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Article

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Racial Differences in PAM50 Subtypes in the Carolina Breast Cancer Study

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Abstract

Background: African American breast cancer patients have lower frequency of hormone receptor–positive (HR+)/human epidermal growth factor receptor 2 (HER2)–negative disease and higher subtype-specific mortality. Racial differences in molecular subtype within clinically defined subgroups are not well understood.

Methods: Using data and biospecimens from the population-based Carolina Breast Cancer Study (CBCS) Phase 3 (2008–2013), we classified 980 invasive breast cancers using RNA expression-based PAM50 subtype and recurrence (ROR) score that reflects proliferation and tumor size. Molecular subtypes (Luminal A, Luminal B, HER2-enriched, and Basal-like) and ROR scores (high vs low/medium) were compared by race (blacks vs whites) and age (≤50 years vs > 50 years) using chi-square tests and analysis of variance tests.

Results: Black women of all ages had a statistically significantly lower frequency of Luminal A breast cancer (25.4% and 33.6% in blacks vs 42.8% and 52.1% in whites; younger and older, respectively). All other subtype frequencies were higher in black women (case-only odds ratio [OR] = 3.11, 95% confidence interval [CI] = 2.22 to 4.37, for Basal-like; OR = 1.45, 95% CI = 1.02 to 2.06, for Luminal B; OR = 2.04, 95% CI = 1.33 to 3.13, for HER2-enriched). Among clinically HR+/HER2- cases, Luminal A subtype was less common and ROR scores were statistically significantly higher among black women.

Conclusions: Multigene assays highlight racial disparities in tumor subtype distribution that persist even in clinically defined subgroups. Differences in tumor biology (eg, HER2-enriched status) may be targetable to reduce disparities among clinically ER+/HER2- cases.

Breast cancer incidence is higher in young black women compared with young white women, and while 2010 Surveillance, Epidemiology, and End Results data showed that across all ages white women had higher incidence (1), recent data from the American Cancer Society suggest that overall incidence rates have converged (2). This convergence could compound breast cancer mortality disparities. Mortality hazard rates among black women vary by subtype but are 20% to 150% higher relative to white women (3,4). Differences are particularly pronounced among hormone receptor (HR)–positive,

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human epidermal growth factor receptor 2 (HER2)–negative patients (4,5). Interventions to reduce these disparities require improved understanding of how tumor-level and patient-level factors interact.

The Carolina Breast Cancer Study Phase 3 (CBCS3, 2008–2013) was initiated to disentangle the role of health service and tumor biological factors in breast cancer disparities (6–8). Beginning in 1993, participants were enrolled in the study via randomized recruitment that oversampled black women and women younger than age 50 years at diagnosis. Research from earlier phases of the Carolina Breast Cancer Study (CBCS1 and 2, 1993–2001) used a five-marker immunohistochemistry panel to show higher prevalence of Basal-like breast cancer (defined as HR- and HER2- and positive for either Ck5/6 or EGFR) and lower prevalence of HR+/HER2- breast cancer among young (age < 50 years) black women (9), findings that have been confirmed in other studies (4,10–12).

Decreased HR+/HER2- disease among young black women could arise from lower screening utilization (13) or from differences in risk factor profiles (14) or both. These factors could also lead to differences in genomic characteristics, even within more clinically homogeneous groups. Of particular interest is whether biological differences in HR+/HER2- tumors of black and white women could account for mortality disparities by race (5). Current guidelines recommend that these patients receive genomic testing as a decision-making tool (15), but few studies have utilized genomic data to characterize racial differences in clinically defined groups (16), and these differences have not been evaluated using biospecimens from population-based studies.

To elucidate differences in tumor biology by race, we used high-throughput RNA profiling methods to determine the PAM50 molecular subtypes of invasive breast cancers from more than 1000 women participating in the population-based CBCS3, with nearly equal numbers of black and white women. We identified PAM50-based intrinsic subtype and classified patients for ROR score based on proliferation (ROR-P) or a combination of proliferation and tumor size (ROR-PT) (17). We also explored Oncotype DX scores by race for a subset of patients who underwent clinical genomic testing. This work evaluates racial differences in the relative frequency of molecular subtypes and assesses whether differences in tumor genomics persist even within clinically defined subgroups.

Methods

Study Population

The Carolina Breast Cancer Study Phase 3 (CBCS3) is the third phase (2008–2013) of a population-based study conducted in North Carolina (NC) that began in 1993; study details and sampling schemes have been described previously (7,18). Briefly, cases of invasive breast cancer between age 20 and 74 years were identified using rapid case ascertainment in cooperation with the NC Central Cancer Registry, with black and young cases (age 20–49 years) oversampled using randomized recruitment (18). Randomized recruitment allows sample weighting to make inferences about the frequency of subtype in the NC source population. Tumor size, stage, node status, estrogen receptor (ER), progesterone receptor (PR), HER2, and Oncotype DX data were abstracted from medical records, and tumor grade was centrally assigned by a single pathologist (JG) using the Nottingham breast cancer grading system (19). The study was approved by the Office of Human Research Ethics at the University of North Carolina at Chapel Hill, and informed consent was obtained from each participant.

Molecular and Clinical Subtyping

Paraffin-embedded tumor blocks were requested from participating pathology laboratories for each case. The study pathologist (JG) reviewed hematoxylin and eosin (H&E) for each tumor, selected a representative tumor block, and circled tumor areas for coring. Ten 10 μM sections were cut from blocks after coring (for immunohistochemistry), and an additional H&E section was obtained and reviewed for tumor cellularity. Only cores with adequate top and bottom tumor cellularity by manual review were selected for RNA analyses. Nanostring assays were performed using two separate 1.0 mm cores. RNA was isolated from cores using Qiagen RNeasy formalin-fixed, paraffin-embedded kit and protocol, with 95% of tumors producing quantifiable RNA. In total, 1122 samples from 1042 participants with invasive breast cancer from CBCS Phase 3 were analyzed using the PAM50 assay. Samples were randomly assigned to three batches for RNA analyses, and the laboratory was blinded to duplicates and all participant characteristics. Two standard samples were included with each batch: an RNA sample comprised of Stratagene (La Jolla, CA) Universal Human Reference and a breast cell-line cocktail comprised of RNA isolates from pooled breast cell lines. To assess batch variability, we calculated Pearson correlation coefficients for pairwise comparisons of these samples across the PAM50 genes. Pearson correlations were 0.980, 0.987, and 0.992 for the Stratagene reference and 0.987, 0.989, and 0.997 for the breast cell line mix. All assays were performed in the Rapid Adoption Molecular (RAM) laboratory at the University of North Carolina.

To classify samples, the NanoStringNorm package in Bioconductor was first used to eliminate samples that did not have sufficient Nanostring data quality (39 of 1122, 3%). The PAM50 predictor (17) was then used to categorize breast tumors as Luminal A, Luminal B, HER2-enriched, Basal-like, and normal-like and to calculate the risk of recurrence (ROR) score with proliferation (ROR-P) and proliferation plus tumor size (ROR-PT). Briefly, each sample was classified based on the subtype centroid with the highest Pearson correlation. Duplicate samples were treated independently during classification; after classification, the sample with the highest PAM50 confidence score was selected for inclusion in patient-level analyses. After excluding patients with normal-like subtype (ie, specimens with insufficient tumor cellularity), patients missing clinical data, and patients with race other than black or white, 980 patients were included in the final analysis. Patients were roughly evenly divided in four groups: black women younger than age 50 years (n = 232), black women age 50 years or older (n = 268), white women younger than age 50 years (n = 236), and white women age 50 years or older (n = 244). Compared with cases not analyzed, included samples were more likely to be at least age 50 years (32.5% vs 52.2%, chi-square $P = 0.01$) and have grade 2 tumors (15.4% vs 34.3%; chi-square $P = 0.01$). Participant characteristics are presented in Supplementary Table 1 (available online).

Statistical Analysis

Biomarker variables, including PAM50-based ROR-P and ROR-PT and Oncotype DX, were used both continuously and categorically. The cutoff points to define high levels were 52.9 for ROR-P,

Table 1. PAM50 subtype by race and age, Carolina Breast Cancer Study Phase 3, 2008–2013

	Race and age, y				
		Black		White	
PAM50 subtype	All cases*	<50 No. (%)	≥50 No. (%)	<50 No. (%)	≥50 No. (%)
Total	980 (100.0)	232 (100.0)	268 (100.0)	236 (100.0)	244 (100.0)
Luminal A†	377 (38.5)	59 (25.4)	90 (33.6)	101 (42.8)	127 (52.1)
Basal-like‡	249 (25.4)	85 (36.6)	84 (31.3)	44 (18.6)	36 (14.8)
HER2-enriched	114 (11.6)	37 (16.0)	29 (10.8)	21 (8.9)	27 (11.1)
Luminal B	194 (19.8)	43 (18.5)	53 (19.8)	57 (24.2)	41 (16.8)
Normal-like	46 (4.7)	8 (3.45)	12 (4.5)	13 (5.5)	13 (5.3)

*Frequency estimates (%) were adjusted for sampling weights from the randomized recruitment design. HER2 = human epidermal growth factor receptor 2.
†Two-sided chi-square comparing Basal-like with non-Basal-like across all age/race categories ($P < .001$).
‡Two-sided chi-square comparing Luminal A to Non–Luminal A across all age/race categories ($P < .001$).

Table 2. Odds ratios for breast cancer subtype by patient characteristics

	Age, y					Race				
PAM50 subtype	≥50 No. (%)	<50 No. (%)	OR* (95% CI)	OR† (95% CI)	P‡	White, No. (%)	Black, No. (%)	OR§ (95% CI)	OR† (95% CI)	P‡
LumA	217 (44.6)	160 (35.8)	1.00 (reference)	1.00 (reference)	.06	228 (50.2)	149 (31.0)	1.00 (reference)	1.00 (reference)	<.001
Basal-like	120 (24.6)	129 (28.7)	1.54 (1.10 to 2.14)	1.08 (0.72 to 1.62)		80 (17.6)	169 (35.2)	3.11 (2.22 to 4.37)	1.93 (1.27 to 2.93)	
LumB	94 (19.3)	100 (22.4)	1.47 (1.04 to 2.09)	1.21 (0.83 to 1.77)		98 (21.6)	96 (20.0)	1.45 (1.02 to 2.06)	1.14 (0.78 to 1.68)	
HER2-enriched	56 (11.5)	58 (13.0)	1.45 (0.95 to 2.22)	1.12 (0.70 to 1.79)		48 (10.6)	66 (13.8)	2.04 (1.33 to 3.13)	1.43 (0.88 to 2.30)	
Normal-like	25	21	–	–		26	20	–	–	

*Adjusted for race. CI = confidence interval; HER2 = human epidermal growth factor receptor 2; OR = odds ratio.
†Additionally adjusted for tumor size (≤2 cm vs > 2 cm), nodal status (negative vs positive), stage (I and II vs III and IV), and grade (I and II vs III).
‡Two-sided chi-square P values exclude 46 cases with normal-like PAM50 subtype and 138 cases with unclassified/missing clinical subtype.
§Adjusted for age.

64.7 for ROR-PT, and 30 for Oncotype DX, per established protocols (17,20). Racial differences by categorical variables, such as clinical characteristics and subtype, were assessed with Pearson chi-square tests or Fisher exact tests. Sampling weights were used to adjust subtype frequency estimates across all estimates, consistent with the randomized recruitment design of the study. Odds ratios (ORs) represent prevalence odds ratios and were estimated using logistic regression, regressing tumor characteristics on population subgroup (age or race groups). Analysis of variance was used to assess statistical differences for continuous variables. All statistical tests were two-sided with an α of .05, and all analyses were performed using SAS version 9.2 (SAS Institute, Inc, Cary, NC). *P* values were not corrected for multiple comparisons because tumor characteristics are not independent.

Results

Breast Cancer Subtypes by Race and Age

In the CBCS Phase 3, racial differences in frequency of molecular subtype were most pronounced for Basal-like and Luminal A breast cancer. As shown in Table 1, the proportion of Basal-like breast cancer as measured by RNA profiling was 25.4% overall (n = 249 cases), but was higher in black women; 36.6% and 31.3% of younger (age < 50 years) and older (age ≥ 50 years) black women, respectively, had Basal-like breast cancer. Basal-like breast cancer comprised less than 20% of cases among white women. The higher frequency of Basal-like tumors in black women was offset by a decrease in the frequency of Luminal A breast cancer.

The lowest frequency of Luminal A breast cancer was observed among young black women (25.4%, n = 59), followed by older black women (33.6%, n = 90), young white women (42.8%, n = 101), and older white women (52.1%, n = 127) (Table 1). The frequency of Luminal B cancers was relatively stable across all four age- and race-defined groups, at approximately 20%. There was a suggestion that HER2-enriched tumors may be more frequent among young black women (16.0%, n = 37) compared with all other groups (8.9%–11.1%); however, the sample size was small, and this difference was not statistically significant. But considering all age groups together, Table 2 shows that compared with white women, black women had statistically significantly higher odds of all three non–Luminal A subtypes: Basal-like (OR = 3.11, 95% confidence interval [CI] = 2.22 to 4.37), Luminal B (OR = 1.45, 95% CI = 1.02 to 2.06), and HER2-enriched breast cancer (OR = 2.04, 95% CI = 1.33 to 3.13). All of these odds ratios were attenuated after adjusting for other clinical covariates (size, node, stage, grade), but the odds ratios for Basal-like breast cancer remained statistically significant. Table 2 also illustrates age associations for breast cancer subtypes. Women younger than age 50 years had higher odds of Basal-like and Luminal B breast cancer (relative to Luminal A), but age associations with PAM50 subtype were not statistically significant after adjusting for clinical covariates.

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Table 3. Relative frequency of clinical features, hormone receptor–positive, HER2-negative cases, Carolina Breast Cancer Study 3 (2008–2013)

		Race and age, y				
		Black		White		
Clinical feature	All cases	<50 No. (%)	≥50 No. (%)	<50 No. (%)	≥50 No. (%)	P*
Tumor size, cm						
≤2	265 (55.1)	36 (40.9)	63 (54.3)	69 (54.8)	97 (64.2)	.006
>2	216 (44.9)	52 (59.1)	53 (45.7)	57 (45.2)	54 (35.8)	
Missing	11	1	3	1	6	
>2 vs ≤2, OR (95% CI)	–	2.60 (1.51 to 4.45)	1.51 (0.92 to 2.48)	1.48 (0.92 to 2.41)	1.00 (reference)	
Nodal status						
Negative	277 (57.5)	41 (46.6)	70 (60.3)	72 (56.7)	94 (62.3)	.11
Positive	205 (42.5)	47 (53.4)	46 (39.7)	55 (43.3)	57 (37.8)	
Missing	10	1	3	0	6	
Positive vs negative, OR (95% CI)	–	1.89 (1.11 to 3.22)	1.08 (0.66 to 1.78)	1.26 (0.78 to 2.04)	1.00 (reference)	
Stage						
I and II	403 (83.6)	71 (80.7)	101 (87.1)	104 (81.9)	127 (84.1)	.60
III and IV	79 (16.4)	17 (19.3)	15 (12.9)	23 (18.1)	24 (15.9)	
Missing	10	1	3	0	6	
III and IV vs I and II, OR (95% CI)	–	1.27 (0.64 to 2.52)	0.79 (0.39 to 1.58)	1.17 (0.63 to 2.19)	1.00 (reference)	
Grade						
I and II	342 (71.6)	54 (62.1)	76 (66.1)	90 (71.4)	122 (81.3)	.006
III	136 (28.5)	33 (37.9)	39 (33.9)	36 (28.6)	28 (18.7)	
Missing	14	2	4	1	7	
III vs I and II OR (95% CI)	–	2.66 (1.47 to 4.84)	2.24 (1.27 to 3.93)	1.74 (0.99 to 3.06)	1.00 (reference)	

*Two-sided chi-square P values exclude participants with missing data. CI = confidence interval; HER2 = human epidermal growth factor receptor 2; OR = odds ratio.

Clinical and Gene Expression Characteristics of HR-Positive, HER2-Negative Cancers

Mortality disparities are greatest among HR+/HER2- cancers (5), so racial differences in clinical, histopathologic, and biomarker data are particularly important for this group. Among HR+/HER2- patients, tumor size and grade varied statistically significantly by race and age (Table 3). Compared with older white women, younger black women had more than twice the odds of a large tumor (OR = 2.60, 95% CI = 1.51 to 4.45), and both younger and older black women had twice the odds of having a high-grade tumor (younger black: OR = 2.66, 95% CI = 1.47 to 4.84; older black: OR = 2.24, 95% CI = 1.27 to 3.93). Stage and node status were not statistically significantly different within strata defined by race or age, although when cross-classifying on both race and age, younger black women had higher odds of being node positive.

Table 4 shows racial differences in biomarker levels and class distributions among HR+/HER2- cases (n = 492), including PAM50 subtype, ROR-P and ROR-PT, and Oncotype DX. ROR-P is a risk of recurrence score based on correlation to PAM50 subtype and a proliferation term. ROR-PT utilizes these correlations and additionally incorporates tumor size (17). ROR-PT is a research version of a test that is utilized clinically in the United States and internationally. PAM50 subtype classification is not reported clinically in the United States but is utilized in Europe. While ROR-P and ROR-PT are correlated, approximately 20% of cases have different categorical scores for the two metrics. PAM50 subtype differed statistically significantly across all race and age strata, and there were statistically significant differences in the percentage of Luminal A breast tumors between black and white women with HR+/HER2- disease. Both older and younger black women were more likely to have non–Luminal A PAM50 subtype, but the association was attenuated and not statistically significant in older black women. The ROR-P and ROR-PT scores differed by race and age, with black women having statistically significantly higher frequency of high-risk tumors for both ROR scores (OR > 1.5 for ROR-P and OR > 2.0 for ROR-PT in both younger and older black women) (Table 5). On a continuous scale, average scores for ROR-P and ROR-PT also differed by race and age ($P < .001$). Associations between age-/race-defined groups and PAM50, ROR-PT, and Oncotype DX were not substantially altered by adjustment for size, grade, node status, or stage.

To evaluate differences in genomic subtype based on race (without further stratifying on age), several supplemental analyses were conducted (Supplementary Table 2, available online). Considering black women vs white women, only about half of black women (51.0%) had Luminal A PAM50 subtype, compared with 59.9% of white women ($P = .05$). Black women overall (combining age groups) had higher odds of a high risk score by ROR-P (OR = 1.31, 95% CI = 0.82 to 2.08), ROR-PT (OR = 1.87, 95% CI = 1.11 to 3.16), and Oncotype DX (OR = 1.63, 95% CI = 0.63 to 4.24), but these racial differences were statistically significant only for ROR-PT (Supplementary Table 2, available online). The higher frequency of ROR-P high among black women was independently validated using data from The Cancer Genome Atlas Project (TCGA) (Supplementary Table 2, available online); in the TCGA, the magnitude of association was similar (OR = 2.87, 95% CI = 1.24 to 6.65). ROR-PT could not be assessed in TCGA because of missing data on tumor size.

Discussion

Using RNA expression data, this analysis classified breast cancer PAM50 subtype in a population-based sample of black and

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Table 4. Relative frequency of biomarker class among clinically defined HR-positive, HER2-negative cases, Carolina Breast Cancer Study 3 (2008–2013)

	Race and age, y					
		Black		White		
Genomic biomarker	All cases	<50 No. (%)	≥50 No. (%)	<50 No. (%)	≥50 No. (%)	*P*
PAM50 subtype						
Luminal A	276 (56.1)	40 (44.9)	66 (55.5)	66 (52.0)	104 (66.2)	.03*
Basal-like	26 (5.3)	7 (7.9)	7 (5.9)	8 (6.3)	4 (2.6)	
Her2-enriched	26 (5.3)	7 (7.9)	7 (5.9)	3 (2.4)	9 (5.7)	
Luminal B	135 (27.4)	30 (33.7)	33 (27.7)	42 (33.1)	30 (19.1)	
Normal-like	29 (5.9)	5 (5.6)	6 (5.0)	8 (6.3)	10 (6.4)	
ROR-P						
Mean score (SD)	29.07 (22.87)	35.44 (22.93)	32.78 (21.28)	28.54 (24.39)	23.08 (21.36)	<.001†
Low	133 (27.0)	16 (18.0)	21 (17.7)	38 (29.9)	58 (36.9)	<.001*
Medium	269 (54.7)	49 (55.1)	78 (65.6)	61 (48.0)	81 (51.6)	
High	90 (18.3)	24 (27.0)	20 (16.8)	28 (22.1)	18 (11.5)	
ROR-PT						
Mean score (SD)	36.71 (24.18)	45.40 (24.16)	40.66 (22.85)	36.34 (24.83)	28.87 (22.36)	<.001†
Low	117 (24.4)	13 (14.8)	18 (15.5)	35 (27.8)	51 (34.0)	<.001*
Medium	296 (61.7)	56 (63.6)	79 (68.1)	74 (58.7)	87 (58.0)	
High	67 (14.0)	19 (21.6)	19 (16.4)	17 (13.5)	12 (8.0)	
Missing	12	1	3	1	7	
Oncotype DX						
Mean score (SD)	18.85 (8.92)	18.83 (9.44)	20.20 (9.69)	17.57 (7.70)	18.96 (9.44)	.54†
Low	92 (48.2)	11 (45.8)	20 (43.5)	28 (51.9)	33 (49.3)	.91*
Medium	80 (41.9)	10 (41.7)	20 (43.5)	23 (42.6)	27 (40.3)	
High	19 (10.0)	3 (12.5)	6 (13.0)	3 (5.6)	7 (10.5)	
Missing	301	65	73	73	90	

*Two-sided chi-square *P* values exclude participants with missing data, and for PAM50 subtype, normal-like cases are excluded. HER2 = human epidermal growth factor receptor 2; HR = hormone receptor; ROR-P = risk of recurrence based on proliferation; ROR-PT = risk of recurrence based on tumor size.
†*P* value calculated using a two-sided analysis of variance test.

white women. As has been previously reported using other molecular methods (9), the results show a strong racial disparity in the frequency of Basal-like breast cancer, affecting both younger and older black women. The higher relative frequency of Basal-like breast cancer in black women is offset by a decreased frequency of Luminal A breast cancers. Black women also had higher frequency of Luminal B and HER2-enriched PAM50 subtype. Mortality disparities among clinically defined HR+/HER2- cases are greater than in other clinical subgroups (5), and these analyses suggest that tumor biology may contribute; black women with clinically defined HR+/HER2- disease are more likely to have aggressive PAM50 subtypes (Luminal B, HER2-enriched, or Basal-like) and high risk of recurrence (ROR) scores.

The potential of genomic biomarkers to guide clinical decision-making has the largest impact among HR+/HER2- cases, where genomic tests are most frequently utilized. Although clinically indistinguishable by standard tests, outcomes vary widely and racial disparities are pronounced in this group (4,5). Several commercial genomic tests are available clinically (Oncotype DX, Mammaprint, Prosigna), and relative to standard clinical markers or immunohistochemical surrogates (21,22), at least one previous observational study has shown that RNA-based subtyping more accurately predicts recurrence and survival (23). Based on the current analysis, all four tumor PAM50 subtypes occur in HR+/HER2- cases, with black women having higher frequency of non–Luminal A (Basal-like, HER2-enriched, and Luminal B) cancer relative to white women with the same clinical profile. The ROR-P and ROR-PT scores that track proliferation and proliferation plus tumor size, respectively, were higher in black women of all ages relative to white women. Quantitative Oncotype DX scores did not vary statistically significantly by race, but there were increased odds of a high risk score among black women overall. When considering categorical risk scores, only the ROR-PT showed statistically significantly higher odds of "high-risk" relative to "low- and medium-risk" tumors. While previous studies have evaluated uptake of Oncotype DX by race (8,24,25), few studies have compared categorical Oncotype DX scores by race among HR+/HER2- cases. Two previous studies found increased odds of high Oncotype DX recurrence score for black vs white women (26,27), with both reporting similar proportions of patients with high Oncotype DX. While racial differences for Oncotype DX scores were not statistically significant in this population, the magnitude and direction of our findings agree with those previous reports. Across multiple genomic biomarkers, high-risk tumors are more common (roughly 10% of the HR+/HER2- population) in black women.

The current analysis suggests that genomic subtyping could have important clinical implications. Among HR+/HER2- patients, higher risk scores in black patients suggest that adherence to American Society of Clinical Oncology guidelines (15) may result in chemotherapy or novel therapeutic approaches for a larger proportion of black patients (ie, because high risk scores are an indication for chemotherapy). Detection of

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Table 5. Odds ratios for association between race, age, and genomic biomarker class, Carolina Breast Cancer Study Phase 3

	Race and age, y			
	Black		White	
Genomic biomarker	<50 OR (95% CI)	≥50 OR (95% CI)	<50 OR (95% CI)	≥50 OR (95% CI)
PAM50 subtype				
Other vs Luminal A	2.40 (1.41 to 4.10)	1.58 (0.97 to 2.57)	1.81 (1.12 to 2.93)	1.00 (reference)
Other vs Luminal A*	1.93 (1.07 to 3.48)	1.43 (0.84 to 2.46)	1.73 (1.02 to 2.92)	1.00 (reference)
ROR-P				
High vs medium/low	2.85 (1.45 to 5.62)	1.56 (0.79 to 3.10)	2.18 (1.15 to 4.17)	1.00 (reference)
High vs medium/low*	2.26 (1.16 to 4.41)	2.50 (1.37 to 4.57)	1.30 (0.77 to 2.20)	1.00 (reference)
ROR-PT				
High vs medium/low	3.17 (1.45 to 6.90)	2.25 (1.05 to 4.86)	1.79 (0.82 to 3.91)	1.00 (reference)
High vs medium/low*	2.29 (1.13 to 4.64)	2.58 (1.37 to 4.87)	1.21 (0.71 to 2.08)	1.00 (reference)
Oncotype DX				
High vs medium/low	1.22 (0.29 to 5.17)	1.29 (0.40 to 4.11)	0.50 (0.12 to 2.05)	1.00 (reference)
High vs medium/low*	0.81 (0.16 to 4.06)	0.66 (0.18 to 2.45)	0.45 (0.10 to 2.01)	1.00 (reference)

*Adjusted for tumor size (≤2 cm vs > 2 cm), nodal status (negative vs positive), stage (I and II vs III and IV), and grade (I and II vs III), except in the case of risk of recurrence based on tumor size (ROR-PT), which was not adjusted for tumor size. ROR-PT explicitly includes size as part of the classifier. CI = confidence interval; OR = odds ratio; ROR-P = risk of recurrence based on proliferation; ROR-PT = risk of recurrence based on tumor size.

HER2-enriched status among clinically HER2- cases may also be targetable, given a recent clinical trial suggesting that these cases benefit from HER2-targeted therapies (28). Disparities in frequency of positive HER2 status by race have been evaluated previously in Surveillance, Epidemiology, and End Results data, and differences between black and white women have been weak (29). However, the current findings suggest that clinical tests may miss some HER2-enriched cancers that would benefit from biologic therapy. In our study, approximately 5% of clinically defined ER+/HER2- cases were HER2-enriched by PAM50 analysis.

The distribution of PAM50 subtype detected in our analysis differs from a previous population-based analysis conducted in the Life After Cancer Epidemiology (LACE) and Pathways studies (30) and suggests a poorer prognostic profile for blacks overall. In LACE/Pathways, roughly half of the tumors were Luminal A, whereas only 38% were Luminal A in CBCS3. Frequency of Luminal B (both studies approximately 20%) and HER2-enriched (13% in LACE/Pathways, 12% in CBCS3) tumors were similar, but CBCS3 had a much higher frequency of Basal-like breast cancer (9.8% in LACE/Pathways vs 25% in CBCS3). The differences between these two studies may reflect national geographic trends or differences in population genetics; a recent Report to the Nation has emphasized geographic variation in incidence of triple-negative breast cancer, with highest incidence in the southeastern United States (31). However, the most compelling differences between LACE/Pathways and CBCS are in race and age composition; namely, LACE/Pathways was predominantly (>75%) older women and fewer than 10% were black. A strength of the CBCS3 for disparities research is oversampling of young women (age < 50 years) and black women.

Our findings should be interpreted in light of some limitations. We used a research version of the PAM50, ROR-P, and ROR-PT and not the clinically approved Prosigna assay (which reports the ROR-PT score). Clinical data for these tests were unavailable. Oncotype DX data were available in the clinical record; however, data were missing for about 60% of HR-positive/HER2-negative patients in our study, limiting statistical power to detect small differences by race and age. While missing data limit comparisons of Oncotype DX and ROR-P/PT results, the Oncotype DX association with race is similar to those reported previously. Furthermore, the statistically significant association between race and ROR-PT persisted when restricting to the group of patients with clinically available Oncotype DX data. Considering PAM50 subtype, we lacked the statistical power to assess race- and age-associated differences in the frequency of HER2-enriched and Luminal B breast cancer. We did observe statistically significant differences in the frequency of both PAM50 subtypes by race, but could not further evaluate whether these differences were stronger for younger vs older black women. Finally, the CBCS3 did not collect screening records for the years prior to diagnosis, so we were unable to classify patients as to mode of detection (screen detected, interval detected, medically detected). Given that screening adherence patterns may differ by race and age and could lead to higher rates of indolent, screen-detected cancers in older white women, relationships between screening patterns and subtype frequency distributions merit further investigation.

Despite some limitations, these data clearly show that even within clinically defined subgroups, there are important biological differences between black and white women's tumors. A persistent high-priority research question is how tumor biological characteristics balance with patient-level variables (such as treatment adherence or access to quality diagnostic information that informs therapy) in the progression of HR+/HER2- breast cancers. CBCS3 recruitment ended in 2013, and survival data are not yet mature enough for analyses of how PAM50 or clinical subtype may mediate survival disparities, but future work will leverage the biological data collected herein, together with detailed risk factor and treatment data, to elucidate how multiple factors work together to produce differences in frequency distributions and overall poorer outcomes in all subtypes of breast cancer for black women.

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