

ARTICLE

The predictive ability of the 313 variant–based polygenic risk score for contralateral breast cancer risk prediction in women of European ancestry with a heterozygous *BRCA1* or *BRCA2* pathogenic variantInge M. M. Lakeman et al.[#]

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 the Consortium of Investigators of Modifiers of *BRCA1/2* (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, hazard ratio (HR) per SD = 1.12, 95% confidence interval (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.

Genetics in Medicine (2021) 23:1726–1737; <https://doi.org/10.1038/s41436-021-01198-7>

INTRODUCTION

Heterozygotes of germline pathogenic variants in *BRCA1* or *BRCA2* (henceforth *BRCA1/2* heterozygotes) have a higher risk of developing contralateral breast cancer than nonheterozygotes.¹ The estimated cumulative 10-year contralateral breast cancer risk varies across studies between 18.5% and 34.2% for *BRCA1* heterozygotes and between 10.8% and 29.2% for *BRCA2* heterozygotes,^{1–6} 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 burden,⁹ 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 studies (GWAS).^{11–13} 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 population.^{14–16} 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 cancer.^{17–19} Furthermore, the PRS derived from the general population has also been shown to be associated with breast cancer risk in *BRCA1/2* heterozygotes.^{20–24}

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 0.630.¹⁴ Among *BRCA2* heterozygotes, this same PRS₃₁₃ was also associated with breast cancer risk, hazard ratio (HR) per SD = 1.31, 95% confidence interval (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 variants.²⁵ Whether variants associated with breast cancer are associated with contralateral breast cancer risk for *BRCA1/2* heterozygotes as well, individually or combined in

[#]A full list of authors and their affiliations appears at the end of the paper.

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 is 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 available.²⁶ CIMBA includes 81 individual studies of which the majority of the participants were ascertained through cancer genetics clinics.²⁶ 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 Fig. 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 OncoArray.²⁷ The remaining participants were genotyped with the Illumina iCOGS array.¹¹ 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 previously;¹⁴ correlation between PRS based on the two genotyping arrays was high.¹⁹ 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 Breast Cancer Association Consortium (BCAC) population controls that were included in the validation data set.¹⁴ 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* heterozygotes.²⁴

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 (Fig. 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 5,923 and 3,752 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-index.³² 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, ten 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 ten 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*

Table 1. Characteristics of the participants.

		<i>BRCA1</i> heterozygotes		<i>BRCA2</i> heterozygotes	
		UBC, <i>n</i> (%)	CBC, <i>n</i> (%)	UBC, <i>n</i> (%)	CBC, <i>n</i> (%)
<i>N</i>		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	<i>N</i>	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)

Table 1 continued

		<i>BRCA1</i> heterozygotes		<i>BRCA2</i> heterozygotes	
		UBC, <i>n</i> (%)	CBC, <i>n</i> (%)	UBC, <i>n</i> (%)	CBC, <i>n</i> (%)
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)
	Noninvasive	–	135 (10)	–	102 (16)
ER status	Positive	–	101 (7)	–	197 (30)
	Negative	–	446 (32)	–	50 (8)
	Unknown	–	855 (61)	–	400 (62)
PRS₃₁₃					
Standardized PRS ₃₁₃ 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)

BC breast cancer, *BRRM* bilateral risk-reducing mastectomy, CBC contralateral breast cancer, ER status estrogen receptor status of the tumor, PRS polygenic risk score, SD standard deviation, UBC unilateral breast cancer.

^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 inches), T2 = > 2 cm–5 cm (> 0.79 –1.97 inches), T3 = > 5 cm (> 1.97 inches), T4 = any size, with direct extension to the chest wall or skin.

^dIncluding neoadjuvant and adjuvant chemotherapy.

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 previously.²³ These external incidence estimates were based on prospective cohort data from three consortia on heterozygotes of pathogenic *BRCA1* and *BRCA2* variants,¹ the International *BRCA1/2* Carrier Cohort Study (IBCCS), the Breast Cancer Family Registry (BCFR), and the Kathleen Cunningham 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 3.5.0.³⁴ 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 (Fig. S3). Patient and tumor characteristics as well as the PRS distributions are shown in Table 1 and Fig. S4.

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 Fig. 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 = 6.0×10^{-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 = 3.2×10^{-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 = 4.9×10^{-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 Fig. 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 = 1.9×10^{-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

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

		<i>BRCA1</i> heterozygotes (ER-negative PRS ₃₁₃)					<i>BRCA2</i> heterozygotes (ER-positive PRS ₃₁₃)				
		UBC cases, <i>n</i>	CBC cases, <i>n</i>	HR ^a	95% CI	<i>P</i>	UBC cases, <i>n</i>	CBC cases, <i>n</i>	HR ^a	95% CI	<i>P</i>
PRS continuous	All CBC	5,189	1,402	1.12	1.06–1.18	5.98×10 ⁻⁵	3,561	647	1.15	1.07–1.25	1.94×10 ⁻⁴
	Invasive CBC	5,324	1,267	1.13	1.07–1.20	3.15×10 ⁻⁵	3,663	545	1.15	1.06–1.25	6.02×10 ⁻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	Main effect	5,189	1,402	1.48	1.15–1.89	2.03×10 ⁻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	4.79×10 ⁻⁸	1,238	268	1.23	1.09–1.38	5.78×10 ⁻⁴
	40–50	1,821	456	0.99	0.90–1.09	0.785	1,306	261	1.19	1.05–1.34	6.91×10 ⁻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	4.32×10 ⁻³	3,207	570	1.16	1.07–1.26	1.99×10 ⁻⁴
	Class II	1,345	374	1.15	1.04–1.28	4.75×10 ⁻³	125	25	0.91	0.65–1.28	0.594

BC1 first primary breast cancer, *CBC* contralateral breast cancer, *CI* confidence interval, *HR* hazard ratio, *PRS* polygenic risk score, *UBC* unilateral breast cancer. ^aHRs for association with breast cancer and the continuous PRS₃₁₃ are reported per standard deviation of the PRS in population-based controls. ^bClass I pathogenic variants result in an unstable or no protein. Class II pathogenic variants yield stable mutant proteins.

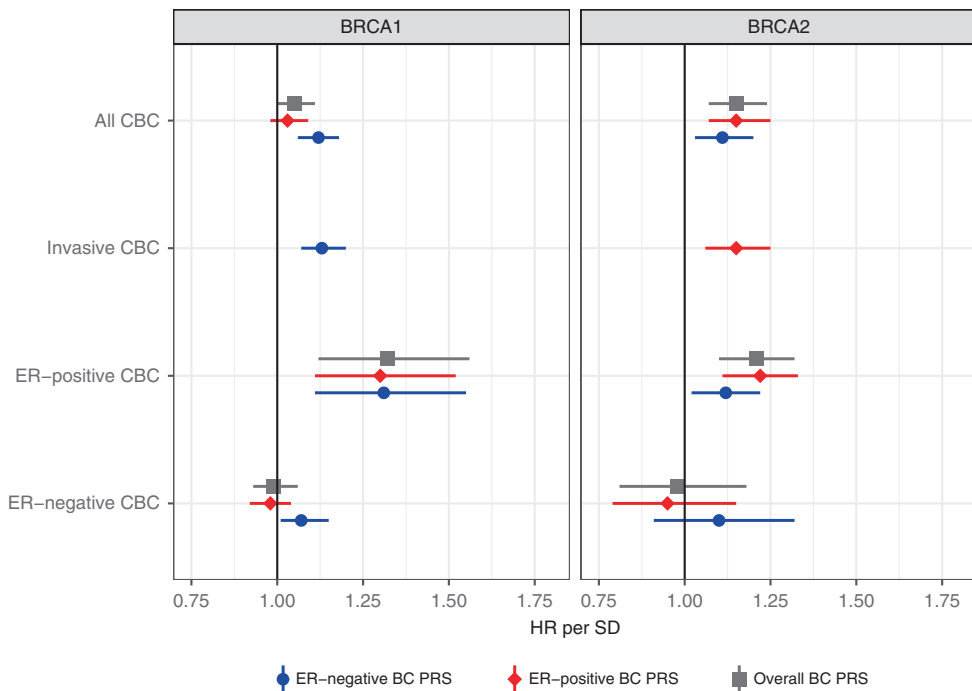


Fig. 1 Association between the PRS and contralateral breast cancer risk for *BRCA1* and *BRCA2* heterozygotes. Effect size of the association between contralateral breast cancer and the three different PRS₃₁₃ after testing for covariates for the following selections: all contralateral breast cancer, 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. CBC contralateral breast cancer, ER estrogen receptor, HR hazard ratio, PRS polygenic risk score, SD standard deviation.

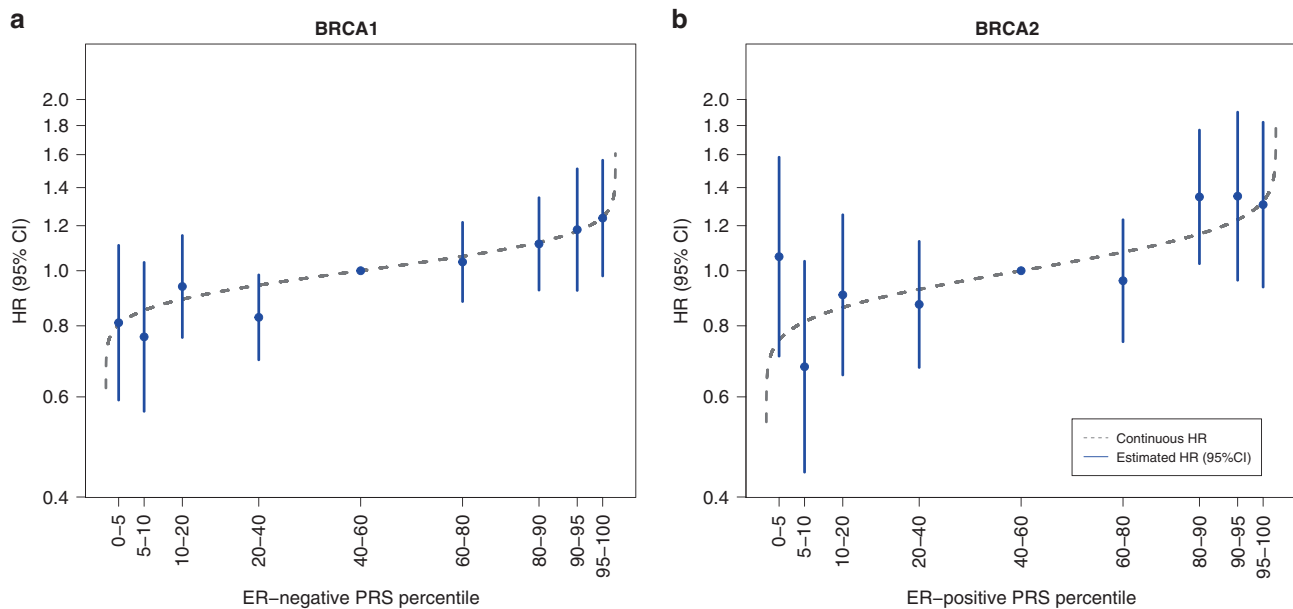


Fig. 2 Association between categories of the PRS and contralateral breast cancer risk for *BRCA1* and *BRCA2* heterozygotes. HRs and 95% CI for percentiles of the ER-negative PRS₃₁₃ for *BRCA1* heterozygotes and the ER-positive PRS₃₁₃ for *BRCA2* heterozygotes, relative to the middle quintile. The PRS 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 gray 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. CI confidence interval, ER estrogen receptor, HR hazard ratio, PRS polygenic risk score.

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 = 6.0×10^{-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 = 2.1×10^{-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 Fig. 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 = 2.2×10^{-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.

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 years 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 nonsignificantly 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 Fig. 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.

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 PRS₃₁₃, while for *BRCA2* heterozygotes, both

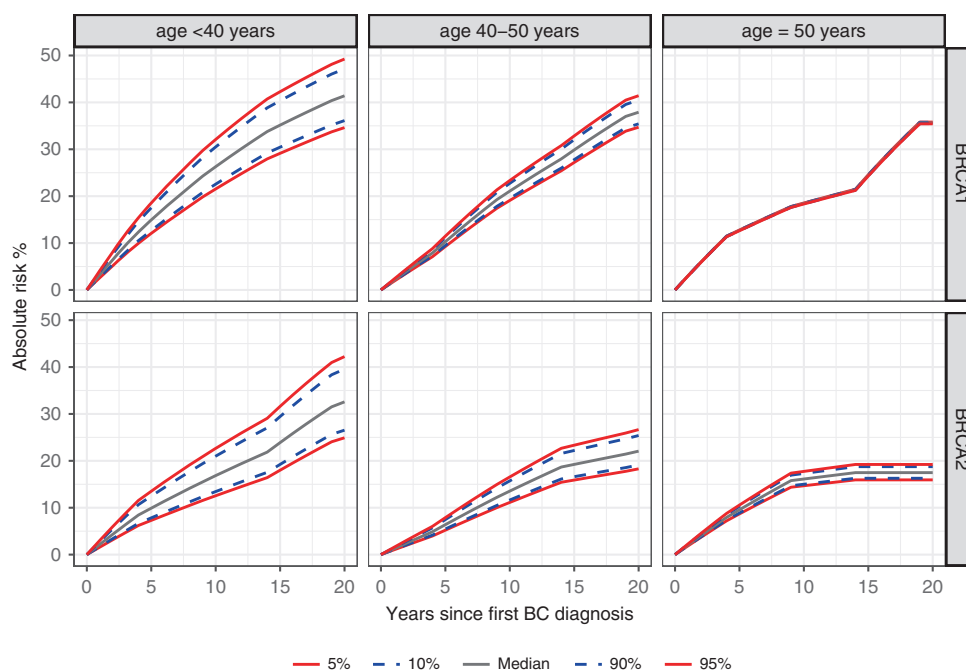


Fig. 3 Absolute contralateral breast cancer risk by PRS percentiles per age category of the first breast cancer diagnosis for *BRCA1* and *BRCA2* heterozygotes. Predicted absolute contralateral breast cancer risks by percentile of the continuous ER-negative PRS₃₁₃ for *BRCA1* heterozygotes and ER-positive 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* heterozygotes.²⁰ 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. BC breast cancer, CBC contralateral breast cancer, PRS polygenic risk score.

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 population,¹⁹ and the lower relative risk for the first cancer in *BRCA1/2* heterozygotes than in the general population.²⁴ 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 nongenetic 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 bias.³⁵ 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 metastases.³⁶

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 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 design:¹ 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 data set 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.

DATA AVAILABILITY

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 (email: ljm26@medschl.cam.ac.uk) to get access to these concept forms (<http://cimba.ccg.medschl.cam.ac.uk/contact/>).

Received: 7 August 2020; Accepted: 26 April 2021;
Published online: 10 June 2021

REFERENCES

- Kuchenbaecker, K. B. et al. Risks of breast, ovarian, and contralateral breast cancer for *BRCA1* and *BRCA2* mutation carriers. *JAMA*. **317**, 2402–2416 (2017).
- Graeser, M. K. et al. Contralateral breast cancer risk in *BRCA1* and *BRCA2* mutation carriers. *J. Clin. Oncol.* **27**, 5887–5892 (2009).
- Mavaddat, N. et al. Cancer risks for *BRCA1* and *BRCA2* mutation carriers: results from prospective analysis of EMBRACE. *J. Natl. Cancer Inst.* **105**, 812–822 (2013).
- Metcalfe, K. et al. Predictors of contralateral breast cancer in *BRCA1* and *BRCA2* mutation carriers. *Br. J. Cancer*. **104**, 1384–1392 (2011).
- Rhiem, K. 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 Res.* **14**, R156 (2012).

- van der Kolk, D. M. 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 Res. Treat.* **124**, 643–651 (2010).
- Lizarraga, I. M., Sugg, S. L., Weigel, R. J. & Scott-Conner, C. E. Review of risk factors for the development of contralateral breast cancer. *Am. J. Surg.* **206**, 704–708 (2013).
- Kramer, I. et al. The influence of adjuvant systemic regimens on contralateral breast cancer risk and receptor subtype. *J. Natl. Cancer Inst.* **111**, 709–718 (2019).
- Carbine, N. E., Lostumbo, L., Wallace, J. & Ko, H. Risk-reducing mastectomy for the prevention of primary breast cancer. *Cochrane Database Syst. Rev.* **4**, Cd002748 (2018).
- van den Broek, A. J. et al. Impact of age at primary breast cancer on contralateral breast cancer risk in *BRCA1/2* mutation carriers. *J. Clin. Oncol.* **34**, 409–418 (2016).
- Michailidou, K. et al. Large-scale genotyping identifies 41 new loci associated with breast cancer risk. *Nat. Genet.* **45**, 353–352 (2013).
- Michailidou, K. et al. Association analysis identifies 65 new breast cancer risk loci. *Nature*. **551**, 92–94 (2017).
- Lilyquist, J., Ruddy, K. J., Vachon, C. M. & Couch, F. J. Common genetic variation and breast cancer risk—past, present, and future. *Cancer Epidemiol. Biomarkers Prev.* **27**, 380–394 (2018).
- Mavaddat, N. et al. Polygenic risk scores for prediction of breast cancer and breast cancer subtypes. *Am. J. Hum. Genet.* **104**, 21–34 (2019).
- Mavaddat, N. et al. Prediction of breast cancer risk based on profiling with common genetic variants. *J. Natl. Cancer Inst.* **107**, djv036 (2015).
- Ho, W. K. et al. European polygenic risk score for prediction of breast cancer shows similar performance in Asian women. *Nat. Commun.* **11**, 3833 (2020).
- Robson, M. E. et al. Association of common genetic variants with contralateral breast cancer risk in the WECARE Study. *J. Natl. Cancer Inst.* **109**, djx051 (2017).
- Sawyer, S. et al. A role for common genomic variants in the assessment of familial breast cancer. *J. Clin. Oncol.* **30**, 4330–4336 (2012).
- Kramer, I. et al. Breast cancer polygenic risk score and contralateral breast cancer risk. *Am. J. Hum. Genet.* **107**, 837–848 (2020).
- Kuchenbaecker, K. B. et al. Evaluation of polygenic risk scores for breast and ovarian cancer risk prediction in *BRCA1* and *BRCA2* mutation carriers. *J. Natl. Cancer Inst.* **109**, djw302 (2017).
- Antoniou, A. C. et al. Common variants in *LSP1*, *2q35* and *8q24* and breast cancer risk for *BRCA1* and *BRCA2* mutation carriers. *Hum. Mol. Genet.* **18**, 4442–4456 (2009).
- Antoniou, A. C. et al. Common breast cancer-predisposition alleles are associated with breast cancer risk in *BRCA1* and *BRCA2* mutation carriers. *Am. J. Hum. Genet.* **82**, 937–948 (2008).
- Antoniou, A. C. et al. Common breast cancer susceptibility alleles and the risk of breast cancer for *BRCA1* and *BRCA2* mutation carriers: implications for risk prediction. *Cancer Res.* **70**, 9742–9754 (2010).
- Barnes, D. R. et al. Polygenic risk scores and breast and epithelial ovarian cancer risks for carriers of *BRCA1* and *BRCA2* pathogenic variants. *Genet. Med.* **22**, 1653–1666 (2020).
- Gail, M. H. & Pfeiffer, R. M. Breast cancer risk model requirements for counseling, prevention, and screening. *J. Natl. Cancer Inst.* **110**, 994–1002 (2018).
- Chenevix-Trench, G. et al. 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 Res.* **9**, 104 (2007).
- Amos, C. I. et al. The OncoArray Consortium: a network for understanding the genetic architecture of common cancers. *Cancer Epidemiol. Biomarkers Prev.* **26**, 126–135 (2017).
- Gaudet, M. M. et al. Identification of a *BRCA2*-specific modifier locus at *6p24* related to breast cancer risk. *PLoS Genet.* **9**, e1003173 (2013).
- Couch, F. J. et al. Genome-wide association study in *BRCA1* mutation carriers identifies novel loci associated with breast and ovarian cancer risk. *PLoS Genet.* **9**, e1003212 (2013).
- Kuchenbaecker, K. B. et al. Associations of common breast cancer susceptibility alleles with risk of breast cancer subtypes in *BRCA1* and *BRCA2* mutation carriers. *Breast Cancer Res.* **16**, 3416 (2014).
- Milne, R. L. et al. Identification of ten variants associated with risk of estrogen-receptor-negative breast cancer. *Nat. Genet.* **49**, 1767–1778 (2017).
- Harrell, F. E. Jr, Lee, K. L. & Mark, D. B. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat. Med.* **15**, 361–387 (1996).
- Azur, M. J., Stuart, E. A., Frangakis, C. & Leaf, P. J. Multiple imputation by chained equations: what is it and how does it work? *Int. J. Methods Psychiatr. Res.* **20**, 40–49 (2011).
- R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria (2019).

35. Dahabreh, I. J. & Kent, D. M. Index event bias as an explanation for the paradoxes of recurrence risk research. *JAMA*. **305**, 822–823 (2011).
36. Begg, C. B. et al. Contralateral breast cancers: Independent cancers or metastases? *Int. J. Cancer*. **142**, 347–356 (2018).
37. Akdeniz, D. et al. Risk factors for metachronous contralateral breast cancer: a systematic review and meta-analysis. *Breast*. **44**, 1–14 (2019).
38. Giardiello, D. et al. Prediction and clinical utility of a contralateral breast cancer risk model. *Breast Cancer Res.* **21**, 144 (2019).

ACKNOWLEDGEMENTS

We acknowledge all the families, clinicians, family doctors, researchers, research nurses, research assistants, and technicians who contribute to the individual studies from which we used the data for this research and paper.

AUTHOR CONTRIBUTIONS

Conceptualization: A.C.C., M.R., M.K.S. Formal analysis: I.M.M.L., A.J.v.d.B., J.A.M.V., D.R.B. Resources: All authors. Supervision: A.C.C., M.R., M.K.S. Writing—original draft: I.M.M.L., A.C.C., M.R., M.K.S. Writing—review & editing: All authors.

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. CIMBA: The CIMBA data management and data analysis were supported by Cancer Research—UK grants C12292/A20861, C12292/A11174. G.C.T. and A.B.S. are NHMRC Research Fellows. iCOGS: the European Community's Seventh Framework Programme under grant agreement number 223175 (HEALTH-F2-2009-223175) (COGS), Cancer Research UK (C1287/A10118, C1287/A 10710, C12292/A11174, C1281/A12014, C5047/A8384, C5047/A15007, C5047/A10692, C8197/A16565), the National Institutes of Health (CA128978) and Post-Cancer GWAS initiative (1U19 CA148537, 1U19 CA148065 and 1U19 CA148112—the GAME-ON initiative), the Department of Defence (W81XWH-10-1-0341), the Canadian Institutes of Health Research (CIHR) for the CIHR Team in Familial Risks of Breast Cancer (CRN-87521), and the Ministry of Economic Development, Innovation and Export Trade (PSR-SIIRI-701), Komen Foundation for the Cure, the Breast Cancer Research Foundation, and the Ovarian Cancer Research Fund. OncoArray: the PERSPECTIVE and PERSPECTIVE I&I projects funded by the Government of Canada through Genome Canada and the Canadian Institutes of Health Research, the Ministère de l'Économie, de la Science et de l'Innovation du Québec through Genome Québec, and the Quebec Breast Cancer Foundation; the NCI Genetic Associations and Mechanisms in Oncology (GAME-ON) initiative and Discovery, Biology and Risk of Inherited Variants in Breast Cancer (DRIVE) project (NIH grants U19 CA148065 and X01HG007492); and Cancer Research UK (C1287/A10118 and C1287/A16563). BCFR: UM1 CA164920 from the National Cancer Institute. The content of this paper does not necessarily reflect the views or policies of the National Cancer Institute or any of the collaborating centers in the Breast Cancer Family Registry (BCFR), nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government or the BCFR. BFB0CC: Lithuania (BFB0CC-LT): Research Council of Lithuania grant SEN-18/2015. BIDMC: Breast Cancer Research Foundation. BMBSA: Cancer Association of South Africa (PI Elizabeth J. van Rensburg). BRI-COH: S. L.N. is partially supported by the Morris and Horowitz Families Professorship. CNIO: Spanish Ministry of Health PI16/00440 supported by FEDER funds, the Spanish Ministry of Economy and Competitiveness (MINECO) SAF2014-57680-R and the Spanish Research Network on Rare diseases (CIBERER). COH-CCGCRN: Research reported in this publication was supported by the National Cancer Institute of the National Institutes of Health under grant number R25CA112486, and RC4CA153828 (PI: J. Weitzel) from the National Cancer Institute and the Office of the Director, National Institutes of Health. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. CONSIT TEAM: Associazione Italiana Ricerca sul Cancro (AIRC; IG2015 number 16732) to P. Peterlongo. DEMOKRITOS: European Union (European Social Fund—ESF) and Greek national funds through the Operational Program “Education and Lifelong Learning” of the National Strategic Reference Framework (NSRF)—Research Funding Program of the General Secretariat for Research & Technology: SYN11_10_19 NBCA. Investing in knowledge society through the European Social Fund. DFKZ: German Cancer Research Center. EMBRACE: Cancer Research UK Grants C1287/A10118 and C1287/A11990. D.G.E. and F.L. are supported by an NIHR grant to the Biomedical Research Centre, Manchester. The Investigators at The Institute of Cancer Research and The Royal Marsden NHS Foundation Trust are supported by an NIHR grant to the Biomedical Research Centre at The Institute of Cancer Research and The Royal Marsden NHS Foundation Trust. R.E. and E.B. are supported by Cancer Research UK Grant C5047/A8385. R.E. is also supported by NIHR support to the Biomedical Research Centre at The Institute of Cancer Research and The Royal Marsden NHS

Foundation Trust. FCCC: A.K.G. was in part funded by the NCI (R01 CA214545), The University of Kansas Cancer Center Support Grant (P30 CA168524), The Kansas Institute for Precision Medicine (P20 GM130423), and the Kansas Bioscience Authority Eminent Scholar Program. A.K.G. is the Chancellors Distinguished Chair in Biomedical Sciences Professorship. FPGMX: A. Vega is supported by the Spanish Health Research Foundation, Instituto de Salud Carlos III (ISCIII), partially supported by FEDER funds through Research Activity Intensification Program (contract grant numbers: INT15/00070, INT16/00154, INT17/00133), and through Centro de Investigación Biomédica en Red de Enfermedades Raras CIBERER (ACCI 2016: ER17P1AC7112/2018); Autonomous Government of Galicia (Consolidation and structuring program: IN607B), and by the Fundación Mutua Madrileña. The German Consortium for Hereditary Breast and Ovarian Cancer (GC-HBOC) is funded by the German Cancer Aid (110837, 70111850, coordinator: Rita K. Schmutzler, Cologne) and the Ministry for Innovation, Science and Research of the State of North Rhine-Westphalia (#323-8.0302.16.02-132142). GEMO: initially funded by the French National Institute of Cancer (INCA, PHRC Ile de France, grant AOR 01 082, 2001-2003, grant 2013-1-BCB-01-ICH-1), the Association “Le cancer du sein, parlons-en!” Award (2004), the Association for International Cancer Research (2008-2010), and the Foundation ARC pour la recherche sur le cancer (grant PJA 20151203365). It also received support from the Canadian Institute of Health Research for the “CIHR Team in Familial Risks of Breast Cancer” program (2008–2013), and the European commission FP7, Project «Collaborative Ovarian, breast and prostate Gene-environment Study (COGS), Large-scale integrating projects» (2009–2013). GEMO is currently supported by the INCa grant SHS-E-SP 18-015. GEORGETOWN: The Survey, Recruitment, and Biospecimen Collection Shared Resource at Georgetown University (NIH/NCI grant P30-CA051008), the Fisher Center for Hereditary Cancer and Clinical Genomics Research, and the Nina Hyde Center for Breast Cancer Research. G-FAST: Bruce Poppe is a senior clinical investigator of FWO. Mattias Van Heetvelde obtained funding from IWT. HCSC: Spanish Ministry of Health PI15/00059, PI16/01292, and CB-161200301 CIBERONC from ISCIII (Spain), partially supported by European Regional Development FEDER funds. HEBOS: Helsinki University Hospital Research Fund, the Finnish Cancer Society and the Sigrid Juselius Foundation. The HEBON study is supported by the Dutch Cancer Organisation grants NKI1998-1854, NKI2004-3088, NKI2007-3756, the Netherlands Organisation of Scientific Research grant NWO 91109024, the Pink Ribbon grants 110005 and 2014-187.WO76, the BBMRI grant NWO 184.021.007/CP46 and the Transcan grant JTC 2012 Cancer 12-054. HRBCP: Hong Kong Sanatorium and Hospital, Dr Ellen Li Charitable Foundation, The Kerry Group Kuok Foundation, National Institute of Health1R 03CA130065, and North California Cancer Center. HUNBOCS: Hungarian Research Grants KTI-OTKA CK-80745, NKFI-OTKA K-112228 and TUDFO/51757/2019-ITM. ICO: Contract grant sponsor: Supported by the Carlos III National Health Institute funded by FEDER funds—a way to build Europe—(PI16/00563, PI19/00553 and CIBERONC); the Government of Catalonia (Pla estratègic de recerca i innovació en salut (PERIS) Project MedPerCan, 2017SGR1282 and 2017SGR496); and CERCA program.IHCC: supported by grant PBZ_KBN_122/P05/2004 and the program of the Minister of Science and Higher Education under the name “Regional Initiative of Excellence” in 2019–2022 project number 002/RID/2018/19 amount of financing 12 000 000 PLN. ILUH: Icelandic Association “Walking for Breast Cancer Research” and by the Landspítali University Hospital Research Fund. INHERIT: Canadian Institutes of Health Research for the “CIHR Team in Familial Risks of Breast Cancer” program—grant CRN-87521 and the Ministry of Economic Development, Innovation and Export Trade—grant # PSR-SIIRI-701. IOVHBOCS: Ministero della Salute and “5x1000” Istituto Oncologico Veneto grant. IPOBCS: Liga Portuguesa Contra o Cancro. kConFab: The National Breast Cancer Foundation, and previously by the National Health and Medical Research Council (NHMRC), the Queensland Cancer Fund, the Cancer Councils of New South Wales, Victoria, Tasmania and South Australia, and the Cancer Foundation of Western Australia. KOHBRA: the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), and the National R&D Program for Cancer Control, Ministry of Health & Welfare, Republic of Korea (HI16C1127; 1020350; 1420190). KUMC: NIGMS P20 GM130423 (to A.K.G.). MAYO: NIH grants CA116167, CA192393 and CA176785, an NCI Specialized Program of Research Excellence (SPORE) in Breast Cancer (CA116201), and a grant from the Breast Cancer Research Foundation. MCGILL: Jewish General Hospital Weekend to End Breast Cancer, Quebec Ministry of Economic Development, Innovation and Export Trade. Marc Tischkowitz is supported by the funded by the European Union Seventh Framework Program (2007Y2013)/European Research Council (Grant No. 310018). MODSQUAD: MH CZ—DRO (MMCI, 00209805) and LM2018125, MEYS—NPS I—LO1413 to LF, and by Charles University in Prague project UNCE204024 (MZ). MSKCC: the Breast Cancer Research Foundation, the Robert and Kate Niehaus Clinical Cancer Genetics Initiative, the Andrew Sabin Research Fund and a Cancer Center Support Grant/Core Grant (P30 CA008748). NAROD: 1R01 CA149429-01. NCI: the Intramural Research Program of the US National Cancer Institute, NIH, and by support services contracts NO2-CP-11019-50, NO2-CP-21013-63 and NO2-CP-65504 with Westat, Inc, Rockville, MD. NICCC: Clalit Health Services in Israel, the Israel Cancer Association and the Breast Cancer Research Foundation (BCRF), NY. NNPIO: the Russian Foundation for Basic Research (grants 17-00-00171, 18-515-45012 and 19-515-25001). NRG

Oncology: U10 CA180868, NRG SDMC grant U10 CA180822, NRG Administrative Office and the NRG Tissue Bank (CA 27469), the NRG Statistical and Data Center (CA 37517) and the Intramural Research Program, NCI. OSUCCG: Ohio State University Comprehensive Cancer Center. PBCS: supported by the "Fondazione Pisa per la Scienza, project nr. 127/2016. Maria A Caligo was supported by the grant: "n. 127/16 Caratterizzazione delle varianti missenso nei geni BRCA1/2 per la valutazione del rischio di tumore al seno" by Fondazione Pisa, Pisa, Italy; SEABASS: Ministry of Science, Technology and Innovation, Ministry of Higher Education (UM.C/HIR/MOHE/06) and Cancer Research Initiatives Foundation. SMC: the Israeli Cancer Association. SWE-BRCA: the Swedish Cancer Society. UCHICAGO: NCI Specialized Program of Research Excellence (SPORE) in Breast Cancer (CA125183), R01 CA142996, 1U01CA161032 and by the Ralph and Marion Falk Medical Research Trust, the Entertainment Industry Fund National Women's Cancer Research Alliance and the Breast Cancer research Foundation. O.I.O. is an ACS Clinical Research Professor. UCLA: Jonsson Comprehensive Cancer Center Foundation; Breast Cancer Research Foundation. UCSF: UCSF Cancer Risk Program and Helen Diller Family Comprehensive Cancer Center. UKFOCR: Cancer Research UK. UPENN: Breast Cancer Research Foundation; Susan G. Komen Foundation for the cure, Basser Research Center for BRCA. UPITT/MWH: Hackers for Hope Pittsburgh. VFCTG: Victorian Cancer Agency, Cancer Australia, National Breast Cancer Foundation. WCP: B.Y.K. is funded by the American Cancer Society Early Detection Professorship (SIOP-06-258-01-COUN) and the National Center for Advancing Translational Sciences (NCATS), grant UL1TR000124.

ETHICS DECLARATION

All participants were recruited by the host institutions under protocols approved by local ethics review boards and provided written informed consent.²⁴

COMPETING INTERESTS

C. Isaacs is consultant to Astra Zeneca, Novartis, Pfizer, Genentech, PUMA, Seattle Genetics, and received research support from Tesaro. The other authors declare no competing interests.

Inge M. M. Lakeman^{1,2,186}, Alexandra J. van den Broek^{3,186}, Juliën A. M. Vos³, Daniel R. Barnes⁴, Julian Adlard⁵, Irene L. Andrusis^{6,7}, Adalgeir Arason^{8,9}, Norbert Arnold^{10,11}, Banu K. Arun¹², Judith Balmaña^{13,14}, Daniel Barrowdale⁴, Javier Benitez^{15,16}, Ake Borg¹⁷, Trinidad Caldés¹⁸, Maria A. Caligo¹⁹, Wendy K. Chung²⁰, Kathleen B. M. Claes²¹, GEMO Study Collaborators*, EMBRACE Collaborators*, J. Margriet Collée²², Fergus J. Couch²³, Mary B. Daly²⁴, Joe Dennis⁴, Mallika Dhawan²⁵, Susan M. Domchek²⁶, Ros Eeles²⁷, Christoph Engel²⁸, D. Gareth Evans^{29,30}, Lidia Feliubadaló³¹, Lenka Foretova³², Eitan Friedman^{33,34}, Debra Frost⁴, Patricia A. Ganz³⁵, Judy Garber³⁶, Simon A. Gayther³⁷, Anne-Marie Gerdes³⁸, Andrew K. Godwin³⁹, David E. Goldgar⁴⁰, Eric Hahnen^{41,42}, Christopher R. Hake⁴³, Ute Hamann⁴⁴, Frans B. L. Hogervorst⁴⁵, Maartje J. Hooning⁴⁶, John L. Hopper⁴⁷, Peter J. Hulick^{48,49}, Evgeny N. Imyanitov⁵⁰, OCGN Investigators*, HEBON Investigators*, KconFab Investigators*, Claudine Isaacs⁵¹, Louise Izatt⁵², Anna Jakubowska^{53,54}, Paul A. James^{55,56}, Ramunas Janavicius^{57,58}, Uffe Birk Jensen⁵⁹, Yue Jiao^{60,61,62}, Esther M. John^{63,64}, Vijai Joseph⁶⁵, Beth Y. Karlan⁶⁶, Carolien M. Kets⁴⁵, Irene Konstantopoulou⁶⁷, Ava Kwong^{68,69,70}, Clémentine Legrand⁷¹, Goska Leslie⁴, Fabienne Lesueur^{60,61,62}, Jennifer T. Loud⁷², Jan Lubiński⁵³, Siranoush Manoukian⁷³, Lesley McGuffog⁴, Austin Miller⁷⁴, Denise Molina Gomes⁷⁵, Marco Montagna⁷⁶, Emmanuelle Mouret-Fourme⁷⁷, Katherine L. Nathanson²⁶, Susan L. Neuhausen⁷⁸, Heli Nevanlinna⁷⁹, Joanne Ngeow Yuen Yie^{80,81}, Edith Olah⁸², Olufunmilayo I. Olopade⁸³, Sue K. Park^{84,85,86}, Michael T. Parsons⁸⁷, Paolo Peterlongo⁸⁸, Marion Piedmonte⁷⁴, Paolo Radice⁸⁹, Johanna Rantala⁹⁰, Gad Rennert⁹¹, Harvey A. Risch⁹², Rita K. Schmutzler^{41,42,93}, Priyanka Sharma⁹⁴, Jacques Simard⁹⁵, Christian F. Singer⁹⁶, Zsofia Stadler⁹⁷, Dominique Stoppa-Lyonnet^{77,98,99}, Christian Sutter¹⁰⁰, Yen Yen Tan¹⁰¹, Manuel R. Teixeira^{102,103}, Soo Hwang Teo^{104,105}, Alex Teulé³¹, Mads Thomassen¹⁰⁶, Darcy L. Thull¹⁰⁷, Marc Tischkowitz^{108,109}, Amanda E. Toland¹¹⁰, Nadine Tung¹¹¹, Elizabeth J. van Rensburg¹¹², Ana Vega^{113,114,115}, Barbara Wappenschmidt^{41,42}, Peter Devilee^{1,116}, Christi J. van Asperen², Jonine L. Bernstein¹¹⁷, Kenneth Offit^{65,97}, Douglas F. Easton^{4,118}, Matti A. Rookus¹¹⁹, Georgia Chenevix-Trench⁸⁷, Antonis C. Antoniou^{4,187}, Mark Robson^{97,187} and Marjanka K. Schmidt^{12,3,119,187}

¹Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands. ²Department of Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands. ³Division of Molecular Pathology, The Netherlands Cancer Institute–Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands. ⁴Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK. ⁵Yorkshire Regional Genetics Service, Chapel Allerton Hospital, Leeds, UK. ⁶Fred A. Litwin Center for Cancer Genetics, Lunenfeld-Tanenbaum Research Institute of Mount Sinai Hospital, Toronto, ON, Canada. ⁷Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada. ⁸Department of Pathology, Landspítali University Hospital, Reykjavík, Iceland. ⁹BMC (Biomedical Centre), Faculty of Medicine, University of Iceland, Reykjavík, Iceland. ¹⁰Department of Gynaecology and Obstetrics, University Hospital of Schleswig-Holstein, Campus Kiel, Christian-Albrechts University Kiel, Kiel, Germany. ¹¹Institute of Clinical Molecular Biology, University Hospital of Schleswig-Holstein, Campus Kiel, Christian-Albrechts University Kiel, Kiel, Germany. ¹²Department of Breast Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, TX, USA. ¹³Hereditary cancer Genetics Group, Vall d'Hebron Institute of Oncology, Barcelona, Spain. ¹⁴Department of Medical Oncology, Vall d'Hebron Barcelona Hospital Campus, University Hospital of Vall d'Hebron, Barcelona, Spain. ¹⁵Centro de Investigación en Red de Enfermedades Raras (CIBERER), Madrid, Spain. ¹⁶Human Cancer Genetics Programme, Spanish National Cancer Research Centre (CNIO), Madrid, Spain. ¹⁷Department of Oncology, Lund University and Skåne University Hospital, Lund, Sweden. ¹⁸Molecular Oncology Laboratory, CIBERONC, Hospital Clínico San Carlos, IdISSC (Instituto de Investigación Sanitaria del Hospital Clínico San Carlos), Madrid, Spain. ¹⁹SOD Genetica Molecolare, University Hospital, Pisa, Italy. ²⁰Departments of Pediatrics and Medicine, Columbia University, New York, NY, USA. ²¹Centre for Medical Genetics, Ghent University, Ghent, Belgium. ²²Department of Clinical Genetics, Erasmus University Medical Center, CA, Rotterdam, The Netherlands. ²³Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA. ²⁴Department of Clinical Genetics, Fox Chase Cancer Center, Philadelphia, PA, USA. ²⁵Cancer Genetics and Prevention Program, University of California San Francisco, San Francisco, CA, USA. ²⁶Basser Center for BRCA, Abramson Cancer

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41436-021-01198-7>.

Correspondence and requests for materials should be addressed to M.K.S.

Reprints and permission information is available at <http://www.nature.com/reprints>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2021

Center, University of Pennsylvania, Philadelphia, PA, USA. ²⁷Oncogenetics Team, The Institute of Cancer Research and Royal Marsden NHS Foundation Trust, London, UK. ²⁸Institute for Medical Informatics, Statistics and Epidemiology, University of Leipzig, Leipzig, Germany. ²⁹Division of Evolution and Genomic Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester Academic Health Science Centre, Manchester, UK. ³⁰North West Genomics Laboratory Hub, Manchester Centre for Genomic Medicine, St Mary's Hospital, Manchester University NHS Foundation Trust, Manchester Academic Health Science Centre, Manchester, UK. ³¹Hereditary Cancer Program, ONCOBELL-IDIBELL-IGTP, Catalan Institute of Oncology, CIBERONC, Barcelona, Spain. ³²Department of Cancer Epidemiology and Genetics, Masaryk Memorial Cancer Institute, Brno, Czech Republic. ³³The Susanne Levy Gertner Oncogenetics Unit, Chaim Sheba Medical Center, Ramat Gan, Israel. ³⁴Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel. ³⁵Schools of Medicine and Public Health, Division of Cancer Prevention & Control Research, Jonsson Comprehensive Cancer Center, UCLA, Los Angeles, CA, USA. ³⁶Cancer Risk and Prevention Clinic, Dana-Farber Cancer Institute, Boston, MA, USA. ³⁷Center for Bioinformatics and Functional Genomics and the Cedars Sinai Genomics Core, Cedars-Sinai Medical Center, Los Angeles, CA, USA. ³⁸Department of Clinical Genetics, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark. ³⁹Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, Kansas City, KS, USA. ⁴⁰Department of Dermatology, Huntsman Cancer Institute, University of Utah School of Medicine, Salt Lake City, UT, USA. ⁴¹Center for Familial Breast and Ovarian Cancer, Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany. ⁴²Center for Integrated Oncology (CIO), Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany. ⁴³Waukesha Memorial Hospital-Pro Health Care, Waukesha, WI, USA. ⁴⁴Molecular Genetics of Breast Cancer, German Cancer Research Center (DKFZ), Heidelberg, Germany. ⁴⁵Family Cancer Clinic, The Netherlands Cancer Institute–Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands. ⁴⁶Department of Medical Oncology, Erasmus MC Cancer Institute, Rotterdam, The Netherlands. ⁴⁷Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, VIC, Australia. ⁴⁸Center for Medical Genetics, NorthShore University HealthSystem, Evanston, IL, USA. ⁴⁹The University of Chicago Pritzker School of Medicine, Chicago, IL, USA. ⁵⁰N.N. Petrov Institute of Oncology, St. Petersburg, Russia. ⁵¹Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC, USA. ⁵²Clinical Genetics, Guy's and St Thomas' NHS Foundation Trust, London, UK. ⁵³Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland. ⁵⁴Independent Laboratory of Molecular Biology and Genetic Diagnostics, Pomeranian Medical University, Szczecin, Poland. ⁵⁵Parkville Familial Cancer Centre, Peter MacCallum Cancer Center, Melbourne, VIC, Australia. ⁵⁶Sir Peter MacCallum Department of Oncology, The University of Melbourne, Melbourne, VIC, Australia. ⁵⁷Hematology, Oncology and Transfusion Medicine Center, Department of Molecular and Regenerative Medicine, Vilnius University Hospital Santariskiu Clinics, Vilnius, Lithuania. ⁵⁸State Research Institute Centre for Innovative Medicine, Vilnius, Lithuania. ⁵⁹Department of Clinical Genetics, Aarhus University Hospital, Aarhus, Denmark. ⁶⁰Genetic Epidemiology of Cancer team, Paris, France. ⁶¹Institut Curie, Paris, France. ⁶²Mines ParisTech, Fontainebleau, France. ⁶³Department of Epidemiology & Population Health, Stanford University School of Medicine, Stanford, CA, USA. ⁶⁴Department of Medicine, Division of Oncology, Stanford Cancer Institute, Stanford University School of Medicine, Stanford, CA, USA. ⁶⁵Clinical Genetics Research Lab, Department of Cancer Biology and Genetics, Memorial Sloan Kettering Cancer Center, New York, NY, USA. ⁶⁶David Geffen School of Medicine, Department of Obstetrics and Gynecology, University of California at Los Angeles, Los Angeles, CA, USA. ⁶⁷Molecular Diagnostics Laboratory, INRASTES, National Centre for Scientific Research 'Demokritos', Athens, Greece. ⁶⁸Hong Kong Hereditary Breast Cancer Family Registry, Cancer Genetics Centre, Happy Valley, Hong Kong. ⁶⁹Department of Surgery, The University of Hong Kong, Pok Fu Lam, Hong Kong. ⁷⁰Department of Surgery, Hong Kong Sanatorium and Hospital, Happy Valley, Hong Kong. ⁷¹Département de Génétique, CHU de Grenoble, Grenoble, France. ⁷²Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA. ⁷³Unit of Medical Genetics, Department of Medical Oncology and Hematology, Fondazione IRCCS Istituto Nazionale dei Tumori di Milano, Milan, Italy. ⁷⁴NRG Oncology, Statistics and Data Management Center, Roswell Park Cancer Institute, Buffalo, NY, USA. ⁷⁵Service de Biologie de la reproduction, Cytogénétique et Génétique Médicale, CHI Poissy–Saint Germain, Poissy, France. ⁷⁶Immunology and Molecular Oncology Unit, Veneto Institute of Oncology IOV–IRCCS, Padua, Italy. ⁷⁷Service de Génétique, Institut Curie, Paris, France. ⁷⁸Department of Population Sciences, Beckman Research Institute of City of Hope, Duarte, CA, USA. ⁷⁹Department of Obstetrics and Gynecology, Helsinki University Hospital, University of Helsinki, Helsinki, Finland. ⁸⁰Cancer Genetics Service, National Cancer Centre, Singapore, Singapore. ⁸¹Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore, Singapore. ⁸²Department of Molecular Genetics, National Institute of Oncology, Budapest, Hungary. ⁸³Center for Clinical Cancer Genetics, The University of Chicago, Chicago, IL, USA. ⁸⁴Department of Preventive Medicine, Seoul National University College of Medicine, Seoul, Korea. ⁸⁵Department of Biomedical Sciences, Seoul National University Graduate School, Seoul, Korea. ⁸⁶Cancer Research Institute, Seoul National University, Seoul, Korea. ⁸⁷Department of Genetics and Computational Biology, QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia. ⁸⁸Genome Diagnostics Program, IFOM–the FIRC Institute of Molecular Oncology, Milan, Italy. ⁸⁹Unit of Molecular Bases of Genetic Risk and Genetic Testing, Department of Research, Fondazione IRCCS Istituto Nazionale dei Tumori (INT), Milan, Italy. ⁹⁰Clinical Genetics, Karolinska Institutet, Stockholm, Sweden. ⁹¹Calit National Cancer Control Center, Carmel Medical Center and Technion Faculty of Medicine, Haifa, Israel. ⁹²Chronic Disease Epidemiology, Yale School of Public Health, New Haven, CT, USA. ⁹³Center for Molecular Medicine Cologne (CMMC), Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany. ⁹⁴Department of Internal Medicine, Division of Medical Oncology, University of Kansas Medical Center, Westwood, KS, USA. ⁹⁵Genomics Center, Centre Hospitalier Universitaire de Québec–Université Laval Research Center, Québec City, QC, Canada. ⁹⁶Dept of OB/GYN and Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria. ⁹⁷Clinical Genetics Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA. ⁹⁸Department of Tumour Biology, INSERM U830, Paris, France. ⁹⁹Université Paris Descartes, Paris, France. ¹⁰⁰Institute of Human Genetics, University Hospital Heidelberg, Heidelberg, Germany. ¹⁰¹Dept of OB/GYN, Medical University of Vienna, Vienna, Austria. ¹⁰²Department of Genetics, Portuguese Oncology Institute, Porto, Portugal. ¹⁰³Biomedical Sciences Institute (ICBAS), University of Porto, Porto, Portugal. ¹⁰⁴Breast Cancer Research Programme, Cancer Research Malaysia, Subang Jaya, Selangor, Malaysia. ¹⁰⁵Department of Surgery, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia. ¹⁰⁶Department of Clinical Genetics, Odense University Hospital, Odense C, Denmark. ¹⁰⁷Department of Medicine, Magee-Womens Hospital, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. ¹⁰⁸Program in Cancer Genetics, Departments of Human Genetics and Oncology, McGill University, Montréal, QC, Canada. ¹⁰⁹Department of Medical Genetics, National Institute for Health Research Cambridge Biomedical Research Centre, University of Cambridge. Vol Box 134, Level 6 Addenbrooke's Treatment Centre, Addenbrooke's Hospital, Cambridge, UK. ¹¹⁰Department of Cancer Biology and Genetics, The Ohio State University, Columbus, OH, USA. ¹¹¹Department of Medical Oncology, Beth Israel Deaconess Medical Center, Boston, MA, USA. ¹¹²Department of Genetics, University of Pretoria, Arcadia, South Africa. ¹¹³Centro de Investigación en Red de Enfermedades Raras (CIBERER), Madrid, Spain. ¹¹⁴Fundación Pública Galega de Medicina Xenómica, Santiago de Compostela, Spain. ¹¹⁵Instituto de Investigación Sanitaria de Santiago de Compostela (IDIS), Complejo Hospitalario Universitario de Santiago, SERGAS, Santiago de Compostela, Spain. ¹¹⁶Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands. ¹¹⁷Department of Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, NY, USA. ¹¹⁸Centre for Cancer Genetic Epidemiology, Department of Oncology, University of Cambridge, Cambridge, UK. ¹¹⁹Division of Psychosocial Research and Epidemiology, The Netherlands Cancer Institute–Antoni van Leeuwenhoek hospital, Amsterdam, The Netherlands. ¹²⁰These authors contributed equally: Inge M. M. Lakeman, Alexandra J. van den Broek. ¹²¹Shared last authors: Antonis C. Antoniou, Mark Robson, Marjanka K. Schmidt. *Lists of authors and their affiliations appears at the end of the paper. ¹²²email: mk.schmidt@nki.nl

GEMO STUDY COLLABORATORS

Yue Jiao^{60,61,62}, Clémentine Legrand⁷¹, Fabienne Lesueur^{60,61,62}, Denise Molina Gomes⁷⁵, Emmanuelle Mouret-Fourme⁷⁷, Dominique Stoppa-Lyonnet^{77,98,99}, Emmanuelle Barouk-Simonet¹⁴⁷, Muriel Belotti⁷⁷, Pascaline Berthet¹⁴⁸, Yves-Jean Bignon¹⁴⁹, Valérie Bonadona¹⁵⁰, Brigitte Bressac-de Paillerets¹⁵¹, Bruno Buecher⁷⁷, Sandrine Caputo^{77,152}, Olivier Caron¹⁵¹, Laurent Castera¹⁴⁸, Virginie Caux-Moncoutier⁷⁷, Chrystelle Colas⁷⁷, Marie-Agnès Collonge-Rame¹⁵³, Isabelle Coupier¹⁵⁴, Antoine de Pauw⁷⁷, Capucine Delnatte¹⁵⁵, Camille Elan⁷⁷, Laurence Faivre^{156,157}, Sandra Fert Ferrer¹⁵⁸, Marion Gauthier-Villars⁷⁷, Paul Gesta¹⁵⁹, Sophie Giraud¹⁶⁰, Lisa Golmard⁷⁷, Claude Houdayer¹⁶¹, Christine Lasset¹⁵⁰, Maïté Laurent⁷⁷, Dominique Leroux⁷¹, Michel Longy¹⁴⁷, Véronique Mari¹⁶², Sylvie Mazoyer¹⁶³, Noura Mebirouk^{60,61,62}, Isabelle Mortemousque¹⁶⁴, Fabienne Prieur¹⁶⁵, Pascal Pujol¹⁵⁴, Claire Saule⁷⁷, Helene Schuster¹⁶⁶, Nicolas Sevenet¹⁴⁷, Hagay Sobol^{167,168}, Johanna Sokolowska¹⁶⁹ and Laurence Venat-Bouvet¹⁷⁰

EMBRACE COLLABORATORS

Julian Adlard⁵, Daniel Barrowdale⁴, Ros Eeles²⁷, D. Gareth Evans^{29,30}, Debra Frost⁴, Louise Izatt⁵², Marc Tischkowitz^{108,109}, Douglas F. Easton^{4,118}, Munaza Ahmed¹²², Julian Barwell¹²³, Angela Brady¹²⁴, Paul Brennan¹²⁵, Carole Brewer¹²⁶, Jackie Cook¹²⁷, Rosemarie Davidson¹²⁸, Alan Donaldson¹²⁹, Alison M. Dunning¹¹⁸, Jacqueline Eason¹³⁰, Diana M. Eccles¹³¹, Helen Gregory¹³², Helen Hanson¹³³, Patricia A. Harrington¹¹⁸, Alex Henderson¹³⁴, Shirley Hodgson¹³⁵, M. John Kennedy¹³⁶, Fiona Laloo¹³⁷, Clare Miller¹³⁸, Patrick J. Morrison¹³⁹, Kai-ren Ong¹⁴⁰, Aoife O'Shaughnessy-Kirwan¹⁴¹, Jo Perkins⁴, Mary E. Porteous¹⁴², Mark T. Rogers¹⁴³, Lucy E. Side¹⁴⁴, Katie Snape¹⁴⁵ and Lisa Walker¹⁴⁶

OCGN INVESTIGATORS

Irene L. Andrulis^{6,7}, Gord Glendon⁶ and Anna Marie Mulligan^{184,185}

HEBON INVESTIGATORS

J. Margriet Collée²², Frans B. L. Hogervorst⁴⁵, Maartje J. Hoening⁴⁶, Carolien M. Kets⁴⁵, Peter Devilee^{1,116}, Christi J. van Asperen², Matti A. Rookus¹¹⁹, Marjanka K. Schmidt^{2,3,119,187}, Cora M. Aalfs¹⁷¹, Muriel A. Adank⁴⁵, Margreet G. E. M. Ausems¹⁷², Marinus J. Blok¹⁷³, Encarna B. Gómez García¹⁷³, Bernadette A. M. Heemskerk-Gerritsen⁴⁶, Antoinette Hollestelle⁴⁶, Agnes Jager⁴⁶, Linetta B. Koppert¹⁷⁴, Marco Koudijs¹⁷⁵, Mieke Krieger⁴⁶, Hanne E. J. Meijers-Heijboer¹⁷¹, Arjen R. Mensenkamp¹⁷⁶, Thea M. Mooij¹⁷⁷, Jan C. Oosterwijk¹⁷⁸, Ans M. W. van den Ouweland²², Frederieke H. van der Baan¹⁷⁷, Annemieke H. van der Hout¹⁷⁸, Lizet E. van der Kolk⁴⁵, Rob B. van der Luijt¹⁷⁵, Carolien H. M. van Deurzen¹⁷⁹, Helena C. van Doorn¹⁸⁰, Klaartje van Engelen¹⁸¹, Liselotte P. van Hest¹⁸², Theo A. M. van Os¹⁷¹, Senno Verhoef⁴⁵, Maartje J. Vogel⁴⁵ and Juul T. Wijnen¹⁸³

KCONFAB INVESTIGATORS

Georgia Chenevix-Trench⁸⁷, Jonathan Beesley⁸⁷, Stephen Fox¹²⁰, Helene Holland⁸⁷, Kelly-Anne Phillips^{47,56,120,121} and Amanda B. Spurdle⁸⁷

¹²⁰Peter MacCallum Cancer Center, Melbourne, Victoria, Australia. ¹²¹Department of Medicine, St Vincent's Hospital, The University of Melbourne, Fitzroy, VIC, Australia. ¹²²North East Thames Regional Genetics Service, Great Ormond Street Hospital for Children NHS Trust, London, UK. ¹²³Leicestershire Clinical Genetics Service, University Hospitals of Leicester NHS Trust, Leicester, UK. ¹²⁴North West Thames Regional Genetics Service, Kennedy Galton Centre, The North West London Hospitals NHS Trust, Middlesex, UK. ¹²⁵Breast Department, KK Women's and Children's Hospital, Singapore, Singapore. ¹²⁶Department of Clinical Genetics, Royal Devon & Exeter Hospital, Exeter, UK. ¹²⁷Sheffield Clinical Genetics Service, Sheffield Children's Hospital, Sheffield, UK. ¹²⁸Department of Clinical Genetics, South Glasgow University Hospitals, Glasgow, UK. ¹²⁹Clinical Genetics Department, St Michael's Hospital, Bristol, UK. ¹³⁰Nottingham Clinical Genetics Service, Nottingham University Hospitals NHS Trust, Nottingham, UK. ¹³¹Faculty of Medicine, University of Southampton, Southampton, UK. ¹³²North of Scotland Regional Genetics Service, NHS Grampian & University of Aberdeen, Foresterhill, Aberdeen, UK. ¹³³Southwest Thames Regional Genetics Service, St George's Hospital, London, UK. ¹³⁴Institute of Genetic Medicine, Centre for Life, Newcastle Upon Tyne Hospitals NHS Trust, Newcastle upon Tyne, UK. ¹³⁵Department of Clinical Genetics, St George's, University of London, London, UK. ¹³⁶Academic Unit of Clinical and Molecular Oncology, Trinity College Dublin and St James's Hospital, Dublin, Ireland. ¹³⁷Department of Population Health Sciences, Weill Cornell Medicine, New York, NY, USA. ¹³⁸Department of Clinical Genetics, Alder Hey Hospital, Liverpool, UK. ¹³⁹Northern Ireland Regional Genetics Centre, Belfast City Hospital, Belfast, UK. ¹⁴⁰West Midlands Regional Genetics Service, Birmingham Women's Hospital Healthcare NHS Trust, Birmingham, UK. ¹⁴¹SingHealth Duke-NUS Breast Centre, Singapore, Singapore. ¹⁴²South East of Scotland Regional Genetics Service, Western General Hospital, Edinburgh, UK. ¹⁴³All Wales Medical Genetics Services, University Hospital of Wales, Cardiff, UK. ¹⁴⁴Princess Anne Hospital, Southampton, UK. ¹⁴⁵Medical Genetics Unit, St George's, University of London, London, UK. ¹⁴⁶Oxford Regional Genetics Service, Churchill Hospital, Oxford, UK. ¹⁴⁷Oncogénétique, Institut Bergonié, Bordeaux, France. ¹⁴⁸Département de Biopathologie, Centre François Baclesse, Caen, France. ¹⁴⁹Laboratoire d'Oncologie moléculaire, Centre de Lutte Contre le Cancer, Centre Jean Perrin, Clermont-Ferrand, France. ¹⁵⁰Unité de Prévention et d'Epidémiologie Génétique, Centre Léon Bérard, Lyon, France. ¹⁵¹Département de médecine-Oncogénétique, Gustave Roussy, Villejuif, France. ¹⁵²Paris Sciences Lettres Research University, Paris, France. ¹⁵³Service de Génétique Biologique, CHRU de Besançon, Besançon, France. ¹⁵⁴Unité d'Oncogénétique, CHU Arnaud de Villeneuve, Montpellier, France. ¹⁵⁵Oncogénétique. Institut de Cancérologie de l'Ouest site René Gauducheau, Saint Herblain, France. ¹⁵⁶Unité d'oncogénétique, Centre de Lutte Contre le Cancer, Centre Georges-François Leclerc, Dijon, France. ¹⁵⁷Centre de Génétique. CHU Dijon, Dijon, France. ¹⁵⁸Laboratoire de Génétique Chromosomique, Hôtel Dieu Centre Hospitalier, Chambéry, France. ¹⁵⁹Service Régional Oncogénétique Poitou-Charentes, CH Niort, Niort, France. ¹⁶⁰Service de Génétique, Groupement Hospitalier Est, Hospices Civils de Lyon, Bron, France. ¹⁶¹Department of Genetics, F76000 and Normandy University, UNIROUEN, Inserm U1245, Normandy Centre for Genomic and Personalized Medicine, Rouen University Hospital, Rouen, France. ¹⁶²Département d'Hématologie-Oncologie Médicale, Centre Antoine Lacassagne, Nice, France. ¹⁶³Lyon Neuroscience Research Center-CRNL, INSERM U1028, CNRS UMR5292, University of Lyon, Lyon, France. ¹⁶⁴Service de Génétique, Hôpital Bretonneau-CHRU, Tours, France. ¹⁶⁵Service de Génétique Clinique Chromosomique et Moléculaire, Hôpital Nord, CHU Saint Etienne, St Etienne, France. ¹⁶⁶Unité d'Oncogénétique, Centre Paul Strauss, Strasbourg, France. ¹⁶⁷Département Oncologie Génétique, Prévention et Dépistage, Institut Paoli-Calmettes, Marseille, France. ¹⁶⁸Marseille Medical School, Aix-Marseille University, Marseille, France. ¹⁶⁹Laboratoire de génétique médicale, Nancy Université, Centre Hospitalier Régional et Universitaire, Vandœuvre-les-Nancy, France. ¹⁷⁰Department of Medical Oncology, CHU Dupuytren, Limoges, France. ¹⁷¹Department of Clinical Genetics, Amsterdam UMC, location AMC, Amsterdam, The Netherlands. ¹⁷²Division Laboratories, Pharmacy and Biomedical Genetics, Department of Genetics, University Medical Center Utrecht, Utrecht, The Netherlands. ¹⁷³Department of Clinical Genetics, Maastricht University Medical Center, Maastricht, The Netherlands. ¹⁷⁴Department of Surgical Oncology, Family Cancer Clinic, Erasmus MC Cancer Institute, Rotterdam, The Netherlands. ¹⁷⁵Department of Medical Genetics, University Medical Center, Utrecht, The Netherlands. ¹⁷⁶Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands. ¹⁷⁷Department of Epidemiology, The Netherlands Cancer Institute, Amsterdam, The Netherlands. ¹⁷⁸Department of Genetics, University Medical Center Groningen, University Groningen, Groningen, The Netherlands. ¹⁷⁹Department of Pathology, Erasmus University Medical Center, Rotterdam, The Netherlands. ¹⁸⁰Department of Gynaecology, Family Cancer Clinic, Erasmus MC Cancer Institute, Rotterdam, The Netherlands. ¹⁸¹Department of Clinical Genetics, VU University Medical Center, Amsterdam, The Netherlands. ¹⁸²Clinical Genetics. Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands. ¹⁸³Department of Human Genetics and Department of Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands. ¹⁸⁴Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada. ¹⁸⁵Laboratory Medicine Program, University Health Network, Toronto, ON, Canada.