Linkage Disequilibrium Mapping of CHEK2: Common Variation and Breast Cancer Risk

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Abbreviations: CHEK2, checkpoint kinase 2; CI, confidence interval; HWE, Hardy-Weinberg equilibrium; kb, kilobase; LD, linkage disequilibrium; MAF, minor allele frequency; OR, odds ratio; SNP, single nucleotide polymorphism; tagSNP, haplotype-tagging single nucleotide polymorphism

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ABSTRACT

Background

Checkpoint kinase 2 (CHEK2) averts cancer development by promoting cell cycle arrest and activating DNA repair in genetically damaged cells. Previous investigation has established a role for the CHEK2 gene in breast cancer aetiology, but studies have largely been limited to the rare 1100delC mutation. Whether common polymorphisms in this gene influence breast cancer risk remains unknown. In this study, we aimed to assess the importance of common CHEK2 variants on population risk for breast cancer by capturing the majority of diversity in the gene using haplotype tagging single nucleotide polymorphisms (tagSNPs).

Methods and Findings

We analyzed 14 common SNPs spanning 52 kilobases (kb) of the CHEK2 gene in 92 Swedish women. Coverage evaluation indicated that these typed SNPs would efficiently convey association signal also from untyped SNPs in the same region. Six of the 14 SNPs predicted well both the haplotypic and single SNP variations within CHEK2. We genotyped these six tagSNPs in 1,577 postmenopausal breast cancer cases and 1,513 population controls, but found no convincing association between any common CHEK2 haplotype and breast cancer risk. The 1100delC mutation was rare in our Swedish population—0.7% in cases and 0.4% in controls—with a corresponding odds ratio for carriers versus noncarriers of 2.26 (95% confidence interval, 0.99–5.15). Estimates of the population frequency and the odds ratio of 1100delC indicate that our sample is representative of a Northern European population.

Conclusions

Notwithstanding the involvement of the CHEK2 gene in breast cancer aetiology, we show that common polymorphisms do not influence postmenopausal breast cancer risk.

The Editors’ Summary of this article follows the references.
Introduction

Breast cancer is overall the most common cancer in women worldwide [1]. Twin and family studies have clearly demonstrated the importance of genetic contribution to breast cancer risk [2,3]. Genetic studies of familial breast cancer have identified several high-penetration mutations in genes such as BRCA1 and BRCA2, but these mutations contribute little to risk at the population level due to their low prevalence. Whilst genetic risk factors for breast cancer in women not carrying any high-penetration mutations are largely unknown, a polygenic model has been suggested to account for residual familial risk [4], which anticipates small effects of low-penetration genetic risk variants in combination with environmental influence.

CHEK2, encoding a cell-cycle checkpoint kinase, is a strong candidate gene for cancer susceptibility. Following phosphorylation of CHEK2 by ATM after DNA damage, the activated CHEK2 protein phosphorylates p53, Cdc25, and BRCA1, thereby arresting the cell cycle and activating DNA repair [5–8]. As recently verified, the cellular surveillance system for DNA damage-checking and repair plays an important role in averting cancer development [9,10].

An association between the CHEK2*1100delC variant and breast cancer risk was initially established in breast cancer families without BRCA1 or BRCA2 mutations [11,12]. This deletion, first detected in a breast cancer case with a family history of the Li-Fraumeni syndrome [13], leads to a premature termination of translation that abolishes CHEK2 kinase activity [14]. Most subsequent population studies of the 1100delC mutation included few carriers and found no significant association with breast cancer risk [12,15–23]. Recently, the association between the 1100delC and breast cancer susceptibility was convincingly replicated in a pooled analysis of 10,860 unselected cases and 9,065 controls from ten studies in five countries [24]. In addition, a more recent study has further demonstrated that CHEK2*1100delC confers an even higher lifetime risk in women with a family history of bilateral breast cancer [25]. Two other putatively functional CHEK2 single nucleotide polymorphisms (SNPs), IVS2+1G-A and I157T, have been studied in relation to breast cancer risk. Some groups have found an increased risk of breast cancer associated with these variants [15,23,26,27], whilst others have not [16,28,29]. These polymorphisms are rare and could only make a limited contribution to the genetic risk for breast cancer at the population level. Of the two common CHEK2 polymorphisms studied so far, neither was associated with breast cancer risk [30]. Thus, whether common variants in CHEK2 are involved in breast cancer etiology remains unknown.

To address this question, we analyzed the association between common haplotypes in the CHEK2 gene and breast cancer risk in a well-defined Swedish population consisting of 1,577 breast cancer cases and 1,513 controls. In addition, we explored interactions between CHEK2 variation and hormone-related risk factors for breast cancer. For comparison with other populations, we also genotyped 1100delC in the entire set of cases and controls.

Methods

Study Population

This study builds on an earlier population-based Swedish case-control study of postmenopausal breast cancer [31–35], with a study base consisting of all Swedish-born women between 50 and 74 years of age that were resident in Sweden between October 1993 and March 1995. During that period, we identified all breast cancer cases at diagnosis through the six regional cancer registries in Sweden, to which reporting of all malignant tumours is mandatory. We randomly selected controls from the Swedish Registry of Total Population that matched the cases in 5-y age strata. Of the eligible cases (3,979) and controls (4,188), 3,345 (84%) cases and 3,454 (82%) controls participated in the initial questionnaire-based study providing detailed information about menopausal hormone use, reproductive history, and other lifestyle factors. From this initial study, we randomly selected 1,500 cases and 1,500 age-frequency matched controls among the postmenopausal participants without any previous malignancy (except carcinoma in situ of the cervix or non-melanoma skin cancer). With the intention of increasing statistical power in subgroup analyses, we further selected all remaining cases and controls (191 cases and 108 controls) who had used menopausal hormones (oestrogen only or any combination of oestrogen and progestin) for at least 4 y, and all (110 cases and 104 controls) with self-reported diabetes mellitus. Additionally, 345 controls that were shared between the initial breast cancer study and an endometrial cancer study with same inclusion criteria were added to our control sample. In total, we selected 1,828 cases and 2,057 controls.

Upon providing informed consent, participants donated whole blood. We collected archived paraffin-embedded, non-cancerous tissue samples for deceased breast cancer cases as well as for those breast cancer cases that declined to donate blood but consented to our use of the tissue. We obtained blood samples and archived tissue samples for 1,521 and 275 breast cancer patients, respectively, and blood samples from 1,524 controls. Reasons for nonparticipation included a lack of interest in research or a negative attitude towards genetic research, old age, and severe disease or death. For deceased cases and cases that consented to our access to tissue, the major reason for nonparticipation was unwillingness or lack of time at the respective pathology departments to retrieve the tissue. Population-based participation rates (taking into account the proportion that did not participate in the questionnaire study) for cases and controls were 73% and 61%, respectively.

This study was approved by the Institutional Review Boards in Sweden and at the National University of Singapore.

DNA Isolation

DNA was extracted by the Swegene laboratories in Malmö (Sweden) from 4 ml of whole blood using the QIAamp DNA Blood Maxi Kit (Qiagen, Valencia, California, United States) according to the manufacturer’s instructions. From non-malignant cells in paraffin-embedded tissue, we extracted DNA using a standard phenol/chloroform/isoamyl alcohol protocol [36]. We successfully isolated DNA from 1,318 (blood) and 272 (tissue) breast cancer patients, and from 1,518 controls.

SNP Markers and Genotyping

We selected SNPs in the CHEK2 gene and its 5-kb flanking sequences from dbSNP (build 123, http://www.ncbi.nlm.nih.gov/SNP) and Celera databases, aiming for an initial marker density of at least one SNP per 5 kb. SNPs were genotyped...
using the Sequenom primer extension-based assay (San Diego, California, United States) following the manufacturer’s instructions. Briefly, multiplex primer extension assays were designed with the SpectroDesigner software. As template, 5 ng of genomic DNA was used in a multiplex PCR reaction. The PCR product was further purified before the primer extension reaction to generate allele-specific base extension products. The base-extension products were detected in the MassARRAY time-of-flight mass spectrometry (MALDI-TOF) system (Sequenom) to determine genotypes. All genotyping results were generated and checked by laboratory staff unaware of case-control status. Only SNPs for which more than 85% of the samples gave a genotype call were used in further analyses. We genotyped 200 randomly selected SNPs in the 92 control samples using both the Sequenom system and the BeadArray system from Illumina (San Diego, California, United States). The genotype concordance was over 99.5%, suggesting high genotyping accuracy.

Linkage Disequilibrium Characterization and TagSNP Selection

To characterize the linkage disequilibrium (LD) pattern, we calculated pairwise $D’$ and $R^2$ values using two-SNP haplotypes inferred via the EH plus program [37]. We reconstructed haplotypes using the PLEM algorithm [38] implemented in the tagSNPs program [39], and selected tagSNPs based on the $R^2$ coefficient. $R^2$ is the squared correlation between true haplotype dosage (number of copies of a haplotype) and the haplotype dosage predicted by tagSNPs. We chose tagSNPs so that common SNP genotypes and common haplotypes were predicted with an $R^2$ value of 0.8 or higher [40]. In order to evaluate our tagSNP performance in capturing unobserved SNPs within CHEK2, and to assess whether we needed a denser set of markers, we performed the SNP-dropping analysis [41]. In brief, each of the 14 genotyped SNPs was dropped in turn, and tagSNPs were selected from the remaining 13 SNPs so that their haplotypes predicted the 13 SNPs with an $R^2$ value of 0.9 [42]. A slightly more stringent value of 0.9 was used here, as we were predicting only SNPs and not haplotypes with our tagSNPs. We then estimated how well the tagSNP haplotypes of the remaining 13 SNPs predicted the dropped SNP, an evaluation that can provide an unbiased and accurate estimate of tagSNP performance [43]. This method assumes the markers to be a random selection of the polymorphisms in the gene. However, some intervals between adjacent genotyped SNPs exceeded 5 kb. We therefore employed additional tests for these intervals. If the tagSNPs selected using only the set of SNPs on one side of the interval predicted the individual SNPs on the other side sufficiently well, then we judged the coverage to be adequate. We had five intervals greater than 5 kb, thus ten sets of predictions. One of these ten was ignored, as there were only two SNPs to the left of the gap.

Statistical Analyses

We first conducted the haplotype-based association analysis, since the tagSNPs were chosen primarily such that their haplotypes can accurately predict common SNPs in the gene. Our testing strategy was to first fit a single model and to assess for each stratum the haplotype-trait association as a global likelihood ratio test (on 7 degrees of freedom). The global test corresponds to carrying out seven tests on 1 degree of freedom each, and applying a correction for multiple hypothesis testing. Only if the global test was significant did we proceed to testing individual haplotype contrasts, adjusting for multiple testing [44]. We first computed expected haplotype dosage using haplotype frequencies estimated for cases and controls combined, assuming Hardy-Weinberg equilibrium (HWE). We then included the haplotype dosage as a covariate in a logistic regression model conditioned on the variables used for sampling: age (3-y age groups), menopausal oestrogen only use (never, < 4 y, or ≥ 4 y), menopausal oestrogen and progestin use (never, < 4 y, or ≥ 4 y) and self-reported diabetes mellitus (yes or no). The appropriateness of the approach, which in imputing haplotypes does not condition on disease status and the nonrandom ascertainment scheme, is argued for by Stram et al. [39]. That is, when $R^2$ values are high, as is the case here, point and interval estimates obtained by this approach will be reasonably accurate. Confounding has been defined as the presence of a common cause to the exposure and the outcome [45], and we believe that the lifestyle and reproductive breast cancer risk factors, shown in Table 1, are unlikely to cause genetic variation in CHEK2. However, to assess whether the risk factors could be intermediates in the causal pathway between CHEK2 and breast cancer, we tested whether there was an association between the tagSNPs and any of the risk factors among the randomly sampled controls using a Kruskal-Wallis test. We also assessed the associations between groups of haplotypes and breast cancer risk. Since there is no biological reason to cluster haplotypes on the basis of their frequency, we employed a Bayesian association mapping approach [46] that clusters haplotypes according to their allelic similarity.

Results

Study Population

Selected characteristics of the participants in the parent questionnaire study and the current genetic study are summarized in Table 1. The distribution of nongenetic factors among cases and controls in the current study were comparable to those in the parent study and reflected established associations [47,48]. The exceptions were long-term menopausal hormone use and diabetes mellitus, which had been consciously oversampled into the current study.

Cases who donated a tissue sample were on average 1.5 y older and were more likely to have been diagnosed with stage 2 or more advanced cancers ($p < 0.0001$) compared to cases who donated a blood sample. However, no significant differences in genotype frequencies were evident between cases who participated via blood or tissue samples (unpublished data). Furthermore, genotype frequencies were similar between tumours cm or smaller in diameter without lymph node involvement and tumours larger than 2 cm or with spread to regional lymph nodes (unpublished data).

LD Pattern and Coverage Estimation

Of 34 successfully genotyped SNPs in CHEK2, 23 were polymorphic (Figure 1 and Table 2), but five of them showed a significant deviation ($p < 0.01$) from HWE and were therefore excluded from further analyses. The five problematic SNPs
were all located within the shared region between CHEK2 and several homologous pseudogenes [49]. Of the remaining 18 polymorphic SNPs, 14 were common (here defined as having a minor allele frequency [MAF] of at least 0.03) and were therefore used to characterize the LD pattern and haplotype structure within CHEK2. The 14 SNPs spanned 52 kb of the gene, which yielded a mean and median density of one SNP per 4 kb and 3.2 kb, respectively. The D' pattern (Figure 1) suggested strong LD across CHEK2 without extensive recombination, and we therefore treated the whole gene as

Table 1. Selected Characteristics of the Postmenopausal Sporadic Breast Cancer Cases and Controls Participating in the Present Study Compared to All Who Answered the Questionnaire in the Parent Study

| Category                              | Characteristic   | Parent Study |          | Present Study |          |
|--------------------------------------|------------------|--------------|----------|---------------|----------|
|                                      |                  | Number of Cases/Controls | Cases | Controls | Number of Cases/Controls | Cases | Controls |
| Age and parity data, case numbers, and mean ± standard deviation | Age (y)          | 2,817/3,111  | 63.4 ± 6.7 | 64.3 ± 6.5 | 1,577/1,513  | 63.3 ± 6.5 | 63.1 ± 6.4 |
|                                      | Age at menarche (y) | 2,557/2,832  | 13.5 ± 1.4 | 13.6 ± 1.4 | 1,430/1,385  | 13.5 ± 1.4 | 13.5 ± 1.4 |
|                                      | Age at menopause (y) | 2,802/3,093  | 50.4 ± 3.5 | 50.0 ± 3.9 | 1,567/1,500  | 50.4 ± 3.5 | 50.0 ± 4.0 |
|                                      | Recent BMI (kg/m²) a | 2,802/3,065  | 25.8 ± 4.2 | 25.5 ± 4.2 | 1,568/1,492  | 25.8 ± 4.1 | 25.5 ± 4.2 |
|                                      | Age at first birth (y) | 2,373/2,753  | 25.3 ± 4.9 | 24.6 ± 4.6 | 1,339/1,366  | 25.4 ± 4.9 | 24.7 ± 4.7 |
|                                      | Parity            | 2,817/3,110  | 1.8 ± 1.2  | 2.1 ± 1.4  | 1,577/1,513  | 1.8 ± 1.2  | 2.2 ± 1.3  |
| Duration of menopausal hormone use, case numbers, and percentages | < 4 y            | 405/330      | 14.6      | 10.8       | 206/190      | 13.2      | 12.8       |
|                                      | ≥ 4 y            | 389/256      | 14.0      | 8.4        | 309b/216b    | 19.8      | 14.5       |
| History, case numbers, and percentages | Self-reported diabetes mellitus (yes/no) | 2,810/2,652 | 6.0       | 6.1        | 1,575/1,398b | 9.0        | 7.8        |
|                                      | Family history (yes/no)c | 2,744/2,607 | 16.0      | 9.2        | 1,538/1,376  | 16.0      | 9.2        |

aOne year prior to diagnosis.
bLong-term users of menopausal hormones and women with diabetes mellitus were oversampled.
cFamily history was defined as having at least one first-degree relative with breast cancer.
BMI, body mass index.
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Figure 1. Exon, SNP, and LD Information for CHEK2
Lane 1 (Chr): Physical position on chromosome 22. Lane 2 (CHEK2): Exon locations. Lane 3 (dbSNP): SNPs downloaded from dbSNP build 124. Lane 4 (Genotyped): SNPs genotyped in our study and their respective frequencies in 92 controls. Accession numbers are given for the 14 common SNPs (MAF ≥ 0.03) that were in HWE, tagSNPs are marked with asterisks. LD grid: pairwise D’ between the 14 common SNPs.
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as ing haplotypes into our subsequent haplotype reconstruction. We identified a total of 31 haplotypes from the 14 SNPs, six of which were common (i.e., had an estimated population frequency of at least 0.03) and accounted for 81% of the chromosomes (Table 3). We selected six tagSNPs from the 14 common SNPs (Figure 1 and Table 2) that could efficiently and accurately predict the eight remaining common SNPs and the six common haplotypes within CHEK2 with an average $R^2$ of 0.94.

Four of the 34 typed CHEK2 SNPs were nonsynonymous coding polymorphisms (Table 2). None of these polymorphisms were common and, except for the 1100delC mutation, were not analyzed further.

Since one cannot assume that tagSNPs capture non-genotyped SNPs as efficiently as genotyped SNPs [50], we used the SNP dropping method [41] to evaluate tagSNP performance. We found that the dropped SNPs were captured with an average $R^2$ of 0.93, with 13 of the 14 dropped SNPs (93%) having an $R^2$ greater than 0.75. Therefore, the six selected tagSNPs could efficiently capture not only all the genotyped but also unobserved common SNPs within CHEK2. Some intervals between adjacent SNPs exceeded 5 kb. We found the average proportion of the variance on one side of the intervals captured by tagSNPs chosen from the other side was at least 0.68 and averaged

Table 2. Summary Data on 34 SNPs in CHEK2 and Its 5-kb Flanking Sequences Successfully Genotyped in 92 Swedish Controls

| SNP Number | dbSNP Name | Region | Amino Acid Change/Conserved SNP | Position | Alleles | Minor Allele Frequency | HWE p-Value | tagSNPs |
|------------|------------|--------|--------------------------------|----------|---------|------------------------|------------|---------|
| 1          | rs5762743  | 3’ downstream | | 27404217 | A | 0 | – | |
| 2          | rs6005836  | 3’ downstream | | 27404332 | A | 0 | – | |
| 3          | rs2881500  | 3’ downstream | | 27405800 | G/A | 0.500 | 0.000 | |
| 4          | rs3963404  | Intron 16–17 | | 27409529 | C/T | 0.500 | 0.000 | |
| 5          | rs4035547  | Intron 16–17 | | 27409670 | C | 0 | – | |
| 6          | rs17882942 | Exon 16 | V483L | 27409685 | C | 0 | – | |
| 7          | rs17883172 | Exon 16 | K472E | 27409718 | G | 0 | – | |
| 8          | rs17883172 | Exon 16 | K472E | 27409718 | G | 0 | – | |
| 9          | rs135424   | Intron 15–16 | | 27410346 | G/A | 0.500 | 0.000 | |
| 10         | rs2346874  | Intron 15–16 | | 27410423 | C/T | 0.494 | 0.000 | |
| 11         | rs4035551  | Intron 15–16 | | 27410584 | G/A | 0.500 | 0.000 | |
| 12         | rs5762746  | Intron 15–16 | Conserved | 27412677 | T/C | 0.494 | 0.000 | |
| 13         | rs5762749  | Intron 14–15 | | 27415591 | C/G | 0.375 | 0.78 | |
| 14         | rs2267130  | Intron 9–10 | | 27424308 | C/T | 0.495 | 0.92 | |
| 15         | rs743181   | Intron 9–10 | | 27427521 | T/C | 0.352 | 0.21 | |
| 16         | rs743185   | Intron 9–10 | | 27427771 | C/T | 0.117 | 0.21 | |
| 17         | rs2073326  | Intron 9–10 | | 27430081 | A/C | 0.016 | 0.87 | |
| 18         | rs1884816  | Intron 8–9 | Conserved | 27431287 | T/C | 0.124 | 0.19 | |
| 19         | rs1884817  | Intron 8–9 | Conserved | 27431499 | G/C | 0.139 | 0.59 | |
| 20         | rs752776   | Intron 7–8 | | 27432783 | G/A | 0.337 | 0.67 | |
| 21         | rs6005843  | Intron 7–8 | | 27432998 | T/C | 0.023 | 0.83 | |
| 22         | rs6005846  | Intron 6–7 | | 27440386 | A/C | 0.011 | 0.92 | |
| 23         | rs2347443  | Intron 6–7 | | 27443774 | G/C | 0.148 | 0.44 | |
| 24         | rs17879961 | Exon 6 | I157T | 27445541 | T/C | 0.006 | 0.96 | |
| 25         | rs4525795  | Intron 4–5 | | 27450390 | C | 0 | – | |
| 26         | rs1262748  | Intron 4–5 | | 27450739 | G | 0 | – | |
| 27         | rs13056673 | Intron 4–5 | | 27453934 | T | 0 | – | |
| 28         | rs738722   | Intron 4–5 | | 27454566 | C/T | 0.319 | 0.14 | |
| 29         | rs17883862 | Exon 4 | P85L | 27455010 | C | 0 | – | |
| 30         | rs5762764  | 5’ UTR-intron 3–4 | | 27457544 | A/G | 0.330 | 0.83 | |
| 31         | rs5762765  | 5’ UTR-intron 3–4 | | 27457587 | G/C | 0.292 | 0.55 | |
| 32         | rs2236141  | 5’ UTR-exon 3 | Conserved | 27462424 | C/T | 0.122 | 0.74 | |
| 33         | rs2236142  | 5’ UTR-exon 3 | Conserved | 27462498 | C/G | 0.313 | 0.65 | |
| 34         | rs7292212  | 5’ UTR-exon 3 | | 27462726 | C | 0 | – | |

Table 3. Common Haplotypes Reconstructed Using 14 SNPs in CHEK2

| Haplotypes | Haplotype Frequency | $R_h^{2b}$ |
|------------|---------------------|------------|
| GGTCCTGAGTAGCC | 0.271 | 0.964 |
| GCCCTGAGG GCCCC | 0.190 | 0.938 |
| ACCCTGGGGAGCC | 0.117 | 0.995 |
| GCCCTGAGGCGTG | 0.103 | 0.887 |
| GCTTCCGGCAGCG | 0.096 | 0.975 |
| GGTCCTGAGGAGCG | 0.030 | 0.854 |

*Non-synonymous variant amino acid exchange or polymorphism located in conserved sequence between human-mouse or human-rat or both.

$^{a}$dbSNP build 124.

$^{b}$Major alleles given first and minor alleles second.

$^{c}$In 92 controls.

$^{d}$From $\chi^2$ test in 92 controls.

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$^a$In 92 controls.

$^b$$R_h^2$ quantifies how well we can predict the number of copies of haplotypes an individual carries using the six tagSNPs.

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a single haplotype block in our subsequent haplotype reconstruction. We identified a total of 31 haplotypes from the 14 SNPs, six of which were common (i.e., had an estimated population frequency of at least 0.03) and accounted for 81% of the chromosomes (Table 3). We selected six tagSNPs from the 14 common SNPs (Figure 1 and Table 2) that could efficiently and accurately predict the eight remaining common SNPs and the six common haplotypes within CHEK2 with an average $R_h^2$ of 0.94.

Four of the 34 typed CHEK2 SNPs were nonsynonymous coding polymorphisms (Table 2). None of these polymorphisms were common and, except for the 1100delC mutation, were not analyzed further.

Since one cannot assume that tagSNPs capture non-genotyped SNPs as efficiently as genotyped SNPs [50], we used the SNP dropping method [41] to evaluate tagSNP performance. We found that the dropped SNPs were captured with an average $R^2$ of 0.93, with 13 of the 14 dropped SNPs (93%) having an $R^2$ greater than 0.75. Therefore, the six selected tagSNPs could efficiently capture not only all the genotyped but also unobserved common SNPs within CHEK2. Some intervals between adjacent SNPs exceeded 5 kb. We found the average proportion of the variance on one side of the intervals captured by tagSNPs chosen from the other side was at least 0.68 and averaged...
0.75. This is likely to be an underestimate, as the markers considered were a much greater distance apart than the polymorphisms in the intervals of interest. Thus we consider such gaps to present little problem and conclude that our tagSNPs effectively capture the common variation present in the gene.

Association Analyses

We successfully genotyped the six tagSNPs in 1,577 breast cancer cases and 1,513 controls. All six tagSNPs were in HWE among the controls (unpublished data). We assessed, among the randomly selected controls, whether any of the tagSNPs were associated with the lifestyle and reproductive breast cancer risk factors shown in Table 1. TAG2 and TAG4 were statistically associated with parity (p = 0.029 and p = 0.009, respectively), which showed a statistically significant trend for allelic dose (TAG2, p = 0.006; TAG4, p = 0.0002).

We identified 19 rare (Table S1) and seven common haplotypes from the six tagSNPs (Table 4). The 19 rare haplotypes were collapsed into a single group in the association analyses. One additional common haplotype was identified in the full set of controls, which was not identified in the preliminary 92 controls. A global test of association, including all common haplotypes and the 19 rare haplotypes as one group in the model, was not statistically significant (likelihood ratio test, p = 0.19). Table 4 presents odds ratios (ORs) and confidence intervals (CIs) for tagSNP haplotypes using the most common haplotype (haplotype 1) as reference. None of the common haplotypes increased the risk of breast cancer, but the comparison between the group of 19 rare haplotypes against the reference showed a slight association with breast cancer risk (OR 1.24; 95% CI, 1.02–1.51), which did not, however, carry over to the global test, in which multiple testing has been formally accounted for. After excluding the 1100delC carriers (n = 28) from the analysis, this association decreased (OR 1.20; 95% CI, 0.98–1.47), whilst the odds ratios for the common haplotypes remained unchanged. Comparing carriers to noncarriers of each haplotype, instead of using haplotype 1 as reference, did not substantially affect the results (unpublished data).

We also tested the association between tagSNP genotypes and breast cancer risk, overall (Table S8) and stratified by other risk factors (unpublished data), but no additional compelling results emerged. Adjusting the models including TAG2 or TAG4, for parity did not change our results.

Analysis of CHEK2*1100delC

The 1100delC mutation, genotyped in 1,510 cases and 1,334 controls, was rare in our Swedish population, with a frequency of 0.4% in the controls (HWE, p = 0.89). The deletion was slightly more common in the cases (0.7%) than in the controls, and the corresponding age- and sampling scheme-adjusted odds ratio for carriers versus noncarriers was 2.26 (95% CI, 0.99–5.15) (Table 5). The 1100delC was exclusively carried on rare haplotypes, which may explain the marginally significant association between the group of 19 rare haplotypes and breast cancer risk (Table 4).

Discussion

Our extensive linkage disequilibrium mapping association study did not provide support for an association between common variations in CHEK2 and breast cancer risk. The 1100delC mutation was more common in cases than in controls in our study population, but the difference was not statistically significant. The genotype frequencies were
similar to those reported in other populations, thus confirming that our study population is representative of other Northern European populations.

The population on which our study was based is relatively large, well defined, and genetically homogenous. We were able to cover the majority of common variations in the CHEK2 gene by genotyping a relatively dense map of markers. We had detailed information about hormone-related risk factors for breast cancer that allowed us to explore gene-environment interactions. We also assessed the statistical power of our study. Until now, the power evaluations of CHEK2 genetic studies have been carried out under the assumption that the genetic risk allele is being directly tested [17]. This approach misleadingly overestimates power, because the susceptibility allele is rarely tested directly. Our detailed understanding of the LD pattern across CHEK2 allowed us to overcome these limitations and estimate power more accurately. To estimate power we used a method described by Chapman et al. [42], which assumes codominant effects at an unobserved locus (assuming 80% power, an \( \alpha \) value of 0.05, and \( R^2 = 0.9 \) for the ability of haplotypes to predict the allele count at the causal locus). We estimate that our study should be able to detect a disease susceptibility locus with an odds ratio of 1.23 if its MAF is 0.5, of 1.25 if MAF = 0.35, of 1.32 if MAF = 0.15, or of 1.55 if MAF = 0.05. Therefore, given the comprehensive coverage of common genetic variants within CHEK2 by our tagSNP approach, it is unlikely that any common disease allele of at least moderate effect was missed in our study.

Selection bias could have arisen in this study, as non-participation was related to severe disease or death. However, CHEK2 genotype frequencies were not significantly different between cases donating tissue samples, representing more ill or deceased patients, and cases donating blood samples. This non-participation thus affected only the generalizability of our study.

The 1100delC variant has not previously been studied in the Swedish population. The variant is rare [15–21,23], except in a few Northern European populations such as Finland and the Netherlands, where moderate frequency of 1% or above has been observed [11,12,22]. The low population frequency of 1100delC in the Swedish population is therefore generally in line with previous data. Furthermore, the corresponding odds ratio for carriers versus noncarriers was 2.26 (95% CI, 0.99–5.15), which is also consistent with the results from other Northern European populations [22,24]. Taken together, the results of our 1100delC analysis confirm that our case-control sample is representative of a Northern European population.

A few pathogenic variants have been identified by sequencing CHEK2, examples of which are 1100delC and I157T [13]. Association analyses of these variants in various different populations suggest that these variants are associated with moderate risk for breast cancer and have a low population frequency [15,23,24,26,27]. The pathological effect of 1100delC was suggested to be due to haploinsufficiency caused by the lost function of the 1100delC truncated protein [51]. In contrast, by introducing a nonconservative amino acid change into the forhead-associated domain of CHEK2, I157T may produce a dominant-negative effect by forming heterodimers with wild-type CHEK2 protein [27]. The I157T mutation could also have a pathological effect by producing a defective protein for binding and phosphorylating its downstream target, Cdc25A, and for binding p53 and BRCA1 [15]. Given the fact that CHEK2 plays a central role in the DNA damage checkpoint and apoptosis pathways [52], the modest effect of the CHEK2 pathogenic variants is surprising. It is however most likely due to the functional redundancy in the crucial DNA damage checkpoint pathway and thus the compensation of defect in CHEK2 function by other members of the pathway. This mechanism was suggested in a recent study showing that the normal degradation of Cdc25A is shown to be maintained in the carriers of the 1100delC variant [51]. Our association study supports other reports [30] indicating that, besides the known pathogenic coding variants, it is unlikely that other common coding or noncoding variants play a significant role in a predisposition to breast cancer. This is consistent with the notion discussed by Cybulski et al. [15] that there are three classes of risk genes for breast cancer: a class of genes in which mutations are rare but confer a very high relative risk (including BRCA1 and BRCA2); a class in which variants are common but confer only modest risk (few if any such genes have been confirmed to date); and a class (including CHEK2) in which mutations are rare but associated with only modest risk. Although the idea is speculative, CHEK2 may exercise a pleiotropic effect as a central player in the DNA damage checkpoint and apoptosis pathways on other biological processes than its role in carcinogenesis, and this may provide necessary selection pressure to maintain its functional variants at a low population frequency.

To our knowledge, this is the most comprehensive evaluation of the association between the genetic variation within CHEK2 and postmenopausal breast cancer risk. However, despite the role of the CHEK2 gene in breast cancer aetiology, we conclude that common variations in CHEK2 do not affect breast cancer risk, at least not in the Swedish population. On the other hand, our study cannot exclude the possible influence of rare genetic variants in CHEK2, such as the CHEK2*1100delC, on breast cancer risk. Investigation of such rare risk alleles in a population-based design requires a much larger sample size than the one utilized in the present study.

Supporting Information

Alternative Language Abstract S1. Swedish Translation of the Abstract
Found at DOI: 10.1371/journal.pmed.0030168.sd001 (22 KB DOC).

Alternative Language Abstract S2. Icelandic Translation of the Abstract
Found at DOI: 10.1371/journal.pmed.0030168.sd002 (21 KB DOC).

Alternative Language Abstract S3. Chinese Translation of the Abstract
Found at DOI: 10.1371/journal.pmed.0030168.sd003 (27 KB DOC).

Table S1. Association of the 19 Rare Haplotypes with Breast Cancer Risk
Found at DOI: 10.1371/journal.pmed.0030168.t001 (49 KB DOC).

Table S2. Common Haplotypes Reconstructed Using Six tagSNPs in CHEK2 in Relation to Breast Cancer Risk, Considering Only Cases Who Eventually Were Diagnosed with a Second Breast Cancer
Found at DOI: 10.1371/journal.pmed.0030168.t002 (38 KB DOC).
Table S3. Common Haplotypes Reconstructed Using Six tagSNPs in CHEK2 in Relation to Breast Cancer Risk, by Family History

Family history was considered positive for participants who had at least one first-degree relative with breast cancer.

Found at DOI: 10.1371/journal.pmed.0030168.s003 (45 KB DOC).

Table S4. Common Haplotypes Reconstructed Using Six tagSNPs in CHEK2 in Relation to Breast Cancer Risk, Stratified by Body Mass Index

Body mass index is calculated as weight/height² (kg/m²).

Found at DOI: 10.1371/journal.pmed.0030168.s004 (52.5 KB DOC).

Table S5. Common Haplotypes Reconstructed Using Six tagSNPs in CHEK2 in Relation to Breast Cancer Risk, Stratified by Age at First Childbirth

Found at DOI: 10.1371/journal.pmed.0030168.s005 (51 KB DOC).

Table S6. Common Haplotypes Reconstructed Using Six tagSNPs in CHEK2 in Relation to Breast Cancer Risk, Stratified by Duration of CHEK2

Common Haplotypes Reconstructed Using Six tagSNPs in CHEK2 in Relation to Breast Cancer Risk, Stratified by Use of Any Kind of Medium-Potency Oestrogens

Found at DOI: 10.1371/journal.pmed.0030168.s006 (51 KB DOC).

Table S7. Common Haplotypes Reconstructed Using Six tagSNPs in CHEK2 in Relation to Breast Cancer Risk, Stratified by Parity

Found at DOI: 10.1371/journal.pmed.0030168.s007 (55 KB DOC).

Table S8. Association between Six tagSNPs in CHEK2 and Breast Cancer Risk

Found at DOI: 10.1371/journal.pmed.0030168.s008 (47 KB DOC).

Accession Numbers

Found at DOI: 10.1371/journal.pmed.0030168.st008 (47 KB DOC).

Table S9. Common Haplotypes Reconstructed Using Six tagSNPs in CHEK2 in Relation to Breast Cancer Risk, Stratified by Body Mass Index

Body mass index is calculated as weight/height² (kg/m²).

Found at DOI: 10.1371/journal.pmed.0030168.s009 (52.5 KB DOC).

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Author contributions. KE, JL, and SW wrote the article. KE and KH performed the statistical analyses with the assistance of JP, MMI, and AS. CB oversaw the genotyping procedures, and KE assisted with the genotyping. KE and YL selected the polymorphisms. JL and SW coordinated the study. KH, JP, KSC, ETL, PH, JL, and SW contributed to conception and design of the project and critically revised the manuscript. All authors read and approved the final manuscript.

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Editors’ Summary

**Background.** Approximately 5% of breast cancer patients have a strong familial risk for the disease. In these families, multiple family members are usually affected, often at an early age (before they are 35). Rare inherited variants in two genes, **BRCA1** and **BRCA2**, are responsible for the high breast cancer risk in most of these “breast cancer families.” Among the other 95% of women with breast cancer who are not known to have inherited one of these high-risk variants, there may still be some inherited susceptibility to the disease: about 10% of women have a “moderate” family history of breast cancer. The inherited genetic factors that are responsible for the more common but weaker susceptibility to breast cancer are so far mostly unknown. These factors could, for example, be common variants in many different genes, each of which on its own cause only a moderately raised risk (although more common variants of the **BRCA1** and **BRCA2** genes do not seem to be associated with breast cancer).

**Why Was This Study Done?** **CHEK2** is another gene that has been associated with familial breast cancer. In some breast cancer families without mutations in **BRCA1** and **BRCA2**, a specific variant of **CHEK2** (called **1100delC**), indicating a deletion at position 1100) occurred frequently in patients with breast cancer. The association between this particular **CHEK2** variant and an increased risk of breast cancer was confirmed in a large study that looked at approximately 10,000 patients with breast cancer and 10,000 controls. However, the **1100delC** variant of **CHEK2** is rare, and scientists want to know whether more common variants of the gene were associated with breast cancer. A number of previous studies that looked at two common variants of **CHEK2** had not given consistent results. This study used six common variants to predict all common variants of **CHEK2** in a group of postmenopausal Swedish women and tested whether any of the six variants were associated with the women having breast cancer.

**What Did the Researchers Do and Find?** They identified the six common variants by analyzing the DNA of the **CHEK2** gene in 92 Swedish women. They then compared the frequency of these common variants between approximately 1,500 women with breast cancer and 1,500 women of the same age and similar lifestyles without breast cancer. They found no association between any of the common variants and the likelihood of the women having breast cancer.

**What Do These Findings Mean?** This study suggests that Swedish women (or those of Northern European descent who have a similar genetic inheritance) who have any of the common variants of the **CHEK2** gene do not have a raised risk of getting breast cancer. However, this finding might not apply in other populations. As in previous studies, this study also found a raised risk of breast cancer for women who carried the rare **1100delC** variant, but the study was too small to test whether there are other rare variants that might also predispose to breast cancer. Overall, scientists have not yet found many common gene variants that increase the risk of getting cancer. But, to test conclusively for associations between genetic variants, both common and rare, and cancer, very large studies will be needed. Understanding how a person’s genetic make-up and their environment and lifestyle influence their risk for certain cancers remains a challenge.

Additional Information. Please access these Web sites via the online version of this summary at http://dx.doi.org/10.1371/journal.pmed.0030168.

- Risk factors for breast cancer from the American Cancer Society
- Summary of information on the **CHEK2** gene from the US National Center for Biotechnology Information
- Information on Breast Cancer molecular biology and genetics from Cancer Research UK
- Information on breast cancer genetics from the UK Public Health Genetics Unit