Association of Genetic Ancestry with Breast Cancer in Ethnically Diverse Women from Chicago

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Abstract

Introduction: Non-Hispanic (nH) Black and Hispanic women are disproportionately affected by early onset disease, later stage, and with more aggressive, higher grade and ER/PR negative breast cancers. The purpose of this analysis was to examine whether genetic ancestry could account for these variation in breast cancer characteristics, once data were stratified by self-reported race/ethnicity and adjusted for potential confounding by social and behavioral factors.

Methods: We used a panel of 100 ancestry informative markers (AIMs) to estimate individual genetic ancestry in 656 women from the “Breast Cancer Care in Chicago” study, a multi-ethnic cohort of breast cancer patients to examine the association between individual genetic ancestry and breast cancer characteristics. In addition we examined the association of individual AIMs and breast cancer to identify genes/regions that may potentially play a role in breast cancer disease disparities.

Results: As expected, nH Black and Hispanic patients were more likely than nH White patients to be diagnosed at later stages, with higher grade, and with ER/PR negative tumors. Higher European genetic ancestry was protective against later stage at diagnosis (OR 0.7 95%CI: 0.54–0.92) among Hispanic patients, and higher grade (OR 0.73, 95%CI: 0.56–0.95) among nH Black patients. After adjustment for multiple social and behavioral risk factors, the association with later stage remained, while the association with grade was not significant. We also found that the AIM SNP rs10954631 on chromosome 7 was associated with later stage (p=0.02) and higher grade (p=0.012) in nH Whites and later stage (p=0.03) in nH Blacks.
Conclusion: Non-European genetic ancestry was associated with later stage at diagnosis in ethnic minorities. The relation between genetic ancestry and stage at diagnosis may be due to genetic factors and/or unmeasured environmental factors that are overrepresented within certain racial/ethnic groups.

Introduction

Race and ethnicity are associated with breast cancer incidence and mortality. Black and Hispanic women are more likely than their White counterparts to present at an earlier age, with different breast cancer characteristics such as later stages of breast cancer, and aggressive tumor that have poor prognoses (e.g., negative hormone receptor status, high grade, nuclear atypia, mitotic index, S-phase fraction, and necrosis) [1–7]. The causes of the racial and ethnic disparity in breast cancer characteristics are likely a result of both genetic and environmental influences. Several environmental factors experienced during life such as inequalities in health and socioeconomic status have been implicated in the racial disparity in breast cancer prognosis [8]. However, these factors do not completely account for these disparities [9, 10]. It has been postulated that certain biologic characteristics of the tumor might account for at least a portion of the disparity in the breast cancer characteristics and survival [11, 12].

Generally, racial/ethnic groups have been categorized by common geographic origins and shared physical characteristics, such as skin color. Black Americans are primarily a mixture of European and African ancestry, whereas Hispanic Americans are generally a mixture of European, African, and Native American ancestral backgrounds. Racial and ethnic categories used in biomedical research tend to conflate genetic, socioeconomic, social and cultural factors that contribute to racial and ethnic health disparities [12, 13].

Genetic heterogeneity within each racial and ethnic grouping may bias associations in genetic association studies, generating both false-positive and false-negative results [14–18]. Variations in the distribution of single nucleotides polymorphisms (SNPs), called ancestry informative markers (AIMs), have been shown to describe the architecture of genome variations between populations [19]. AIMS have been used to test the potential role of genetics in disease disparities within admixed populations [15, 20, 21]. The genetic ancestry proportions assigned to each individual, as opposed to membership of one racial group, serves as a proxy for the genetic ancestral background of the individual and can be used to assess relations between genetic ancestry and breast cancer characteristics. The finding of an association between genetic ancestry and a particular outcome may implicate genetic factors in the differential expression among racial groups.

The relationship between genetic ancestry and breast cancer characteristics has been previously investigated in several studies [20, 22–24], but whether genetic
ancestry contributes to these differences is still unconfirmed. We use a sociodemographically diverse sample from a population-based study to determine if genetic ancestry, estimated using AIMs, was associated with breast cancer characteristics, after accounting for self-reported race/ethnicity.

Materials and Methods

Study population

The study protocol was approved by the University of Illinois at Chicago Institutional Review Board (IRB#2010-0519). All samples were collected with written informed consent from each participant. The University of Illinois at Chicago Institutional Review Board approved the consent forms. Cases were a subset from the parent study “Breast Cancer Care in Chicago” (BCCC). Details of this study have been published elsewhere [25]. Briefly, eligible female patients were between 30 and 79 years of age at diagnosis, resided in Chicago, had a first primary in situ or invasive breast cancer, were diagnosed between October 1, 2005 and February 31, 2008, and self-identified as either non-Hispanic White, non-Hispanic Black or Hispanic. All diagnosing facilities in the greater Chicago area (N=56) were visited on a monthly basis and all eligible newly diagnosed cases were ascertained. Certified tumor registrars employed by the Illinois State Cancer Registry (ISCR) reviewed pathology records, the hospital tumor registry or both, depending on the protocol at each hospital. Patients were further screened for eligibility and scheduled for interviews if eligible and interested. The 90 minute interview was administered either in English or Spanish-as appropriate- using computer-assisted personal interview procedures. The final interview response rate was 56%, representing 989 completed interviews among eligible patients (397 nH White, 411 nH Black, and 181 Hispanic, and response rates 51%, 59% and 66%, respectively). The interview included questions pertaining to the process of discovery, diagnosis, and treatment of the patient’s breast cancer, as well as questions about healthcare-seeking behaviour, sociodemographic background, and known or suspected breast cancer risk factors (i.e., age at menarche, parity, age at first full-term pregnancy, breastfeeding, use of oral contraceptives, use of hormone replacement therapy, family history of breast cancer). Women were asked about the presence of any health problems or existing conditions (i.e., comorbidities) that required seeing a doctor or healthcare practitioner on regular basis at the time of breast cancer diagnosis. Upon completion of the interview, 848 patients consented to allow abstraction of their medical records, including access to their ISCR data and to provide biological samples. Of these, 666 (67%) provided a blood sample.

Self-reported Race/ethnicity

Self-reported race/ethnicity was defined through separate self-identification of Hispanic ethnicity (yes/no) and race (White/Black). Racial/ethnic groups were
categorized as follows: non-Hispanic White (nH White), non-Hispanic Black (nH Black) and Hispanic. Ethnicity was defined as Hispanic if the patient self-identified as Hispanic, reported a Latin American country of origin, or reported a Latin American country of origin for both biological parents.

Global Genetic Ancestry

DNA from blood was genotyped for 100 AIMs using the Sequenom MassARRAY iPLEX platform. The AIMs panel consisted of carefully selected autosomal markers that were previously identified and validated for estimating continental ancestry information in admixed populations [26–28]. All 100 AIMs were genotyped using the Sequenom MassARRAY genotyping platform with iPLEXchemistry according to manufacturer’s recommendations. Briefly, iPLEX assays were designed utilizing the Sequenom Assay Design software, allowing for single base extension designs used for multiplexing. PCR and unextended primer sequences may be found within the supplementary materials. Multiplex assays were performed to amplify 5 ng of genomic DNA by polymerase chain reaction (PCR). PCR reactions were treated with shrimp alkaline phosphatase (SAP) to neutralize unincorporated dNTPs. Subsequently, a post-PCR single base extension reaction was performed for each multiplex reaction using concentrations of 0.625 μM for low mass primers and 1.25 μM for high mass primers. Reactions were diluted with 16 μl of H₂O and fragments were purified with resin, spotted onto Sequenom SpectroCHIP microarrays and scanned by MALDI-TOF mass spectrometry. Individual SNP genotype calls were generated using Sequenom TYPER software, which automatically calls allele specific peaks according to their expected masses. Genotyping quality control for all SNPs was assessed using blinded duplicate genotyping for 60 DNA samples. A genotype concordance rate of 99% was observed for all markers. Genotyping call rates exceeded 98.5% for all individuals included in the analyses.

Individual admixture estimates for each study participant were calculated using a model-based clustering method as implemented in the program STRUCTURE v2.3 [29]. STRUCTURE 2.3 was run using parental population genotypes from west Africans, Europeans, and Native Americans [26] under the admixture model using the Bayesian Markov chain Monte Carlo method (K=3, assuming three founding populations) and a burn-in length of 30 000 for 70 000 repetitions. Ten cases that self-reported as European American and had more than 70% West African genetic ancestry were excluded. After the exclusions, genotype information was available for a total of 656 cases.

Statistical analysis

This analysis is based on 656 patients with valid genotyping results (255 NH White, 277 African Americans and 124 Hispanic). Stage at diagnosis, hormone receptor status and histologic grade were abstracted from the patient’s medical records. Stage at diagnosis was categorized using the American Joint Committee...
on Cancer (AJCC) categories of 0, 1, 2, and 3 and 4. Hormone receptor was defined as positive if tumor contained estrogen (ER) and/or progesterone (PR) receptors, and negative in the absence of both receptor types. Histologic grade was defined as low, intermediate and high. Among the 656 with biological samples, stage at diagnosis was available for 643 cases, histological grade for 575 cases, ER and PR data was available for 600 cases. Later stage at diagnosis was defined as stage 2, 3, 4 vs. 0, 1. Higher grade was defined as grade intermediate and high versus low. ER/PR negative breast cancer was defined as being negative for both ER and PR. As the determination of the Human Epidermal Growth Factor Receptor 2 (Her2) status was not a standard procedure when the BCCC cases were ascertained, we have Her2 status for only 362 cases and only 60 cases with triple negative in our population. Therefore, we excluded Her2 status in the present analysis.

Body mass index (BMI) was calculated as measured weight (kg) divided by measured height (m) squared. Area-level measures of socioeconomic status were based on two well-established measures of neighborhood structural characteristics: concentrated disadvantage and concentrated affluence. The concentrated disadvantage variable was constructed using the following variables derived from the U.S. Census: percent below poverty; percent unemployed; percent receiving public assistance; percent in female-headed households; percent under age 18; and percent African-American. The concentrated affluence variable was constructed using the following Census-derived variables: percent of families with incomes above $75,000; percent of adults with a college education; and percent of the civilian labor force employed in professional or managerial occupations.

Baseline characteristics of the population were compared across self-reported racial/ethnic groups using Chi-square statistics tests for categorical variables and ANOVA for continuous variables. Mean genetic ancestry was estimated as the average of the individual genetic ancestry estimates within self-reported racial/ethnic group. We used logistic regression to examine the association between genetic ancestry and breast cancer characteristics within self-reported racial/ethnic group. Genetic ancestry variables were divided equally into fifths at the quintiles within self-reported racial ethnic groups. Separate models were run for each self-reported racial/ethnic group (nH White, nH Black and Hispanic), ancestry (European, West-African, and Native American) and tumor characteristic (later stage, higher grade, ER/PR negative) to estimate the odds ratio and 95% confidence interval for the highest to the lowest fifth of genetic ancestry. The choice of quintiles was based on the assumption that if there was an effect of ancestry it was likely to be monotonic such that the effect would increase with increasing ancestry. We performed several other categorizations for modeling in ordinal logistic regression and they gave similar results. The regression models were adjusted for health insurance, income, education, concentrated disadvantage, concentrated affluence, nulliparity, and age at first and last birth. All reported p-values are two-sided. Statistical analyses were conducted using Stata version 11 (College Station, TX). The association between SNPs in our AIMs panel (Table S1) and breast cancer characteristics were tested using PLINK after removing individuals with >5% missing genotypes and adjusting for corresponding genetic
ancestry category, health insurance, income, education, disadvantage, affluence, nulliparity, and age at first and last birth. We run logistic regression models stratified by self-reported race/ethnicity adjusting for genetic ancestry. For nH Black cases, we adjusted for West African Ancestry and for Hispanic cases we adjusted for both West African Ancestry and Native American ancestry. We did not need to adjust for European genetics ancestry among nH White cases.

Results

Baseline characteristics of the cohort

The tumor and demographic characteristics of the final cohort which includes a total of 250 White, 273 Black, and 120 Hispanic women are summarized in Table 1. The mean age at diagnosis was 55 years (range 25 to 78 years). Racial/ethnic disparities in breast cancer characteristics were apparent in this population, as nH Black and Hispanic women were diagnosed at a later stage, higher grade and with a higher proportion of ER/PR negative tumors, compared to nH Whites. In addition, a greater proportion of nH Black and Hispanic women were overweight/obese, had more co-morbidities, were less likely to have their cancer detected through screening mammography, had a lower level of education and income, and less likely to have private insurance than nH Whites. The distribution of tumor characteristics and breast cancer risk factors of this subset was similar to the full cohort (Table S2).

The distribution of estimated West African, European and Native American ancestry varied among the three self-reported racial groups (Figure 1). The predominant genetic ancestry proportion among White cases was the European genetic ancestry, with a mean of 90% (± SD 11%). The predominant genetic ancestry among Black cases was West African genetic ancestry, with a mean of 79% (± SD 13%). Hispanic women had a wide range of European (mean 45%), Native American (mean 37%) and West African (mean 18%) genetic ancestry representing a highly admixed group.

 Genetic ancestry and Breast cancer:

We examined the association between genetic ancestry (modeled as an ordinal variable in fifths) and tumor characteristics within self-reported racial/ethnic groups (Table 2). Greater European ancestry (top quintile versus lowest quintile) was protective against late stage (Hispanics: OR 0.70, 95%, 0.54–0.92; nH Blacks: OR 0.88, 95%CI: 0.75–1.05). Among Hispanics it was also protective against higher grade (OR 0.73, 95% CI: 0.56–0.95%). On the other hand, greater West-African ancestry was associated with higher grade (OR 1.36, 95%CI: 1.05–1.77) and later stage (OR 1.13, 95%CI: 0.95–1.34) among nH Blacks. Native American ancestry was associated with later stage at diagnosis (OR 1.36, 95%CI: 1.04–1.79).

The associations between genetic ancestry and breast cancer characteristics were generally not attenuated after adjustment for social and behavioral factors as the
|                          | Total | nH White | nH Black | Hispanic | p-value |
|--------------------------|-------|----------|----------|----------|---------|
|                          | n     | %        | %        | %        |         |
| **Age, mean(± SD)**     | 656   | 55(11)   | 56(11)   | 53(11)   | 0.138   |
| **Age at first birth, mean(± SD)** | 656   | 26(±6)   | 21(±5)   | 23(±6)   | <0.0001 |
| **Age at last birth, mean(± SD)** | 656   | 31(±6)   | 29(±6)   | 31(±6)   | <0.0001 |
| **Stage at diagnosis (n=643)** |       |          |          |          |         |
| 0,1 (early stage)       | 374   | 67       | 55       | 48       | 0.0004  |
| 2,3,4 (late stage)      | 269   | 33       | 45       | 52       |         |
| **Histologic grade (n=575)** |       |          |          |          |         |
| Low/intermediate        | 348   | 67       | 55       | 61       | 0.025   |
| High                    | 227   | 34       | 45       | 39       |         |
| **ER/PR status (n=600)** |       |          |          |          |         |
| ER and/or PR Positive   | 474   | 86       | 55       | 61       | <0.001  |
| Double negative         | 126   | 14       | 45       | 39       |         |
| **Her2 overexpression (n=362)** |       |          |          |          |         |
| No                      | 305   | 90       | 78       | 86       | 0.028   |
| Yes                     | 57    | 10       | 22       | 14       |         |
| **Body mass index (kg/m²) (n=652)** |       |          |          |          |         |
| Normal weight (18.5–24.9) | 202   | 49       | 20       | 20       | <0.0001 |
| Overweight (25.0–29.9)  | 194   | 22       | 29       | 48       |         |
| Obese (>30.0)           | 256   | 29       | 52       | 32       |         |
| **Any co-morbidities (n=656)** |       |          |          |          |         |
| No                      | 286   | 49       | 37       | 48       | 0.007   |
| Yes                     | 370   | 51       | 63       | 52       |         |
| **Nulliparity (n=656)** |       |          |          |          |         |
| Yes                     | 133   | 37       | 10       | 7        | 0.007   |
| No                      | 523   | 63       | 90       | 93       |         |
| **Menopausal status (n=649)** |       |          |          |          |         |
| No                      | 132   | 17       | 20       | 27       | 0.105   |
| Yes                     | 517   | 83       | 80       | 73       |         |
| **Mode of Breast cancer detection (n=656)** |       |          |          |          |         |
| Screen detected         | 336   | 60       | 45       | 45       | 0.003   |
| Symptomatic             | 320   | 40       | 55       | 55       |         |
| **Education (n=655)**   |       |          |          |          |         |
| less than High school   | 120   | 4        | 20       | 44       | <0.0001 |
| High school             | 138   | 15       | 27       | 21       |         |
| some college            | 397   | 81       | 53       | 35       |         |
| **Annual household Income (n=655)** |       |          |          |          |         |
| less than $30,000       | 263   | 17       | 56       | 57       | <0.0001 |
| $30,000 to $75,000      | 277   | 52       | 38       | 37       |         |
| Greater than $75,000    | 102   | 31       | 6        | 7        |         |
| **Insurance category (n=656)** |       |          |          |          |         |
| No outpatient insurance | 84    | 7        | 14       | 23       | <0.0001 |
| Public                  | 125   | 4        | 31       | 23       |         |
unadjusted and adjusted point estimates were similar, but CI widened and lost statistical significance at p<0.05 (Table 2).

We also examined the association of individual SNPs in our AIMs panel (Table S2) and breast cancer characteristics among our three self-reported racial/ethnic categories. In unadjusted model, two AIMs were significantly associated in nH Whites after Bonferroni correction (p<0.0005). SNPs rs11073967 on chromosome 15 was associated with later stage (p=9.9×10⁻⁵), and rs10954631 on chromosome 7 was associated with ER/PR negative status (p=2.6×10⁻⁴). However, only rs10954631 remained statistically significant after adjusting for social and behavioral factors (p=1.1×10⁻⁴). The rs10954631 SNP was also associated with other breast cancer characteristics: later stage (p=0.02) and higher grade (p=0.012) in nH White as well as with later stage (p=0.03) in nH Black. Data summarised in Table S3 (Stage at diagnosis), Table S4 (Grade at diagnosis) and Table S5 (ER_PR positivity).

**Discussion**

We used ancestry informative markers to estimate individual genetic ancestry in a multi-racial cohort of breast cancer patients and examined the association between genetic ancestry and breast cancer characteristics after accounting for self-reported race/ethnicity.

In our population of incident breast cancer patients, nH Whites and Blacks had 79% European and 90% West African ancestries respectively. Hispanics, however, had a broader heterogeneous mixture of West African, European, and Native American ancestries. While this ethnic category represents individuals who, for most part, share a common language, it actually encompasses groups of individuals that differ in terms of their genetic ancestry proportions [32, 33]. The average West African ancestry we estimated in our sample of Hispanics was 18%, which is much higher than that observed in Mexicans and Mexican Americans (4–7%) [20, 34], and is more similar to Puerto Ricans (21%) [35].

When assessing the relationship between genetic ancestry and breast cancer characteristics, we found that Native American genetic ancestry was associated with later stage at diagnosis and West African genetic ancestry was associated with higher grade in ethnic minorities. These associations could not be explained by multiple social and behavioral risk factors. The finding that genetic ancestry is associated with stage of breast cancer suggests that genetic factors may play a role...
in the observed breast cancer disparities. Alternatively, this could be due, in part to the strong correlation between genetic ancestry and self-reported race/ethnicity.

Our results are consistent with Fejerman et al. [22] and Palmer et al [23] who also found that genetic ancestry was associated with stage at diagnosis among nH Blacks. Similar to Reding et al [24], but in contrast to Fejerman et al and Palmer
et al, we did not find an association between ER/PR status and West African genetic ancestry among nH Blacks. Our sample size may have limited our ability to detect associations between ancestry and ER/PR status in nH Blacks. However, we did observe several associations between ancestry and breast cancer characteristics that have not been previously reported. For instance, higher West African ancestry increased the odds of higher grade among nH Blacks. However, after adjustment for multiple social and behavioral risk factors, the point estimate was similar to unadjusted models but lost statistical significance at \( p = 0.05 \). We

| Ancestry/Subgroup | Unadjusted | Adjusted* |
|-------------------|------------|-----------|
|                   | n | OR (95% CI) | OR (95% CI) |
| Later Stage at Diagnosis | | | |
| European Ancestry | | | |
| Among nH Whites | 250 | 0.97 (0.80–1.17) | 1.13 (0.88–1.47) |
| Among nH Blacks | 273 | 0.88 (0.75–1.05)* | 0.84 (0.70–1.02)* |
| Among Hispanics | 120 | 0.70 (0.54–0.92)*** | 0.58 (0.41–0.80)*** |
| West African Ancestry | | | |
| Among nH Blacks | 273 | 1.13 (0.95–1.34)* | 1.15(0.97–1.41)* |
| Native American Ancestry | | | |
| Among Hispanics | 120 | 1.36 (1.04–1.79)** | 1.35 (1–1.83)** |
| Higher Grade Disease | | | |
| European Ancestry | | | |
| Among nH Whites | 224 | 1.06 (0.86–1.31) | 1.05 (0.79–1.41) |
| Among nH Blacks | 242 | 0.73 (0.56–0.95)** | 0.79(0.6–1.05)* |
| Among Hispanics | 109 | 1.04 (0.69–1.56) | 0.95 (0.57–1.56) |
| West African Ancestry | | | |
| Among nH Blacks | 242 | 1.36 (1.05–1.77)** | 1.26 (0.95–1.67)* |
| Native American Ancestry | | | |
| Among Hispanics | 109 | 1.07 (0.70–1.62) | 1.23 (0.73–2.06) |
| ER/PR Negative Status | | | |
| European Ancestry | | | |
| Among nH Whites | 228 | 1.18 (0.90–1.56) | 1.20 (0.81–1.78) |
| Among nH Blacks | 240 | 0.97 (0.82–1.18) | 1.04 (0.84–1.29) |
| Among Hispanics | 110 | 1.13 (0.81–1.56) | 1.05 (0.75–1.49) |
| West African Ancestry | | | |
| Among nH Blacks | 240 | 0.84 (0.65–1.10) | 1.03 (0.83–1.28) |
| Native American Ancestry | | | |
| Among Hispanics | 110 | 1.04 (0.74–1.46) | 1.10 (0.76–1.60) |

*\( p < 0.20 \),
**\( p < 0.10 \),
***\( p < 0.05 \),
****\( p < 0.01 \).

OR, odds ratio from logistic regression comparing the highest versus the lowest fifth of the subsample distribution.

*Adjusted for health insurance, income, education, disadvantage, affluence, nulliparity, and age at first and last birth.

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also observed an association between Native American ancestry and stage at diagnosis that was not previously seen in Hispanic women from the US or Mexico. Our results differ from those of Fejerman and colleagues [20] who analyzed the effect of ancestry on stage, grade and other tumor characteristics in U.S. Latinas and did not find any statistically significant associations. The differences between our study and those of Fejerman et al. [20] could be attributed to variation in the case populations. The Fejerman et al study consisted of Hispanic women from the San Francisco Bay area while our study in the Chicago metropolitan area contained a larger proportion of Hispanic women from the Caribbean. As previously stated, the proportion of Native American and West African genetic ancestry is significantly different between Hispanics of Mexican origin and those from the Caribbean, thus genetic ancestry in each group might be a proxy for a different risk factor. This highlights why investigators should not generalize findings across all Hispanic populations.

We examined the association of individual AIMs markers with breast cancer characteristics to identify whether these markers are in linkage disequilibrium with regions that may potentially play a role in breast cancer disease disparities. We found that the SNP rs10954631 on chromosome 7 was associated with later stage (p=0.02) and higher grade (p=0.012) in nH White women as well as with later stage (p=0.03) in nH Black women.

SNP rs10954631 is located in the KIAA1549 gene which is about 2 mb downstream of the BRAF oncogene. The KIAA1549 gene is often fused to the BRAF in cases of pilocytic astrocytoma [36]. This SNP is located in an interesting region of chromosome 7 as several associations with breast cancer were observed. The 7q34 region on chromosome 7 was shown to be associated with lobular breast cancer specific predisposition [37]. SNP rs10954631 is located about 500 kb downstream of Transcription intermediary factor 1α (TIF-1α) gene -also known as TRIM24. Overexpression of the TRIM24/IF-1 gene in breast cancer is associated with poor prognosis and worse survival [38]. Gross Cystic Disease Fluid Protein-15(GCDFP-15)/Prolactin-Inducible Protein (PIP) expression is associated with invasive breast cancer [39]. There are also several genes close to this region that are associated with many types of cancer including breast cancer, EPH receptor B6 (EPHB) [40, 41] and Transient receptor potential vanilloid 6 (TRPV6) [42]. Further analysis of this region is needed.

A major strength of this study is the use of a sociodemographically diverse sample that capture the three major racial/ethnic groups from a population-based study to assess the relationship between genetic ancestry and breast cancer. Nonetheless there are limitations to these analyses. In addition to the relatively small sample size, potential misclassification of ER/PR status, grade and stage might tend to alter observed associations in unpredictable ways, by either attenuating or biasing associations away from the null. Finally, it is important to emphasize that the association of stage at diagnosis with genetic ancestry does not necessarily represent racially distributed genetic factors. It was not possible to adjust completely for all the myriad ways in which social, behavioral, and health care access differences that could have contributed to tumor characteristics, and as
such we cannot interpret the adjusted relations of genetic ancestry with tumor characteristics as being the sole result of ancestral origin. Nonetheless, our inability to adjust away these associations by including multiple social and health care access variables in our models leaves open the possibility that differences in genetic ancestry contribute to racial/ethnic disparities in tumor characteristics.

Conclusion
Differences in breast cancer aggression among different racial and ethnic groups have been previously reported, but whether genetic ancestry contributes to these differences remain unknown. Our study reveals that genetic ancestry plays a role in breast cancer. We used diverse samples from a population-based study and found that non-European genetic ancestry was associated with later stage and grade at diagnosis in ethnic minorities. Future studies investigating the relation between genetic ancestry and stage at diagnosis are warranted. As this relationship may be due to genetic factors and/or unmeasured environmental factors that are overrepresented within certain racial/ethnic groups.

Supporting Information
Table S1. Ancestry Informative Marker Panel.
doi:10.1371/journal.pone.0112916.s001 (DOCX)
Table S2. Descriptive and tumor characteristics of the full BCCC cohort stratified by self-reported race/ethnicity.
doi:10.1371/journal.pone.0112916.s002 (DOCX)
Table S3. Table S3 Association of Grade at diagnosis and ancestry.
doi:10.1371/journal.pone.0112916.s003 (XLSX)
Table S4. Association of Stage at diagnosis and ancestry.
doi:10.1371/journal.pone.0112916.s004 (XLSX)
Table S5. Association of ER_PR status and ancestry.
doi:10.1371/journal.pone.0112916.s005 (XLSX)

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Author Contributions
Conceived and designed the experiments: UA GR RK. Performed the experiments: UA ES AM EB. Analyzed the data: UA GR RK ES KB AM AS CP. Wrote the paper: UA GR RK ES KB AM AS CP.
References

1. Barcenas CH, Wells J, Chong D, French J, Looney SW, et al. (2010) Race as an independent risk factor for breast cancer survival: breast cancer outcomes from the medical college of georgia tumor registry. Clinical breast cancer 10: 59–63.

2. Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V (2007) 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 109: 1721–1728.

3. Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, et al. (2006) Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA: the journal of the American Medical Association 295: 2492–2502.

4. Cunningham JE, Montero AJ, Garrett-Mayer E, Berkel HJ, Ely B (2010) Racial differences in the incidence of breast cancer subtypes defined by combined histologic grade and hormone receptor status. Cancer causes & control: CCC 21: 399–409.

5. Gapstur SM, Dupuis J, Gann P, Collilla S, Winchester DP (1996) Hormone receptor status of breast tumors in black, Hispanic, and non-Hispanic white women. An analysis of 13,239 cases. Cancer 77: 1465–1471.

6. Lantz PM, Mujahid M, Schwartz K, Janz NK, Fagerlin A, et al. (2006) The influence of race, ethnicity, and individual socioeconomic factors on breast cancer stage at diagnosis. American Journal of Public Health 96: 2173–2178.

7. O’Brien KM, Cole SR, Tse CK, Perou CM, Carey LA, et al. (2010) Intrinsic breast tumor subtypes, race, and long-term survival in the Carolina Breast Cancer Study. Clinical cancer research: an official journal of the American Association for Cancer Research 16: 6100–6110.

8. Whitman S, Ansell D, Orsi J, Francois T (2011) The racial disparity in breast cancer mortality. Journal of community health 36: 588–596.

9. Albain KS, Unger JM, Crowley JJ, Coltman CA Jr, Hershman DL (2009) Racial disparities in cancer survival among randomized clinical trials patients of the Southwest Oncology Group. Journal of the National Cancer Institute 101: 984–992.

10. Newman LA, Griffith KA, Jatoi I, Simon MS, Crowe JP, et al. (2006) Meta-analysis of survival in African American and white American patients with breast cancer: ethnicity compared with socioeconomic status. Journal of clinical oncology: official journal of the American Society of Clinical Oncology 24: 1342–1349.

11. Ademuyiwa FO, Edge SB, Erwin DO, Orom H, Ambrosone CB, et al. (2011) Breast cancer racial disparities: unanswered questions. Cancer Res 71: 640–644.

12. Wallace TA, Martin DN, Ambs S (2011) Interactions among genes, tumor biology and the environment in cancer health disparities: examining the evidence on a national and global scale. Carcinogenesis 32: 1107–1121.

13. Cooper RS, Kaufman JS, Ward R (2003) Race and genomics. N Engl J Med 348: 1166–1170.

14. Bonilla C, Boxill LA, Donald SA, Williams T, Sylvester N, et al. (2005) The 8818G allele of the agouti signaling protein (ASIP) gene is ancestral and is associated with darker skin color in African Americans. Human Genetics 116: 402–406.

15. Caulfield T, Fullerton SM, Ali-Khan SE, Arbour L, Burchard EG, et al. (2009) Race and ancestry in biomedical research: exploring the challenges. Genome medicine 1: 8.

16. Choudhry S, Coyle NE, Tang H, Salari K, Lind D, et al. (2006) Population stratification confounds genetic association studies among Latinos. Human Genetics 118: 652–664.

17. Shriner MD, Parra EJ, Dios S, Bonilla C, Norton H, et al. (2003) Skin pigmentation, biogeographical ancestry and admixture mapping. Human genetics 112: 387–399.

18. Tsai HJ, Choudhry S, Naqvi M, Rodriguez-Cintron W, Burchard EG, et al. (2005) Comparison of three methods to estimate genetic ancestry and control for stratification in genetic association studies among admixed populations. Human Genetics 118: 424–433.

19. Kittles RA, Weiss KM (2003) Race, ancestry, and genes: implications for defining disease risk. Annual review of genomics and human genetics 4: 33–87.
20. Fejerman L, John EM, Huntsman S, Beckman K, Choudhry S, et al. (2008) Genetic ancestry and risk of breast cancer among U.S. Latinas. Cancer research 68: 9723–9728.

21. Giri VN, Egleston B, Ruth K, Uzzo RG, Chen DY, et al. (2009) Race, genetic West African ancestry, and prostate cancer prediction by prostate-specific antigen in prospectively screened high-risk men. Cancer prevention research (Philadelphia, Pa) 2: 244–250.

22. Fejerman L, Haiman CA, Reich D, Tandon A, Deo RG, et al. (2009) An admixture scan in 1,484 African American women with breast cancer. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 18: 3110–3117.

23. Palmer JR, Ruiz-Narvaez EA, Rotimi CN, Cupples LA, Cozier YC, et al. (2013) Genetic susceptibility Loci for subtypes of breast cancer in an african american population. Cancer Epidemiol Biomarkers Prev 22: 127–134.

24. Reding KW, Carlson CS, Kahsai O, Chen CC, McDavid A, et al. (2012) Examination of ancestral informative markers and self-reported race with tumor characteristics of breast cancer among Black and White women. Breast Cancer Research and Treatment 134: 801–809.

25. Rauscher GH, Ferrans CE, Kaiser K, Campbell RT, Calhoun EE, et al. (2010) Misconceptions about breast lumps and delayed medical presentation in urban breast cancer patients. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 19: 640–647.

26. Kosoy R, Nassir R, Tian C, White PA, Butler LM, et al. (2009) Ancestry informative marker sets for determining continental origin and admixture proportions in common populations in America. Human mutation 30: 69–78.

27. Nassir R, Kosoy R, Tian C, White PA, Butler LM, et al. (2009) An ancestry informative marker set for determining continental origin: validation and extension using human genome diversity panels. BMC genetics 10: 39.

28. Torres JB, Stone AC, Kittles R (2013) An anthropological genetic perspective on Creolization in the Anglophone Caribbean. American Journal of Physical Anthropology 151: 135–143.

29. Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 164: 1567–1587.

30. Sampson JH, Morenoff JD, Earls F (1999) Beyond social capital:spatial dynamics of collective efficacy for children. American Sociological Review 64: 633–660.

31. Browning CR, Cagney KA (2002) Neighborhood structural disadvantage, collective efficacy, and self-rated physical health in an urban setting. Journal of Health and Social Behavior 43: 383–399.

32. Bryc K, Auton A, Nelson MR, Oksenberg JR, Hauser SL, et al. (2010) Genome-wide patterns of population structure and admixture in West Africans and African Americans. Proc Natl Acad Sci U S A 107: 786–791.

33. Haile RW, John EM, Levine AJ, Cortessis VK, Unger JB, et al. (2012) A review of cancer in u.s. Hispanic populations. Cancer prevention research (Philadelphia, Pa) 5: 150–163.

34. Fejerman L, Romieu I, John EM, Lazcano-Ponce E, Huntsman S, et al. (2010) European ancestry is positively associated with breast cancer risk in Mexican women. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 19: 1074–1082.

35. Amirian E, Liu Y, Scheurer ME, El-Zein R, Gilbert MR, et al. (2010) Genetic variants in inflammation pathway genes and asthma in glioma susceptibility. Neuro-oncology 12: 444–452.

36. Jones DT, Kocialkowski S, Liu L, Pearson DM, Backlund LM, et al. (2008) Tandem duplication producing a novel oncogenic BRAF fusion gene defines the majority of pilocytic astrocytomas. Cancer Research 68: 8673–8677.

37. Sawyer E, Roylance R, Petridis C, Brook MN, Nowinski S, et al. (2014) Genetic predisposition to in situ and invasive lobular carcinoma of the breast. PLoS Genet 10: e1004285.

38. Chambon M, Orsetti B, Berthe ML, Bascoul-Mollevi C, Rodriguez C, et al. (2011) Prognostic significance of TRIM24/TIF-1alpha gene expression in breast cancer. American Journal of Pathology 178: 1461–1469.
39. Darb-Esfahani S, von Minckwitz G, Denkert C, Ataseven B, Hogel B, et al. (2014) Gross cystic disease fluid protein 15 (GCDFP-15) expression in breast cancer subtypes. BMC Cancer 14: 546.

40. Bhushan L, Kandpal RP (2011) EphB6 receptor modulates micro RNA profile of breast carcinoma cells. PLoS One 6: e22484.

41. Fox BP, Kandpal RP (2009) EphB6 receptor significantly alters invasiveness and other phenotypic characteristics of human breast carcinoma cells. Oncogene 28: 1706–1713.

42. Lehen’kyi V, Raphael M, Prevarskaya N (2012) The role of the TRPV6 channel in cancer. J Physiol 590: 1369–1376.