



Universiteit
Leiden
The Netherlands

The path to individualised breast cancer screening

Lakeman, I.M.M.

Citation

Lakeman, I. M. M. (2022, June 14). *The path to individualised breast cancer screening*. Retrieved from <https://hdl.handle.net/1887/3420638>

Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/3420638>

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

CHAPTER 5

5

The predictive ability of the 313-variant-based polygenic risk score for contralateral breast cancer risk prediction in women of European ancestry with a heterozygote *BRCA1* or *BRCA2* pathogenic variant

Inge M.M. Lakeman*, Alexandra J. van den Broek*, Juliën A.M. Vos, Daniel R. Barnes, Julian Adlard, Irene L. Andrulis, Adalgeir Arason, Norbert Arnold, Banu K. Arun, Judith Balmaña, Daniel Barrowdale, Javier Benitez, Ake Borg, Trinidad Caldés, Maria A. Caligo, Wendy K. Chung, Kathleen B.M. Claes, GEMO Study Collaborators, EMBRACE Collaborators, J. Margriet Collée, Fergus J. Couch, Mary B. Daly, Joe Dennis, Mallika Dhawan, Susan M. Domchek, Ros Eeles, Christoph Engel, D. Gareth Evans, Lidia Feliubadaló, Lenka Foretova, Eitan Friedman, Debra Frost, Patricia A. Ganz, Judy Garber, Simon A. Gayther, Anne-Marie Gerdes, Andrew K. Godwin, David E. Goldgar, Eric Hahnen, Christopher R. Hake, Ute Hamann, Frans B.L. Hogervorst, Maartje J. Hooning, John L. Hopper, Peter J. Hulick, Evgeny N. Imyanitov, OCGN Investigators, HEBON Investigators, GEMO Investigators, Claudine Isaacs, Louise Izatt, Anna Jakubowska, Paul A. James, Ramunas Janavicius, Uffe Birk Jensen, Yue Jiao, Esther M. John, Vijai Joseph, Beth Y. Karlan, Carolien M. Kets, Irene Konstantopoulou, Ava Kwong, Clémentine Legrand, Goska Leslie, Fabienne Lesueur, Jennifer T. Loud, Jan Lubiński, Siranoush Manoukian, Lesley McGuffog, Austin Miller, Denise Molina Gomes, Marco Montagna, Emmanuelle Mouret-Fourme, Katherine L. Nathanson, Susan L. Neuhausen, Heli Nevanlinna, Joanne Ngeow Yuen Yie, Edith Olah, Olufunmilayo I. Olopade, Sue K. Park, Michael T. Parsons, Paolo Peterlongo, Marion Piedmonte, Paolo Radice, Johanna Rantala, Gad Rennert, Harvey A. Risch, Rita K. Schmutzler, Priyanka Sharma, Jacques Simard, Christian F. Singer, Zsofia Stadler, Dominique Stoppa-Lyonnet, Christian Sutter, Yen Yen Tan, Manuel R. Teixeira, Soo Hwang Teo, Alex Teulé, Mads Thomassen, Darcy L. Thull, Marc Tischkowitz, Amanda E. Toland, Nadine Tung, Elizabeth J. van Rensburg, Ana Vega, Barbara Wappenschmidt, Peter Devilee, Christi J. van Asperen, Jonine L. Bernstein, Kenneth Offit, Douglas F. Easton, Matti A. Rookus, Georgia Chenevix-Trench, Antonis C. Antoniou#, Mark Robson#, and Marjanka K. Schmidt#

* Contributed equally to this manuscript

Shared last authors

Abstract

Purpose: To evaluate the association between a previously published 313-variant-based breast cancer (BC) polygenic risk score (PRS₃₁₃) and contralateral breast cancer (CBC) risk, in *BRCA1* and *BRCA2* pathogenic variant heterozygotes.

Methods: We included women of European ancestry with a prevalent first primary invasive BC (*BRCA1*=6,591 with 1,402 prevalent CBC cases; *BRCA2*=4,208 with 647 prevalent CBC cases) from CIMBA, a large international retrospective series. Cox regression analysis was performed to assess the association between overall and ER-specific PRS₃₁₃ and CBC risk.

Results: For *BRCA1* heterozygotes the estrogen receptor (ER)-negative PRS₃₁₃ showed the largest association with CBC risk, HR per SD=1.12, 95%CI [1.06-1.18], C-index=0.53; for *BRCA2* heterozygotes, this was the ER-positive PRS₃₁₃, HR=1.15, 95%CI [1.07-1.25], C-index=0.57. Adjusting for family history, age at diagnosis, treatment or pathological characteristics for the first BC did not change association effect sizes. For women developing first BC <age 40 years, the cumulative PRS₃₁₃ 5th and 95th percentile 10-year CBC risks were 22% and 32% for *BRCA1* and 13% and 23% for *BRCA2* heterozygotes, respectively.

Conclusion: The PRS₃₁₃ can be used to refine individual CBC risks for *BRCA1/2* heterozygotes of European ancestry, however the PRS₃₁₃ needs to be considered in the context of a multifactorial risk model to evaluate whether it might influence clinical-decision-making.

Introduction

Heterozygotes of germline pathogenic variants in *BRCA1* or *BRCA2* (henceforth: *BRCA1/2* heterozygotes) have a higher risk of developing contralateral breast cancer than non-heterozygotes¹. The estimated cumulative 10-year contralateral breast cancer risk varies across studies between 18.5%-34.2% for *BRCA1* heterozygotes and between 10.8%-29.2% for *BRCA2* heterozygotes¹⁻⁶, compared to 4-6% in the population^{7,8}. Whether or not to undergo a risk-reducing contralateral mastectomy, which is an invasive intervention and associated with side effects such as postoperative surgical complications, inability to breast feed in the future and psychosocial burden⁹, is an important and difficult decision for *BRCA1/2* heterozygotes who have been just confronted with their first breast cancer diagnosis. Precise individualized risk estimates could facilitate decision making for these women.

Two important factors influencing contralateral breast cancer risk in *BRCA1/2* heterozygotes are the age at diagnosis of the first breast tumor and a family history of breast cancer^{2,4,5,10}. The effect of family history on contralateral breast cancer risk suggests a role for other genetic factors. In the last decade, more than 180 common low risk variants have been associated with breast cancer risk in Genome Wide Association Studies¹¹⁻¹³. Individually, these variants are associated with small increases in risk, but when combined as polygenic risk scores (PRS) they may improve disease-related risk stratification for women of European and Asian ancestry in the population¹⁴⁻¹⁶. A limited number of studies have shown that variants associated with the risk of a first primary breast cancer are also associated with the risk of contralateral breast cancer¹⁷⁻¹⁹. Furthermore, the PRS derived from the general population has also been shown to be associated with breast cancer risk in *BRCA1/2* heterozygotes²⁰⁻²⁴.

The most predictive, well validated PRS, for breast cancer in the general population is based on 313 breast cancer-associated variants (PRS₃₁₃); it showed an association with breast cancer in ten prospective studies with an odds ratio (OR) per standard deviation (SD) of 1.61 and an area under the receiver-operator characteristic curve of 0.630¹⁴. Among *BRCA2* heterozygotes, this same PRS₃₁₃ was also associated with breast cancer risk, hazard ratio (HR) per SD=1.31, 95%CI [1.27-1.36]²⁴. Among *BRCA1* heterozygotes, the largest association with breast cancer risk was found using the estrogen receptor (ER)-negative PRS₃₁₃ (which uses the same variants but with weights adapted to provide better prediction for ER-negative disease), HR=1.29, 95%CI [1.25-1.33]²⁴. Although these effect sizes were smaller than those for the general population, the 313-variant-based PRS could have a substantial impact on the high absolute risks²⁴, associated with *BRCA1/2* pathogenic variants²⁵. Whether variants associated with breast cancer are associated with contralateral breast cancer risk for *BRCA1/2* heterozygotes as well, individually or combined in a PRS,

has not been investigated previously. If so, the PRS may be useful to guide choices for risk management, especially regarding invasive risk-reducing contralateral mastectomy. In this study, we investigated whether the 313-variant-based PRS for breast cancer are associated with contralateral breast cancer risk among women of European ancestry with pathogenic variants in *BRCA1/2* and explored the implications for contralateral breast cancer risk prediction for these women.

Materials and Methods

Study participants

We used retrospective cohort data from heterozygotes participating in the Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA)²⁶. Briefly, CIMBA participants are heterozygotes of pathogenic variants in *BRCA1* or *BRCA2* who are 18 years or older at the time of inclusion and have phenotypic data available²⁶. CIMBA includes eighty-one individual studies of which the majority of the participants were ascertained through cancer genetics clinics²⁶. Although studies in CIMBA include individuals of non-European ancestry, our analyses were, due to power considerations (small numbers available for analyses and expected lower estimates for the PRS₃₁₃ in Asian ancestry based on results of women in the general breast cancer population¹⁹), restricted to women of European ancestry with available array genotyping data (31,195 women of 67 studies).

Women were eligible for this retrospective analysis if they developed an invasive primary breast tumor without metastatic disease at least 1 year before the baseline age. Women without information about metastatic disease were assumed to have no metastatic disease (n=9,242 of whom 2,140 had a known negative lymph node status). Baseline age was defined as the age at local ascertainment (97%), or when this was not known, age at genetic testing (2%) or age at last follow-up (1%). Women were excluded if no information was available about the age at baseline or if they had developed synchronous contralateral breast cancer. Synchronous contralateral breast cancer was defined as contralateral breast cancer within one year after the first primary breast cancer, which was based on the exact date of cancer diagnosis or, if this was not available, on the age at diagnosis. A schematic overview of the selection is shown in Figure S1. In total, 6,591 women with *BRCA1* and 4,208 women with *BRCA2* pathogenic variants were included in this study, among whom 1,402 *BRCA1* heterozygotes and 647 *BRCA2* heterozygotes have had contralateral breast cancer. The diagnosis of primary and contralateral breast cancer was confirmed by pathology records, tumor registry data or medical records by the individual studies. Available phenotypic information for all participants is shown in Table 1, including the number of participants for whom the information was not available for

each of the variables. Information about the ER-status of the first primary breast cancer compared to the contralateral breast cancer is shown in Table S1.

Genotyping and Polygenic Risk Score calculation

For most of the participants, genotyping was performed with the Illumina OncoArray²⁷. The remaining participants were genotyped with the Illumina iCOGS array¹¹. Details about the quality control procedures and correlation between the arrays have been described previously^{19, 24, 28-31}. European ancestry was determined using genetic data and multidimensional scaling. More detailed information about the genotyping and PRS calculation is provided in the supplementary methods.

We used the 313-variant-based PRS for breast cancer developed in an independent study using data from the general population as described previously¹⁴; correlation between PRS based on the two genotyping arrays was high¹⁹. The PRS for overall breast cancer (PRS₃₁₃) and two ER-specific PRS, the ER-positive PRS₃₁₃ and ER-negative PRS₃₁₃ were calculated. The variants and their corresponding weights used in the PRS as published previously¹⁴, and the imputation quality are listed in Table S2. The three PRS were standardized to the mean from all CIMBA participants, including both unaffected and affected women, and to the SD in BCAC population controls which were included in the validation dataset¹⁴. Using these SDs, the HR estimates for the associations of the standardized PRS₃₁₃ in our study are directly comparable with the OR estimates reported in the BCAC population-based study¹⁴ and the HR estimates reported for primary breast cancer in *BRCA1* and *BRCA2* heterozygotes²⁴.

Statistical analysis

To assess the associations between the three PRS and contralateral breast cancer risk in *BRCA1/2* heterozygotes, Cox-regression analyses were performed. The time at risk was started one year after the first breast cancer diagnosis based on the exact date or if not available, on the age of developing the first breast tumor. Time at risk of participants was censored at age at baseline, i.e., end of follow-up in these analyses, prophylactic contralateral mastectomy, or death, whichever was earlier (Figure S2). Incidence of a metachronous contralateral breast cancer, invasive or *in situ*, before baseline was considered as an event in the main analyses. The proportional hazard assumption was evaluated by using Schoenfeld residuals against the transformed time. A sensitivity analysis was performed considering invasive contralateral breast cancer only as an event. Women who developed an *in situ* contralateral breast cancer were censored at the age at diagnosis of the *in situ* contralateral breast cancer. Furthermore, a sensitivity analysis was performed including information about distant relapse, which was available for 1,725 *BRCA1* and 1,450 *BRCA2* heterozygotes. In total 55 *BRCA1* heterozygotes and 101 *BRCA2* heterozygotes were censored at the age of distant relapse of which 13 and 11 women

were excluded from the analyses, respectively, because they developed distant relapse in the year before the baseline age.

Analyses were stratified by country (Table S3), adjusted for birth cohort (quartiles of the observed distribution), and clustered on family membership using a unique family-identifier to account for the inclusion of related individuals. For *BRCA1* and *BRCA2* respectively, there were 5923 and 3752 clusters of which 554 and 362 clusters had more than one participant. The main analyses assessed the association with the PRS as a continuous covariate. We evaluated the linearity of the association using restricted cubic splines with three knots, which showed no evidence for violation of the linearity assumption. The discriminatory ability of the best performing PRS was evaluated by Harrell's C-index³². C-indexes were calculated stratified by country and clustered on family membership.

The influence of possible confounding variables on the observed associations was assessed using the PRS exhibiting the largest associations. Possible confounding variables included breast cancer family history, age at diagnosis of the first breast cancer, pathological characteristics and treatment of the first breast cancer. Each variable was added to the model one by one and in addition, a full model that included all possible confounders together was fitted. If the addition of a variable resulted in a change of more than 10% in the log HR, the variable was retained as a covariate in the final Cox-regression model. To avoid excluding many participants with missing data for one of these included variables (Table 1), missing data were imputed using Multiple Imputation by Chained Equations (MICE)³³. Imputation was started with the least missing variable and progressed in order of increased amount of missing data. Using this method, 10 complete data sets for analyses were created and mean parameter estimates were derived.

Secondary analyses were performed for ER-positive and ER-negative cases only, based on the ER-status of the contralateral breast cancer, after imputation as described above. The average number of ER-positive and ER-negative cases in the 10 imputed data sets is shown in Table S4. In these analyses the event of interest was either ER-positive or ER-negative contralateral breast cancer. Contralateral breast cancer cases with the alternative ER-status were censored at the age of contralateral breast cancer.

The interaction between the PRS with the age at first breast cancer diagnosis was tested in the final model, treating the PRS as a continuous variable. Furthermore, the effect size of the PRS was evaluated for groups based on the age at first primary breast cancer diagnosis (<40 years; 40 to 50 years; ≥ 50 years)^{1, 20}. The association of the PRS and contralateral breast cancer risk was tested separately for heterozygotes of pathogenic variants that lead to unstable or no protein (class I) and heterozygotes of pathogenic variants that lead to mutant stable protein (class II). Finally, analyses were performed to test the association

between a categorized PRS and contralateral breast cancer risk to establish whether the results were consistent with those under a continuous PRS model. The categories were defined on the basis of the distribution of the PRS in unilateral breast cancer cases, using PRS percentiles (0-5th, 5th-10th, 10th-20th, 20th-40th, 40th-60th (reference), 60th-80th, 80th-90th, 90th-95th, 95th-100th).

Cumulative risks

Absolute contralateral breast cancer risks were calculated at percentiles of the best-performing continuous PRS for both *BRCA1* and *BRCA2* heterozygotes, using the log HR per SD and including an interaction term with the continuous age at first breast cancer diagnosis (at age 35; 45 and 55 for the corresponding age groups as described below). For this purpose, we constrained the incidence of contralateral breast cancer, by age at first breast cancer and in years after the first breast cancer, and averaged over all PRS categories to agree with external contralateral breast cancer incidence estimates, as described previously²³. These external incidence estimates were based on prospective cohort data from three consortia on heterozygotes of pathogenic *BRCA1* and *BRCA2* variants¹, the International *BRCA1/2* Carrier Cohort Study (IBCCS), the Breast Cancer Family Registry (BCFR), and the Kathleen Cuninghame Foundation Consortium for Research Into Familial Breast Cancer (kConFab). Because the contralateral breast cancer incidences vary with the age of first breast cancer diagnosis, incidences were calculated for three different groups based on the age of the first breast cancer diagnosis (<40 years, 40 to 50 years, ≥50 years)¹.

All statistical tests were performed with R version 3.5.0³⁴. Statistical significance was defined as a two-sided p-value <0.05.

Results

In the analyses, 6,591 *BRCA1* and 4,208 *BRCA2* heterozygotes of European ancestry who had developed an invasive first primary breast cancer before entry in CIMBA were identified. The median follow-up time was 6.0 and 5.4 years for *BRCA1* and *BRCA2* heterozygotes, respectively. In total, 1,402 *BRCA1* and 647 *BRCA2* heterozygotes were diagnosed with a metachronous contralateral breast cancer before enrollment in CIMBA. The cumulative 10-year risk of developing contralateral breast cancer in this cohort was 25%, 95%CI [23.5%-26.4%] and 18.8%, 95%CI [17.1%-20.5%] for *BRCA1* and *BRCA2* heterozygotes, respectively (Figure S3). Patient and tumor characteristics as well as the PRS distributions are shown in Table 1 and Figure S4.

Table 1. Characteristics of the participants

		<i>BRCA1</i> heterozygotes		<i>BRCA2</i> heterozygotes	
		UBC, n (%)	CBC, n (%)	UBC, n (%)	CBC, n (%)
N		5,189	1,402	3,561	647
Genotyping Array	iCOGS	895 (17)	200 (14)	383 (11)	80 (12)
	OncoArray	4,294 (83)	1,202 (86)	3,178 (89)	567 (88)
Birth cohort	<1920	25 (0.5)	8 (0.6)	23 (0.6)	9 (1)
	1920-1929	143 (3)	46 (3)	121 (3)	30 (5)
	1930-1939	392 (8)	130 (9)	341 (10)	99 (15)
	1940-1949	1,060 (20)	386 (28)	793 (22)	172 (27)
	1950-1959	1,540 (30)	452 (32)	1,104 (31)	202 (31)
	1960-1969	1,354 (26)	298 (21)	822 (23)	115 (18)
	≥1970	675 (13)	82 (6)	357 (10)	20 (3)
Variant class^a	I	3,354 (65)	904 (64)	3,207 (90)	570 (88)
	II	1,345 (26)	374 (27)	125 (4)	25 (4)
	III	490 (9)	124 (9)	229 (6)	52 (8)
BRRM		160 (3)	0	101 (3)	0
Deceased	N	44 (0.8)	12 (0.9)	19 (0.5)	2 (0.3)
Family history^b	No BC	583 (11)	175 (12)	289 (8)	78 (12)
	1 BC	906 (17)	270 (19)	760 (21)	127 (20)
	≥ 2 BC	1,250 (24)	363 (26)	1,120 (31)	210 (32)
	Unknown	2,450 (47)	594 (42)	1,392 (39)	232 (36)
Characteristics of first BC					
Age at diagnosis	Mean	41.8	38.5	44.5	41.8
	Range	19-82	19-68	18-85	21-75
ER status	Positive	570 (11)	92 (7)	1,302 (37)	182 (28)
	Negative	1,738 (33)	402 (29)	424 (12)	61 (9)
	Unknown	2,881 (56)	908 (65)	1,835 (52)	404 (62)
Node status	Positive	797 (15)	182 (13)	781 (22)	119 (18)
	Negative	1,544 (30)	441 (31)	877 (25)	151 (23)
	Unknown	2,848 (55)	779 (56)	1,903 (53)	377 (58)
Tumor size^c	T1	1,261 (24)	314 (22)	842 (24)	136 (21)
	T2	771 (15)	211 (15)	553 (16)	87 (13)
	T3	67 (13)	12 (0.9)	78 (2)	8 (1)
	T4	16 (0.5)	2 (0.1)	22 (0.6)	2 (0.3)
	Unknown	3,074 (59)	863 (62)	2,066 (58)	414 (64)
Chemotherapy^d	Yes	1,099 (21)	236 (17)	821 (23)	123 (19)
	No	576 (11)	212 (15)	503 (14)	129 (20)
	Unknown	3,514 (68)	954 (68)	2,237 (63)	395 (61)
Adjuvant hormone therapy	Yes	493 (10)	125 (9)	795 (22)	111 (17)
	No	1,103 (21)	288 (21)	474 (13)	135 (21)
	Unknown	3,593 (69)	989 (71)	2,292 (64)	401 (62)
Adjuvant trastuzumab therapy	Yes	11 (0.2)	1 (0.1)	20 (0.6)	0 (0)
	No	1,161 (22)	351 (25)	983 (28)	218 (34)
	Unknown	4,017 (77)	1,050 (75)	2,558 (72)	429 (66)
Radiotherapy	Yes	1,090 (21)	277 (20)	797 (22)	158 (24)
	No	535 (10)	141 (10)	420 (12)	84 (13)
	Unknown	3,564 (69)	984 (70)	2,344 (66)	405 (63)

Characteristics of CBC

Age at diagnosis	Mean	-	47.3	-	51.24
	Range	-	26-80.5	-	23.8-86
Invasiveness	Invasive	-	1,267 (90)	-	545 (84)
	Non-invasive	-	135 (10)	-	102 (16)
ER-status	Positive	-	101 (7)	-	197 (30)
	Negative	-	446 (32)	-	50 (8)
	Unknown	-	855 (61)	-	400 (62)
PRS₃₁₃					
Standardized PRS₃₁₃ mean (SD)	Overall BC	0.08 (1.01)	0.13 (1.01)	0.09 (1.02)	0.27 (1.04)
	ER-positive BC	0.07 (1.01)	0.09 (1.01)	0.08 (1.01)	0.27 (1.03)
	ER-negative BC	0.09 (1.00)	0.23 (0.99)	0.07 (1.02)	0.23 (1.07)

^aVariant class: I=unstable or no protein, II= stable mutant protein, III= consequence unknown.

^bFamily history was defined as the number of first- or second- degree relatives affected with BC, ranging from 0 to ≥ 2 .

^cTumor size: T1= ≤ 2 cm (≤ 0.79 in), T2= > 2 cm-5cm (> 0.79 -1.97in), T3= > 5 cm (> 1.97 in), T4=any size, with direct extension to the chest wall or skin.

^dIncluding neoadjuvant and adjuvant chemotherapy

Abbreviations: BC, Breast Cancer; BRRM, Bilateral Risk Reducing Mastectomy; CBC, Contralateral Breast Cancer; ER-status, Estrogen Receptor status of the tumor; N, Number; PRS, Polygenic Risk Score; SD, Standard Deviation; UBC, Unilateral Breast Cancer

PRS and contralateral breast cancer risk

Results of the association analyses between the PRS and contralateral breast cancer risk are shown in Table 2, Table S4 and Figure 1.

BRCA1 heterozygotes

For *BRCA1* heterozygotes the ER-negative PRS₃₁₃ showed the largest association with all contralateral breast cancer, HR per SD=1.12, 95%CI [1.06-1.18], p-value=6.0x10⁻⁵, C-index 0.53, 95%CI [0.51-0.55]. There was no evidence of violation of the proportional hazard assumption, p-value=0.840.

Neither sequential inclusion of possible confounders, nor including all these confounders in one model, changed the log HR estimate for the ER-negative PRS₃₁₃ association more than 10% when compared with the model with no confounders (Table S5).

Considering only invasive contralateral breast cancer as the event of interest resulted in a similar association with the ER-negative PRS₃₁₃, HR per SD=1.13, 95%CI [1.07-1.20], p-value=3.2x10⁻⁵.

Censoring at distant metastasis relapse, if applicable, did not change the effect size of the ER-negative PRS₃₁₃, HR per SD=1.12, 95%CI [1.06-1.18], p-value=4.9x10⁻⁵.

The HR-estimates for association with contralateral breast cancer for different quantiles of the ER-negative PRS₃₁₃, were consistent with the predicted HRs from the model using the continuous ER-negative PRS₃₁₃ (Table 2 and Figure 2).

For ER-positive contralateral breast cancer as event, the PRS₃₁₃ showed the largest association, HR per SD=1.32, 95%CI [1.12-1.56], p-value=0.002. For ER-negative contralateral breast cancer as event, only the ER-negative PRS₃₁₃ showed a significant association, HR per SD=1.07, 95%CI [1.01-1.15], p-value=0.036 (Table S4).

BRCA2 heterozygotes

For *BRCA2* heterozygotes the largest association was seen with the ER-positive PRS₃₁₃, HR per SD=1.15, 95%CI [1.07-1.25], p-value=1.9x10⁻⁴, C-index 0.57, 95%CI [0.54-0.59]. There was no evidence of violation of the proportional hazard assumption, p-value=0.300.

Neither sequential inclusion of possible confounders, nor including all these confounders in one model, changed the log HR estimate for the ER-positive PRS₃₁₃ association more than 10% when compared with the model with no confounders (Table S5).

Considering only invasive contralateral breast cancer as the event of interest resulted in a similar association, HR per SD for the ER-positive PRS₃₁₃=1.15, 95%CI [1.06-1.25], p-value=6.0x10⁻⁴.

Censoring at distant metastasis relapse, if applicable, did not change the effect size of the ER-positive PRS₃₁₃, HR per SD=1.15, 95%CI [1.07-1.24], p-value=2.1x10⁻⁴.

The HR estimates for association with contralateral breast cancer for different quantiles of the ER-positive PRS₃₁₃, were consistent with the predicted estimates using the continuous PRS₃₁₃ (Table 2 and Figure 2).

The ER-positive PRS₃₁₃ showed the largest association with ER-positive contralateral breast cancer for *BRCA2* heterozygotes, HR per SD=1.22, 95%CI [1.11-1.33], p-value=2.2x10⁻⁵ (Table S4). None of the PRS showed significant associations with ER-negative contralateral breast cancer for *BRCA2* heterozygotes, but the ER-negative PRS₃₁₃ exhibited the largest HR estimate, HR per SD=1.10, 95%CI [0.91-1.32], p-value=0.346.

Table 2: Results of association analyses between the PRS₃₁₃ and contralateral breast cancer risk

	BRCA1 heterozygotes [ER-negative PRS ₃₁₃]				BRCA2 heterozygotes [ER-positive PRS ₃₁₃]					
	UBC cases, n	CBC cases, n	HR ^a	95% CI	P	UBC cases, n	CBC cases, n	HR ^a	95% CI	P
PRS continuous										
All CBC	5,189	1,402	1.12	1.06-1.18	5.98x10 ⁻⁵	3,561	647	1.15	1.07-1.25	1.94x10 ⁻⁴
Invasive CBC	5,324	1,267	1.13	1.07-1.20	3.15x10 ⁻⁵	3,663	545	1.15	1.06-1.25	6.02x10 ⁻⁴
Categorical PRS percentiles										
0-5	260	48	0.81	0.59-1.11	0.188	166	28	1.06	0.71-1.58	0.782
5-10	259	54	0.77	0.57-1.03	0.082	198	26	0.68	0.44-1.04	0.074
10-20	519	131	0.94	0.76-1.15	0.544	355	51	0.91	0.66-1.25	0.554
20-40	1,038	230	0.83	0.70-0.98	0.031	697	108	0.87	0.68-1.13	0.295
40-60 [reference]	1,037	282	1.00			695	123	1.00		
60-80	1,038	313	1.04	0.88-1.22	0.664	734	128	0.96	0.75-1.23	0.748
80-90	519	170	1.11	0.92-1.34	0.255	358	90	1.35	1.03-1.77	0.030
90-95	259	82	1.18	0.92-1.51	0.185	178	46	1.35	0.96-1.90	0.082
95-100	260	92	1.24	0.98-1.56	0.074	180	47	1.31	0.94-1.82	0.116
PRS*age BC1 continuous	5,189	1,402	1.48	1.15-1.89	2.03x10 ⁻³	3,561	647	1.53	1.11-2.12	0.010
Interaction effect			0.99	0.99-1.00	0.025			0.99	0.99-1.00	0.089
PRS effect per age group										
<40	2,339	815	1.22	1.14-1.31	4.79x10 ⁻⁸	1,238	268	1.23	1.09-1.38	5.78x10 ⁻⁴
40-50	1,821	456	0.99	0.90-1.09	0.785	1,306	261	1.19	1.05-1.34	6.91x10 ⁻³
≥50	1,029	131	1.03	0.86-1.24	0.715	1,017	118	0.97	0.81-1.15	0.698
Variant class^b										
Class I	3,354	904	1.11	1.03-1.18	4.32x10 ⁻³	3,207	570	1.16	1.07-1.26	1.99x10 ⁻⁴
Class II	1,345	374	1.15	1.04-1.28	4.75x10 ⁻³	125	25	0.91	0.65-1.28	0.594

^a HRs for association with breast cancer and the continuous PRS₃₁₃ are reported per standard deviation of the PRS in population-based controls.

^b Class I pathogenic variants result in an unstable or no protein. Class II pathogenic variants yield stable mutant proteins.

Abbreviations: BC1, First primary Breast Cancer; CBC, Contralateral Breast Cancer; CI, Confidence Interval; HR, Hazard Ratio; PRS, Polygenic Risk Score; UBC, Unilateral Breast Cancer.

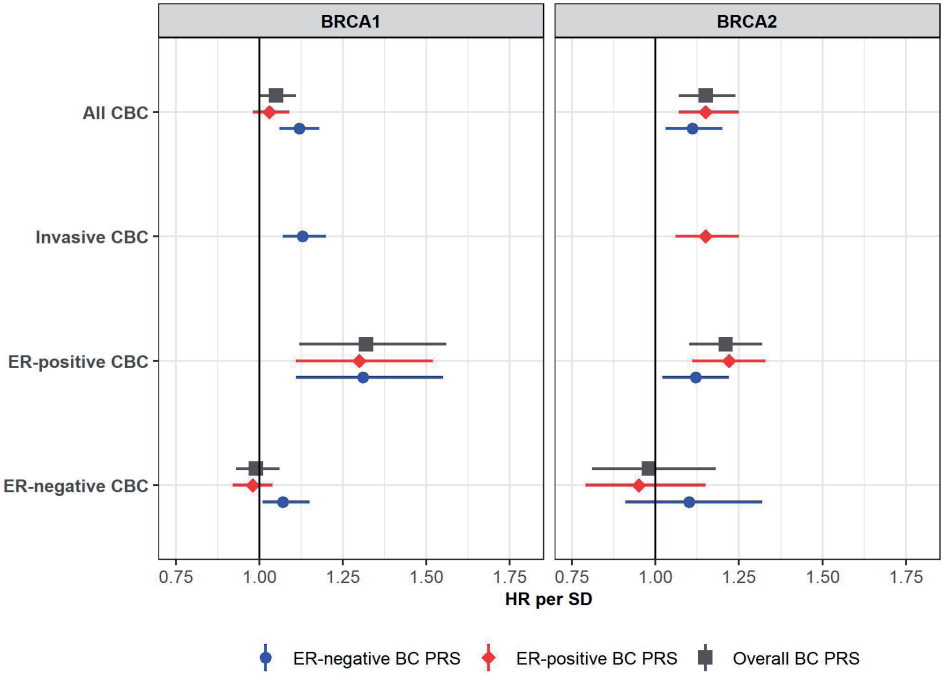


Figure 1: Association between the PRS and contralateral breast cancer risk for BRCA1 and BRCA2 heterozygotes

The figure includes the effect size of the association between contralateral breast cancer and the three different PRS313 after testing for covariates for the following selections: all contralateral breast cancer, invasive contralateral breast cancer only, ER-negative contralateral breast cancer, and ER-positive contralateral breast cancer. The numbers of unilateral and contralateral breast cancer cases and effect sizes are shown in Table 2 and Table S4.

Abbreviations: CBC, Contralateral Breast Cancer; ER, Estrogen Receptor; HR, Hazard Ratio; PRS, Polygenic Risk Score; SD, Standard Deviation.

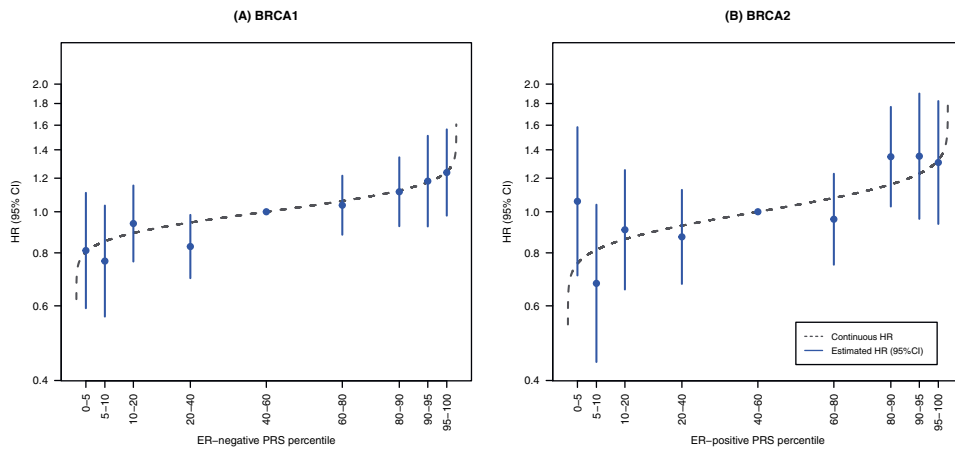


Figure 2: Association between categories of the PRS and contralateral breast cancer risk for BRCA1 and BRCA2 heterozygotes

HRs and 95%CI for percentiles of the ER-negative PRS₃₁₃ for BRCA1 heterozygotes and the ER-positive PRS₃₁₃ for BRCA2 heterozygotes, relative to the middle quintile. The PRS percentile groups were 0-5%, 5-10%, 10-20%, 20-40%, 40-60% [reference], 60-80%, 80-90%, 90-95%, and 95-100% based on the distribution in unilateral breast cancer cases. The numbers and corresponding effect sizes are shown in Table 2. The grey line represents the distribution based on the HR of the continuous ER-negative PRS₃₁₃ and ER-positive PRS₃₁₃ and the distribution in unilateral breast cancer cases of BRCA1 and BRCA2 heterozygotes respectively.

Abbreviations: CI, Confidence Interval; ER, Estrogen Receptor; HR, Hazard Ratio; PRS, Polygenic Risk Score.

Interaction with age at first breast cancer diagnosis

A significant interaction between the age at first breast cancer diagnosis and the ER-negative PRS₃₁₃ was found for *BRCA1* heterozygotes: HR per year=0.99, 95%CI [0.99-1.00], p-value=0.025. For *BRCA2* heterozygotes a similar magnitude of interaction was observed with the ER-positive PRS₃₁₃, although the interaction was not significant, HR per year=0.99, 95%CI [0.99-1.00], p-value=0.09.

Categorizing age at first breast cancer diagnosis for *BRCA1* heterozygotes resulted in HRs per SD of the ER-negative PRS₃₁₃ of 1.22, 95%CI [1.14-1.31], 0.99, 95%CI [0.90-1.09] and 1.03, 95%CI [0.86-1.24] for ages <40 years, 40-50 years and ≥50 year respectively. For *BRCA2* heterozygotes the corresponding estimates for ER-positive PRS₃₁₃ were 1.23, 95%CI [1.09-1.38], 1.19, 95%CI [1.05-1.34] and 0.97, 95%CI [0.81-1.15] respectively (Table 2).

Analyses by predicted variant effect on protein expression

For *BRCA1* heterozygotes, the HRs for association between the ER-negative PRS₃₁₃ and contralateral breast cancer risk were similar for heterozygotes of pathogenic variants, which lead to a stable mutant protein (class II) compared with those leading to no protein

or an unstable protein (class I). For *BRCA2* heterozygotes, the ER-positive PRS₃₁₃ effect size for the association with contralateral breast cancer risk was non-significantly smaller among heterozygotes of a pathogenic variant that lead to a stable mutant protein, although statistical power to detect these associations was low and the confidence intervals overlap with the overall estimate (Table 2).

Cumulative risks

Estimate cumulative contralateral breast cancer risks, by categories of age at diagnosis of the first breast cancer are shown in Figure 3. The largest risk difference was seen for women with a first breast cancer diagnosis before the age of 40, with *BRCA1* heterozygotes at the 5th percentile of the ER-negative PRS₃₁₃ having a 10- and 20-year risk of 22% and 35% compared with 32% and 49% at the 95th percentile, respectively. For *BRCA2* heterozygotes, the 10- and 20-year risks in this category were 13% and 25% at the 5th percentile of the ER-positive PRS₃₁₃ compared with 23% and 42% for women at the 95th percentile.

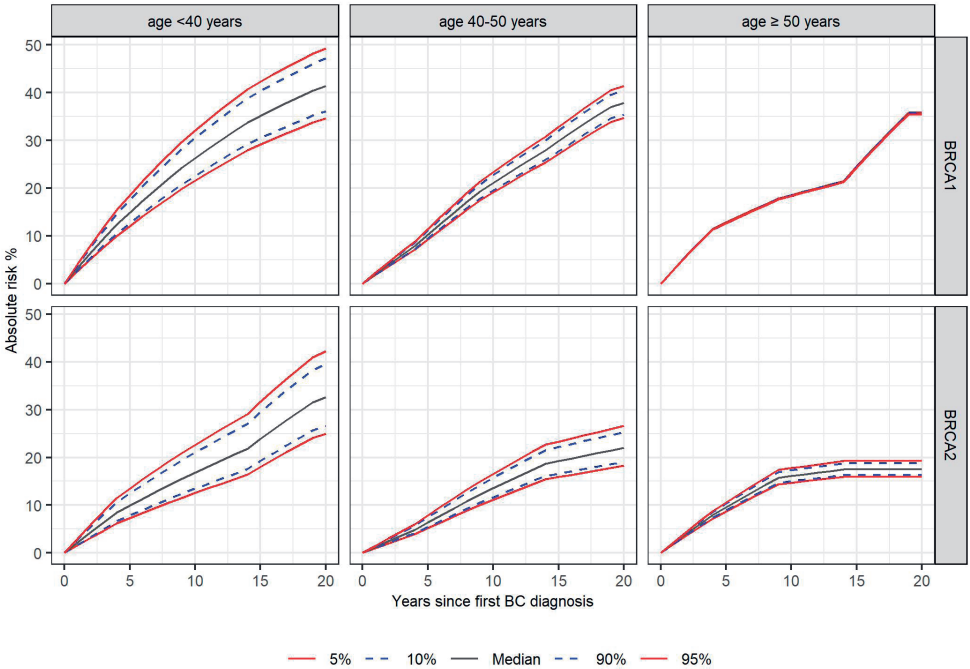


Figure 3: Absolute contralateral breast cancer risk by PRS percentiles per age category of the first breast cancer diagnosis for BRCA1 and BRCA2 heterozygotes

Predicted absolute contralateral breast cancer risks by percentile of the continuous ER-negative PRS₃₁₃ for BRCA1 heterozygotes and ER-positive PRS₃₁₃ for BRCA2 heterozygotes. The assumed contralateral breast cancer incidences were from a study that estimated breast cancer incidence in a large prospective cohort of BRCA1 and BRCA2 heterozygotes²⁰. The age categories were based on

the age at diagnosis of the first primary breast tumor. Risks were calculated including the interaction between the PRS and the continuous age of first breast cancer diagnosis. The lines for different percentiles of the PRS are overlapping for the age category ≥ 50 year for *BRCA1* heterozygotes. Abbreviations: BC, Breast Cancer; CBC, Contralateral Breast Cancer; PRS, Polygenic Risk Score.

Discussion

In this study we investigated the associations between an established PRS based on 313 variants for primary first breast cancer and contralateral breast cancer risks among *BRCA1* and *BRCA2* heterozygotes of European ancestry enrolled in the large international retrospective CIMBA cohort. We showed significant albeit modest associations among both *BRCA1* and *BRCA2* heterozygotes between the PRS and contralateral breast cancer risk. For *BRCA1* heterozygotes, the largest association was seen with the ER-negative PRS_{313'}, while for *BRCA2* heterozygotes, both the PRS₃₁₃ and ER-positive PRS₃₁₃ showed similar associations with contralateral breast cancer risk that were somewhat larger than the ER-negative PRS₃₁₃ association. These findings are consistent with previous studies on the effects of disease-specific PRS on the first breast cancers in *BRCA1* and *BRCA2* heterozygotes^{20,24} and with the higher relative prevalence of ER-negative and ER-positive contralateral breast cancers respectively, in this cohort.

For both *BRCA1* and *BRCA2* heterozygotes, the strength of the association was greater for ER-positive contralateral breast cancers compared with ER-negative contralateral breast cancers (in the case of *BRCA1*, even if the ER-negative PRS was used), although most of the confidence intervals overlapped. The effect sizes for the PRS are also larger for ER-positive disease in the general population, perhaps because ER-positive disease is commoner and the power to identify genetic variants has been greater for ER-positive disease. With larger data sets, it should be possible to develop better subtype specific PRS for contralateral breast cancer.

Although we found clear associations between the PRS and contralateral breast cancer risk, the magnitude of these associations (expressed in terms of HRs) were smaller than previously reported for the first breast cancers. For *BRCA1* heterozygotes, the HR per SD for the association between the ER-negative PRS₃₁₃ and breast cancer was 1.29, 95%CI [1.25-1.33]²⁴, compared with 1.12, 95%CI [1.06-1.18] for contralateral breast cancer in this study. For *BRCA2* heterozygotes, the HR per SD for the association between the ER-positive PRS₃₁₃ and breast cancer was 1.31, 95%CI [1.26-1.36]²⁴, compared with 1.15, 95%CI [1.07-1.24] for contralateral breast cancer in this study. This lower relative risk is consistent with a general pattern of a lower relative risk in a higher risk population, as seen in, the lower relative risk for contralateral breast cancer than first breast cancer in the general population¹⁹, and the lower relative risk for the first cancer in *BRCA1/2* heterozygotes than in the general

population²⁴. The attenuated estimate might be explained by several factors, some of which are speculative. *BRCA1/2* pathogenic variant heterozygotes in this study were selected based on having a first breast cancer; these women will have on average a higher PRS, but also higher frequencies of other genetic and non-genetic risk factors than women who do not develop breast cancer at all. This can lead to a weaker association with the PRS as women with the largest PRS may have lower risks due to other factors, a phenomenon related to index event bias³⁵. There could also be negative interactions between the PRS effect and other risk factors (for example, treatment factors). However, in this study, we have shown that adjustment for the known contralateral breast cancer risk factors did not change the effect size of the PRS, which was also shown in population-based studies^{17,19}. Finally, although we tried to exclude potential early metastases misdiagnosed as second primaries by excluding women who developed a contralateral breast cancer the first year after the primary diagnosis, it is possible that a small percentage of contralateral breast cancers were metastases³⁶.

A limitation of this study is that participants were recruited through clinical genetic centers, resulting in ascertainment bias, as individuals are more likely to have a strong family of breast cancer and/or be affected at a young age in order to be referred for testing. This was a historical cohort in which follow-up was prior to entry into CIMBA, so that all cases are prevalent. Therefore, the breast cancer patients included in the analyses are likely to be at higher contralateral breast cancer risk when compared with the general *BRCA1/2* heterozygote breast cancer population. Indeed, the estimated 20-year risks of developing contralateral breast cancer in this study were higher compared to a previously published study with a prospective design¹: 47% versus 40% for *BRCA1* heterozygotes and 40% versus 26% for *BRCA2* heterozygotes, respectively. While this is unlikely to introduce a significant bias in the relative risk estimates, a prospective cohort would clearly be preferable, although this will take several years to achieve. Finally, the PRS was developed using data sets of women of European ancestry, since our dataset included insufficient samples of women of other ancestries, and our results were exclusively based on women of European ancestry. Therefore, caution is required when applying this to non-European ancestry populations. However, a population study found clear associations between the PRS, based on the same 313 variants or a subset of these variants, and (contralateral) breast cancer also in women of Asian ancestry. The effect size of these associations were slightly weaker, possibly reflecting the fact that this PRS was developed in a cohort of women of European ancestry^{16,19}. These results suggest that there might be an association with the PRS as well in *BRCA1/2* heterozygotes of Asian ancestry. Future studies including a sufficient number of individuals of Asian ancestry are needed to confirm this statement.

Although the relative risks of the PRS for contralateral breast cancer were modest, differences in the PRS may still have an important effect on the absolute risk, which is

high. *BRCA1* and *BRCA2* heterozygotes under age 40 at first breast cancer, at the 5th and 95th percentile of the PRS differed by 10% in 10-year contralateral breast cancer risk. These absolute risk differences are modest, but might be of relevance for the choices regarding preventive surgery if incorporated into a multifactorial model that includes other predictive factors, such as family history and adjuvant systemic treatment of the first breast cancer^{37, 38}. In the context of such a comprehensive model, further research is needed to investigate whether the PRS would contribute to the choices that women make for follow-up or preventive surgery.

To summarize, we have investigated the associations between PRS based on 313 variants with contralateral breast cancer risk in a large international series of *BRCA1/2* heterozygotes. We found that the PRS is associated with contralateral breast cancer risk in both *BRCA1* and *BRCA2* heterozygotes of European ancestry and that PRS can be used to refine estimates of contralateral breast cancer risks in these women. However, for women with a first breast cancer after the age of 50, PRS may be of less value in the prediction of the contralateral breast cancer risk. Incorporating risk factors other than PRS and including ER-specific estimates may further improve contralateral breast cancer risk prediction. Before implementation in a diagnostic setting, our results should be validated in a prospective cohort of *BRCA1* and *BRCA2* heterozygotes.

Acknowledgements and funding

This work was supported by the Alpe d'HuZes/Dutch Cancer Society (KWF Kankerbestrijding) project 6253 and Dutch Cancer Society (KWF Kankerbestrijding) project UL2014-7473. See online version for further details.

We acknowledge all the families, clinicians, family doctors, researchers, research nurses, research assistants, and technicians who contribute to the individual studies of which we used the data for this research and manuscript. See online version for further details.

Ethics Statement

All participants were recruited by the host institutions under protocols approved by local ethics review boards and provided written informed consent²⁴.

Disclosure of potential conflicts of interest

Claudine Isaacs is consultant to Astra Zeneca, Novartis, Pfizer, Genentech, PUMA, Seattle Genetics and received research support from Tesaro.

Data availability statement

The CIMBA data is available on request. To receive access to the data, a concept form must be submitted, which will then be reviewed by the CIMBA Data Access Coordination Committee (DACC). Please contact Lesley McGuffog (e-mail: ljm26@medschl.cam.ac.uk), to get access to these concept forms (<http://cimba.ccge.medschl.cam.ac.uk/contact/>).

References

1. Kuchenbaecker KB, Hopper JL, Barnes DR, et al. Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. *Jama*. Jun 20 2017;317(23):2402-2416. doi:10.1001/jama.2017.7112
2. Graeser MK, Engel C, Rhiem K, et al. Contralateral breast cancer risk in BRCA1 and BRCA2 mutation carriers. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. Dec 10 2009;27(35):5887-92. doi:10.1200/jco.2008.19.9430
3. Mavaddat N, Peock S, Frost D, et al. Cancer risks for BRCA1 and BRCA2 mutation carriers: results from prospective analysis of EMBRACE. *Journal of the National Cancer Institute*. Jun 5 2013;105(11):812-22. doi:10.1093/jnci/djt095
4. Metcalfe K, Gershman S, Lynch HT, et al. Predictors of contralateral breast cancer in BRCA1 and BRCA2 mutation carriers. *British journal of cancer*. Apr 26 2011;104(9):1384-92. doi:10.1038/bjc.2011.120
5. Rhiem K, Engel C, Graeser M, et al. The risk of contralateral breast cancer in patients from BRCA1/2 negative high risk families as compared to patients from BRCA1 or BRCA2 positive families: a retrospective cohort study. *Breast cancer research : BCR*. Dec 7 2012;14(6):R156. doi:10.1186/bcr3369
6. van der Kolk DM, de Bock GH, Leegte BK, et al. Penetrance of breast cancer, ovarian cancer and contralateral breast cancer in BRCA1 and BRCA2 families: high cancer incidence at older age. *Breast cancer research and treatment*. Dec 2010;124(3):643-51. doi:10.1007/s10549-010-0805-3
7. Lizarraga IM, Sugg SL, Weigel RJ, Scott-Conner CE. Review of risk factors for the development of contralateral breast cancer. *American journal of surgery*. Nov 2013;206(5):704-8. doi:10.1016/j.amjsurg.2013.08.002
8. Kramer I, Schaapveld M, Oldenburg HSA, et al. The Influence of Adjuvant Systemic Regimens on Contralateral Breast Cancer Risk and Receptor Subtype. *Journal of the National Cancer Institute*. Jul 1 2019;111(7):709-718. doi:10.1093/jnci/djz010
9. Carbine NE, Lostumbo L, Wallace J, Ko H. Risk-reducing mastectomy for the prevention of primary breast cancer. *The Cochrane database of systematic reviews*. Apr 5 2018;4:Cd002748. doi:10.1002/14651858.CD002748.pub4
10. van den Broek AJ, van 't Veer LJ, Hooning MJ, et al. Impact of Age at Primary Breast Cancer on Contralateral Breast Cancer Risk in BRCA1/2 Mutation Carriers. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. Feb 10 2016;34(5):409-18. doi:10.1200/jco.2015.62.3942
11. Michailidou K, Hall P, Gonzalez-Neira A, et al. Large-scale genotyping identifies 41 new loci associated with breast cancer risk. *NatGenet*. 4/2013 2013;45(4):353-2. Not in File. doi:ng.2563 [pii];10.1038/ng.2563 [doi]
12. Michailidou K, Lindstrom S, Dennis J, et al. Association analysis identifies 65 new breast cancer risk loci. *Nature*. Oct 23 2017;doi:10.1038/nature24284

13. Lilyquist J, Ruddy KJ, Vachon CM, Couch FJ. Common Genetic Variation and Breast Cancer Risk - Past, present, and future. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. Jan 30 2018;doi:10.1158/1055-9965.epi-17-1144
14. Mavaddat N, Michailidou K, Dennis J, et al. Polygenic Risk Scores for Prediction of Breast Cancer and Breast Cancer Subtypes. *American journal of human genetics*. Jan 3 2019;104(1):21-34. doi:10.1016/j.ajhg.2018.11.002
15. Mavaddat N, Pharoah PD, Michailidou K, et al. Prediction of breast cancer risk based on profiling with common genetic variants. *J Natl Cancer Inst*. 5/2015 2015;107(5)Not in File. doi:djv036 [pii];10.1093/jnci/djv036 [doi]
16. Ho WK, Tan MM, Mavaddat N, et al. European polygenic risk score for prediction of breast cancer shows similar performance in Asian women. *Nat Commun*. Jul 31 2020;11(1):3833. doi:10.1038/s41467-020-17680-w
17. Robson ME, Reiner AS, Brooks JD, et al. Association of Common Genetic Variants With Contralateral Breast Cancer Risk in the WECARE Study. *Journal of the National Cancer Institute*. Oct 1 2017;109(10)doi:10.1093/jnci/djx051
18. Sawyer S, Mitchell G, McKinley J, et al. A role for common genomic variants in the assessment of familial breast cancer. *J Clin Oncol*. 12/10/2012 2012;30(35):4330-4336. Not in File. doi:JCO.2012.41.7469 [pii];10.1200/JCO.2012.41.7469 [doi]
19. Kramer I, Hooning MJ, Mavaddat N, et al. Breast Cancer Polygenic Risk Score and Contralateral Breast Cancer Risk. *American journal of human genetics*. Nov 5 2020;107(5):837-848. doi:10.1016/j.ajhg.2020.09.001
20. Kuchenbaecker KB, McGuffog L, Barrowdale D, et al. Evaluation of Polygenic Risk Scores for Breast and Ovarian Cancer Risk Prediction in BRCA1 and BRCA2 Mutation Carriers. *Journal of the National Cancer Institute*. Jul 01 2017;109(7)doi:10.1093/jnci/djw302
21. Antoniou AC, Sinilnikova OM, McGuffog L, et al. Common variants in LSP1, 2q35 and 8q24 and breast cancer risk for BRCA1 and BRCA2 mutation carriers. *Human molecular genetics*. Nov 15 2009;18(22):4442-56. doi:10.1093/hmg/ddp372
22. Antoniou AC, Spurdle AB, Sinilnikova OM, et al. Common breast cancer-predisposition alleles are associated with breast cancer risk in BRCA1 and BRCA2 mutation carriers. *American journal of human genetics*. Apr 2008;82(4):937-48. doi:10.1016/j.ajhg.2008.02.008
23. Antoniou AC, Beesley J, McGuffog L, et al. Common breast cancer susceptibility alleles and the risk of breast cancer for BRCA1 and BRCA2 mutation carriers: implications for risk prediction. *Cancer research*. Dec 1 2010;70(23):9742-54. doi:10.1158/0008-5472.Can-10-1907
24. Barnes DR, Rookus MA, McGuffog L, et al. Polygenic risk scores and breast and epithelial ovarian cancer risks for carriers of BRCA1 and BRCA2 pathogenic variants. *Genetics in Medicine*. 2020/10/01 2020;22(10):1653-1666. doi:10.1038/s41436-020-0862-x
25. Gail MH, Pfeiffer RM. Breast Cancer Risk Model Requirements for Counseling, Prevention, and Screening. *Journal of the National Cancer Institute*. Sep 1 2018;110(9):994-1002. doi:10.1093/jnci/djy013

26. Chenevix-Trench G, Milne RL, Antoniou AC, Couch FJ, Easton DF, Goldgar DE. An international initiative to identify genetic modifiers of cancer risk in BRCA1 and BRCA2 mutation carriers: the Consortium of Investigators of Modifiers of BRCA1 and BRCA2 (CIMBA). *Breast cancer research : BCR*. 2007;9(2):104. doi:10.1186/bcr1670
27. Amos CI, Dennis J, Wang Z, et al. The OncoArray Consortium: A Network for Understanding the Genetic Architecture of Common Cancers. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. Jan 2017;26(1):126-135. doi:10.1158/1055-9965.epi-16-0106
28. Gaudet MM, Kuchenbaecker KB, Vijai J, et al. Identification of a BRCA2-specific modifier locus at 6p24 related to breast cancer risk. *PLoS genetics*. 2013;9(3):e1003173. doi:10.1371/journal.pgen.1003173
29. Couch FJ, Wang X, McGuffog L, et al. Genome-wide association study in BRCA1 mutation carriers identifies novel loci associated with breast and ovarian cancer risk. *PLoS genetics*. 2013;9(3):e1003212. doi:10.1371/journal.pgen.1003212
30. Kuchenbaecker KB, Neuhausen SL, Robson M, et al. Associations of common breast cancer susceptibility alleles with risk of breast cancer subtypes in BRCA1 and BRCA2 mutation carriers. *Breast cancer research : BCR*. Dec 31 2014;16(6):3416. doi:10.1186/s13058-014-0492-9
31. Milne RL, Kuchenbaecker KB, Michailidou K, et al. Identification of ten variants associated with risk of estrogen-receptor-negative breast cancer. *Nature genetics*. Dec 2017;49(12):1767-1778. doi:10.1038/ng.3785
32. Harrell FE, Jr., Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Statistics in medicine*. Feb 28 1996;15(4):361-87. doi:10.1002/(sici)1097-0258(19960229)15:4<361::Aid-sim168>3.0.Co;2-4
33. Azur MJ, Stuart EA, Frangakis C, Leaf PJ. Multiple imputation by chained equations: what is it and how does it work? *International journal of methods in psychiatric research*. Mar 2011;20(1):40-9. doi:10.1002/mpr.329
34. R_Core_Team_(2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
35. Dahabreh IJ, Kent DM. Index Event Bias as an Explanation for the Paradoxes of Recurrence Risk Research. *Jama*. 2011;305(8):822-823. doi:10.1001/jama.2011.163 %J JAMA
36. Begg CB, Ostrovnaya I, Geyer FC, et al. Contralateral breast cancers: Independent cancers or metastases? *International journal of cancer*. Jan 15 2018;142(2):347-356. doi:10.1002/ijc.31051
37. Akdeniz D, Schmidt MK, Seynaeve CM, et al. Risk factors for metachronous contralateral breast cancer: A systematic review and meta-analysis. *Breast (Edinburgh, Scotland)*. Apr 2019;44:1-14. doi:10.1016/j.breast.2018.11.005
38. Giardiello D, Steyerberg EW, Hauptmann M, et al. Prediction and clinical utility of a contralateral breast cancer risk model. *Breast cancer research : BCR*. Dec 17 2019;21(1):144. doi:10.1186/s13058-019-1221-1

Supplementary figures and tables

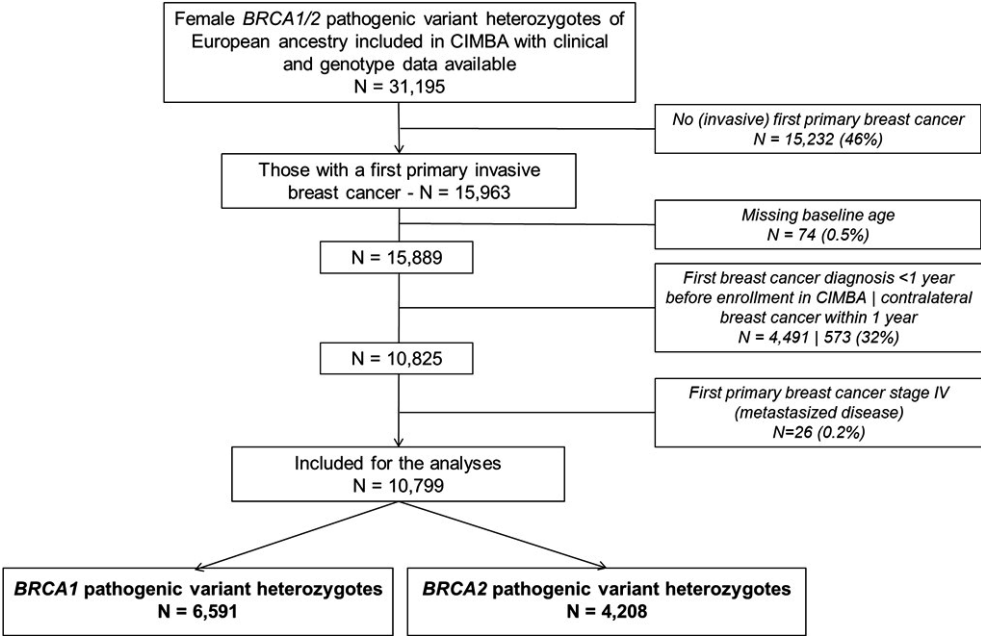


Figure S1: Flow chart of the inclusion of CIMBA participants
Flow chart of the inclusion and exclusion of CIMBA participants for this study.
Abbreviation: N, Number

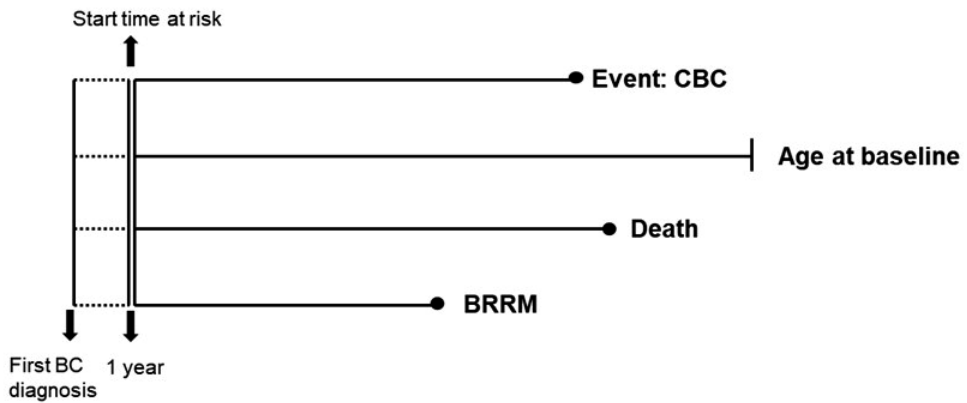


Figure S2: Time at risk in the association analyses

The time at risk was assumed to start one year after the first breast cancer. Participants were censored at (i) age at baseline, (ii) bilateral risk reducing mastectomy or (iii) death, whichever was earlier. Baseline age was defined as the age at local ascertainment (97%), or when this was not known, age at genetic testing (2%) or age at last follow-up (1%). Incidence of a metachronous contralateral breast cancer, invasive or *in situ*, before baseline was considered as an event in the main analyses. Abbreviations: BC, Breast Cancer; BRRM, Bilateral Risk Reducing Mastectomy; CBC, Contralateral Breast Cancer.

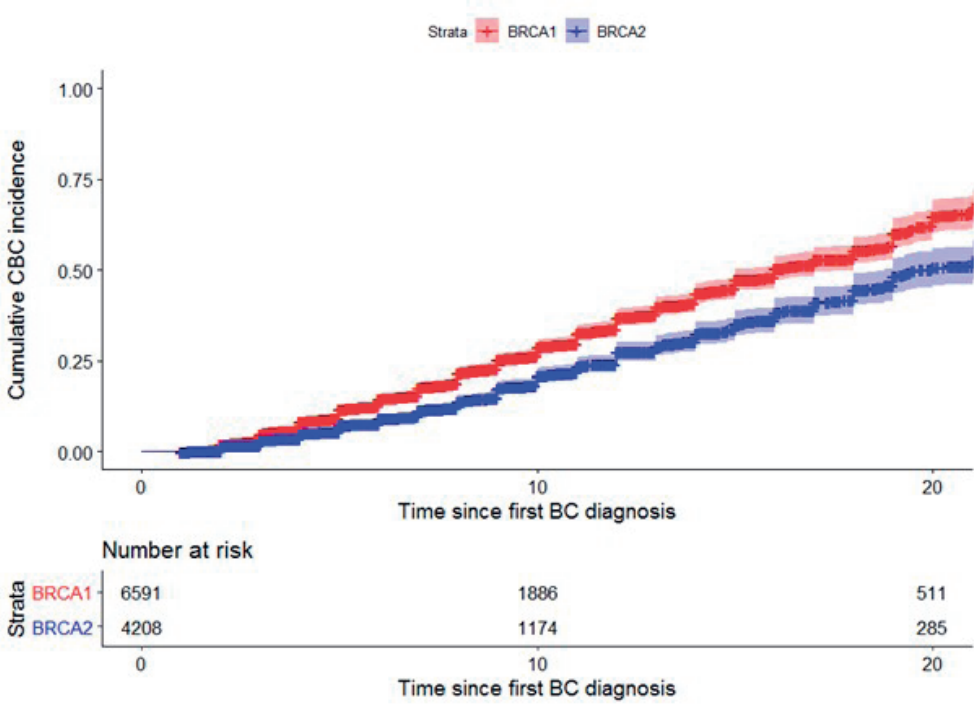


Figure S3: Cumulative contralateral breast cancer incidence for *BRCA1* and *BRCA2* heterozygotes since the first breast cancer diagnosis

Plot of the cumulative contralateral breast cancer incidence for *BRCA1* (red) and *BRCA2* (blue) pathogenic variant heterozygotes. Confidence intervals are shown with the transparent red and blue color. The time of follow-up started at the age of first primary invasive breast cancer diagnosis. Abbreviations: BC, Breast Cancer; CBC, Contralateral Breast Cancer.

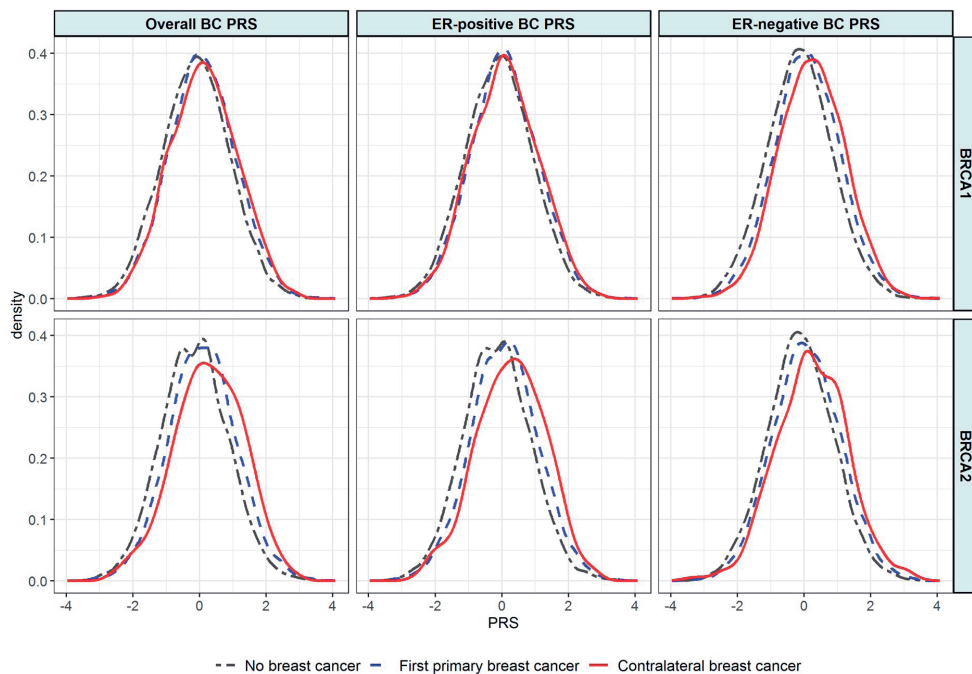


Figure S4: Distribution of the overall breast cancer, ER-positive and ER-negative PRS₃₁₃ for *BRCA1* and *BRCA2* heterozygotes without breast cancer, with a first primary breast cancer and with contralateral breast cancer

Density plots of the standardized PRS distributions for *BRCA1* and *BRCA2* heterozygotes. The distributions are shown for CIMBA participants who did not develop breast cancer (grey two-dashed line), who developed an invasive first primary breast cancer only (blue dashed line, selection shown in Figure S1) and who developed a metachronous contralateral breast cancer (red solid line). The number of included women for these groups were 8,837, 5,189, and 1,402 for *BRCA1* heterozygotes and 5,665, 3,561, and 647 for *BRCA2* heterozygotes.

Abbreviations: BC, Breast Cancer; ER, Estrogen Receptor; PRS, Polygenic Risk Score.

Table S1: Estrogen receptor status of the first primary breast tumor and the contralateral breast tumor

	ER-status BC1	ER-status CBC		
		ER-positive	ER-negative	Unknown
<i>BRC A1</i> heterozygotes	ER-positive	25	42	25
	ER-negative	29	256	117
	Unknown	47	148	713
<i>BRC A2</i> heterozygotes	ER-positive	100	19	63
	ER-negative	16	18	27
	Unknown	81	13	310

Abbreviations: BC1, first primary Breast Cancer; CBC, Contralateral Breast Cancer; ER, Estrogen Receptor.

Table S2: 313 variants included in the polygenic risk score

See online material. First nine columns of the table were published by Mavaddat et al.¹

Table S3: Country of origin of included CIMBA participants

Country of origin		<i>BRCA1</i> heterozygotes	<i>BRCA2</i> heterozygotes
Group ^a	Country		
Africa	South Africa	29	70
America	Brazil	0	1
	Canada	209	103
	United States of America	1266	735
Asia	Israel	60	52
	Qatar	0	1
Australia	Australia	355	269
Eastern Europe	Albania	1	0
	Czech Republic	41	0
	Hungary	120	36
	Latvia	9	0
	Lithuania	62	6
	Poland	217	0
	Russia	12	0
Northwestern Europe	Austria	179	77
	Belgium	128	43
	Denmark	224	171
	Ireland	1	1
	Finland	46	44
	France	677	565
	Germany	762	394
	Iceland	0	102
	Netherlands	440	196
	Sweden	177	24
	United Kingdom	702	614
Southern Europe	Greece	99	13
	Italy	472	285
	Portugal	23	58
	Spain	280	348

^a Groups for country used in the cox-regression analyses

Table S4: Results of the association analyses between the PRS and contralateral breast cancer risk

Outcome	PRS ₃₁₃					BRCA1 heterozygotes					BRCA2 heterozygotes										
	Overall BC	ER-positive	ER-negative	Overall BC	ER-positive	ER-negative	Overall BC	ER-positive	ER-negative	Overall BC	ER-positive	ER-negative	Overall BC	ER-positive	ER-negative						
All CBC	5,189	1,402	1,05	1.00-1.11	0.059	3,561	647	1.15	1.07-1.24	2.33x10 ⁻⁴	5,189	1,402	1.03	0.98-1.09	0.208	3,561	647	1.15	1.07-1.25	1.94x10 ⁻⁴	
ER-positive			1.12	1.06-1.18	5.98x10 ⁻⁵			1.11	1.03-1.20	0.005			1.12	1.06-1.18	5.98x10 ⁻⁵			1.11	1.03-1.20	0.005	
ER-negative																					
ER-positive CBC	6,312 ^a	279 ^a	1.32	1.12-1.56	0.002	3,701 ^a	507 ^a	1.21	1.10-1.32	4.19x10 ⁻⁵	6,312 ^a	279 ^a	1.30	1.11-1.52	0.002	3,701 ^a	507 ^a	1.22	1.11-1.33	2.15x10 ⁻⁵	
ER-positive			1.31	1.11-1.55	0.003			1.12	1.02-1.22	0.014			1.31	1.11-1.55	0.003			1.12	1.02-1.22	0.014	
ER-negative																					
ER-negative CBC	5,468 ^a	1123 ^a	0.99	0.93-1.06	0.859	4,068 ^a	140 ^a	0.98	0.81-1.18	0.809	5,468 ^a	1123 ^a	0.98	0.92-1.04	0.491	4,068 ^a	140 ^a	0.95	0.79-1.15	0.628	
ER-positive			1.07	1.01-1.15	0.036			1.10	0.91-1.32	0.346			1.07	1.01-1.15	0.036			1.10	0.91-1.32	0.346	
ER-negative																					

^a Average number over 10 imputed datasets

Abbreviations: BC, Breast Cancer; CBC, Contralateral Breast Cancer; CI, Confidence Interval; ER, Estrogen Receptor; HR, Hazard Ratio; PRS, Polygenic Risk Score; UBC, Unilateral Breast Cancer.

Table S5: Results of the change in effect size of the association between the PRS and contralateral breast cancer risk, using multivariable Cox Regression models

Added variable	BRCA1 heterozygotes; ER-negative PRS ₃₁₃			BRCA2 heterozygotes; ER-positive PRS ₃₁₃						
	β^a	% change	HR ^a	95% CI	β^b	% change	HR ^b	95% CI	P	
Base model^c	0.111	ref	1.12	1.06-1.18	5.98x10 ⁻⁵	0.143	ref	1.15	1.07-1.25	1.94x10 ⁻⁴
Family history	0.112	1.10	1.12	1.06-1.18	4.43x10 ⁻⁵	0.143	0.26	1.15	1.07-1.25	2.53x10 ⁻⁴
Age of BC1	0.112	1.03	1.12	1.06-1.18	4.32x10 ⁻⁵	0.151	5.01	1.16	1.08-1.26	1.29x10 ⁻⁴
Tumor characteristics BC1										
ER-status	0.111	0.04	1.12	1.06-1.18	4.28x10 ⁻⁵	0.141	1.68	1.15	1.07-1.24	3.73x10 ⁻⁴
Node status	0.112	0.69	1.12	1.06-1.18	4.65x10 ⁻⁵	0.145	1.27	1.16	1.07-1.25	2.21x10 ⁻⁴
Tumor size	0.111	0.01	1.12	1.06-1.18	5.36x10 ⁻⁵	0.147	2.24	1.16	1.07-1.25	1.95x10 ⁻⁴
Therapy BC1										
Chemotherapy	0.110	0.70	1.12	1.06-1.18	5.97x10 ⁻⁵	0.143	0.04	1.15	1.07-1.25	2.53x10 ⁻⁴
Hormone	0.111	0.10	1.12	1.06-1.18	5.15x10 ⁻⁵	0.144	0.14	1.15	1.07-1.25	2.48x10 ⁻⁴
Trastuzumab	0.111	0.02	1.12	1.06-1.18	5.22x10 ⁻⁵	0.143	0.23	1.15	1.07-1.25	2.57x10 ⁻⁴
Radiotherapy	0.111	0.09	1.12	1.06-1.18	5.29x10 ⁻⁵	0.143	0.18	1.15	1.07-1.25	2.56x10 ⁻⁴
Full model	0.114	2.24	1.12	1.07-1.18	4.50x10 ⁻⁵	0.150	4.37	1.16	1.07-1.26	2.06x10 ⁻⁴
variables combined										

^a Effect size of the ER-negative PRS₃₁₃

^b Effect size of the ER-positive PRS₃₁₃

^c Cox regression model for the association between the PRS and contralateral breast cancer, stratified by country, clustered on family membership, and adjusted for birth cohort (quartiles of the observed distribution).

Abbreviations: BC1, first primary Breast Cancer; CI, Confidence Interval; HR, Hazard Ratio; PRS, Polygenic Risk Score

Supplementary references

1. Mavaddat N, Michailidou K, Dennis J, et al. Polygenic Risk Scores for Prediction of Breast Cancer and Breast Cancer Subtypes. *American journal of human genetics*. Jan 3 2019;104(1):21-34. doi:10.1016/j.ajhg.2018.11.002

