

CHEK2 1100delC and male breast cancer in the Netherlands

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Abstract Mutations in the breast cancer susceptibility genes *BRCA1*, *BRCA2*, and *CHEK2* are known risk factors for female breast cancer. Mutations in *BRCA1* and *BRCA2* also are associated with male breast cancer (MBC). Similarly, it had been suggested in the original *CHEK2* identification report that the *CHEK2* 1100delC mutation confers an increased risk for MBC. Here, we have evaluated the risk of *CHEK2* 1100delC for MBC by genotyping *CHEK2* 1100delC in 23 familial and 71 unselected Dutch MBC cases. None of the 23 familial MBC cases carried the *CHEK2* 1100delC mutation. In contrast, *CHEK2* 1100delC was present in 3 of the 71 (4.2%) unselected MBC cases, which was significantly more prevalent than the 1.1% Dutch population frequency assessed in 1,692 individuals ($P = 0.05$, OR = 4.1, 95% CI 1.2–14.3). Our data suggest

that, in the Netherlands, *CHEK2* 1100delC is associated with an increased risk for MBC.

Keywords Cancer susceptibility · *CHEK2* · *CHK2* · Male breast cancer

Introduction

Male breast cancer (MBC) is a rare disease that accounts for less than 1% of all breast cancers. Major risk factors for MBC are Klinefelter syndrome, disorders associated with high estrogen levels, testicular disorders, and a family history of breast cancer. Germ line mutations in the breast cancer susceptibility genes *BRCA2* and *BRCA1* account for approximately 4–14% of familial MBC cases, with higher frequencies found in *BRCA2* founder populations [1]. Apart from *BRCA2* and *BRCA1* mutations, mutations in the androgen receptor, the *CYP17* and the *PTEN* genes have also been implicated in MBC [1–3]. Yet, it is still unclear what the underlying predisposition in the majority of MBC cases is.

In 2002, we and others identified the *CHEK2* 1100delC mutation as a breast cancer susceptibility allele [4, 5]. We estimated that female *CHEK2* 1100delC carriers had a twofold increased breast cancer risk compared with non-carriers, classifying the mutation as a moderate-risk breast cancer susceptibility allele [6]. In addition to a female breast cancer risk, 4 of 33 (12%) index cases of families with at least one MBC case were *CHEK2* 1100delC positive suggesting that the mutation also might confer a risk for MBC [4]. Thus far, the prevalence of *CHEK2* 1100delC in MBC has been evaluated in 12 additional studies [7–18]. Yet, only 3 of 627 genotyped samples carried the *CHEK2* 1100delC mutation, raising doubts on the association of

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CHEK2 1100delC with MBC. Evaluation of *CHEK2* 1100delC in MBC is however severely hampered by the moderate cancer risks associated with *CHEK2* 1100delC, the large differences in *CHEK2* 1100delC population frequencies and the rarity of the MBC phenotype.

In the Netherlands, the relatively high *CHEK2* 1100delC population frequency of 1.1% previously had allowed the identification of *CHEK2* 1100delC as a breast cancer allele and a colorectal cancer allele [4, 19, 20]. Here, we have exploited our unique opportunity to assess *CHEK2* 1100delC-associated cancer risks by screening *CHEK2* 1100delC in 94 Dutch MBC cases.

Materials and methods

Male breast cancer cases and controls

The evaluated MBC cohort consisted of 23 familial and 71 unselected MBC cases, together including all available MBC cases registered at Erasmus MC. All familial breast cancer cases were ascertained through the Rotterdam Family Cancer Clinic at Erasmus MC (Table 1). A family history of MBC was defined by the presence of at least two breast cancer cases in first- or second-degree relatives of whom at least one was a man (21 families) or by the presence of at least one MBC case who had two or more first-degree relatives diagnosed with cancer types other than breast carcinoma (2 families). Screening of all 23 MBC families had identified *BRCA2* and *BRCA1* germ line mutations in five and three families, respectively. The *CHEK2* 1100delC mutation was tested in blood-derived DNA of 14 MBC cases and in 17 first-degree relatives of 9 MBC cases of whom no DNA was available (Table 1). Unselected MBC cases included all available MBC cases from the Erasmus MC Pathology archive (16 paraffin-embedded tumors), the Rotterdam Family Cancer Clinic (3 blood-derived DNA

samples), and the Rotterdam Medical Oncology Tumor Bank (52 fresh-frozen tumors). None of the unselected MBC cases had been screened for *BRCA1* or *BRCA2* mutations. Of note, 25 fresh-frozen MBC tumors had also been part of a previous study that had evaluated the clinical relevance of steroid hormones and factors involved in the urokinase system among male and female breast cancers [21]. The Medical Ethical Review Board at Erasmus MC has approved the study, which was carried out according to the Code of Conduct of the Federation of Medical Scientific Societies in the Netherlands (<http://www.fmwv.nl/>).

Population controls were previously reported and included a hospital-based cohort of 967 spouses of heterozygous cystic fibrosis mutation carriers registered by the department of Clinical Genetics at Erasmus MC, a population-based cohort of 460 persons without cancer after age 55 years from the Erasmus Rotterdam Health and the Elderly Study (ERGO) and a cohort of 265 randomly selected blood donors [6, 22].

CHEK2 1100delC genotyping

CHEK2 1100delC genotyping was performed on DNA by allele-specific oligonucleotide hybridization, with confirmation of mutant cases by long-range PCR and sequencing of an independent template, as described [4].

Statistics

The difference between *CHEK2* 1100delC frequency in cancer cases and controls among *CHEK2* 1100delC carriers versus non-carriers was tested by using Fisher's Exact Test. Odds ratios (OR) and corresponding 95% confidence intervals (95% CI) were calculated using Woolf's method. All statistical analyses were performed with STATA statistical package, release 10 (STATA Corp, College Station, TX).

Results and discussion

Genotyping of *CHEK2* 1100delC identified 3 of 94 MBC cases (3.2%) as heterozygous carriers of the *CHEK2* 1100delC mutation (Table 2). All three *CHEK2* 1100delC cases were identified among the 71 unselected MBC cases (4.2%, Table 2), which was significantly more prevalent compared with the 1.1% Dutch population frequency ($P = 0.05$, OR = 4.1, 95% CI 1.2–14.3). Our results thus suggest that *CHEK2* 1100delC is associated with an increased risk for MBC in the Dutch population.

Age at diagnosis and cytosolic estrogen receptor (ER) and progesterone receptor (PgR) levels were available for 50 unselected MBC cases including all three *CHEK2* 1100delC

Table 1 Inclusion criteria of familial MBC cases

| Inclusion criteria MBC families | Mutation status | | |
|--|---------------------|--------------|--------------|
| | Non- <i>BRCA1/2</i> | <i>BRCA1</i> | <i>BRCA2</i> |
| ≥1 MBC with ≥1 FBC 1st/2nd DGR (MBC tested) | 9 | 1 | 2 |
| ≥1 MBC with ≥1 FBC 1st/2nd DGR (BRC in 1st DGR tested) | 4 | 2 | 3 |
| ≥1 MBC with ≥2 non-BRC 1st DRG (MBC tested) | 2 | – | – |
| Total (23) | 15 | 3 | 5 |

FBC, female breast cancer; MBC, male breast cancer; non-BRC, cancer types other than breast carcinoma; 1st/2nd DGRs, first- or second-degree relatives

Table 2 Summary of reported *CHEK2* 1100delC genetic screens in male breast cancer cases

| Reference | Population | <i>CHEK2</i> 1100delC+/total tested (%) | | | |
|----------------------|------------------|---|----------------------|--------------------|-------------------|
| | | Population controls | Unselected MBC cases | Familial MBC cases | All MBC cases |
| Present study | NL | 18/1,692 (1.1) | 3/71 (4.2) | 0/23 | 3/94 (3.2) |
| [4] | UK/NL/US/Germany | 18/1,620 (1.1) | | 4/33 (12.1) | 4/33 (12.1) |
| [13, 18] | Finland | 26/1,885 (1.4) | 2/132 (1.5) | 0/18 | 2/150 (1.3) |
| [9, 14, 17] | UK | 20/3,749 (0.5) | 0/79 | 1/90 (1.1) | 1/169 (0.6) |
| [11, 14, 15] | USA | 8/2,261 (0.4) | 0/109 | 0/19 | 0/128 |
| [16] | Israel | 0/146 | 0/31 | 0/23 | 0/54 |
| [10] | Italy | 0/263 | 0/77 | 0/25 | 0/102 |
| [8] | Germany | 6/1,315 (0.5) | | 0/12 | 0/12 |
| [12] | Australia | 1/736 (0.1) ^a | | 0/7 | 0/7 |
| [7] | South Korea | n.a. | | 0/5 | 0/5 |
| | Total | | 5/499 | 5/255 | 10/754 |

The summary includes data from the present study and 13 studies that have thus far screened *CHEK2* 1100delC in MBC cases

NL, the Netherlands; UK, United Kingdom; USA, United States of America; MBC, male breast cancer

^a Controls were obtained from Ref. [6]

n.a., not available

Bold data indicate our data in the present study

MBC cases. The *CHEK2* 1100delC MBC cases were diagnosed with breast cancer at age 64, 66, and 73 years, which was not different from the average age at diagnosis of 67 years among 47 *CHEK2* 1100delC negative MBC cases. All three *CHEK2* 1100delC cases were ER positive as were 41 of 47 (87%) *CHEK2* 1100delC negative cases. Similar, two of the three *CHEK2* 1100delC MBC cases (67%) were PgR positive compared to 37 of 47 (78%) *CHEK2* 1100delC negative cases. Clinical follow-up including tumor stage, lymph node involvement, local and/or distant metastasis data were available for two *CHEK2* 1100delC and 33 *CHEK2* 1100delC negative unselected MBC cases. Overall, the median follow-up time of MBC cases still alive was 47 months (range 2–165 months). The first *CHEK2* 1100delC case was diagnosed with a lymph node-negative T1 breast tumor and the second case had a lymph node-positive T4 breast tumor. Both cases died of bone metastasis, 24 and 36 months after their diagnosis, respectively. In contrast, distant metastasis at diagnosis and during follow-up was reported in 12 of 33 (36%) *CHEK2* 1100delC negative MBC cases and only two of these had a sole relapse to the bone. In agreement with previous reports on bone metastatic disease and ER status, all four MBC cases diagnosed with bone metastasis were ER positive, affirming the strong association between relapse to bone and positive ER status [23, 24].

In the initial *CHEK2* 1100delC identification report, 4 unrelated *CHEK2* 1100delC cases had been identified among 33 (12%) high-risk *BRCA1/BRCA2* negative MBC families, suggesting an estimated 10-fold increased MBC risk for *CHEK2* 1100delC MBC carriers [4]. Yet, here we did not identify *CHEK2* 1100delC among the 23 familial MBC cases

analyzed. The analyzed familial MBC cohort included eight confirmed *BRCA1/BRCA2* positive MBC cases (Table 1). These cases were a priori unlikely to carry *CHEK2* 1100delC as the *CHEK2* 1100delC mutation is quite uncommon among *BRCA1/BRCA2* mutation carriers [4, 6]. Additionally, four *BRCA1/BRCA2* negative families had been included for which the MBC case was not available for *CHEK2* 1100delC genotyping (Table 1). As a whole, we thus had 11 *BRCA1/BRCA2* negative familial MBC cases available for *CHEK2* 1100delC analysis, a too low number to establish a possible correlation between *CHEK2* 1100delC and familial *BRCA1/BRCA2* negative breast cancer.

In addition to our previous 4 of 33 MBC cases [4], thus far, only three *CHEK2* 1100delC cases have been identified worldwide among the additional 627 MBC cases genotyped (Table 2) [7–18]. In contrast to the initial *CHEK2* 1100delC identification report [4] and our current report, this low frequency of 0.5% (3 of 627 MBC cases) had suggested that *CHEK2* 1100delC is not a risk allele for MBC. However, most of the reported studies had low *CHEK2* 1100delC population frequencies and also had used small MBC cohorts [4, 7–18], resulting in a lack of statistical power to detect *CHEK2* 1100delC-associated cancer risks. Interestingly, the Finnish *CHEK2* 1100delC MBC study had comparable power to our study, but failed to identify a *CHEK2* 1100delC related MBC risk [13, 18]. Similarly, we previously had identified *CHEK2* 1100delC as a risk allele for Dutch colorectal cancer cases whereas no such risk could be detected for Finnish colorectal cancer cases [20, 25]. These observations might suggest the presence of additional susceptibility alleles in the Dutch population that are absent

in the Finnish population. One might speculate that the same putative Dutch cancer susceptibility allele acts in synergy with *CHEK2* 1100delC to confer a higher risk for colorectal cancer as well as male breast cancer. Or, are there two or more cancer susceptibility alleles present in the Dutch population that, together with *CHEK2* 1100delC, are responsible for the Dutch *CHEK2* 1100delC phenotype of female breast cancer, male breast cancer, and colorectal cancer?

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