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ORIGINAL REPORT

*CHEK2**1100delC Heterozygosity in Women With Breast Cancer Associated With Early Death, Breast Cancer–Specific Death, and Increased Risk of a Second Breast Cancer

Maren Weischer, Børge G. Nordestgaard, Paul Pharoah, Manjeet K. Bolla, Heli Nevanlinna, Laura J. van't Veer, Montserrat Garcia-Closas, John L. Hopper, Per Hall, Irene L. Andrulis, Peter Devilee, Peter A. Fasching, Hoda Anton-Culver, Diether Lambrechts, Maartje Hooning, Angela Cox, Graham G. Giles, Barbara Burwinkel, Annika Lindblom, Fergus J. Couch, Arto Mannermaa, Grethe Grenaker Alnæs, Esther M. John, Thilo Dörk, Henrik Flyger, Alison M. Dunning, Qin Wang, Taru A. Muranen, Richard van Hien, Jonine Figueroa, Melissa C. Southey, Kamila Czene, Julia A. Knight, Rob A.E.M. Tollenaar, Matthias W. Beckmann, Argyrios Ziogas, Marie-Rose Christiaens, Johanna Margriet Collée, Malcolm W.R. Reed, Gianluca Severi, Frederik Marme, Sara Margolin, Janet E. Olson, Veli-Matti Kosma, Vessela N. Kristensen, Alexander Miron, Natalia Bogdanova, Mitul Shah, Carl Blomqvist, Annegien Broeks, Mark Sherman, Kelly-Anne Phillips, Jingmei Li, Jianjun Liu, Gord Glendon, Caroline Seynaeve, Arif B. Ekici, Karin Leunen, Mieke Kriege, Simon S. Cross, Laura Baglietto, Christof Sohn, Xianshu Wang, Vesa Kataja, Anne-Lise Børresen-Dale, Andreas Meyer, Douglas F. Easton, Marjanka K. Schmidt, and Stig E. Bojesen

Author affiliations appear at the end of this article.

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Corresponding author: Stig E. Bojesen, MD, PhD, DMSci, Department of Clinical Biochemistry, Herlev Hospital, Copenhagen University Hospital, Herlev Ringvej 75, DK-2730 Herlev, Denmark; e-mail: stig.egil.bojesen@regionh.dk.

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ABSTRACT

Purpose
We tested the hypotheses that *CHEK2**1100delC heterozygosity is associated with increased risk of early death, breast cancer–specific death, and risk of a second breast cancer in women with a first breast cancer.

Patients and Methods
From 22 studies participating in the Breast Cancer Association Consortium, 25,571 white women with invasive breast cancer were genotyped for *CHEK2**1100delC and observed for up to 20 years (median, 6.6 years). We examined risk of early death and breast cancer–specific death by estrogen receptor status and risk of a second breast cancer after a first breast cancer in prospective studies.

Results
*CHEK2**1100delC heterozygosity was found in 459 patients (1.8%). In women with estrogen receptor–positive breast cancer, multifactorially adjusted hazard ratios for heterozygotes versus noncarriers were 1.43 (95% CI, 1.12 to 1.82; log-rank $P = .004$) for early death and 1.63 (95% CI, 1.24 to 2.15; log-rank $P < .001$) for breast cancer–specific death. In all women, hazard ratio for a second breast cancer was 2.77 (95% CI, 2.00 to 3.83; log-rank $P < .001$) increasing to 3.52 (95% CI, 2.35 to 5.27; log-rank $P < .001$) in women with estrogen receptor–positive first breast cancer only.

Conclusion
Among women with estrogen receptor–positive breast cancer, *CHEK2**1100delC heterozygosity was associated with a 1.4-fold risk of early death, a 1.6-fold risk of breast cancer–specific death, and a 3.5-fold risk of a second breast cancer. This is one of the few examples of a genetic factor that influences long-term prognosis being documented in an extensive series of women with breast cancer.

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INTRODUCTION

Breast cancer is the most common cancer among women. Treatment and clinical management of breast cancer has improved considerably during the last few decades, but breast cancer remains a potentially fatal disease, partly because the prognostication at diagnosis is still insufficient. Thus, there is need to identify biomarkers associated with poor prognosis and to adjust surveillance in women at risk accordingly.

*CHEK2**1100delC is a founder mutation carried by 0.5% to 1.6% of individuals of Northern and Eastern European descent.[1,2] It is inherited from the parents and present in the germline DNA, is not a tumor marker, and encodes a truncated CHEK2 protein. In the cell nucleus, normal CHEK2 is activated in response to DNA double-strand breakage,

and this protein controls cell cycle, DNA repair, and apoptosis.[3,4] Individuals heterozygous for *CHEK2**1100delC have a two- to three-fold increased risk of breast cancer.[1,2,5-9] However, it is unknown whether *CHEK2**1100delC heterozygous women with breast cancer differ in their prognosis from noncarriers.

Here we examined the overall risk of early death, risk of breast cancer–specific death, and risk of a second breast cancer in *CHEK2**1100delC heterozygotes and noncarriers in 25,571 white women of Northern and Eastern European descent who had breast cancer by using data from 22 studies conducted in 12 countries (Appendix Table A1, online only). We also re-estimated the risk of (first) breast cancer in 25,571 breast cancer cases and 30,056 controls.

PATIENTS AND METHODS

Participant Selection and Study Design

Prospective design. From the studies participating in the Breast Cancer Association Consortium, we included women who had been tested for *CHEK2**1100delC, including noncarriers or heterozygotes for *CHEK2**1100delC (Appendix Table A1; Appendix Fig A1, online only). The majority of the samples were genotyped in a single, prospective experiment by using a standard assay (see Table A1, Genotyping, online only). Women of self-reported non-European ancestry were excluded. Women with a first invasive breast cancer were eligible for inclusion if information was available on death, breast cancer–specific death, or diagnosis of a second breast cancer during follow-up. Second breast cancer was defined as a contralateral breast cancer. Only 14 *CHEK2**1100delC homozygotes were identified, and these were excluded a priori from all analyses. For this study, we included 25,571 women for whom there was available information on early death, for 24,345 women on breast cancer–specific death, and for 25,094 on diagnosis of a second breast cancer (Appendix Fig A1).

Case-control design. For the case-control analysis, each participating study included controls from the same population as previously described (Appendix Table A1). The 22 participating studies included 30,056 controls.

Genotyping. Participants from 21 of the 22 studies were genotyped by using the same TaqMan-based assay.[10] A 162-bp fragment flanking the *CHEK2**1100delC mutation was amplified by polymerase chain reaction by using forward primer 5′-GGCAGACTATGTTAATCTTTTTATTTTATGG-3′ and reverse primer 5′-CAAGAACTTCAGGCGCCAAGT-3′. *CHEK2**1100delC carrier status was detected by using the following probes: wild-type allele 5′-VIC-TTTAGATTACTGATTTTGGGC-3′ and mutated allele 5′-FAM-TTAGATTATGATTTTGGGCAC-3′. A positive, negative, and nontemplate control were included in each run. In the majority of studies, heterozygote status was validated by using sequencing.[9-11] Of all participants available for genotyping, 98.6% were successfully genotyped.

Statistical Analysis

We used the statistical software STATA (STATA/SE for Windows, version 12.1; STATA, College Station, TX). We used the χ^2 test for categorical characteristics and Kruskal-Wallis one-way analysis of variance for continuous characteristics to test for differences in epidemiologic and tumor characteristics between *CHEK2**1100delC heterozygotes and noncarriers.

Prospective studies. We plotted cumulative incidences of early death, breast cancer–specific death, and second breast cancer as a function of time after the first breast cancer diagnosis and tested for differences between *CHEK2**1100delC heterozygotes and noncarriers by using log-rank statistics. For breast cancer–specific death and second breast cancer, any death was considered as a competing event when plotting the cumulative incidence curves by using the Fine-Gray method. The women were observed from time of diagnosis of the first breast cancer. However, most studies included prevalent cases, and time under observation began from the date of blood sampling (left truncation). This provides a valid test of association and, provided the proportional hazards assumption is not violated, an unbiased estimate of the hazard ratio.[12] Follow-up ended at the end point of interest, death, or end of follow-up, whichever came first. We used Cox proportional hazard regression to calculate hazard ratios with 95% CIs for early death, breast cancer–specific death, and a second breast cancer. The proportional hazard assumption was assessed visually by plotting ln(−ln(survival)) versus ln(age). Hazard ratios for early death and breast cancer–specific death were stratified by study and were adjusted for epidemiologic and tumor characteristics: age at and year of diagnosis, family history (positive, negative, missing), body mass index (< 18.5, 18.5 to 24.9, 25.0 to 29.9, ≥ 30.0 kg/m^2, missing), menopausal status (premenopausal, postmenopausal, missing), tumor size (< 20, 20-50, > 50 cm, missing), lymph node status (positive, negative, missing), tumor differentiation grade (good, moderate, poor, missing), progesterone receptor status (positive, negative, missing), and human epidermal growth factor receptor 2 status (positive, negative, missing). In overall survival analyses, we adjusted for the changes in risk over time by estrogen receptor status[13] by modeling the effect of estrogen receptor status (positive, negative, missing) to change as a function of years of follow-up. In the multifactorially adjusted regression models, participants with missing information were assigned a missing value category. We tested for interactions between *CHEK2**1100delC and epidemiologic and tumor characteristics by using a likelihood ratio test, excluding women for whom the relevant information was missing. Hazard ratio for a second breast cancer was stratified by study and adjusted for age at and year of diagnosis of the first breast cancer, study, and family history.

Case-control study. We used logistic regression to calculate odds ratios with 95% CIs of a first breast cancer by *CHEK2**1100delC heterozygosity for each individual study, for all studies overall, separated by estrogen receptor status, adjusting for age at diagnosis of cases and age at ascertainment of controls on a continuous scale. We tested for heterogeneity across all studies and across estrogen receptor status by using the *metan* command.

Ethics

All studies were approved by their institutional review committees, and written informed consent was obtained from all participants.

RESULTS

Among 25,571 women with invasive breast cancer, 459 (1.8%) were *CHEK2**1100delC heterozygous and 25,112 (98.2%) were noncarriers (Table 1). Over a median follow-up period of 6.6 years, we observed 124 (27%) deaths, 100 (22%) breast cancer–specific deaths, and 40 (9%) second breast cancers among *CHEK2**1100delC heterozygotes; corresponding numbers among noncarriers were 4,864 (19%), 2,732 (11%), and 607 (2%), respectively. At the time of diagnosis, heterozygotes versus noncarriers were on average 4 years younger ($P < .001$), more often had a positive family history ($P < .001$), were more likely to be premenopausal ($P < .001$), and had a higher frequency of estrogen receptor–positive ($P < .001$) and progesterone receptor–positive ($P = .01$) tumors. We observed no differences in year of diagnosis, body mass index, tumor size, lymph node status, tumor grade, or human epidermal growth factor receptor 2 status between heterozygotes and noncarriers. Although the amount of missing data was substantial, the frequency of missing information was similar for most characteristics between *CHEK2**1100delC heterozygotes and noncarriers.

Analysis by Estrogen Receptor Status

The hazards of early death and breast cancer–specific death by estrogen receptor status were not proportional over time. Within each estrogen receptor stratum, however, the hazards by *CHEK2**1100delC carrier status were proportional; hence, subsequent analyses of these end points were performed separated by estrogen receptor status. There was no evidence for interaction between *CHEK2**1100delC

Table 1. Epidemiologic and Tumor Characteristics at Time of Diagnosis of the First Breast Cancer

Characteristic	*CHEK2**1100delC Genotype: Noncarriers No.	Noncarriers %	Heterozygotes No.	Heterozygotes %	*P*
No. of patients with breast cancer	25,112		459		
Age at diagnosis, years					< .001
Median	54		50		
IQR	46-63		43-59		
Year of diagnosis	2000		2000		.49
IQR	1996-2003		1997-2004		
Familial history					< .001
Negative	6,652	26	70	15	
Positive	2,531	10	60	13	
Missing	15,929	63	329	72	
Body mass index, kg/m^2					.94
< 18.5	347	1	5	1	
18.5-24.9	9,136	36	126	27	
25.0-29.9	6,479	26	89	19	
≥ 30.0	3,478	14	43	9	
Missing	5,672	23	196	43	
Menopausal status					< .001
Premenopausal	7,231	29	142	31	
Postmenopausal	13,101	52	142	31	
Missing	4,780	19	175	38	
Tumor size, mm					.65
< 20	10,769	43	182	40	
20-50	7,894	31	148	32	
> 50	717	3	13	3	
Missing	5,732	23	116	25	
Lymph node status					.60
Negative	8,853	35	141	31	
Positive	6,451	26	110	24	
Missing	9,808	39	208	45	
Tumor grade					.13
Well differentiated	4,641	18	71	15	
Moderately differentiated	9,634	38	191	42	
Poorly differentiated/undifferentiated	6,215	25	104	23	
Missing	4,622	18	93	20	
Estrogen receptor status					< .001
Positive	14,234	57	290	63	
Negative	4,320	17	41	9	
Missing	6,558	26	128	28	
Progesterone receptor status					
Positive	10,739	43	212	46	.01
Negative	5,864	23	83	18	
Missing	8,509	34	164	36	
Human epidermal growth factor receptor 2					
Positive	1,577	6	34	7	.69
Negative	6,420	26	128	28	
Missing	17,115	68	297	65	

NOTE. *P* values were calculated by using χ^2 test for categorical characteristics and Kruskal-Wallis one-way analysis of variance tests for age and calendar year of diagnosis, excluding patients with breast cancer who had missing values.
Abbreviation: IQR, interquartile range.

carrier status and estrogen receptor status on the risk of early death ($P = .39$) or on breast cancer–specific death ($P = .28$) when we excluded women with missing information on estrogen receptor status.

Early Death

Cumulative incidence and risk of early death, overall and separated by estrogen receptor status, following a first breast cancer by *CHEK2**1100delC carrier status is shown in Figure 1. Among women with estrogen receptor–positive breast cancer, *CHEK2**1100delC heterozygotes had increased incidence of early death compared with noncarriers (log-rank test $P = .004$) with a multifactorially adjusted hazard ratio of 1.43 (95% CI, 1.12 to 1.82). Among women with estrogen receptor–negative breast cancer, incidence of early death was similar for *CHEK2**1100delC heterozygotes and noncarriers

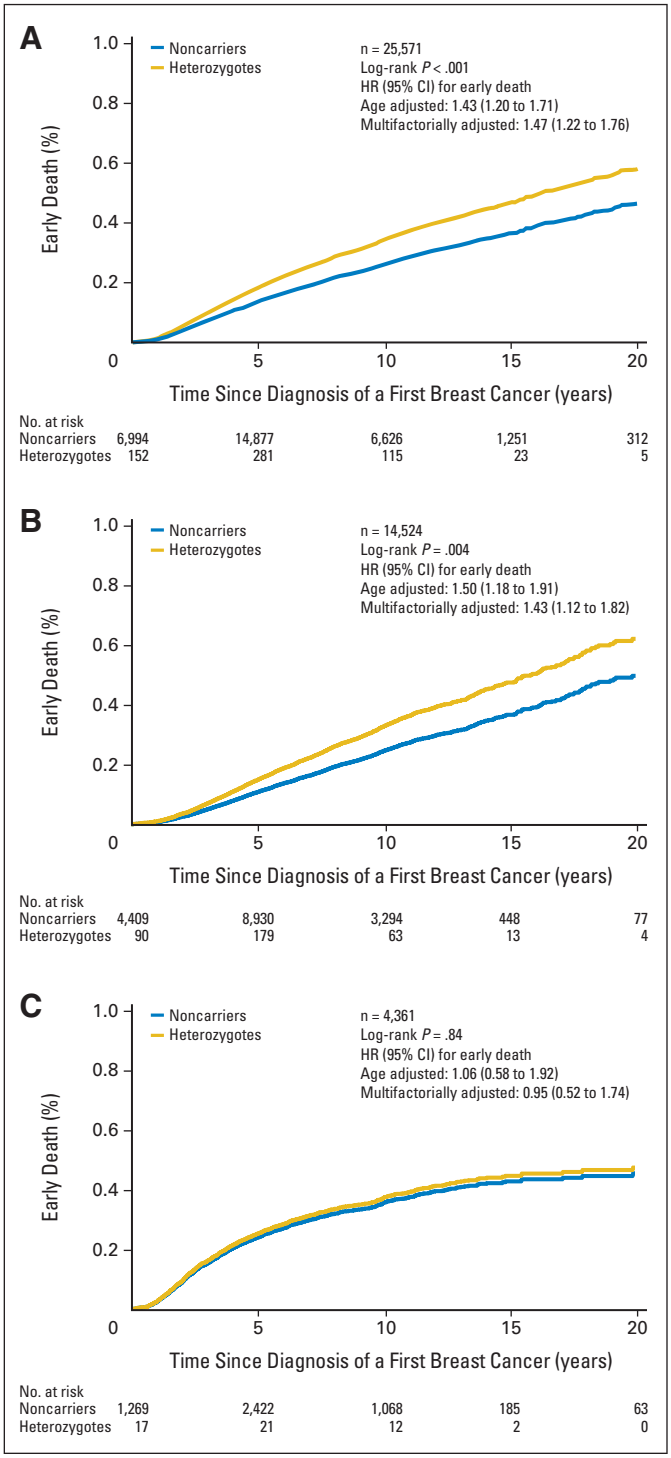

Fig 1. Cumulative incidence of early death according to *CHEK2**1100delC carrier status for all participants, separated by estrogen receptor status: (A) all patients; (B) estrogen receptor–positive patients; (C) estrogen receptor–negative patients. Patients were included at time of blood sampling following a first breast cancer and observed until death or end of follow-up, whichever came first. Multifactorially adjusted hazard ratio (HR) for early death in heterozygotes versus noncarriers stratified by study adjusted for age at diagnosis, year of diagnosis, body mass index, menopausal status, tumor size, lymph node status, progesterone receptor status, and human epidermal growth factor receptor 2.

(log-rank test $P = .84$) with a multifactorially adjusted hazard ratio of 0.95 (95% CI, 0.52 to 1.74).

In women with estrogen receptor–positive tumors, we observed no interactions for risk of early death between *CHEK2**1100delC carrier status and age at and year of diagnosis, family history, menopausal status, tumor size, lymph node status, tumor grade, or progesterone receptor status; we observed borderline significant interactions with body mass index ($P = .02$), lymph node status ($P = .05$), and human epidermal growth factor receptor 2 status ($P = .05$; Table 2); however, if these P values for tests of interaction were corrected for 10 parallel tests by using the Bonferroni method, none were significant (required P value = .05/10 = .005).

Breast Cancer–Specific Death

Cumulative incidence and risk of breast cancer–specific death, overall and separated by estrogen receptor status, following a first breast cancer by *CHEK2**1100delC carrier status is shown in Figure 2. Among women with estrogen receptor–positive breast cancer, *CHEK2**1100delC heterozygotes had increased risk of breast cancer–specific death compared with noncarriers (log-rank test $P < .001$) with a multifactorially adjusted hazard ratio of 1.63 (95% CI, 1.24 to 2.15). Among women with estrogen receptor–negative breast cancer, risk of breast cancer–specific death was similar for *CHEK2**1100delC heterozygotes and noncarriers (log-rank test $P = .71$) with a multifactorially adjusted hazard ratio of 1.09 (95% CI, 0.56 to 2.14).

Second Breast Cancer

Cumulative incidence and risk of second breast cancer, overall and separated by estrogen receptor status, following a first breast cancer by *CHEK2**1100delC carrier status is shown in Figure 3. Among women with estrogen receptor–positive breast cancer, *CHEK2**1100delC heterozygotes had an increased risk of second breast cancer compared with noncarriers (log-rank test $P < .001$) with a multifactorially adjusted hazard ratio of 3.52 (95% CI, 2.35 to 5.27). We observed no second breast cancers among *CHEK2**1100delC heterozygous women with estrogen receptor–negative first breast cancer; however, the cumulative incidence did not differ between *CHEK2**1100delC heterozygous and noncarriers (log-rank $P = .29$). A test for interaction between *CHEK2**1100delC and estrogen receptor status on risk of second breast cancer was not possible.

First Breast Cancer

We estimated the odds ratio of a first breast cancer for *CHEK2**1100delC heterozygotes versus noncarriers in 25,571 cases and 30,056 controls (Fig 4). Age-adjusted odds ratio of breast cancer for heterozygotes versus noncarriers was 3.01 (95% CI, 2.53 to 3.58) for all studies combined; the test for heterogeneity of estimates across studies gave $P = .06$. In analyses separated by estrogen receptor status, the corresponding odds ratios were 3.47 (95% CI, 2.87 to 4.18) for estrogen receptor–positive and 1.54 (95% CI, 1.09 to 2.17) for estrogen receptor–negative breast cancer; these two estimates were different ($P < .001$).

DISCUSSION

In 25,571 white women of European ancestry with a first breast cancer that was estrogen receptor–positive who were observed for a median of 6.6 years, we found that *CHEK2**1100delC heterozygosity was

Table 2. Risk of Early Death After a First Estrogen Receptor–Positive Breast Cancer, Separated by Epidemiologic and Tumor Characteristics

Characteristic	No. of Patients With Breast Cancer	No. of Deaths	*CHEK2**1100delC Heterozygotes *v* Noncarriers				Interaction Test *P*†
			Age Adjusted		Multifactorially Adjusted		
			HR	95% CI	HR	95% CI	
All	14,524	2,545	1.50	1.18 to 1.91	1.43	1.12 to 1.82	
Age at diagnosis, years							.64
< 53	6,040	1,011	1.38	0.99 to 1.92	1.29	0.92 to 1.80	
≥ 53	8,484	1,534	1.61	1.14 to 2.28	1.59	1.12 to 2.25	
Year of diagnosis							.24
Before 2000	6,395	1,498	1.74	1.30 to 2.33	1.67	1.24 to 2.24	
2000 or after	6,956	703	1.10	0.60 to 2.00	1.16	0.63 to 2.12	
Missing	1,173	344	1.35	0.74 to 2.48	1.28	0.70 to 2.37	
Family history							.13
Negative	4,416	843	1.49	0.84 to 2.64	1.59	0.89 to 2.84	
Positive	1,693	305	0.79	0.35 to 1.80	0.90	0.39 to 2.08	
Missing	8,415	1,397	1.73	1.31 to 2.29	1.52	1.15 to 2.01	
Body mass index, kg/m^2							.02
< 18.5	208	45	—		—		
18.5-24.9	5,483	808	2.19‡	1.44 to 3.34	2.38‡	1.56 to 3.65	
25.0-29.9	3,721	597	1.27	0.74 to 2.19	1.30	0.74 to 2.26	
≥ 30.0	1,933	385	2.43	1.21 to 4.50	2.31	1.21 to 4.41	
Missing	3,179	710	1.12	0.73 to 1.72	0.96	0.62 to 1.48	
Menopausal status							.46
Premenopausal	4,025	536	1.76	1.13 to 2.74	1.76	1.12 to 2.77	
Postmenopausal	8,209	1,450	1.60	1.09 to 2.35	1.48	1.00 to 2.17	
Missing	2,290	559	1.19	0.77 to 1.83	1.14	0.74 to 1.76	
Tumor size, mm							.70
< 20	7,389	860	1.31	0.83 to 2.07	1.24	0.79 to 1.98	
20-50	5,028	1,214	1.51	1.07 to 2.15	1.39	0.97 to 1.98	
> 50	412	146	1.29	0.48 to 3.44	1.37	0.47 to 4.04	
Missing	1,695	325	1.81	1.02 to 3.23	2.09	1.15 to 3.77	
Lymph node status							.05
Negative	5,697	673	1.14	0.64 to 2.03	1.13	0.63 to 2.02	
Positive	4,279	1,027	1.98	1.39 to 2.81	2.08	1.45 to 2.98	
Missing	4,548	845	1.31	0.88 to 1.96	1.10	0.74 to 1.65	
Tumor grade							.22
Well differentiated	3,355	380	2.17	1.18 to 4.01	2.46	1.33 to 4.56	
Moderately differentiated	6,566	1,067	1.53	1.07 to 2.20	1.55	1.08 to 2.23	
Poorly differentiated/undifferentiated	2,653	662	1.27	0.76 to 2.10	1.13	0.67 to 1.88	
Missing	1,950	436	1.19	0.66 to 2.13	1.12	0.61 to 2.03	
Progesterone receptor status							.71
Positive	10,210	1,640	1.44	1.06 to 1.95	1.38	1.01 to 1.87	
Negative	2,528	568	1.24	0.72 to 2.14	1.08	0.62 to 1.88	
Missing	1,786	337	2.07	1.14 to 3.77	2.08	1.12 to 3.86	
Human epidermal growth factor receptor 2							.05
Positive	989	168	0.57	0.18 to 1.81	0.49	0.15 to 1.61	
Negative	5,103	862	1.44	0.98 to 2.10	1.33	0.90 to 1.95	
Missing	8,432	1,515	1.71	1.24 to 2.37	1.73	1.25 to 2.41	

NOTE. Multifactorial adjustment included age at diagnosis, year of diagnosis, family history, body mass index, menopausal status, tumor size, lymph node status, tumor grade, progesterone receptor status, human epidermal growth factor receptor 2 status, while stratifying for study.

†*P* values are for test of interaction in the multifactorially adjusted model between *CHEK2**1100delC genotype and categories of characteristics with known values, while excluding women for whom the relevant information was missing.

‡No deaths among *CHEK2**1100delC heterozygotes in this subgroup.

associated with a 1.4-fold risk of early death, a 1.6-fold risk of breast cancer–specific death, and a 3.5-fold risk of a second breast cancer. The poorer survival in *CHEK2**1100delC heterozygotes has been suggested previously,[14,15] but this study provides much stronger evidence for this association and demonstrates that it is restricted to estrogen receptor–positive disease.[11,16,17] We also obtained an estimate for the relative risk of breast cancer in *CHEK2**1100delC heterozygotes similar to that estimated previously.[1,2,5-9] This is one of the few examples of a genetic factor influencing long-term prognosis documented in an extensive series of women with breast cancer, and it raises the possibility that other genetic factors influencing breast cancer prognosis could be identified, given sufficiently large, well-conducted studies.

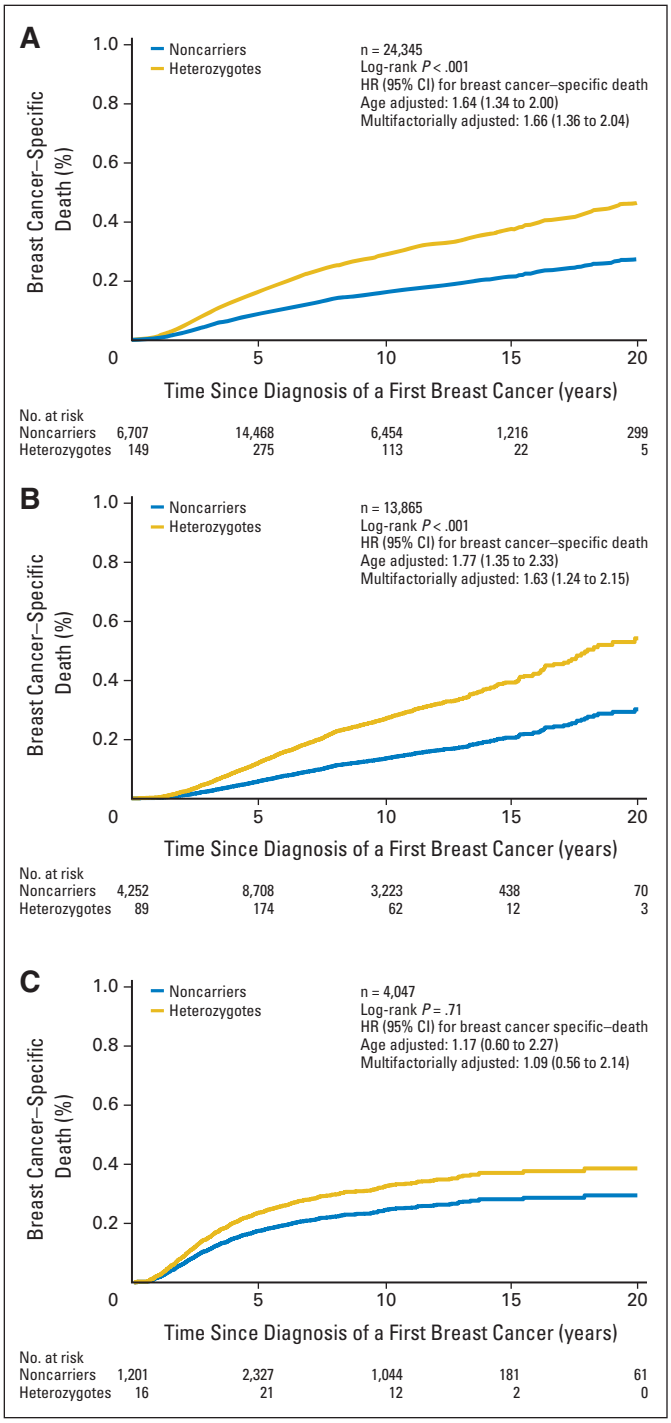

Fig 2. Cumulative incidence of breast cancer–specific death according to *CHEK2**1100delC carrier status for all participants, separated by estrogen receptor status: (A) all patients; (B) estrogen receptor–positive patients; (C) estrogen receptor–negative patients. Patients were included at time of blood sampling following a first breast cancer and were observed until death or end of follow-up, whichever came first. Other causes of death were considered as a competing event. Multifactorially adjusted hazard ratio (HR) for breast cancer–specific death in heterozygotes versus noncarriers stratified by study and adjusted for age at diagnosis, year of diagnosis, body mass index, menopausal status, tumor size, lymph node status, progesterone receptor status, and human epidermal growth factor receptor 2.

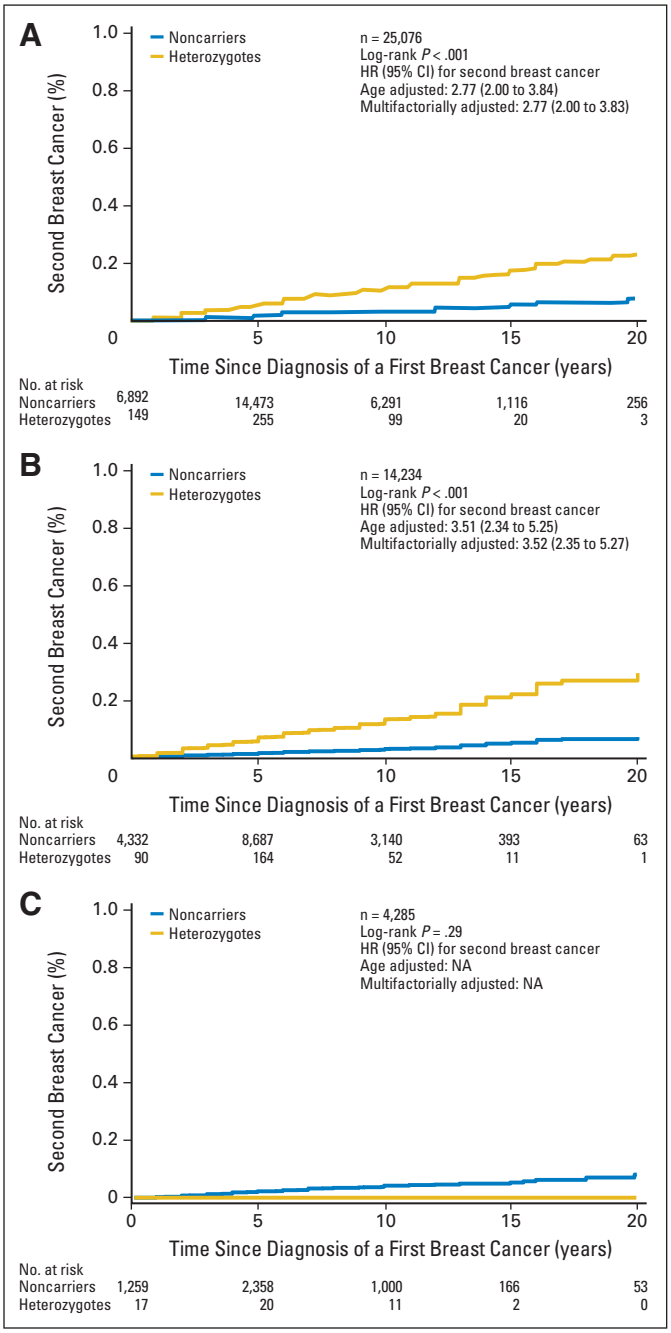

Fig 3. Cumulative incidence of second breast cancer according to *CHEK2**1100delC carrier status for all participants, separated by estrogen receptor status: (A) all patients; (B) estrogen receptor–positive patients; (C) estrogen receptor–negative patients. Patients were included at time of blood sampling following a first breast cancer and were observed until death, diagnosis of a second breast cancer, or end of follow-up, whichever came first. Any death was considered as a competing event. Multifactorially adjusted hazard ratio (HR) for second breast cancer in heterozygotes versus noncarriers stratified by study was adjusted for age at diagnosis of the first breast cancer, study, year of diagnosis of the first breast cancer, and family history. Because we observed no second breast cancers among the 41 *CHEK2**1100delC heterozygous women with estrogen receptor–negative first breast cancer, an HR could not be calculated in these women. N/A, not applicable.

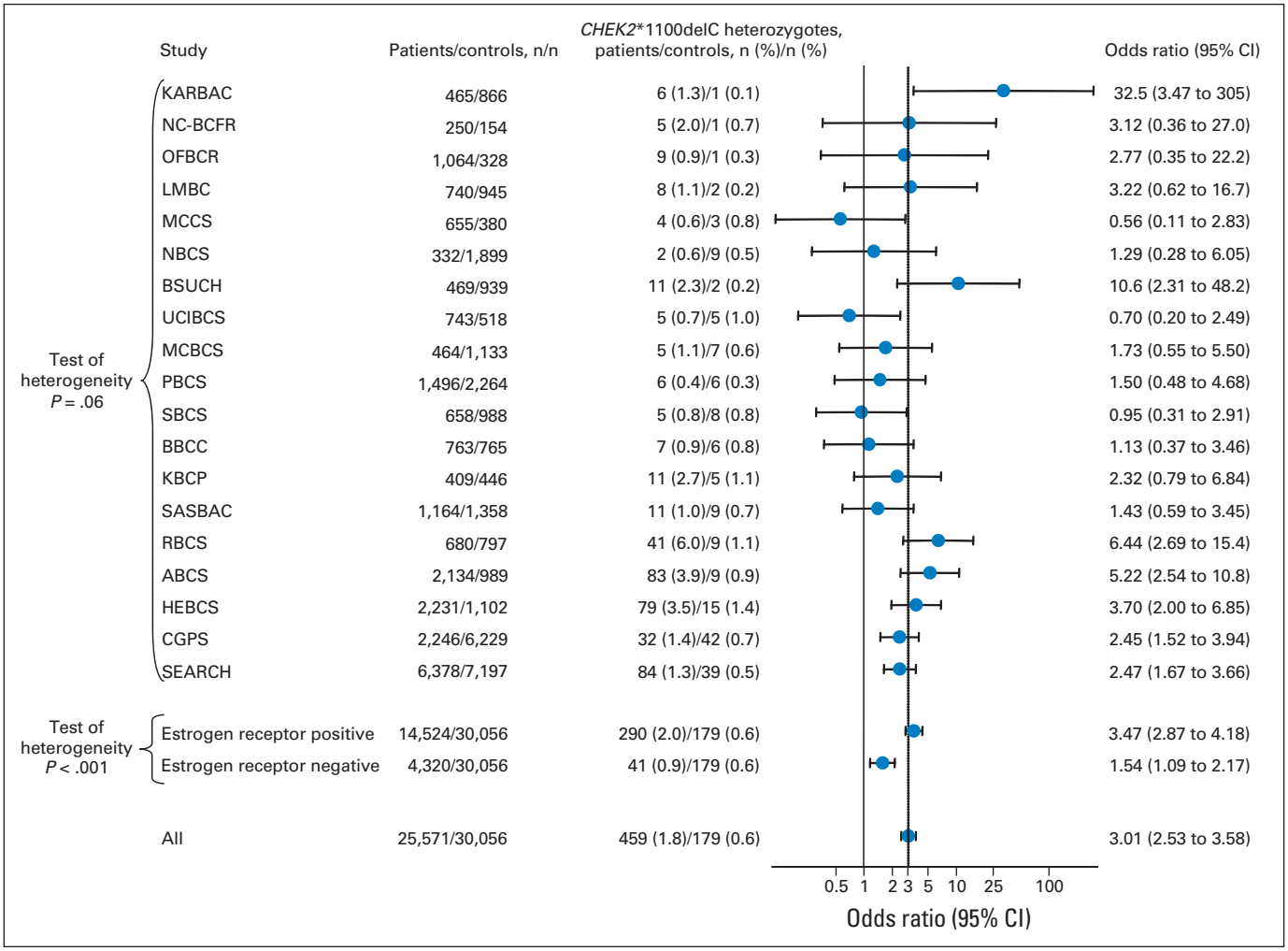

Fig 4. Risk of a first breast cancer by *CHEK2**1100delC carrier status in individual studies ranked by statistical power for all studies combined, separated by estrogen receptor status. The combined odds ratios were adjusted for age at diagnosis (cases) or ascertainment (controls). The combined studies odds ratio included participants from all 22 studies, including Australian Breast Cancer Family Study (ABCFS) and Leiden University Medical Centre Breast Cancer Study (ORIGO), which are not shown individually. These two studies had no heterozygous controls, and therefore odds ratios could not be calculated for these individual studies. Odds ratio was not calculated for Hannover Breast Cancer Study (HABCS) because their case series with follow-up data was strongly biased toward *CHEK2* mutation carriers.[15] ABCS, Amsterdam Breast Cancer Study; BBCC, Bavarian Breast Cancer Cases and Controls; BSUCH, Breast Cancer Study of the University Clinic of Heidelberg; CGPS, Copenhagen General Population Study; HEBCS, Helsinki Breast Cancer Study; KARBAC, Karolinska Breast Cancer Study; KBCP, Kuopio Breast Cancer Project; LMBC, Leuven Multidisciplinary Breast Centre; MCBCS, Mayo Clinic Breast Cancer Study; MCCS, Melbourne Collaborative Cohort Study; NBCS, Norwegian Breast Cancer Study; NC-BCFR, Northern California Breast Cancer Family Registry; OFBCR, Ontario Familial Breast Cancer Registry; PBCS, National Cancer Institute Polish Breast Cancer Study; RBCS, Rotterdam Breast Cancer Study; SASBAC, Singapore and Sweden Breast Cancer Study; SBCS, Sheffield Breast Cancer Study; SEARCH, Study of Epidemiology and Risk Factors in Cancer Heredity; UCIBCS, University of California at Irvine Breast Cancer Study.

The 2.8-fold risk of a second breast cancer in all *CHEK2**1100delC heterozygous versus noncarrier women was similar to the estimated odds ratios of a first breast cancer in a previous meta-analysis[1] and the odds ratio found in this study. This finding is supported by other smaller studies of selected patients with breast cancer. The increased risk of second breast cancer is believed to be largely a result of inherited susceptibility, and this result is consistent with the model that *CHEK2**1100delC combines with other risk factors to confer increased susceptibility.[11,16-18]

Strengths of this study include the large sample size and the participation of 22 centers in 12 countries. Moreover, the large majority of genotypes were generated in a single experiment by using one single assay, minimizing the possibility for bias as a result of differential genotyping by disease status. Furthermore, the long duration of follow-up and the detailed records on a second breast cancer and death allowed us to observe the associations between *CHEK2**1100delC carrier status and these end points beyond the usually reported 5 years after diagnosis of a first breast cancer.

One factor limiting the clinical application of our finding is that *CHEK2**1100delC appears to be confined to white individuals of Northern or Eastern European origin, and our findings are therefore unlikely to be directly applicable to populations with other origins. Other inactivating *CHEK2* mutations have been reported in other populations, but further studies would be required to confirm whether these are also associated with a poor prognosis. In addition,

65% of the women were missing human epidermal growth factor receptor 2 status, 25% to 30% estrogen receptor status, 40% to 45% lymph node status, 25% tumor size, and 60% to 70% family history, all characteristics known to be associated with survival. Importantly, however, the frequency of the missing information was similar between *CHEK2**1100delC heterozygotes and noncarriers for most of these characteristics, as expected, given that *CHEK2**1100delC was unknown to the woman and her physician when the information was collected and that germline genotypes are distributed at random during meiosis and therefore typically are not confounded by lifestyle or treatment.[19,20] Therefore, although the amount of missing data has limited our statistical power in some analyses, we do not believe that it reflects inherent biases likely to distort our results. Another important limitation of the study is the absence of treatment information. It is therefore theoretically possible that the reason for the lack of a significant survival difference in the estrogen receptor–negative group in *CHEK2**1100delC heterozygotes versus noncarriers is that whatever negative prognostic impact heterozygosity has, it is overcome by the administration of chemotherapy specific for this group. However, any choice of therapy was blinded to *CHEK2**1100delC status. Finally, the number of second breast cancers is small, which might indicate insufficient ascertainment but may also indicate that the number is unlikely to have been inflated by recurrences registered as second breast cancers.

These results raise the question of whether *CHEK2**1100delC testing should be offered to white women of Northern or Eastern European descent with an estrogen receptor–positive first breast cancer. The high risk of a second breast cancer is comparable to that at which women with a strong family history of breast cancer would be offered prophylactic surgery. However, this observational study cannot provide a specific recommendation on whether prophylactic surgery is warranted. Prolonged duration of antiestrogen therapy might be warranted, particularly since it may result in a substantial reduction in the risk of second cancer. Furthermore, in *CHEK2**1100delC heterozygous versus noncarrier women, the risk of an estrogen receptor–positive breast cancer was three-fold for both the first and second breast cancer, although the effect was less pronounced for estrogen receptor–negative breast cancer. This finding is supported by an earlier study[14] and could have implications for prevention of breast cancer in *CHEK2**1100delC heterozygous women. An analysis of treatment subgroups and *CHEK2**1100delC status would hopefully provide insight into the mechanism, and therefore potentially affect clinical use of *CHEK2**1100delC mutation testing. It would also have been of interest to know whether the estrogen receptor–positive breast cancers of *CHEK2**1100delC heterozygotes were highly proliferative since women with these breast cancers have a relatively poor prognosis despite their tumors being estrogen receptor–positive. Although the test of interaction between tumor grade and *CHEK2**1100delC status was insignificant for the women with estrogen receptor–positive breast cancers, the highest hazard ratios for early death were found for the well-differentiated tumors. Further studies should focus on the mechanism(s) behind the present observations and hopefully will provide sufficient evidence to guide prophylaxis and treatment of *CHEK2**1100delC heterozygous women.

In conclusion, approximately one in 50 women with breast cancer is *CHEK2**1100delC heterozygous, and testing for this mutation can identify women at increased risk of early death or breast cancer–specific death and of developing a second breast cancer. This is one of the few examples of a genetic factor that influences long-term prognosis being documented in an extensive series of women with breast cancer.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Maren Weischer, Børge G. Nordestgaard, Stig E. Bojesen
Financial support: Børge G. Nordestgaard, Paul Pharoah, Per Hall, Douglas F. Easton
Administrative support: Manjeet K. Bolla, Qin Wang, Douglas F. Easton
Provision of study materials or patients: All authors
Collection and assembly of data: All authors
Data analysis and interpretation: Maren Weischer, Børge G. Nordestgaard, Paul Pharoah, Douglas F. Easton, Marjanka K. Schmidt, Stig E. Bojesen
Manuscript writing: All authors
Final approval of manuscript: All authors

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Affiliations

Maren Weischer, Børge G. Nordestgaard, Henrik Flyger, and Stig E. Bojesen, University of Copenhagen, Copenhagen, Denmark; Paul Pharoah, Manjeet K. Bolla, Alison M. Dunning, Qin Wang, Mitul Shah, and Douglas F. Easton, University of Cambridge, Cambridge; Montserrat Garcia-Closas, Institute of Cancer Research, London; Angela Cox, Malcolm W.R. Reed, and Simon S. Cross, University of Sheffield, Sheffield, United Kingdom; Heli Nevanlinna, Taru A. Muranen, and Carl Blomqvist, University of Helsinki and Helsinki University Central Hospital, Helsinki; Arto Mannermaa, Veli-Matti Kosma, and Vesa Kataja, University of Eastern Finland; Arto Mannermaa, Veli-Matti Kosma, and Vesa Kataja, Kuopio University Hospital, Kuopio, Finland; Laura J. van 't Veer, Richard van Hien, Annegien Broeks, and Marjanka K. Schmidt, Netherlands Cancer Institute, Amsterdam; Peter Devilee and Rob A.E.M. Tollenaar, Leiden University Medical Center, Leiden; Maartje Hooning, Johanna Margriet Collée, and Mieke Kriege, Erasmus University Medical Center; Caroline Seynaeve, Erasmus Medical Center-Daniel den Hoed Cancer Center, Rotterdam, the Netherlands; John L. Hopper, Graham G. Giles, Melissa C. Southey, Gianluca Severi, and Laura Baglietto, University of Melbourne; Graham G. Giles, Gianluca Severi, and Laura Baglietto, the Cancer Council Victoria, Melbourne; Kelly-Anne Phillips, Peter MacCallum Cancer Centre and University of Melbourne, East Melbourne, Victoria, Australia; Per Hall, Annika Lindblom, Kamila Czene, and Sara Margolin, Karolinska Institutttet, Stockholm, Sweden; Irene L. Andrulis, Julia A. Knight, and Gord Glendon, Mount Sinai Hospital, Toronto, Ontario, Canada; Peter A. Fasching, Matthias W. Beckmann, and Arif B. Ekici, Friedrich-Alexander University Erlangen-Nuremberg; Peter A. Fasching and Matthias W. Beckmann, Comprehensive Cancer Center Erlangen-Nuremberg, Erlangen; Barbara Burwinkel, Frederik Marme, and Christof Sohn, University of Heidelberg; Barbara Burwinkel, German Cancer Research Center, Heidelberg; Thilo Dörk, Natalia Bogdanova, and Andreas Meyer, Hannover Medical School, Hannover, Germany; Peter A. Fasching, University of California at Los Angeles, Los Angeles; Hoda Anton-Culver and Argyrios Ziogas, University of California Irvine, Irvine; Esther M. John and Vessela N. Kristensen, Cancer Prevention Institute of California, Fremont, CA; Diether Lambrechts, Vesalius Research Center, Vlaams Instituut voor Biotechnologie and Katholieke Universiteit (KU) Leuven; Marie-Rose Christiaens and Karin Leunen, University Hospital Leuven, KU Leuven, Leuven, Belgium; Fergus J. Couch, Janet E. Olson, and Xianshu Wang, Mayo Clinic, Rochester, MN; Grethe Grenaker Alnæs and Anne-Lise Børresen-Dale, Norwegian Radium Hospital, Oslo University Hospital, University of Oslo, Oslo, Norway; Jonine Figueroa and Mark Sherman, National Cancer Institute, Bethesda, MD; Alexander Miron, Dana-Farber Cancer Institute, Boston, MA; and Jingmei Li and Jianjun Liu, Genome Institute of Singapore, Singapore.

