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Citation

Mavaddat, N., Dorling, L., Carvalho, S., Allen, J., Gonzalez-Neira, A., Keeman, R., ... Easton, D. F. (2022). Pathology of tumors associated with pathogenic germline variants in 9 breast cancer susceptibility genes. *Jama Oncology*, 8(3). doi:10.1001/jamaoncol.2021.6744

Version: Publisher's Version

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Downloaded from: <https://hdl.handle.net/1887/3276870>

Note: To cite this publication please use the final published version (if applicable).

Pathology of Tumors Associated With Pathogenic Germline Variants in 9 Breast Cancer Susceptibility Genes

Breast Cancer Association Consortium

 Supplemental content

IMPORTANCE Rare germline genetic variants in several genes are associated with increased breast cancer (BC) risk, but their precise contributions to different disease subtypes are unclear. This information is relevant to guidelines for gene panel testing and risk prediction.

OBJECTIVE To characterize tumors associated with BC susceptibility genes in large-scale population- or hospital-based studies.

DESIGN, SETTING, AND PARTICIPANTS The multicenter, international case-control analysis of the BRIDGES study included 42 680 patients and 46 387 control participants, comprising women aged 18 to 79 years who were sampled independently of family history from 38 studies. Studies were conducted between 1991 and 2016. Sequencing and analysis took place between 2016 and 2021.

EXPOSURES Protein-truncating variants and likely pathogenic missense variants in *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *CHEK2*, *PALB2*, *RAD51C*, *RAD51D*, and *TP53*.

MAIN OUTCOMES AND MEASURES The intrinsic-like BC subtypes as defined by estrogen receptor, progesterone receptor, and ERBB2 (formerly known as *HER2*) status, and tumor grade; morphology; size; stage; lymph node involvement; subtype-specific odds ratios (ORs) for carrying protein-truncating variants and pathogenic missense variants in the 9 BC susceptibility genes.

RESULTS The mean (SD) ages at interview (control participants) and diagnosis (cases) were 55.1 (11.9) and 55.8 (10.6) years, respectively; all participants were of European or East Asian ethnicity. There was substantial heterogeneity in the distribution of intrinsic subtypes by gene. *RAD51C*, *RAD51D*, and *BARD1* variants were associated mainly with triple-negative disease (OR, 6.19 [95% CI, 3.17-12.12]; OR, 6.19 [95% CI, 2.99-12.79]; and OR, 10.05 [95% CI, 5.27-19.19], respectively). *CHEK2* variants were associated with all subtypes (with ORs ranging from 2.21-3.17) except for triple-negative disease. For *ATM* variants, the association was strongest for the hormone receptor (HR)⁺ERBB2⁻ high-grade subtype (OR, 4.99; 95% CI, 3.68-6.76). *BRCA1* was associated with increased risk of all subtypes, but the ORs varied widely, being highest for triple-negative disease (OR, 55.32; 95% CI, 40.51-75.55). *BRCA2* and *PALB2* variants were also associated with triple-negative disease. *TP53* variants were most strongly associated with HR⁺ERBB2⁺ and HR⁻ERBB2⁺ subtypes. Tumors occurring in pathogenic variant carriers were of higher grade. For most genes and subtypes, a decline in ORs was observed with increasing age. Together, the 9 genes were associated with 27.3% of all triple-negative tumors in women 40 years or younger.

CONCLUSIONS AND RELEVANCE The results of this case-control study suggest that variants in the 9 BC risk genes differ substantially in their associated pathology but are generally associated with triple-negative and/or high-grade disease. Knowing the age and tumor subtype distributions associated with individual BC genes can potentially aid guidelines for gene panel testing, risk prediction, and variant classification and guide targeted screening strategies.

JAMA Oncol. 2022;8(3):e216744. doi:10.1001/jamaoncol.2021.6744
Published online January 27, 2022.

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Breast cancer (BC) is a heterogeneous disease; different subtypes are associated with distinct biology, prognosis, and potential for therapy.¹⁻³ There is evidence that inherited genetic predisposition contributes to this heterogeneity.^{4,5} However, data for detailed analysis of tumor pathologies that are associated with most BC susceptibility genes have been limited, particularly in population-based studies. Recent results from 2 large-scale sequencing studies, BRIDGES⁶ and CARRIERS,⁷ found evidence of an association with BC risk for germline protein-truncating variants (PTVs) and/or rare missense variants (MSVs) in 9 genes: *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *CHEK2*, *PALB2*, *RAD51C*, *RAD51D*, and *TP53*. Women carrying variants in these genes may be offered enhanced screening, including by magnetic resonance imaging, risk-reducing surgery, chemoprevention, and genetic counselling; knowledge of germline gene variants also affects treatment.⁸ Intrinsic BC subtypes have been defined on the basis of patterns of gene expression; these include luminal-A, which defines a subset of hormone receptor-positive tumors that are associated with a good 5-year prognosis, and luminal-B, ERBB2-enriched and basal tumors with poorer prognosis.^{2,9} Gene expression data are not routinely available in diagnostic laboratories, but large-scale epidemiological studies can use subtypes based on immunochemical markers to define intrinsic-like surrogates that are broadly associated with the molecular subtypes.^{10,11} In this article, we use data from BRIDGES to assess associations between variants in these genes and pathological features of nonmetastasized breast tumors relevant to prognosis and/or distinct therapeutic options. We further quantify the contribution of rare BC susceptibility genes to the development of distinct BC subtypes in women of different ages.

Methods

Studies and Inclusion Criteria

The BRIDGES study included women with BC and unaffected control participants who were participating in the Breast Cancer Association Consortium (<https://bcac.ccge.medschl.cam.ac.uk/>; eTable 1 in Supplement 1). The analyses presented in this article are based on cases from the subset of population-based or hospital-based studies that were sampled independently of family history, together with population-matched control participants (38 studies). Women aged between 18 and 79 years were included. Pathology information from the first primary invasive BC was considered. Cases in which the index tumor was the second tumor and patients with metastases at initial diagnosis were excluded.¹² All studies were approved by the relevant ethical review boards, and participants provided written informed consent.

Laboratory Methods, Variant Calling, and Classification

We focused on 9 genes with evidence of an association with BC.⁶ We considered PTVs for all 9 genes, and rare (carrier frequency <0.1%) MSVs in *BRCA1*, *BRCA2* and *TP53* that were likely pathogenic according to adaptations of the American College of Medical Genetics guidelines.⁶ Approximately 80% of *CHEK2*

Key Points

Question What breast tumor characteristics are associated with rare pathogenic protein truncating or missense variants in breast cancer susceptibility genes?

Findings In this case-control study involving 46 387 control participants and 42 680 women with a diagnosis of breast cancer, pathology features (eg, tumor subtype, morphology, size, TNM stage, and lymph node involvement) associated with rare germline (likely) pathogenic variants in 9 different breast cancer susceptibility genes were studied. Substantial differences in tumor subtype distribution by gene were found.

Meaning The results of this study suggest that tumor subtypes differ by gene; these findings can potentially inform guidelines for gene panel testing, risk prediction in unaffected individuals, variant classification, and understanding of breast cancer etiology.

PTVs were c.1100delC. The *TP53* PTV and MSV carriers were considered together. Carriers of *BRCA1* and *BRCA2* PTVs were excluded from the analyses of other genes. Carriers of PTVs in *BRCA1* and *BRCA2* and women who harbored a pathogenic variant in more than 1 non-*BRCA* gene were also excluded. *Non-carriers* were defined as women without PTVs or MSVs in any of the genes. Further details are provided in the eMethods in Supplement 1.

Tumor Pathology

Pathology information was based on histology and immunohistochemistry results from medical records, rescored whole slides, or tumor microarrays that were curated in the Breast Cancer Association Consortium database, version 12.^{13,14} Markers included estrogen receptor (ER), progesterone receptor (PR), and erb-b2 receptor tyrosine kinase 2 (ERBB2, formerly known as HER2) status, which was denoted as positive or negative; histological grade (grades 1, 2, and 3); morphology; tumor size (<2, 2-5, or >5 cm); lymph node involvement (yes/no); and TNM stage (I, II, and III). For the purposes of this analysis, we defined 5 clinically relevant intrinsic subtypes based on available immunohistochemistry and grade: HR⁺ERBB2⁻ low (/intermediate) grade, HR⁺ERBB2⁺, and HR⁺ERBB2⁻ high grade, HR⁻ERBB2⁺ and triple negative (TN). Grades 1 and 2 were considered low-grade and grade 3 high-grade disease (eTable 2 in Supplement 1).^{11,12,15,16}

Statistical Analysis

Analyses were based on estimating the odds ratios (ORs) associated with carrying any PTV (or pathogenic MSV) in each gene. First, complete-case analyses based on all available data were conducted. Case-control analyses were used to estimate the OR for developing a tumor of a particular subtype according to single markers and case-only analyses to evaluate the evidence for differences by subtype. Logistic regression was used for binary characteristics and multinomial logistic regression for multicategory tumor characteristics. For multicategory outcomes, a model in which the log(OR) varied linearly with the outcome level was also fitted. Analyses were adjusted for age (defined as age at diagnosis for patients and

age at interview for control participants) and country of origin of the study.

To evaluate heterogeneity of risk by intrinsic tumor subtypes, we first imputed missing pathology variables using Multiple Imputation by Chained Equations. Intrinsic subtypes were constructed for each of 100 imputed data sets, and the results of multinomial regression for each imputed data set were pooled. We also compared these data with results obtained after imputing tumor pathology using an expectation-maximization (EM) algorithm (eMethods in [Supplement 1](#)).¹⁷ We investigated interactions with age for each gene according to tumor subtype by including an age $\times$ variant product term in the model and also estimated the proportion of BC cases, by age-group and intrinsic subtype, for pathogenic variants in each gene.

Associations between (likely) pathogenic variant carrier status and tumor size and lymph node status were evaluated. Analyses were also conducted that included size, lymph node status, and intrinsic subtype in the same model and PR status and the HR-positive subtypes in the same model.

Gene-specific cumulative risks for each subtype were calculated by combining age-specific OR estimates with 2016 UK population incidence rates as a baseline and accounting for competing risk of not developing BC of a different subtype. Age-specific and gene-specific subtype proportions for tumor subtypes included in the risk prediction algorithm BOADICEA¹⁸ were also calculated (eMethods in [Supplement 1](#)).

Analyses were conducted using RStudio, version 1.2.5033 (RStudio); Stata, version 14.2 (StataCorp); and GFortran. Statistical significance was set at $P < .05$.

Results

Study Characteristics

The study comprised 46 387 control participants and 42 680 women with a diagnosis of BC from 22 countries, with mean (SD) ages at interview and diagnosis of 55.1 (11.9) and 55.8 (10.6) years, respectively (eTable 3 in [Supplement 1](#)). Numbers of variant carriers by gene are shown in eTable 4 in [Supplement 1](#) and patterns of missingness in pathology data in eTable 5 in [Supplement 1](#) and eTable 1 in [Supplement 2](#); for ER status, 18%; grade, 18%; PR status, 32%; and ERBB2 status, 43% of data were missing. There was no association between missingness and genotype.

Single marker analyses were based on complete data (eFigure 1 in [Supplement 1](#) and eTables 2 and 3 in [Supplement 2](#)). The remaining analyses were carried out following imputation of missing data.

Distribution of Intrinsic Tumor Subtypes and Age Trends

The PTVs in all 9 BC genes showed evidence of variation in the ORs among the 5 intrinsic subtypes ([Figure 1](#), [Figure 2](#); eFigures 2-5 and eTables 6 and 7 in [Supplement 1](#); eTable 4 in [Supplement 2](#)). For *BRCA1* PTV carriers, the OR was highest (OR, 55.32; 95% CI, 40.51-75.55) for TN disease, much lower for HR⁺ERBB2⁻ low-grade disease (OR, 3.26; 95% CI, 2.21-4.80) and HR⁺ERBB2⁺ disease (OR, 2.27; 95% CI, 1.16-4.45), and

intermediate for HR⁺ERBB2⁻ high-grade and HR⁻ERBB2⁺ disease (OR, 13.5 [95% CI, 9.16-19.90] and OR, 9.85 [95% CI, 5.71-17.02], respectively). Associations between *BRCA2* PTVs and intrinsic subtypes were more homogeneous across subtypes, with higher ORs associated with HR⁺ERBB2⁻ high-grade disease (OR, 11.53; 95% CI, 8.92-14.90) and TN tumors (OR, 10.07; 95% CI, 7.61-13.32). For *ATM*, the association was strongest for HR⁺ERBB2⁻ high-grade tumors (OR, 4.99; 95% CI, 3.68-6.76). *CHEK2* PTVs were associated with similar ORs with all subtypes except TN, for which there was no evidence of association. *PALB2* PTVs were associated with all subtypes, but with higher ORs for HR⁺ERBB2⁻ high-grade (OR, 9.43; 95% CI, 6.24-14.25) and TN disease (OR, 8.05; 95% CI, 5.17-12.53).

The PTVs in *RAD51C*, *RAD51D*, and *BARD1* were most strongly associated with TN disease (OR, 6.19 [95% CI, 3.17-12.12]; OR, 6.19 [95% CI, 2.99-12.79]; and OR, 10.05 [95% CI, 5.27-19.19], respectively). *RAD51D* PTVs were also associated with HR⁺ERBB2⁻ high-grade tumors.

Similar to PTVs, carriers of *BRCA1* MSV were strongly enriched for TN disease (eTable 4 in [Supplement 2](#) and eFigure 6 in [Supplement 1](#)). The ORs were higher than those for PTVs for all of the intrinsic subtypes, although these differences were not statistically significant. For *BRCA2*, the ORs for MSVs and PTVs were similar. *TP53* variants were associated with HR⁻ERBB2⁺ and HR⁺ERBB2⁺ but not TN disease.

A decline in the ORs with increasing age was observed for *BRCA1* and *BRCA2*; this trend was similar for all subtypes (OR, 0.96 per year for both genes; $P = 7.05 \times 10^{-7}$ and 3.14×10^{-11} for *BRCA1* [95% CI, 0.94-0.98] and *BRCA2* [95% CI, 0.95-0.97], respectively; eTable 4 in [Supplement 2](#)). The ORs also declined with age for *CHEK2*, but the trend was much weaker. There was no evidence of a decline in the ORs for *ATM*, *BARD1*, *RAD51C*, or *RAD51D*, but the confidence limits for the last 3 genes were wide.

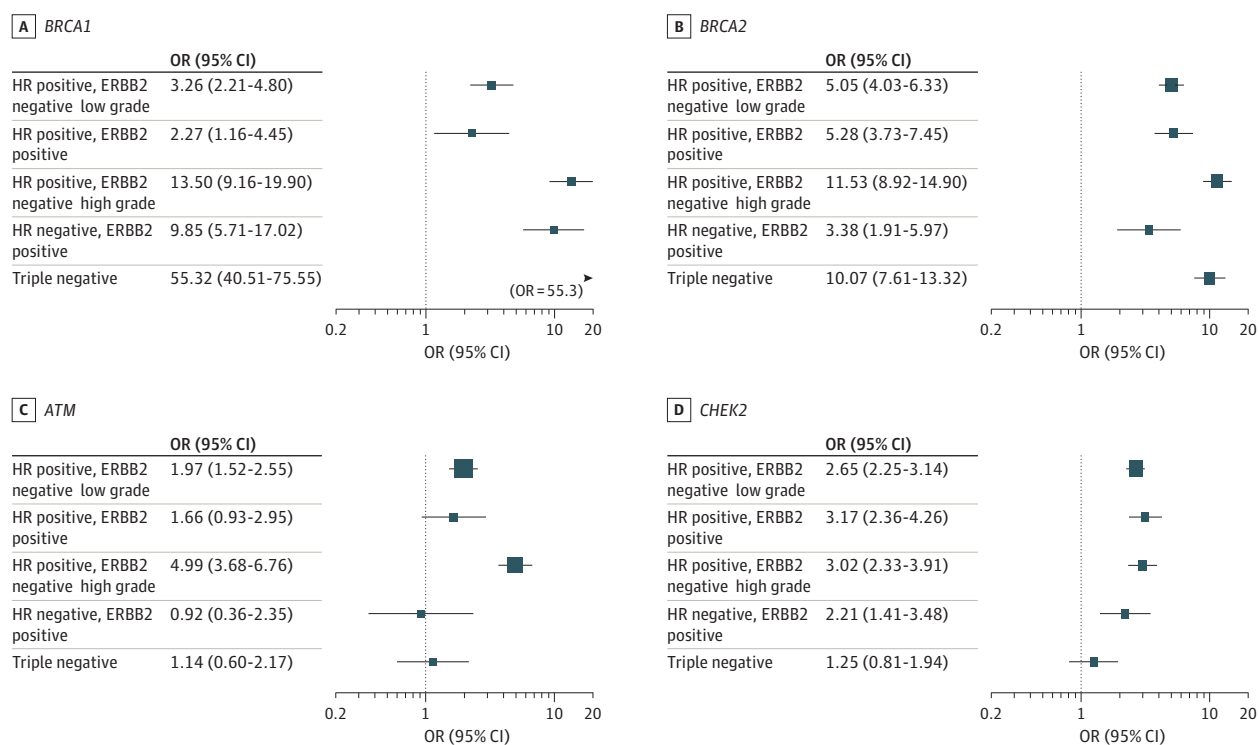
We further stratified HR-positive subtypes by PR expression to determine whether carrier status was associated with PR. For *BRCA1*, the ORs were lowest for ER⁺, PR⁺ tumors compared with other categories (eTable 8 in [Supplement 1](#)). Consistent with this observation, *BRCA1* PTV carriers were more likely to be PR negative, even after adjusting for intrinsic subtype. There was also some weak evidence for *BRCA2* PTVs and PR negativity, but no evidence for the other genes.

Association Between Breast Cancer Susceptibility Genes and Other Prognostic Factors

The PTVs in *BRCA2*, *CHEK2*, and *PALB2* were associated with larger tumor size, lymph node involvement, and higher stage at diagnosis (eFigure 1 in [Supplement 1](#)). The individual associations with larger tumor size and lymph node involvement remained significant after adjusting for intrinsic subtypes. The association between PTVs in all 9 genes with intrinsic subtypes remained similar after including size and lymph node status in the model (eTable 5 in [Supplement 2](#)).

For each gene, most BCs were carcinoma no special type (ductal carcinoma); in aggregate, 71% of tumors in carriers and 68% in noncarriers were ductal carcinoma. *BRCA1* tumors were less likely to be lobular than ductal (OR, 0.40; 95% CI, 0.25-0.63) but more likely to be medullary than nonmedullary (OR,

Figure 1. Association Odds Ratios (ORs) for Protein-Truncating Variant Carrier Status in Breast Cancer Susceptibility Genes *BRCA1*, *BRCA2*, *ATM*, and *CHEK2* and Intrinsic Subtypes of Breast Cancer



Multiple Imputation by Chained Equations imputation was conducted as described in the Methods and intrinsic subtypes constructed for each imputed data set. Multinomial logistic regression was conducted with intrinsic subtypes

as the outcome variable, adjusting by age at diagnosis/interview and country, and the results of these analyses were pooled. These results are also shown in eTable 4 in Supplement 2. HR indicates hormone receptor.

5.24; 95% CI, 3.34-8.22) (eTables 2 and 3 in Supplement 2). *TP53* tumors were more likely to be mixed lobular and ductal than ductal carcinoma (OR, 7.01; 95% CI, 3.04-16.17; $P = 5 \times 10^{-6}$). Otherwise, tumors associated with variations in the other BC genes were not enriched for any particular morphology.

Prevalence of Pathogenic Variants According to Subtypes and Age

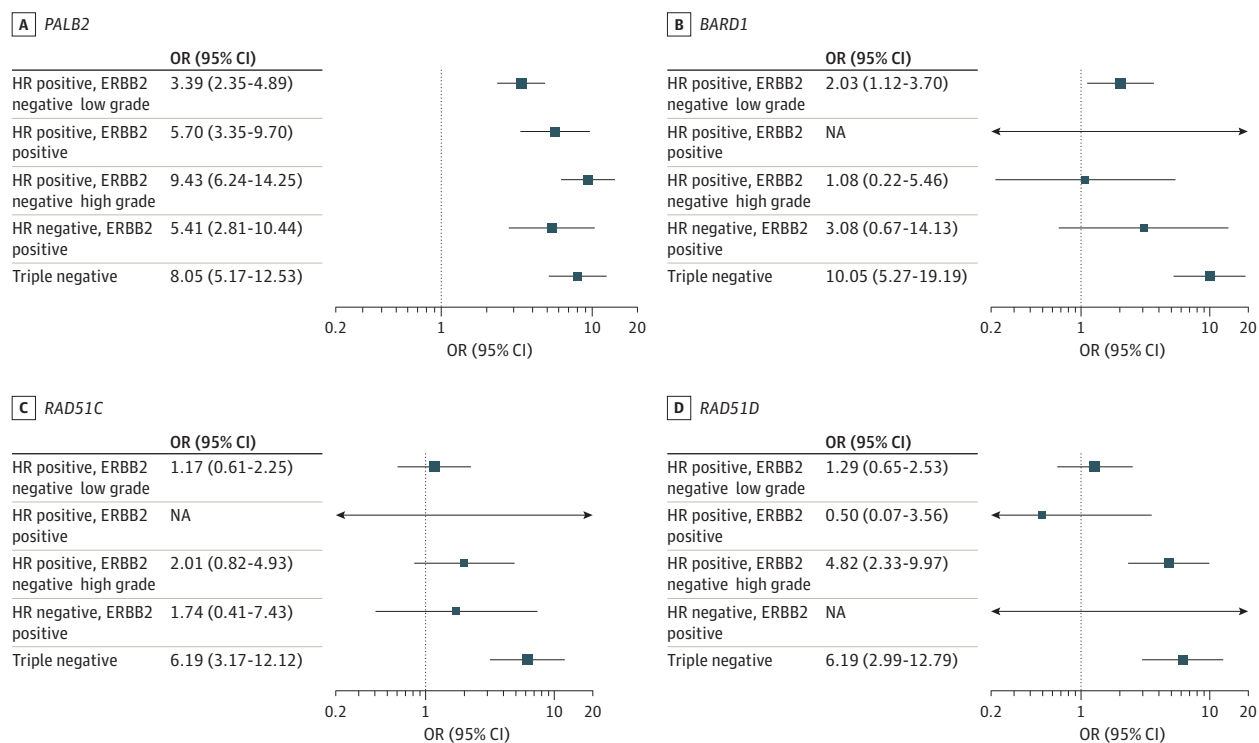
We assessed the association between rare variants in BC susceptibility genes with the burden of disease in women of different ages (eFigures 7-12 and eTable 7 in Supplement 1). Together, the 9 genes were associated with 14.4% of all tumors in women 40 years or younger but less than 4% in women older than 60 years. Among younger women, the prevalence of variants combined was higher among women with TN and HR⁺ERBB2⁻ high-grade tumors than those with other subtypes. The highest prevalence (27.3%) was among women 40 years or younger with TN tumors, mainly driven by *BRCA1* (eFigure 7 in Supplement 1). The combined prevalence of pathogenic variants was close to or exceeded 10% for all subtypes in women younger than 40 years and for TN and HR⁺ERBB2⁻ high-grade disease in women aged 40 to 59 years. Although *TP53*-related tumors comprised only a small proportion of ERBB2-positive disease, approximately 70% of *TP53* tumors among women 40 years or younger were ERBB2-positive.

Age-Specific Cumulative Risk of Developing Intrinsic BC Tumor Subtypes

Estimated cumulative risks according to intrinsic subtypes are shown in Figure 3 and Figure 4. The estimated risk for TN tumors was highest for *BRCA1* (40% by age 80 years), and 7% to 12% for *BRCA2*, *BARD1*, *PALB2*, *RAD51C*, and *RAD51D*. In contrast, the highest risks for HR⁺ERBB2⁻ low-grade disease were associated with *BRCA2* (22%) followed by *PALB2* and *CHEK2*.

Discussion

This case-control study evaluated the pathology of BCs developing in carriers of PTVs and/or rare MSVs in 9 BC susceptibility genes: *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *CHEK2*, *PALB2*, *RAD51C*, *RAD51D*, and *TP53* in a large multicenter collaborative study comprising population-based and hospital-based studies. The pattern of intrinsic subtypes and markers of tumor aggressiveness differed between carriers of variants in individual BC susceptibility genes and noncarriers. As expected,^{19,20} BC in *BRCA1* carriers were strongly enriched for TN tumors, with TN disease representing approximately 60% of all tumors and approximately 70% of tumors in women 40 years and younger. However, the risks for all other subtypes were also increased (ORs, 2.27-13.5). For *BRCA2*, the highest

Figure 2. Association Odds Ratios (ORs) for Protein-Truncating Variant Carrier Status in Breast Cancer Susceptibility Genes *PALB2*, *BARD1*, *RAD51C*, and *RAD51D* and Intrinsic Subtypes of Breast Cancer

Multiple Imputation by Chained Equations imputation was conducted as described in the Methods and intrinsic subtypes constructed for each imputed data set. Multinomial logistic regression was conducted with intrinsic subtypes

as the outcome variable, adjusting by age at diagnosis/interview and country, and the results of these analyses were pooled. These results are also shown in eTable 4 in Supplement 2. HR indicates hormone receptor; NA, not applicable.

ORs were for HR⁺ERBB2⁻ high-grade and TN disease, which was consistent with the strong association with ERBB2 negativity.^{21,22} The most common subtype (43% of cases) was HR⁺ERBB2⁻ low (/intermediate)-grade disease, but a clear excess of TN disease (approximately 18% of tumors) was apparent, even at younger ages. Subtype-specific associations for *BRCA1* and *BRCA2* MSVs were similar to those for PTVs in the corresponding genes.

Although the ORs were lower, the pattern of intrinsic subtypes for *PALB2* carriers was very similar to that for *BRCA2* carriers (Figures 1 and 2), with variation in both genes being associated with ERBB2 negativity and TN disease.²³ This similarity may reflect the closely associated functions of *PALB2* and *BRCA2* in the DNA damage response.²⁴

Conversely, the profile of intrinsic subtypes associated with *BARD1* carriers was similar to *BRCA1* carriers, with an excess of TN tumors (40% of cases), albeit the overall risk was much lower. Consistent with this observation, *Bard1* and *Brcal* knockout mice have similar phenotypes.²⁵ *BARD1* and *BRCA1* proteins form a stable complex, the heterodimer coordinating a range of cellular pathways to maintain genomic stability. Although *BRCA1* requires *BARD1* for stability and tumor suppressor functions, *BARD1* also plays distinct roles in cell cycle progression.^{25,26}

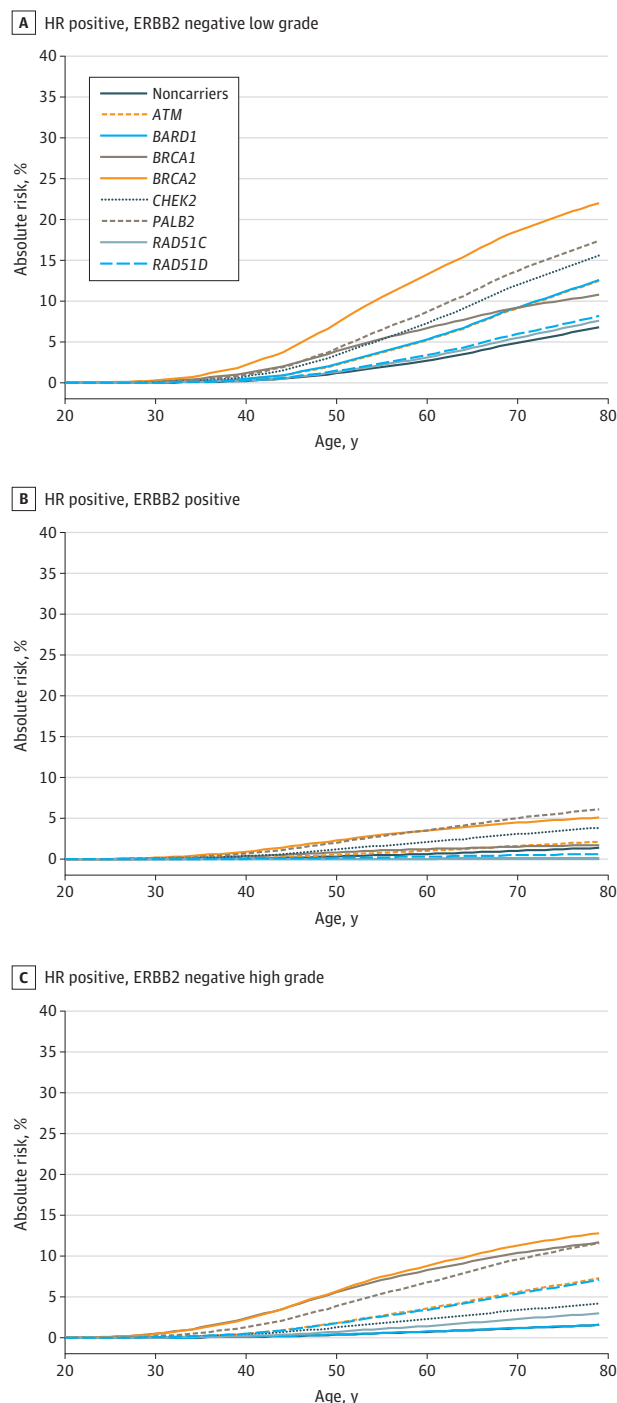
Carriers of PTVs in *ATM* and *CHEK2* were more strongly associated with ER-positive disease, but this study highlights

some differences. For *ATM*, the association was particularly strong for HR⁺ERBB2⁻ high-grade tumors (OR, 4.99; 95% CI, 3.68-6.76), with weaker associations for the other HR-positive subtypes (although HR⁺ERBB2⁻ low-grade tumors were still the most common). An association with the luminal B subtype has been reported previously in a small data set ($n = 28$).²⁷ *CHEK2* was associated with a similar OR for all the HR-positive subtypes and increased risk of HR⁺ERBB2⁺, but not TN disease. *ATM* plays a central role in the activation of DNA damage response and cell cycle checkpoint control, while *CHEK2* is involved downstream of *ATM* in cell cycle arrest, apoptosis, and DNA repair.^{28,29}

RAD51C and *RAD51D* are known ovarian cancer susceptibility genes and are more recently associated with BC,^{6,30} in particular with TN disease.^{4,31,32} In the present study, ORs for TN disease for PTVs in both genes were approximately 6.0. The subtype distribution of *RAD51C* is similar to *RAD51D*, reflecting their closely associated functions. We did observe an excess of HR⁺ERBB2⁻ high-grade tumors in *RAD51D* but not *RAD51C* carriers; however, the numbers of PTV carriers were small and we cannot exclude the subtype distributions being similar.

TP53 tumors were strongly enriched for ERBB2-positive subtypes (46% of cases), which was consistent with earlier studies in patients with or without Li-Fraumeni syndrome^{33,34} and examination of patients identified by multigene panel testing.³⁵ We also observed an association with mixed ductal

Figure 3. Estimates of Cumulative Risks of Breast Cancer by Age and Hormone Receptor (HR)-Positive Subtype for Protein-Truncating Variants in 8 Breast Cancer Susceptibility Genes



Age-, gene-, and subtype-specific cumulative risks were calculated as described in the Methods and eMethods in Supplement 1. Baseline incidence rates were derived from UK breast cancer incidence rates for 2016 (<https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/breast-cancer/incidence-invasive>).

and lobular morphology, tumors that comprise distinct but clonally related morphological components.³⁶

Pathogenic PTVs and MSVs in these 9 BC susceptibility genes were disproportionately associated with more aggressive BC, particularly among younger women. Carriers of rare genetic variants in the 9 genes constituted almost a third of women who received a diagnosis at or younger than 40 years of TN disease and approximately 16% of women with HR⁺ERBB2⁺ high-grade disease. All genes except *CHEK2* were more strongly associated with high-grade disease. Across genes, 27% to 72% of tumors were grade 3 (eTable 2 in Supplement 2). Previously studies have suggested that tumors in carriers of rare PTVs are larger^{23,37-40} and more likely to be identified as interval rather than screen-detected cancers.³⁷ In the present study, *BRCA2*-, *CHEK2*-, and *PALB2*-associated tumors were larger and more likely to be lymph node positive.

Despite the strong enrichment of TN disease for many of the genes, most carriers will still develop HR⁺ disease. With the exception of *BRCA1*, the most common subtype for all genes was HR⁺ERBB2⁻ low (/intermediate)-grade disease (Figures 3 and 4). However, these absolute risk projections indicate average subtype-specific risks, while individual risk prediction should also consider polygenic modifiers, family history, and lifestyle and reproductive factors, as well as the risk of developing cancers at other sites.⁴¹ The age- and subtype-specific risk estimates (eTable 6 in Supplement 2 and eFigure 13 in Supplement 1) may be used to refine BC risk prediction algorithms, such as BOADICEA.⁴¹

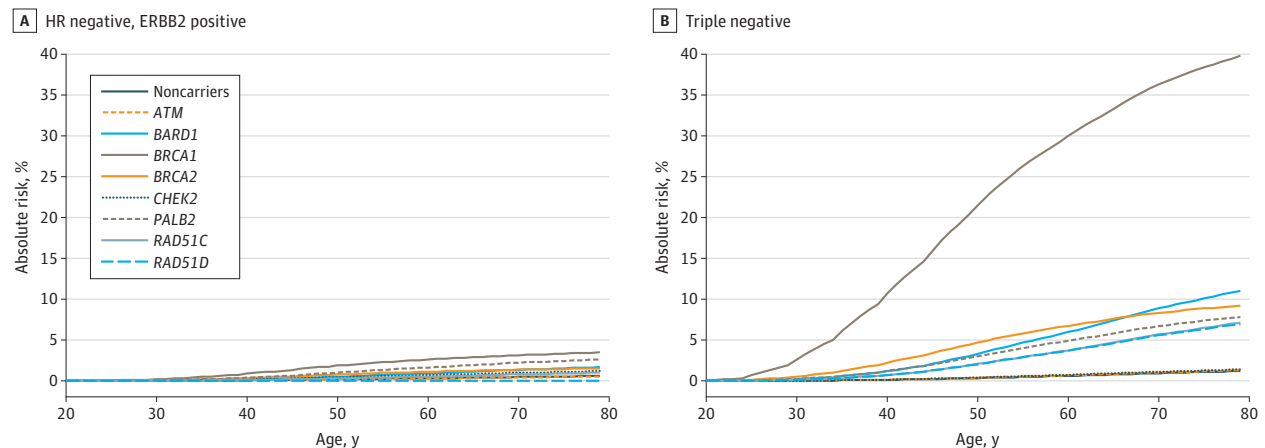
These results may also inform guidelines for eligibility for gene panel sequencing and BC surveillance in the general population. The combined prevalence of pathogenic variants in any of the 9 genes reached 10% for TN cases in those who received a diagnosis when younger than 60 years and HR⁺ERBB2⁺ high-grade and HR⁺ERBB2⁺ cases in those who received a diagnosis at 40 years or younger (in HR⁺ERBB2⁺ cases, the prevalence was 9.4%). These are slight underestimates of the true frequency because some variants deleterious to gene function, notably large gene rearrangements, will have been missed in the targeted sequencing.⁶

Tumor characteristics can also be used in determining whether variants of uncertain significance are likely to be pathogenic based on the assumption that the tumor characteristics of pathogenic variants of uncertain significance will be similar to known pathogenic variants.⁴² Therefore, these data should improve the precision of variant classification algorithms and extend them to a larger set of genes.

Strengths and Limitations

The strengths of this study are its large sample size (42 680 cases and 46 387 control participants) and sampling of cases independent of family history, while most earlier investigations have involved women who were ascertained in genetics clinics and selected based on family history, genotype, or pathology. The large sample size allowed us to obtain unbiased estimates of ORs and age interaction effects, while the sampling framework provided results that are particularly relevant as gene panel testing becomes applied at a general population level. Cases and control participants underwent sequencing on the same platform and using a single variant calling algorithm. We analyzed a comprehensive set of variables and their associa-

Figure 4. Estimates of Cumulative Risks of Breast Cancer by Age and Hormone Receptor (HR)–Negative Subtype for Protein-Truncating Variants in 8 Breast Cancer Susceptibility Genes



Age-, gene-, and subtype-specific cumulative risks were calculated as described in the Methods and eMethods in Supplement 1. Baseline incidence rates were derived from UK breast cancer incidence rates for 2016 (<https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/breast-cancer/incidence-invasive>).

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tions. Finally, the results found using 2 different imputation methods, Multiple Imputation by Chained Equations and an EM algorithm were consistent (eTable 9 in Supplement 1).

Despite the large size of this study, the sample size with complete pathology data was still limited for some genes. For example, ERBB2 status was missing for approximately 43% of samples, although missingness is likely to be random with respect to genotype, and imputation methods performed well. There was also minor heterogeneity in definition of stage, grade, and cutoffs for ER, PR, and ERBB2 across studies. The subtypes defined by immunohistochemical markers do not align perfectly with intrinsic subtypes defined by expression profiles, such as PAM-50,^{43,44} but such data are not available in large-scale epidemiological studies or routine practice. Fi-

nally, most participants were of European descent, and larger studies of women from other racial and ethnic groups will be important.

Conclusions

This case-control study suggests that rare variants in BC susceptibility genes display marked heterogeneity with respect to tumor phenotype, but also similarities between genes that are consistent with known biological functions. This present study provides detailed quantification of subtype-specific BC risks; these can potentially improve risk prediction models and breast cancer prevention strategies.

ARTICLE INFORMATION

Accepted for Publication: October 7, 2021.

Published Online: January 27, 2022.
doi:10.1001/jamaoncol.2021.6744

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Author Contributions: Dr Easton had full access to all of the data in the study and takes responsibility

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Obtained funding: Devilee, Teo, Easton, Schmidt.

Administrative, technical, or material support: Bolla, Wang, Keeman.

Conflict of Interest Disclosures: Dr Mavaddat reported grants from the University of Cambridge, European Union Horizon 2020, Wellcome Trust, Genome Canada, Canadian Institutes of Health Research, and National Cancer Institute during the conduct of the study. Dr Allen reported grants from EU Horizons 2020 during the conduct of the study. Dr Bolla reported grants from Cancer Research UK and the National Institutes of Health (NIH) during the conduct of the study. Dr Andrulis reported grants from NIH during the conduct of the study. Dr Briceno reported grants from Universidad de la Sabana during the conduct of the study. Dr Fasching reported personal fees from Roche, Lilly, Novartis, Pierre Fabre, Gilead, Seagen, Eisai, Daiichi Sankyo, AstraZeneca, Merck Sharp & Dohme, and Pfizer and grants from Cepheid and BioNtech during the conduct of the study. Dr Giles reported grants from National Health and Medical Research Council during the conduct of the study. Dr Hahnen reported personal fees from AstraZeneca outside the submitted work. Dr Hartikainen reported grants from Cancer Foundation Finland during the conduct of the study. Ms Morra reported being paid by an EU grant during the conduct of the study. Dr Park-Simon reported being a recipient of the Claudia von Schilling Stiftung Award during the conduct of the study. Dr Schmutzler reported grants from German Cancer Aid during the conduct of the study. Dr Southey reported grants from National Health and Medical Research Council (NHMRC) (Australia) during the conduct of the study. Dr Spurdle reported grants from the NHMRC during the conduct of the study. Dr Pharoah reported grants from Cancer Research UK during the conduct of the study. Dr Kvist reported grants from European Union Horizon 2020 research and innovation program BRIDGES during the conduct of the study. Dr Nevanlinna reported grants from Helsinki University Hospital, Sigrid Juselius Foundation, and Cancer Foundation Finland during the conduct of the study as well as honorarium from AstraZeneca outside the submitted work. Dr Camp reported grants from the University of Utah, Huntsman Cancer Foundation, and the National Cancer Institute. Dr Schmidt reported grants from EU during the conduct of the study. Dr Easton reported grants from the European Commission and Wellcome Trust outside the submitted work. No other disclosures were reported.

Funding/Support: The BRIDGES panel sequencing was supported by the European Union Horizon 2020 research and innovation program BRIDGES (grant 634935) and the Wellcome Trust (v203477/Z/16/Z). The Breast Cancer Association Consortium (BCAC) is funded by the European Union's Horizon 2020 Research and Innovation Programme (grants 634935 and 633784 for BRIDGES and B-CAST,

respectively) and the PERSPECTIVE I&I project, which is funded by the Government of Canada through Genome Canada and the Canadian Institutes of Health Research, the Ministère de l'Économie et de l'Innovation du Québec, through Genome Québec, the Quebec Breast Cancer Foundation. Additional funding for BCAC is provided via the Confluence project, which is funded with intramural funds from the National Cancer Institute Intramural Research Program. The ABCS study was supported by the Dutch Cancer Society (grants NKI 2007-3839; 2009 4363). The ACP study is funded by the Breast Cancer Research Trust, UK. Prof Muir and Dr Lophatananon are supported by the National Institute of Health Research (NIHR) Manchester Biomedical Research Centre, the Allan Turing Institute under the Engineering and Physical Sciences Research Council grant EP/N510129/1. The work of the BBCC was partly funded by ELAN-Fond of the University Hospital of Erlangen. For BIGGS, Dr Saloustros is supported by the NIHR Comprehensive Biomedical Research Centre, Guy's & St. Thomas' National Health Service (NHS) Foundation Trust in partnership with King's College London. Dr Tomlinson is supported by the Oxford Biomedical Research Centre. The BREast Oncology GALician Network (BREGAN) is funded by Acción Estratégica de Salud del Instituto de Salud Carlos III FIS grant PI12/02125/Cofinanciado FEDER; Acción Estratégica de Salud del Instituto de Salud Carlos III FIS Intrastad (grant PI13/01136), and grant PI17/00918/Cofinanciado FEDER. Funding was received from Xerencia de Xestión Integrada de Vigo-SERGAS, Instituto de Salud Carlos III grant 10CSA012E and Consellería de Industria Programa Sectorial de Investigación Aplicada grant EC11-192. The BSUCH study was supported by the Dietmar-Hopp Foundation, the Helmholtz Society, and the German Cancer Research Center. The CCGP is supported by funding from the University of Crete. The CECILE study was supported by Fondation de France, Institut National du Cancer, Ligue Nationale contre le Cancer, Agence Nationale de Sécurité Sanitaire, de l'Alimentation, de l'Environnement et du Travail, and Agence Nationale de la Recherche. The CGPS was supported by the Chief Physician Johan Boserup and Lise Boserup Fund, the Danish Medical Research Council, and Herlev and Gentofte Hospital. The CNIO-BCS was supported by the Instituto de Salud Carlos III, the Red Temática de Investigación Cooperativa en Cáncer, and grants from the Asociación Española Contra el Cáncer and the Fondo de Investigación Sanitario (PI11/00923 and PI12/00070). Dr Osorio is partially supported by FIS PI19/00640 supported by FEDER funds and the Spanish Network on Rare Diseases (CIBERER). COLBCCC is supported by the German Cancer Research Center. Dr Torres was in part supported by a postdoctoral fellowship from the Alexander von Humboldt Foundation. PROCAS is funded from NIHR grant PGfAR 0707-10031. Prof Evans and Dr Howell are supported by the NIHR Manchester Biomedical Research Centre (IS-BRC-1215-20007). The German Consortium of Hereditary Breast and Ovarian Cancer is supported by the German Cancer Aid (grants 110837 and 70114178) and Federal Ministry of Education and Research, Germany (grant 01GY1901). This work was also funded by the European Regional Development Fund and Free State of Saxony, Germany (Leipzig Research Centre for Civilization Diseases project numbers

713-241202, 713-241202, 14505/2470, 14575/2470). The GENICA was funded by the Federal Ministry of Education and Research Germany grants 01KW9975/5, 01KW9976/8, 01KW9977/0, and 01KW0114, the Robert Bosch Foundation, Deutsches Krebsforschungszentrum (DKFZ), the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr University Bochum, and the Department of Internal Medicine, Evangelische Kliniken Bonn gGmbH, Johanniter Krankenhaus, Bonn, Germany. Generation Scotland received core support from the Chief Scientist Office of the Scottish Government Health Directorates (CZD/16/6) and the Scottish Funding Council (HR03006) and is currently supported by the Wellcome Trust (216767/Z/19/Z). Genotyping of the GS:SFHS samples was carried out by the Genetics Core Laboratory at the Edinburgh Clinical Research Facility, University of Edinburgh, Scotland and was funded by the Medical Research Council UK and the Wellcome Trust (104036/Z/14/Z). Funding for identification of cases and contribution to BCAC funded in part by the Wellcome Trust Seed Award. The GESBC was supported by the Deutsche Krebshilfe e. V. and the German Cancer Research Center. The HABCS study was supported by the Claudia von Schilling Foundation for Breast Cancer Research, Lower Saxonian Cancer Society, German Research Foundation (grant Do761/10-1), and the Rudolf Bartling Foundation. The HEBCS was financially supported by the Helsinki University Hospital Research Fund, the Finnish Cancer Society, and the Sigrid Juselius Foundation. The HMBCS was supported by a grant from the German Research Foundation (Do761/10-1) and the Rudolf Bartling Foundation. The HUBCS was supported by a grant from the German Federal Ministry of Research and Education (RUS08/017). Drs Bermisheva and Khushnutdinova were supported by the megagrant from the Government of Russian Federation (No. 075-15-2021-595) and Saint Petersburg State University (grant 602368366). Financial support for KARBAC was provided through the regional agreement on medical training and clinical research between Stockholm County Council and Karolinska Institutet, Swedish Cancer Society, Gustav V Jubilee foundation, and Bert von Kantzows foundation. The KARMA study was supported by Märit and Hans Rausing's Initiative Against Breast Cancer. The KBCP was financially supported by the government funding of Kuopio University Hospital, Cancer Fund of North Savo, the Finnish Cancer Organizations, and by the strategic funding of the University of Eastern Finland. The kConFab is supported by a grant from the National Breast Cancer Foundation and previously by the National Health and Medical Research Council (NHMRC), Queensland Cancer Fund, Cancer Councils of New South Wales, Victoria, Tasmania, and South Australia, and the Cancer Foundation of Western Australia. Financial support for the AOCS was provided by the US Army Medical Research and Materiel Command (DAMD17-01-1-0729), Cancer Council Victoria, Queensland Cancer Fund, Cancer Council New South Wales, Cancer Council, South Australia, Cancer Foundation of Western Australia, Cancer Council, Tasmania, and the National Health and Medical Research Council of Australia (NHMRC 400413, 400281, and 199600). Dr Chenevix-Trench is supported by the NHMRC. The MARIE study was supported by the Deutsche Krebshilfe e.V. (70-2892-BR I, 106332, 108253,

108419, 110826, and 110828), Hamburg Cancer Society, German Cancer Research Center, and the Federal Ministry of Education and Research Germany (01KH0402). The MASTOS study was supported by Cyprus Research Promotion Foundation grants O104/13 and O104/17 and the Cyprus Institute of Neurology and Genetics. The Melbourne Collaborative Cohort Study (MCCS) cohort recruitment was funded by VicHealth and Cancer Council Victoria. The MCCS was further augmented by Australian National Health and Medical Research Council grants 209057, 396414, and 1074383 and by infrastructure provided by Cancer Council Victoria. Cases and their vital status were ascertained through the Victorian Cancer Registry and the Australian Institute of Health and Welfare, including the National Death Index and the Australian Cancer Database. The MBCSG is supported by funds from the Italian Association for Cancer Research to Drs Radice and Peterlongo. The MYBRCA is funded by research grants from the Wellcome Trust grant (v203477/Z/16/Z) and the Malaysian Ministry of Higher Education (UM.C/HIR/MOHE/06) and Cancer Research Malaysia. The NBCS has received funding from the K.G. Jebsen Centre for Breast Cancer Research, the Research Council of Norway grant 193387/V50 (to Drs Børresen-Dale and Kristensen) and grant 193387/H10 (to Drs Børresen-Dale and Kristensen), South Eastern Norway Health Authority (grant 39346 to Dr Børresen-Dale), and the Norwegian Cancer Society (to Drs Børresen-Dale and Kristensen). The Ontario Familial Breast Cancer Registry (OFBCR) was supported by grant UO1CA164920 from the US National Cancer Institute of the National Institutes of Health. The PBCS was funded by Intramural Research Funds of the National Cancer Institute. Genotyping for PLCO was supported by the Intramural Research Program of the National Institutes of Health. The SASBAC study was supported by funding from the Agency for Science, Technology and Research of Singapore, the National Institutes of Health, and the Susan G. Komen Breast Cancer Foundation. SEARCH is funded by Cancer Research UK (grants C490/A10124 and C490/A16561) and supported by the NIHR Biomedical Research Centre at the University of Cambridge. The University of Cambridge has received salary support for Prof Pharoah from the NHS in the East of England through the Clinical Academic Reserve. SGBCC is funded by the National Research Foundation Singapore, NUS start-up Grant, National University Cancer Institute Singapore Centre Grant, Breast Cancer Prevention Programme, Asian Breast Cancer Research Fund and the NMRC Clinician Scientist Award. Population-based controls were from the Multi-Ethnic Cohort funded by grants from the Ministry of Health, Singapore, National University of Singapore and National University Health System. SKKDKFZS is supported by the German Cancer Research Center. The SZBCS was supported by grant PBZ_KBN_122/PO5/2004 and the program of the Minister of Science and Higher Education under the name "Regional Initiative of Excellence" in 2019-2022 project number 002/RID/2018/19. UBSCS was supported by funding from National Cancer Institute grant R01CA163353 (Dr Camp) and the Women's Cancer Center at the Huntsman Cancer Institute. Data collection for UBSCS was supported by the Utah Population Database and Utah Cancer Registry. Dr Spurdle was supported by an NHMRC Investigator Fellowship (APP177524).

Data Sharing Statement: Requests for individual-level data used in these analyses should be made via the BCAC data access coordinating committee (bcac@medschl.cam.ac.uk).

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