 susceptibility loci of breast cancer in women of African ancestry. Carcinogenesis. 2012; 33(4):835–40. [PubMed: 22357627]
101. Michailidou K, Hall P, Gonzalez-Neira A, et al. Large-scale genotyping identifies 41 new loci associated with breast cancer risk. Nat Genet. 2013; 45(4):353–61. 361e1–2. [PubMed: 23535729]
102. Garcia-Closas M, Couch FJ, Lindstrom S, et al. Genome-wide association studies identify four ER negative-specific breast cancer risk loci. Nat Genet. 2013; 45(4):392–8. 398e1–2. [PubMed: 23535733]
103. Zheng W, Cai Q, Signorello LB, et al. Evaluation of 11 breast cancer susceptibility loci in African-American women. Cancer Epidemiol Biomarkers Prev. 2009; 18(10):2761–4. [PubMed: 19789366]
104. Ruiz-Narvaez EA, Rosenberg L, Rotimi CN, 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(2):525–30. [PubMed: 20140701]

105. Hutter CM, Young AM, Ochs-Balcom HM, 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(9):1950–9. [PubMed: 21795501]

106. Haiman CA, Chen GK, Vachon CM, et al. A common variant at the TERT-CLPTM1L locus is associated with estrogen receptor-negative breast cancer. Nat Genet. 2011; 43(12):1210–4. [PubMed: 22037553]

107. Newman B, Moorman PG, Millikan R, et al. The Carolina Breast Cancer Study: integrating population-based epidemiology and molecular biology. Breast Cancer Res Treat. 1995; 35(1):51–60. [PubMed: 7612904]

108. McGee SA, Durham DD, Tse CK, et al. Determinants of breast cancer treatment delay differ for african american and white women. Cancer Epidemiol Biomarkers Prev. 2013; 22(7):1227–38. [PubMed: 23825306]

109. Ambrosone CB, Ciupak GL, Bandera EV, et al. Conducting Molecular Epidemiological Research in the Age of HIPAA: A Multi-Institutional Case-Control Study of Breast Cancer in African-American and European-American Women. J Oncol. 2009; 2009:871250. [PubMed: 19865486]

110. Bandera EV, Chandran U, Zirpoli G, et al. Rethinking sources of representative controls for the conduct of case-control studies in minority populations. BMC Med Res Methodol. 2013; 13:71. [PubMed: 23721229]

111. Rosenberg L, Rao RS, Palmer JR. A case-control study of acetaminophen use in relation to the risk of first myocardial infarction in men. Pharmacoepidemiol Drug Saf. 2003; 12(6):459–65. [PubMed: 14513659]

112. 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(2):117–22. [PubMed: 15018884]

113. Kolonel LN, Henderson BE, Hankin JH, et al. A multiethnic cohort in Hawaii and Los Angeles: baseline characteristics. Am J Epidemiol. 2000; 151(4):346–57. [PubMed: 10695593]

114. Khoury MJ, Wacholder S. Invited commentary: from genome-wide association studies to gene-environment-wide interaction studies--challenges and opportunities. Am J Epidemiol. 2009; 169(2):227–30. discussion 234–5. [PubMed: 19022826]

Table 1

Cases and controls in the AMBER Consortium, by subtype and study, 2013

	Case-control studies		Cohort studies		Total with questionnaire data available
	CBCS	WCHS	BWHS	MEC	
Breast cancer cases	1,535	1,088	2,063	1,053	5,739
ER+	841	555	848	623	2,867
ER−	587	225	444	224	1,480
ER unknown	107	308	771	206	1,392
ER−, PR−, HER2−	336	130	153	—	619
Age at diagnosis <40	227	125	178	0	530
Age at diagnosis ≥40	1,308	963	1,885	1,053	5,209
Controls	788	975	8,298	4,212	14,273