CHEK2*1100delC Genotyping for Clinical Assessment of Breast Cancer Risk: Meta-Analyses of 26,000 Patient Cases and 27,000 Controls

Maren Weischer, Stig Egel Bojesen, Christina Ellervik, Anne Tybjærg-Hansen, and Børge Grønne Nordestgaard

ABSTRACT

Purpose
CHEK2*1100delC heterozygosity may be associated with an increased risk of breast cancer; however, it is unclear whether the evidence is sufficient to recommend genotyping in clinical practice.

Patients and Methods
We identified studies on CHEK2*1100delC heterozygosity and the risk of unselected, early-onset, and familial breast cancer through comprehensive, computer-based searches of PubMed, EMBASE, and Web of Science. Aggregated risk estimates were compared with previous estimates for BRCA1 and BRCA2 mutation heterozygotes.

Results
By using fixed-effect models for CHEK2*1100delC heterozygotes versus noncarriers, we found aggregated odds ratios of 2.7 (95% CI, 2.1 to 3.4) for unselected breast cancer, 2.6 (95% CI, 1.3 to 5.5) for early-onset breast cancer, and 4.8 (95% CI, 3.3 to 7.2) for familial breast cancer. For familial breast cancer, this corresponds to a cumulative risk of breast cancer at age 70 years in CHEK2*1100delC heterozygotes of 37% (95% CI, 26% to 56%), which compares with similar previous estimates of 57% (95% CI, 47% to 66%) for BRCA1 mutation heterozygotes and 49% (95% CI, 40% to 57%) for BRCA2 mutation heterozygotes.

Conclusion
These meta-analyses emphasize that CHEK2*1100delC is an important breast cancer–predisposing gene, which increases the risk three- to five-fold. Because the cumulative risk of breast cancer at age 70 years among familial patient cases for CHEK2*1100delC heterozygotes is almost as high as that for BRCA1 and BRCA2 mutation heterozygotes, genotyping for CHEK2*1100delC should be considered together with BRCA1 and BRCA2 mutation screening in women with a family history of breast cancer.

J Clin Oncol 26:542-548. © 2008 by American Society of Clinical Oncology

INTRODUCTION
CHEK2 (cell cycle checkpoint kinase 2) acts as a tumor suppressor in the nucleus, where it blocks cell proliferation and initiates DNA repair in response to DNA double-strand breakage. A dysfunctional variant, CHEK2*1100delC, has lost this ability.

CHEK2*1100delC is primarily present in individuals of Northern and Eastern European descent. Studies in these populations have reported an increased breast cancer risk in heterozygotes versus noncarriers in some, but not all studies. Among positive studies, the effect has been demonstrated in patients with unselected, early-onset, and familial breast cancer. However, it is unclear whether the evidence is sufficient to recommend genotyping for CHEK2*1100delC among women with familial breast cancer to improve risk prediction. Unlike mutation screening of the BRCA1 and BRCA2 genes, testing for CHEK2*1100delC generally has not reached clinical practice.

In patients with breast cancer, CHEK2*1100delC heterozygosity has been associated with a double risk of developing a second breast cancer and a shorter recurrence-free survival rate compared with noncarriers. This effect is independent of breast tumor characteristics.

In this study, we performed meta-analyses of studies on CHEK2*1100delC heterozygosity and the risk of breast cancer among patient cases with unselected, early-onset, and familial breast cancer. We also compared these results with a recent meta-analysis on the risk of breast cancer for women with mutations in BRCA1 or BRCA2.
PATIENTS AND METHODS

Search Strategy and Selection Criteria

Prospective cohort and case-control studies of CHEK2*1100delC and the risk of breast cancer published before March 1, 2007, were identified through computer-based searches of PubMed, EMBASE, and Web of Science by using the keywords CHEK2, CHEK2*1100delC and CHK2 alone and in combination with breast cancer. This search identified 42 studies.3-18,22-47 Inclusion criteria for the meta-analyses were women with unilateral breast cancer, which could not be attributed to a known multicancer syndrome, breast cancer–free women as controls, a Northern or Eastern European descent, available for CHEK21100delC genotype, and a BRCA1 and BRCA2 mutation–negative or unknown carrier status. Each patient case and control counted only once. Among the 42 initially identified studies, we excluded studies of ethnic groups other than those of Northern or Eastern European descent,22-24,34,38,40,42,43,45,47 studies of breast cancer that occurred as part of a multicancer syndrome,31,35,36 and studies of men with breast cancer.3,9,14,46 Among the remaining 16 studies, parts of the data from six studies were excluded because of the ethnicity of participant subgroups,4,14,16 the presence of men as CHEK2*1100delC heterozygotes in subgroups,4 the presence of CHEK2*1100delC homozygotes,15 or the publishing of subgroup data.9,11 Studies were classified according to study authors as unselected (including the general population), early-onset (age < 51 years at diagnosis), or familial breast cancer. We classified breast cancer as familial if the studies used the word familial. These studies mainly used standard international criteria that required for fulfillment a woman with breast cancer together with at least one first- and one second-degree relative with breast cancer, a male relative with breast cancer, or at least one patient case of female breast cancer and one patient case of ovarian cancer among relatives. In some studies, patients with breast cancer could be classified into more than one group. In these studies, data subgroups were classified separately or, if not possible, were classified according to the most prevalent patient-group. Patient cases with known BRCA1 or BRCA2 mutations were excluded from the analysis of familial breast cancer.3,8,17 None of the early-onset patient cases were BRCA1 or BRCA2 positive. Eventually, we identified 12 studies of unselected breast cancer,1,4,5,8,15,17,18 four of early-onset breast cancer,3,9,11,14,17 and nine of familial breast cancer.1,3,9,10,12,17,18 Patient cases and controls counted only once, though several studies had more than one group of patients with breast cancer and only one control group. To overcome this, we divided or accumulated controls into as many subgroups as there were groups of patients with breast cancer.1,3,9,10,12,17,18 In articles with more than one patient population within the same category or more than one control group, or vice versa, we

Table 1. Characteristics of Studies on CHEK2*1100delC Heterozygosity and Risk of Breast Cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Overall Heterozygosity</th>
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<tbody>
<tr>
<td></td>
<td>Patient Cases</td>
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<tr>
<td></td>
<td>No.</td>
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<tr>
<td>Unselected breast cancer</td>
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<td>636</td>
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<td>De Jong et al 200416</td>
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<td>Pereira et al 200416</td>
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<tr>
<td>CHEK2 Consortium 200417</td>
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<td>Van Binst et al 200418</td>
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<td>Kleib et al 200515</td>
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<tr>
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<td>Early-onset breast cancer</td>
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<td>Friedrichsen et al 200414</td>
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<td>Familial breast cancer</td>
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<td>71</td>
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<tr>
<td>Bernstein et al 200622</td>
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NOTE: Included studies are listed in chronologic order. Participant number may vary from original publication because of exclusion of study subgroups. Abbreviations: CC, case-control study; PR, prospective study; GP, general population; UK, United Kingdom; NL, the Netherlands; USA, United States of America; FIN, Finland; dHPLC, denaturing-high-performance liquid chromatography; D, Germany; AUS, Australia; DGGE, denaturing gradient gel electrophoresis; ASO/RFLP-PCR, allele-specific oligonucleotide/restriction fragment length polymorphism–polymerase chain reaction; CSGE, conformation-sensitive gel electrophoresis. 

*GE Healthcare, Uppsala, Sweden.
†Applied Biosystems, Foster City, CA.
‡Seuenom, San Diego, CA.
pooled patient cases or controls, respectively.\textsuperscript{3,4,8,9,17} Data from a single group differed from that in the original publication, because individuals who were of other than Northern and Eastern European descent were excluded after personal communication with the authors (A. Sigurdson, March 2007).\textsuperscript{16} Information on the previous history of all types of cancer was not available in patient cases and controls. Articles were scrutinized, including data abstraction performed independently by two of the authors (M.W. and C.E.), and discrepancies were solved by discussion with a third author (B.G.N.).

**Data Abstraction**

From each study, information on study design, geographic location, ethnicity, sex, age, definition, and numbers of patient cases and controls, the genotyping methods and the frequencies of genotypes were extracted.

**Statistical Analysis**

We used STATA statistical software (version 9.2; STATA Corp, College Station, TX). We evaluated the genotype distribution among patient cases and controls and found no departure from the Hardy-Weinberg equilibrium. Odds ratios (ORs) for CHEK2*1100delC heterozygotes versus noncarriers were calculated as fixed and random-effect measures by using Mantel-Haenszel statistics. In the fixed-effect model, we assumed that all studies come from a common population and that the effect size is not significantly different among the different studies. In the random-effects model, we incorporate the random variation within the studies and the variation among the different studies. We tested for publication bias graphically by using funnel plots, in which the log(OR) is plotted against the standard error (log[OR]) to form a simple scatter plot. Asymmetric plots indicated publication bias.

**Risk Comparison With BRCA1 and BRCA2 Mutation Heterozygotes**

We compared the current aggregated risk estimates of familial breast cancer in white women who were heterozygous for CHEK2*1100delC with that of white women who had any BRCA1 or BRCA2 mutation.\textsuperscript{21} To convert between ORs as a measure of relative risk to a cumulative breast cancer risk at age 70 years, we used a value of 7.8% cumulative risk at age 70 years and an OR of 1.0 for the average white women in the general population.\textsuperscript{45} The relative risk of breast cancer for women with any BRCA1 or BRCA2 mutation was estimated as the cumulative incidence at age 70 years divided by 7.8%. The cumulative risk of breast cancer at age 70 years for women who were heterozygous for CHEK2*1100delC was calculated as the aggregated OR multiplied by 7.8% (OR \times 7.8%).

**Results**

Characteristics of the comprised total of 26,488 patient cases and of 27,402 controls in the studies are listed in Table 1.

**Unselected Breast Cancer**

By using fixed and random-effect models, we found aggregated ORs for breast cancer of 2.7 (95% CI, 2.1 to 3.4) and of 2.4 (95% CI, 1.8 to 3.2), respectively, for CHEK2*1100delC heterozygotes versus noncarriers in studies of patients with unselected breast cancer (Fig 1). The funnel plot showed no evidence of publication bias. I\textsuperscript{2}, the fraction of variation in the aggregated OR attributable to study heterogeneity, was only 8% among studies of patients with unselected breast cancer. In meta-analyses, the finding of a significant association often depends on a few large studies; however, the overall OR when leaving out the two largest studies\textsuperscript{39} was 1.8 (95% CI, 1.2 to 2.5) when using both fixed and random-effect models.

**Early-Onset Breast Cancer**

By using fixed and random-effect models, we found aggregated ORs for breast cancer of 2.6 (95% CI, 1.3 to 5.5) and 2.7 (95% CI, 1.3 to 5.6), respectively, for CHEK2*1100delC heterozygotes versus noncarriers in studies of patients with early-onset breast cancer (age < 51 years).
years; Fig 2). The funnel plot showed no evidence of publication bias. I² was 0% among these studies.

**Familial Breast Cancer**

By using fixed and random-effect models, we found aggregated ORs for breast cancer of 4.8 (95% CI, 3.3 to 7.2) and 4.6 (95% CI, 3.1 to 6.8), respectively, for CHEK2*1100delC heterozygotes versus non-carriers in studies of patients with familial breast cancer (Fig 3). The funnel plot showed no evidence of publication bias. I² was 0% among these studies.

**Risk Comparison With BRCA1 and BRCA2 Mutation Heterozygotes**

Figure 4 compares the estimates of relative risk and cumulative risk of developing breast cancer before age 70 years by CHEK2*1100delC heterozygosity, on the basis of the present paper, with that of women with any BRCA1 or BRCA2 mutation, as reported in a very recent meta-analysis. Among women with familial breast cancer, women heterozygous for CHEK2*1100delC and those heterozygous for BRCA1 and BRCA2 mutations had relative risks of breast cancer of 4.8 (95% CI, 3.3 to 7.2), 7.3 (95% CI, 6.0 to 8.5), and 6.3 (95% CI, 5.1 to 7.3), respectively. The equivalent cumulative breast cancer risks at age 70 years were 37% (95% CI, 26% to 56%), 57% (95% CI, 47% to 66%), and 49% (95% CI, 40% to 57%), respectively.

**DISCUSSION**

The present meta-analyses of 16 studies, including a total of 26,488 patient cases and 27,402 controls, provide the most comprehensive analyses of a single breast cancer–predisposing mutation so far. The meta-analyses emphasize that CHEK2*1100delC heterozygosity increases the risk of breast cancer three- to five-fold, which supports previous individual studies conducted in patients with unselected, early-onset, and familial breast cancer. The studies used for the present meta-analyses showed no evidence of publication bias and little or no evidence of heterogeneity.

Interestingly, among patients with unselected and early-onset breast cancer, we found CHEK2*1100delC heterozygosity conferred a two- to three-fold increased risk of developing breast cancer. In comparison, a familial history of breast cancer in both a mother and a sister only conferred a 2.5-fold risk. This is less than the 4.8-fold risk found in our meta-analysis of patients with familial breast cancer for CHEK2*1100delC heterozygotes. With a cumulative risk of breast cancer at age 70 years of 7.8% in the average white woman in the general population, this equals an estimated 37% cumulative risk of developing breast cancer by age 70 years for CHEK2*1100delC heterozygotes. This is almost as high as the previously reported cumulative risk at age 70 years of 57% and 49% among BRCA1 and BRCA2 mutation heterozygotes, respectively.21 Given this evidence, physicians should no longer hesitate to offer CHEK2*1100delC genotyping and counseling together with BRCA1 and BRCA2 mutation screening in women with a familial history of breast cancer, especially in women of Northern or Eastern European descent.

A common limitation of meta-analyses is study heterogeneity—the problem of comparing apples and oranges. We have tried to avoid this by excluding studies of individuals not of Northern or Eastern European descent, because CHEK2*1100delC is known to be largely absent in most other populations.22,23,34,38,40,42,43,45,47 We found minimal evidence of study heterogeneity (I² = 8%) in our study of patients with unselected breast cancer. Heterogeneity is often caused by variation in the environmental and genetic background of study participants, which is unavoidable when combining many studies; however, according to Higgins et al, an I² less than 25% in meta-analysis is regarded as low. We found no heterogeneity in studies of early-onset and familial breast cancer, presumably because the number of included studies was lower.

Furthermore, meta-analyses are often subject to publication bias. However, we performed a comprehensive literature search with no language restriction; after selection of comparable studies, there was no evidence of publication bias when testing for this by a visual inspection of funnel plots. Finally, meta-analyses are often dominated by a few large studies, which markedly reduces the evidence from smaller studies. We therefore calculated the overall OR among patients with unselected breast cancer with and without the two largest studies and, in both instances, found that CHEK2*1100delC heterozygosity was associated with an increased risk of breast cancer.

Finally, on the basis of the results of our meta-analysis, we also estimated that heterozygosity of CHEK2*1100delC among women
with a family history of breast cancer conveys a clinical relevant (37%) cumulative risk of breast cancer. This only represents a rough estimate by multiplying the aggregated OR of 4.8 with the reported cumulative risk of breast cancer by age 70 years in white women in the general population of 7.8%. Therefore, it could be argued that such a conclusion is misleading. Indeed, prospective studies of families with the \textit{CHEK2} variant are needed to better estimate the true disease penetrance of the \textit{CHEK2}*1100delC variant.

The offer of new genetic tests to individuals who may have an increased risk of an inherited disease is often postponed unnecessarily because of a longstanding paradigm in medical genetics that only accepts monogenic inheritance with close to 100% penetrance as valid for the counseling of patients. However, what constitutes risk of the development of a disease like breast cancer in an individual has, with time, proven to be complex. Even the presence of \textit{BRCA1} or \textit{BRCA2} mutations does not provide patients and their doctors with a clear yes or no answer,\textsuperscript{21} because—as is also true for \textit{CHEK2}—the penetrance of a mutation in these genes is less than 100%. Therefore, it no longer seems reasonable to offer screening for \textit{BRCA1} and \textit{BRCA2} mutations without simultaneously testing for \textit{CHEK2}*1100delC.

Ten years after its discovery,\textsuperscript{51} it is now time to accept \textit{CHEK2} as a clinically useful third breast cancer gene and to share our knowledge with individual women seeking advice on the risk of breast cancer. Women with a familial history of breast cancer could be informed of an estimated 37% risk of developing breast cancer by age 70 years if positive for \textit{CHEK2}*1100delC, whereas \textit{CHEK2}*1100delC–positive women without a familial history of breast cancer could be informed of a 21% risk of developing breast cancer by age 70 years. For women in the general population, absolute, 10-year risk estimates for breast cancer can be provided on the basis of \textit{CHEK2}*1100delC heterozygosity, age, hormone replacement therapy, and body mass index.\textsuperscript{3}
42. Rajkumar T, Soumtrita N, Nancy NK, et al: BRCA1, BRCA2 and CHEK2 (1100 del C) germline mutations in hereditary breast and ovarian cancer
48. National Cancer Institute: Probability of developing breast cancer by race. http://canques.seer.cancer.gov/cgi-bin/cq_submit?dir=devcan2003&db=1&rpt=TAB&sel=1^2^3^10^&x=Starting%20Age^0,1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20&z=Race^1,2,3&dec=3&title=Probability+of+Developing+Cancer~For+Breast+Cancer+by+Race,+Females~SEER+17+Registries+for+2001-2003&template=faststats, 2007

Acknowledgment
We thank J.P. Strueming, MD, and Alice J. Sigurdson, PhD, from the U.S. Radiologic Technologist (USRT) study group for providing us with information on ethnicity and on the CHEK2*1100delC carrier status of their study participants.