Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Association of the *CHEK2* c.1100delC variant, radiotherapy, and systemic treatment with contralateral breast cancer risk and breast cancer-specific survival

Anna Morra
The Netherlands Cancer Institute
Maartje A. C. Schreurs
Erasmus MC Cancer Institute
Irene L. Andrulis
Lunenfeld-Tanenbaum Research Institute of Mount Sinai Hospital
Hoda Anton-Culver
University of California Irvine
Annelie Augustinsson
Lund University
Matthias W. Beckmann
University Hospital Erlangen
Sabine Behrens
German Cancer Research Center (DKFZ)
Stig E. Bojesen
Copenhagen University Hospital
Manjeet K. Bolla
University of Cambridge
Hiltrud Brauch
Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology
Annegien Broeks
The Netherlands Cancer Institute
Saundra S. Buys
University of Utah
Nicola J. Camp
University of Utah
Jose E. Castelao
Instituto de Investigación Sanitaria Galicia Sur (IISGS), Xerencia de Xestion Integrada de Vigo-SERGAS
Melissa H. Cessna
Intermountain Healthcare

Jenny Chang-Claude
German Cancer Research Center (DKFZ)
Wendy K. Chung
Columbia University
NBCS Collaborators
Sarah V. Colonna
University of Utah
Fergus J. Couch
Mayo Clinic
Angela Cox
University of Sheffield
Simon S. Cross
University of Sheffield
Kamila Czene
Karolinska Institutet
Mary B. Daly
Fox Chase Cancer Center
Joe Dennis
University of Cambridge
Peter Devilee
Leiden University Medical Center
Thilo Dörk
Hannover Medical School
Alison M. Dunning
University of Cambridge
Miriam Dwek
University of Westminster
Douglas F. Easton
University of Cambridge
Diana M. Eccles
University of Southampton
Mikael Eriksson
Karolinska Institutet
D. Gareth Evans
University of Manchester, Manchester Academic Health Science Centre
Peter A. Fasching
University Hospital Erlangen
Tanja N. Fehm
University Hospital Düsseldorf, Heinrich-Heine University Düsseldorf

Jonine D. Figueroa
The University of Edinburgh
Henrik Flyger
Copenhagen University Hospital
Marike Gabrielson
Karolinska Institutet
Manuela Gago-Dominguez
Fundación Pública Galega de Medicina Xenómica, Instituto de Investigación Sanitaria de Santiago de Compostela (IDIS), Complejo Hospitalario Universitario de Santiago, SERGAS
Montserrat García-Closas
National Cancer Institute, National Institutes of Health, Department of Health and Human Services
José A. García-Sáenz
Instituto de Investigación Sanitaria San Carlos (IdISSC), Centro Investigación Biomédica en Red de Cáncer (CIBERONC)
Jeanine Genkinger
Columbia University
Felix Grassmann
Health and Medical University
Melanie Gündert
German Cancer Research Center (DKFZ)
Eric Hahnen
Faculty of Medicine and University Hospital Cologne, University of Cologne
Christopher A. Haiman
University of Southern California
Ute Hamann
German Cancer Research Center (DKFZ)
Patricia A. Harrington
University of Cambridge
Jaana M. Hartikainen
University of Eastern Finland
Reiner Hoppe
Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology
John L. Hopper
The University of Melbourne
Richard S. Houlston
The Institute of Cancer Research
Anthony Howell
University of Manchester
ABCTB Investigators

University of Sydney

kConFab Investigators

Peter MacCallum Cancer Center

Anna Jakubowska

Pomeranian Medical University

Wolfgang Janni

University Hospital Ulm

Helena Jernström

Lund University

Esther M. John

Stanford University School of Medicine

Nichola Johnson

The Institute of Cancer Research

Michael E. Jones

The Institute of Cancer Research

Vessela N. Kristensen

Oslo University Hospital and University of Oslo

Allison W. Kurian

Stanford Cancer Institute, Stanford University School of Medicine

Diether Lambrechts

KU Leuven

Loic Le Marchand

University of Hawaii Cancer Center

Annika Lindblom

Karolinska Institutet

Jan Lubiński

Pomeranian Medical University

Michael P. Lux

University Hospital Erlangen

Arto Mannermaa

University of Eastern Finland

Dimitrios Mavroudis

University Hospital of Heraklion

Anna Marie Mulligan

University of Toronto

Taru A. Muranen

University of Helsinki

Heli Nevanlinna

University of Helsinki

Ines Nevelsteen
Leuven Cancer Institute, University Hospitals Leuven
Patrick Neven
Leuven Cancer Institute, University Hospitals Leuven
William G. Newman
University of Manchester, Manchester Academic Health Science Centre
Nadia Obi
University Medical Center Hamburg-Eppendorf
Kenneth Offit
Memorial Sloan Kettering Cancer Center
Andrew F. Olshan
University of North Carolina at Chapel Hill
Tjoung-Won Park-Simon
Hannover Medical School
Alpa V. Patel
American Cancer Society
Paolo Peterlongo
IFOM ETS - The AIRC Institute of Molecular Oncology
Kelly-Anne Phillips
Peter MacCallum Cancer Centre
Dijana Plaseska-Karanfilska
MASA
Eric C. Polley
Mayo Clinic
Nadege Presneau
University of Westminster
Katri Pylkäs
University of Oulu
Brigitte Rack
University Hospital Ulm
Paolo Radice
Fondazione IRCCS Istituto Nazionale dei Tumori (INT)
Muhammad U. Rashid
German Cancer Research Center (DKFZ)
Valerie Rhenius
University of Cambridge
Mark Robson
Memorial Sloan Kettering Cancer Center
Atocha Romero

Hospital Universitario Puerta de Hierro

Emmanouil Saloustros

University Hospital of Larissa

Elinor J. Sawyer

King's College London

Rita K. Schmutzler

Faculty of Medicine and University Hospital Cologne, University of Cologne

Sabine Schuetze

University Hospital Ulm

Christopher Scott

Mayo Clinic

Mitul Shah

University of Cambridge

Snezhana Smichkoska

Ss. Cyril and Methodius University in Skopje, Medical Faculty, University Clinic of Radiotherapy and Oncology

Melissa C. Southey

Monash University

William J. Tapper

University of Southampton

Lauren R. Teras

American Cancer Society

Rob A.E.M. Tollenaar

Leiden University Medical Center

Katarzyna Tomczyk

The Institute of Cancer Research

Ian Tomlinson

The University of Edinburgh

Melissa A. Troester

University of North Carolina at Chapel Hill

Celine M. Vachon

Mayo Clinic

Elke M. van Veen

University of Manchester, Manchester Academic Health Science Centre

Qin Wang

University of Cambridge

Camilla Wendt

Karolinska Institutet

Hans Wildiers

Leuven Cancer Institute, University Hospitals Leuven
Robert Winqvist
University of Oulu
Argyrios Ziogas
University of California Irvine
Per Hall
Karolinska Institutet
Paul D.P. Pharoah
University of Cambridge
Muriel A. Adank
The Netherlands Cancer Institute - Antoni van Leeuwenhoek hospital
Antoinette Hollestelle
Erasmus MC Cancer Institute
Marjanka K. Schmidt
The Netherlands Cancer Institute - Antoni van Leeuwenhoek hospital
Maartje J. Hooning (m.hooning@erasmusmc.nl)
Erasmus MC Cancer Institute

Research Article

Keywords: CHEK2 c.1100delC germline genetic variant, radiotherapy, systemic treatment, contralateral breast cancer risk, survival

Posted Date: February 13th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2569372/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Breast cancer (BC) patients with a germline *CHEK2* c.1100delC variant have an increased risk of contralateral BC (CBC) and worse BC-specific survival (BCSS) compared to non-carriers. We aimed to assess the associations of *CHEK2* c.1100delC, radiotherapy, and systemic treatment with CBC risk and BCSS.

Analyses were based on 82,701 women diagnosed with invasive BC including 963 *CHEK2* c.1100delC carriers; median follow-up was 9.1 years. Differential associations of treatment by *CHEK2* c.1100delC status were tested by including interaction terms in a multivariable Cox regression model. A multi-state model was used for further insight into the relation between *CHEK2* c.1100delC status, treatment, CBC risk and death.

There was no evidence for differential associations of therapy with CBC risk by *CHEK2* c.1100delC status The strongest association with reduced CBC risk was observed for the combination of chemotherapy and endocrine therapy [HR(95%CI): 0.66 (0.55–0.78)]. No association was observed with radiotherapy. Results from the multi-state model showed shorter BCSS for *CHEK2* c.1100delC carriers versus non-carriers also after accounting for CBC occurrence [HR(95%CI) :1.30 (1.09–1.56)].

In conclusion, systemic therapy was associated with reduced CBC risk irrespective of *CHEK2* c.1100delC status. Moreover, *CHEK2* c.1100delC carriers had shorter BCSS, which appears not to be fully explained by their CBC risk.

(Main MS: 3201 words)

Novelty And Impact

Based on 82,701 breast cancer patients, including 963 *CHEK2* c.1100delC carriers we found no differential association of radiotherapy or systemic treatment with contralateral breast cancer risk by *CHEK2* c.1100delC status. We observed a shorter breast cancer-specific survival for *CHEK2* c.1100delC carriers, after accounting for contralateral breast cancer occurrence and characteristics of the first breast cancer. This work may inform decisions concerning the harms and benefits of treatment of *CHEK2* c.1100delC carriers in clinical practice.

Introduction

Breast cancer (BC) has the highest incidence in women worldwide [1]. One of the germline variants that confer a moderate increased BC risk is the *CHEK2* c.1100delC variant [2–4], which is found in approximately 0.7% of the Northern and Western European populations [5]. Overall, carriers of this variant are diagnosed at a younger age than non-carriers [4] and the majority develops BCs that are estrogen receptor (ER)- and progesterone receptor (PR)-positive and human epidermal growth factor receptor 2 (HER2)-negative [3, 6]. Although this BC subtype has the most favorable prognosis in the general BC

population [7], *CHEK2* c.1100delC carriers have a higher risk of developing contralateral breast cancer (CBC) and worse survival [3, 4, 6, 8, 9] compared to non-carriers.

Reasons behind these differences are still unclear. A possible explanation is that *CHEK2* c.1100delC carriers have a different response to treatment compared to non-carriers, e.g., their normal tissue might experience more harm from radiotherapy. *CHEK2* c.1100delC carriers have a functional deficiency in checkpoint kinase 2 (CHK2), a kinase that controls phosphorylation of downstream factors, such as BRCA1 and BRCA2 [10]. This leads to a reduced BRCA1/2 function, impaired DNA repair and increased risk of BC [11]. A recent study showed that the *CHEK2* c.1100delC variant also disrupts the apoptosis of BC cells, causing unchecked proliferation and contributing to a poorer prognosis [12]. Radiotherapy has been shown to increase the risk of CBC in the general BC population, especially in younger patients [13]. Treatment with radiotherapy causes DNA strand breaks, which are less likely to be repaired in *CHEK2* c.1100delC carriers [14]. While this might be beneficial for the treatment of the first primary cancer, which is likely to have lost both functional CHEK2 alleles, and cannot repair DNA strand breaks at all, carriers might be more prone to developing a CBC [15]. One case-only study showed a non-significant increased risk for developing CBC after treatment with radiotherapy in *CHEK2* c.1100delC carriers versus non-carriers but due to the small study size the effects in the younger population could not be investigated [16]. Only one other small study reported on the association between radiotherapy and CBC risk by *CHEK2* c.1100delC status [8].

On the other hand, less is known about whether the effects of systemic therapy on CBC risk and survival differ by CHEK2 c.1100delC status. A population-based study showed a significant decrease in CBC risk following chemotherapy and endocrine therapy in general BC [17]. One single-hospital study also found a decreased risk of CBC after chemotherapy use in *CHEK2* c.1100delC carriers, and did not find evidence for a differential association by *CHEK2* c.1100delC status [18]. This study also found no evidence for a differential impact of chemotherapy on survival [18].

Given this uncertainty, our aim was to assess, within a large international cohort, potential differential associations of treatment given for the first primary BC (i.e. radiotherapy, chemotherapy and endocrine therapy) by *CHEK2* c.1100delC status with CBC risk, and to investigate whether the worse breast cancer-specific survival (BCSS) so far reported in carriers is explained solely by the increased CBC risk.

Materials And Methods

Study population

We used data from the Breast Cancer Association Consortium (BCAC), selected women of European ancestry, diagnosed with a first primary invasive BC between 1980 and 2018; exclusion criteria are shown in Fig. 1. The main analyses were based on 82,701 BC patients from 58 BCAC studies (Table S1). All

individual studies were approved by the appropriate institutional review boards and/or medical ethical committees. Written informed consent was obtained from all study participants.

Previous analyses investigating the relationship between *CHEK2* c.1100delC status, risk of CBC, and mortality have been based on a subset of patients genotyped with Taqman [3, 4]. In particular, the current study includes most carriers from the Weischer et al. study (n = 459) [4] and from the Kriege et al. study (n = 193) [18], but is based on a larger number of BC patients and includes updated follow-up data.

Data collection

Data included information about *CHEK2* c.1100delC status, vital status at last follow-up, CBC occurrence, age and year of diagnosis of the first primary BC, tumor characteristics of the first primary BC and CBC, as well as treatment given for the first primary BC (Tables 1 and 2). In particular, all relevant clinical-pathological and treatment information, as well as outcome information, was collected by individual studies and harmonized by the BCAC Survival, Pathology and Treatment Working Group at the Netherlands Cancer Institute, Amsterdam, the Netherlands, in collaboration with the individual studies before incorporation into the BCAC database (version 13, May 2021). *CHEK2* c.1100delC status was obtained from five different sources: BRIDGES sequencing data [19], Taqman and iPLEX genotyping [3, 4, 20], and imputed genotypes from OncoArray [21] or iCOGS [22] as described in the Supplementary Methods.

Table 1
Clinical, tumor and treatment characteristics for the first primary BC by *CHEK2* c.1100delC carrier status.

Characteristics	Non-carriers	*CHEK2* c.1100delC carriers	P-value
Number of patients, n	81,738	963	
Number of patients diagnosed with CBC, n (%)	1,757 (2.1)	59 (6.1)	
Number of patients diagnosed with ipsilateral BC, n (%)*	517 (0.6)	6 (0.6)	
Total FU time, years (IQR)	9.2 (5.3–13.6)	9.6 (5.5–13.9)	
Clinical risk factors			
Age at diagnosis, y, median (IQR)	56 (47–64)	52 (44–61)	< 0.001
Age at diagnosis, n (%)			< 0.001
< 40 years	9,471 (11.6)	171 (17.8)	
40–50 years	19,978 (24.4)	277 (28.8)	
50–60 years	23,044 (28.2)	266 (27.6)	
> 60 years	29,245 (35.8)	249 (25.9)	
Year of diagnosis, n (%)			< 0.001
1980–1989	2,259 (2.8)	48 (5.1)	
1990–1999	20,055 (24.8)	297 (31.3)	
2000–2009	45,910 (56.7)	492 (51.8)	
≥ 2010	12,781 (15.8)	113 (11.9)	
Missing, n	733	13	
Tumor characteristics			
Tumor size, n (%)			0.01
≤ 2 cm	40,263 (63.0)	421 (58.6)	
> 2 and ≤ 5 cm	20,977 (32.8)	273 (38.0)	
> 5 cm	2,718 (4.3)	24 (3.3)	
Missing, n	17,780	245	

Percentages are only on observed, non-missing data, and may not total 100 because of rounding. * Data component not actively collected in BCAC. Abbreviations: CBC = contralateral breast cancer; CT = chemotherapy; ER = estrogen receptor; ET = endocrine therapy; PR = progesterone receptor; HER2 = human epidermal growth factor receptor 2.

Characteristics	Non-carriers	*CHEK2* c.1100delC carriers	P-value
Lymph node status, n (%)			< 0.001
Negative	42,079 (61.4)	439 (54.8)	
Positive	26,456 (38.6)	362 (45.2)	
Missing, n	13,203	162	
Grade, n (%)			0.01
Grade 1	12,572 (19.1)	112 (15.3)	
Grade 2	31,594 (48.1)	388 (53.0)	
Grade 3	21,536 (32.8)	232 (31.7)	
Missing, n	16,036	231	
Morphology, n (%)			0.16
Ductal	52,127 (74.0)	659 (77.5)	
Lobular	10,596 (15.0)	116 (13.7)	
Medullary	619 (0.9)	3 (0.4)	
Mixed (ductal & lobular)	3,032 (4.3)	37 (4.4)	
Mucinous	895 (1.3)	7 (0.8)	
Papillary	160 (0.2)	22 (0.1)	
Tubular	908 (1.3)	1 (0.6)	
Other	2,111 (3.0)	5 (2.6)	
Missing, n	11,290	113	
ER status, n (%)			< 0.001
Negative	13,918 (20.4)	93 (11.8)	
Positive	54,481 (79.7)	694 (88.2)	
Missing, n	13,339	176	

Percentages are only on observed, non-missing data, and may not total 100 because of rounding. * Data component not actively collected in BCAC. Abbreviations: CBC = contralateral breast cancer; CT = chemotherapy; ER = estrogen receptor; ET = endocrine therapy; PR = progesterone receptor; HER2 = human epidermal growth factor receptor 2.

Characteristics	Non-carriers	*CHEK2* c.1100delC carriers	P-value
PR status, n (%)			< 0.001
Negative	19,128 (32.1)	169 (24.5)	
Positive	40,548 (68.0)	520 (75.5)	
Missing, n	22,062	274	
HER2 status, n (%)			0.55
Negative	37,395 (83.5)	418 (82.5)	
Positive	7,376 (16.5)	89 (17.6)	
Missing, n	36,967	456	
Treatment			
Surgery, n (%)			< 0.001
Breast conserving surgery	23,706 (43.3)	244 (36.3)	
Mastectomy	16,129 (29.4)	259 (38.5)	
Type unknown	15,330 (27.6)	169 (25.2)	
Missing, n	26,573	291	
Radiotherapy, n (%)			0.36
No	13,163 (26.0)	181 (27.6)	
Yes	37,479 (74.0)	474 (72.4)	
Missing, n	31,096	308	
Systemic therapy, n (%)			< 0.001
No systemic therapy	4,996 (11.2)	94 (17.0)	
CT, no ET	7,501 (16.8)	88 (15.9)	
ET, no CT	16,976 (38.1)	153 (27.7)	
Both CT and ET	15,116 (33.9)	218 (39.4)	
Missing, n	37,149	410	

Percentages are only on observed, non-missing data, and may not total 100 because of rounding. * Data component not actively collected in BCAC. Abbreviations: CBC = contralateral breast cancer; CT = chemotherapy; ER = estrogen receptor; ET = endocrine therapy; PR = progesterone receptor; HER2 = human epidermal growth factor receptor 2.

Characteristics	Non-carriers	*CHEK2* c.1100delC carriers	P-value
Trastuzumab, n (%)			0.96
No	37,466 (95.4)	478 (95.2)	
Yes	1,819 (4.6)	24 (4.8)	
Missing, n	42,453	461	

Percentages are only on observed, non-missing data, and may not total 100 because of rounding. * Data component not actively collected in BCAC. Abbreviations: CBC = contralateral breast cancer; CT = chemotherapy; ER = estrogen receptor; ET = endocrine therapy; PR = progesterone receptor; HER2 = human epidermal growth factor receptor 2.

Table 2
Contralateral breast cancer risk (hazard ratio) by treatment for first primary breast cancer and *CHEK2* c.1100delC status. Stratified by time since first primary breast cancer diagnosis.

	Total follow-up time			**< 5-year follow-up**			**> 5 years follow-up**		
No of patients	82,701			73,354			62,688		
No of CBC events	1,816			656			1,160		
	HR (95%CI)	P-value	P-int	HR (95%CI)	P-value	P-int	HR (95%CI)	P-value	P-int
CHEK2 c.1100delC status	2.37 (1.82–3.08)	< 0.001		3.08 (2.12–4.48)	< 0.001		1.93 (1.33–2.80)	< 0.001	
Radiotherapy			0.31			0.30			0.77
No radiotherapy	ref			ref			ref		
Radiotherapy	1.07 (0.94–1.21)	0.33		0.98 (0.81–1.19)	0.84		1.12 (0.96–1.31)	0.16	
Systemic therapy			0.46			0.70			0.39
No systemic therapy	ref			ref			ref		
CT, no ET	0.77 (0.62–0.96)	0.02		0.58 (0.41–0.83)	0.003		0.90 (0.70–1.15)	0.39	
ET, no CT	0.70 (0.58–0.83)	< 0.001		0.62 (0.46–0.84)	0.002		0.73 (0.59–0.91)	0.005	
Both CT and ET	0.65 (0.55–0.78)	< 0.001		0.50 (0.37–0.68)	< 0.001		0.75 (0.62–0.93)	0.007	

Adjusted for age at diagnosis, ER status, tumor size, nodal status and grade of first primary breast cancer. Abbreviations: CT = chemotherapy; ET = endocrine therapy; P-int = P-value for the comparison of a model including an interaction term between *CHEK2* c.1100delC status and a specific treatment (radiation or systemic treatment) with a model without any interaction term.

Statistical analyses

Multiple imputation, performed using R package MICE (version 3.13.0), was used to handle missing values in clinical and pathological variables. Details are given in the Supplementary Methods and Table

S2. Descriptive statistics are shown as mean ± standard deviation (SD) or median and interquartile range (IQR). We used Pearson's $\chi 2$ test for categorical data and Kruskal-Wallis test for continuous data to calculate differences in patients' characteristics. The primary study outcomes were time to CBC and BCSS (time to death due to BC).

Hazard ratios (HRs) and 95% confidence intervals (CIs) for the association of treatment given for the first primary BC (radiotherapy and/or type of systemic treatment) and *CHEK2* c.1100delC status with time to CBC were estimated via Cox regression models allowing for delayed entry, stratified by country and adjusted for age at first primary BC diagnosis, tumor size, nodal status, grade and ER status. Since ER status is known to violate the proportionality hazards assumption and because the majority of *CHEK2* c.1100delC carriers develop ER-positive BC, we performed an additional main analysis restricted to patients diagnosed with a first primary ER-positive BC. We assumed that patients with unknown CBC status did not develop a CBC during follow-up, and that for CBC cases with unknown time from first primary BC to CBC diagnosis, CBC occurrence was at last available follow-up.

Time at risk started either three months after first primary BC diagnosis or at study entry if entry was more than three months after first primary BC diagnosis, and ended at time of CBC, death or last follow-up, whichever came first. We tested for potential differential association of adjuvant and/or neo-adjuvant therapy on CBC risk according to *CHEK2* c.1100delC status by including an interaction term between treatment (radiotherapy or systemic treatment) variable and *CHEK2* c.1100delC status in the model. CBC risk analyses were stratified by two follow-up time intervals: i) the first 5 years after BC diagnosis and ii) starting 5 years after BC diagnosis.

To gain further insight into the relation between *CHEK2* c.1100delC status, treatment given for the first primary BC, CBC risk and death, we used a multi-state model in the framework of the Cox model, with diagnosis of the first primary BC as initial state, diagnosis of CBC as intermediate (transient) state, and death due to BC, death due to other causes, and death due to unknown causes as absorbing states (Fig. 2), as specified in the Supplementary Methods.

The main CBC risk and multi-state analyses were performed on imputed datasets. Complete-case analyses (excluding study subjects with missing values in any of the variables included in the models) were performed as sensitivity analyses. Additional analyses were restricted to: a) patients diagnosed with first primary BC from 2000 onwards to reduce heterogeneity in treatment regimens; b) patients diagnosed at age 40 or younger to see if the association with radiotherapy was stronger in this subgroup, as reported previously in the general BC population [13].

Results

Patients carrying the *CHEK2* c.1100delC variant were diagnosed at a younger age and earlier years. The tumors of carriers were larger at time of diagnosis, were more often lymph node-positive, grade 2, and ER- and PR-positive than in non-carriers; also some differences in treatment were observed (Table 1).

Contralateral breast cancer

CHEK2 c.1100delC carriers were diagnosed at younger age and in earlier calendar years, both for the first primary tumor as well as for the CBC. Overall, the characteristics of the CBC were similar between the non-carriers and carriers (Table S3). However, *CHEK2* c.1100delC carriers more often had positive nodes at CBC diagnosis than non-carriers (p = 0.02).

CBC risk by treatment and CHEK2 c.1100delC carrier status

There was no evidence for a differential association of *CHEK2* c.1100delC status by radiotherapy [Tables 2–3: P-value for interaction = 0.31 in all patients and P-value for interaction = 0.99 in ER-positive patients] or systemic therapy [P-value for interaction = 0.46 in all patients and P-value for interaction = 0.68 in ER-positive patients]. Moreover, we did not find an association with radiotherapy on CBC risk [HR (95%CI): 1.07 (0.94–1.21), P = 0.33 in all BC patients and 1.07 (0.92–1.25), P = 0.35 in ER-positive BC patients]. Regarding systemic therapy, we observed that chemotherapy alone [HR (95%CI): 0.77 (0.62–0.96), P = 0.02 in all BC patients and 0.73 (0.52–1.03), P = 0.07 in ER-positive BC patients], endocrine therapy alone [HR (95%CI): 0.70 (0.58–0.83), P < 0.001 in all BC patients and 0.66 (0.54–0.81), P < 0.001 in ER-positive BC patients] and the combination of both [HR (95%CI): 0.65 (0.55–0.78), P < 0.001 in all BC patients and 0.65 (0.52–0.82), P < 0.001 in ER-positive BC patients] had a protective association with CBC risk compared to women who did not receive any systemic therapy as part of their treatment.

Table 3

Contralateral breast cancer risk (hazard ratio) by treatment for first primary BC and *CHEK2* c.1100delC status in ER-positive BC patients. Stratified by time since first primary breast cancer diagnosis.

	Total follow-up time			**< 5-year follow-up**			**> 5 years follow-up**		
No of patients	55,175			51,146			41,269		
No of CBC events	1,133			427			706		
	HR (95%CI)	P-value	P-int	HR (95%CI)	P-value	P-int	HR (95%CI)	P-value	P-int
CHEK2 c.1100delC status	2.55 (1.87–3.48)	< 0.001		3.42 (2.24–5.22)	< 0.001		1.94 (1.22–3.08)	0.005	
Radiotherapy			0.99			0.47			0.41
No radiotherapy	ref			ref			ref		
Radiotherapy	1.07 (0.92–1.25)	0.35		1.04 (0.81–1.34)	0.75		1.09 (0.90–1.32)	0.36	
Systemic therapy			0.68			0.91			0.96
No systemic therapy	ref			ref			ref		
CT, no ET	0.73 (0.52–1.03)	0.07		0.62 (0.38–1.03)	0.06		0.80 (0.52–1.23)	0.31	
ET, no CT	0.66 (0.54–0.81)	< 0.001		0.55 (0.40–0.77)	< 0.001		0.73 (0.57–0.94)	0.02	
Both CT and ET	0.65 (0.52–0.82)	< 0.001		0.48 (0.34–0.69)	< 0.001		0.77 (0.58–1.03)	0.08	

Adjusted for age at diagnosis, nodal status, size category and grade of first primary breast cancer. Abbreviations: CT = chemotherapy; ET = endocrine therapy; P-int = P-value for the comparison of a model including an interaction term between *CHEK2* c.1100delC status and a specific treatment (radiotherapy or systemic treatment) with a model without any interaction term.

Results of analyses for patients diagnosed at the age of 40 years or younger or for patients diagnosed from 2000 onwards were in line with the results of the main analyses (Tables S4-S5). Complete-case analyses results were consistent with the corresponding results of the imputed data analyses (Tables S6-S9), except for the association with radiotherapy in patients diagnosed at the age of 40 years or younger. For these patients, radiotherapy was significantly associated with increased CBC risk in the complete-

case analysis with follow-up starting 5 years after diagnosis of the first primary BC [Table S7; HR (95%CI): 2.12 (1.06–4.22), P = 0.03]. In addition, interaction terms between treatments and *CHEK2* c.1100delC status could not be properly estimated in some of the complete-case analyses, due to insufficient data. These included, among others, the analysis based on all patients with follow-up starting at 5 years after BC diagnosis; the analysis restricted to patients diagnosed at the age of 40 years or younger and based on the total follow-up; and the analysis restricted to ER-positive BC with follow-up starting 5 years after BC diagnosis (Tables S10-S12).

CHEK2 c.1100delC carrier status, CBC and survival trajectories

CHEK2 c.1100delC carriers versus non-carriers had an almost 2.4 fold risk of developing a CBC [HR (95%CI): 2.37 (1.82–3.08), P < 0.001 in all patients and 2.55 (1.87–3.48), P < 0.001 in patients with an ER-positive first primary BC; Table 4] and a 1.3-fold risk of BC death after censoring for CBC occurrence [HR (95%CI): 1.30 (1.09–1.56), P = 0.003 in all patients and 1.38 (1.12–1.71), P = 0.003 in patients with an ER-positive first primary BC; Table 4]. There was no evidence for association of *CHEK2* c.1100delC carrier status with other transitions. Results from the analyses restricted to patients diagnosed with first primary BC from 2000 onwards were in line with the results from the main analyses (Table S15).

Table 4

Multi-state model in all breast cancer patients and in patients diagnosed with a first primary ER-positive breast cancer: Hazard ratio for the comparison of *CHEK2* c.1100delC carriers versus non-carriers for each transition.

Analysis	Transition	Description	HR (95% CI)	P	Cases	Events
All BC patients	1	First primary BC -> CBC	2.37 (1.82–3.08)	< 0.001	82,701	1,816
	2	First primary BC -> BC death	1.30 (1.09–1.56)	0.003		7,467
	3	First primary BC -> death not due to BC	1.00 (0.75–1.34)	0.98		4,247
	4	First primary BC -> death of unknown cause	1.07 (0.76–1.49)	0.70		3,548
	5	CBC -> BC death	1.23 (0.72–2.10)	0.46	1,816	281
	6	CBC -> death not due to BC	0.60 (0.14–2.52)	0.49		124
	7	CBC -> death of unknown cause	1.21 (0.41–3.53)	0.73		94
Patients diagnosed with primary ER-positive BC	1	First primary BC -> CBC	2.55 (1.87–3.48)	< 0.001	55,175	1,133
	2	First primary BC -> BC death	1.38 (1.12–1.71)	0.003		4,266
	3	First primary BC -> death not due to BC	1.13 (0.81–1.56)	0.47		2,817
	4	First primary BC -> death of unknown cause	0.97 (0.63–1.48)	0.87		2,090

Abbreviations: HR = hazard ratio; CI = confidence interval; BC = breast cancer; CBC = contralateral breast cancer. The models included age at first primary BC diagnosis, nodal status, tumor size, grade, radiotherapy and systemic treatment given for the first primary BC as covariates. The model based on all BC patient included ER status of the first primary BC as additional covariate. Baseline hazards were allowed to vary across country and transition. All the estimates from the model are shown in Tables S13-S14.

Analysis	Transition	Description	HR (95% CI)	P	Cases	Events
	5	CBC -> BC death	1.49 (0.79–2.81)	0.21	1,133	167
	6	CBC -> death not due to BC	0.89 (0.20–4.06)	0.89		80
	7	CBC -> death of unknown cause	0.61 (0.14–2.79)	0.53		55

Abbreviations: HR = hazard ratio; CI = confidence interval; BC = breast cancer; CBC = contralateral breast cancer. The models included age at first primary BC diagnosis, nodal status, tumor size, grade, radiotherapy and systemic treatment given for the first primary BC as covariates. The model based on all BC patient included ER status of the first primary BC as additional covariate. Baseline hazards were allowed to vary across country and transition. All the estimates from the model are shown in Tables S13-S14.

Regarding treatment, radiotherapy was associated with a protective association against death due to causes other than BC or unknown causes, while there was no significant association with BC-specific death (Tables S13-S15). Endocrine therapy alone was associated with a significantly decreased risk of BC-specific death (particularly in patients diagnosed with an ER-positive first primary BC) and with a highly significantly decreased risk of death due to unknown causes. The combination of endocrine therapy and chemotherapy was associated with decreased risk of BC death (in patients diagnosed with an ER-positive first primary BC), with risk of death due to causes other than BC and had the strongest protective association against death due to unknown causes (Tables S14). The corresponding complete-case analyses showed similar patterns of association (Tables S16-S18).

Discussion

The main goal of this study was to assess potential differential associations of treatment by *CHEK2* c.1100delC status with CBC risk, and to investigate if the poorer survival in *CHEK2* c.1100delC carriers may be explained alone by the occurrence of CBC. The Breast Cancer Association Consortium provided a unique resource of 963 carriers of this single *CHEK2* variant to study this question in more detail.

These data did not support the hypothesis of differential associations of treatment with CBC risk by *CHEK2* c.1100delC status. As expected, systemic therapy was found to decrease CBC risk, with the strongest association in the first five years after first primary BC diagnosis, when endocrine therapy is likely to be ongoing [17, 23]. Overall, we did find that the combination of endocrine therapy with chemotherapy resulted in the largest reduction in CBC risk, which has been previously reported [17]. The lack of evidence for a differential association of systemic therapy with CBC risk by *CHEK2* c.1100delC

status suggests that carriers experience a similar beneficial effect as non-carriers. This is in line with previous studies in *CHEK2* c.1100delC carriers [18, 24, 25].

Also, we did not find a significant association of radiotherapy with CBC risk. This lack of association is in contrast with previous studies in sporadic BC patients, which showed that radiotherapy is a contributor to CBC risk, especially when treatment was administered at a younger age [13, 26–28]. One explanation for this might be the change of radiation techniques over time. However, analyses restricted to patients diagnosed from the year 2000 onwards, when treatment regimens were expected to be more homogeneous, showed similar results as were found in the main analyses. Therefore, although observational – and non-randomized – studies like the present cannot rebut hypotheses of causality, these changes are unlikely to be the reason behind the lack of association between radiotherapy and CBC risk in our study.

In line with previous studies [3, 4] we found a greater than two-fold increased risk of CBC in *CHEK2* c.1100delC carriers compared to non-carriers. This is consistent with the reported increase in risk of a first primary BC [2, 19], suggesting that genetic variants that predispose to the development of a first primary BC will also predispose to the development of a CBC. We also observed a shorter BCSS in *CHEK2* c.1100delC carriers compared to non-carriers, after accounting for CBC occurrence, age at diagnosis of the first primary BC and tumor characteristics. This suggests that the shorter BCSS in *CHEK2* c.1100delC carriers versus non-carriers is partly explained by a component other than the established prognostic factors. Moreover, *CHEK2* c.1100delC carriers were on average diagnosed in earlier calendar years compared to non-carriers. Therefore, carriers probably received less efficacious chemotherapy and endocrine therapy compared to non-carriers, which could have affected survival.

The main strengths of our study are the large sample size, including information about tumor pathology, treatment, time to CBC and survival, and a median follow-up of over 9 years. In addition, the use of a multi-state model provides important advantages compared to individual survival models with different endpoints. By modeling all events of interest together, the multi-state model gives insight on how intermediate events, such as CBC, affects survival. Moreover, it allows estimation of transition-specific treatment and covariates effects, thereby providing insight on whether and to what extent the effects change across transitions and corresponding endpoints. Most of the studies were hospital or population based and most BC patients unaware of a *CHEK2* variant, which we determined in the research setting. Therefore, it is highly unlikely that knowledge of carrier status could have affected clinical data collection.

There are some limitations to our study that need to be acknowledged. Between studies there was minor heterogeneity in the definition of stage, grade and cut-offs for ER, PR, and HER2 status, which would have affected both carriers and non-carriers to a similar extent and is unlikely to have impacted our conclusions. Many of the variables related to tumor characteristics and treatment had large proportions of missing values. Complete-case analyses have less power to detect the associations of interest and might be biased if case data are not missing completely at random [29]. We addressed the missing data problem by employing multiple imputation [29], which should provide unbiased estimates, provided data

are missing at random and that imputation models are correctly specified. Analyses restricted to complete-case data yielded results that were mostly consistent with the results based on imputed data. In addition, in some complete-case analyses the number of *CHEK2* c.1100delC carriers was too low to properly estimate the interaction terms. This underlines the importance of the analyses based on imputed data, which avoids losses in the number of cases and events in the analyses. We also did not consider type of chemotherapy or endocrine therapy in the analyses, nor had we information about ovarian function suppression. Moreover, information about the occurrence of primary ipsilateral BCs was very limited and could not be properly accounted for in our analyses. However, based on the available information, there was no difference in the proportion of ipsilateral BC between *CHEK2* c.1100delC carriers and non-carriers (0.6% in both groups) and is unlikely to have had a major impact on our BCSS results. An additional limitation was the lack of information on cause of death for about 25% of those who had died. This would result in a loss of power to detect associations with BCSS in case most of the deaths of unknown causes were due to BC. However, this would, at worst, dilute our results rather than leading to false-positive significant associations with BCSS. Finally, while we accounted for several established BC prognostic factors in our analyses, we cannot exclude the presence of residual bias affecting to some extent our results. An example of such bias is known as “indication bias”, which applies to the presence of an indication which causes or affects the outcome of interest [30]. This could explain some of the unexpected results for the association of radiotherapy and systemic treatment with death-related outcomes, in case treatment decisions are influenced by the presence/absence of certain conditions or morbidities in such a way that patients receiving the treatment are less likely to die from other causes than BC. While indication bias could have affected the treatment-related effects on mortality, it is less likely to be an issue for the association of *CHEK2* c.1100delC status and treatment with CBC risk and survival.

In conclusion, the results of our study did not provide evidence for differential associations with radiation or systemic therapy by *CHEK2* c.100delC status on CBC risk. This suggests that associations with these treatments on CBC risk are similar between carriers and non-carriers. Furthermore, we confirmed the presence of a risk component for BC-specific death in *CHEK2* c.1100delC carriers which is not explained by CBC occurrence or characteristics of the first primary BC. Genotyping of *CHEK2* c.1100delC in patients of ongoing clinical trials would allow the evaluation of treatment response in detail and determine any impact of the *CHEK2* c.1100delC variant on the efficacy of BC treatment. In addition, studies focusing on for examples the molecular copy number aberration profile of *CHEK2*-related tumors should further shed light on potential biological mechanisms underlying the observed increased CBC risk and possible worse survival in CHEK2 c.1100delCcarriers.

Abbreviations

BC: breast cancer

BCAC: Breast Cancer Association Consortium

BCSS: breast cancer-specific survival

CBC: contralateral breast cancer

CI: confidence interval

CMF: Cyclophosphamide Methotrexate Fluorouracil

CT: chemotherapy

ER: estrogen receptor

ET: endocrine therapy

HER2: human epidermal growth factor receptor 2

HR: hazard ratio

IQR: interquartile range

P: p-value

PR: progesterone receptor

SD: standard deviation

Declarations

Acknowledgements

We thank all the individuals who took part in these studies and all the researchers, clinicians, technicians and administrative staff who have enabled this work to be carried out. ABCFS thank Maggie Angelakos, Judi Maskiell, Gillian Dite. ABCS thanks the Blood bank Sanquin, The Netherlands. ABCTB Investigators: Christine Clarke, Deborah Marsh, Rodney Scott, Robert Baxter, Desmond Yip, Jane Carpenter, Alison Davis, Nirmala Pathmanathan, Peter Simpson, J. Dinny Graham, Mythily Sachchithananthan. Samples are made available to researchers on a non-exclusive basis. BCEES thanks Allyson Thomson, Christobel Saunders, Terry Slevin, BreastScreen Western Australia, Elizabeth Wylie, Rachel Lloyd. BIGGS thanks Niall McInerney, Gabrielle Colleran, Andrew Rowan, Angela Jones. The BREOGAN study would not have been possible without the contributions of the following: Manuela Gago-Dominguez, Jose Esteban Castelao, Angel Carracedo, Victor Muñoz Garzón, Alejandro Novo Domínguez, Maria Elena Martinez, Sara Miranda Ponte, Carmen Redondo Marey, Maite Peña Fernández, Manuel Enguix Castelo, Maria Torres, Manuel Calaza (BREOGAN), José Antúnez, Máximo Fraga and the staff of the Department of Pathology and Biobank of the University Hospital Complex of Santiago-CHUS, Instituto de Investigación Sanitaria de Santiago, IDIS, Xerencia de Xestion Integrada de Santiago-SERGAS; Joaquín González-Carreró and the staff of the Department of Pathology and Biobank of University Hospital Complex of Vigo, Instituto de

Investigacion Biomedica Galicia Sur, SERGAS, Vigo, Spain. The BSUCH study acknowledges the Principal Investigator, Barbara Burwinkel, and, thanks Peter Bugert, Medical Faculty Mannheim. CCGP thanks Styliani Apostolaki, Anna Margiolaki, Georgios Nintos, Maria Perraki, Georgia Saloustrou, Georgia Sevastaki, Konstantinos Pompodakis. CGPS thanks staff and participants of the Copenhagen General Population Study. For the excellent technical assistance: Dorthe Uldall Andersen, Maria Birna Arnadottir, Anne Bank, Dorthe Kjeldgård Hansen. The Danish Cancer Biobank is acknowledged for providing infrastructure for the collection of blood samples for the cases. CNIO-BCS thanks Guillermo Pita, Charo Alonso, Nuria Álvarez, Pilar Zamora, Primitiva Menendez, the Human Genotyping-CEGEN Unit (CNIO). Investigators from the CPS-II cohort thank the participants and Study Management Group for their invaluable contributions to this research. They also acknowledge the contribution to this study from central cancer registries supported through the Centers for Disease Control and Prevention National Program of Cancer Registries, as well as cancer registries supported by the National Cancer Institute Surveillance Epidemiology and End Results program. The authors would like to thank the California Teachers Study Steering Committee that is responsible for the formation and maintenance of the Study within which this research was conducted. A full list of California Teachers Study (CTS) team members is available at https://www.calteachersstudy.org/team. DIETCOMPLYF thanks the patients, nurses and clinical staff involved in the study. The DietCompLyf study was funded by the charity Against Breast Cancer (Registered Charity Number 1121258) and the NCRN. We thank the participants and the investigators of EPIC (European Prospective Investigation into Cancer and Nutrition). ESTHER thanks Hartwig Ziegler, Sonja Wolf, Volker Hermann, Christa Stegmaier, Katja Butterbach. FHRISK and PROCAS thank NIHR for funding. The GENICA Network: Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, and University of Tübingen, Germany [RH, HB, Wing-Yee Lo], Department of Internal Medicine, Johanniter GmbH Bonn, Johanniter Krankenhaus, Bonn, Germany [Yon-Dschun Ko, Christian Baisch], Institute of Pathology, University of Bonn, Germany [Hans-Peter Fischer], Molecular Genetics of Breast Cancer, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany [Ute Hamann], Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr University Bochum (IPA), Bochum, Germany [Thomas Brüning, Beate Pesch, Sylvia Rabstein, Anne Lotz]; and Institute of Occupational Medicine and Maritime Medicine, University Medical Center Hamburg-Eppendorf, Germany [Volker Harth]. HEBCS thanks Johanna Kiiski, Carl Blomqvist, Taru A. Muranen, Kristiina Aittomäki, Kirsimari Aaltonen, Karl von Smitten, Irja Erkkilä. KARMA and SASBAC thank the Swedish Medical Research Counsel. KBCP thanks Eija Myöhänen. kConFab/AOCS wish to thank Heather Thorne, Eveline Niedermayr, all the kConFab research nurses and staff, the heads and staff of the Family Cancer Clinics, and the Clinical Follow Up Study (which has received funding from the NHMRC, the National Breast Cancer Foundation, Cancer Australia, and the National Institute of Health (USA)) for their contributions to this resource, and the many families who contribute to kConFab. The following are kConFab Investigators: David Amor, Lesley Andrews, Yoland Antill, Rosemary Balleine, Jonathan Beesley, Ian Bennett, Michael Bogwitz, Leon Botes, Meagan Brennan, Melissa Brown, Michael Buckley, Jo Burke, Phyllis Butow, Liz Caldon, Ian Campbell, Michelle Cao, Anannya Chakrabarti, Deepa Chauhan, Manisha Chauhan, Georgia Chenevix-Trench, Alice Christian, Paul Cohen, Alison Colley, Ashley Crook, James Cui, Eliza Courtney, Margaret Cummings, Sarah-Jane Dawson, Anna DeFazio, Martin

Delatycki, Rebecca Dickson, Joanne Dixon, Ted Edkins, Stacey Edwards, Gelareh Farshid, Andrew Fellows, Georgina Fenton, Michael Field, James Flanagan, Peter Fong, Laura Forrest, Stephen Fox, Juliet French, Michael Friedlander, Clara Gaff, Mike Gattas, Peter George, Sian Greening, Marion Harris, Stewart Hart, Nick Hayward, John Hopper, Cass Hoskins, Clare Hunt, Paul James, Mark Jenkins, Alexa Kidd, Judy Kirk, Jessica Koehler, James Kollias, Sunil Lakhani, Mitchell Lawrence, Jason Lee, Shuai Li, Geoff Lindeman, Lara Lipton, Liz Lobb, Sherene Loi, Graham Mann, Deborah Marsh, Sue Anne McLachlan, Bettina Meiser, Roger Milne, Sophie Nightingale, Shona O'Connell, Sarah O'Sullivan, David Gallego Ortega, Nick Pachter, Jia-Min Pang, Gargi Pathak, Briony Patterson, Amy Pearn, Kelly Phillips, Ellen Pieper, Susan Ramus, Edwina Rickard, Bridget Robinson, Mona Saleh, Anita Skandarajah, Elizabeth Salisbury, Christobel Saunders, Jodi Saunus, Rodney Scott, Clare Scott, Adrienne Sexton, Andrew Shelling, Peter Simpson, Melissa Southey, Amanda Spurdle, Jessica Taylor, Renea Taylor, Heather Thorne, Alison Trainer, Kathy Tucker, Jane Visvader, Logan Walker, Rachael Williams, Ingrid Winship, Mary Ann Young, Milita Zaheed. LMBC thanks Gilian Peuteman, Thomas Van Brussel, EvyVanderheyden and Kathleen Corthouts. MABCS thanks Milena Jakimovska (RCGEB “Georgi D. Efremov”), Snezhana Smichkoska, Emilija Lazarova, Marina Iljoska (University Clinic of Radiotherapy and Oncology), Katerina Kubelka-Sabit, Dzengis Jasar, Mitko Karadjozov (Adzibadem-Sistina Hospital), Andrej Arsovski and Liljana Stojanovska (Re-Medika Hospital) for their contributions and commitment to this study. MARIE thanks Petra Seibold, Nadia Obi, Sabine Behrens, Ursula Eilber and Muhabbet Celik. MBCSG (Milan Breast Cancer Study Group): Siranoush Manoukian, Bernard Peissel, Jacopo Azzollini, Erica Rosina, Daniela Zaffaroni, Bernardo Bonanni, Irene Feroce, Mariarosaria Calvello, Aliana Guerrieri Gonzaga, Monica Marabelli and the personnel of the Cogentech Cancer Genetic Test Laboratory. The MCCS was made possible by the contribution of many people, including the original investigators, the teams that recruited the participants and continue working on follow-up, and the many thousands of Melbourne residents who continue to participate in the study. The MISS study group acknowledges the former Principal Investigator, professor Håkan Olsson. We thank the coordinators, the research staff and especially the MMHS participants for their continued collaboration on research studies in breast cancer. MSKCC thanks Marina Corines, Lauren Jacobs. The following are NBCS Collaborators: Kristine K. Sahlberg (PhD), Anne-Lise Børresen-Dale (Prof. Em.), Inger Torhild Gram (Prof.), Karina Standahl Olsen (Ass. Prof.), Olav Engebråten (MD), Bjørn Naume (MD), Jürgen Geisler (MD), OSBREAC and Grethe I. Grenaker Alnæs (MSc). For NHS and NHS2 the study protocol was approved by the institutional review boards of the Brigham and Women’s Hospital and Harvard T.H. Chan School of Public Health, and those of participating registries as required. We would like to thank the participants and staff of the NHS and NHS2 for their valuable contributions as well as the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. The authors assume full responsibility for analyses and interpretation of these data. OBCS thanks Arja Jukkola-Vuorinen, Mervi Grip, Saila Kauppila, Meeri Otsukka, Leena Keskitalo and Kari Mononen for their contributions to this study. The OFBCR thanks Teresa Selander, Nayana Weerasooriya and Steve Gallinger. ORIGO thanks E. Krol-Warmerdam, and J. Blom for patient accrual, administering questionnaires, and managing clinical information. The LUMC survival data were retrieved from the Leiden hospital-based cancer registry system (ONCDOC) with the help of Dr. J. Molenaar. PBCS thanks Louise Brinton, Mark Sherman, Neonila

Szeszenia-Dabrowska, Beata Peplonska, Witold Zatonski, Pei Chao, Michael Stagner. The ethical approval for the POSH study is MREC /00/6/69, UKCRN ID: 1137. We thank staff in the Experimental Cancer Medicine Centre (ECMC) supported Faculty of Medicine Tissue Bank and the Faculty of Medicine DNA Banking resource. The authors wish to acknowledge the roles of the Breast Cancer Now Tissue Bank in collecting and making available the samples and/or data, and the patients who have generously donated their tissues and shared their data to be used in the generation of this publication. PREFACE thanks Sonja Oeser and Silke Landrith. The RBCS thanks Jannet Blom, Saskia Pelders, Wendy J.C. Prager – van der Smissen, and the Erasmus MC Family Cancer Clinic. SBCS thanks Sue Higham, Helen Cramp, Dan Connley, Ian Brock, Sabapathy Balasubramanian and Malcolm W.R. Reed. We thank the SEARCH and EPIC teams. SKKDKFZS thanks all study participants, clinicians, family doctors, researchers and technicians for their contributions and commitment to this study. We thank the SUCCESS Study teams in Munich, Duessldorf, Erlangen and Ulm. SZBCS thanks Ewa Putresza. UBCS thanks all study participants, the ascertainment, laboratory and research informatics teams at Huntsman Cancer Institute and Intermountain Healthcare, and Justin Williams, Brandt Jones, Myke Madsen, Stacey Knight and Kerry Rowe for their important contributions to this study. UCIBCS thanks Irene Masunaka. UKBGS thanks Breast Cancer Now and the Institute of Cancer Research for support and funding of the Generations Study, and the study participants, study staff, and the doctors, nurses and other health care providers and health information sources who have contributed to the study. We acknowledge NHS funding to the Royal Marsden/ICR NIHR Biomedical Research Centre.

Conflict of interests

Matthias W. Beckmann and Peter A. Fasching conduct research funded by Amgen, Novartis and Pfizer (not related to this study). Peter A. Fasching received Honoraria from Roche, Novartis and Pfizer (not related to this study). Allison W. Kurian's institution received a research funding from Myriad genetics for an unrelated project (not related to this study). Emmanouil Saloustros reported the following: honoraria from Amgen Hellas, Pfizer Hellas, and IPSEN (not related to this study); support from Merck Greece and Pfizer Hellas for attending meetings (not related to this study); he participated in advisory boards in Greece (MSD Greece, AstraZeneca Greece, Gilead Sciences Hellas, Pfizer Hellas, Genesis Pharma), not related to this study; he is a PI in sponsored clinical trials: MSD Greece (not related to this study). The other authors declare no conflict of interest.

Data availability statement

The datasets analyzed during the current study are not publicly available due to protection of participant privacy and confidentiality, and ownership of the contributing institutions, but may be made available in an anonymized form via the corresponding author on reasonable request and after approval of the involved institutions. To receive access to the data, a concept form must be submitted, which will then be reviewed by the BCAC Data Access Coordination Committee (DACC); see http://bcac.ccge.medschl.cam.ac.uk/bcacdata/.

Ethics statement

All individual studies were approved by the appropriate institutional review boards and/or medical ethical committees. Written informed consent was obtained from all study participants.

Author contributions

M.J.H. and M.K.S. conceived the study. A.M. and M.A.C.S. performed the main data analyses. A.M., M.A.C.S., M.A.A, A.H., M.J.H. and M.K.S interpreted the data. A.M., M.A.C.S., M.J.H. and M.K.S drafted the initial manuscript. Q.W., M.K.B., and J.D. provided database support. All authors contributed to the critical revision and editing of the final version of the manuscript. All authors were involved in the data generation or provision and read and approved the final manuscript. The work reported in the paper has been performed by the authors, unless clearly specified in the text.

Funding

BCAC is funded by the European Union's Horizon 2020 Research and Innovation Programme (grant numbers 634935 and 633784 for BRIDGES and B-CAST respectively), and the PERSPECTIVE I&I project, funded by the Government of Canada through Genome Canada and the Canadian Institutes of Health Research, the Ministère de l'Économie et de l'Innovation du Québec through Genome Québec, the Quebec Breast Cancer Foundation. The EU Horizon 2020 Research and Innovation Programme funding source had no role in study design, data collection, data analysis, data interpretation or writing of the report. Additional funding for BCAC is provided via the Confluence project which is funded with intramural funds from the National Cancer Institute Intramural Research Program, National Institutes of Health.

Genotyping of the OncoArray was funded by the NIH Grant U19 CA148065, and Cancer Research UK Grant C1287/A16563 and the PERSPECTIVE project supported by the Government of Canada through Genome Canada and the Canadian Institutes of Health Research (grant GPH-129344) and, the Ministère de l'Économie, Science et Innovation du Québec through Genome Québec and the PSRSIIRI-701 grant, and the Quebec Breast Cancer Foundation. Funding for iCOGS came from: the European Community's Seventh Framework Programme under grant agreement n° 223175 (HEALTH-F2-2009-223175) (COGS), Cancer Research UK (C1287/A10118, C1287/A10710, 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, and Komen Foundation for the Cure, the Breast Cancer Research Foundation, and the Ovarian Cancer Research Fund.

The BRIDGES panel sequencing was supported by the European Union Horizon 2020 research and innovation program BRIDGES (grant number, 634935) and the Wellcome Trust (v203477/Z/16/Z).

The Australian Breast Cancer Family Study (ABCFS) was supported by grant UM1 CA164920 from the National Cancer Institute (USA). The content of this manuscript 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 USA Government or the BCFR. The ABCFS was also supported by the National Health and Medical Research Council of Australia, the New South Wales Cancer Council, the Victorian Health Promotion Foundation (Australia) and the Victorian Breast Cancer Research Consortium. J.L.H. is a National Health and Medical Research Council (NHMRC) Senior Principal Research Fellow. M.C.S. is a NHMRC Senior Research Fellow. The ABCS study was supported by the Dutch Cancer Society [grants NKI 2007-3839; 2009 4363]. The Australian Breast Cancer Tissue Bank (ABCTB) was supported by the National Health and Medical Research Council of Australia, The Cancer Institute NSW and the National Breast Cancer Foundation. The AHS study is supported by the intramural research program of the National Institutes of Health, the National Cancer Institute (grant number Z01-CP010119), and the National Institute of Environmental Health Sciences (grant number Z01-ES049030). The work of the BBCC was partly funded by ELAN-Fond of the University Hospital of Erlangen. The BBCS is funded by Cancer Research UK and Breast Cancer Now and acknowledges NHS funding to the NIHR Biomedical Research Centre, and the National Cancer Research Network (NCRN). The BCEES was funded by the National Health and Medical Research Council, Australia and the Cancer Council Western Australia and acknowledges funding from the National Breast Cancer Foundation (JS). For the BCFR-NY, BCFR-PA, BCFR-UT this work was supported by grant UM1 CA164920 from the National Cancer Institute. The content of this manuscript 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. The BCINIS study is supported in part by the Breast Cancer Research Foundation (BCRF). For BIGGS, ES is supported by NIHR Comprehensive Biomedical Research Centre, Guy's & St. Thomas' NHS Foundation Trust in partnership with King's College London, United Kingdom. IT is supported by the Oxford Biomedical Research Centre. The BREast Oncology GAlician Network (BREOGAN) is funded by Acción Estratégica de Salud del Instituto de Salud Carlos III FIS PI12/02125/Cofinanciado and FEDER PI17/00918/Cofinanciado FEDER; Acción Estratégica de Salud del Instituto de Salud Carlos III FIS Intrasalud (PI13/01136); Programa Grupos Emergentes, Cancer Genetics Unit, Instituto de Investigacion Biomedica Galicia Sur. Xerencia de Xestion Integrada de Vigo-SERGAS, Instituto de Salud Carlos III, Spain; Grant 10CSA012E, Consellería de Industria Programa Sectorial de Investigación Aplicada, PEME I + D e I + D Suma del Plan Gallego de Investigación, Desarrollo e Innovación Tecnológica de la Consellería de Industria de la Xunta de Galicia, Spain; Grant EC11-192. Fomento de la Investigación Clínica Independiente, Ministerio de Sanidad, Servicios Sociales e Igualdad, Spain; and Grant FEDER-Innterconecta. Ministerio de Economia y Competitividad, Xunta de Galicia, Spain. The BSUCH study was supported by the Dietmar-Hopp Foundation, the Helmholtz Society and the German Cancer Research Center (DKFZ). CCGP is supported by funding from the University of Crete. The CECILE study was supported by Fondation de France, Institut National du Cancer (INCa), Ligue Nationale contre le Cancer, Agence Nationale de Sécurité Sanitaire, de l'Alimentation, de l'Environnement et du Travail (ANSES), Agence Nationale de la Recherche (ANR). The CGPS was supported by the Chief Physician Johan Boserup and Lise Boserup Fund, the Danish Medical Research Council, and Herlev and Gentofte Hospital. The American Cancer Society funds the creation, maintenance, and updating of the

CPS-II cohort. The California Teachers Study (CTS) and the research reported in this publication were supported by the National Cancer Institute of the National Institutes of Health under award number U01-CA199277; P30-CA033572; P30-CA023100; UM1-CA164917; and R01-CA077398. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Cancer Institute or the National Institutes of Health. The collection of cancer incidence data used in the California Teachers Study was supported by the California Department of Public Health pursuant to California Health and Safety Code Section 103885; Centers for Disease Control and Prevention's National Program of Cancer Registries, under cooperative agreement 5NU58DP006344; the National Cancer Institute's Surveillance, Epidemiology and End Results Program under contract HHSN261201800032I awarded to the University of California, San Francisco, contract HHSN261201800015I awarded to the University of Southern California, and contract HHSN261201800009I awarded to the Public Health Institute. The opinions, findings, and conclusions expressed herein are those of the author(s) and do not necessarily reflect the official views of the State of California, Department of Public Health, the National Cancer Institute, the National Institutes of Health, the Centers for Disease Control and Prevention or their Contractors and Subcontractors, or the Regents of the University of California, or any of its programs. The University of Westminster curates the DietCompLyf database funded by Against Breast Cancer Registered Charity No. 1121258 and the NCRN. The coordination of EPIC is financially supported by the European Commission (DG-SANCO) and the International Agency for Research on Cancer. The national cohorts are supported by: Ligue Contre le Cancer, Institut Gustave Roussy, Mutuelle Générale de l'Education Nationale, Institut National de la Santé et de la Recherche Médicale (INSERM) (France); German Cancer Aid, German Cancer Research Center (DKFZ), Federal Ministry of Education and Research (BMBF) (Germany); the Hellenic Health Foundation, the Stavros Niarchos Foundation (Greece); Associazione Italiana per la Ricerca sul Cancro-AIRC-Italy and National Research Council (Italy); Dutch Ministry of Public Health, Welfare and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), Statistics Netherlands (The Netherlands); Health Research Fund (FIS), PI13/00061 to Granada, PI13/01162 to EPIC-Murcia, Regional Governments of Andalucía, Asturias, Basque Country, Murcia and Navarra, ISCIII RETIC (RD06/0020) (Spain); Cancer Research UK (14136 to EPIC-Norfolk; C570/A16491 and C8221/A19170 to EPIC-Oxford), Medical Research Council (1000143 to EPIC-Norfolk, MR/M012190/1 to EPIC-Oxford) (United Kingdom). The ESTHER study was supported by a grant from the Baden Württemberg Ministry of Science, Research and Arts. Additional cases were recruited in the context of the VERDI study, which was supported by a grant from the German Cancer Aid (Deutsche Krebshilfe). FHRISK and PROCAS are funded from NIHR grant PGfAR 0707-10031. DGE, AH and WGN are supported by the NIHR Manchester Biomedical Research Centre (IS-BRC-1215-20007). The GC-HBOC (German Consortium of Hereditary Breast and Ovarian Cancer) is supported by the German Cancer Aid (grant no 110837 and 70114178, coordinator: Rita K. Schmutzler, Cologne) and the Federal Ministry of Education and Research, Germany (grant no 01GY1901). This work was also funded by the European Regional Development Fund and Free State of Saxony, Germany (LIFE - Leipzig Research Centre for Civilization Diseases, project numbers 713-241202, 713-241202, 14505/2470, 14575/2470). The GENICA was funded by the Federal Ministry of Education and Research (BMBF) Germany grants 01KW9975/5, 01KW9976/8, 01KW9977/0 and

01KW0114, the Robert Bosch Foundation, Stuttgart, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr University Bochum (IPA), Bochum, as well as the Department of Internal Medicine, Johanniter GmbH Bonn, Johanniter Krankenhaus, Bonn, Germany. The GESBC was supported by the Deutsche Krebshilfe e. V. [70492] and the German Cancer Research Center (DKFZ). The HABCS study was supported by the Claudia von Schilling Foundation for Breast Cancer Research, by the Lower Saxonian Cancer Society, and by the Rudolf Bartling Foundation. The HEBCS was financially supported by the Helsinki University Hospital Research Fund, the Sigrid Juselius Foundation and the Cancer Foundation Finland. Financial support for KARBAC was provided through the regional agreement on medical training and clinical research (ALF) between Stockholm County Council and Karolinska Institutet, the Swedish Cancer Society, The Gustav V Jubilee foundation and Bert von Kantzows foundation. The KARMA study was supported by Märit and Hans Rausings Initiative Against Breast Cancer. The KBCP was financially supported by the special Government Funding (VTR) of Kuopio University Hospital grants, Cancer Fund of North Savo, the Finnish Cancer Organizations, and by the strategic funding of the University of Eastern Finland. kConFab and the kConFab Follow-Up Study have been supported by grants from Cancer Australia (809195 and 1100868), the Australian National Breast Cancer Foundation (IF 17 kConFab), the National Health and Medical Research Council (454508, 288704, and 145684), the Queensland Cancer Fund, the Cancer Councils of New South Wales, Victoria, Tasmania, and South Australia, and the Cancer Foundation of Western Australia. K-AP is a National Health and Medical Research Council Leadership Fellow (Australia) (1195294). Financial support for the AOCS was provided by the United States Army Medical Research and Materiel Command [DAMD17-01-1-0729], Cancer Council Victoria, Queensland Cancer Fund, Cancer Council New South Wales, Cancer Council South Australia, The Cancer Foundation of Western Australia, Cancer Council Tasmania and the National Health and Medical Research Council of Australia (NHMRC; 400413, 400281, 199600). G.C.T. and P.W. are supported by the NHMRC. RB was a Cancer Institute NSW Clinical Research Fellow. LMBC is supported by the 'Stichting tegen Kanker'. DL is supported by the FWO. The MABCS study is funded by the Research Centre for Genetic Engineering and Biotechnology "Georgi D. Efremov", MASA. The MARIE study was supported by the Deutsche Krebshilfe e.V. [70-2892-BR I, 106332, 108253, 108419, 110826, 110828], the Hamburg Cancer Society, the German Cancer Research Center (DKFZ) and the Federal Ministry of Education and Research (BMBF) Germany [01KH0402]. MBCSG is supported by grants from the Italian Association for Cancer Research (AIRC). The MCBCS was supported by the NIH grants R35CA253187, R01CA192393, R01CA116167, R01CA176785 a NIH Specialized Program of Research Excellence (SPORE) in Breast Cancer [P50CA116201], and the Breast Cancer Research Foundation. The Melbourne Collaborative Cohort Study (MCCS) cohort recruitment was funded by VicHealth and Cancer Council Victoria. The MCCS was further augmented by Australian National Health and Medical Research Council grants 209057, 396414 and 1074383 and by infrastructure provided by Cancer Council Victoria. Cases and their vital status were ascertained through the Victorian Cancer Registry and the Australian Institute of Health and Welfare, including the National Death Index and the Australian Cancer Database. The MEC was supported by NIH grants CA63464, CA54281, CA098758, CA132839 and CA164973. The MISS study was supported by funding from ERC-2011-294576 Advanced grant, Swedish Cancer Society CAN

2018/675, Swedish Research Council, Local hospital funds, Berta Kamprad Foundation FBKS 2021-19, Gunnar Nilsson. The MMHS study was supported by NIH grants CA97396, CA128931, CA116201, CA140286 and CA177150. MSKCC is supported by grants from the Breast Cancer Research Foundation and Robert and Kate Niehaus Clinical Cancer Genetics Initiative The NBCS has received funding from the K.G. Jebsen Centre for Breast Cancer Research; the Research Council of Norway grant 193387/V50 (to A-L Børresen-Dale and V.N. Kristensen) and grant 193387/H10 (to A-L Børresen-Dale and V.N. Kristensen), South Eastern Norway Health Authority (grant 39346 to A-L Børresen-Dale) and the Norwegian Cancer Society (to A-L Børresen-Dale and V.N. Kristensen). The Northern California Breast Cancer Family Registry (NC-BCFR) and Ontario Familial Breast Cancer Registry (OFBCR) were supported by grant U01CA164920 from the USA National Cancer Institute of the National Institutes of Health. The content of this manuscript 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 USA Government or the BCFR. The Carolina Breast Cancer Study (NCBCS) was funded by Komen Foundation, the National Cancer Institute (P50 CA058223, U54 CA156733, U01 CA179715), and the North Carolina University Cancer Research Fund. The NHS was supported by NIH grants P01 CA87969, UM1 CA186107, and U19 CA148065. The NHS2 was supported by NIH grants UM1 CA176726 and U19 CA148065. The OBCS was supported by research grants from the Finnish Cancer Foundation, the Academy of Finland (grant number 250083, 122715 and Center of Excellence grant number 251314), the Finnish Cancer Foundation, the Sigrid Juselius Foundation, the University of Oulu, the University of Oulu Support Foundation and the special Governmental EVO funds for Oulu University Hospital-based research activities. The ORIGO study was supported by the Dutch Cancer Society (RUL 1997-1505) and the Biobanking and Biomolecular Resources Research Infrastructure (BBMRI-NL CP16). The PBCS was funded by Intramural Research Funds of the National Cancer Institute, Department of Health and Human Services, USA. Genotyping for PLCO was supported by the Intramural Research Program of the National Institutes of Health, NCI, Division of Cancer Epidemiology and Genetics. The PLCO is supported by the Intramural Research Program of the Division of Cancer Epidemiology and Genetics and supported by contracts from the Division of Cancer Prevention, National Cancer Institute, National Institutes of Health. The POSH study is funded by Cancer Research UK (grants C1275/A11699, C1275/C22524, C1275/A19187, C1275/A15956 and Breast Cancer Campaign 2010PR62, 2013PR044. The RBCS was funded by the Dutch Cancer Society (DDHK 2004-3124, DDHK 2009-4318). The SASBAC study was supported by funding from the Agency for Science, Technology and Research of Singapore (A*STAR), the US National Institute of Health (NIH) and the Susan G. Komen Breast Cancer Foundation. The SBCS was supported by Sheffield Experimental Cancer Medicine Centre and Breast Cancer Now Tissue Bank. SEARCH is funded by Cancer Research UK [C490/A10124, C490/A16561] and supported by the UK National Institute for Health Research Biomedical Research Centre at the University of Cambridge. The University of Cambridge has received salary support for PDPP from the NHS in the East of England through the Clinical Academic Reserve. The SZBCS was 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. UBCS was supported by funding from National

Cancer Institute (NCI) grant R01 CA163353 (to N.J. Camp) and the Women's Cancer Center at the Huntsman Cancer Institute (HCI). Data collection for UBCS was supported by the Utah Population Database (UPDB) and Utah Cancer Registry (UCR). Partial support for UPDB datasets was provided by the University of Utah HCI and the HCI Cancer Center Support grant, P30 CA2014 from the National Cancer Institute. UCR is funded by the National Cancer Institute's SEER Program, Contract No. HHSN261201800016I, the US Centers for Disease Control and Prevention's National Program of Cancer Registries, Cooperative Agreement No. NU58DP006320, with additional support from the University of Utah and Huntsman Cancer Foundation. The UCIBCS component of this research was supported by the NIH [CA58860, CA92044] and the Lon V Smith Foundation [LVS39420]. The UKBGS is funded by Breast Cancer Now and the Institute of Cancer Research (ICR), London. ICR acknowledges NHS funding to the NIHR Biomedical Research Centre. The US3SS study was supported by Massachusetts (K.M.E., R01CA47305), Wisconsin (P.A.N., R01 CA47147) and New Hampshire (L.T.-E., R01CA69664) centers, and Intramural Research Funds of the National Cancer Institute, Department of Health and Human Services, USA. The USRT Study was funded by Intramural Research Funds of the National Cancer Institute, Department of Health and Human Services, USA.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021;71:209-49.
2. CHEK2 Breast Cancer Case-Control Consortium. CHEK2*1100delC and susceptibility to breast cancer: a collaborative analysis involving 10,860 breast cancer cases and 9,065 controls from 10 studies. Am J Hum Genet 2004;74:1175-82.
3. Schmidt MK, Hogervorst F, van Hien R, Cornelissen S, Broeks A, Adank MA, Meijers H, Waisfisz Q, Hollestelle A, Schutte M, van den Ouweland A, Hooning M, et al. Age- and Tumor Subtype-Specific Breast Cancer Risk Estimates for CHEK2*1100delC Carriers. J Clin Oncol 2016;34:2750-60.
4. Weischer M, Nordestgaard BG, Pharoah P, Bolla MK, Nevanlinna H, Van't Veer LJ, Garcia-Closas M, Hopper JL, Hall P, Andrulis IL, Devilee P, Fasching PA, et al. CHEK2*1100delC heterozygosity in women with breast cancer associated with early death, breast cancer-specific death, and increased risk of a second breast cancer. J Clin Oncol 2012;30:4308-16.
5. Thompson D, Seal S, Schutte M, McGuffog L, Barfoot R, Renwick A, Eeles R, Sodha N, Houlston R, Shanley S, Klijn J, Wasielewski M, et al. A multicenter study of cancer incidence in CHEK2 1100delC mutation carriers. Cancer Epidemiol Biomarkers Prev 2006;15:2542-5.
6. de Bock GH, Schutte M, Krol-Warmerdam EM, Seynaeve C, Blom J, Brekelmans CT, Meijers-Heijboer H, van Asperen CJ, Cornelisse CJ, Devilee P, Tollenaar RA, Klijn JG. Tumour characteristics and prognosis of breast cancer patients carrying the germline CHEK2*1100delC variant. J Med Genet 2004;41:731-5.

7. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, et al. Molecular portraits of human breast tumours. Nature 2000;406:747-52.
8. Broeks A, de Witte L, Nooijen A, Huseinovic A, Klijn JG, van Leeuwen FE, Russell NS, van't Veer LJ. Excess risk for contralateral breast cancer in CHEK2*1100delC germline mutation carriers. Breast Cancer Res Treat 2004;83:91-3.
9. Greville-Heygate SL, Maishman T, Tapper WJ, Cutress RI, Copson E, Dunning AM, Haywood L, Jones LJ, Eccles DM. Pathogenic Variants in CHEK2 Are Associated With an Adverse Prognosis in Symptomatic Early-Onset Breast Cancer. JCO Precis Oncol 2020;4.
10. Ertych N, Stolz A, Valerius O, Braus GH, Bastians H. CHK2-BRCA1 tumor-suppressor axis restrains oncogenic Aurora-A kinase to ensure proper mitotic microtubule assembly. Proc Natl Acad Sci U S A 2016;113:1817-22.
11. Zannini L, Delia D, Buscemi G. CHK2 kinase in the DNA damage response and beyond. J Mol Cell Biol 2014;6:442-57.
12. Ansari N, Shahrabi S, Khosravi A, Shirzad R, Rezaeean H. Prognostic Significance of CHEK2 Mutation in Progression of Breast Cancer. Lab Med 2019;50:e36-e41.
13. Akdeniz D, Schmidt MK, Seynaeve CM, McCool D, Giardiello D, van den Broek AJ, Hauptmann M, Steyerberg EW, Hooning MJ. Risk factors for metachronous contralateral breast cancer: A systematic review and meta-analysis. Breast 2019;44:1-14.
14. Thompson LH. Recognition, signaling, and repair of DNA double-strand breaks produced by ionizing radiation in mammalian cells: the molecular choreography. Mutat Res 2012;751:158-246.
15. Bartek J, Lukas J. Chk1 and Chk2 kinases in checkpoint control and cancer. Cancer Cell 2003;3:421-9.
16. Broeks A, Braaf LM, Huseinovic A, Nooijen A, Urbanus J, Hogervorst FB, Schmidt MK, Klijn JG, Russell NS, Van Leeuwen FE, Van 't Veer LJ. Identification of women with an increased risk of developing radiation-induced breast cancer: a case only study. Breast Cancer Res 2007;9:R26.
17. Kramer I, Schaapveld M, Oldenburg HSA, Sonke GS, McCool D, van Leeuwen FE, Van de Vijver KK, Russell NS, Linn SC, Siesling S, van Oordt CWMV, Schmidt MK. The Influence of Adjuvant Systemic Regimens on Contralateral Breast Cancer Risk and Receptor Subtype. Jnci-Journal of the National Cancer Institute 2019;111:709-18.
18. Kriege M, Hollestelle A, Jager A, Huijts PE, Berns EM, Sieuwerts AM, Meijer-van Gelder ME, Collee JM, Devilee P, Hooning MJ, Martens JW, Seynaeve C. Survival and contralateral breast cancer in CHEK2 1100delC breast cancer patients: impact of adjuvant chemotherapy. Br J Cancer 2014;111:1004-13.
19. Dorling L, Carvalho S, Allen J, Gonzalez-Neira A, Luccarini C, Wahlstrom C, Pooley KA, Parsons MT, Fortuno C, Wang Q, Bolla MK, Dennis J, et al. Breast Cancer Risk Genes - Association Analysis in More than 113,000 Women. New England Journal of Medicine 2021;384:428-39.
20. Adank MA, Jonker MA, Kluijt I, van Mil SE, Oldenburg RA, Mooi WJ, Hogervorst FB, van den Ouweland AM, Gille JJ, Schmidt MK, van der Vaart AW, Meijers-Heijboer H, et al. CHEK2*1100delC

homozygosity is associated with a high breast cancer risk in women. J Med Genet 2011;48:860-3.

21. Amos CI, Dennis J, Wang Z, Byun J, Schumacher FR, Gayther SA, Casey G, Hunter DJ, Sellers TA, Gruber SB, Dunning AM, Michailidou K, et al. The OncoArray Consortium: A Network for Understanding the Genetic Architecture of Common Cancers. Cancer Epidemiol Biomarkers Prev 2017;26:126-35.
22. Bahcall O. COGS project and design of the iCOGS array. Nature Genetics 2013.
23. Early Breast Cancer Trialists' Collaborative G. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet 2005;365:1687-717.
24. Mellemkjaer L, Dahl C, Olsen JH, Bertelsen L, Guldberg P, Christensen J, Borresen-Dale AL, Stovall M, Langholz B, Bernstein L, Lynch CF, Malone KE, et al. Risk for contralateral breast cancer among carriers of the CHEK2*1100delC mutation in the WECARE Study. Br J Cancer 2008;98:728-33.
25. Kriege M, Jager A, Hollestelle A, Berns EM, Blom J, Meijer-van Gelder ME, Sieuwerts AM, van den Ouweland A, Collee JM, Kroep JR, Martens JW, Hooning MJ, et al. Sensitivity to systemic therapy for metastatic breast cancer in CHEK2 1100delC mutation carriers. J Cancer Res Clin Oncol 2015;141:1879-87.
26. Hooning MJ, Aleman BM, Hauptmann M, Baaijens MH, Klijn JG, Noyon R, Stovall M, van Leeuwen FE. Roles of radiotherapy and chemotherapy in the development of contralateral breast cancer. J Clin Oncol 2008;26:5561-8.
27. Stovall M, Smith SA, Langholz BM, Boice JD, Jr., Shore RE, Andersson M, Buchholz TA, Capanu M, Bernstein L, Lynch CF, Malone KE, Anton-Culver H, et al. Dose to the contralateral breast from radiotherapy and risk of second primary breast cancer in the WECARE study. Int J Radiat Oncol Biol Phys 2008;72:1021-30.
28. Boice JD, Jr., Harvey EB, Blettner M, Stovall M, Flannery JT. Cancer in the contralateral breast after radiotherapy for breast cancer. N Engl J Med 1992;326:781-5.
29. van Buuren S. Flexible imputation of missing dataed. Boca Raton, FL: CRC Press, 2012.
30. Kyriacou DN, Lewis RJ. Confounding by Indication in Clinical Research. JAMA 2016;316:1818-19.

Figures

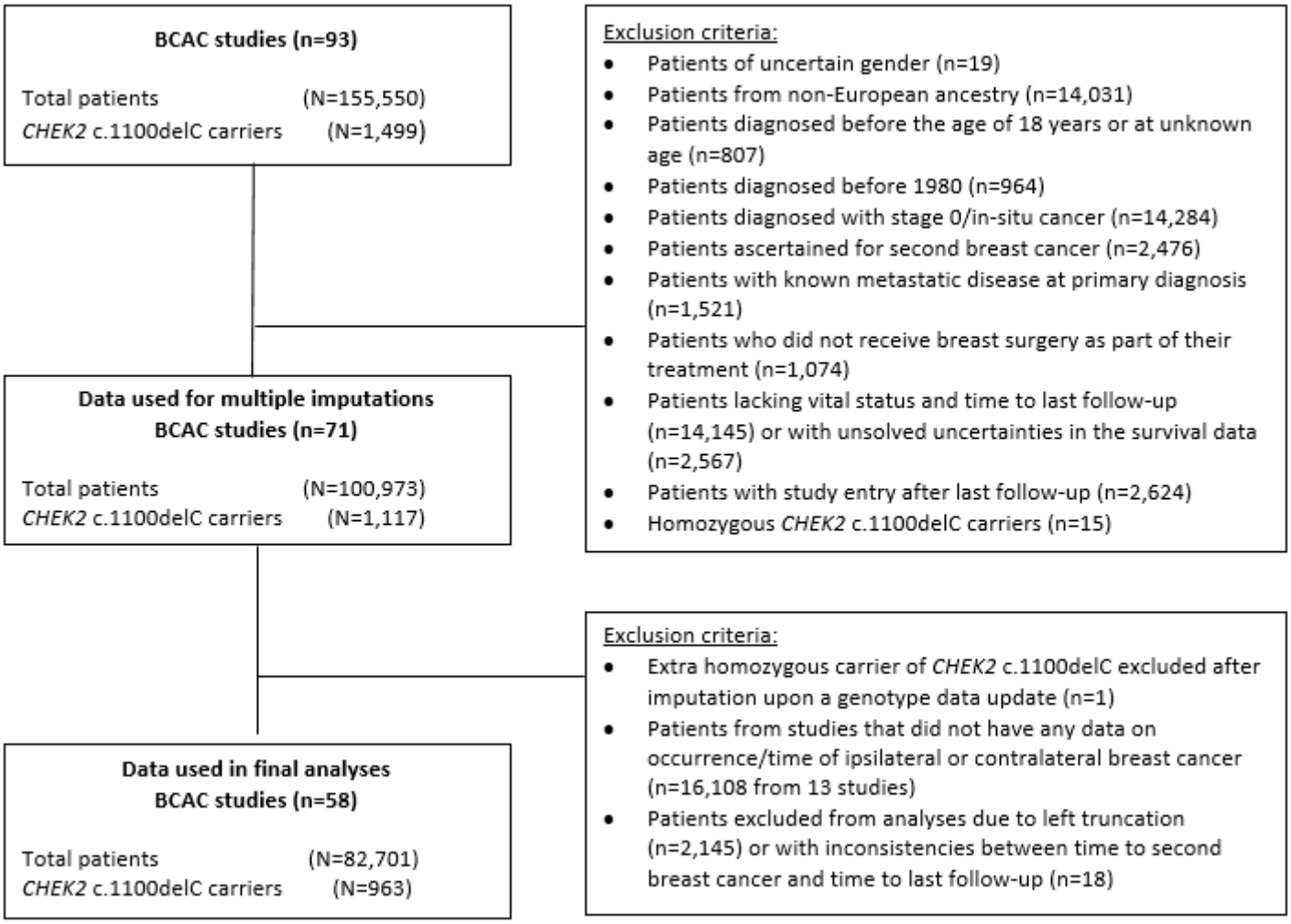

Figure 1

Data flowchart of inclusion and exclusion of patients with breast cancer from the Breast Cancer Association Consortium (BCAC) database

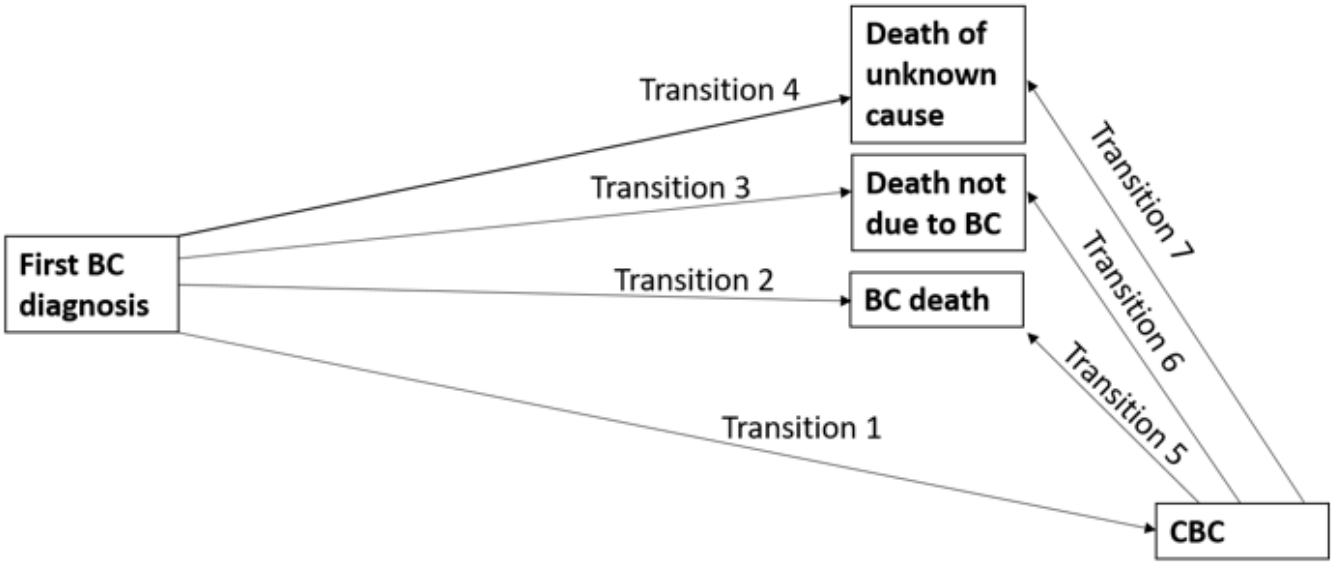

Figure 2

Graphical representation of the multi-state model

Abbreviations: BC=breast cancer, CBC=contralateral breast cancer

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.docx
- SupplementaryTablesS2S18.docx
- Supplementarymethods.docx