CHEK2*1100delC homozygosity is associated with a high breast cancer risk in women

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Abstract

Background Mutations in the CHEK2 gene confer a moderately increased breast cancer risk. The risk for female carriers of the CHEK2*1100delC mutation is 2-fold increased. Breast cancer risk for carrier women is higher in a familial breast cancer setting which is due to coinheritance of additional genetic risk factors. This study investigated the occurrence of homozygosity for the CHEK2*1100delC allele among familial breast cancer cases and the associated breast cancer risk.

Methods and results Homozygosity for the CHEK2*1100delC allele was identified in 8/2554 Dutch independent familial non-BRCA1/2 breast cancer cases. The genotype relative risk for breast cancer of homozygous and heterozygous familial breast cancer cases was 101.34 (95% CI: 4.47-121,000) and 4.04 (95% CI: 0.88-21.0), respectively. Female homozygotes appeared to have a greater than 2-fold increased breast cancer risk compared to familial CHEK2*1100delC heterozygotes ($p=0.044$). These results and the occurrence of multiple primary tumors in 7/10 homozygotes indicate a high cancer risk in homozygous women from non-BRCA1/2 families.

Conclusions Intensive breast surveillance is therefore justified in these homozygous women. It is concluded that diagnostic testing for bi-allelic mutations in CHEK2 is indicated in non-BRCA1/2 breast cancer families, especially in populations with a relatively high prevalence of deleterious mutations in CHEK2.
The CHEK2 kinase functions in the DNA damage response pathway and, activated by ATM phosphorylates a variety of targets such as CDC25A/C, BRCA1 and p53.

Both truncating and some missense mutations in CHEK2 confer an increased breast cancer risk. Besides rare CHEK2 germline mutations, five founder mutations in CHEK2 have been identified with a variable prevalence in different populations. The p.S428F mutation has a prevalence of 1.37% in the Ashkenazi Jewish population, p.I157T is most prevalent in Slavic populations (~5.0%), IVS+2G>A has been found in German and Polish populations (~0.3%), the del5395 in the Polish population, and CHEK2*1100delC with highest prevalence in Northern Europe. The CHEK2*1100delC mutation results in a frameshift leading to a premature termination at codon 381. In the Netherlands the prevalence in the general population is 1.1%, 2.5% in unselected breast cancer cases and up to 4.9% in familial breast cancer cases.

The population-based breast cancer risk for a female CHEK2*1100delC carrier is consistently associated with odds ratio’s between 1.5-3.0, corresponding to an estimated lifetime risk for breast cancer of approximately 20-25%.

Although arguably influenced by ascertainment biases, publications have shown that female heterozygous CHEK2*1100delC carriers from a familial breast cancer setting have a higher lifetime breast cancer risk of 37%. Since females from western populations have an average lifetime risk of ~10%, this suggests that these females have a 3-fold increased breast cancer risk. This increased risk in a familial setting is most likely explained by a polygenic model in which other genetic risk factors present in these families contribute to the individual risk of these heterozygous cases.

Clinical utility of CHEK2*1100delC mutation testing is currently under debate. We were interested whether homozygous female CHEK2*1100delC carriers have a higher cancer risk than heterozygous carriers. Since the prevalence of the CHEK2*1100delC allele is high in the Netherlands we are in the unique position to address this question. The prevalence of homozygous individuals in the general population is estimated to be approximately 1 in 33,000 individuals.

Genomic DNA of 2,554 independent breast cancer patients from familial non-BRCA1/2 breast cancer cohorts of three Dutch Clinical Genetics Centres were genotyped for the CHEK2*1100delC mutation. For the majority of families, DNA from only one affected individual was available. If DNA was available from multiple breast cancer patients of a family, the case with the earliest onset of breast cancer was selected. The frequency of the CHEK2*1100delC allele in the general population was estimated based on the genotypes of 3,267 controls. No homozygous and 37 heterozygous individuals were detected amongst controls. Of these controls, 2,059 were derived from two previous Dutch studies and the remaining 1,208 controls.
were genotyped for this study. Material of patients was used following institutional guidelines; breast cancer cases gave informed consent to screen for susceptibility genes. DNA from irresversibly anonymized controls was used following the code for Proper Secondary Use of Human Tissue.

Heterozygosity for the CHEK2*1100delC mutation was identified in 112/2554 female breast cancer cases (4.4%) and we found 8/2554 cases to be homozygous for the allele (0.3%). Homozygosity for CHEK2*1100delC was confirmed by sequencing. We estimated the genotype relative risk (GRR) based on the observed allele frequencies in our cases and controls and under the assumption of the Hardy-Weinberg equilibrium (HWE) in the total population. The estimated GRR for heterozygous and homozygous familial breast cancer cases were 4.04 (95% confidence interval (CI): 0.88-21.0) and 101.34 (95% CI: 4.47-121,000), respectively. The GRR for homozygotes is much higher than that of heterozygotes but the confidence intervals are broad and overlapping. We therefore tested and confirmed the hypothesis that the breast cancer risk of homozygous females was more than twice the risk of heterozygous CHEK2*1100delC females (p-value = 0.044), showing that two CHEK2*1100delC alleles result in a more than additive risk. For detailed information about the statistical methods and analysis see appendix. If we interpret the results in a conservative way, it indicates that the breast cancer risk for homozygous CHEK2*1100delC female carriers is increased more than 4-fold, that is, more than twice the risk of heterozygous carriers in the population. Since the risk estimates are based on the comparison of risks within a familial cohort, the risk of familial homozygous cases is likely to be more than 6-fold, that is, more than twice the risk of heterozygous familial cases. However, even the conservative estimate justifies intensive breast cancer surveillance.

We then tested all available siblings (blood DNA or DNA from paraffin embedded material when deceased) of the 8 homozygous cases for the presence of CHEK2*1100delC homozygosity (for pedigrees see Figure 1). Of the total of 26 individuals tested in the generation concerned, we identified a total of 12 homozygous individuals (two males, 10 females, including our initial eight probands), and 12 heterozygous individuals (four males, eight females). All 10 female homozygotes had breast cancer; 7/10 had multiple primary tumors; four had bilateral breast cancer and four were additionally affected with colon cancer, ovarian cancer, uterine cancer or melanoma respectively (Table 1). Of note, no homozygosity was found in any of the six unaffected females. One homozygous male had a thymoma at age 47 and the other homozygous male was cancer free at age 54 years.

In the proband generation 12 heterozygous CHEK2*1100delC carriers were identified of whom four were affected with cancer. Of the 36 siblings in total 10 (five
Figure 1 Pedigrees of CHEK2*1100delC homozygous breast cancer patients

The initial eight probands with CHEK2*1100delC homozygosity (+/+) are indicated by arrows. Genotyping results are mentioned (+/- = heterozygosity and -/- = wild-type) above each individual. Individuals with breast cancer (B) are shown as filled circles, with the age at diagnosis. Other cancers are indicated beneath the relevant individuals. Carc = carcinoma of unknown type, Co = colon, Cx = cervical, Eso = esophageal, Leu = leukemia, Lu = lung, Mel = melanoma (Oc = ocular; is = in situ), Ov = ovarian, Pa = pancreatic, Pe = renal, Sarc = chondrosarcoma, Sk = skin, Thym = thymoma, Ut = uterine, and d = age of death. Family 4 was lost to follow-up (NA = not available). We obtained informed consent from all homozygous females and their family members following institutional guidelines. Additional test results are indicated below the individual involved.
males and five females) were not available for testing (six from one family, family 4).

Table 1 shows the age of onset and histology of the breast cancer tumors in homozygous females. Median age of diagnosis of (first) breast cancer for the homozygous females was 47 years old (average age 44 years).

Of the 12 available tumors, 11 were grade 2-3 invasive ductal carcinoma. Nine out of 12 (75%) breast cancers were estrogen (ER) positive and eight progesterone (PR) positive tumors, confirming the predominantly hormone positive tumors observed in heterozygote carriers of the CHEK2*1100delC mutation (Table 1).12 Remarkably, HER2 staining was positive in six out of twelve tumors (50%) in comparison to the known 20-25% in breast cancer tumors in the population.

Figure 1 shows the pedigrees of the CHEK2*1100delC homozygous carriers. Additional DNA testing, besides BRCA1/2 screening, was performed in some families to exclude other known tumor syndromes (for test results see pedigrees). No other DNA diagnosis could be made except for the homozygosity for the CHEK2*1100delC allele in these families.

In previous studies, homozygosity for the CHEK2*1100delC allele was once described in a Dutch male with colorectal cancer at age 52 and in a patient from German/Welsh descent who was bilaterally affected with breast cancer at age 47 and 61 and had an uterine sarcoma at age 58.5,13 In an independent bilateral breast cancer study we enrolled one additional family with homozygosity of the CHEK2*1100delC mutation and identified one homozygous female with bilateral breast cancer age 50 and 56 (both ER/PR positive, HER2 negative), and colon cancer at age 59, and two homozygous males of whom one died of colon cancer at age 32. These cases match well with our findings reported here. It is remarkable that colon cancer thus far has been described (present study combined with literature and our additional family) in four of the 17 homozygous individuals, with ages of onset of 32, 43, 59 and 52 years. Publications variably showed a higher prevalence of heterozygosity for the CHEK2*1100delC mutation in colorectal cohorts.14,15 Systematic studies are needed to evaluate the association between colorectal cancer or other cancers and CHEK2*1100delC homozygosity.

This is the first paper to describe the incidence of homozygosity for the CHEK2*1100delC in a large familial non-BRCA1/2 breast cancer cohort.

In summary, CHEK2*1100delC homozygous females in a familial breast cancer setting have a high breast cancer risk. A conservative estimate shows that the risk is more than 4-fold higher than the risk of women in the general population. However, since the breast cancer risks are based on the GRR estimates of heterozygous and homozygous women within a familial breast cancer cohort, the risk for homozygous
Table 1 Tumor characteristics for CHEK2*1100delC homozygous female carriers

<table>
<thead>
<tr>
<th>Family (member)</th>
<th>Cancer</th>
<th>Age</th>
<th>Tumor type</th>
<th>Grade</th>
<th>ER</th>
<th>PR</th>
<th>HER2</th>
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<tr>
<td>1 (A)</td>
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<td>+</td>
<td>+</td>
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<tr>
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<td>BC R</td>
<td>56</td>
<td>IDC</td>
<td>2</td>
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<td>+</td>
<td>-</td>
</tr>
<tr>
<td>1 (B)</td>
<td>BC L</td>
<td>63</td>
<td>IDC</td>
<td>2</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
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<td>BC R</td>
<td>67</td>
<td>IDC</td>
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<td>-</td>
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<td>Endometrioid</td>
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</tr>
<tr>
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<td>45</td>
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</tr>
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<td>3</td>
<td>BC R</td>
<td>29</td>
<td>ILC+LCIS</td>
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<td>ILC</td>
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Cancer type, side, and age at diagnosis for each CHEK2*1100delC homozygous female carrier is mentioned by family. Breast cancers were revised for tumor type, differentiation grade, and hormone receptor status. BC=breast cancer, L=left, R=right, NA=not available, IDC=invasive ductal carcinoma, ILC=invasive lobular carcinoma, DCIS=ductal carcinoma in situ, LCIS=lobular carcinoma in situ, ER=estrogen receptor, PR=progesterone receptor, HER2=HER2/Neu receptor expression.

*Original report.
*1100delC homozygosity is associated with a high breast cancer risk in women

women in this setting is likely more than 6-fold increased. The GRR estimate of 101.34 (95% CI: 4.47-121,000) for homozygotes also supports a breast cancer risk substantially higher than four. It is reasonable to assume that similar risks will apply for women that are homozygous or compound heterozygous for other deleterious mutations in CHEK2, such as IVS+2G>A and del5395. Further studies are needed to elucidate if this also applies for missense variants such as p.I157T and p.S428F. Interestingly, the phenotype of homozygous CHEK2*1100delC individuals is different from bi-allelic mutation carriers in the other known moderate breast cancer risk genes which cause specific syndromes. Recessive inheritance of mutations in PALB2 and BRII1 result in Fanconi anemia and in ATM results in ataxia telangiectasia.

We conclude that diagnostic testing for mutations in CHEK2 has clinical utility in a familial breast cancer setting, especially in populations where these risk alleles are relatively prevalent. Based on our study we propose that women who are homozygous or compound heterozygous for deleterious mutations in CHEK2 are eligible for intensive breast surveillance in a specialist environment concordant with guidelines for female BRCA1/2 mutation carriers. If our data are replicated and a level of risk similar to BRCA1/2 carriers is further validated in homozygous CHEK2 carriers, these women should also have access to preventive mastectomy after adequate counselling.

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References


Appendix Statistical Analysis

In this appendix we describe the estimation of genotype relative risks for breast cancer, the construction of confidence intervals for these relative risks and a statistical test for testing the alternative hypothesis whether the breast cancer risk of homozygous females for the CHEK2*1100delC allele is more than two times the breast cancer risk of heterozygous females with the CHEK2*1100delC allele in familial breast cancer families. We first introduce some notation.

1. Notation

Denote the genotype frequencies among “familial breast cancer cases” as 
\[ p_{aa} = P(aa | \text{Case}) \], \[ p_{aA} = P(aA | \text{Case}) \] and \[ p_{AA} = P(AA | \text{Case}) \], with \( a \) the CHEK2*1100delC allele and \( A \) the wild type allele. We assume that the allele frequencies in the total population are in Hardy-Weinberg equilibrium (HWE) and it is therefore sufficient to introduce only notation for the allele frequencies and not the genotype frequencies; under the HWE-assumption the genotype frequencies can be written in terms of the allele frequencies. Denote the \( a \) and \( A \) allele frequencies in the total population as \( q_a \) and \( q_A \), respectively. To estimate the genotype relative risks for breast cancer and to test the alternative hypothesis as stated above two
data-sets are available. The first data-set contains the genotypes of \( n = 2554 \) women with breast cancer from breast cancer families. The numbers of these women with genotypes \( aa, aA \) and \( AA \) are denoted as \( N_{aa}, N_{aA} \) and \( N_{AA} \), respectively. The vector \( (N_{aa}, N_{aA}, N_{AA}) \) has a multinomial distribution with parameters \( n \) and probability vector \( (p_{aa}, p_{aA}, p_{AA}) \). Note that \( N_{aa} \) given \( N_{AA} \), \( N_{aA} \) has a binomial distribution with parameters \( n - N_{AA} \) and 
\( \frac{p_{aa}}{p_{aa} + p_{aA}} \). Of course, similar distributions hold for the other conditional combinations. Of the \( n = 2554 \) cases, 2434 were homozygous for the wild type allele \( (n_{AA} = 2434) \), 112 cases were heterozygous \( (n_{aA} = 112) \) and 8 cases were homozygous for the CHEK2*1100delC allele \( (n_{aa} = 8) \).

The second data-set consists of 3267 controls. Since we assume HWE in the total population it is sufficient to consider allele frequencies in stead of genotype frequencies. In total \( m = 6534 = 2 \times 3267 \) alleles were typed in the control group.

Denote the number of \( A \)-alleles as \( M_a \) and the number of \( a \)-alleles as \( M_a \).

Then \( M_a \) has a binomial distribution with parameters \( m \) and \( q_a \) (under HWE).

We found the following numbers: 37 CHEK2*1100delC alleles \( (m_a = 37) \) and 6497 wild type alleles \( (m_a = 6497) \).
1.1 Estimation of the Genotype Relative Risk (GRR)

By Bayes’ rule and under the assumption of HWE in the total population the genotype relative risks can be written in terms of the parameters just defined:

\[
GRR_{aa} = \frac{P(\text{Case} | aa)}{P(\text{Case} | AA)} = \frac{P(aa | \text{Case}) P(AA)}{P(AA | \text{Case}) P(aa)} = \frac{p_{aa} (1-q_a)^2}{p_{aa} q_a^2}
\]

\[
GRR_{aA} = \frac{P(\text{Case} | aA)}{P(\text{Case} | AA)} = \frac{P(aA | \text{Case}) P(AA)}{P(AA | \text{Case}) P(aA)} = \frac{p_{aA} (1-q_a)^2}{p_{aA} 2q_a(1-q_a)}
\]

Both relative risks can be estimated by replacing the genotype and allele frequencies in the formulas by the fractions found among the cases and the controls. This yields the estimates \(\hat{GRR}_{aa} = 101.34\) and \(\hat{GRR}_{aA} = 4.04\).

1.2 Test for relative risk

In the following we describe how to test the alternative hypothesis that the breast cancer risk for homozygous females is at least two times the risk for heterozygous females in familial breast cancer families; so we test the hypotheses

\[H_0 : \frac{P(\text{Case} | aa)}{P(\text{Case} | aA)} \leq 2 \quad \lor \quad H_1 : \frac{P(\text{Case} | aa)}{P(\text{Case} | aA)} > 2,\]

with (under HWE)

\[
\frac{P(\text{Case} | aa)}{P(\text{Case} | aA)} = \frac{p_{aa} 2(1-q_a)q_a}{p_{aA} q_a^2}.
\]
Like before this relative risk can be estimated as

\[
\frac{N_{aa}}{N_{at}} \frac{2(1-M_a/m)(M_a/m)}{(M_a/m)^2}
\]

if \( M_a > 0, N_{at} > 0 \). We therefore use the test-statistic

\[
T = \frac{N_{aa}}{N_{at}} \frac{2(1-M_a/m)(M_a/m)}{(M_a/m)^2} 1_{[M_a > 0]} \mid N_{at} = n_{at}
\]

with \( T = \infty \) if \( N_{at} = 0 \), to test the hypotheses as just given. The distribution of the test-statistic \( T \) depends on the unknown parameters, since \( N_{aa} \mid N_{at} = n_{at} \) and \( N_{at} \mid N_{at} = n_{at} \) have binomial distributions with parameters, respectively, equal to,

\[
n - n_{at} \quad \text{and} \quad p_{at}/(p_{aa} + p_{at}) = 1/(1 + p_{at}/p_{aa})
\]

and \( n - n_{at} \) and \( p_{at}/(p_{aa} + p_{at}) = 1/(p_{at}/p_{aa} + 1) \). Moreover \( M_a \) has a binomial distribution with parameters \( m \) and \( q_a \).

Suppose we observed the value \( t \) for the test-statistic \( T \), the p-value is equal to

\[
\sup_{H_0} P_{H_0}(T \geq t \mid N_{at} = n_{at})
\]

where the supremum is taken over the parameter set under the null hypothesis:

\[
\{(q_a, \lambda) = \frac{p_{at}}{p_{aa}}; \lambda \frac{2(1-q_a)q_a}{q_a^2} \leq 2, q_a \in (0,1), \lambda > 0\}
\]

The p-value can now be computed as (for \( t > 0 \), otherwise the p-value equals 1)

\[
\sup_{H_0} P_{H_0} \left( \frac{N_{aa}}{N_{at}} \frac{2(1-M_a/m)(M_a/m)}{(M_a/m)^2} 1_{[M_a > 0]} \geq t \mid N_{at} = n_{at} \right)
\]
The p-value is found by maximizing the sum of probabilities with respect to $\lambda$ and $q_x$. We found a p-value of 0.044.

1.3 Construction of 95% confidence intervals for $GRR_{aa}$ and $GRR_{aA}$

Next we briefly describe the construction of a 95% confidence interval for the genotype relative risk $GRR_{aa}$. The 95% confidence interval for $GRR_{aa}$ contains all values $\tau$ for which the following null hypothesis is not rejected.

\[ \sup_{H_0} \sum_{x=1}^n P_{H_0}(N_{aA} \geq \frac{2(1 - M_{a}/m)(M_{a}/m)}{(M_{a}/m)^2} | M_a = x, N_{aA} = n_{aA}) P_{H_0}(M_a = x) \]

\[ = \sup_{H_0} \sum_{x=1}^n P_{H_0}(N_{aA} \geq \frac{(x/m)^{1/2}}{2(1 - x/m)} | M_a = x, N_{aA} = n_{aA}) P_{H_0}(M_a = x) \]

\[ = \sup_{H_0} \sum_{x=1}^n P_{H_0}(N_{aA} \geq \frac{(x/m)^{1/2}}{2(1 - x/m)} | M_a = x, N_{aA} = n_{aA}) P_{H_0}(M_a = x) \]

Since $N_{aA} | N_{aA} = n_{aA}$ has a binomial distribution with parameters $n-n_{aA}$ and probability $1/(\lambda+1)$ and $M_a$ has a binomial distribution with parameters $m$ and $q_x$, the sum of probabilities can be computed for given values of $q_x$ and $\lambda$.

Since $N_{aA} | N_{aA} = n_{aA}$ has a binomial distribution with parameters $n-n_{aA}$ and probability $1/(\lambda+1)$ and $M_a$ has a binomial distribution with parameters $m$ and $q_x$, the sum of probabilities can be computed for given values of $q_x$ and $\lambda$.

Since $N_{aA} | N_{aA} = n_{aA}$ has a binomial distribution with parameters $n-n_{aA}$ and probability $1/(\lambda+1)$ and $M_a$ has a binomial distribution with parameters $m$ and $q_x$, the sum of probabilities can be computed for given values of $q_x$ and $\lambda$.
\[ H_0 : \frac{P_{aa}}{P_{AA}} \frac{(1-q_{a})^2}{q_a^2} = \tau \quad \vee \quad H_1 : \frac{P_{aa}}{P_{AA}} \frac{(1-q_{a})^2}{q_a^2} \neq \tau \]

at the level of 5%.

The test statistic used is given by
\[
T = \frac{N_{aa} (1-M_{a}/m)^2}{N AA (M_{a}/m)^2} I_{M_{a} > 0} \mid N_{aa} = n_{aa}
\]

with \( T = \infty \) if \( N_{aa} = 0 \). The \( p \)-value and thus the values of \( \tau \) for which the null hypothesis is not rejected can be computed similarly as before. This resulted in a 95% confidence interval for \( GRR_{\infty}^{aA} \): \([4.47, 121000]\).

The construction of the 95% confidence interval for \( GRR_{\infty}^{aA} \) is similar; we found the interval \([0.878, 21.02]\).