Survival and contralateral breast cancer in *CHEK2* 1100delC breast cancer patients: impact of adjuvant chemotherapy

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Background: We assessed the sensitivity to adjuvant chemotherapy in *cell cycle checkpoint kinase 2* (*CHEK2*) vs non-*CHEK2* breast cancer patients by comparing the contralateral breast cancer incidence and distant disease-free and breast cancer-specific survival between both groups, stratified for adjuvant chemotherapy.

Methods: One Dutch hereditary non-*BRCA1/2* breast cancer patient cohort ($n = 1220$) and two Dutch cohorts unselected for family history ($n = 1014$ and $n = 2488$, respectively) were genotyped for *CHEK2* 1100delC. Hazard ratios for contralateral breast cancer, distant disease-free and breast cancer-specific death for mutation carriers vs noncarriers were calculated using the Cox proportional hazard method, stratified for adjuvant chemotherapy.

Results: The *CHEK2* mutation carriers ($n = 193$) had an increased incidence of contralateral breast cancer (multivariate hazard ratio 3.97, 95% confidence interval 2.59–6.07). Distant disease-free and breast cancer-specific survival were similar in the first 6 years in mutation carriers compared with noncarriers, but diverted as of 6 years after breast cancer diagnosis (multivariate hazard ratios and 95% confidence intervals 2.65 (1.79–3.93) and 2.05 (1.41–2.99), respectively). No significant interaction between *CHEK2* and adjuvant chemotherapy was observed.

Conclusions: The *CHEK2* 1100delC-associated breast cancer is associated with a higher contralateral breast cancer rate as well as worse survival measures beyond 6 years after diagnosis. No differential sensitivity to adjuvant chemotherapy was observed in *CHEK2* patients.

In 2002, the *cell cycle checkpoint kinase 2* (*CHEK2*) gene was identified as the first moderate-risk susceptibility gene for breast cancer. The *CHEK2* 1100delC mutation has been associated with a two- to three-fold increased risk of developing breast cancer in women (Meijers-Heijboer *et al*, 2002; The CHEK2-Breast Cancer Consortium, 2002; Weischer *et al*, 2008; Cybulski *et al*, 2011). The frequency of the *CHEK2* 1100delC mutation is relatively high in the Netherlands, being ∼1% in the general population, 2–4% in unselected breast cancer patients and 5–6% in breast cancer

patients with a family history of breast cancer (The CHEK2-Breast Cancer Consortium, 2002; The CHEK2 Breast Cancer Case-Control Consortium, 2004; Hollestelle *et al*, 2010).

The CHEK2 kinase acts as a tumour suppressor and plays a critical role in DNA damage repair. Because of a compromised DNA repair mechanism, it has been hypothesised that *CHEK2* mutation carriers might have an increased sensitivity to chemotherapeutic agents causing double-strand DNA breaks, such as platinum or anthracyclines (Nevanlinna and Bartek, 2006).

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Previous studies showed that *CHEK2* 1100delC-associated breast cancer patients had mainly oestrogen receptor (ER)-positive tumours (de Bock *et al*, 2006; Nagel *et al*, 2012; Weischer *et al*, 2012), and a higher incidence of contralateral breast cancer compared with noncarriers (de Bock *et al*, 2004; Schmidt *et al*, 2007; Weischer *et al*, 2012). In addition, a worse disease-free and overall survival has been observed in *CHEK2* 1100delC-associated breast cancer patients compared with patients without this mutation (de Bock *et al*, 2004; Schmidt *et al*, 2007; Weischer *et al*, 2012). In the overall breast cancer population, the risk of contralateral breast cancer is reduced by adjuvant chemotherapy (Bertelsen *et al*, 2008; Reding *et al*, 2010). Data on the impact of adjuvant chemotherapy on the contralateral breast cancer risk and survival of *CHEK2* 1100delC-associated breast cancer patients *vs* noncarriers have not been reported yet. If *CHEK2* 1100delC mutation carriers are indeed more sensitive to chemotherapy due to a defective DNA repair mechanism, the differences in contralateral breast cancer incidence and in distant disease-free and breast cancer-specific survival in *CHEK2* mutation carriers compared with noncarriers would be most pronounced in breast cancer patients not treated with adjuvant chemotherapy.

In this study we therefore evaluated the contralateral breast cancer rate, distant disease-free survival and breast cancer-specific survival in breast cancer patients with the *CHEK2* 1100delC mutation compared with patients without this mutation, stratified for adjuvant treatment with chemotherapy.

MATERIALS AND METHODS

Three different cohorts of women with invasive early breast cancer were included in this study. Overall inclusion criteria were: age at diagnosis of first breast cancer < 80 years, diagnosis of first breast cancer after 1970, no distant metastases at or within 3 months after primary diagnosis, no proven *BRCA1* or *BRCA2* mutation and follow-up data available.

The first cohort (*n* = 1220) consisted of breast cancer patients ascertained through the Rotterdam Family Cancer Clinic. All selected patients were tested negative for *BRCA1* and *BRCA2* mutations. We refer to this cohort as the 'non-*BRCA1/2*' cohort.

The second cohort (*n* = 1014, after exclusion of 64 patients already included in the non-*BRCA1/2* cohort) consisted of patients enrolled in the ORIGO study, designed to investigate the percentage of *BRCA1/2* mutations in an unselected breast cancer population. This study concerned a consecutive series of breast cancer patients. Data on contralateral breast cancer rate and survival for *CHEK2* mutation carriers *vs* non-carriers have been previously described (de Bock *et al*, 2004). We refer to this cohort as 'ORIGO'.

The third cohort was drawn from the Rotterdam Medical Oncology Tumour bank (RMOT) database (*n* = 2488, after exclusion of 114 patients already included in the non-*BRCA1/2* cohort and 1 patient already included in the ORIGO cohort). The RMOT database included breast cancer patients mainly diagnosed in the South Western region of the Netherlands from whom fresh frozen breast tumour specimens were submitted to the Erasmus MC for routine ER and progesterone receptor (PgR) measurement between 1978 and 2004. We refer to this cohort as 'RMOT'.

Part of the non-*BRCA1/2* cohort (57%) and the entire ORIGO cohort were also included in the study of Weischer *et al* (2012), investigating survival and contralateral breast cancer risk in *CHEK2* 1100delC mutation carriers. For the current study, follow-up of the patients in both cohorts has been updated to July 2010.

For all eligible patients of the three cohorts, data on patient and tumour characteristics, treatment and follow-up were collected from the medical records. The study was approved by the Institutional Review Board.

Genotyping. DNA was isolated from peripheral blood for patients from the non-*BRCA1/2* and ORIGO cohorts and from freshly frozen tumour tissue for patients from the RMOT cohort (The CHEK2 Breast Cancer Case-Control Consortium, 2004; Riaz *et al*, 2012). The *CHEK2* 1100delC mutation status was determined by either allele-specific oligonucleotide hybridisation or Taqman genotyping as described earlier (Meijers-Heijboer *et al*, 2002; Jekimovs *et al*, 2005). A number of samples (*n* = 783) were analysed with both methods, showing a concordance of 100% between the two methods.

Statistical analyses. Differences in patient and tumour characteristics and types of treatment between *CHEK2* 1100delC mutation carriers and noncarriers were compared by *t*-test (continuous variables) or χ^2 test (categorical variables). Events of interest were metachronous contralateral breast cancer (diagnosed > 3 months after diagnosis of the first breast cancer), distant metastases and breast cancer-specific death. For analyses regarding metachronous contralateral breast cancer, the non-*BRCA1/2* cohort was excluded, because this cohort probably is enriched with cases with bilateral breast cancer cases as contralateral breast cancer is one of the criteria for referral for genetic testing for *BRCA1/2* mutations and therefore might overestimate the contralateral breast cancer incidence. Kaplan–Meier curves were constructed for contralateral breast cancer rate, distant disease-free survival and breast cancer-specific survival for *CHEK2* 1100delC mutation carriers and noncarriers separately. Censoring events overall were death not related to breast cancer, last medical contact or end of this study (1 July 2010), and for contralateral breast cancer rate it also included death because of breast cancer and preventive bilateral or contralateral mastectomy. Differences in outcome between *CHEK2* 1100delC mutation carriers and noncarriers were tested by the log rank test.

We calculated univariate and multivariate hazard ratios (HRs) and 95% confidence intervals (CIs) for the above-mentioned events for mutation carriers *vs* noncarriers by the Cox proportional hazard method. Models were adjusted for patient, tumour and

Table 1. The *CHEK2* 1100delC status in the three different breast cancer patient cohorts

Age	Total *n*	Carriers		Noncarriers	
		n	%	*n*	%
Non-*BRCA1/2* familial					
All	1220	77	6.3	1143	93.7
< 40	409	23	5.6	386	94.4
40–50	474	26	5.5	448	94.5
⩾ 50	337	28	8.3	309	91.7
ORIGO (unselected for family history)					
All	1014	29	2.9	985	97.1
< 40	106	3	2.8	103	97.2
40–50	283	11	3.9	272	96.1
⩾ 50	625	15	2.4	610	97.6
RMOT (unselected for family history)					
All	2488	87	3.5	2401	96.5
< 40	236	11	4.7	225	95.3
40–50	628	22	3.5	606	96.1
⩾ 50	1624	54	3.3	1570	96.7
Total	4722	193	4.1	4529	95.9

Abbreviations: *CHEK2* = cell cycle checkpoint kinase 2; RMOT = Rotterdam Medical Oncology Tumour bank.

treatment factors, including age at and year of diagnosis, cohort (non-*BRCA1/2*, ORIGO, RMOT), tumour size (T1, T2, T3 or T4, unknown), nodal status (negative, positive, unknown), hormone receptor status (negative, positive, unknown), differentiation grade (1, 2, 3, unknown), type of surgery (lumpectomy, mastectomy, none or unknown), adjuvant chemotherapy (yes, no, unknown), adjuvant hormonal therapy (yes, no, unknown) and radiotherapy (yes, no, unknown), and the models for distant disease-free and breast cancer-specific survival also for contralateral breast cancer as time-dependent variable. Tumour ER and PgR analyses were

performed by ligand binding assay or enzyme-linked immunoas-say. The cutoff for ER and PgR positivity was $10\,fmol\,mg^{-1}$ cytosolic protein or $\geqslant 10\%$ immunopositive cells. A negative hormone receptor status was defined as negativity for both ER and PgR, and a positive hormone receptor status as positivity for ER and/or PgR. Hormone receptor status was considered as unknown if information on both ER and PgR status was lacking, or in case of negative ER and unknown PgR status.

The proportional hazard assumption was assessed by using an extended Cox model with time-depending covariables. A likelihood

Table 2. Patient and tumour characteristics of *cell cycle checkpoint kinase 2* (*CHEK2*) 1100delC mutation carriers and noncarriers					
	Carrier		**Noncarrier**		
	n	%	*n*	%	*P*-value
Number of patients	193		4529		
Median follow-up in years	6.8 (0.6–31.9)		7.2 (0.1–38.9)		
Age at breast cancer diagnosis					
Median (years)	50.0		51.4		0.003
Mean (years)	50.1		52.8		
Range (years)	22.5–78.0		18.7–79.9		
<40	37	19.2	714	15.8	0.14
40–50	59	30.6	1326	29.3	
⩾50	97	50.3	2489	55.0	
Year of diagnosis					
<1990	58	30.1	1590	35.1	0.04
1990–2000	83	43.0	1996	44.1	
>2000	52	26.9	943	20.8	
Synchronous contralateral breast cancer					
No	189	97.9	4443	98.1	0.87
Yes	4	2.1	86	1.9	
T stage					
T1	95	51.9	2074	47.2	0.23
T2	72	39.3	1886	42.9	
T3	11	6.0	264	6.0	
T4	5	2.7	168	3.8	
Unknown	10	(5.2)	137	(3.0)	
N stage					
Negative	105	57.1	2388	53.9	0.31
1–3	35	19.0	1096	24.7	
4+	44	23.9	949	21.4	
Unknown	9	(4.7)	96	(2.1)	
Differentiation grade					
1	21	14.7	370	10.5	0.11
2	52	36.4	1155	32.6	
3	70	49.0	2014	56.9	
Unknown	50	(25.9)	990	(21.9)	
Hormone receptor status					
Negative	9	5.5	759	19.3	<0.001
Positive	157	94.5	3173	80.7	
Unknown	30	(15.5)	597	(13.2)	
HER2/neu					
Negative	73	76.0	1886	80.7	0.26
Positive	23	24.0	452	19.3	
Unknown	97	(50.2)	2191	(48.3)	

ratio test was used to test whether an extended model was significantly different compared with the proportional hazard model. The extended models for *CHEK2* and tumour size were significant for both distant metastases ($P = 0.003$ and $P = 0.002$, respectively) and breast cancer-specific death ($P = 0.02$ and $P = 0.04$), indicating that the hazards for distant metastases and breast cancer-specific death by *CHEK2* 1100delC and tumour size were not proportional over time. Because the survival curves for distant disease-free and breast cancer-specific survival for *CHEK2* mutation carriers and noncarriers were similar over the first 6 years, but started to diverge after 6 years (Figure 2A and D), we chose for an extended Cox model with tumour size as time-dependent variable and that contains a heaviside function (Kleinbaum and Klein, 2005), together with the *CHEK2* 1100delC variable, with a cutoff point of 6 years. The corresponding model then provided two hazard ratios for *CHEK2* 1100delC, one for the first 6 years and one for beyond 6 years after breast cancer diagnosis.

Analyses for contralateral breast cancer distant disease-free and breast cancer-specific survival were stratified for adjuvant treatment with chemotherapy (yes/no). Analyses for distant disease-free survival were also stratified for type of adjuvant chemotherapy (anthracycline-based/nonanthracycline-based) and adjuvant hormonal therapy (yes/no).

Interaction terms between *CHEK2* 1100delC mutation status and administration of adjuvant chemotherapy (yes/no) for the different end points were calculated. In addition, the prognostic impact of age at diagnosis, tumour size, nodal status, ER status, chemotherapy and hormonal therapy were analysed separately for *CHEK2* 1100delC and non-*CHEK2* patients.

All analyses were performed using SPSS (version 20.0; SPSS, Inc., Chigago, IL, USA) and Stata (version 12; Stat Corporation, College Station, TX, USA). A two-sided $P < 0.05$ was considered as statistically significant.

RESULTS

In total, 193 (4.1%) of the 4722 included female breast cancer patients tested positive for the *CHEK2* 1100delC mutation (Table 1). The percentage of *CHEK2* 1100delC mutations was highest in the non-*BRCA1/2* cohort (6.3%, $n = 77$) *vs* 2.9% ($n = 29$) in the ORIGO and 3.5% ($n = 87$) in the RMOT cohort, respectively.

Patient and tumour characteristics of the 193 *CHEK2* 1100delC mutation carriers and the 4529 non-carriers are shown in Table 2. The median follow-up time was 6.8 years for mutation carriers and 7.2 years for noncarriers. The *CHEK2* 1100delC-associated breast cancer patients were significantly younger at diagnosis than the noncarriers (median 50.0 *vs* 51.4 years). In addition, mutation carriers were more often diagnosed with breast cancer after 2000 (26.9% *vs* 20.8%), probably reflecting the higher percentage of *CHEK2* 1100delC mutation carriers in the non-*BRCA1/2* cohort, as patients in this cohort were more often diagnosed after 2000 (51%) compared with patients from the RMOT cohort ($<1\%$) and ORIGO cohort (37%; data not shown in tables). Breast cancers in mutation carriers were more often hormone receptor positive compared with cancers in noncarriers (94.5% *vs* 80.7%). No significant differences in tumour size, nodal status, differentiation grade and her2 expression were observed between *CHEK2* 1100delC mutation carriers and noncarriers.

Types of treatment are shown in Table 3. Significantly less *CHEK2*-associated breast cancer patients received radiotherapy compared with the noncarriers (62.0% *vs* 71.3%). This observation could not be explained by differences in surgical therapy (lumpectomy *vs* mastectomy), TN stage or age at diagnosis, but might be partly explained by differences in the year of diagnosis between the two groups (see Table 2). Adjuvant chemotherapy was

administered to 31% of the patients, mainly consisting of anthracycline-containing regimens, and 24% of the patients received adjuvant endocrine therapy, mainly consisting of tamoxifen. This was not significant different between *CHEK2* mutation carriers and noncarriers.

Contralateral breast cancer risk. The incidence of metachronous contralateral breast cancer was higher in *CHEK2* 1100delC mutation carriers than in noncarriers, with a 10-year risk of 28.9% *vs* 8.5%, respectively (Table 4 and Figure 1). In a multivariate analysis, the risk of contralateral breast cancer remained higher in mutation carriers compared with noncarriers (HR 3.97, 95% CI 2.59–6.07).

If stratified for adjuvant chemotherapy, the increased contralateral breast cancer rate for *CHEK2* 1100delC-associated breast cancer patients was observed in both the group treated with and without chemotherapy (HR multivariate 6.95, 95% CI 3.21–15.00 and 3.15, 95% CI 1.86–5.35, respectively). Although the HR was higher for

Table 3. Types of treatment in *CHEK2* 1100delC mutation carriers and noncarriers

	Carrier		Noncarrier		
	n	%	*n*	%	*P*-value
Surgical therapy					
None	0	0	10	0.2	0.80
Lumpectomy	88	47.1	2087	46.4	
Mastectomy	99	52.9	2405	53.4	
Unknown	6		27		
Adjuvant systemic therapy					
None	117	63.2	2646	59.3	0.42
Only chemotherapy	32	17.3	826	18.5	
Only hormonal therapy	12	6.5	441	9.9	
Chemo- and hormonal therapy	24	13.0	547	12.3	
Unknown	8		69		
(Neo)-adjuvant chemotherapy					
None	129	69.4	3089	69.0	0.41
Anthracyclines	38	20.4	838	18.7	
Anthracyclines/taxanes	0	0	68	1.5	
Other	16	8.6	435[a]	9.7	
Unknown type	3	1.6	45	1.0	
Unknown if treated	7		54		
Adjuvant hormonal therapy					
None	143	77.3	3392	76.0	0.46
LHRH/ other	6	3.2	84[b]	1.9	
Tamoxifen	33	17.8	812	18.2	
Aromatase inhibitor	1	0.5	29	0.6	
Sequential tamoxifen/aromatase inhibitor	2	1.1	120	2.7	
Unknown type	0	0	27	0.6	
Unknown if treated	8		65		
Radiotherapy					
No	73	38.0	1298	28.7	0.006
Yes	119	62.0	3220	71.3	
Unknown	1		11		

Abbreviations: *CHEK2* = *cell cycle checkpoint kinase 2*; LHRH = luteinising-hormone-releasing hormone.
[a]Four patients received taxanes.
[b]Four patients were treated with radiotherapy of the ovaries or oophorectomy.

patients treated with adjuvant chemotherapy, no significant interaction between the *CHEK2* 1100delC mutation and adjuvant chemotherapy was observed (HR for interaction 2.15; $P = 0.10$; not shown in Tables). Stratifying for adjuvant endocrine therapy was not possible because only 19 *CHEK2* 1100delC-associated breast cancer patients were treated with hormonal therapy. No contralateral breast cancers were observed in these 19 patients.

Survival. The data regarding distant disease-free survival are shown in Table 5 and Figure 2A–C and regarding breast cancer-specific survival in Table 5 and Figure 2D–F. The Kaplan–Meier curves showed a nonsignificant decreased distant disease-free ($P = 0.06$) and breast cancer-specific survival ($P = 0.14$) for *CHEK2*-associated patients compared with non-*CHEK2* patients (Figure 2A and D).

As can be observed in the Kaplan–Meier curves, the distant disease-free (Figure 2A) and breast cancer-specific survival (Figure 2D) were similar over the first 6 years after breast cancer diagnosis for *CHEK2* 1100delC mutation carriers and noncarriers and started to diverge after 6 years. In the first 6 years after breast cancer diagnosis, the multivariate hazard ratios for *CHEK2* vs non-*CHEK2* breast cancer patients were not significant for distant disease-free (1.08; 95% CI 0.83–1.42) and breast cancer-specific survival (1.00, 95% CI 0.71–1.41). Survival outcomes beyond 6 years after breast cancer diagnosis, however, were significantly worse for *CHEK2* mutation carriers compared with noncarriers. Multivariate HRs for the period beyond 6 years after breast cancer diagnosis were 2.65 (95% CI 1.79–3.93) for distant disease-free and 2.05 (95% CI 1.41–2.99) for breast cancer-specific survival, respectively.

If we stratified our analyses for adjuvant chemotherapy, a significantly worse distant disease-free ($P = 0.002$) and a trend for a worse breast cancer-specific survival ($P = 0.05$) were observed for *CHEK2*-associated compared with non-*CHEK2*-associated breast cancer patients in the chemotherapy group (Figure 2C and F) that was not observed in the group not treated with chemotherapy ($P = 0.52$ and $P = 0.40$, respectively, Figure 2B and E). However, in both treatment groups, the group treated with and the group treated without adjuvant chemotherapy, we observed in the first 6 years after breast cancer diagnosis no significant differences in distant disease-free survival between *CHEK2* mutation carries and noncarriers (HR multivariate 1.41, 95% CI 0.91–2.16 and HR

multivariate 0.98, 95% CI 0.69–1.38, respectively; Table 5), whereas beyond 6 years after breast cancer diagnosis, a significantly worse distant disease-free survival in *CHEK2* mutation carriers compared with noncarriers was observed for both the adjuvant chemotherapy group (HR 4.54, 95% CI 2.41–8.65) and the group without adjuvant chemotherapy (2.31, 95% CI 1.39–3.83). Although the HRs were higher in the adjuvant chemotherapy group compared with the group without adjuvant chemotherapy, no significant interaction was observed between *CHEK2* 1100delC and adjuvant chemotherapy (HR interaction 1.51; $P = 0.08$; data not shown in tables).

If analysing patients treated with anthracycline-based chemotherapy and nonanthracycline-based chemotherapy separately, we observed in both groups a significantly worse distant disease-free survival beyond 6 years after breast cancer diagnosis for *CHEK2* mutation carriers compared with noncarriers (HR multivariate 4.16, 95% CI 1.69–10.22 and 4.73 95% CI 1.85–12.09, respectively; Table 5).

In addition, stratifying for treatment with hormonal therapy (yes/no) had no major impact on the hazard ratio for disease-free survival. The *CHEK2*-associated breast cancer patients had a significantly worse distant disease-free survival than noncarriers beyond 6 years after diagnosis, independently of treatment with adjuvant hormonal therapy (HR 3.24, 95% CI 1.16–9.03) or without adjuvant hormonal therapy (HR 2.88, 95% CI 1.88–4.41; Table 5).

For breast cancer-specific survival a similar phenomenon was observed; only beyond 6 years after breast cancer diagnosis a significantly worse breast cancer-specific survival was observed for *CHEK2* mutation carriers vs noncarriers in both adjuvant chemotherapy groups (i.e., the group treated with and without adjuvant chemotherapy). In the first 6 years after breast cancer diagnosis, we observed no significant difference in breast cancer-specific survival between *CHEK2* mutation carriers and noncarriers (Figure 2D–F and Table 5).

In Table 6, multivariate HRs with respect to breast cancer-specific survival are presented for several prognostic factors, separately for *CHEK2* and non-*CHEK2* patients. Age at diagnosis, tumour size and nodal status appeared to be prognostic factors in both groups, although the prognostic value of age at diagnosis was not significant in the *CHEK2* group. Interestingly, ER status appeared only to be a prognostic factor in the non-*CHEK2* group

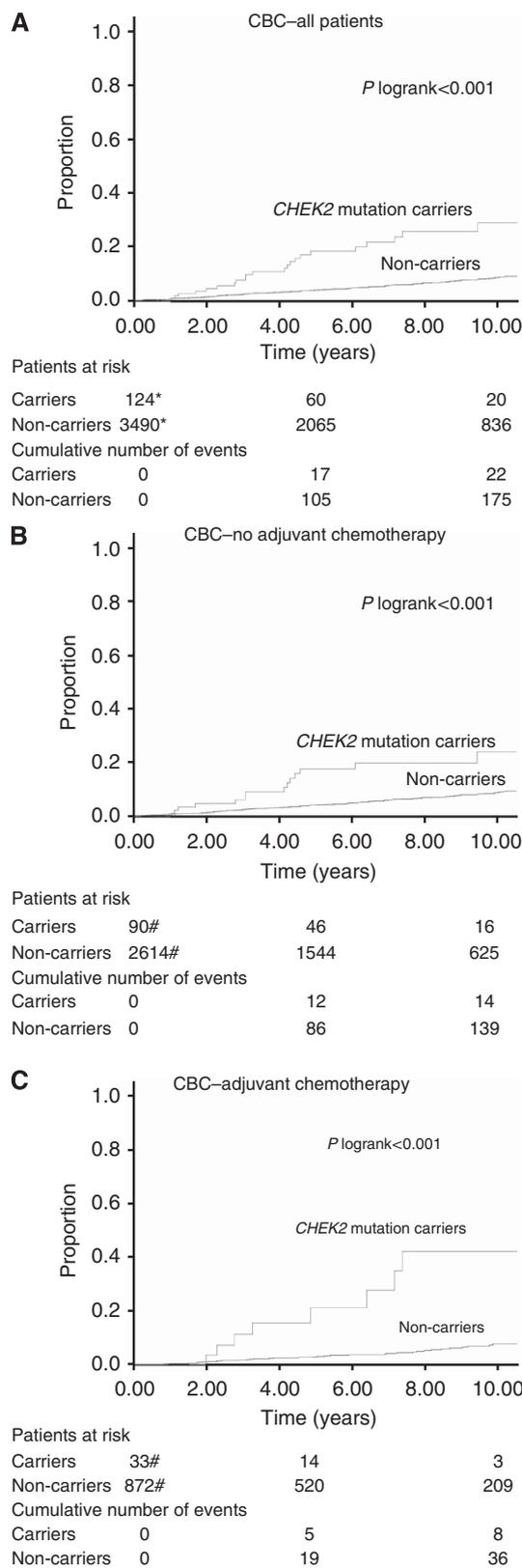| Table 4. Rate of metachronous contralateral breast cancer[a]					
	Carrier	**Noncarrier**		**HR univariate**	**HR multivariate**
	% (95% CI)	**% (95% CI)**	**P-value**	**HR (95% CI)**	**HR (95% CI)**
All patients					
5 Years	18.3 (11.7–28.0)	3.8 (3.1–4.5)	<0.001	4.07 (2.66–6.21)	3.97 (2.59–6.07)[b]
10 Years	28.9 (19.1–42.1)	8.5 (7.2–9.7)			
No adjuvant chemotherapy					
5 Years	17.7 (10.3–29.3)	4.2 (2.3–5.0)	<0.001	3.18 (1.87–5.40)	3.15 (1.86–5.35)[b]
10 Years	24.1 (14.1–39.2)	8.8 (7.3–10.2)			
Adjuvant chemotherapy					
5 Years	21.9 (9.1–42.2)	2.8 (1.7–4.2)	<0.001	7.35 (3.42–15.08)	6.95 (3.21–15.00)[b]
10 Years	42.1 (22.6–77.4)	7.4 (5.3–10.6)			

Abbreviations: CHEK2 = cell cycle checkpoint kinase 2; CI = confidence interval; HR = hazard ratio.
[a] For analyses regarding contralateral breast cancer (CBC), the non-BRCA1/2 cohort was excluded, because this cohort was probably enriched with patients with CBC.
[b] Adjusted for year of diagnosis, cohort and type of surgery. Other covariates, that is, age at diagnosis, tumour size, nodal status, hormone receptor status, differentiation grade, type of surgery, type of chemotherapy, hormonal therapy and radiotherapy, had an impact of <10% on the HR.

(HR 0.58, 95% CI 0.45–0.75) and not in the *CHEK2* group (HR 1.11, 95% CI 0.43–2.84). Administration of adjuvant chemotherapy (HR 0.70 95% CI 0.35–1.41) and of adjuvant hormonal therapy (HR 0.62, 95% CI 0.30–1.28) had a nonsignificant favourable effect on breast cancer-specific survival in the *CHEK2* group.

A CBC–all patients

Patients at risk

Carriers	124*	60	20
Non-carriers	3490*	2065	836

Cumulative number of events

Carriers	0	17	22
Non-carriers	0	105	175

B CBC–no adjuvant chemotherapy

Patients at risk

Carriers	90#	46	16
Non-carriers	2614#	1544	625

Cumulative number of events

Carriers	0	12	14
Non-carriers	0	86	139

C CBC–adjuvant chemotherapy

Patients at risk

Carriers	33#	14	3
Non-carriers	872#	520	209

Cumulative number of events

Carriers	0	5	8
Non-carriers	0	19	36

DISCUSSION

In this study, we observed a worse distant disease-free and breast cancer-specific survival for *CHEK2* 1100delC-associated compared with noncarrier patients, becoming apparent beyond 6 years after breast cancer diagnosis. Furthermore, we confirmed the higher incidence of contralateral breast cancer in breast cancer patients carrying the *CHEK2* 1100delC mutation, as observed in earlier studies (de Bock *et al*, 2004; Schmidt *et al*, 2007; Weischer *et al*, 2012). Both observations were not influenced by chemotherapy, suggesting no differential efficacy of chemotherapy in *CHEK2* 1100delC mutation carriers.

Interestingly, the worse distant disease-free and breast cancer-specific survival in *CHEK2* 1100delC mutation carriers was only observed beyond 6 years after breast cancer diagnosis, rather than immediately after breast cancer diagnosis. Although earlier studies also observed or suggested a worse (distant disease-free) survival in *CHEK2* 1100delC mutation carriers (de Bock *et al*, 2004; Schmidt *et al*, 2007; Weischer *et al*, 2012), none of these studies showed the phenomenon that we observed. In view of these discordant results, we further performed analyses in the three patient cohorts, and found that the trend for a worse distant disease-free and breast cancer-specific survival beyond 6 years was only observed in the RMOT cohort, whereas in the other two cohorts the survival became worse as of diagnosis (significant for the ORIGO cohort and nonsignificant for the non-*BRCA1/2* cohort; data not shown). A reason for this different observation in the RMOT compared with the non-*BRCA1/2* and ORIGO cohorts might be different patient characteristics in the RMOT cohort, such as the higher age at diagnosis (median 55 years compared with 44 and 52 years, respectively) and earlier diagnose years, resulting in a lower percentage of patients treated with adjuvant chemotherapy (19% *vs* 52% and 37%, respectively) and adjuvant hormonal therapy (14% *vs* 36% and 35%, respectively) and other types of treatment (more often CMF and less often anthracycline-based or taxane-based regimens). As adjuvant hormonal therapy is generally given for a period of 5 years, it can be hypothesised that the worse survival beyond 6 years in *CHEK2*-associated breast cancer patients might be an effect of the hormonal therapy. However, evidence for this hypothesis is not found in this study as we observed in both groups (i.e., the group treated with and the group treated without adjuvant hormonal therapy) a worse survival in *CHEK2* mutation carriers compared with noncarriers more than 6 years after breast cancer diagnosis. It can also be hypothesised that the worse distant disease-free and breast cancer-specific survival for *CHEK2* mutation carriers after 6 years is caused by the higher incidence of contralateral breast cancer in this group. However, this worse distant disease-free and breast cancer-specific survival remained if we censored at time of metachronous contralateral breast cancer and/or local recurrences (data not shown) and if we included contralateral breast cancer as time-dependent covariable in the extended Cox models, indicating that the observed worse survival measures are not only due to the higher incidence of contralateral breast cancer and/or local recurrences in the *CHEK2* group. As the

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Figure 1. Contralateral breast cancer rate for *CHEK2*-positive (green line) and *CHEK2*-negative patients (blue line) in (A) all patients, (B) patients not treated with systemic therapy and (C) patients treated with systemic therapy. *Patients with synchronous contralateral breast cancer and patients from the non-BRCA1/2 cohort were excluded for this analysis regarding metachronous contralateral breast cancer.
#In (B and C), stratified for adjuvant chemotherapy, one additional carrier and four noncarriers for whom treatment with adjuvant chemotherapy was unknown were excluded. The full colour version of this figure is available at *British Journal of Cancer* online.

Table 5. Distant disease-free and breast cancer-specific survival				
	HR <6 years after diagnosis		HR ⩾6 years after diagnosis	
	Univariate	Multivariate	Univariate	Multivariate
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Distant disease-free survival				
All patients	1.02 (0.78–1.33)	1.08 (0.83–1.42)[a]	2.15 (1.45–3.18)	2.65 (1.79–3.93)[a]
No adjuvant chemotherapy	0.90 (0.64–1.27)	0.98 (0.69–1.38)[b]	1.98 (1.19–3.28)	2.31 (1.39–3.83)[b]
Adjuvant chemotherapy	1.38 (0.90–2.13)	1.41 (0.91–2.16)[c]	3.64 (1.94–6.81)	4.54 (2.41–8.56)[c]
Anthracycline-based chemotherapy	1.58 (0.92–2.72)	1.59 (0.92–2.75)[c]	3.64 (1.55–8.55)	4.16 (1.69–10.22)[c]
Nonanthracycline-based chemotherapy	1.23 (0.61–2.50)	1.16 (0.57–2.35)[c]	3.76 (1.49–9.47)	4.73 (1.85–12.09)[c]
No adjuvant hormonal therapy	1.01 (0.75–1.36)	1.08 (0.80–1.45)[a]	2.43 (1.59–3.71)	2.88 (1.88–4.41)[a]
Adjuvant hormonal therapy	1.22 (0.66–2.23)	1.36 (0.74–2.51)[a]	2.79 (1.01–7.73)	3.24 (1.16–9.03)[a]
Breast cancer-specific survival				
All patients	0.96 (0.68–1.35)	1.00 (0.71–1.41)[b]	1.75 (1.20–2.54)	2.05 (1.41–2.99)[b]
No adjuvant chemotherapy	0.92 (0.60–1.41)	1.00 (0.65–1.53)[b]	1.68 (1.03–2.73)	2.03 (1.25–3.32)[b]
Adjuvant chemotherapy	1.10 (0.61–1.97)	1.05 (0.59–1.88)[b]	2.33 (1.29–4.21)	2.55 (1.40–4.64)[b]

Abbreviations: CI = confidence interval; HR = hazard ratio.
[a] Adjusted for tumour size and chemotherapy, and other covariates had an impact of <10% on the HR.
[b] Adjusted for tumour size, and other covariates had an impact of <10% on the HR.
[c] Adjusted for tumour size and hormonal therapy, and other covariates had an impact of <10% on the HR.

worse distant disease-free and breast cancer-specific survival cannot be explained by a reduced sensitivity to systemic therapy and by the higher incidence of contralateral breast cancer, we suppose that a different tumour biology might play a role. More research is needed into tumour biology of *CHEK2* breast cancers.

The CHEK2 kinase acts as a tumour suppressor and plays a critical role in DNA damage repair. In response to double-strand DNA breaks, CHEK2 is activated by ataxia telangiectasia mutated (ATM) and involved in cell cycle control, DNA repair and apoptosis. The CHEK2 kinase phosphorylates TP53 and BRCA1, whereupon BRCA1 represses the nonhomologous end-joining pathway and activates the homologous recombination repair pathway (Nevanlinna and Bartek, 2006; Tung and Silver, 2011; Roeb *et al*, 2012). Based on the role of CHEK2 kinase in activating the repair mechanism of double-strand DNA breaks and the role of CHEK2 in the coordination of the mitotic spindle assembly, we hypothesised that *CHEK2* 1100delC mutation carriers might be more sensitive to chemotherapy. For *BRCA1*-associated breast cancer patients, there is growing evidence from *in vitro* studies on BRCA1-deficient cells and retrospective clinical studies on *BRCA1* mutation carriers suggesting a higher sensitivity to chemotherapeutic agents causing double-strand DNA breaks, such as platinum or anthracyclines (Kriege *et al*, 2009; Bayraktar and Gluck, 2012). However, the results found in the current study do not support the hypothesis that *CHEK2* mutation carriers have an increased sensitivity to chemotherapy, neither for anthracycline-based regimens nor for nonanthracycline-based regimens. In contrast, the observations on the worse distant disease-free and breast cancer-specific survival and the higher contralateral breast cancer rate in *CHEK2* mutation carriers being most pronounced in the group treated with chemotherapy suggest that *CHEK2* mutation carriers are rather less than more sensitive to chemotherapy compared with noncarrier breast cancer patients. However, as the interaction between *CHEK2* and adjuvant chemotherapy is not significant, we cannot prove a decreased sensitivity to adjuvant chemotherapy in *CHEK2* breast cancer patients. It might be that differences in patient and tumour characteristics between the groups treated with and without adjuvant chemotherapy may have influenced the results of the

analyses stratified for adjuvant chemotherapy. Patients treated with adjuvant chemotherapy were younger at diagnosis, were more often diagnosed after 2000 (anthracycline-based chemotherapy being common), had a larger tumour size, had more often node positive and hormone receptor negative tumours and were more often treated with mastectomy and hormonal therapy than patients not treated with adjuvant chemotherapy. It might be that especially younger or node-positive *CHEK2*-associated breast cancer patients have a worse prognosis compared with noncarriers.

The increased risk of contralateral breast cancer in *CHEK2* 1100delC mutation carriers was observed in patients treated with adjuvant chemotherapy as well as in patients not receiving adjuvant chemotherapy. A factor to take into account hereby is radiotherapy, as it was suggested that *CHEK2* mutation carriers might be more sensitive to ionising radiation (Broeks *et al*, 2004, 2007; Mellemkjaer *et al*, 2008). However, in multivariate analyses we found an increased risk of contralateral breast cancer in both patients treated with (HR 4.12 95% CI 2.49–6.83) and without radiotherapy (HR 3.17 95% CI 1.36–7.35; data not shown).

The strengths of our study include the relatively large sample size, the known tumour and treatment data and the homogeneous population from one country. To our knowledge, this is in fact the largest study on survival in *CHEK2* 1100delC breast cancer patients with available treatment data enabling to investigate the effect of adjuvant chemotherapy in this data set.

We are however aware of some limitations. First, this is a retrospective study and consequently treatment was provided on indication, according to the Dutch guidelines existing for the respectively treatment years. Although treatment guidelines have changed over time, in general, patients with the worst prognosis (based on age at diagnosis and tumour stage/characteristics) received adjuvant chemotherapy. This probably is the reason why patients receiving adjuvant chemotherapy had a worse distant disease-free survival (median 6.5 and 15.0 years for *CHEK2* mutation carriers and noncarriers, respectively; Figure 2C) compared with patients not receiving adjuvant chemotherapy (11.0 and 16.8 years for mutation carriers and noncarriers, respectively; Figure 2B).

Another possible limitation is longevity bias in the non-*BRCA1/2* group, developed by selection of preferably long-living patients
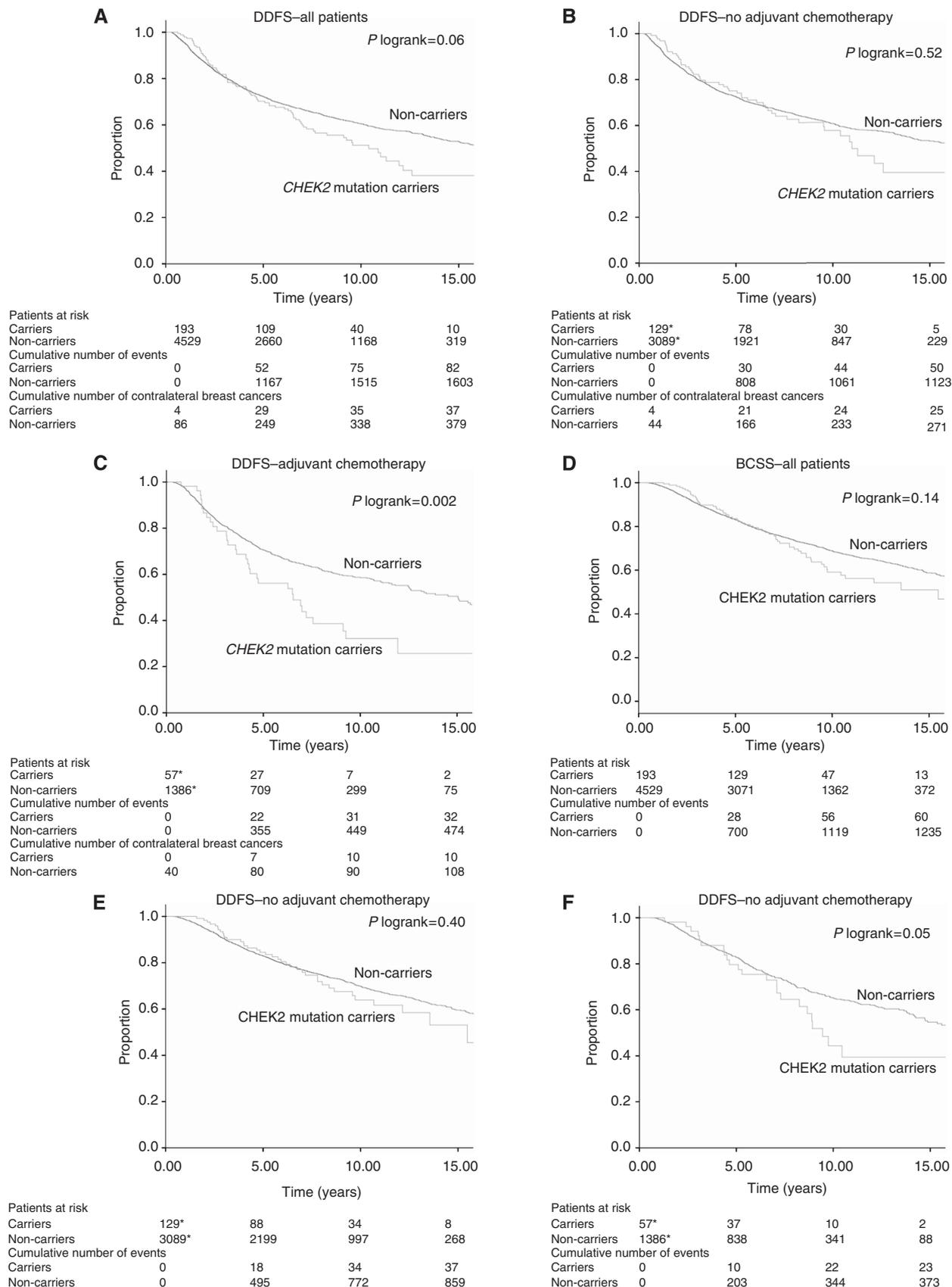
Figure 2. Distant disease-free survival for *CHEK2*-positive (green line) and *CHEK2*-negative patients (blue line) in (**A**) all patients, (**B**) patients not treated with adjuvant chemotherapy and (**C**) patients treated with adjuvant chemotherapy, and breast cancer-specific survival for *CHEK2*-positive (green line) and *CHEK2*-negative patients (blue line) in (**D**) all patients, (**E**) patients not treated with adjuvant chemotherapy and (**F**) patients treated with adjuvant chemotherapy. *In (**B**, **C**, **E** and **F**) stratified for adjuvant chemotherapy, an additional 7 carriers and 54 noncarriers for whom treatment with adjuvant chemotherapy was unknown were excluded.

Table 6. Prognostic factors for breast cancer-specific survival, separately for the *CHEK2* and non-*CHEK2* group

	Carrier	Noncarrier
	HR (95% CI)	HR (95% CI)
Age at diagnosis		
Per year older	0.99 (0.97–1.01)	0.99 (0.99–0.996)
Tumour size		
T1	1	1
T2, 3 and 4	4.29 (2.34–7.87)	2.46 (1.98–3.06)
Tumour size × time (in years)		0.95 (0.92–0.98)
Nodal status		
Negative	1	1
Positive	2.28 (1.17–4.46)	2.72 (2.36–3.15)
ER status		
Negative	1	1
Positive	1.11 (0.43–2.84)	0.58 (0.45–0.75)
Chemotherapy		
No	1	1
Yes	0.70 (0.35–1.41)	0.68 (0.58–0.80)
Hormonal therapy		
No	1	1
Yes	0.62 (0.30–1.28)	0.89 (0.76–1.05)

Abbreviations: *CHEK2* = *cell cycle checkpoint kinase 2*; CI = confidence interval; ER = oestrogen receptor; HR = hazard ratio.

who were tested for *BRCA1/2* mutations (long) after their breast cancer diagnosis. After stratifying for cohort, we indeed observed the longest survival within the non-*BRCA1/2* cohort. However, we also performed left-truncated analyses, and the results were similar as those from the initial analyses, with a worse distant disease-free and breast cancer-specific survival beyond 6 years after diagnosis for *CHEK2* mutation carriers compared with noncarriers (data not shown).

Finally, genetic testing for *CHEK2* 1100delC within the RMOT cohort was performed on DNA isolated from tumour tissue and not from peripheral blood. It is possible that some germline *CHEK2* 1100delC mutations were missed because the mutant *CHEK2* allele was lost in the tumour instead of the wild-type allele (Sodha *et al*, 2002; Kilpivaara *et al*, 2003). Moreover, a detected *CHEK2* 1100delC mutation in the tumour might have been acquired somatically, being different from a germline mutation. Importantly, there were no discrepancies in DNA analyses test results of *CHEK2* status observed when comparing blood and tumour sample test of 115 patients.

Currently, genotyping for *CHEK2* is not yet performed at genetic testing for breast cancer as it is not considered to have clinical consequences. Moreover, implementation of *CHEK2* genotyping (and especially presymptomatic testing of relatives) is complicated by the observation that in breast cancer families the breast cancer risk does not segregate completely with the presence or absence of the 1100delC mutation (The CHEK2 Breast Cancer Case-Control Consortium, 2004). However, the high incidence of contralateral breast cancer in *CHEK2* 1100delC mutation carriers found in the current study (10-year risk 24.1%) and in earlier studies is comparable to the incidence of contralateral breast cancer that we observed in *BRCA1* and *BRCA2* mutation carriers in an

earlier study (10-year risk 25% and 20%, respectively) (Brekelmans *et al*, 2006) and in line with observations of other groups (10-year risk ranging between 13 and 37%) (Haffty *et al*, 2002; Metcalfe *et al*, 2004; Robson *et al*, 2005; Pierce *et al*, 2006; Graeser *et al*, 2009; van der Kolk *et al*, 2010). Based on this increased contralateral breast cancer rate, affected *BRCA1/2* mutation carriers have a more intensive follow-up scheme for contralateral breast cancer detection including MRI or can opt for a risk-reducing contralateral mastectomy, even though it is unknown whether such a risk-reducing contralateral mastectomy can improve survival (Tung, 2011). In our opinion, this follow-up regimen should also be offered to *CHEK2* 1100delC mutation carriers after breast cancer diagnosis. In addition, as the population frequency of the *CHEK2* 1100delC mutation in the Netherlands is high, it may be relevant to discuss the implementation of genotyping for *CHEK2* in breast cancer patients. Moreover, genetic testing for *CHEK2* 1100delC and may be other variants may also be considered in other populations where the mutation frequency is relevantly high such as Finland, Sweden, United Kingdom and North America (Hollestelle *et al*, 2010).

In conclusion, *CHEK2* 1100delC mutation carriers had a significantly higher incidence of contralateral breast cancer compared with noncarriers that may have consequences for follow-up strategies in clinical practice. Distant disease-free and breast cancer-specific survival were similar over the first 6 years after diagnosis, but became worse beyond 6 years after diagnosis for *CHEK2* 1100delC mutation carriers compared with noncarrier BC patients. No significant interaction between adjuvant chemotherapy and a *CHEK2* 1100delC mutation was observed regarding contralateral breast cancer or distant disease-free survival.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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