ONLINE MUTATION REPORT

Germline *CHEK2**1100delC mutations in breast cancer patients with multiple primary cancers

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CHEK2 is a human homologue of yeast Cds1 and Rad53 which encodes the cell cycle checkpoint kinase. CHEK2 is activated by phosphorylation by ATM in response to double-strand DNA breaks.^{1,2} Activated CHEK2 in turn phosphorylates and activates p53, which triggers cell cycle arrest at G1 or apoptosis.^{3,4} CHEK2 also phosphorylates Cdc25C, preventing cellular entry into mitosis after DNA damage.⁵ Thus, CHEK2 plays an important role in the network of cell cycle regulation and DNA damage repair, which are crucial processes in preventing cancer development.

*CHEK2**1100delC leads to premature termination of translation and abolishes the kinase activity of the encoded protein. Although an initial report described germline *CHEK2* mutations in Li-Fraumeni syndrome (LFS),⁶ subsequent studies have shown that *CHEK2**1100delC is a low penetrance variant conferring an increased susceptibility to breast cancer with a relative risk of 2.0, not a high penetrance allele responsible for LFS.⁶⁻⁷ In addition, *CHEK2**1100delC also may be associated with other cancers. A recent study suggested that *CHEK2**1100delC may contribute significantly to familial prostate cancer ($p=0.02$).⁸ Another study suggests that *CHEK2**1100delC is over-represented in breast cancer families with colorectal cancers (hereditary breast and colorectal cancer, HBC) as compared with families with breast cancer only (non-HBC).⁹ We previously reported that women with multiple primary cancers (breast and any other cancer) are more likely to have *BRCA1* and *BRCA2* mutations than matched individuals with breast cancer only.¹⁰ Here we asked whether a similar sample of women with multiple primary cancers, one of which was breast cancer, had an excess of *CHEK2**1100delC compared to controls. Thus, we examined the frequency of *CHEK2**1100delC in constitutional DNA from 161 women with multiple primary cancers (one primary was always breast cancer) as compared to a matched control population without cancer.

METHODS

Patient and control populations

A total of 161 women with breast and at least one other non-breast cancer were evaluated. Some 96 patients were recruited from the Cancer Risk Evaluation Program (CREP) at the Hospital of the University of Pennsylvania; patients were either self or physician referred to CREP because of a perceived elevated risk of inherited susceptibility to breast cancer. A total of 65 patients were recruited from the Medical Oncology and Radiation Oncology clinics at the University of Pennsylvania regardless of family history based on the presence of multiple primary cancers where one cancer was breast cancer. The average age of breast cancer diagnosis was 52.1 years. All non-breast malignancies were considered, including non-melanoma skin cancers. A total of 132 (82.0%) women had two primary cancers, 29 (18.0%) had three or more primary cancers, and 41 (25.5%) had non-melanoma skin cancers. Some 109 of the cases were Caucasian, five were African Americans, and 47 were of unknown race. Table 1

Key points

- *CHEK2**1100delC is a low penetrance allele which confers susceptibility to female breast cancer with a relative risk of 2.0.
- Given that previous reports described *CHEK2* mutations in patients with multiple cancers and a link between *CHEK2**1100delC and families with both breast and colon cancers, we investigated whether *CHEK2**1100delC is more frequent in women with both a primary breast cancer and a primary non-breast cancer of any type than matched controls without cancer.
- Constitutional DNA from 161 multiple primary cancer patients were analysed for *CHEK2**1100delC by direct sequencing using intron based primers. This allele was found in 3/161 (1.9%) women with multiple primary cancers, which is not significantly higher than the controls at 1/153 (0.7%) ($p=0.623$).
- These data suggest that it is unlikely that *CHEK2**1100delC plays a major role in conferring susceptibility to multiple primary cancers, breast cancer being one of the primary cancers.

provides a description of the cancers reported in these women.

The 153 control individuals were selected from primary care clinics in the same referral catchment area as the cases, or were female spouses marrying into families with breast cancer ascertained for linkage analysis from the same geographic area. All the controls were Caucasian. All cases and controls provided informed consent approved by the Institutional Review Board at the University of Pennsylvania. Personal and family histories of all cancers were recorded, including age of diagnosis. Pathological reports were obtained on all probands and on other family members when possible.

Mutation analysis

DNA was extracted from peripheral blood mononuclear cells and stored in TE at 4°C. Exon 10 of *CHEK2* was amplified by PCR of genomic DNA. The primer sequences are: forward 5'-AGCCTACGTGCTCTCTTGA-3'; reverse: 5'-GCAGTCCACAGCACGGTTAT-3'. PCR amplification was performed in a final volume of 50 µl containing 120 ng of DNA, 1×buffer (1.5 mM Mg²⁺, 10 mM Tris-HCl, and 50 mM KCl (pH 8.3)), 0.2 mM each of dCTP, dATP, dGTP, and dTTP, each primer at 0.4 µM, and 1.0 U Taq polymerase (Boehringer Mannheim,

Abbreviations: CREP, Cancer Risk Evaluation Program; HBC, hereditary breast and colorectal cancer; LFS, Li-Fraumeni syndrome

Family 1575 **Family 1674** **Family 1785** **Family CC39**

Figure 1 Pedigrees of families of all *CHEK2**1100delC carriers. Carriers are denoted by arrows. Type of cancer and age at diagnosis are noted where available. Br, breast; Lymph, lymphoma; Ov, ovarian, Pros, prostate.

Mannheim, Germany). Annealing temperature was 56°C. Variants were identified by direct sequencing using the ABI Prism BigDye Terminator (Perkin-Elmer Applied Biosystems, Foster City, CA, USA) and the ABI Prism 3100 DNA Analyzer (Perkin-Elmer Applied Biosystems).

Statistical analyses

Differences in the frequency of *CHEK2**1100delC between the multiple primary cancer and control groups were assessed using Fisher's exact test. Logistical regression was used to calculate the age-adjusted odds ratio as the age difference

Table 1 Non-breast primary cancers in families with multiple primary cancers

Cancer	No. of individuals (%)*
Non-melanoma skin	41 (25%)
Melanoma	23 (14%)
Colorectal	24 (15%)
Ovarian	16 (10%)
Thyroid	14 (9%)
Endometrial	13 (8%)
Hodgkin disease	9 (6%)
Cervical	8 (5%)
Others†	45 (28%)

*A total of 29 patients had two or more non-breast cancers, so the number of cancers does not equal the number of patients and the total percent exceeds 100%.

†Other primary cancers include leukaemia, brain, non-Hodgkin lymphoma, pancreatic, sarcoma, bladder, uterine, kidney, lung, head and neck, genital tract, duodenum, renal, thymus, intestine, and papillary adenocarcinoma.

between the multiple primary cancer and control groups was considered.

RESULTS

CHEK2*1100delC was detected in three of 161 cases (1.9%) and in one of 153 (0.7%) controls (p = 0.623). The age-adjusted odds ratio was 0.41 (95% CI: 0.04 to 4.11). No significant difference in the frequency of CHEK2*1100delC was found between the multiple primary cancer and control populations. The pedigrees of the families of all CHEK2*1100delC mutation carriers are shown in fig 1. The CHEK2*1100delC carrier from the control population was 58 years old, had a sister diagnosed with breast cancer at age 54, and had six other family members diagnosed with various other cancers. Of the three CHEK2*1100delC carriers with multiple primary cancers, the carrier from family 1785 was ascertained in a hospital based breast cancer treatment clinic; she was diagnosed with Hodgkin disease at age 21, thyroid cancer (NOS) at age 49, and breast cancer at age 49. Her mother was diagnosed with breast cancer at age 60 (fig 1). The other two CHEK2*1100delC mutation carriers were identified in our cancer risk evaluation clinic. The first of these individuals (family 1575) was diagnosed with ovarian cancer at age 62 and breast cancer at age 72; there are five other women in this family with breast cancer who ranged in age at diagnosis from 25 to 81 years (median 47 years). The other CHEK2*1100delC carrier ascertained from the risk evaluation clinic (family 1674) was diagnosed with bilateral breast cancer at 45 and 56 years of age, and ovarian cancer at age 79; a sister and two brothers were diagnosed with breast cancer at 44, 67, and 73 years of age, respectively. None of the CHEK2*1100delC carriers has a detectable BRCA1 or BRCA2 mutation. There was no significant difference in the frequency of CHEK2*1100delC between the multiple primary cancer population and control population when patients with non-melanoma skin cancers were removed from the analysis (p = 0.323). However, CHEK2*1100delC was found in two of 16 women with ovarian cancer as a second primary cancer (p = 0.021 as compared to controls).

DISCUSSION

We reported previously that CHEK2*1100delC is a low penetrance breast cancer susceptibility allele with a high level of statistical significance (p = 0.00000003).⁷ Others have suggested that CHEK2*1100delC is also associated with a multiple cancer phenotype in specific types of families and individuals.⁵⁻⁹ In particular, CHEK2*1100delC has been associated with a familial breast and colorectal cancer phenotype, with a prevalence in families with non-BRCA1/

BRCA2 associated breast cancer and HBCC reported to be as high as 18.2%.⁹ In contrast, we found no evidence for a significant increase in CHEK2*1100delC frequency in our multiple primary cases, including the 24 cases in our sample set who had both breast and colorectal cancer. Interestingly, the frequency of CHEK2*1100delC in the current study was found to be significantly higher in women with ovarian cancer as a second primary cancer. CHEK2*1100delC also was observed at a significantly higher frequency in families with both breast and ovarian cancer in the original report of CHEK2*1100delC as a low penetrance breast cancer susceptibility allele, however individual women with both breast and ovarian cancer were not evaluated.⁷ In summary, we provide evidence that germline variant CHEK2*1100delC is rarely associated with the presence of multiple primary cancers where one cancer is breast cancer. However, since CHEK2*1100delC is a low penetrance allele, it is possible that studies with larger sample sets could identify a small contribution from CHEK2*1100delC in multiple primary cancers. Moreover, as our numbers of each cancer type and breast cancer in this study were small, larger studies of homogeneous multiple primary cancers could uncover an association between CHEK2*1100delC in specific subsets of women with breast cancer and another primary cancer, possibly ovarian cancer.

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