ORIGINAL ARTICLE

Tumour characteristics and prognosis of breast cancer patients carrying the germline *CHEK2**1100delC variant

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Background: The germline *CHEK2**1100delC variant has been associated with breast cancer in multiple case families where involvement of *BRCA1* and *BRCA2* has been excluded.

Methods: We have investigated the tumour characteristics and prognosis of carriers of this germline variant by means of a prospective cohort study in an unselected cohort of 1084 consecutive patients with primary breast cancer. Data were collected for 34 patients with a germline *CHEK2**1100delC mutation and for 102 patients without this mutation, stratified by age and date of diagnosis of the first primary breast cancer (within 1 year).

Results: Carriers developed steroid receptor positive tumours (oestrogen receptor (ER): 91%; progesterone receptor (PR): 81%) more frequently than non-carriers (ER: 69%; PR: 53%; $p=0.04$). Mutation carriers more frequently had a female first or second degree relative with breast cancer ($p=0.03$), or had any first or second degree relative with breast or ovarian cancer ($p=0.04$). Patients with the *CHEK2* variant had a more unfavourable prognosis regarding the occurrence of contralateral breast cancer (relative risk (RR)=5.74; 95% confidence interval (CI) 1.67 to 19.65), distant metastasis-free survival (RR=2.81; 95% CI 1.20 to 6.58), and disease-free survival (RR=3.86; 95% CI 1.91 to 7.78). As yet, no difference with respect to overall survival has been found at a median follow up of 3.8 years.

Conclusion: We conclude that carrying the *CHEK2**1100delC mutation is an adverse prognostic indicator for breast cancer. If independently confirmed by others, intensive surveillance, and possibly preventive measures, should be considered for newly diagnosed breast cancer cases carrying the *CHEK2**1100delC variant.

About 15% of all breast cancer patients have a positive family history for this disease.¹ A polygenic model to explain this familial aggregation has been proposed,² and several genes have been identified to date. Mutated alleles of *BRCA1*, *BRCA2*, and *TP53* confer highly increased breast cancer risks, but each gene accounts for only a small fraction of the familial clustering and the total breast cancer incidence.^{3–6} Other known genes, such as *ATM*, and unknown genes are believed to confer much lower breast cancer risks.^{5–6} However, as their mutated alleles are believed to be common in the general population, they may account for a substantial proportion of breast cancer cases. Recently, it has been found that a specific mutation in the *CHEK2* gene, termed *CHEK2**1100delC, is associated with breast cancer occurring in multiple-case families in which the involvement of *BRCA1* and *BRCA2* has been excluded.^{7–9} *CHEK2* is involved in cell cycle control and DNA repair through its ability to phosphorylate p53, Cdc25c, and *BRCA1*,¹⁰ a function which is abrogated by the 1100delC mutation. This mutation has a population frequency of approximately 1%, and has been suggested to confer an approximately twofold elevated risk of breast cancer.^{7–8} In this study, we investigated the characteristics of *CHEK2**1100delC associated tumours, and the prognosis of patients with such breast cancers, in a prospectively collected cohort containing 1084 consecutive unselected patients with primary breast cancer.

METHODS

Patient population and study design

Patients were consecutively recruited irrespective of their family history from three centres in south west Netherlands (two academic cancer centres in Leiden and Rotterdam, and

one general hospital in Leiden) between October 1, 1996 and July 1, 2002. Patients with a previous primary breast cancer were excluded from the study. There was no selection for age at diagnosis in the Leiden hospitals, while in the Rotterdam Cancer Centre there was an upper limit of 70 years of age. The median follow up time for the total group was 3.8 years (range 0.2–6.4 years; mean 3.6 years); for those without any event the median follow up time was 3.6 years (range 0.2–5.7 years; mean 3.5 years).

We have investigated the tumour characteristics and prognosis of carriers of this germline variant by means of a prospective cohort study of these 1084 unselected consecutive patients with primary breast cancer. Because survival data were not directly available and had to be retrieved from clinical files, the tumour characteristics of the first primary breast cancer and the prognosis in *CHEK2**1100delC carriers were compared with the characteristics of a stratified sample of non-carriers (three carrier patients per non-carrier patient). Non-carrier patients were stratified for age and date of diagnosis of the first primary breast cancer (within 1 year). Non-carriers were randomly selected from all non-carriers who were eligible for a particular carrier. The following were used as parameters for prognosis: disease-free survival, incidence of contralateral breast tumours, local-regional recurrences and distant metastases, and overall survival.

The Medical Ethical Review Boards of the involved centres approved the study protocol, under the condition that written informed consent was obtained from the patients.

Abbreviations: ER, oestrogen receptor; PR, progesterone receptor; RR, relative risk

Data collection and methods

Clinical and pathology data (including oestrogen receptor (ER) and progesterone receptor (PR) status) of the patients were retrieved from medical files and pathology reports. Information on follow up (including second malignancies) was retrieved from the records of the *CHEK2**1100delC

positive cases and the non-carriers. The data were collected by investigators without prior knowledge of *CHEK2* status.

From each breast cancer patient, in the period between diagnosis and surgery, a detailed family cancer history was obtained, including data from first and second degree relatives, and a blood sample for DNA testing. There were no familial interrelationships (up to second degree relatives) between any of the patients included in this study.

The *CHEK2**1100delC mutation was determined as described previously.⁸ All positive results were confirmed by sequence analysis. *BRCA1/2* mutations were determined as also described previously,¹¹ with slight modifications to include frequently mutated regions of *BRCA2*.

Statistical analysis

Baseline clinical-pathological characteristics and the family history of patients with a *CHEK2**1100delC mutation were compared to those from non-carriers by using χ^2 tests and, if applicable, *t* tests. The clinical-pathological characteristics of the first breast cancer were analysed.

Events in patients with breast cancer and a germline *CHEK2**1100delC mutation were compared to those in patients without the *CHEK2**1100delC mutation by estimating Kaplan-Meier survival probabilities for disease-free survival, survival without contralateral breast cancer, distant metastasis-free survival, and overall survival for patients with breast cancer and a *CHEK2**1100delC mutation as compared to patients without this mutation. The date of diagnosis of the first breast cancer was used as the moment of entry. Differences between the two groups were tested by using the log rank test. In the survival probability curves data were censored at 5 years of follow up.

Univariate Cox regression analysis was then performed for the following events: occurrence of contralateral tumours, local-regional recurrences, distant metastases, other malignancies, or death, or any combination of these events. To test the assumption of proportional hazards, a Cox proportional hazard model was performed in which an interaction term of *CHEK2* status and a time dependent covariate was added.¹² A significant effect of that interaction term denotes the presence of a time dependent effect and thus a violation of the proportional hazards assumption. Since age was one of the matching variables, a stratified Cox regression analysis was performed. Age was grouped into 10 year intervals and was used as a stratification variable. To explore the options for multivariate analysis, univariate Cox regression analyses were performed for each of the covariates from tables 1 and 2 (excluding age) for each event. *p* Values under 5% were considered as statistically significant. All analyses were performed with SPSS version 11.01.

RESULTS

Patient and tumour characteristics

The germline *CHEK2**1100delC mutation was found in 34 out of 1084 patients (3.1%) with primary breast cancer. None of the patients with a *CHEK2**1100delC mutation had a *BRCA1* or *BRCA2* mutation. Patients with a *CHEK2* mutation were significantly younger than patients without this mutation (49.0 v 53.2 years; *p* = 0.03).

When comparing patients with a *CHEK2* mutation with the matched controls, a significant difference between the two groups was only found regarding steroid receptor status (table 1). In the patients with a germline *CHEK2**1100delC mutation, 91% of the tumours were ER positive in contrast to 69% of the matched controls (*p* = 0.03), and 81% were PR positive compared to 53% of the matched controls (*p* = 0.04). No significant differences between the two groups were found with respect to tumour size, histological subtype,

Table 1 Clinical-pathological characteristics of patients with (n = 34) and without (n = 102) a *CHEK2**1100delC mutation at the time of diagnosis of the first primary breast cancer

	<i>CHEK2</i> mutation present (n = 34)	<i>CHEK2</i> mutation absent (n = 102)	Diff.
Age (mean; SD; range)	49.0; 11.6; 25–83	48.4; 11.9; 23–86	<i>t</i> = 0.26; <i>p</i> = 0.80
Tumour size			
2 cm or less	16 (53%)	49 (53%)	
More than 2 cm	14 (47%)	43 (47%)	$\chi^2 = 0.00$
Unknown	4	10	<i>p</i> = 0.99
Axillary nodal status			
Negative	14 (40%)	43 (44%)	
Positive	19 (60%)	53 (56%)	$\chi^2 = 0.06$
Unknown	1	6	<i>p</i> = 0.81
Tumour stage			
DCIS	2 (7%)	5 (6%)	
I–II	22 (73%)	74 (87%)	
III–IV	6 (20%)	6 (7%)	$\chi^2 = 4.08$
Unknown	4	17	<i>p</i> = 0.13
Histological tumour type			
Ductal invasive	25 (73%)	79 (84%)	
Lobular invasive	4 (12%)	8 (9%)	
DCIS	2 (6%)	5 (5%)	
Other	3 (9%)	3 (2%)	$\chi^2 = 3.50$
Unknown	–	7	<i>p</i> = 0.32
Oestrogen receptor status			
Negative	2 (9%)	22 (31%)	
Positive	21 (91%)	48 (69%)	$\chi^2 = 4.67$
Unknown	11	32	<i>p</i> = 0.03
Progesterone receptor status			
Negative	3 (19%)	25 (47%)	
Positive	13 (81%)	27 (53%)	$\chi^2 = 4.35$
Unknown	19	50	<i>p</i> = 0.04
Grade†			
Good (grade I)	4 (19%)	12 (15%)	
Moderate (grade II)	11 (52%)	34 (44%)	
Bad (grade III)	6 (29%)	32 (41%)	$\chi^2 = 1.09$
Unknown	13	24	<i>p</i> = 0.58
Surgical procedure			
Breast conserving therapy	17 (52%)	40 (40%)	
Breast conserving therapy with sentinel node procedure	1 (3%)	5 (5%)	
Mastectomy	14 (42%)	50 (49%)	
Mastectomy with sentinel node procedure	1 (3%)	4 (4%)	
Other	–	2 (2%)	$\chi^2 = 2.01$
Unknown	1	1	<i>p</i> = 0.74
Resection line involved			
No	27 (87%)	81 (88%)	
Yes	4 (13%)	11 (12%)	$\chi^2 = 0.02$
Unknown	3	10	<i>p</i> = 0.89
Adjuvant systemic therapy			
No	13 (40%)	39 (39%)	
Chemotherapy	9 (27%)	18 (18%)	
Endocrine therapy	3 (9%)	13 (13%)	
Combination of both	8 (24%)	30 (30%)	$\chi^2 = 1.66$
Unknown	1	2	<i>p</i> = 0.65
Adjuvant radiotherapy			
No	9 (27%)	29 (29%)	
Yes	24 (73%)	71 (71%)	$\chi^2 = 0.04$
Unknown	1	2	<i>p</i> = 0.85

Diff., difference.

†According to Bloom-Richardson's grading.

grade, or surgical procedure, or in choice of adjuvant systemic therapy and radiotherapy.

Table 2 shows the family histories of the patients in the study, stratified according to *CHEK2**1100delC carrier status. Mutation carriers more frequently had a first or second degree female relative with breast cancer ($p = 0.03$) or had any first or second degree relative with breast or ovarian cancer ($p = 0.04$). There were no apparent associations with cases who had first degree relatives with breast cancer at any age, first degree relatives with breast cancer at a younger age, or with cases who had ovarian cancer or male cancer in the family pedigree.

Prognosis and survival

In patients with and without the *CHEK2**1100delC mutation, significant differences were observed regarding disease-free survival (log rank 12.26; $p = 0.005$), contralateral breast cancer-free survival (log rank 10.51; $p = 0.001$), and distant metastasis-free survival (log rank 4.33; $p = 0.04$). No difference in overall survival has been observed as yet (log rank 0.38; $p = 0.54$). At 5 year follow up, 62% of the patients with a *CHEK2**1100delC mutation showed an event in contrast to only 37% of the non-carriers (fig 1A); contralateral breast cancer had occurred in 26% (9% in the non-carriers; fig 1B) and distant metastasis in 43% (28% in the non-carriers; fig 1C). At the median follow up (3.8 years), an event had occurred in 56% of the carriers (8% in the non-carriers; fig 1A); contralateral breast cancer had occurred in 26% (4% in the non-carriers; fig 1B) and distant metastasis in 29% (13% in the non-carriers; fig 1C).

There was no violation of the assumption of proportional hazards regarding any event ($p = 0.596$), contralateral breast cancer ($p = 0.510$), locoregional breast cancer ($p = 0.992$), distant metastasis ($p = 0.315$), other malignancies ($p = 0.553$), or death ($p = 0.529$). The relative risks (RR) and

95% confidence intervals (95% CI) for patients with a *CHEK2**1100delC mutation in comparison to non-carriers to develop a specific event are summarised in table 3. The highest relative risk (RR = 5.74; 95% CI 1.67 to 19.65) was found to be the development of contralateral breast cancer. Significant differences were also found with respect to the occurrence of distant metastasis (RR = 2.81; 95% CI 1.20 to 6.58) and any of the mentioned events (RR = 3.86; 95% CI 1.91 to 7.78).

Because none of the covariates from tables 1 and 2 (excluding age) yielded any statistically significant contribution (results not presented) no multivariate analyses were performed.

DISCUSSION

In our series of breast cancer patients we found a prevalence of 3.1% of the *CHEK2**1100delC mutation, which is somewhat higher than reported earlier.^{7,8} This may have been caused by one centre (the Rotterdam Cancer Centre) only including patients diagnosed before the age of 70. Another explanation is that in our population the allele frequency of the *CHEK2**1100delC mutation is higher. In both our study and that by Vahteristo *et al*,⁷ the prevalence of the *CHEK2**1100delC mutation was higher in breast cancer patients with a family history of breast cancer (3.9% and 3.1%, respectively) than in patients without such a family history (2.4% and 1.5%, respectively). Among cases with a strong family history (derived from non-*BRCA1/2* families self referred to family cancer clinics) the *CHEK2**1100delC mutation is even more prevalent.⁷⁻⁹ Taken together, these facts suggest that the *CHEK2**1100delC mutation is associated with a positive family history of breast cancer, unrelated to *BRCA1/2* mutations. It has been suggested that the *CHEK2**1100delC mutation acts together with an other as yet unidentified breast cancer susceptibility gene in these families.^{8,9}

The patients in our study who carried the *CHEK2**1100delC mutation more frequently developed steroid receptor positive tumours than did patients without this mutation. In this regard, these tumours appear more like *BRCA2* related tumours than *BRCA1* related tumours. Receptor positive tumours generally show a better prognosis than receptor negative tumours,¹³ but the *CHEK2**1100delC positive patients in our study had a less favourable prognosis than non-carriers stratified for age and year of diagnosis, even though there were no significant differences in application of adjuvant systemic therapy. However, there is a marginal but statistically insignificant excess of lower grade cancer in the *CHEK2**1100delC cases as compared to the non-*CHEK2**1100delC cases (70% *v* 59%). It therefore seems that the *CHEK2**1100delC carriers represent a small but distinct group of breast cancer cases, like those carrying *BRCA2* mutations but quite unlike those carrying *BRCA1* mutations in terms of histological grade and receptor status, and unlike the average sporadic breast cancer case in terms of prognosis.¹⁴⁻²⁰

Whether this is due to loss of function of the *CHEK2* protein in these cancers remains to be seen. The relationship between 1100delC carrier status and loss of heterozygosity at the gene locus is complex, but very few breast tumours from 1100delC carriers show *CHEK2* protein expression.^{7,9} Although the role of *CHEK2* as an upstream activator of p53 following DNA damage has been controversial, there is some suggestion that its loss could impair DNA-damage induced apoptosis.²¹

With respect to prognosis, the sixfold risk (RR = 5.98) in contralateral breast cancer in patients with a *CHEK2**1100delC mutation compared to matched controls was most remarkable (table 3). This is almost twice as high

Table 2 Family histories of patients with (n = 34) and without (n = 102) the *CHEK2**1100delC mutation

	<i>CHEK2</i> mutation present (n = 34)	<i>CHEK2</i> mutation absent (n = 102)	Difference
Any breast or ovarian cancer in first or second degree relative	19 (56%)	37 (36%)	$\chi^2 = 4.05$ $p = 0.04$
Female breast cancer in first degree relative	7 (21%)	15 (15%)	$\chi^2 = 0.65$ $p = 0.42$
Female breast cancer in first or second degree relative	19 (56%)	35 (34%)	$\chi^2 = 4.96$ $p = 0.03$
Number of female breast cancer cases			
0	15 (44%)	67 (66%)	
1	13 (38%)	25 (24%)	$\chi^2 = 5.02$ $p = 0.08$
2 or more	6 (18%)	10 (10%)	$\chi^2 = 0.51$ $p = 0.47$
Female breast cancer in first or second degree relative under age 60	9 (26%)	21 (21%)	
Female breast cancer in first or second degree relative under age 50	6 (17%)	16 (15%)	$\chi^2 = 0.07$ $p = 0.79$
Female breast cancer in first or second degree relative under age 40	2 (6%)	9 (9%)	$\chi^2 = 0.30$ $p = 0.59$
Female breast cancer in first or second degree relative under age 30	0 (0%)	3 (3%)	$\chi^2 = 1.02$ $p = 0.31$
Male breast cancer in first or second degree relative	0 (0%)	0 (0%)	–
Ovary cancer in first or second degree relative	1 (3%)	5 (5%)	$\chi^2 = 0.23$ $p = 0.63$
Ovary and breast cancer in one first or second degree relative	0 (0%)	2 (1.5%)	$\chi^2 = 0.68$ $p = 0.41$

Figure 1 Probabilities of disease-free survival (A), survival without contralateral breast cancer (B), distant metastasis-free survival (C), and overall survival (D) for patients with breast cancer and the *CHEK2**1100delC mutation (n = 34) compared to patients with breast cancer but without this mutation (n = 102).

as found for carriers of a *BRCA1/2* mutation,^{14–18} and difficult to reconcile with the only moderately elevated overall breast cancer risk conferred by this mutation. Yet our finding is supported by the studies of Vahteristo *et al*⁷ and Broeks *et al*²² which also revealed a six times higher prevalence of *CHEK2**1100delC carriers among patients with bilateral

as compared to unilateral breast cancer. However, we cannot exclude that *CHEK2**1100delC carriers carry risk alleles at other, as yet unknown, breast cancer susceptibility genes.²³

Another remarkable finding of our study is that *CHEK2**1100delC mutation carriers are at higher risk for the development of distant metastasis (RR = 2.81). This result suggests that *CHEK2**1100delC associated tumours are more malignant relative to other breast tumours. However, thus far we have found no statistically significant effect of the *CHEK2**1100delC variant on overall survival, although this may have been due to the short follow up. In fact, the survival curves tend to diverge at 5 years of follow up (fig 1D).

Factors other than the *CHEK2**1100delC mutation might have caused the more malignant behaviour of tumours in carrier patients. Due to the small numbers, we were not able to analyse this in a multivariate model. A larger study should address the contribution of this *CHEK2* variant to tumour characteristics and would also allow comparison of subgroups of patients, for example carrier versus non-carrier patients, both with metastases in follow up.

To our knowledge, this is the first report on the clinical behaviour of *CHEK2**1100delC associated breast tumours in a consecutive series of unselected patients with breast cancer. Because the *CHEK2**1100delC mutation seems to increase breast cancer risk only slightly with a mean earlier onset of 4 years, and because co-segregation with breast cancer in families where this variant occurs is not strong,^{7–9} DNA testing for this mutation has not yet been introduced into daily clinical management of breast cancer. In addition, very few breast cancers can be attributed to the *CHEK2**1100delC mutation. As a result, we identified only a small number of carriers in our patient cohort, for which follow up was also relatively short. But if our findings of a more unfavourable prognosis for patients with *CHEK2**1100delC related tumours are confirmed independently in other larger patient cohorts, *CHEK2* status may become an important determinant when

Table 3 Events in patients with breast cancer and the *CHEK2**1100delC mutation (n = 34) compared to those without this mutation (n = 102)

	<i>CHEK2</i> mutation present (n = 34)	<i>CHEK2</i> mutation absent (n = 102)	RR	95% CI
Any event	16 (47%)	21 (21%)	3.86	1.91 to 7.78
Contralateral breast cancer*	7 (21%)*	4 (4%)	5.74	1.67 to 19.65
Local-regional recurrence†	0 (0%)	5 (5%)	0.03	0.00 to 284
Distant metastasis	10 (29%)	17 (16%)	2.81	1.20 to 6.58
Other malignancy‡	1 (3%)	1 (1%)	3.19	0.20 to 51.09
Deaths§	4 (12%)	9 (9%)	1.76	0.52 to 5.93

Relative risks (RR) (univariate) and 95% CI are shown.
*One patient (without the *CHEK2* mutation) had a local recurrence in addition to contralateral breast cancer. Two other patients (one patient with the *CHEK2* mutation) had a later diagnosis of distant metastasis.
†Including one case with ipsilateral second breast cancer.
‡Other malignancies were: adenocarcinoma of the colon and chondrosarcoma grade I (in patients with the *CHEK2* mutation); and fibrous meningioma (in a patient without the *CHEK2* mutation). Both patients with a *CHEK2* mutation also had a contralateral breast cancer while the patient with the chondrosarcoma was later diagnosed with metastatic disease.
§All deaths were related to breast cancer and preceded by distant metastasis.
¶Two of these were synchronic breast cancers (contralateral breast cancer within 1 week).

considering intensive surveillance and/or preventive measures, especially since the laboratory test can be carried out cost effectively if large numbers are tested.

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