Breast Cancer Risk in Women from Ghana Carrying Rare Germline Pathogenic Mutations

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ABSTRACT

Background: Risk estimates for women carrying germline mutations in breast cancer susceptibility genes are mainly based on studies of European ancestry women.

Methods: We investigated associations between pathogenic variants (PV) in 34 genes with breast cancer risk in 871 cases [307 estrogen receptor (ER)-positive, 321 ER-negative, and 243 ER-unknown] and 1,563 controls in the Ghana Breast Health Study (GBHS), and estimated lifetime risk for carriers. We compared results with those for European, Asian, and African American ancestry women.

Results: The frequency of PV in GBHS for nine breast cancer genes was 8.38% in cases and 1.22% in controls. Relative risk estimates for overall breast cancer were: (OR, 13.70; 95% confidence interval (CI), 4.03–46.51) for *BRCA1*, (OR, 7.02; 95% CI, 3.17–15.54) for *BRCA2*, (OR, 17.25; 95% CI, 2.15–138.13) for *PALB2*, 5 cases and no controls carried *TP53 PVs*, and 2.10, (0.72–6.14) for moderate-risk genes combined (*ATM, BARD1, CHEK2, RAD51C, RAD52D*). These estimates were similar to those previously reported in other populations and were modified by ER status. No other genes evaluated had mutations associated at $P < 0.05$ with overall risk. The estimated lifetime risks for mutation carriers in *BRCA1*, *BRCA2*, and *PALB2* and moderate-risk genes were 18.4%, 9.8%, 22.4%, and 3.1%, respectively, markedly lower than in Western populations with higher baseline risks.

Conclusions: We confirmed associations between PV and breast cancer risk in Ghanaian women and provide absolute risk estimates that could inform counseling in Ghana and other West African countries.

Impact: These findings have direct relevance for breast cancer genetic counseling for women in West Africa.

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Introduction

Gene panel testing for breast cancer predisposition is widely used in high-income countries; however, susceptibility gene risk estimates are often imprecise and derived primarily from family-based studies in European ancestry populations (1–3). Recent publications from three large studies in women of European, Asian, African American (AA), and other racial/ethnic groups in the U.S. have enhanced our understanding of the role genetic factors in breast cancer etiology (4–6). The Breast Cancer Risk after Diagnostic Gene Sequencing (BRIDGES) study performed germline DNA panel testing for 34 putative breast cancer susceptibility genes in 60,466 cases and 53,461 controls of European and Asian ancestry (4). This study identified the most clinically useful genes for genetic testing as four high-risk [relative risk (RR) >4] genes, *BRCA1, BRCA2*, *PALB2*, and *TP53*; five moderate-risk (RR 2–4) genes, *ATM*, *BARD1*, *CHEK2*, *RAD51C*, and *RAD51D*, and provided precise RR estimates for overall and estrogen receptor (ER)-defined breast cancer. Palmer and colleagues investigated AA women (5,054 cases and 4,993 controls) and found similar results as reported by BRIDGES based on germline DNA panel testing of 23 genes (5). The Cancer Risk Estimates Related to Susceptibility (CARRIERS) study used the same sequencing panel as Palmer and colleagues to investigate a multiethnic U.S. population (78% non-Hispanic White, 14% non-Hispanic Black, 4% Asian, 3% Hispanic, and 2% other) of 32,247 breast cancer cases and 32,544 controls and also reported similar results as BRIDGES (6). Notably, most AA

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women in Palmer and colleagues overlapped with the CARRIERS study population.

Although breast cancer incidence historically has been low in West African compared with Western countries, incidence rates in West Africa are rising. West African women are disproportionately affected by early-onset/ER-negative breast cancer, and low survival rates mainly due to advanced stages at diagnosis (7–9). To date, there are limited data on the association of germline mutations in breast cancer susceptibility genes with disease risk in West Africa (10–13). Population-based studies are needed to clarify the role of pathogenic mutations in breast cancer genes in African women to improve our understanding of breast cancer etiology and prevention strategies. In this report, we used the 34 gene panel from BRIDGES to sequence 871 breast cancer cases and 1,563 controls in the Ghana Breast Health Study (GBHS; ref. 14–16). We estimated ORs of overall and ER-defined breast cancer for protein truncating variants (PTVs) and pathogenic missense variants, and we compared them with published estimates in European, Asian, and AA populations (4–6). We also provide population-specific lifetime breast cancer risk estimates for mutation carriers in Ghana.

Materials and Methods

Study population

The GBHS has been described in detail elsewhere (14–16). In brief, the GBHS is a population-based case–control study of breast cancer conducted in Accra and Kumasi, Ghana. The study enrolled breast cancer cases and frequency matched population-based controls using census-based sampling of women between the ages of 18 to 74 years of age. Cases were women recommended for a biopsy based on the suspicion of malignancy or presenting at a study hospital for treatment of pathologically documented breast cancer within the previous year. Controls included women who reported never having been diagnosed with breast cancer (14–16). We performed gene panel sequencing on 1,077 cases and 1,993 controls with an available source of germline DNA. After quality control, we included 871 breast cancer cases (829 pathologically confirmed invasive, 12 pathologically confirmed *in situ*, and 30 considered malignant based on clinical manifestations) and 1,563 controls (Supplementary Fig. S1). The study was approved by the Special Studies Institutional Review Board of the NCI (Rockville, MD), the Ghana Health Service Ethical Review Committee and institutional review boards at the Noguchi Memorial Institute for Medical Research (Accra, Ghana), the Kwame Nkrumah University of Science and Technology (Kumasi, Ghana), the School of Medical Sciences at Komfo Anokye Teaching Hospital (Kumasi, Ghana), and Westat (Rockville, MD). All participants provided written informed consent.

Laboratory methods, variant calling, and classification

Details on the laboratory methods, variant calling, and classification have been published (4). In brief, we analyzed a gene panel of 34 known or suspected breast cancer susceptibility genes. Library preparation was conducted using the Fluidigm Juno 192.48 system at the Centre for Cancer Genetic Epidemiology, University of Cambridge, Cambridge, United Kingdom. Amplified products were combined into barcoded libraries of 768 samples, each of which was run on a single lane of an Illumina HiSeq4000. Each sample was sequenced to an average depth of 349 reads, in the target region.

Variant calling was performed using VarDict (17); comparison with other callers indicated that this had much better specificity for this type of targeted sequencing (18). We applied the following filters at the VCF level: Phred-scaled sequencing quality assessment of the bases contributing to the variant (QUAL) <30, allele fraction <0.2 and mean mapping quality (MQMEAN) <60, mean number of mismatches per read (NM) >2.0, AFxBase Depth <7.5. Variants failing any of these filters were removed. PTVs were defined as frameshifting insertions/deletions, stop/gain or canonical splice variants as classified by the Emsembl Variant Effect Predictor (19), except for variants in the last exon of each gene, which were excluded from the primary analysis. Missense variants defined as pathogenic or likely pathogenic in ClinVar by two or more clinical laboratories (Ambry Genetics, SCRP, Invitae, GeneDx, Counsyl, InSiGHT) were considered pathogenic, the same criteria as applied by Palmer and colleagues in the study of AA women (5).

Statistical analysis

We used Fisher exact test to compare carrier frequencies in GBHS to carrier frequencies in BRIDES and Palmer and colleagues. We used logistic regression to perform burden analyses to estimate ORs and 95% confidence intervals (CI) associated with carrying a pathogenic variant (PV) in each gene adjusting for age and family history. Because risk associations for PTVs and PVs in the moderate-risk genes (ATM, BARD1, CHEK2, RAD51C, and RAD51D) were found to be similar by BRIDGES (4), and the GBHS sample size was too small to evaluate each gene separately, we tested for associations with breast cancer risk for these genes combined. Disease endpoints considered were overall (invasive or *in situ*), ER-defined, and triple-negative (TN, defined as being negative for ER, PR, and HER2) breast cancer. Case-only analyses were used to estimate heterogeneity P values (P_{het}) in associations between carrying a PV with risk of ER-defined breast cancer. We compared frequencies and risk estimates of PTVs in breast cancer risk genes with those reported among European and Asian ancestries in the BRIDGES study (4), and the frequencies and risk estimates associated with all PVs in aggregate (defined as either PTVs and pathogenic missense variants) in breast cancer risk genes with those reported among AA and a multiethnic (primarily non-Hispanic White) U.S. population by CARRIERS (5, 6). For comparisons with the BRIDGES studies, we were able to compare overall breast cancer risk estimates for carrying PTVs in *ATM, BARD1, BRCA1, BRCA2, CHEK2, MSH6, NF1, PALB2, PTEN, RAD51C, RAD51D*, and *TP53* separately with European and Asian ancestry women; BRIDGES did not report risk estimates for other genes separately by ancestry group (4). We generated z-scores and corresponding P values to compare risk estimates across studies (P-diff). Population attributable risks (PAR) for pathogenic mutations were calculated on the basis of the mutation frequencies in the control population and OR estimates.

Lifetime absolute risk estimates of overall and ER-defined breast cancer within the 18 to 74 years age range, the oldest age in the GBHS (female life expectancy in Ghana is 64.4 years; ref. 20), for women carrying a PV were generated using the Individualized Coherent Absolute Risk Estimator tool (iCARE; ref. 21). Absolute risk estimation using iCARE requires the ORs for disease risk, age-specific disease incidence rates and competing mortality rates and an individual level reference dataset of risk factors representing the underlying population (21). We used the estimated ORs for risk of breast cancer for carriers of PVs in *BRCA1, BRCA2, PALB2*, and the combined moderate-risk genes. We leveraged the census-based sampling strategy to generate sampling fractions and estimate breast cancer incidence rates in Accra and Kumasi, as previously described in greater detail (22). The population distribution of carrying a PV was estimated using the controls from our study, which provided the reference dataset for absolute risk estimation. The overall absolute risk estimation further accounted for competing mortality due to causes other than breast cancer using the

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competing mortality rates derived from published estimates of overall mortality (20) and the mortality due to breast cancer published by GLOBOCAN 2018 (23). We further derived 95% CIs for lifetime absolute risk of breast cancer using bootstrap resampling. This approach assumed the disease incidence rates and competing mortality rates to be known with certainty. The log-OR estimating the association of each carrier type with overall breast cancer or subtype-specific breast cancer asymptotically follows a normal distribution by the theory of maximum likelihood estimation. Each bootstrap iteration sampled the log-ORs and sampled the reference dataset with replacement. The CIs were calculated using the 2.5 and 97.5 percentiles of the lifetime absolute risk distribution derived from 1000 bootstrap iterations.

A P value threshold of 0.05 was used to denote statistically significant associations. Analyses were performed in R (version 3.4.2) and SAS (version 9.4).

Results

The GBHS study population included 871 breast cancer cases [307 ER-positive (48.9%), 321 ER-negative (51.1%), 243 without ER tumor status data, and 172 (28%) TN] and 1,563 controls. The mean (SD) age (in years) of cases was slightly older [50.8 (12.0)] than controls [45.8 (12.7)], reflecting controls being initially frequency matched to women with a suspicion of breast cancer prior to diagnosis confirmation (Supplementary Table S1).

Frequency of PTV and missense PVs

Among the 34 genes investigated, the percentage of controls carrying at least one PTV across all genes was 3.97% and for overall, ER-positive, ER-negative, and TN cases the percentages were 11.94%, 13.68%, 12.46%, and 15.52%, respectively (Supplementary Table S2; Supplementary Fig. S2). Supplementary Table S3 lists the identified PVs.

Figure 1 and Supplementary Table S4 shows PTV frequencies in GBHS and BRIDGES for the 9 genes (*ATM, BARD1, BRCA1, BRCA2, CHEK2, PALB2, RAD51C, RAD51D*, and *TP53*) that were associated with breast cancer risk at a Bayesian False Discovery Probability (BFDP) <5% in BRIDGES (4). The frequency of carrying at least one PTV in these nine genes was 1.15% in controls and 7.58%, 8.14%, and 8.41% in overall, ER-positive, and ER-negative disease, respectively. Women in GBHS had approximately two times the frequency of PTVs in *BRCA1* and *BRCA2* than women of European or Asian ancestry in the BRIDGES study (4) for both controls and overall breast cancer cases; however, differences were statistically significant only for overall breast cancer. For mutation frequencies in ER-defined disease, the largest differences were for PTVs in ER-positive cases for *BRCA2* and *PALB2*. There was also a higher frequency of *TP53* PTVs for ER-negative cases in GBHS than BRIDGES. No controls carried a *TP53* PTV, and no cases or controls carried a *CHEK2* PTV. Seven cases and one control carried missense variants classified as pathogenic in *ATM*, *BRCA1*, *CHEK2*, and *TP53* (Supplementary Tables S5 and S6).

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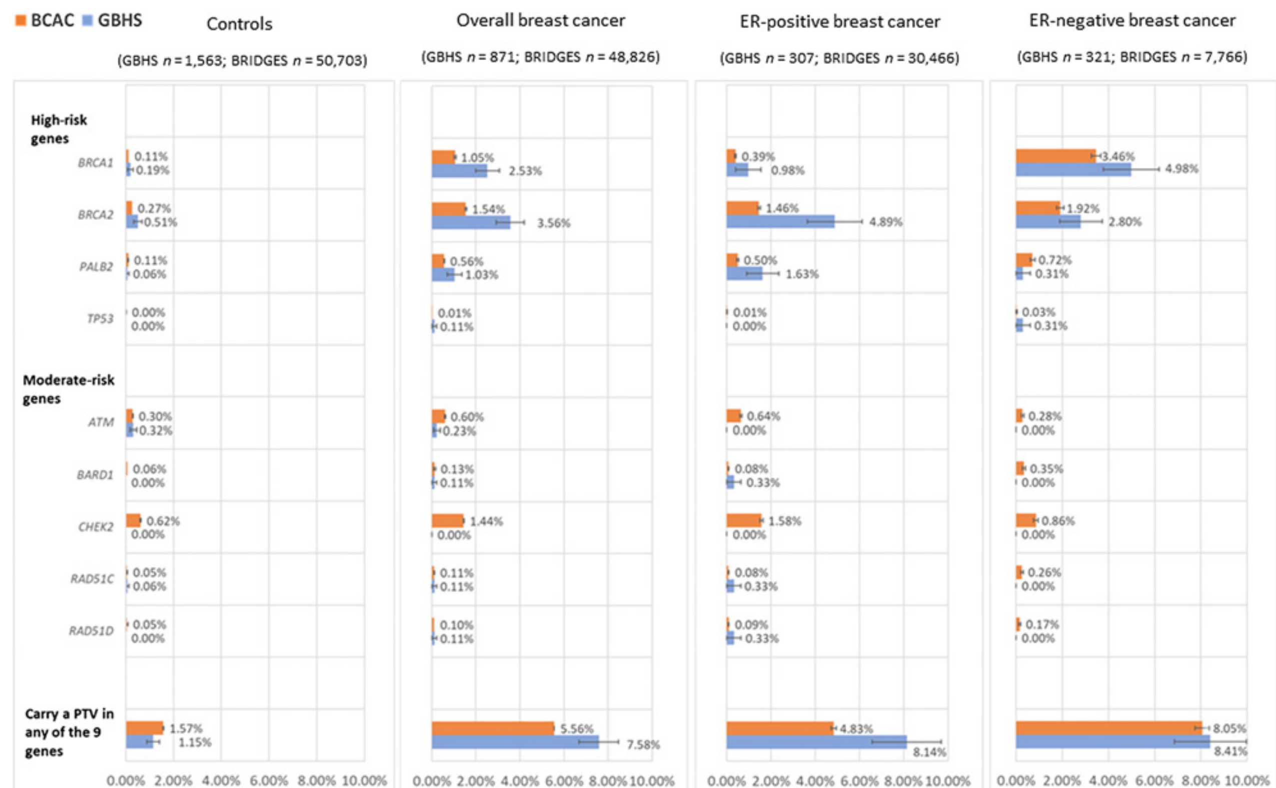

1. Breast Cancer Association Consortium. Breast Cancer Risk Genes — Association Analysis in More than 113,000 Women. New England Journal of Medicine 2021.

Figure 1.

Comparison of the frequency of PTVs in breast cancer genes for controls and breast cancer cases from the GBHS and the BRIDGES study (European and Asian ancestries combined). Includes genes with PTVs reported associated with risk of breast cancer at a BFDP < 5% in BRIDGES[1]. See Supplementary Table S4 for more details.

Figure 2.

Comparison of the frequency of pathogenic mutations in putative susceptibility breast cancer genes in controls and breast cancer cases from the GBHS and the CARRIERS study in AA women[1]. See Supplementary Table S7 for more details

Figure 2 shows PV frequencies in the 9 breast cancer genes for GBHS and AA women in Palmer and colleagues (PTVs and pathogenic missense variants were combined for comparison with Palmer and colleagues; ref. 5). This figure shows a higher mutation frequency in GBHS cases and controls for *BRCA1* and *BRCA2*, in ER-positive cases for *PALB2*, and in ER-negative cases for *TP53*, like the comparisons with European or Asian ancestry women in BRIDGES. No pathogenic *TP53* mutations were found in controls from either study. Notably, GBHS controls had approximately 10 times the frequency of *BRCA1* pathogenic mutations compared with AA controls (**Fig. 2**; Supplementary Table S7; ref. 5).

PV carrier frequencies were higher in GBHS cases who reported a breast cancer family history in a first-degree relative at the time of interview, compared with those without such a history. Carrying a PV in *BRCA1* and *BRCA2* was not associated ($P < 0.05$) with age at diagnosis for overall, ER-positive, or ER-negative disease (Supplementary Fig. S3; Supplementary Tables S8 and S9).

Comparisons of mutation frequencies with genes not found associated with breast cancer at BFDP <5% by BRIDGES (4) are shown in Supplementary Tables S4 and S7. Comparisons of mutation frequencies between GBHS and a multiethnic U.S. population in CARRIERS (6) is not shown; however, they were reported to be similar to BRIDGES (4) and Palmer and colleagues (5), except for *BRCA1* pathogenic mutations in controls that were markedly low in AA controls (5).

Relative risk of breast cancer for mutation carriers

Among the 34 genes investigated, we estimated associations with breast cancer risk for mutation carriers in 14 genes, as there were no carriers in cases and/or controls for the remaining 20 genes (**Fig. 3**; Supplementary Table S2). We identified significant associations at $P <$ 0.05 between PTVs and overall breast cancer risk for 3 high-risk genes: *BRCA1* (OR, 13.17; 95% CI, 3.86–44.91), *BRCA2* (OR, 7.02; 95% CI, 3.17–15.54), and *PALB2* (OR, 17.25; 95% CI, 2.15–138.13). The magnitude of association for these genes was like those in women of European or Asian ancestry in BRIDGES (**Fig. 4**; Supplementary Table S10; ref. 4). PTVs in *BRCA1* were more strongly associated with ER-negative (OR, 28.62; 95% CI, 8.15–100.57) and TN disease (OR, 41.67; 95% CI, 11.53–150.54) than ER-positive (OR, 5.43; 95% CI, 1.08–27.43) disease (ER-positive vs. ER-negative case-only $P_{het} = 0.01$). PTVs in *BRCA2* were more strongly associated with ER-positive (OR, 10.32; 95% CI, 4.27–24.96) than ER-negative (OR, 5.62; 95% CI, 2.12–14.88) and TN (OR, 7.96; 95% CI, 2.82–22.47) disease, but differences were not statistically significant (ER-positive vs. ER-negative case-only $P_{het} = 0.16$; **Figure 4**; Supplementary Table S2). ER-specific associations for *BRCA1* and *BRCA2* were consistent with European and Asian populations in BRIDGES (P-diff>0.20, **Figure 4**, Supplementary Table S10) (4). *PALB2* was more strongly associated with ER-positive than ER-negative disease [(OR, 28.02; 95% CI, 3.17–247.74) and (OR, 5.02; 95% CI, 0.31–82.59) respectively], but estimates were imprecise, and differences were not statistically significant (case-only $P_{het} = 0.12$; Supplementary Table S2). *PALB2* ER-specific estimates were not significantly different from those reported by BRIDGES (4). The magnitude of the associations for *BRCA1, BRCA2*, and *PALB2* for carrying a PV in these genes was also like AA women and the multiethnic U.S. population in CARRIERS. Pathogenic mutations in *ATM* and *RAD51C* were not significantly associated with breast cancer risk, although the magnitude of the estimated ORs was like those reported by BRIDGES, Palmer and colleagues and CARRIERS (**Fig. 4**; Supplementary Table S10). ORs could not be estimated for *BARD1, CHEK2, RAD51D*, and *TP53* as no carriers were identified in cases or controls. Notably, five cases but no controls (cases = 0.57% vs. controls = 0.0%; $P = 0.006$) were identified carrying a PV in *TP53*. The magnitude of the estimated OR

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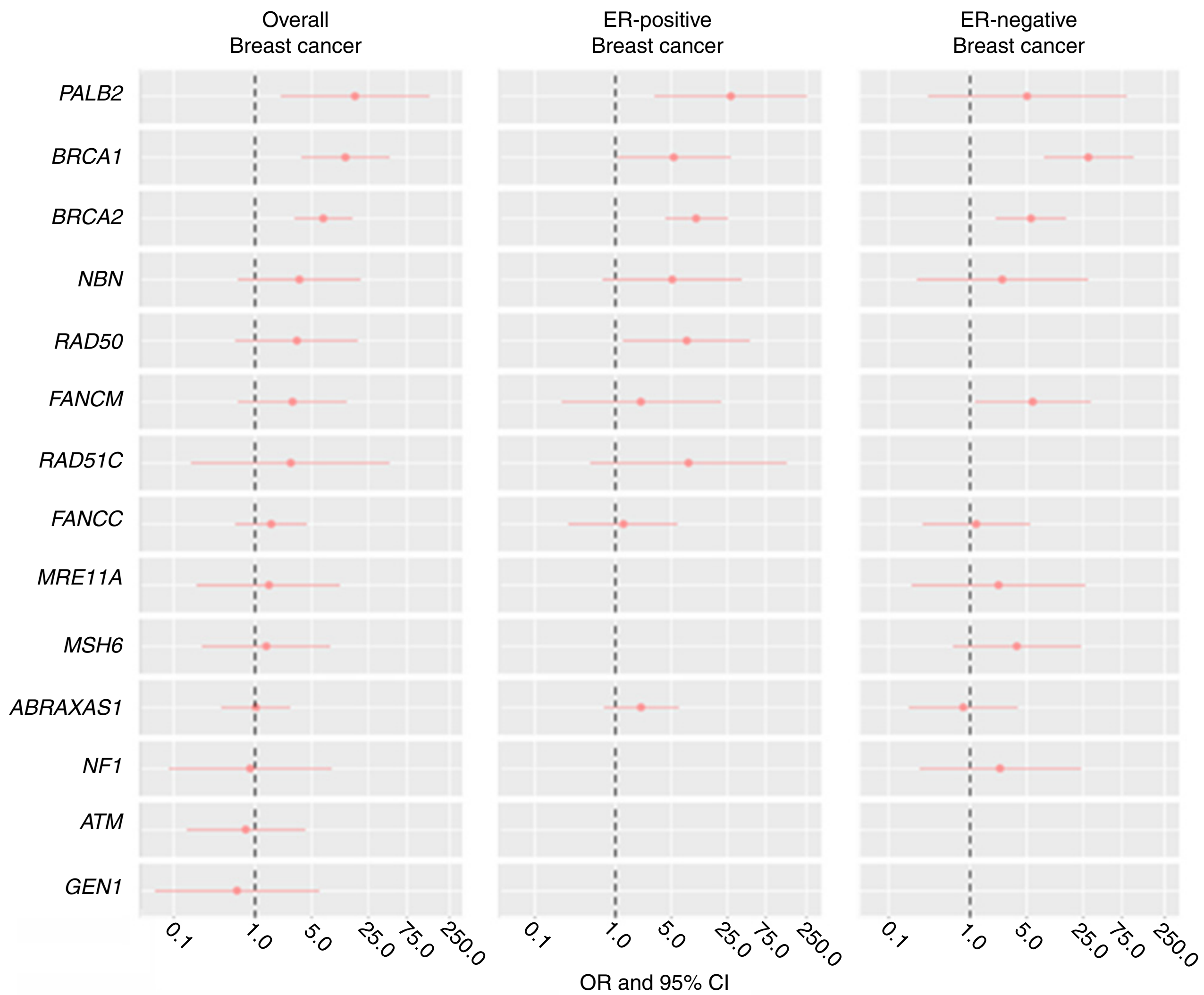

1. Genes with no OR and 95% CI are due to small sample size of cases and/or controls carrying PTVs in these genes. Sample sizes for genes not shown (controls/overall cases; *P*): *AKT1* (0/0), *BABAM2* (0/1; $P = 0.36$), *BARD1* (0/1; $P = 0.36$), *BRIP1* (2/0; $P = 0.54$), *CDH1* (0/1; $P = 0.36$), *CHEK2* (0/0), *EPCAM* (0/1; $P = 0.36$), *MEN1* (0/0), *MLH1* (0/0), *MSH2* (0/0), *MUTYH* (3/0; $P = 0.56$), *PIK3CA* (0/0), *PMS2* (0/3; $P = 0.05$), *PTEN* (0/0), *RAD51D* (0/1; $P = 0.36$), *RECQL* (0/0), *RINT1* (0/2; $P = 0.13$), *STK11* (0/0), *TP53* (0/1; $P = 0.36$), *XRCC2* (0/1; $P = 0.35$)

Figure 3.
Association between PTVs in putative susceptibility genes and risk of breast cancer in the GBHS. The figure shows ORs with 95% CIs, indicated by bars. Genes are ordered by the size of estimated odds ratio for overall breast cancer. Showing 14 of 34[1] genes of which ORs and 95% CI could be estimated. See Supplementary Table S2 for more details.

for overall breast cancer among women carrying a PV in any of the combined moderate-risk genes, *ATM*, *BARD1*, *CHEK2*, *RAD51C*, and *RAD51D*, was similar to other populations (OR, 2.10; 95% CI, 0.72–6.14; Supplementary Table S10; refs. 4–6). Only 1 ER-negative case carried a PV in the combined moderate-risk genes (Supplementary Table S2). Among other investigated genes, *RAD50* was associated with risk of ER-positive disease (OR, 7.89; 95% CI, 1.29–48.20), in contrast to results from BRIDGES and CARRIERS (P-diffs = 0.03 and 0.02, respectively). *FANCM* was associated with risk of ER-negative disease (OR, 5.91; 95% CI, 1.17–29.91; ref. 4), consistent with BRIDGES and CARRIERS results (P-diff>0.08; Supplementary Fig. S4; Supplementary Table S10). Evidence for associations with risk for mutations in other putative risk genes was weaker.

PAR and lifetime absolute risk of breast cancer for mutation carriers in 9 established breast cancer genes

Supplementary Table S11 shows the PAR estimates for *BRCA1* and *BRCA2* pathogenic mutations in Ghana for overall, ER-positive, and ER-negative disease, based on mutation frequencies in the control population and OR estimates. PAR estimates in Ghana might be higher

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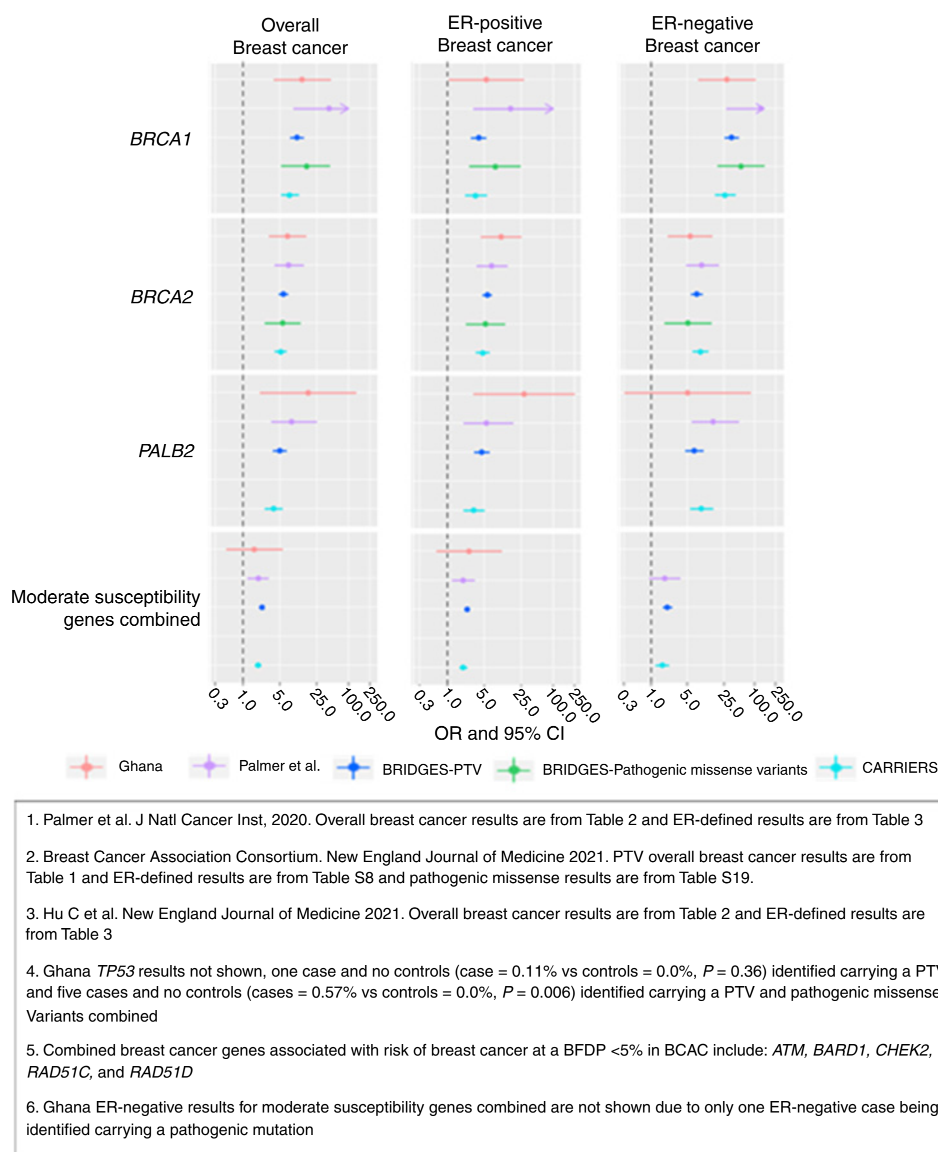

Figure 4.
Associations between PTVs in the GBHS, AAs (PTVs and pathogenic missense variants combined) as reported by Palmer et al.[1], Europeans and Asian ancestries in the BRIDGES[2], and in a multiethnic U.S. population as reported by CARRIERS[3] (PTVs and pathogenic missense variants combined) for high risk breast cancer genes (*BRCA1, BRCA2*, PALB2, and TP53[4)] and five[5,6] moderate susceptibility breast cancer risk genes combined. The figure shows ORs with 95% CIs, indicated by bars. See Supplementary Table S10 for more details.

than in other populations, however CIs were wide and overlapped with PAR estimates using data from previously published studies of larger sample sizes (4, 5).

The lifetime absolute risk for overall breast cancer among women carrying a PV in *BRCA1, BRCA2, PALB2*, and moderate-risk genes combined (*ATM, BARD1, CHEK2, RAD51C*, and *RAD51D*) was: 18.4% (95% CI, 6.0%–48.6%), 9.8% (95% CI, 4.5%–19.5%), 22.4% (95% CI, 3.5%–78.6%), and 3.1% (95% CI, 1.1%–8.4%), respectively. *BRCA1* mutation carriers had a higher lifetime risk for developing ER-negative (17.3%; 95% CI, 5.6%–41.1%) than ER-positive (3.7%; 0.9%–14.3%) disease, while *BRCA2* and *PALB2* mutation carriers had higher lifetime risks for ER-positive (7.4%; 3.1%–15.4% and 19.1%; 2.4%–72.3%, respectively) than ER-negative (3.8%; 1.5%–8.8% and 3.2%; 0.2%–27.0%) disease. Mutation carriers for moderate-risk genes had a lifetime risks for ER-positive disease of 2.5% (0.7%–7.8%; **Fig. 5**). An estimate of lifetime risk for ER-negative disease for mutation carriers in moderate-risk genes is not shown due to only 1 ER-negative case being found carrying a PV in the combined moderate-risk genes. As a sensitivity analysis, we estimated lifetime risks using OR estimates from BRIDGES while keeping Ghana age-specific disease incidence rates and competing mortality rates. Estimates were similar except for *PALB2* (Supplementary Table S12), likely due to the lack of precision of the OR estimate from the GBHS.

Discussion

In this study in Ghanaian women, we confirmed breast cancer risk associations for PVs in four established high-risk genes (*BRCA1, BRCA2, PALB2*, and *TP53*) and provided evidence consistent with moderate-risk associations for *ATM, BARD1, CHEK2, RAD51C*, and *RAD51D*. Relative risks estimates were of similar magnitude to results published in large studies in women of European, Asian, AA and a multiethnic (primarily non-Hispanic White) U.S. population (4–6). However, Ghanaian women were about twice as likely to carry pathogenic mutations in *BRCA1* or *BRCA2*. The estimated lifetime risks for overall breast cancer among carriers were lower for Ghanaian

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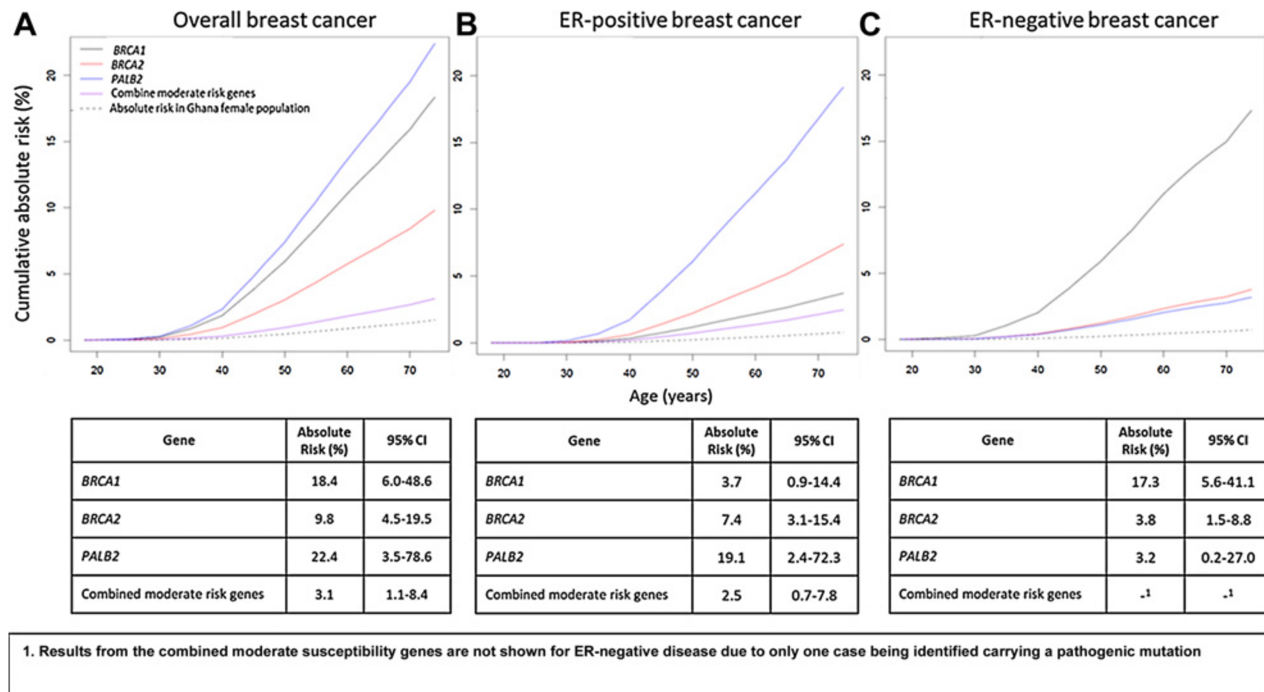

Gene	Absolute Risk (%)	95% CI
BRCA1	18.4	6.0-48.6
BRCA2	9.8	4.5-19.5
PALB2	22.4	3.5-78.6
Combined moderate risk genes	3.1	1.1-8.4

Gene	Absolute Risk (%)	95% CI
BRCA1	3.7	0.9-14.4
BRCA2	7.4	3.1-15.4
PALB2	19.1	2.4-72.3
Combined moderate risk genes	2.5	0.7-7.8

Gene	Absolute Risk (%)	95% CI
BRCA1	17.3	5.6-41.1
BRCA2	3.8	1.5-8.8
PALB2	3.2	0.2-27.0
Combined moderate risk genes	-[1]	-[1]

Figure 5.

Estimates of absolute risk of (**A**) overall, (**B**) ER-positive, and (**C**) ER-negative breast cancer through age 74 in women from Ghana who are carriers of pathogenic mutations in three high-risk genes (*BRCA1, BRCA2, PALB2)*, and five susceptibility genes combined (*ATM, BARD1, CHEK2, RAD51C*, and *RAD51D*). See Supplementary Table S12 for more.

women than for AA (5), European ancestry women in the United Kingdom (4), and non-Hispanic Whites in the United States (6).

By using the same gene panel, methodology and bioinformatic pipelines as in the BRIDGES study, we were able to make direct comparisons between estimates in GBHS and this large study of European and Asian ancestry women (cases = 60,466 and controls = 53,461 controls; ref. 4). This gene panel was also similar to that used by Palmer and colleagues and CARRIERS, allowing for comparisons with AA women (cases = 5,054 and controls = 4,993) and a multiethnic U.S. population (cases = 32,247 and controls = 32,544), respectively (5, 6). Although OR estimates for carrying a PV in established high- and moderate-risk genes were similar across populations (4–6), OR estimates in GBHS were less precise due to the smaller sample size. Notably, the OR estimates for AA *BRCA1* carriers reported by Palmer and colleagues were also imprecise due to only 1 of 4,925 controls (0.02%) carrying a *BRCA1* pathogenic mutation (5). Compared with other studies, the Carolina Breast Cancer Study also reported a low *BRCA1* carrier frequency among controls, 1 in 1,635 (0.06%, AA = 592, European Americans = 1,016, other ancestry = 27; ref. 24). *BRCA1* and *BRCA2* carrier frequencies among GBHS controls are more like those from the Nigeria Breast Cancer Study (NBCS), which reported 3 in 997 (0.3%) and 4 in 997 (0.4%) controls to be *BRCA1* and *BRCA2* carrier, respectively (10). Adedokun and colleagues found in a study from Uganda and Cameroon 2 in 185 (1.08%) hospital-based controls to be *BRCA1* carriers, while no controls were found to be *BRCA2* carriers (13). Collectively, these findings suggest that women from West Africa more frequently are carriers for PVs in *BRCA1* and *BRCA2*. However, the control populations in the GBHS, NBCS, and Adedokun and colleagues were on average approximately 10 to 15 years younger than controls in BRIDGES, Palmer and colleagues, and CARRIERS (4–6, 10, 13). These age differences across populations could at least partly explain differences in PV carrier frequency distributions. In addition, information on prior breast cancer screening was collected on control women in the GBHS, but they were not screened at study enrollment to identify subclinical breast cancer, which could have resulted in overestimation of pathogenic mutation frequencies.

GBHS ER-positive cases more often carried PVs in *BRCA2* and *PALB2* compared with ER-positive cases of European, Asian or AA ancestry (4, 5). However, GBHS ER-negative carrier frequencies were similar to other populations, except for a higher *TP53* carrier frequency in GBHS than AA ER-negative cases (5). Our finding suggesting PV in *RAD50* being associated with risk of ER-positive disease contrasted with results from the BRIDGES and CARRIERS studies, thus this finding require replication in larger studies (4, 6). Palmer and colleagues did not report results for *RAD50* (5). Previous, reports found pathogenic mutations in *BRCA1, BRCA2*, and combined breast cancer genes were more common among women diagnosed at younger than older ages (4–6, 10). We did not find age associated with *BRCA1* and *BRCA2*, possibly due to our study having a smaller sample size and/or a younger, more narrower age range (median diagnosis age = 51 years; range = 18–74 years) than most other populations. Notably, 59.2% of NBCS cases were less than 50 years of age, which reported *BRCA1* mutations were more common in women diagnosed at younger ages (10).

We were not able to obtain risk estimates for all the individual moderate-risk genes due to our limited sample size, thus we combined these genes to test for associations with breast cancer risk. Among the combined moderate-risk genes, we found modest evidence of an association with risk of ER-positive disease. For ER-negative

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cases we found only 1 case carrying a pathogenic missense variant [p. Ala2622Val] in *ATM*. Notably, BRIDGES, Palmer and colleagues, and CARRIERS found *RAD51C* and *RAD51D* to be most strongly associated with risk for ER-negative disease. BRIDGES and CARRIERS also found *BARD1* most strongly associated with risk of ER-negative disease, while Palmer and colleagues found no association with risk for *BARD1* (4–6). For *CHEK2*, we did not identify PVs among GBHS cases; however, one control carried a pathogenic missense variant [p. Arg145Trp]. PVs in *CHEK2* were reported among 0.2% of cases from Nigeria (10) and 1.1% of cases from Cameroon, but none were the c.1100delC mutation (25). In BRIDGES, nearly 80% of *CHEK2* PTVs were accounted for by c.1100delC, a founder mutation variant in Northwestern European populations (4, 25). This variant accounted for 47% and 33% of *CHEK2* PTVs found among AA cases and controls, respectively, possibly resulting from European population admixture among AAs (5).

BRIDGES estimated lifetime cumulative risks by age 80 years for *BRCA1*, *BRCA2*, and *PALB2* PTV carriers in the UK of approximately 55%, 45%, and 42%, respectively (4). These results were similar to findings from CARRIERS, which reported cumulative absolute risks by age 85 for non-Hispanic Whites in the U.S. of approximately 50% for PVs in *BRCA1* or *BRCA2*, and 32% for variants in *PALB2*. Palmer and colleagues estimated lifetime cumulative risks by age 85 for *BRCA2* and *PALB2* in AA women of 58% and 30%, respectively (5). Palmer and colleagues did not report a lifetime risk for *BRCA1* because only 1 control was identified carrying a PV in the gene. In contrast, we estimated much lower lifetime risks for overall breast cancer by age 74 for Ghanaian women in Kumasi and Accra carrying pathogenic mutations in *BRCA1*, *BRCA2*, and *PALB2*. On the basis of the figures reporting the lifetime absolute risk in BRIDGES (**Fig. 3**), CARRIERS (**Fig. 1**), and Palmer and colleagues (**Fig. 2**), the absolute risk at age 74 in these populations were only slightly lower than the lifetime risks (4–6). The lower lifetime risks found in the GBHS, despite the similar OR, can be explained by the lower overall breast cancer incidence rates and higher competing mortality rates in Ghana compared with the UK and AA populations (20, 26–28). Differences in rates could be partly explained by younger demographics in Ghana compared with higher income countries and differences in reproductive patterns (29, 30). Differences can also be explained by overdiagnosis or detection of indolent tumors in higher income countries with widespread mammographic screening programs (31–33), compared with only opportunistic screening in Ghana (14, 34). This will be particularly relevant for ER-positive tumors that are more likely to be screen-detected than ER-negative tumors (35). However, we could not compare ER-specific absolute risk across populations because they were not reported for other ancestry populations (4–6).

Our estimated lifetime risks for mutation carriers could be used to inform genetic counseling of women in Ghana and other West African countries. However, our lifetime risk estimates were based on incidence rates estimates from two major cities, Kumasi and Accra, and they may not reflect estimates in more rural regions of Ghana. Other barriers for implementation of genetic testing and counseling services in these countries need to be addressed, including consideration of psycho-social and cultural factors, burden to the health care systems, and guidelines for clinical management of mutation carriers developed for local environments (36–38).

Our findings indicate that OR estimates for pathogenic mutations in established high- and moderate-risk breast cancer genes are similar across populations. However, overall lifetime risks for PV carriers are lower in Ghana due to lower underlying rates for overall breast cancer than in Western populations. The role of other putative breast cancer genes remains unclear and larger studies in West Africa are needed to increase the precision of risk estimates.

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