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Article

Contribution of Germline Predisposition Gene Mutations to Breast Cancer Risk in African American Women

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Abstract

Background: The risks of breast cancer in African American (AA) women associated with inherited mutations in breast cancer predisposition genes are not well defined. Thus, whether multigene germline hereditary cancer testing panels are applicable to this population is unknown. We assessed associations between mutations in panel-based genes and breast cancer risk in 5054 AA women with breast cancer and 4993 unaffected AA women drawn from 10 epidemiologic studies. **Methods:** Germline DNA samples were sequenced for mutations in 23 cancer predisposition genes using a QIAseq multiplex amplicon panel. Prevalence of mutations and odds ratios (ORs) for associations with breast cancer risk were estimated with adjustment for study design, age, and family history of breast cancer. **Results:** Pathogenic mutations were identified in 10.3% of women with estrogen receptor (ER)-negative breast cancer, 5.2% of women with ER-positive breast cancer, and 2.3% of unaffected women. Mutations in *BRCA1*, *BRCA2*, and *PALB2* were associated with high risks of breast cancer (OR = 47.55, 95% confidence interval [CI] = 10.43 to >100; OR = 7.25, 95% CI = 4.07 to 14.12; OR = 8.54, 95% CI = 3.67 to 24.95, respectively). *RAD51D* mutations were associated with high risk of ER-negative disease (OR = 7.82, 95% CI = 1.61 to 57.42). Moderate risks were observed for *CHEK2*, *ATM*, *ERCC3*, and *FANCC* mutations with ER-positive cancer, and *RECQL* mutations with all breast cancer. **Conclusions:** The study identifies genes that predispose to breast cancer in the AA population, demonstrates the validity of current breast cancer testing panels for use in AA women, and provides a basis for increased referral of AA patients for cancer genetic testing.

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In recent years, there have been notable advances in knowledge of both prevalence and penetrance of germline inactivating mutations in genes that are associated with a moderate (relative risk 2–4) or high (relative risk >4) risk of breast cancer (1,2). In addition to *BRCA1* and *BRCA2* (3), at least 10 other genes [*ATM* (4,5), *BARD1* (6,7), *CDH1* (8), *CHEK2* (9), *NF1* (10), *PALB2* (11,12), *PTEN* (13), *RAD51C* (14), *RAD51D* (15), and *TP53* (16)] have been associated with moderate or high risk of breast cancer in women of European ancestry (EA). This information has been used to inform cancer risk management such as prophylactic surgery or enhanced screening and is increasingly being used to guide targeted treatments.

However, limited data are available from women of African ancestry, including from African American (AA) women, who have a higher incidence of breast cancer at young ages, a higher incidence of estrogen receptor (ER)-negative breast cancer, and a 42% higher breast cancer mortality rate than non-Hispanic white women (17,18). A recent study of African ancestry women reported relative risks for *BRCA1* and *BRCA2* only (19). Stable estimates of the prevalence of pathogenic mutations in AA women and the magnitude of associations between mutations and breast cancer risk are not available, despite being critical for informing appropriate recommendations for genetic testing and for counseling on preventive strategies. To fill these critical gaps, we assembled 5054 AA women with breast cancer and 4993 unaffected AA women from 10 epidemiologic studies and conducted uniform targeted multigene panel testing.

Methods

Study Sample

Participants were drawn from 5 prospective cohort studies, 3 case-control studies, 1 case-cohort study, and 1 case-only study (see Table 1; Supplementary Table 1, available online). The Northern California Breast Cancer Family Registry (27) preferentially selected women with a family history of breast cancer and the Black Women: Etiology and Survival of TNBC study (26) and the University of California Irvine Breast Cancer Study (28) preferentially selected women with breast cancer at young ages. Participants in all other studies were unselected with regard to family history or young age at diagnosis. For the cohort studies, 1 to 2 unaffected AA women were selected from among all unaffected women at the time of case patient diagnosis, matched to case patients on age. Most biospecimen samples in the cohort studies (83%) were obtained either before breast cancer diagnosis or less than a year after diagnosis. An additional study, the NIEHS Sister Study (31), which enrolled women who had a sister with breast cancer, contributed data only to stratified analyses restricted to women with a family history of breast cancer. Further descriptions of the studies are given in Supplementary Materials, including Supplementary Table 1 (available online). Institutional review boards at the Mayo Clinic and all contributing sites approved the research. All participants provided written informed consent.

DNA Sequencing

Genomic DNA samples were subjected to multiplex amplicon-based analysis of 746 target regions covering all coding regions and consensus splice sites from 37 cancer predisposition genes using a QIAseq (QIAGEN) (32) custom panel. These genes were selected because of inclusion on commercial hereditary cancer genetic testing panels or because of previous reports suggesting associations with breast, ovarian, endometrial, colorectal, or pancreatic cancer (2,33–35). The QIAseq protocol was optimized for high-throughput robotic processing of DNA samples and validated as previously described (36). Libraries were individually bar coded by dual indexing and sequenced in pools of 768 on a HiSeq4000. Median sequence read depth was 200X. Twenty-three genes previously implicated in breast cancer were evaluated for this study: *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CDKN2A*, *CHEK2*, *ERCC3*, *FANCC*, *FANCM*, *MLH1*, *MRE11A*, *MSH2*, *MSH6*, *NBN*, *NF1*, *PALB2*, *PTEN*, *RAD51C*, *RAD51D*, *RECQL*, and *TP53* (Supplementary Table 2, available online).

Table 1. Characteristics of study participants by breast cancer status*

Participant characteristics	Affected (n = 5054) No. (%)	Unaffected (n = 4993) No. (%)
Age in years, mean (SD)	54.4 (12.0)	55.2 (11.4)
Age in categories, y		
18–40	604 (12.0)	526 (10.6)
41–50	1411 (28.0)	1124 (22.6)
51–60	1452 (28.9)	1781 (35.8)
61–70	1039 (20.7)	1002 (20.2)
≥71	739 (14.7)	816 (16.4)
Missing	16 (0.32)	15 (0.30)
Study		
BWHS	1425 (28.2)	2871 (57.5)
CPS3	32 (0.6)	78 (1.6)
CPSII	58 (1.1)	48 (1.0)
CTS	55 (1.1)	50 (1.0)
MEC	681 (13.5)	702 (14.1)
BEST	397 (7.9)	0 (0.0)
NC-BCFR	667 (13.2)	54 (1.1)
UCIBCS	74 (1.5)	14 (0.3)
WCHS	1611 (31.9)	1120 (22.4)
WWHS	54 (1.1)	56 (1.1)
First-degree family history of breast cancer		
No	4038 (81.8)	4403 (89.2)
Yes	897 (18.2)	531 (10.8)
Unknown	119	59
Estrogen receptor status		
Negative	1340 (30.6)	—
Positive	3038 (69.4)	—
Unknown	676	—
Triple-negative breast cancer		
No	3370 (83.7)	—
Yes	654 (16.3)	—
Unknown	1030	—

*BWHS = Black Women's Health Study (20,21); CPS3 = Cancer Prevention Study 3 (22); CPSII = Cancer Prevention Study II (23); CTS = California Teachers Study (24); MEC = Multiethnic Cohort (25); BEST = Black Women: Etiology and Survival of TNBC (26); NC-BCFR = Northern California Breast Cancer Family Registry (27); UCIBCS = University of California Breast Cancer Study (28); WCHS = Women Circle of Health (29); WWHS = Wisconsin Women's Health Study (30).

Bioinformatics Analysis

FASTQ files of DNA sequences were generated for each sample from pools of 768 using dual indexing. Reads were trimmed with Cutadapt v1.10 and aligned with bwa-mem v0.7.10. Sequence realignment, recalibration, haplotype calling, and

Table 2. Frequency of pathogenic mutations in known or suspected breast cancer susceptibility genes and associations with breast cancer risk in African American women

Gene	Affected (n = 5054) No. of mutated alleles (mutation frequency, %)	Unaffected (n = 4993) No. of mutated alleles (mutation frequency, %)	OR* (95% CI)	P†
ATM	39 (0.77)	16 (0.32)	1.81 (1.00 to 3.44)	.058
BARD1	7 (0.14)	8 (0.16)	0.78 (0.26 to 2.27)	.64
BRCA1	81 (1.60)	1 (0.02)	47.55 (10.43 to >100)	<.001
BRCA2	98 (1.94)	12 (0.24)	7.25 (4.07 to 14.12)	<.001
BRIP1	9 (0.18)	6 (0.12)	1.24 (0.42 to 3.95)	.70
CDH1	4 (0.08)	2 (0.04)	1.53 (0.27 to 12.02	.64
CDKN2A	1 (0.02)	—	—‡	—
CHEK2	19 (0.38)	6 (0.12)	3.23 (1.31 to 9.16)	.016
ERCC3	14 (0.28)	9 (0.18)	2.40 (1.04 to 5.86)	.044
FANCC	21 (0.42)	10 (0.20)	2.03 (0.93 to 4.69)	.084
FANCM	13 (0.26)	11 (0.22)	1.14 (0.48 to 2.72)	.76
MLH1	—	1 (0.02)	—	—
MSH2	—	—	—	—
MRE11A	2 (0.04)	3 (0.06)	—	—
MSH6	4 (0.08)	2 (0.04)	1.77 (0.31 to 13.74)	.53
NBN	7 (0.14)	10 (0.20)	0.58 (0.20 to 1.59)	.29
NF1	6 (0.12)	1 (0.02)	—	—
PALB2	53 (1.05)	5 (0.10)	8.54 (3.67 to 24.95)	<.001
PTEN	—	—	—	—
RAD51C	9 (0.18)	3 (0.06)	3.00 (0.86 to 13.85)	.11
RAD51D	8 (0.16)	2 (0.04)	2.85 (0.66 to 19.90)	.21
RECQL	16 (0.32)	5 (0.10)	3.04 (1.15 to 9.54)	.036
TP53	5 (0.10)	1 (0.02)	—	—
Total	416 (8.23)	114 (2.28)		

*Odds ratios (ORs) adjusted for study design, age, and first-degree family history of breast cancer. Reference group is women who have no mutations in the given gene. CI = confidence interval.
†Two-sided *P* values from logistic regression analysis.
‡ORs not calculated because of small numbers of mutations.

depth of coverage were conducted using GATK v3.4–46. Nucleotide reads of greater than 20X was set as the quality control threshold for coverage, and 99.8% of samples had sequencing coverage above 20X for more than 90% of target nucleotides. Samples with high levels of homozygosity were excluded. Annotation of variants was provided through the BioR toolkit (37) leveraging dbNSFP v3.0 (38), ClinVar (39), and CAVA (40). Variants were viewed and filtered with VCF-Miner (41). Bam files of classified pathogenic variants were viewed by Integrative Genomics Viewer. All loss of function variants [nonsense, frameshift, consensus splice sites (+/-1 or 2)] and any intronic or missense variants defined as pathogenic or likely pathogenic in ClinVar by 2 or more clinical laboratories (Ambry Genetics, SCRP, InVitae, GeneDX, Counsyl, InSiGHT) were considered pathogenic. All suspected mosaic somatic variants (allele ratio > 80:20) and truncating variants in the last 55 bp of the penultimate exon or last exon that potentially avoid nonsense-mediated mRNA decay and do not influence known functional domains were excluded. Variants positioned after established cutoffs for protein function (eg, *BRCA2* p. Tyr3208X) were excluded. Reduced penetrance variants, all *CHEK2* missense variants, and variants with minor allele frequency greater than 0.3%, other than common founder mutations (eg, *CHEK2* c.1100delC), were excluded.

Statistical Analysis

Frequencies of mutations in each gene were estimated in affected and unaffected women for all studies combined. Associations between combined mutations in each gene and breast cancer risk were assessed using logistic regression adjusted for age, first-degree family history of breast cancer, and study design (cohort, case control, other). Separate analyses restricted to ER-positive, ER-negative, and triple-negative breast cancer (TNBC) (ER-negative, progesterone receptor negative, HER2-negative) status, and analyses stratified by age and family history were also conducted. A case-only logistic regression analysis for enrichment of mutations by ER-positive relative to ER-negative status was conducted. All analyses were performed in R (version 3.4.2), and all tests were two-sided. A *P* value of less than .05 was considered statistically significant. Lifetime absolute risk of breast cancer was estimated for carriers of mutations in specific genes by combining odds ratio (OR) estimates with age-specific AA breast cancer incidence rates from the Surveillance, Epidemiology, and End Results program and age-specific mortality rates from the Centers for Disease Control and Prevention in a competing risk model (Supplementary Materials, available online) (42).

Results

As shown in Table 1, mean age at diagnosis for women with breast cancer was 54.4 years, and 40% were diagnosed at age 50 years or younger. Eighteen percent of affected and 10.8% of unaffected women had a first-degree relative with breast cancer. Tumor ER status was available for 86% of breast cancers: 1340 (30.6%) were ER-negative and 3038 (69.4%) ER-positive.

Table 3. Frequency of pathogenic mutations in known or suspected breast cancer susceptibility genes and associations with breast cancer risk in African American women, in population-based studies*

Gene	Affected (n = 3916) No. of mutated alleles (mutation frequency, %)	Unaffected (n = 4925) No. of mutated alleles (mutation frequency, %)	OR†(95% CI)	P‡
ATM	28 (0.72)	16 (0.33)	1.81 (0.97 to 3.48)	.067
BARD1	7 (0.18)	8 (0.16)	0.98 (0.34 to 2.80)	.97
BRCA1	41 (1.05)	1 (0.02)	42.79 (9.24 to >100)	<.001
BRCA2	72 (1.84)	12 (0.24)	7.31 (4.08 to 14.29)	<.001
BRIP1	6 (0.15)	6 (0.12)	1.14 (0.34 to 3.79)	.83
CHEK2	15 (0.38)	6 (0.12)	3.17 (1.26 to 9.06)	.020
ERCC3	13 (0.33)	9 (0.18)	2.35 (1.01 to 5.76)	.051
FANCC	16 (0.41)	10 (0.20)	2.24 (1.02 to 5.18)	.049
FANCM	11 (0.28)	11 (0.22)	1.17 (0.49 to 2.82)	.72
NBN	4 (0.10)	9 (0.18)	0.51 (0.13 to 1.63)	.28
PALB2	39 (1.00)	5 (0.10)	8.37 (3.56 to 24.57)	<.001
RAD51C	7 (0.18)	3 (0.06)	2.95 (0.80 to 13.72)	.12
RAD51D	6 (0.15)	2 (0.04)	3.06 (0.67 to 21.50)	.18
RECQL	12 (0.31)	5 (0.10)	2.94 (1.07 to 9.37)	.047
Total	277 (7.07)	103 (2.09)		

*Studies that did not preferentially enroll cases based on family history or age; studies included were Black Women's Health Study, Cancer Prevention Study II, Cancer Prevention Study 3, California Teachers Study, Multiethnic Cohort, Women Circle of Health, and Wisconsin Women's Health Study. CI = confidence interval.
†Odds ratios (ORs) adjusted for study design, age, and first-degree family history of breast cancer. Reference group is women who have no mutations in the given gene.
‡Two-sided *P* values from logistic regression analysis.

Pathogenic mutations in the 23 genes tested were identified in 416 (8.2%) of 5054 affected women and 114 (2.3%) of 4993 unaffected women (Table 2). Among women with breast cancer, 81 (1.6%) had mutations in *BRCA1*, 98 (1.9%) in *BRCA2*, and 53 (1.0%) in *PALB2*. In contrast, only 1 mutation in *BRCA1* (0.02%), 12 (0.24%) in *BRCA2*, and 5 (0.10%) in *PALB2* were observed in unaffected women. We compared *BRCA1* mutations classified as benign or as variants of unknown significance and found similar frequencies in affected vs unaffected women (6.0% vs 6.5% for benign and 5.2% vs 4.5% for variants of unknown significance; data not shown), suggesting that the low frequency of *BRCA1* mutations in unaffected women was not caused by sequencing issues. Mutations in genes associated with complex syndromes including *CDH1*, *NF1*, *PTEN*, and *TP53* were observed in 15 (0.30%) affected and 4 (0.08%) unaffected women. When we restricted to the 12 genes previously shown to confer moderate or high risk of breast cancer in women of EA (*ATM*, *BARD1*, *BRCA1*, *BRCA2*, *CDH1*, *CHEK2*, *NF1*, *PALB2*, *PTEN*, *RAD51C*, *RAD51D*, and *TP53*) (1, 2), mutations were identified in 6.5% of affected and 1.1% of unaffected women.

Several recurrent mutations were observed, including 8 that accounted for 51% of the 81 mutations in *BRCA1* among affected women (Supplementary Table 3, available online). The most frequent recurrent *BRCA1* mutation (c.815_824dup10) has been previously reported as being of African origin (43). The 5 most common mutations in *BRCA2* accounted for only 22% of all mutations. The c.1100delC *CHEK2* recurrent mutation from non-Hispanic whites accounted for 9 of 19 and 2 of 6 *CHEK2* mutations in affected and unaffected women, respectively; c.1354C>T, p. Arg452X in *ERCC3* accounted for 6 of 14 and 5 of 9 *ERCC3* mutations in affected and unaffected, respectively; c.355_360delTCTCATinsA in *FANCC* accounted for 13 of 21 and 7 of 10 *FANCC* mutations in affected and unaffected, respectively; and c.3323delA accounted for 8 of 53 and 1 of 5 *PALB2* mutations in affected and unaffected, respectively.

As shown in Table 2, statistically significant associations with increased breast cancer risk were observed for 6 genes (*BRCA1*, *BRCA2*, *CHEK2*, *ERCC3*, *PALB2*, and *RECQL*). In addition, the odds ratio for an association of breast cancer with mutations in *ATM* was 1.81 (95% CI = 1.00 to 3.44). Odds ratios were greater than 4.0 for *BRCA1* (OR = 47.55, 95% CI = 10.43 to 842), *BRCA2* (OR = 7.25, 95% CI = 4.07 to 14.12), and *PALB2* (OR = 8.54, 95% CI = 3.67 to 24.95). Protein truncating mutations in *CHEK2* (OR = 3.23, 95% CI = 1.31 to 9.16), *ERCC3* (OR = 2.40, 95% CI = 1.04 to 5.86), and *RECQL* (OR = 3.04, 95% CI = 1.15 to 9.54) were associated with moderately increased risks of breast cancer. In an analysis restricted to population-based studies (Table 3), results were similar, and further control for individual study did not materially change the odds ratio estimates (Supplementary Table 4, available online). In a sensitivity analysis that excluded affected women if samples were provided more than a year after breast cancer diagnosis (excluded n = 837), odds ratios were essentially unchanged (eg, OR = 31.9, 95% CI = 6.7 to >100 for *BRCA1*; OR = 7.1, 95% CI = 3.9 to 14.2 for *BRCA2*; and OR = 9.7, 95% CI = 4.1 to 28.9 for *PALB2*).

Table 4 provides mutation data and association results separately for ER-negative and ER-positive AA breast cancer. Pathogenic mutations were identified in 10.3% of women with ER-negative breast cancer, 5.2% of women with ER-positive breast cancer, and 2.3% of unaffected women. *BRCA1*, *BRCA2*, and *PALB2* mutations were associated with increased risks of ER-negative breast cancer (Table 4) and TNBC (Supplementary Table 5, available online). There was evidence of association for both *RAD51D* (OR = 7.82, 95% CI = 1.61 to 57.42) and *RAD51C* (OR = 4.23, 95% CI = 0.88 to 22.72) with increased risk of ER-negative breast cancer (Table 4). *BRCA1* ($P < .001$), *PALB2* ($P = .003$), and *RAD51D* ($P = .015$) mutations were more strongly associated with ER-negative than ER-positive breast cancer (Supplementary Table 6, available online), although odds ratios for *BRCA1* and *PALB2* were above 5.0 for both ER subtypes. *ATM*,

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Table 4. Associations between pathogenic mutations in breast cancer predisposition genes and estrogen receptor tumor subtype in African American women

Gene	Estrogen receptor-negative breast cancer			Estrogen receptor-positive breast cancer		
	Affected (n = 1340) No. of mutated alleles (mutation frequency, %)	OR* (95% CI)	P†	Affected (n = 3038) No. of mutated alleles (mutation frequency, %)	OR* (95% CI)	P†
ATM	6 (0.44)	1.00 (0.34 to 2.60)	.99	28 (0.92)	2.08 (1.09 to 4.08)	.029
BARD1	3 (0.22)	1.21 (0.25 to 4.54)	.78	3 (0.10)	0.66 (0.14 to 2.41)	.55
BRCA1	60 (4.48)	129.7 (28.0 to >100)	<.001	19 (0.63)	15.58 (3.09 to >100)	.008
BRCA2	31 (2.31)	9.38 (4.76 to 19.62)	<.001	57 (1.88)	6.83 (3.67 to 13.72)	<.001
BRIP1	3 (0.22)	1.72 (0.34 to 7.23)	.47	4 (0.13)	0.85 (0.20 to 3.30)	.82
CDH1	3 (0.22)	4.75 (0.69 to 40.6)	.47	1 (0.03)	—	—
CHEK2	2 (0.15)	—‡	—	14 (0.46)	4.02 (1.52 to 11.86)	.007
ERCC3	3 (0.22)	1.95 (0.42 to 6.81)	.33	10 (0.33)	2.76 (1.08 to 7.10)	.032
FANCC	5 (0.37)	2.01 (0.60 to 6.01)	.23	14 (0.46)	2.42 (1.00 to 5.97)	.050
NF1	2 (0.15)	—	—	4 (0.13)	9.94 (1.37 to >100)	.045
PALB2	22 (1.64)	15.57 (6.09 to 47.97)	< .001	22 (0.72)	5.44 (2.07 to 17.13)	.001
RAD51C	4 (0.30)	4.23 (0.88 to 22.72)	.071	4 (0.13)	2.22 (0.47 to 11.71)	.31
RAD51D	6 (0.44)	7.82 (1.61 to 57.42)	.018	2 (0.07)	—	—
RECQL	4 (0.30)	2.44 (0.56 to 9.99)	.21	8 (0.26)	2.65 (0.83 to 9.20)	.10
TP53	—	—	—	5 (0.16)	9.24 (1.29 to >100)	.053

*Odds ratios (ORs) adjusted for study design, age, and first-degree family history of breast cancer. Reference group is women who have no mutations in a given gene. CI = confidence interval.
†Two-sided P values from logistic regression analysis.
‡ORs not calculated because of small numbers of mutations.

CHEK2, *ERCC3*, and *FANCC* were associated with ER-positive breast cancer only.

In an effort to confirm validity of the sequencing, we compared mutation frequencies in the AA unaffected women with frequencies in approximately 7500 gnomAD African/African American reference samples (Supplementary Table 7, available online). Comparing AA women with breast cancer with gnomAD African/African American controls yielded odds ratios of 2.4, 2.6, and 2.0 for *ERCC3*, *FANCC*, and *RECQL*, respectively, consistent with the results from the primary analysis, and the odds ratio for *BRCA1* was 13.4, based on 9 controls in gnomAD (Supplementary Table 7, available online).

Results of association analyses stratified on age (younger than 50 years and 50 years and older) (Supplementary Table 8, available online) and by first-degree family history of breast cancer (Supplementary Tables 9 and 10, available online) are also provided. Associations were generally stronger in younger women. Odds ratios were similar after exclusion of women with a family history of breast cancer.

Mutation frequencies in patient categories defined by age and first-degree family history of breast cancer are presented in Figure 1 and Supplementary Table 11, available online. As expected, a high proportion of AA women with breast cancer diagnosed before age 40 years and with a first-degree family history of breast cancer had *BRCA1* mutations, and the prevalence of *BRCA1* mutations decreased sharply with age at diagnosis. Overall, there was a higher frequency of *BRCA1* mutations in women with ER-negative breast cancer (4.5%) than in women with ER-positive cancer (0.63%). In contrast, women with ER-negative breast cancer and those with ER-positive breast cancer had similar frequencies of *BRCA2* mutations.

When mutations in any of the 12 genes that have been shown to be associated with moderate or high increase in breast cancer risk (2) were considered, mutation frequencies were strikingly different for ER-negative and ER-positive breast cancer (Figure 1; Supplementary Table 11, available online). In every age group, the prevalence of mutations was approximately twofold higher for ER-negative than for ER-positive disease. Among women aged 40 years and younger, 21% of all those with ER-negative cancer carried a mutation; among women older than 60 years of age, the proportion was 7.0%. As expected, the prevalence of mutations was highest among women with a first-degree family history of breast cancer.

Characteristics of breast cancers from AA women in the population-based studies were similar to characteristics of breast cancers reported to Surveillance, Epidemiology, and End Results registries for AA women during the same time period, as shown in Supplementary Table 12 (available online). Based on mutation frequency and relative risk, we were able to compute estimated absolute risks of breast cancer in *BRCA2*, *PALB2*, *CHEK2*, and *ATM* AA mutation carriers; absolute risks were 48%, 58%, 30%, and 21% by age 85 years, respectively (Figure 2).

Discussion

The present findings come from the first large study aimed at identifying breast cancer predisposition genes in an African ancestry population, with 5054 affected and 4993 unaffected AA women. Pathogenic mutations in the 12 genes for which there is the most evidence of association (2), primarily from EA populations, were identified in 6.5% of AA women with breast cancer and 1.1% of unaffected AA women. The frequency of pathogenic mutations was especially high (10.3%) in women with ER-negative breast cancer. This study provides the first estimates of breast cancer risk in African ancestry women associated with predisposition genes other than *BRCA1* and *BRCA2*. The observed associations confirm the utility of current hereditary cancer multigene testing panels for AA women.

We could not accurately estimate relative risk of breast cancer for women with mutations in the *BRCA1* gene because only

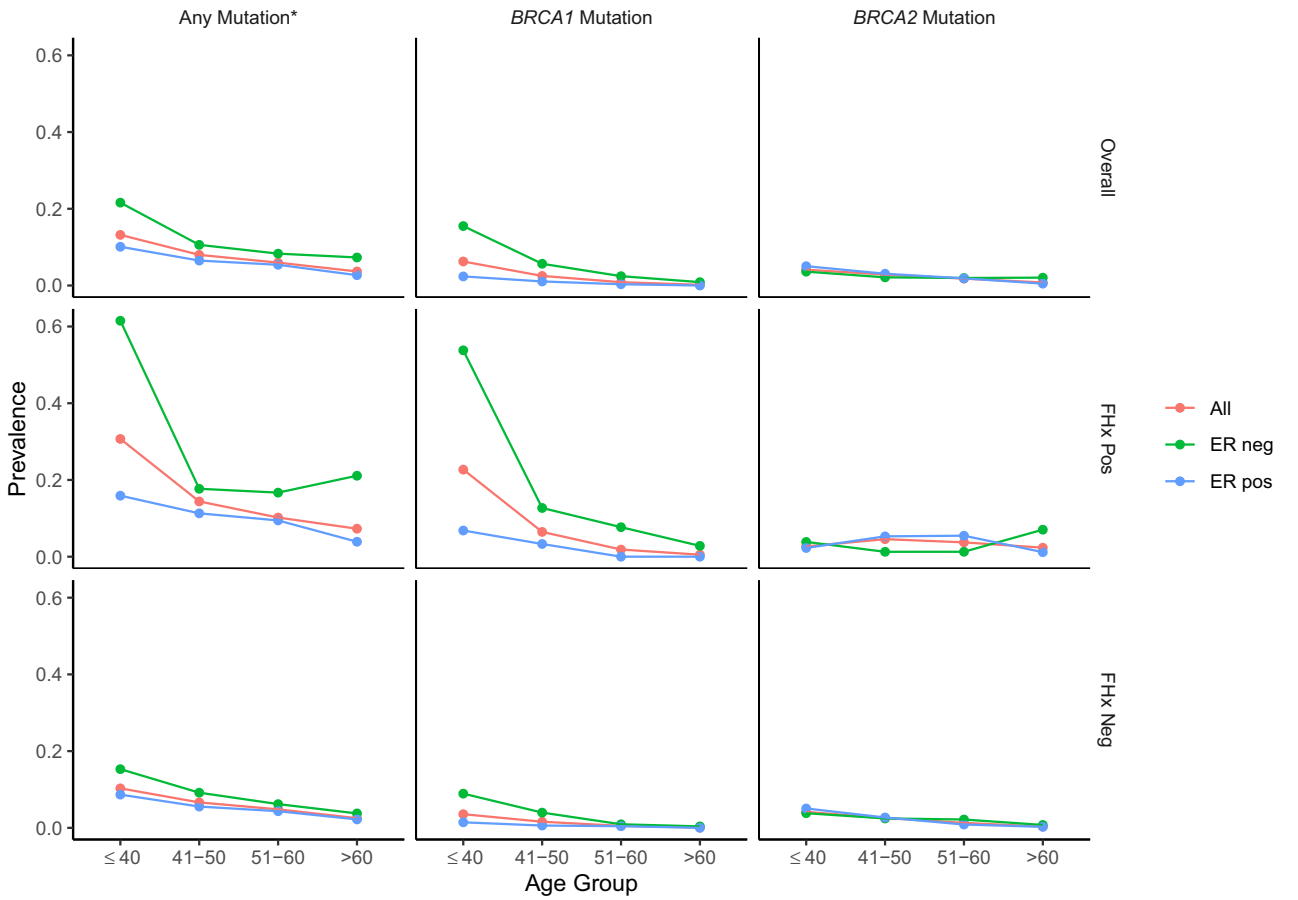

Figure 1. Prevalence of mutations in women with any breast cancer, estrogen receptor-negative (ER-neg) breast cancer, and ER-positive (ER-pos) breast cancer, according to age (in years) at diagnosis and first-degree family history (FHx) of breast cancer. *Any mutation indicates any pathogenic mutation in 12 known breast cancer predisposition genes (*ATM*, *BARD1*, *BRCA1*, *BRCA2*, *CDH1*, *CHEK2*, *NF1*, *PALB2*, *PTEN*, *RAD51C*, *RAD51D*, and *TP53*).

1 (age 52 years) of the 4993 unaffected women had a pathogenic mutation in *BRCA1* (frequency 0.02%). Sequencing problems were excluded as the reason for the low number of mutations in unaffected women. Because the expected number of mutations is quite small, the result could be attributed to unstable estimates. Alternatively, this finding may indicate that *BRCA1* mutations are highly penetrant in women of African ancestry. There are limited data on *BRCA1* mutation frequency in African ancestry women without breast cancer. The earliest reports on mutation frequencies in breast cancer susceptibility genes among AA women with breast cancer were based on small numbers of patients, ranging from 30 to 483 (44–49). Among 213 unaffected women in a US case-control study (44), no *BRCA1* mutations were identified, and among 1089 unselected women in the Bahamas (50), only 1 (0.09%) had a mutation. We examined data from the gnomAD database (noncancer gnomAD v2.1) for African ancestry women without cancer; only 3 individuals out of approximately 10 000 screened had a *BRCA1* mutation. Similarly, in data from the Exome Aggregation Consortium African ancestry population, with exclusion of The Cancer Genome Atlas cases, no *BRCA1* mutations were observed, whereas the prevalence of *BRCA2* mutations was 0.16% (51). These very low frequencies are in line with our results. In contrast, 3 of 997 control patients (0.3%) in a Nigerian case-control study (19) had pathogenic mutations in *BRCA1*, perhaps due to their younger ages and higher proportion of TNBCs.

This study also yielded data suggesting that mutations in *ERCC3* and *FANCC* are associated with increased risk of breast cancer in the AA population. Previous studies of associations between these genes and breast cancer risk were focused on the c.1354C>T, p. Arg452X *ERCC3* recurrent mutation in the Ashkenazi Jewish population (52) and the c.355_360delTCTCATinsA mutation in *FANCC* in EA women (53). However, in the current study, other pathogenic mutations in these genes contributed to the associations. For *RECQL*, mutations observed in the present study (c.1667_1667 + 3delAGTA) were previously associated with increased risk of breast cancer in a Polish population (54). Furthermore, we noted that the estimated relative risk for mutations in *ATM* was lower than previous findings in EA populations (2). Given the potential for multiple testing effects, further analyses of these genes in the AA population will be needed to verify the associations with breast cancer risk.

The present study included 1340 women with ER-negative breast cancer, allowing for ER-specific analyses as well as analyses of TNBC. Our results are generally consistent with an earlier report from sequencing of germline DNA from 2148 TNBC patients unselected for family history of breast cancer (55,56) and 8753 TNBC patients subjected to clinical testing (56), most of European ancestry. The present sample was underpowered for assessment of associations of TNBC with mutations in *BARD1* and *BRIP1*, which were reported in the earlier research (56,57).

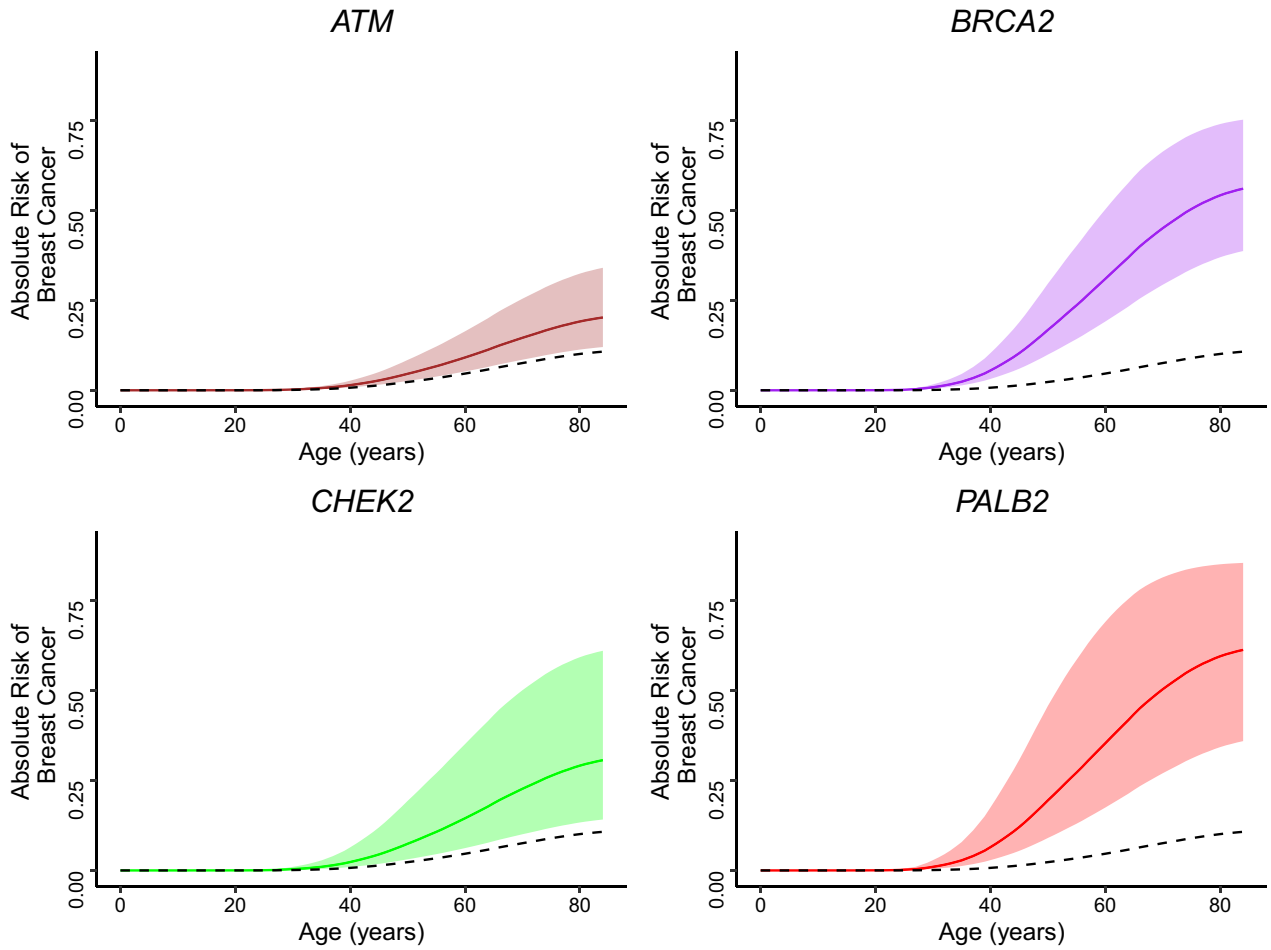

Figure 2. Estimates of absolute risk of breast cancer through age 85 years in African American women who are carriers of pathogenic mutations in 4 specific genes. **Dotted line** in each figure represents absolute risk of US population of African American women regardless of carrier status.

The major limitation of the present study is sample size. Although there were 5 times as many affected women as in the next largest study of African ancestry breast cancer (19), confidence intervals were wide, preventing certainty about the magnitude of associations. However, inclusion of a sizable number of affected women without a family history of breast cancer was a strength of the study. This allowed estimation of the prevalence of mutations within age and family history strata—information that is critical for establishing genetic testing guidelines for AA women. Although samples were from multiple studies, sequencing of all samples was performed at a single site under uniform conditions. Most study participants came from either population-based breast cancer case-control studies or prospective cohort studies in which affected and unaffected women arose from the same population. All analyses were controlled for study design, first-degree family history of breast cancer, and age.

In summary, there are several key findings from the present study. First, multiple genes previously established as breast cancer susceptibility genes in EA populations are also of importance for breast cancer in AA women. Second, mutations were identified in approximately 2 times as many women with ER-negative breast cancer as compared with women with ER-positive cancer; these findings support the importance of genetic testing in AA women with ER-negative breast cancer or TNBC, which disproportionately occurs in AA women. Finally, mutations in *ERCC3*, *FANCC*, and *RECQL* may be associated with AA breast cancer risk, although further studies are needed to confirm these findings.

Multiple studies have demonstrated that rates of *BRCA1* and *BRCA2* testing in the United States are substantially lower in AA than EA women (58–62). Disparities in testing are partly driven by differences in recommendations given to AA women, possibly because of misconceptions among physicians about the prevalence of mutations and associated risks in AA women (62). Genetic test results could impact decisions about risk-reducing surgeries or genetic testing of family members. The present results demonstrate, for the first time, the validity and utility of gene-panel testing, beyond *BRCA1* and *BRCA2*, for breast cancer in AA women. Testing will be particularly valuable for women diagnosed with ER-negative and/or TNBC and their families.

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References

1. Easton DF, Pharoah PD, Antoniou AC, et al. Gene-panel sequencing and the prediction of breast-cancer risk. *N Engl J Med*. 2015;372(23):2243–2257.
2. Couch FJ, Shimelis H, Hu C, et al. Associations between cancer predisposition testing panel genes and breast cancer. *JAMA Oncol*. 2017;3(9):1190–1196.
3. King MC, Marks JH, Mandell JB, et al. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. *Science*. 2003;302(5645):643–646.
4. Goldgar DE, BCFR, Healey S, Dowty JG, et al. Rare variants in the ATM gene and risk of breast cancer. *Breast Cancer Res*. 2011;13(4):R73.
5. Renwick A, Thompson D, Seal S, et al. Breast Cancer Susceptibility Collaboration (UK). ATM mutations that cause ataxia-telangiectasia are breast cancer susceptibility alleles. *Nat Genet*. 2006;38(8):873–875.
6. Baer R. Luring BRCA1 to the scene of the crime. *Cancer Cell*. 2013;23(5):565–567.
7. Brzovic PS, Keeffe JR, Nishikawa H, et al. Binding and recognition in the assembly of an active BRCA1/BARD1 ubiquitin-ligase complex. *Proc Natl Acad Sci USA*. 2003;100(10):5646–5651.
8. Pharoah PD, Guilford P, Caldas C. Incidence of gastric cancer and breast cancer in CDH1 (E-cadherin) mutation carriers from hereditary diffuse gastric cancer families. *Gastroenterology*. 2001;121(6):1348–1353.
9. Weischer M, Nordestgaard BG, Pharoah P, et al. CHEK2*1100delC heterozygosity in women with breast cancer associated with early death, breast cancer-specific death, and increased risk of a second breast cancer. *J Clin Oncol*. 2012;30(35):4308–4316.
10. Madanikia SA, Bergner A, Ye X, et al. Increased risk of breast cancer in women with NF1. *Am J Med Genet A*. 2012;158A(12):3056–3060.
11. Tischkowitz M, Capanu M, Sabbaghian N, et al. WECARE Study Collaborative Group. Rare germline mutations in PALB2 and breast cancer risk: a population-based study. *Hum Mutat*. 2012;33(4):674–680.
12. Antoniou AC, Casadei S, Heikkinen T, et al. Breast-cancer risk in families with mutations in PALB2. *N Engl J Med*. 2014;371(6):497–492.
13. Tan MH, Mester JL, Ngeow J, et al. Lifetime cancer risks in individuals with germline PTEN mutations. *Clin Cancer Res*. 2012;18(2):400–407.
14. Meindl A, Hellebrand H, Wiek C, et al. Germline mutations in breast and ovarian cancer pedigrees establish RAD51C as a human cancer susceptibility gene. *Nat Genet*. 2010;42(5):410–414.
15. Loveday C, Turnbull C, Ramsay E, et al. Breast Cancer Susceptibility Collaboration (UK). Germline mutations in RAD51D confer susceptibility to ovarian cancer. *Nat Genet*. 2011;43(9):879–882.
16. Gonzalez KD, Noltner KA, Buzin CH, et al. Beyond Li Fraumeni Syndrome: clinical characteristics of families with p53 germline mutations. *J Clin Oncol*. 2009;27(8):1250–1256.
17. DeSantis CE, Miller KD, Goding Sauer A, et al. Cancer statistics for African Americans, 2019. *CA Cancer J Clin*. 2019;69(3):211–233.
18. Dunn BK, Agurs-Collins T, Browne D, et al. Health disparities in breast cancer: biology meets socioeconomic status. *Breast Cancer Res Treat*. 2010;121(2):281–292.
19. Zheng Y, Walsh T, Gulsuner S, et al. Inherited breast cancer in Nigerian women. *J Clin Oncol*. 2018;36(28):2820–2825.
20. Rosenberg L, Adams-Campbell L, Palmer JR. The Black Women's Health Study: a follow-up study for causes and preventions of illness. *J Am Med Womens Assoc (1972)*. 1995;50(2):56–58.
21. 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):127–134.
22. Patel AV, Jacobs EJ, Dudas DM, et al. The American Cancer Society's Cancer Prevention Study 3 (CPS-3): recruitment, study design, and baseline characteristics. *Cancer*. 2017;123(11):2014–2024.
23. Calle EE, Rodriguez C, Jacobs EJ, et al. The American Cancer Society Cancer Prevention Study II Nutrition Cohort: rationale, study design, and baseline characteristics. *Cancer*. 2002;94(2):500–511.
24. Bernstein L, Allen M, Anton-Culver H, et al. High breast cancer incidence rates among California teachers: results from the California Teachers Study (United States). *Cancer Causes Control*. 2002;13(7):625–635.
25. Kolonel LN, Henderson BE, Hankin JH, et al. A multiethnic cohort in Hawaii and Los Angeles: baseline characteristics. *Am J Epidemiol*. 2000;151(4):346–357.
26. Pal T, Bonner D, Cragun D, et al. A high frequency of BRCA mutations in young black women with breast cancer residing in Florida. *Cancer*. 2015;121(23):4173–4180.
27. John EM, Sangaramoorthy M, Koo J, et al. Enrollment and biospecimen collection in a multiethnic family cohort: the Northern California site of the Breast Cancer Family Registry. *Cancer Causes Control*. 2019;30(4):395–408.
28. Anton-Culver H, Cohen PF, Gildea ME, et al. Characteristics of BRCA1 mutations in a population-based case series of breast and ovarian cancer. *Eur J Cancer*. 2000;36(10):1200–1208.
29. 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;2009:1–15.
30. Trentham-Dietz A, Sprague BL, Hampton JM, et al. Modification of breast cancer risk according to age and menopausal status: a combined analysis of five population-based case-control studies. *Breast Cancer Res Treat*. 2014;145(1):165–175.
31. Sandler DP, Hodgson ME, Deming-Halverson SL, et al. Sister Study Research Team. The Sister Study Cohort: baseline methods and participant characteristics. *Environ Health Perspect*. 2017;125(12):127003.
32. Lange V, Bohme I, Hofmann J, et al. Cost-efficient high-throughput HLA typing by MiSeq amplicon sequencing. *BMC Genomics*. 2014;15(1):63.
33. Buys SS, Sandbach JF, Gammon A, et al. A study of over 35,000 women with breast cancer tested with a 25-gene panel of hereditary cancer genes. *Cancer*. 2017;123(10):1721–1730.
34. Kurian AW, Li Y, Hamilton AS, et al. Gaps in incorporating germline genetic testing into treatment decision-making for early-stage breast cancer. *J Clin Oncol*. 2017;35(20):2232–2239.
35. Susswein LR, Marshall ML, Nusbaum R, et al. Pathogenic and likely pathogenic variant prevalence among the first 10,000 patients referred for next-generation cancer panel testing. *Genet Med*. 2016;18(8):823–832.
36. Hu C, Hart SN, Polley EC, et al. Association between inherited germline mutations in cancer predisposition genes and risk of pancreatic cancer. *JAMA*. 2018;319(23):2401–2409.
37. Kocher JP, Quest DJ, Duffy P, et al. The Biological Reference Repository (BioR): a rapid and flexible system for genomics annotation. *Bioinformatics*. 2014;30(13):1920–1922.
38. Liu X, Wu C, Li C, et al. dbNSFP v3.0: a one-stop database of functional predictions and annotations for human nonsynonymous and splice-site SNVs. *Hum Mutat*. 2016;37(3):235–241.
39. Landrum MJ, Lee JM, Benson M, et al. ClinVar: public archive of interpretations of clinically relevant variants. *Nucleic Acids Res*. 2016;44(D1):D862–8.
40. Munz M, Ruark E, Renwick A, et al. CSN and CAVA: variant annotation tools for rapid, robust next-generation sequencing analysis in the clinical setting. *Genome Med*. 2015;7(1):76.
41. Hart SN, Duffy P, Quest DJ, et al. VCF-Miner: GUI-based application for mining variants and annotations stored in VCF files. *Brief Bioinform*. 2016;17(2):346–351.
42. Chatterjee N, Shi J, Garcia-Closas M. Developing and evaluating polygenic risk prediction models for stratified disease prevention. *Nat Rev Genet*. 2016;17(7):392–406.
43. Rebbeck TR, Friebel TM, Friedman E, et al. EMBRACE. Mutational spectrum in a worldwide study of 29,700 families with BRCA1 or BRCA2 mutations. *Hum Mutat*. 2018;39(5):593–620.
44. Malone KE, Daling JR, Doody DR, et al. Prevalence and predictors of BRCA1 and BRCA2 mutations in a population-based study of breast cancer in white and black American women ages 35 to 64 years. *Cancer Res*. 2006;66(16):8297–8308.
45. John EM, Miron A, Gong G, et al. Prevalence of pathogenic BRCA1 mutation carriers in 5 US racial/ethnic groups. *JAMA*. 2007;298(24):2869–2876.
46. Nanda R, Schumm LP, Cummings S, et al. Genetic testing in an ethnically diverse cohort of high-risk women: a comparative analysis of BRCA1 and BRCA2 mutations in American families of European and African ancestry. *JAMA*. 2005;294(15):1925–1933.

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47. Fackenthal JD, Zhang J, Zhang B, et al. High prevalence of BRCA1 and BRCA2 mutations in unselected Nigerian breast cancer patients. *Int J Cancer*. 2012; 131(5):1114–1123.
48. Haffty BG, Silber A, Matloff E, et al. Racial differences in the incidence of BRCA1 and BRCA2 mutations in a cohort of early onset breast cancer patients: African American compared to white women. *J Med Genet*. 2005; 43(2):133–137.
49. Frank TS, Deffenbaugh AM, Reid JE, et al. Clinical characteristics of individuals with germline mutations in BRCA1 and BRCA2: analysis of 10,000 individuals. *J Clin Oncol*. 2002;20(6):1480–1490.
50. Trottier M, Lunn J, Butler R, et al. Prevalence of founder mutations in the BRCA1 and BRCA2 genes among unaffected women from the Bahamas. *Clin Genet*. 2016;89(3):328–331.
51. Maxwell KN, Domchek SM, Nathanson KL, Robson ME. Population frequency of germline BRCA1/2 mutations. *J Clinical Oncol*. 2016;34(34):4183–4185.
52. Vijai J, Topka S, Villano D, et al. A recurrent ERCC3 truncating mutation confers moderate risk for breast cancer. *Cancer Discov*. 2016;6(11):1267–1275.
53. Thompson ER, Doyle MA, Ryland GL, et al. Exome sequencing identifies rare deleterious mutations in DNA repair genes FANCC and BLM as potential breast cancer susceptibility alleles. *PLoS Genet*. 2012;8(9):e1002894.
54. Cybulski C, Carrot-Zhang J, Kluzniak W, et al. Germline RECQL mutations are associated with breast cancer susceptibility. *Nat Genet*. 2015;47(6): 643–646.
55. Couch FJ, Hart SN, Sharma P, et al. Inherited mutations in 17 breast cancer susceptibility genes among a large triple-negative breast cancer cohort unselected for family history of breast cancer. *J Clin Oncol*. 2015;33(4):304–311.
56. Shimelis H, LaDuca H, Hu C, et al. Triple-negative breast cancer risk genes identified by multigene hereditary cancer panel testing. *J Natl Cancer Inst*. 2018;110(8):855–862.
57. Lek M, Karczewski KJ, Minikel EV, et al. Exome Aggregation Consortium. Analysis of protein-coding genetic variation in 60,706 humans. *Nature*. 2016; 536(7616):285–291.
58. Jagsi R, Griffith KA, Kurian AW, et al. Concerns about cancer risk and experiences with genetic testing in a diverse population of patients with breast cancer. *J Clin Oncol*. 2015;33(14):1584–1591.
59. Armstrong K, Micco E, Carney A, et al. Racial differences in the use of BRCA1/2 testing among women with a family history of breast or ovarian cancer. *JAMA*. 2005;293(14):1729–1736.
60. Levy DE, Byfield SD, Comstock CB, et al. Underutilization of BRCA1/2 testing to guide breast cancer treatment: black and Hispanic women particularly at risk. *Genet Med*. 2011;13(4):349–355.
61. Susswein LR, Skrzynia C, Lange LA, et al. Increased uptake of BRCA1/2 genetic testing among African American women with a recent diagnosis of breast cancer. *J Clin Oncol*. 2008;26(1):32–36.
62. McCarthy AM, Bristol M, Domchek SM, et al. Health care segregation, physician recommendation, and racial disparities in BRCA1/2 testing among women with breast cancer. *J Clin Oncol*. 2016;34(22):2610–2618.