Frequency of the *CHEK2* 1100delC Mutation among Women with Breast Cancer: An International Study

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Abstract

A founder allele in the *CHEK2* gene (1100delC) has been associated with an elevated risk of breast cancer. This allele is responsible for the majority of *CHEK2*-associated breast cancers in women from northern European countries; however, within Europe, it seems to be rare in countries that are close to the Mediterranean. The frequency of the 1100delC allele has not been measured in non-White populations. We measured the frequency of the *CHEK2* founder allele in 3,882 breast cancer patients and 8,609 controls from various countries. The allele was not seen among Asian patients (from Pakistan or the Philippines) and was present in 1 of 155 cases from Brazil. Among White women, the allele was present in 1.5% of 825 familial cases of breast cancer and in 0.7% of 1,106 patients with nonfamilial breast cancer. The allele was equally frequent in Jewish and non-Jewish patients. We estimate that the *CHEK2* 1100delC allele is associated with an odds ratio of 2.6 for breast cancer, which corresponds to a lifetime risk of ~24% in Ontario. [Cancer Res 2008;68(7):2154–7]

Introduction

Following the positional cloning of *BRCA1* in 1994 (1) and of *BRCA2* in 1995 (2), there has been continued interest in the identification of new genes for hereditary breast cancer. These genes may be roughly divided into two categories—genes with rare alleles, which are associated with a high relative risk of breast cancer (i.e., highly penetrant), and genes with relatively common alleles, which are associated with modest relative risks of breast cancer (i.e., low penetrance genes). It has been suggested that genes in the latter class might account for significant proportion of cancers in the general population.

CHEK2 is one of a few genes that have been clearly associated with an elevated breast cancer risk. A founder allele of *CHEK2* (1100delC) predisposes to breast cancer in Europe and North America (3, 4) and other *CHEK2* alleles have been implicated in breast carcinogenesis in Finland (5) and in Poland (6). In the initial *CHEK2* study, the 1100delC variant was found in 5.1% of individuals with familial breast cancer (but with no *BRCA1* or *BRCA2* mutation), compared with 1.1% of healthy control subjects (3). In a large follow-up study, 10,860 breast cancer cases from five countries (United Kingdom, the Netherlands, Finland, Germany, and Australia) were studied (4). The highest prevalence of the 1100delC mutation was found among cases from the Netherlands (3.5%) followed by Finland (2.2%), the United Kingdom (1.2%), Germany (0.8%), and Australia (0.7%). The frequencies among controls also varied widely, from 1.8% in the Netherlands to 0.1% in Australia. Elsewhere, the mutation frequency has been estimated to be 0.5% in cases from Poland (6) and 0% in cases from Spain (7). As a whole, these studies suggest that the frequency of the 1100delC mutation varies between countries and that the highest mutation rates are seen in northern European countries. To date, no studies have been conducted in non-European populations. We estimated the prevalence of the *CHEK2* 1100delC mutation in 3,882 breast cancer patients belonging to various ethnic groups, including women from Asia and South America.

Materials and Methods

DNA samples were available from women who presented for genetic evaluation to the Women's College Research Institute in Toronto. We also included women who were enrolled in a number of breast cancer studies. For some studies, patients were unselected hospital-based cases of breast cancer. For other studies, patients were preselected on the basis of age of diagnosis, family history, ethnic group, or personal history of cancer. The study was given ethical approval at the Sunnybrook and Women's College Health Sciences Centre and the University of Toronto, and all patients provide written informed consent.

In addition, 8,609 controls were tested. These women were unaffected with breast cancer. A large number of controls were women who attended a screening clinic for healthy women at Women's College Hospital or were female students who attended the University of Toronto. For some hospital-based studies, a control group of non–breast cancer patients was also collected and was available for study. French Canadian controls consisted of DNA extracted from cord blood specimens from unselected newborn infants from the Quebec City region (8). Detailed information on ethnic group was taken from all

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Table 1. *CHEK2* 1100delC mutations in breast cancer cases and controls by ethnicity

Group	Cases			Controls			Odds ratio	*P*
	Total	Positive	Number	Total	Positive	Number		
Jewish	320	4	1.3%	180	0	0%	—	—
French Canadian	560	4	0.7%	6460	19	0.3%	2.4	0.11
Other White	1566	15	1.0%	1223	3	0.3%	3.9	0.03
All Whites	2449	23	0.9%	7863	22	0.3%	3.4	$<10^{-4}$
Brazil	155	1	0.7%	377	0	0%		
Pakistan	376	0	0%	—	—	—		
Filipino	342	0	0%	7	0	0%		
Other/mixed/unknown	560	4	0.7%	322	0	0%		

cases and controls, and individuals were classified according to ethnic group: White women were divided into Ashkenazi Jewish, French Canadian, and other White.

The genomic DNA was extracted from the peripheral blood with PureGene DNA Isolation Kit (Gentra Systems) according to the protocol provided. Two sets of PCR primers were used to screen for the *CHEK2* 1100de1C mutation. Specific primers for the *CHEK2* exon 10 on chromosome 22 were designed to avoid all other homologous sequences in the genome (forward primer 5′-TTAATTTAAGCAAAATTAAATGTC-3′: reverse primer 5′-GGCATGGTGGTGTGCATC-3′). The PCR products were then re-amplified with nested primers, which were designed to amplify the region encompassing the site of *CHEK2* 1100delC. The forward primer contained one base substitution to generate a restriction site for restriction enzyme *Sca*I within the wild-type allele after PCR amplification. The forward primer sequence was 5′-CCCTTTTGTACTGAATTTTAGAGTA-3′ (a T to G substitution at position 1097). The reverse primer was 5′-ACAAGAACTTCAGGCGCCAAGTAG-3. Then, the 116-bp PCR products from the second amplification were digested with the restriction enzyme *Sca*I and incubated overnight at 37°C (2.5 units per sample, Roche Molecular Biochemicals), which digests only the wild-type allele but not the mutant allele. Restriction enzyme digest products were separated on 3% agarose gels visualized with ethidium bromide. The wild-type allele is cut into bands of 92 and 24 bp (the 24-bp band usually runs out off the gel). The mutant allele cannot be cut by the enzyme. Mutations were

Table 2. Description of cases with *CHEK2* mutations

Pedigree	ID no.	Ethnicity	First cancer	Second cancer(s)	Age of diagnosis of breast cancer (y)	Breast cancers in first-degree relatives	Breast cancers in all relatives
BCP066	23246	Irish/Scottish	Breast		48	1	2
BRP0085	24114	Brazilian	Breast		—	—	—
CR2800	4717	English/Dutch	Breast		29	4	4
CR3569	11072	Norwegian	Breast		51	2	5 (1 male)
CR4420	13442	Unknown	Breast		40	1	1
CR4522	17809	German/Scottish/Swedish	Breast		39	1	2
CR4731	20571	Unknown	Breast		46	2	2
CR4911	24925	Ashkenazi Jewish	Breast		46	1	1
EW069	13122	Jewish	Breast		44	0	1
EW154	1369	Irish	Breast		41	1	6
EW190	1362	Irish	Breast		35	1	2
FC0071	4321	Unknown	Breast		39	1	3
FRC0065	25866	French Canadian	Breast		38	—	—
FRC0251	28666	French Canadian	Breast		49	—	—
FRC0387	29613	French Canadian	Breast		38	—	—
MG1031.0	4580	Unknown	Breast	Breast	—	2	3
MTRL790	785	German/Welsh	Breast	Breast/Uterine	47	2	5
PMH223	3455	Finnish	Breast	Thyroid	65	0	0
PMH1024	3857	German	Breast		—	0	1
PMH1304	10108	Scottish	Breast		65	0	0
PMH1316	10204	British	Breast	Colon/Appendix	55	0	1
PMH1488	10535	Irish	Breast	Skin	70	0	0
W9071	895	Dutch/Scottish	Breast		39	2	3
W9173	3672	English/Irish	Breast	Cervical	49	1	3
W9348	3489	Ashkenazi Jewish	Breast		54	0	0
W9724	14666	French Canadian/Scottish	Breast	Breast	33	0	0
W9747	16528	Dutch	Breast	Hodgkin	52	1	2
W9819	20663	Ashkenazi Jewish	Breast		—	1	2

Table 3. *CHEK2* mutations by age and family history, White women

Age (y)	Familial			Nonfamilial		
	Total	Positive	%	Total	Positive	%
<39	216	6	2.8%	180	2	1.1%
40-49	280	4	1.4%	297	1	0.3%
50-59	167	1	0.6%	271	3	1.1%
60+	154	0	0.0%	345	1	0.3%
All	825	12	1.5%	1106	8	0.8%

NOTE: Age of diagnosis was missing on 23 subjects.

confirmed by direct sequencing with Cy5/Cy5.5 Dye Primer Cycle Sequencing Kit and OpenGene Automated DNA Sequencing System (Visible Genetics).

Results

A total of 3,882 women with breast cancer was tested for the 1100delC *CHEK2* mutation. The women were participants in a number of different studies that were designed to estimate the frequencies of various cancer-predisposing alleles in various populations. In some studies, patients were unselected, but for others, patients were selected on the basis of ethnic group, age of diagnosis, a positive family history of cancer, or a personal history of cancer. Therefore, the results are presented separately for the different ethnic groups and for subgroups defined by age of onset, family history, and past cancer history.

A mutation was found in 23 of 2,449 (0.9%) White breast cancer patients. These 23 women were almost all from North America (with European origins). Among Whites, the mutation frequencies were similar for French Canadian patients (0.7%; 4 of 560), Jewish patients (1.3%; 4 of 320), and White women of other ethnic origins (1.0%; 15 of 1,566); the frequency of the mutation in unselected controls was 0.3% for both the French Canadian and other White groups. There was no mutation detected among the Jewish controls, but this sample was relatively small (n = 180).

Brazilian women represent a mixed group, with ancestors of African, Portuguese, Hispanic, and other European origins. One mutation was found among 155 Brazilian patients (0.7%). No mutation was seen among 718 Asian patients (342 Filipino, 376 Pakistani). The mutation prevalences and odds ratios for breast cancer for the different ethnic groups are presented in Table 1. A detailed analysis of the ethnicity of the CHEK2-positive families revealed a dominance of families of English, Scottish, and Irish descent (12 of 28 families; Table 2).

Because the 1100delC mutation seemed to be limited to White patients, the remaining analyses are restricted to 2,449 White women with breast cancer (Brazil excluded). The prevalence of mutations in White breast cancer patients, categorized by age and family history, are presented in Table 3. Among the White patients, 825 women had a family history of breast cancer (one or more first- or second-degree relatives with breast cancer) and 1,106 had no family history. Family history data was missing from 518 cases. White women with familial breast cancer were five times more likely to harbor a *CHEK2* mutation than were White controls (odds ratio, 5.2; 95% confidence interval, 2.6-10.5; P < 0.0001). White women with nonfamilial breast cancer were 2.6 times more likely to harbor a mutation than were unaffected White controls (odds ratio, 2.6; 95% confidence interval, 1.1-5.8; P = 0.05).

Of the White women, 1,588 had previously been tested for the presence of a *BRCA1* or *BRCA2* mutation (Table 4). No *CHEK2* mutation was seen among 307 breast cancer patients with a BRCA mutation. The prevalences of *CHEK2* mutations were 1.4% among those women with no BRCA mutation detected (18 of 1,280) and 0.5% among those who were untested for BRCA mutations (4 of 858). Among the familial cases without a BRCA mutation, the prevalence of *CHEK2* mutations was 2.4% (11 of 453).

One proband was homozygous for the CHEK 1100delC mutation. Homozygosity was confirmed by two assays, first by the restriction enzyme assay and subsequently by DNA sequencing. The proband was diagnosed with bilateral breast cancer (ages 47 and 61 years) and with uterine sarcoma at age 58 years.

We examined the family histories of the 28 probands with a *CHEK2* mutation. Sixteen of the probands with a mutation had a positive family history of breast cancer (58%). We identified 51 cases of breast cancer among the first-, second-, and third-degree relatives of the 28 probands. Of these, 11 were tested for the *CHEK2* mutation (the others were unavailable for testing or were deceased). Only 5 of the 11 affected relatives who were tested for *CHEK2* were also positive for the mutation.

A mutation was present in 1.2% of women diagnosed with breast cancer under the age of 50 years, versus 0.6% of women diagnosed after the age of 50 years (P = 0.14). One hundred thirty-seven of the 2,449 patients had multiple primary breast cancers (5.6%), and 359 patients had cancer at a second site (14.7%). The frequency of *CHEK2* mutations in women with multiple primary breast cancer (0.7%) was similar to that of women with a single case of breast cancer (0.8%). The frequency of the 1100delC mutation in women with breast cancer and another cancer was 1.7% (odds ratio, 2.1; P = 0.13).

In summary, White ethnicity, age of onset below age 50 years, and a positive cancer family history were risk factors for the presence of a *CHEK2* mutation. Mutations were equally frequent in women with unilateral and bilateral breast cancer and were not found in women with a BRCA mutation.

Discussion

We estimated the prevalence of the *CHEK2* 1100delC mutation in breast cancer patients from various ethnic groups. The first goal of this study was to identify populations for which appreciable numbers of women with breast cancer carry the *CHEK2* 1100delC allele and which might benefit from genetic testing. Our second goal was to estimate the magnitude of the risk increase associated with this single mutation in these populations. We included patients from several Asian countries and from Brazil. We did not identify a mutation in patients from Pakistan and the Philippines

Table 4. *CHEK2* mutations by BRCA status and by multiple primary cancer status, White women only (n = 2,449)

One breast cancer	1,953	16	0.8%
Multiple breast cancers	137	1	0.7%
Breast and other cancer	359	6	1.7%
BRCA mutation positive	307	0	0.0%
BRCA mutation negative	1,280	18	1.4%
Not tested	858	4	0.5%

(therefore, controls from these countries were not tested). The founder mutation was restricted to women of European origin, including Ashkenazi Jews and French Canadian women. The mutation was found in a single woman from Brazil (of mixed European ancestry). This particular mutation is the most important founder allele in European countries and other *CHEK2* variants do not seem to make a substantial contribution to breast cancer susceptibility in western Europe or North America (9–11). However, it is possible that other *CHEK2* variants are responsible for the predisposition in other countries. For example, a splice-site mutation in *CHEK2* IVS2+G>A has been found to be associated with breast cancer predisposition in Poland (12), and, in Ashkenazi Jewish women, the *CHEK2* S428F variant increases breast cancer risk by ~2-fold (13).

The observed relative risk of breast cancer associated with the 1100delC mutation in White women with familial breast cancer was ~5-fold. However, the 1100delC allele did not segregate completely with the presence of breast cancer in the families. Incomplete segregation is characteristic of two-gene models for cancer and can be an impediment to gene mapping through linkage analysis (14). For studies of genes in this class, it may be best to conduct association studies of familial cases. For familial cases, the odds ratio was about 5 and an effect of this size is amenable to an association study, even if the allele frequency is relatively rare. However, it is important to note, for counseling purposes, that observing a 5-fold risk does not imply that unaffected women from these same families face a 5-fold risk of breast cancer; their lifetime risk is actually similar to that estimated for nonfamilial cases (below). The observed odds ratio of 5 is a consequence of the case-control design—that is, any gene which is associated with a substantial increase in the risk of breast cancer is more likely to be present in a familial case than in a nonfamilial case because it will result in familial aggregation; for example, if a genetic variant doubles the risk of breast cancer in whomever it is present, it is more likely to be present in a patient with an affected sister than in a singleton case.

From our nonfamilial cases, we estimate a relative risk of 2.6 associated with the 1100delC mutation. Based on the risk to age 74 years for breast cancer in Ontario of 9% (15), this corresponds to a penetrance of roughly 23% penetrance to age 74 years. Previously, the increased risk for breast cancer associated with the *CHEK2* 1100delC mutation has been estimated at between 1.4- and 4.7-fold. In a similar study of unselected breast cancer cases from New York, Offit and colleagues (11) found the frequency of the allele among 300 cases to be 1.0% and among 1,665 controls to be 0.3%. These figures are similar to ours. In Offit et al.'s study and ours, there was no difference in the allele frequency between Ashkenazi and non-Ashkenazi controls. Because of the penetrance of ~25%, and because most of these breast cancer are estrogen receptor positive, some authors have advocated that unaffected *CHEK2* carriers be offered tamoxifen as chemoprevention (16).

In previous studies, patients with a mutation in *BRCA1* or *BRCA2* were excluded from *CHEK2* analysis under the premise that the familial predisposition had been explained. Our data are consistent with this assumption; we found no *CHEK2* mutation in 307 White women with breast cancer and a BRCA mutation. It is unlikely that women with a BRCA mutation will be found to harbor a *CHEK2* mutation. However, it is impractical to offer screening for *CHEK2* as a standalone genetic test; it is better to add the test for selected *CHEK2* mutations as part of a wider mutation screen, at least for patients with breast cancer from women with northern and eastern European origins. The additional cost for adding this single allele to a mutation panel is minimal, and a single-step multiallelic approach is more practical than a sequential approach. Our study was of a single allele of *CHEK2*—it is possible that this allele is very rare in non-European populations but that other *CHEK2* alleles may be founder alleles elsewhere. It is important that ethnic-specific studies be conducted in different populations of breast cancer patients to confirm or exclude *CHEK2* as a contributing factor.

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