# Breast cancer polygenic risk score and contralateral breast cancer risk

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## Abstract

Previous research has shown that polygenic risk scores (PRS) can be used to stratify women according to their risk of developing primary invasive breast cancer. This study aimed to evaluate the association between a recently validated PRS of 313 germline variants ($PRS_{313}$) and contralateral breast cancer (CBC) risk. We included 56,068 women of European ancestry diagnosed with first invasive breast cancer from 1990 onwards with follow-up from the Breast Cancer Association Consortium. Metachronous CBC risk (N=1,027) according to the distribution of the $PRS_{313}$ was quantified using Cox regression analyses. We assessed $PRS_{313}$ interaction with age at first diagnosis, family history, morphology, ER-, PR-, and HER2-status, and (neo)adjuvant therapy. In Asian studies, with limited follow-up, CBC risk associated with $PRS_{313}$ was assessed using logistic regression for 340 women with CBC compared with 12,133 women with unilateral breast cancer. Higher $PRS_{313}$ was associated with increased CBC risk: hazard ratio per standard deviation (SD)=1.25 (95%CI=1.18-1.33) for Europeans, and an OR per SD=1.15 (95%CI=1.02-1.29) for Asians. The absolute lifetime risks of CBC, accounting for death as competing risk, were 12.4% for European women at the $10^{th}$ percentile and 20.5% at the $90^{th}$ percentile of the $PRS_{313}$. We found no evidence of confounding by, or interaction with patient characteristics, characteristics of the primary tumor, or treatment. The C-index for the $PRS_{313}$ alone was 0.563 (95%CI=0.547-0.586). In conclusion, the $PRS_{313}$ is an independent factor associated with CBC risk, and may be incorporated in CBC risk prediction models to help improve stratification of patients and optimize surveillance and treatment strategies.

## Introduction

Due to the high incidence of breast cancer and improving survival, an increasing number of breast cancer survivors are at risk of developing contralateral breast cancer (CBC). The 10-year cumulative incidence of CBC is ~4%[1; 2], however estimates vary widely depending on factors such as germline genetics, family history, and (neo)adjuvant systemic therapy for the first breast cancer[3]. The risk of developing CBC is particularly high in women with rare mutations in certain genes including *BRCA1, BRCA2,* and *CHEK2*, with approximately two- to fourfold higher risks reported compared with women without these mutations[3].

Recently, genome-wide association studies (GWAS) have identified multiple common germline variants that are associated with first primary breast cancer risk[4; 5]. These are associated with small differences in risk individually, but their combined effects can be summarized in a polygenic risk score (PRS), which has been shown to stratify women according to their risk of developing breast cancer[6-9]. Using a large GWAS dataset from the Breast Cancer Association Consortium (BCAC), we previously developed and validated a 313-variant PRS ($PRS_{313}$) among women of European descent. In independent prospective studies, this $PRS_{313}$ predicted the risk of primary invasive breast cancer with an odds ratio (OR) per standard deviation (SD) of 1.61 (95% confidence interval (95%CI)=1.57-1.65)[7]. The $PRS_{313}$ has also been externally validated using the UK Biobank cohort.

The aim of the current study was to evaluate the association between $PRS_{313}$ and CBC risk, using data from BCAC. Other studies have shown associations between risk of CBC and both a 67-variant PRS[10] and individual variants[11], but not yet with $PRS_{313}$, the most extensively validated PRS. Further, the dataset currently evaluated is larger than those previously tested. We carried out two types of analyses. We conducted a cohort study among studies of European ancestry women with follow-up data available, and performed Cox regression analyses to

estimate hazard ratios (HRs) for CBC. Potential confounding and interaction with patient characteristics, characteristics of the primary tumor, or treatment were tested. In addition, to directly compare with the OR reported for $PRS_{313}$ and first breast cancer, we selected case-case series and performed logistic regression analyses comparing the $PRS_{313}$ distribution in women with CBC versus those with unilateral breast cancer. These analyses were conducted separately in European and Asian women (follow-up was too limited to perform a cohort study for the Asian population). Use of $PRS_{313}$ may lead to more accurate CBC risk prediction to support decision making for women who may or may not benefit from additional surveillance and risk-reducing treatment strategies.

## Material and Methods

### Study subjects

*Case-case series*

We selected women who were diagnosed with breast cancer and women without any diagnosis of breast cancer from the BCAC including all women of European ancestry, based on genotyping data, selecting only those studies which reported on CBC (62 studies) (Figure S1A, Table S1-S2). BCAC database version freeze 12 was used. All women diagnosed with invasive breast cancer as a first cancer were included in the analysis; the small number of tumors with unknown invasiveness were considered invasive (Table S2). In the case-case series, a CBC was defined as a breast cancer (in situ or invasive) in the contralateral breast irrespective of the time since the first breast cancer. The case-case series comprised 81,000 women with unilateral breast cancer, 3,607 women with CBC, and 62,830 women without any diagnosis of breast cancer (Figure S1A). We also compared women with unilateral breast cancer to women without any diagnosis of breast cancer to reproduce the estimate that was previously reported for first breast cancer risk[7] in our study selection.

We selected for a separate analysis women of Asian ancestry of the BCAC data comprising 12,133 women with unilateral breast cancer, 340 women with CBC, and 13,398 women without any diagnosis of breast cancer from eight studies (Figure S1B, Table S2).

*European cohort*

In the European cohort we used metachronous CBC as the outcome, defined as a breast cancer in the contralateral breast (in situ or invasive) diagnosed at least three months after the first breast cancer. We used a cut-off of three months to reduce the likelihood that these CBCs represent metastases rather than true second primary tumors. We selected all women diagnosed with breast cancer from the European case-case series and excluded four studies

that did not provide follow-up information on vital status (Figure S1A). We did not include Asian women since follow-up was too limited in these studies. We additionally excluded 6,207 women with no follow-up and 2,208 women who developed synchronous CBC, distant metastasis, or who died or last known to be alive within three months after the first breast cancer diagnosis. Since BCAC also included prevalent cases, we excluded 3,796 women who developed CBC or were censored before study entry. The case-case series included women diagnosed between 1947 and 2018. In the European cohort, we excluded 2,235 women who were diagnosed with their first breast cancer before 1990 or who had missing year of first diagnosis. We restricted to women diagnosed from 1990 onwards so that diagnostic procedures and treatment would be more representative of current practice. Moreover, clinico-pathological, treatment and follow-up data were more complete after 1990. In addition, we excluded 16 studies (9,783women) without information about metachronous CBC events (Figure S1A). After these exclusions, the cohort for this analysis comprised data from 42 studies, including 56,068 women with invasive breast cancer among whom 1,027 metachronous CBC occurred (Table S2).

All individuals provided written informed consent, and all studies were approved by the relevant institutional review boards. BCAC data were centrally harmonized and cleaned in communication with the study data managers and principal investigators. Data collection for individual studies is described in Table S1.

**Genotyping and PRS**

DNA samples from participants were genotyped using the iCOGS array[12; 13] or the OncoArray[4; 14], with genotypes for variants not on the arrays estimated by imputation[4; 13]. The $PRS_{313}$ was calculated as a weighted sum of the minor allele dosages; the variant selection and weights are as given by Mavaddat et al.[7]. We also calculated estimates for a previously published $PRS_{77}$[6], and estrogen receptor (ER)-specific PRSs (ER-positive $PRS_{313}$ and ER-negative $PRS_{313}$)[7]. The

ER-specific PRSs were constructed by defining subtype-specific weights for the 313 variants using a hybrid approach[7]. Variants and corresponding coefficients used to construct the PRS are shown in Table S3. We standardized the PRS in our analyses by dividing it by the SD of the PRS of the controls ($PRS_{77}$ SD=0.45; $PRS_{313}$ SD=0.61; ER-positive $PRS_{313}$ SD=0.65; ER-negative $PRS_{313}$ SD=0.59) exactly as was done in the analyses of the PRS and first breast cancer risk[6; 7]. This allows a direct comparison of the magnitude of the CBC relative risk estimation to that of the first breast cancer.

For samples genotyped with both OncoArray and iCOGS array (9,071 samples), OncoArray data were used in preference as the imputation quality was generally higher. The intraclass correlation coefficient (ICC) between the PRS derived from the two platforms was 0.99 (95%CI=0.99-0.99) for the $PRS_{77}$, and 0.96 (95%CI=0.95-0.96) for $PRS_{313}$ (Figure S2). Given the high correlation between the two platforms, PRS measures from both platforms were used in the analyses without adjustment.

**Statistical analysis**

*European cohort*

The primary outcome in the European cohort was the development of metachronous CBC. Cox proportional hazards models were used to estimate HRs for metachronous CBC risk by PRS, stratified by country. Since previous studies have shown that age at first breast cancer diagnosis is an important predictor of CBC[3], the analyses were performed with attained age as the time scale. Time at risk started three months after the first breast cancer diagnosis and ended at the age of CBC diagnosis, distant metastasis (where available), death, or end of follow-up, whichever came first. For patients that had a study entry more than three months after first breast cancer diagnosis, follow-up started at the age of study entry. We also performed a fixed-effect meta-analysis of country-specific effects using the STATA command

*metan*. We performed a fixed-effect meta-analysis over a random-effect meta-analysis since there was no evidence for heterogeneity in effect sizes between countries (I-squared=0%, Figure S3). For some analyses, only invasive CBC was used as the outcome; in these analyses we censored on in situ CBC. Separate analyses were conducted for ER-positive CBC (censored on ER-negative- and ER-unknown CBC) and ER-negative CBC (censored on ER-positive- and ER-unknown CBC).

We evaluated the linearity of the association between $PRS_{313}$ per unit SD and CBC risk using restricted cubic splines with three knots. There was no evidence for violation of the linearity assumption. Therefore, in the main analysis, the $PRS_{313}$ was treated as a continuous covariate, and estimated the HR per unit SD of the $PRS_{313.}$ Violation of the proportional hazard assumption was assessed by inspection of the Schoenfeld residuals[15]. As a second analysis, we used the per SD log HR of the $PRS_{313}$ to calculate the predicted HR at different percentiles of the $PRS_{313}$, compared to the $50^{th}$ percentile. Third, the $PRS_{313}$ was categorized into percentile groups ($0^{th}$ to $10^{th}$, $10^{th}$ to $20^{th}$, $20^{th}$ to $40^{th}$, $40^{th}$ to $60^{th}$, $60^{th}$ to $80^{th}$, $80^{th}$ to $90^{th}$, $90^{th}$ to $100^{th}$) to illustrate the differences between $PRS_{313}$ subgroups, with the middle quintile ($40^{th}$ to $60^{th}$) as the reference.

We also performed multivariable Cox regression analyses to determine whether the log HR of CBC risk by PRS changed when adjusting for year of first breast cancer diagnosis, family history of breast cancer in a first degree relative, and several clinical characteristics of the first breast cancer such as nodal status, tumor size, morphology, ER-, progesterone receptor (PR)- and human epidermal growth factor receptor 2 (HER2)-status, (neo)adjuvant chemotherapy, adjuvant endocrine therapy, and radiotherapy. These analyses were performed in all patients, a complete case set (excluding patients with unknown values for the covariates), and in a set excluding studies oversampling cases with family history. Potential effect modification of the

$PRS_{313}$ effect by the same variables was evaluated by fitting interaction terms in different models using complete case sets, including the standardized $PRS_{313}$, modifier, and interaction.

The discriminative ability of different models; ([model 1] $PRS_{313}$ alone, [model 2] other risk factors (the adjustment variables from the multivariable Cox regression analyses), [model 3] $PRS_{313}$ + other risk factors) was calculated using Harrell's C-index[16]. Since no standard performance measures are currently available to account for left-truncated follow-up time (*i.e*., to start analyses at age at study entry), we used time since first breast cancer as the time scale to calculate the C-index.

*Absolute risks*

Absolute risks of developing CBC at $PRS_{313}$ percentiles were calculated using the estimated log HRs per SD from the breast cancer cohort (BCAC) under the log-linear model, assuming the PRS is normally distributed. The $PRS_{313}$- and age-specific incidences were constrained to the age-specific CBC incidences from women diagnosed with a first invasive breast cancer in the period 2003-2010 from the Netherlands Cancer Registry (NCR)[1]. The procedure for constraining the incidences has been previously described[17]. The age-specific CBC incidences were calculated overall and for age-specific groups, censoring on death and distant metastasis. We used data from the NCR since this registry has complete coverage of all newly diagnosed cancers in the Netherlands. The NCR cohort included all females aged ≥18 years and follow-up for second cancers was complete until February 1, 2016[1]. We then applied the competing risk of dying on the absolute CBC risks. The absolute CBC risk ($AR_g$) by age $t$ in $PRS_{313}$ category $g$, taking into account the competing risk of dying was calculated by:

$$AR_g(t) = \sum_{u=0}^{t-1} \mu_g(u) S_g(u) S_m(u)$$

Where $\mu_g\ (t)$ is the CBC incidence associated with $PRS_{313}$ category $g$, $S_g\ (t)$ the probability of being free of CBC to age $t$, and $S_m\ (t)$ the probability of surviving to age $t$.

*Case-case series*

For the case-case series (European and Asian), logistic regression models were used to estimate the ORs for CBC risk (comparing with unilateral breast cancer) and for unilateral breast cancer risk (comparing with women without any diagnosis of breast cancer) associated with $PRS_{313}$. All analyses were adjusted for age and country (Table S1). For all unilateral- and contralateral breast cancer patients we used age at first breast cancer diagnosis, and for women without any diagnosis of breast cancer we used age at baseline questionnaire.

For direct comparison with the estimate reported for $PRS_{313}$ and first breast cancer, we also performed logistic regression analyses in the same BCAC study participants included in the validation of the association between $PRS_{313}$ and first breast cancer risk[7]. This validation set comprised a subsample from 24 studies and included 3,781 women with unilateral breast cancer, 94 women with CBC, and 3,753 women without any diagnosis of breast cancer (Table S2). For this analysis, we adjusted for 10 principal components, in line with Mavaddat et al.[7].

For European women who had follow-up time available more than three months after the first breast cancer diagnosis, a sensitivity analysis was performed for metachronous CBC (1,702 CBCs). We also did a separate analysis for invasive CBC (N=3,246), by excluding CBC in situ.

All P-values are two sided; tests with P<.05 are referred to as statistically significant. Analyses were performed using STATA, version 13.1 (StataCorp) and R version 3.3.2.

## Results

*European (cohort) Cox regression analyses*

The European cohort included 56,068 women diagnosed with first invasive breast cancer with 1,027 metachronous CBC events. Median follow-up was 8.4 years. Patient, tumor, and treatment characteristics are summarized in Table S4.

The associations between the different PRSs and CBC risk are shown in Table 1. The HR for CBC per SD of $PRS_{313}$ was 1.25 (95%CI=1.18-1.33). For comparison, the HR per SD for $PRS_{77}$ was 1.21 (95%CI=1.14-1.29). Women within the $0^{th}$ to $10^{th}$ and the $90^{th}$ to $100^{th}$ percentile of the $PRS_{313}$ had 0.59-fold (95%CI=0.45-0.78) and 1.38-fold (95%CI=1.13-1.69) risks of CBC, respectively, compared with women within the $40^{th}$ to $60^{th}$ percentile (Figure 1, Table S5). The predicted HRs of CBC for women at the $10^{th}$ and $90^{th}$ percentile of the $PRS_{313}$ were 0.75 and 1.33, respectively, compared to the $50^{th}$ percentile (Figure 1). Since we observed evidence of departure from the proportional hazards assumption (P=0.02)[15], we also calculated HRs stratified for follow-up duration (<five and ≥five years). The HR by SD of the $PRS_{313}$ was 1.21 (95%CI=1.10-1.32) for CBC diagnosed ≤five years after first breast cancer diagnosis (CBC N=428), and 1.28 (95%CI=1.18-1.38) for CBC diagnosed >five years after first diagnosis (CBC N=599).

The HR per SD of $PRS_{313}$ for ER-positive invasive CBC was 1.38 (95%CI=1.23-1.55), compared to a HR per SD of the ER-positive $PRS_{313}$ of 1.37 (95%CI=1.22-1.54) (Table 1). For ER-negative invasive CBC, the HR per SD was 0.92 (95%CI=0.75-1.12) for $PRS_{313}$ and 1.06 (95%CI=0.86-1.30) for the ER-negative $PRS_{313}$.

Sensitivity analysis using the overall $PRS_{313}$ showed a HR per SD of 1.24 (95%CI=1.16-1.32) for invasive CBC risk. When we used time since first breast cancer as the time scale, we found

similar results (HR per SD=1.25, 95%CI=1.18-1.33). Meta-analysis of country-specific effects showed a HR per SD of 1.25 (95%CI=1.18-1.33) for CBC risk by $PRS_{313}$ (Figure S3).

The association between the $PRS_{313}$ and CBC risk did not change when adjusting for patient, tumor, and treatment characteristics, nor when excluding studies oversampling cases with a family history (Table S6). When considering potential modifiers of the effect of the $PRS_{313}$ on CBC risk (Table 2), we found that the HR was the lowest in women aged <40 years at first breast cancer diagnosis (HR per SD=1.13; 95%CI=0.98-1.31), and tended to increase with age, although these effects were not statistically significant ($P_{heterogeneity}$=.26; $P_{trend}$=.05). We found no indication for effect modification by family history ($P_{heterogeneity}$=.63), morphology ($P_{heterogeneity}$=.14), ER-status ($P_{heterogeneity}$=.13), PR-status (P=.26), HER2-status ($P_{heterogeneity}$=.42), chemotherapy ($P_{heterogeneity}$=.60), endocrine therapy ($P_{heterogeneity}$=.79), or radiotherapy ($P_{heterogeneity}$ =.40) (Table 2).

The C-index was 0.563 (95%CI=0.547-0.586) for the model only including $PRS_{313}$, 0.605 (95%CI=0.591-0.629) for the model only including other risk factors, and 0.623 (95%CI=0.608-0.645) for the complete model (Table 3).

*Absolute risks*

Based on the HR estimates for $PRS_{313}$, the predicted CBC risk by age 80 years was 12.4% at the $10^{th}$ percentile of the $PRS_{313}$, compared with 20.5% at the $90^{th}$ percentile of the $PRS_{313}$ (Figure 2), accounting for death as competing risk. When death was not taken into account as competing risk, the corresponding predicted risks by age 80 were 17.0% at the 10% percentile and 27.9% at the $90^{th}$ percentile of the $PRS_{313}$ (Figure S4). Table 4 shows the five- and 10-year cumulative CBC risks by $PRS_{313}$ for different age groups, accounting for death as competing risk (Table S7 shows results without competing risks).

*European and Asian (case-case series) logistic regression analyses*

Figure 3 shows the distribution of the $PRS_{313}$ per SD in the European case-case series. Median $PRS_{313}$ was -0.4 (interquartile range [IQR]=1.35) for control women without any diagnosis of breast cancer (N=81,000), 0.2 (IQR=1.36) for women with unilateral breast cancer (N=62,830), and 0.5 (IQR=1.40) for women with CBC (N=3,607). The OR for unilateral breast cancer per SD of the $PRS_{313}$, compared to control women, was 1.82 (95%CI=1.80-1.84) (Table S8). The OR for CBC per SD of $PRS_{313}$, compared to unilateral breast cancer, was 1.30 (95%CI=1.26-1.35) .

In sensitivity analyses, the OR per SD of $PRS_{313}$ was 1.27 (95%CI=1.21-1.33) for metachronous CBC and the OR per SD was 1.29 (95%CI=1.24-1.33) for invasive CBC, compared to unilateral breast cancer. When analyses were restricted to the validation set of Mavaddat et al[7], the OR for unilateral breast cancer per SD of the $PRS_{313}$ was 1.67 (95%CI=1.59-1.76) compared to control women, and the OR for CBC per SD of $PRS_{313}$ was 1.39 (95%CI=1.13-1.70) compared to unilateral breast cancer (Table S8).

For women of Asian descent, the OR for unilateral breast cancer per SD of the $PRS_{313}$ was 1.56 (95%CI=1.52-1.60) compared to control women, and the OR for CBC per SD of $PRS_{313}$ was 1.15 (95%CI=1.02-1.29) compared to women with unilateral breast cancer (Table S8).

## Discussion

Previous studies have shown that a PRS, summarizing the effects of common germline variants, can be used to stratify women with respect to their risk to develop a primary breast cancer[6-9]. In this study, we observed a clear association between the $PRS_{313}$ and CBC risk in women of both European and Asian ancestry. The association was observed in both the case-case series and the European cohort. The HRs per SD of CBC for women at the $10^{th}$ and $90^{th}$ percentile of the continuous predicted $PRS_{313}$ were 0.75 and 1.33, respectively, compared to the $50^{th}$ percentile. This translates to absolute risks at the $10^{th}$ and the $90^{th}$ percentile of the $PRS_{313}$ of 12.4% and 20.5%, respectively, by age 80 years. We estimated a C-index for the $PRS_{313}$, summarizing its discriminatory ability, of 0.563 in the European cohort.

One previous study has investigated the effect of a PRS, including 67 variants, and CBC risk[10]. This study found a risk ratio of 1.75 (95%CI=1.41-2.18) for women in the upper quartile of the PRS compared with women in the lowest quartile. To facilitate comparison, we performed a similar analysis in our case-case series, showing an OR of 1.98 (95%CI=1.79-2.18), adjusted for country and age at first diagnosis, for women in the upper quartile of the $PRS_{313}$. This indicates the $PRS_{313}$ improves stratification relative to PRSs including fewer variants. Moreover, in our European cohort, the C-index for the PRS alone improved from 0.547 (95%CI=0.536-0.575) for the previously reported $PRS_{77}$[6] to 0.563 (95%CI=0.547-0.586) for the $PRS_{313}$.

We found no evidence that the association between the $PRS_{313}$ and CBC risk was confounded by family history, adjuvant therapy, morphology, age, or tumor receptor status of the first breast cancer, nor that there was effect modification by those factors. The absence of notable effect modification is in line with the abovementioned study of a 67-variant PRS and CBC risk; no heterogeneity in association was found by age, family history, morphology, ER-status, and adjuvant treatment[10].

To provide an external validation of our findings, we examined data from UK Biobank, which includes many women diagnosed with breast cancer with data available on the $PRS_{313}$ (Supplemental Note). Unfortunately, UK Biobank has no information available on the laterality of the tumor, and it is, therefore, not possible to distinguish between contralateral and ipsilateral breast cancers. We therefore performed analyses using any second breast cancer as the endpoint. This secondary analysis did confirm the association between the $PRS_{313}$ and second breast cancer risk (HR per SD=1.13, 95%CI=1.01-1.27), but with a lower estimate than in our European cohort. The lower estimate may be explained by the inclusion of the ipsilateral breast cancers, which may be more likely to be recurrences than new primary breast cancers compared to CBCs. Indeed, when we used ipsilateral breast cancer as the outcome in our European cohort, we found no association with the $PRS_{313}$ (HR=1.02, 95%CI=0.90-1.15).

The association between the $PRS_{313}$ and CBC risk (OR per SD=1.30; 95%CI=1.26-1.35) in the BCAC database was weaker (expressed in terms of an OR) than was found for first breast cancer among independent prospective studies (OR per SD=1.61; 95%CI=1.57-1.65). Under a simple polygenic model, the relative risk would be expected to be similar for the second breast cancer. The attenuated estimate for CBC might however be explained by several factors. Some attenuation of the estimate might have been due to dilution in the end-point definition, *i.e.*, if some of the CBCs were metastases. Previous studies investigating the clonal relatedness of first breast cancers and CBCs using tumor sequencing have shown that 6-12% of CBCs represent metastases[18; 19]. This hypothesis would be consistent with our finding of a slightly stronger association between the $PRS_{313}$ and late CBCs, diagnosed >five years after the first breast cancer, than for early CBCs, diagnosed ≤five years after the first cancer, since the latter are more likely to be metastases. In addition, 3-5% of the breast cancer patients will have a mutation in the *BRCA1 or BRCA2 gene*[20; 21]*,* who have high CBC risks. It has been shown that

the relative risk associated with PRS is lower (for the first breast cancer) for women with a *BRCA1* and *BRCA2* mutation than in the general population[22], diluting the overall relative risk for CBC. More generally, it is possible that the CBC association may be attenuated due to the effect of other, unmeasured, genetic or other risk factors. If the risks are high, cases with higher $PRS_{313}$ will have, on average, lower values of other risk factors, due to elimination of the highest risk individuals, again attenuating the CBC association. Finally, given the limited information on family history in our dataset, the estimate could have been biased due to a family history effect not detected in our data.

There was some suggestion that the relative risk associated with $PRS_{313}$ decreased with younger age, ($P_{trend}$=.05), and, specifically, was lower for women aged <40 years (HR per SD=1.13; 95%CI=0.98-1.31). Interestingly, Mavaddat et al[7] also found a lower relative risk below age 40 for first breast cancer. This effect may reflect the different characteristics of breast cancers at young ages, both in terms of germline susceptibility and pathology[23; 24]. For example, the proportion of ER-negative breast cancers is higher at young ages, and the PRS is less predictive for ER-negative disease[6; 7; 24].

In the logistic regression analyses in Asian women, the association between the $PRS_{313}$ and CBC risk was slightly weaker than in European women. This finding is consistent with a recent analysis investigating the association between a 287-variant PRS and first breast cancer risk in the Asian population[25], which showed an attenuated OR in Asian women (OR=1.52, 95%CI=1.49-1.56) compared to European women (OR=1.61, 95%CI=1.57-1.66). The lower estimate for Asian women might reflect the fact the $PRS_{313}$ was developed in European populations, and the different LD structure in Asians may attenuate the association since the variants in the PRS are likely to be surrogates for the causal variants. Other explanations for the attenuated estimate may be the slightly younger age at first breast cancer diagnosis and the

higher proportion ER-negative CBCs in Asian women compared to European women in our study. Finally, the imputation quality for variants was somewhat lower, on average, for the Asian than for the European dataset, with three variants on OncoArray and four variants on ICOGs with an imputation quality score<0.3 (Table S3). Nevertheless, we included those variants in the PRS for both European and Asian women, to keep the PRS comparable between ethnicities and studies. Future studies including larger numbers of Asian women, and women of other ethnicities, are needed to generate population-specific PRSs and to validate our findings in these groups.

A major strength of this study is the very large sample size in the BCAC dataset, including genotype information for ~150,000 women and a large number of CBC events. A limitation of this study is missing data on the patient, tumor, and treatment characteristics, which reduces the power of the multivariable Cox regression analyses and interaction analyses. In addition, registration of CBC was not complete; the 10-year cumulative CBC incidence was 2.2% in the BCAC dataset, compared to 3.8% using complete data from the Netherlands Cancer Registry[1]. For this reason, we estimated relative risk estimates using the BCAC data and applied these to external registry data to obtain absolute risk estimates. The underreporting of CBC should not bias our HR estimates, given that the event rate is low and reporting of CBC is unlikely to be related to the $PRS_{313}$. Moreover, we reran the cohort analysis in the subset of countries with a 10-year cumulative CBC incidence ≥3.0% in the BCAC dataset, and the estimates were very similar to the main analyses (HR per SD=1.23, 95%CI=1.14-1.33) (Figure S3).

In conclusion, the $PRS_{313}$ is predictive for the development of CBC. We found no evidence for confounding or effect modification by other previously established CBC risk factors. The $PRS_{313}$ is therefore likely to be an independent risk factor for CBC. Since the predictive ability of the PRS on its own is modest, it should be combined with other breast cancer risk factors to provide

more useful CBC risk prediction models. More accurate risk prediction will help identify women at high CBC risk who will benefit from additional surveillance and/or risk reducing mastectomy, and equally important, to identify those women at low risk in order to avoid unnecessary surgeries.

## Supplemental Data

Supplemental data include four figures, eight tables, Supplemental Note and acknowledgements.

## Data and Code Availability

Data used in this manuscript may be requested through the original providers. Data of the Breast Cancer Association Consortium may be requested for non-profit research through an application procedure with the Breast Cancer Association Consortium; more information: http://bcac.ccge.medschl.cam.ac.uk/bcacdata/. Data of the UK Biobank needs to be requested through UK Biobank; more information: https://www.ukbiobank.ac.uk/researchers/

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## Declaration of Interests

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**Figure 1. Estimates for contralateral breast cancer risk by percentile categories of the 313-variant PRS ($PRS_{313}$)**

The figure shows the hazard ratios per SD and 95% confidence intervals for percentiles of the $PRS_{313}$ relative to the middle quintile (underlying table can be found in Table S5). The solid line denotes the estimates for contralateral breast cancer risk with the $PRS_{313}$ fitted as a continuous covariate. Coefficients to construct the $PRS_{313}$ are shown in Table S3. The $PRS_{313}$ was standardized by SD=0.61, in line with Mavaddat et al.[7]. The analyses were performed with attained age as time scale. PRS = polygenic risk score, SD = standard deviation

**Figure 2. Predicted contralateral breast cancer risk by percentile of the 313-variant PRS ($PRS_{313}$) with death as competing risk**

Coefficients to construct the $PRS_{313}$ are shown in Table S3. The $PRS_{313}$ was standardized by SD=0.61, in line with Mavaddat et al.[7] The CBC incidences were calculated based on incidence data from the Netherlands Cancer Registry[1] and relative risks estimated as described in the Material and Methods. PRS = polygenic risk score, CBC = contralateral breast cancer

**Figure 3. Distribution of the 313-variant PRS ($PRS_{313}$) in 62,830 control women without any diagnosis of breast cancer, 81,000 women with unilateral breast cancer, and 3,607 women with contralateral breast cancer**

Coefficients to construct the $PRS_{313}$ are shown in Table S3. The $PRS_{313}$ was standardized by SD=0.61, in line with Mavaddat et al.[7]. PRS = polygenic risk score, BC = breast cancer, CBC = contralateral breast cancer, SD = standard deviation

**Table 1. Association between PRSs and contralateral breast cancer risk in the European cohort (N=56,068)**

| Polygenic risk score (PRS) | No. of CBC | HR per unit SD[a] | 95%CI | P-value |
|---|---|---|---|---|
| $PRS_{77}$ [b] | | | | |
| All CBC | 1,027 | 1.21 | 1.14-1.29 | <.001 |
| Invasive CBC | 923 | 1.21 | 1.13-1.29 | <.001 |
| $PRS_{313}$ [b] | | | | |
| All CBC | 1,027 | 1.25 | 1.18-1.33 | <.001 |
| Invasive CBC | 923 | 1.24 | 1.16-1.32 | <.001 |
| ER-positive invasive CBC[d] | 275 | 1.38 | 1.23-1.55 | <.001 |
| ER-negative invasive CBC[d] | 97 | 0.92 | 0.75-1.12 | .39 |
| ER-positive $PRS_{313}$ [b,c] | | | | |
| All CBC | 1,027 | 1.23 | 1.16-1.31 | <.001 |
| Invasive CBC | 923 | 1.22 | 1.15-1.30 | <.001 |
| ER-positive invasive CBC[d] | 275 | 1.37 | 1.22-1.54 | <.001 |
| ER-negative $PRS_{313}$ [b,c] | | | | |
| All CBC | 1,027 | 1.25 | 1.17-1.33 | <.001 |
| Invasive CBC | 923 | 1.24 | 1.16-1.33 | <.001 |
| ER-negative invasive CBC[d] | 97 | 1.06 | 0.86-1.30 | .58 |

Abbreviations: PRS = polygenic risk score, No. = number, CBC = contralateral breast cancer, HR = hazard ratio, CI = confidence interval, ER = estrogen receptor, SD = standard deviation

[a] All analyses were performed with attained age as time scale

[b] Coefficients to construct the PRSs are shown in Table S3. All PRSs were standardized by the same SD as was used by Mavaddat et al.[7]. The SD was 0.45 for overall breast cancer $PRS_{77}$, 0.61 for overall breast cancer $PRS_{313}$, 0.65 for ER-positive $PRS_{313}$, and 0.59 for ER-negative $PRS_{313}$

[c] ER-specific PRSs were constructed using a hybrid method, as described by Mavaddat et al.[7]

[d] Patients with ER-unknown CBC (N=551) were censored in these analyses

**Table 2. Association between the 313-variant PRS ($PRS_{313}$) and contralateral breast cancer risk for subgroups**

| Subgroups | No. of patients | No. of CBC | HR per unit SD[a,b] | 95%CI | P-value | $P_{heterogeneity}$[c,d] | $P_{trend}$[c,e] |
|---|---|---|---|---|---|---|---|
| All patients | 56,068 | 1,027 | 1.25 | 1.18-1.33 | <.001 | - | - |
| Age at first breast cancer diagnosis (years) | | | | | | .26 | .05 |
| <40 | 5,877 | 171 | 1.13 | 0.98-1.31 | .09 | | |
| 40-49 | 11,928 | 265 | 1.25 | 1.11-1.41 | <.001 | | |
| 50-59 | 16,882 | 320 | 1.22 | 1.09-1.36 | <.001 | | |
| 60+ | 21,381 | 271 | 1.36 | 1.21-1.52 | <.001 | | |
| Family history (first degree relative) | | | | | | .63 | - |
| no | 33,623 | 618 | 1.26 | 1.16-1.36 | <.001 | | |
| yes | 10,369 | 302 | 1.22 | 1.09-1.36 | <.001 | | |
| Morphology | | | | | | .14 | - |
| ductal | 37,324 | 621 | 1.21 | 1.12-1.31 | <.001 | | |
| lobular | 5,878 | 118 | 1.32 | 1.10-1.59 | .002 | | |
| mixed (ductal and lobular) | 2,174 | 46 | 1.52 | 1.15-2.02 | .004 | | |
| other | 3,344 | 70 | 1.20 | 0.96-1.50 | .11 | | |
| ER-status | | | | | | .13 | - |
| negative | 9,527 | 194 | 1.13 | 0.98-1.30 | .08 | | |
| positive | 38,090 | 670 | 1.28 | 1.19-1.38 | <.001 | | |
| PR-status | | | | | | .26 | - |
| negative | 13,098 | 244 | 1.16 | 1.03-1.32 | .02 | | |
| positive | 27,044 | 554 | 1.27 | 1.17-1.38 | <.001 | | |
| HER2-status | | | | | | .42 | - |
| negative | 23,787 | 352 | 1.29 | 1.17-1.44 | <.001 | | |
| positive | 4,969 | 60 | 1.45 | 1.13-1.85 | .004 | | |
| (Neo)adjuvant chemotherapy | | | | | | .60 | - |
| no | 18,110 | 361 | 1.28 | 1.16-1.42 | <.001 | | |
| yes | 18,559 | 363 | 1.24 | 1.12-1.37 | <.001 | | |
| (Neo)adjuvant endocrine therapy | | | | | | .79 | - |
| no | 10,781 | 242 | 1.28 | 1.13-1.44 | <.001 | | |
| yes | 27,322 | 460 | 1.30 | 1.19-1.43 | <.001 | | |
| Radiotherapy | | | | | | .40 | - |
| no | 11,023 | 188 | 1.33 | 1.15-1.53 | <.001 | | |
| yes | 29,142 | 617 | 1.24 | 1.15-1.34 | <.001 | | |

Abbreviations: PRS = polygenic risk score, No. = number, CBC = contralateral breast cancer, HR = hazard ratio, CI = confidence interval, ER = estrogen receptor, PR = progesterone receptor, HER2 = human epidermal growth factor receptor 2

[a] HR for CBC risk by unit SD of $PRS_{313}$. All analyses were performed with attained age as time scale

[b] Coefficients to construct the $PRS_{313}$ are shown in Table S3. The $PRS_{313}$ was standardized by standard deviation=0.61, in line with Mavaddat et al.[7]

[c] The interaction between the $PRS_{313}$ and each subgroup was tested in different models including the standardized $PRS_{313}$, modifier, and interaction. Patients with unknown values were excluded from these analyses. Since attained age was used as time scale in all models, the model with age at first breast cancer only included the $PRS_{313}$ and interaction

[d] P for interaction based on test for heterogeneity across categories

[e] P for interaction based on a trend test with age as continuous variable

**Table 3. Discriminatory ability (C-index) of the 313-variant PRS ($PRS_{313}$) and other risk factors for contralateral breast cancer risk in the European cohort**

| | C-index (95%CI)[a,b] |
|---|---|
| *Model 1*<br>$PRS_{313}$[c] alone | 0.563 (0.547-0.586) |
| *Model 2*<br>Other risk factors[d] | 0.605 (0.591-0.629) |
| *Model 3*<br>$PRS_{313}$[c] + other risk factors[d] | 0.623 (0.608-0.645) |

Abbreviations: PRS = polygenic risk score, CI = confidence interval

[a] The Harrell's C-index was obtained by the STATA stcox postestimation command 'estat concordance', using time since first breast cancer on the time scale without taking delayed entry (prevalent cases) into account. We did not consider delayed-entry since no standard performance measures are currently available in the statistical literature to account for left-truncated follow-up time. The median of delayed entry was 0.4 years (standard deviation=2.7) in our study

[b] The 95% CIs were obtained by use of the 'somersd' package in STATA

[c] Coefficients to construct the $PRS_{313}$ are shown in Table S3. The $PRS_{313}$ was standardized by SD=0.61, in line with Mavaddat et al.[7]

[d] Including age at first diagnosis, year of first diagnosis, family history for breast cancer in a first degree relative, and clinical characteristics of the first breast cancer (nodal status, tumor size, differentiation grade, morphology, estrogen receptor status, human epidermal growth factor receptor 2 status, chemotherapy, endocrine therapy, radiotherapy)

**Table 4. Five- and ten-year cumulative risks of contralateral breast cancer by the 313-variant PRS ($PRS_{313}$) for different age groups with death as competing risk**

| | 5-year cumulative CBC risks (%) range by age | | | | | 10-year cumulative CBC risks (%) range by age | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Age at first breast cancer diagnosis (years)** | **$5^{th}$ percentile $PRS_{313}$** | **$10^{th}$ percentile $PRS_{313}$** | **$50^{th}$ percentile $PRS_{313}$** | **$90^{th}$ percentile $PRS_{313}$** | **$95^{th}$ percentile $PRS_{313}$** | **$5^{th}$ percentile $PRS_{313}$** | **$10^{th}$ percentile $PRS_{313}$** | **$50^{th}$ percentile $PRS_{313}$** | **$90^{th}$ percentile $PRS_{313}$** | **$95^{th}$ percentile $PRS_{313}$** |
| 30-34 | 1.9-3.1 | 2.1-3.4 | 2.7-4.5 | 3.6-5.9 | 4.0-6.5 | 3.1-4.1 | 3.4-4.5 | 4.5-5.9 | 5.9-7.7 | 6.5-8.5 |
| 35-39 | 0.8-2.1 | 0.9-2.3 | 1.2-3.0 | 1.5-3.9 | 1.7-4.3 | 2.1-3.5 | 2.3-3.8 | 3.0-5.0 | 3.9-6.6 | 4.3-7.2 |
| 40-44 | 1.5-2.8 | 1.7-3.1 | 2.2-4.1 | 2.9-5.3 | 3.2-5.9 | 2.8-4.6 | 3.1-5.0 | 4.1-6.6 | 5.3-8.6 | 5.9-9.4 |
| 45-49 | 1.4-2.5 | 1.5-2.7 | 2.0-3.6 | 2.6-4.7 | 2.9-5.2 | 2.5-3.9 | 2.7-4.3 | 3.6-5.6 | 4.7-7.4 | 5.2-8.1 |
| 50-54 | 1.4-2.8 | 1.5-3.0 | 1.9-4.0 | 2.6-5.2 | 2.8-5.8 | 2.8-4.5 | 3.0-4.9 | 4.0-6.4 | 5.2-8.4 | 5.8-9.3 |
| 55-59 | 1.6-3.1 | 1.8-3.4 | 2.3-4.5 | 3.1-5.9 | 3.4-6.5 | 3.1-4.8 | 3.4-5.2 | 4.5-6.9 | 5.9-9.0 | 6.5-9.9 |
| 60-64 | 1.7-3.3 | 1.9-3.6 | 2.5-4.7 | 3.3-6.2 | 3.6-6.8 | 3.3-5.0 | 3.6-5.4 | 4.7-7.1 | 6.2-9.3 | 6.8-10.2 |
| 65-70 | 1.5-3.2 | 1.6-3.5 | 2.1-4.6 | 2.8-6.1 | 3.1-6.7 | 3.2-4.1 | 3.5-4.5 | 4.6-5.9 | 6.1-7.7 | 6.7-8.5 |

Abbreviations: PRS = polygenic risk score, CBC = contralateral breast cancer
Coefficients to construct the $PRS_{313}$ are shown in Table S3. The $PRS_{313}$ was standardized by SD=0.61, in line with Mavaddat et al[7]. The CBC incidences for each age group were calculated based on incidence data from the Netherlands Cancer Registry[1] and relative risks estimated as described in the Material and Methods. Death was taken into account as competing risk.