The CHEK2*1100delC Variant Acts as a Breast Cancer Risk Modifier in Non-BRCA1/BRCA2 Multiple-Case Families

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ABSTRACT

The frame-shifting mutation 1100delC in the cell-cycle-checkpoint kinase 2 gene (CHEK2) has been reported to be associated with familial breast cancer in families in which mutations in BRCA1 and BRCA2 were excluded. To investigate the role of this variant as a candidate breast cancer susceptibility allele, we determined its prevalence in 237 breast cancer patients and 331 healthy relatives derived from 71 non-BRCA1/BRCA2 multiple-case early onset breast cancer families. Twenty-seven patients (11.4%) were carrying the CHEK2*1100delC variant. At least one carrier was found in 15 of the 71 families (21.1%). There was no evidence of cosegregation between the variant and breast cancer, but carrier patients developed breast cancer earlier than did noncarriers. We studied CHEK2 protein expression in 111, and loss of heterozygosity at CHEK2 in 88 breast tumors from these patients. Twelve of 15 tumors from carriers showed absent protein expression as opposed to 3 of 76 tumors from noncarriers (P < 0.001). CHEK2 loss of heterozygosity was associated with absence of protein expression but not with 1100delC carrier status. Thus, selecting 27 breast cancer cases with a strong familial background not accounted for by BRCA1 or BRCA2 strongly enriches for carriers of CHEK2*1100delC. Our results support a model in which CHEK2*1100delC interacts with an as yet unknown gene (or genes) to increase breast cancer risk.

INTRODUCTION

First-degree female relatives of a breast cancer patient have an ~2-fold increased risk to develop breast cancer (1). Germ-line mutations in the BRCA1 and BRCA2 genes account for <5% of this familial risk (2, 3). To explain the remainder of familial risk, various genetic models have been proposed. Models incorporating a single third hypothetical gene, BRCA3, or a number of common low penetrance genes with additive effect seem to fit equally well, although the latter fitted best when the known effects of parity on breast cancer risk were included (3, 4). A mutation 1100delC in CHEK2 has been proposed recently to be a low-penetration breast cancer susceptibility allele (5, 6). CHEK2 is located on chromosome 22 and encodes the human orthologue of yeast Cds1 and Rad53, which are G2 checkpoint kinases (7). CHEK2 is involved in cell cycle control and DNA repair through its ability to phosphorylate p53, Cdc25c, and BRCA1. The CHEK2*1100delC variant is a protein-truncating mutation that abrogates the kinase activity of the protein. It occurs in 0.3–1.4% of the general population and 0.3–1.7% of breast cancer patients (11.4%) were carrying the CHEK2*1100delC variant. At least one carrier was found in 15 of the 71 families (21.1%). There was no evidence of cosegregation between the variant and breast cancer, but carrier patients developed breast cancer earlier than did noncarriers. We studied CHEK2 protein expression in 111, and loss of heterozygosity at CHEK2 in 88 breast tumors from these patients. Twelve of 15 tumors from carriers showed absent protein expression as opposed to 3 of 76 tumors from noncarriers (P < 0.001). CHEK2 loss of heterozygosity was associated with absence of protein expression but not with 1100delC carrier status. Thus, selecting 27 breast cancer cases with a strong familial background not accounted for by BRCA1 or BRCA2 strongly enriches for carriers of CHEK2*1100delC. Our results support a model in which CHEK2*1100delC interacts with an as yet unknown gene (or genes) to increase breast cancer risk.

MATERIALS AND METHODS

Families. Families were ascertained through the Clinical Genetic Centres in Leiden, Rotterdam, and Nijmegen, as well as through the Netherlands Foundation for the Detection of Hereditary Tumors. Families were eligible if they were at least three cases of breast cancer diagnosed before the age of 60 from whom genotypes could be determined or inferred. DCIS or LCIS before the age of 60 as first primary cancer were also considered eligible diagnoses. Families with cases of ovarian cancer or male breast cancer were excluded, and occurrences of other cancer types were ignored. Seventeen of these 71 families were also part of the previous study identifying the 1100delC*CHEK2 variant as a low-penetration breast cancer susceptibility gene (5). The 71 families selected contained a total of 384 breast cancer patients, 297 of which diagnosed before the age of 60, 2 of which occurred in spouses (excluded from the statistical analysis), and 5 of which had in situ cancer (4 DCIS and 1 LCIS) only. There was one family where the third case diagnosed under 60 was in situ cancer (combined DCIS/LCIS at age 53). Pathology reports were retrieved for 260 patients (68%). For another 84 patients, diagnoses were confirmed by medical records, and retrieval of pathology reports was still in progress at the time when this study was finalized. For the remaining 40 cases, breast cancer diagnoses were ascertained by family interview only. Blood samples and paraffin-embedded tumor tissues were collected after obtaining written informed consent. The institutional ethical committees of all of the hospitals involved approved this study.

BRCA1 and BRCA2 Mutation Testing. In each family, the youngest breast cancer patient from whom a blood sample was available was tested for mutations in the BRCA1 and BRCA2 genes (and for many families the next youngest as well). The different Clinical Genetic Centres applied a variety of methodologies. The large central exons (exon 11 in BRCA1 and BRCA2, exon 10 of BRCA2) were scanned by partial thromboplastin time (13). The small exons were scanned for mutations by denaturing gradient gel electrophoresis or direct sequencing. All of the laboratories specifically assayed the presence of large founder deletions in BRCA1 by deletion junction-PCR (14). For cases where scanning was still in progress at the time of sampling for the purpose of this research, we performed conformation-sensitive gel electrophoresis (15) covering all of the coding regions of both genes. This identified 10 different variants of uncertain clinical significance and 12 different polymorphisms. None of these were cosegregating with breast cancer or the CHEK2*1100delC variant.

Genotyping of the CHEK2*1100delC Variant. The DNA sequence of exon 10 of CHEK2, where the 1100delC resides, is present in multiple

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7 The abbreviations used are: CHEK2, cell-cycle checkpoint kinase 2 gene; DCIS, ductal carcinoma in situ; LCIS, lobular carcinoma in situ; LOH, loss of heterozygosity; AIF, allelic imbalance factor; df, degrees of freedom.
CHEK2 AS A BREAST CANCER RISK MODIFIER

We investigated 71 families with a phenotype of early onset breast cancer, defined as having at least 3 cases diagnosed before the age of 60, and no cases of ovarian or male breast cancer. Mutations in BRCA1 and BRCA2 were excluded in at least the youngest breast cancer case from which a blood sample was available. These families contained a total of 384 breast cancer patients. We collected DNA samples from 237 patients, including all of those with in situ cancer, as well as from 331 family members without breast cancer and 54 spouses. Of the 622 individuals we were thus able to assay for the presence of the CHEK2*1100delC variant, we found 41 (6.6%) to be carriers (Table 1). The prevalence among breast cancer patients was 11.4% (27 of 237), which was significantly higher than the prevalence of the variant in healthy female family members (6 of 212; \( \chi^2 = 12.047; df = 1; P < 0.001 \)). Three carriers were known with in situ cancer (2 DCIS and 1 LCIS). Fifteen families (21.1%) had at least 1 positive individual for this variant. One of these was a family in which the only identifiable carrier was a woman with in situ cancer (DCIS; Fig. 1). The proportion of families in which at least 1 individual carried the CHEK2 variant increased to 31.8% in families with >5 breast cancer patients diagnosed under 60 (Table 1). However, this trend was not statistically significant (\( \chi^2 = 2.6; df = 2; P = 0.272 \)). In addition, CHEK2-positive families had on average slightly more blood-sampled cases than CHEK2-negative families (3.8 versus 3.2; data not shown). Although not a statistically significant difference, this indicates that the odds of detecting the variant is dependent on the number of blood-sampled breast cancer cases in a family.

In the 15 1100delC+/CHEK2-positive families we defined the youngest carrier breast cancer case as the index patient. Under the null hypothesis of complete random Mendelian inheritance, we predicted that 12.9375 of the 54 affected relatives would be carrier of the variant. We observed 12 carriers, so that the null hypothesis could not be rejected.

We performed LOH analysis in 89 archival breast tumor tissues from 88 breast cancer cases from these 71 families, at four markers mapping to either side of CHEK2 (Table 2). LOH at CHEK2 was found in 11 tumors, 3 of which derived from 2 CHEK2*1100delC carriers. In all 3 of the tumors, we could demonstrate that the lost allele was derived from the nontransmitting parent (data not shown). Although the 1100delC variant occurred 2.7 times more frequently among cases showing LOH at CHEK2, this difference was not statistically significant (\( \chi^2 = 1.239; df = 2; P = 0.538 \)).

A tissue microarray with 111 tumors from 111 cases was stained with a mouse monoclonal antibody against the human CHEK2 protein. Examples of obtained staining patterns are shown in Fig. 2.
noted in a previous study (19) there was considerable variability in the percentage of normal cells that were positive. CHEK2 protein expression was absent in 12 of 15 tumors from \textit{CHEK2}^{*1100delC} carriers (80.0%; Table 3). False-negative staining was considered unlikely, because in 6 of 12 tumors from \textit{CHEK2}^{*1100delC} carriers the stromal component stained normally. Notably, the one tumor showing moderate protein expression was an \textit{in situ} carcinoma (DCIS) from a patient from family RUL154 (Fig. 1). In comparison, only 3 of 76 tumors (3.9%) from noncarriers showed an absent CHEK2 protein expression ($\chi^2 = 15.879$; $df = 6$; $P = 0.014$).

The mean age of diagnosis of the first primary tumor of \textit{CHEK2}^{*1100delC} carrier patients was not significantly different from that in noncarriers (48.3 versus 50.6 years; $P = 0.30$). However, any age difference may have been masked by our selection for early onset breast cancer. Indeed, in a Kaplan-Meier analysis the age of onset distribution between the two groups was different ($P < 0.0001$). It is unlikely that this effect is confounded by differences in tumor grade because the percentage of grade III tumors was higher in noncarriers than in carriers (22 of 81 versus 1 of 9). A Cox-regression analysis revealed an odds ratio of 2.1 (95% confidence interval, 1.393–3.166; $P < 0.001$) for carriers to develop breast cancer relative to noncarriers (derived from \textit{CHEK2}^{*1100delC} positive and \textit{CHEK2}^{*1100delC} negative families). Among the 237 genotyped breast cancer patients in our cohort, 35 (14.8%) were known to have had a second primary breast cancer. Five of these (14.3%) were positive for the \textit{CHEK2} variant. Of the 202 patients with one primary breast cancer, 22 tested positive (10.9%). This difference was not statistically significant.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
LOH at \textit{CHEK2} & Number of cases & \textit{CHEK2} carriers & \% \\
\hline
Positive$^a$ & 11 & 3 & 27.3 \\
Suspected$^b$ & 29 & 5 & 17.2 \\
Negative & 29 & 3 & 10.3 \\
Unknown$^c$ & 20 & 3 & 15.0 \\
Totals & 89 & 14 & 14.0 \\
\hline
\end{tabular}
\caption{LOH at \textit{CHEK2}}
\end{table}

$^a$ Cases in which at least one proximal and one distal marker showed AIF $\equiv 1.70$ without interruption by a marker showing an AIF $< 1.70$.

$^b$ Cases in which LOH was found only proximal or distal of \textit{CHEK2}.

$^c$ Cases in which one of the reactions failed.
DISCUSSION

We found the CHEK2*1100delC variant in 11.4% of the breast cancer cases belonging to a highly selected group of families. This prevalence was substantially higher than reported previously by others. Two earlier studies (5, 6) found familial breast cancer cases from families that were not linked to BRCA1 or BRCA2, and found a prevalence of 5.1% and 5.5%, respectively. The families we studied are highly selected in several ways. First, they contain at least 3 breast cancer cases diagnosed before age 60 (the average number of breast cancer cases per family was 5.4). Second, they were selected against cases of ovarian and male breast cancer. Third, they all tested negative for mutations in BRCA1 and BRCA2. On the basis of population incidence, the odds that 3 cases in a family occur under 60 by chance alone are very low, and, thus, they likely have a genetic basis. Hence, in this group of families we suspect an enrichment of a gene (or genes) that may confer substantial breast cancer risks (12). However, because we and others (5, 6) found no or weak evidence for cosegregation between CHEK2*1100delC and breast cancer, CHEK2 is an unlikely candidate for such a gene. It is possible that other, more high-risk mutations in CHEK2 exist that could account for these cases, but this has thus far not been substantiated by more comprehensive mutation scanning of the gene (9, 10, 20, 21).

A more likely explanation for the data presented here is a model in which CHEK2*1100delC interacts with an as yet unknown rare gene (or genes) to confer breast cancer risks comparable with those conferred by BRCA1 or BRCA2. Selecting for families caused by this rare gene would also enrich for CHEK2*1100delC carriers, which would act like a modifier of the breast cancer risk. The CHEK2 Consortium, studying families of Dutch, German, United Kingdom, and North American origin, found the prevalence of the 1100delC variant to increase in families with ≥4 cases (5), but the Finnish study found the highest prevalence among non-BRCA1/2 cases with a moderate family history (6). We also found weak evidence for increasing prevalence of CHEK2*1100delC among families with a more extensive family history of breast cancer. Even among populations with an apparently overall lower prevalence of the 1100delC variant (8), this enrichment is observed. The higher allele frequency in Northern Europe as opposed to North America might be due to a founder effect of CHEK2*1100delC. The proposed risk modifying effect of CHEK2*1100delC is also supported by our finding that carriers in our families develop breast cancer systematically earlier than do noncarriers. Although this may be a peculiarity of this selected group of patients, a similar age-effect has been noted for genetic variants in AR, HRAS1, RAD51, and AIB1 in carriers of BRCA1 or BRCA2 mutations (22–25).

Alternatively, breast cancer in these families has a polygenic basis involving multiple interacting low-penetrance alleles (26), one of which is the CHEK2*1100delC variant. The CHEK2*1100delC is approximately twice as prevalent among unselected breast cancer cases than among controls, suggesting it is a low-risk allele in its own right (5, 6). In keeping with this, we found that CHEK2*1100delC is associated with breast cancer, but it was unable to explain the majority of breast cancer cases in these families. A role for CHEK2 inactivation in breast tumor development is nonetheless supported by the highly significant association we found between CHEK2*1100delC carrier status and an absence of protein expression in the breast tumors. This confirms results obtained by others (6, 19) irrespective of minor differences in interpretation of immunohistochemical staining patterns among these studies. It would also explain the slightly earlier age of onset of breast cancer in 1100delC carriers, as these individuals only need to inactivate the wild-type allele whereas noncarriers would need to inactivate both copies of the gene. Paradoxically, the breast tumors of CHEK2 carriers do not significantly more frequently show LOH at CHEK2. Hence, LOH may not be the only mechanism inactivating the wild-type allele, although the association between LOH and an absent protein expression we observed does indicate it is involved in some cases. Alternative mechanisms include promoter hypermethylation (27) and somatic mutations, but the roles of both appear to be marginal in breast cancer (19, 28). Conceivably, other components of the pathway(s) regulating the expression and/or stability of CHEK2 protein are disturbed in these cases.

An association with bilateral disease, but only a marginal trend toward earlier age of diagnosis was reported in one study (6). In our cohort of cases we found an association between CHEK2 carrier status and earlier age of diagnosis but not between carrier status and multiple primary tumors. This could be a peculiarity of the selected families. Conceivably, many cases not carrying the CHEK2 variant are carriers of another gene defect that predisposes them strongly to develop breast cancer. In combination with a long retrospective follow-up time, this may have masked the subtle effect of CHEK2 on risk.

In conclusion, we find a strong association between CHEK2*1100delC prevalence and breast cancer family history. Our results provide support for the hypothesis that this variant modifies the cancer risk conferred by an as yet unknown gene (or genes). Given the cancer occurrence in the families described here, this gene is expected to cause breast cancer risks comparable with those conferred by BRCA1 and BRCA2. At this point it is in our opinion not appropriate to offer a predictive test for CHEK2 in a clinical setting. The exact relative risk conferred by CHEK2*1100delC is not clear, but likely modest in comparison with BRCA1 and BRCA2. In addition, estimates of breast cancer risk are difficult to make in these families, because the type of interaction (multiplicative or additive) and the role of other factors are presently unknown. Selecting for families with at least one carrier of the CHEK2*1100delC might reduce the genetic heterogeneity likely to exist among non-BRCA1/BRC2A2 families and facilitate the mapping of this breast cancer susceptibility gene by classical linkage analysis.

ACKNOWLEDGMENTS

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REFERENCES


Table 3 Chek2 protein expression according to 1100delC carrier status and LOH

<table>
<thead>
<tr>
<th>Variable</th>
<th>Absent</th>
<th>Weak</th>
<th>Moderate</th>
<th>Strong</th>
<th>Total</th>
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<tr>
<td>CHEK2 +</td>
<td>12</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>CHEK2 -</td>
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<td>41</td>
<td>27</td>
<td>5</td>
<td>76</td>
</tr>
<tr>
<td>LOH +</td>
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<td>7</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>LOH suspected</td>
<td>7</td>
<td>12</td>
<td>8</td>
<td>1</td>
<td>28</td>
</tr>
<tr>
<td>LOH unknown</td>
<td>4</td>
<td>9</td>
<td>5</td>
<td>1</td>
<td>19</td>
</tr>
</tbody>
</table>

a CHEK2 +, carriers of the 1100delC variant.
b CHEK2 -, noncarrier.
c LOH+, at least one proximal and one distal marker showed AIF ≥ 1.70 without interruption by a marker showing an AIF < 1.70.
d LOH suspected, one distal or proximal marker showed an AIF < 1.70 while the closest marker on the other side of CHEK2 was uninformative.
e LOH -, no LOH was found.
f LOH unknown, one of the reactions failed.
CHEK2 AS A BREAST CANCER RISK MODIFIER

CHEK2 is a key mediator of the DNA damage response pathway. Mutations in CHEK2 have been associated with an increased risk of breast cancer, particularly in noncarriers of BRCA1 or BRCA2 mutations. These mutations act as risk modifiers, and their impact on cancer risk is influenced by other genetic and environmental factors. The study of CHEK2 variants in breast cancer susceptibility is crucial for understanding the genetic basis of cancer risk and for developing personalized risk assessment strategies.


The CHEK2*1100delC Variant Acts as a Breast Cancer Risk Modifier in Non-BRCA1/BRCA2 Multiple-Case Families


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