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


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.

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. All positive results were confirmed by sequence analysis. BRCA1/2 mutations were determined as also described previously, 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 χ² 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. 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 ± 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,
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 = 0.009), 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.6,7 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 Valtteristo 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.8,9 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 another as yet unidentified breast cancer susceptibility gene in these families.6,9

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,10,11 but the CHEK2*1100delC mutation 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 marginally but statistically insignificant excess of lower grade cancer in the CHEK2*1100delC cases as compared to non-CHEK2*1100delC cases (70% v 59%). It therefore seems that the CHEK2*1100delC cases 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.12-20

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.10 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>
<thead>
<tr>
<th>CHEK2 mutation present (n = 34)</th>
<th>CHEK2 mutation absent (n = 102)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any breast or ovarian cancer in first or second degree relative</td>
<td>0.04</td>
<td>4.05</td>
</tr>
<tr>
<td>Female breast cancer in first degree relative</td>
<td>0.02</td>
<td>0.65</td>
</tr>
<tr>
<td>Female breast cancer in first or second degree relative</td>
<td>0.03</td>
<td>0.49</td>
</tr>
<tr>
<td>Number of female breast cancer cases</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>0</td>
<td>15 (44%)</td>
<td>67 (66%)</td>
</tr>
<tr>
<td>1</td>
<td>13 (38%)</td>
<td>25 (24%)</td>
</tr>
<tr>
<td>2 or more</td>
<td>6 (18%)</td>
<td>10 (10%)</td>
</tr>
<tr>
<td>Female breast cancer in first or second degree relative under age 60</td>
<td>0.08</td>
<td>0.50</td>
</tr>
<tr>
<td>Female breast cancer in first or second degree relative under age 50</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>Female breast cancer in first or second degree relative under age 40</td>
<td>0.07</td>
<td>0.51</td>
</tr>
<tr>
<td>Female breast cancer in first or second degree relative</td>
<td>0.07</td>
<td>0.30</td>
</tr>
<tr>
<td>Male breast cancer in first or second degree relative</td>
<td>0.07</td>
<td>0.59</td>
</tr>
<tr>
<td>Ovary cancer in first or second degree relative</td>
<td>0.07</td>
<td>0.23</td>
</tr>
<tr>
<td>Ovary and breast cancer in one first or second degree relative</td>
<td>0.07</td>
<td>0.68</td>
</tr>
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</table>
as found for carriers of a BRCA1/2 mutation, 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 al6 and Broeks et al7 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.

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, 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

<table>
<thead>
<tr>
<th>CHEK2 mutation</th>
<th>CHEK2 mutation</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>present (n = 34)</td>
<td>absent (n = 102)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any event</td>
<td></td>
<td>16</td>
<td>4 (47%)</td>
</tr>
<tr>
<td>Contralateral breast cancer*</td>
<td>7 (21%)*</td>
<td>21</td>
<td>4 (21%)</td>
</tr>
<tr>
<td>Local-regional recurrence†</td>
<td>0 (0%)</td>
<td>5</td>
<td>5 (5%)</td>
</tr>
<tr>
<td>Distant metastasis</td>
<td>10 (29%)</td>
<td>17</td>
<td>17 (16%)</td>
</tr>
<tr>
<td>Other malignancy‡</td>
<td>1 (3%)</td>
<td>1</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Death§</td>
<td>4 (12%)</td>
<td>9</td>
<td>9 (9%)</td>
</tr>
</tbody>
</table>

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.
†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.

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).
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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