Variants in *CHEK2* Other than 1100delC Do Not Make a Major Contribution to Breast Cancer Susceptibility

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We recently reported that a sequence variant in the cell-cycle–checkpoint kinase *CHEK2* (*CHEK2* 1100delC) is a low-penetrance breast cancer–susceptibility allele in noncarriers of *BRCA1* or *BRCA2* mutations. To investigate whether other *CHEK2* variants confer susceptibility to breast cancer, we screened the full *CHEK2* coding sequence in *BRCA1/2*-negative breast cancer cases from 89 pedigrees with three or more cases of breast cancer. We identified one novel germline variant, R117G, in two separate families. To evaluate the possible association of R117G and two germline variants reported elsewhere, R145W and I157T with breast cancer, we screened 737 *BRCA1/2*-negative familial breast cancer cases from 605 families, 459 *BRCA1/2*-positive cases from 335 families, and 723 controls from the United Kingdom, the Netherlands, and North America. All three variants were rare in all groups, and none occurred at significantly elevated frequency in familial breast cancer cases compared with controls. These results indicate that 1100delC may be the only *CHEK2* allele that makes an appreciable contribution to breast cancer susceptibility.

DNA damage results in activation of cell-cycle checkpoints that block proliferation and initiate DNA repair processes. Defects of these checkpoint pathways can lead to genomic instability and susceptibility to cancer. Cell-cycle–checkpoint kinase 2 (*CHEK2*, also known as "*CHK2*" [MIM 604373]), is a key mediator of cellular responses to DNA damage (Zhou and Elledge 2000; Bartek et al. 2001). Following double-strand DNA breaks, *CHEK2* is activated through phosphorylation by ATM (MIM 208900) (Matsuoka et al. 1998, 2000). Activated CHEK2 phosphorylates critical cell-cycle proteins, including p53 (MIM 191170), Cdc25C (MIM 157680), Cdc25A (MIM 116947), and BRCA1 (MIM 113705), which promote cell-cycle arrest and activation of DNA repair (Zeng et al. 1998; Chehab et al. 2000; Lee et al. 2000; Falck et al. 2001). We recently reported that *CHEK2* 1100delC, a truncating variant that abrogates the kinase activity of the protein, is a low-penetrance breast cancer–susceptibility allele (The *CHEK2*-Breast Cancer Consortium 2002). *CHEK2* 1100delC was present in 1.1% of healthy control subjects, compared with 5.1% of subjects with breast cancer from *BRCA1/2*-negative families, including 13.5% of subjects from families with male breast cancer. We estimated that *CHEK2* 1100delC confers an ~2-fold increased breast cancer risk in women and 10-fold risk in men (The *CHEK2*-Breast Cancer Consortium 2002). A study of Finnish breast cancer cases reported a *CHEK2* 1100delC frequency of 1.4% in controls and 5.5% in *BRCA1/2*-negative familial breast cancer cases, independently supporting this observation (Vahteristo et al. 2002).

CHEK2 1100delC was originally reported in a family with Li-Fraumeni syndrome (LFS [MIM 151623]) that included three cases of breast cancer (Bell et al. 1999). Screening of cancer cases from LFS and families afflicted with Li-Fraumeni–like syndrome (LFL) revealed two additional *CHEK2* germline sequence variants in individuals with breast cancer (Bell et al. 1999; Lee et al. 2001). R145W was initially reported in the HCT15 colorectal

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cancer cell line and, subsequently, in the germline of an individual with sarcoma at 20 years of age, breast cancer at 42 years of age, and a family history consistent with LFL (Bell et al. 1999; Lee et al. 2001). The breast tumor showed loss of the wild-type *CHEK2* allele. CHEK2 R145W has been shown to be deficient in kinase activity, binding and phosphorylation of Cdc25A, and ATM-dependent phosphorylation and, thus, is plausibly associated with the cancer susceptibility in this individual (Wu et al. 2001; Li et al. 2002). A second reported variant in *CHEK2*, I157T, has wild-type kinase activity but is deficient in binding and phosphorylation of Cdc25A and in binding to BRCA1 and p53 (Falck et al. 2001; Wu et al. 2001; Li et al. 2002). I157T has been reported in families with LFS, LFL, and breast cancer (Bell et al. 1999; Allinen et al. 2001; Bougeard et al. 2001; Lee et al. 2001; Vahteristo et al. 2001; Sullivan et al. 2002). However, I157T has also been detected in control populations at frequencies of 0%–6.5% (Bell et al. 1999; Allinen et al. 2001; Lee et al. 2001; Vahteristo et al. 2001). Thus, it is unclear whether I157T is a neutral polymorphism or confers a small increased risk of cancer.

To identify additional *CHEK2*-sequence variants that may confer susceptibility to breast cancer, we screened the full coding sequence of *CHEK2* in one case subject with breast cancer and negative for mutations in *BRCA1/2* from each of 89 families (from the United Kingdom, the Netherlands, or North America) with at least three cases of breast cancer. These families are referred to as the Variant Ascertainment Set. The families with breast cancer were ascertained from genetics clinics, and samples were obtained with approval of the Local Ethics Research Committee/Institutional Review Board. The 76 families with breast cancer from the United Kingdom and the United States each consisted of a minimum of three cases of breast cancer in first- or second-degree relatives who were diagnosed prior to age 60 years. The 13 families with breast cancer from the Netherlands consisted of a minimum of three cases of breast cancer in first- or second-degree relatives, of whom at least one was diagnosed prior to age 60 years. Nine Dutch families included at least three cases of breast cancer diagnosed prior to age 60 years. At least one affected individual from each of the 89 families was screened by Conformation Sensitive Gel Electrophoresis (CSGE) (Ganguly et al. 1993) for the full coding sequence and intron-exon boundaries of *BRCA1* and BRCA2 and was negative for mutations. In addition, families from the Netherlands were shown to be negative for rearrangements found elsewhere in the Dutch population (Petrij-Bosch et al. 1997). For the *CHEK2* whole gene screen, a single genomic DNA sample from each family was analyzed by CSGE. Exons 10–14 were amplified in a long-range PCR to avoid the partial nonprocessed genomic copies of *CHEK2* (Sodha et al. 2002). The long-range PCR was variably successful in these analyses, but complete data was obtained from a minimum of 76 families for each of exons 10–13 and from 59 families for exon 14.

In total, we identified three sequence variants of *CHEK2* in the 89 cases. Five cases carried the synonymous A252G (E84E) variant that has been reported in several other *CHEK2* screens and is a likely neutral polymorphism with no elevated risk of breast cancer (Bell et al. 1999; Hofmann et al. 2001; Ingvarsson et al. 2002; Reddy et al. 2002; Sullivan et al. 2002). Six cases harbored *CHEK2* 1100delC and have been reported elsewhere (The *CHEK2*-Breast Cancer Consortium 2002). One novel variant, R117G, was detected in two separate families, one from the United Kingdom and one from the Netherlands (fig. 1; table 1).

To evaluate the breast cancer risk associated with R117G and two variants, I157T and R145W, reported elsewhere in the germline of breast cancer cases, we used a study design similar to that employed in our previous analysis of *CHEK2* 1100delC, in which the frequency of the variant in case subjects with a family history of the disease was compared with that in control subjects. Although conventional association studies to detect low-penetrance susceptibility alleles compare population-based series of case with control subjects, use of familial cases markedly improves the power to detect an effect.

For each variant, we screened 737 breast cancer cases from 605 families negative for *BRCA1/2* mutations, 459 cases from 335 families positive for *BRCA1/2,* and 723 control subjects from the United Kingdom, North America, and the Netherlands by means of high stringency, allele-specific oligonucleotide (ASO) hybridization, with sequencing of positive cases (table 1). These series are referred to as "the Variant Evaluation Set" and did not include any cases from the families screened in the Variant Ascertainment Set. The families with breast cancer each contained at least two individuals with breast cancer or one individual with breast cancer and one individual with ovarian cancer who were first- or second-degree relatives of one another. At least one of the individuals with breast cancer was diagnosed before age 60 years. Families were ascertained through cancer genetics clinics in the relevant countries. The United Kingdom control subjects were children from the North Cumbria Community Genetics Project study from the northwest of the United Kingdom (Chase et al. 1998). The Dutch control subjects were spouses of cystic fibrosis heterozygotes from the southwest of the Netherlands. The North American control subjects were neighborhood control subjects from a breast cancer case-control study in the Philadelphia area or spouses marrying-in to families with breast cancer ascertained for linkage analysis from the same area. Each variant was detected by PCR amplification of the relevant exon, application of PCR products to nylon filters, and hybridization under

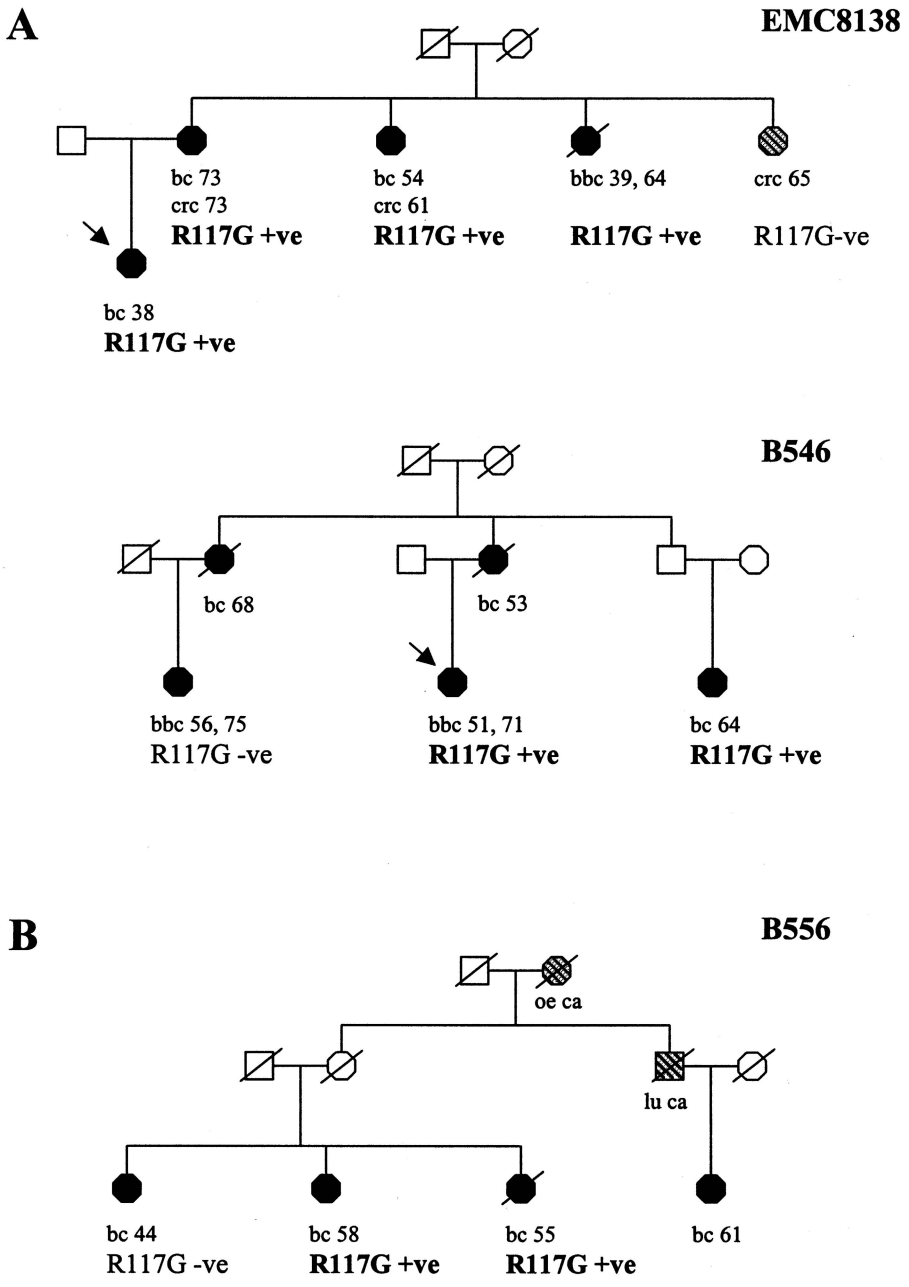

Figure 1 Abridged familial pedigrees with breast cancer who were positive for *CHEK2* R117G. *A*, Pedigrees positive for R117G, as identified in the Variant Ascertainment Set. The index individual screened is indicated with an arrow. *B*, Pedigrees positive for R117G, as identified in the Variant Evaluation Set; all three individuals were screened as part of the analyses. Filled symbols indicate individuals with breast cancer. Hatched symbols indicate individuals with cancers other than breast cancer. bc = breast cancer; bbc = bilateral breast cancer; crc = colorectal cancer; lu ca = lung cancer; oe ca = esophageal cancer. The age at diagnosis is indicated after the tumor type.

high stringency of ^{32}P end-labeled oligonucleotides complementary to mutant and the wild-type sequence (table 2). Every filter contained both a negative and a positive control and was scored independently by three individuals, as described elsewhere (The *CHEK2*-Breast Cancer Consortium 2002).

In our analyses of the Variant Evaluation Set, we identified both R117G and I157T in 2/737 *BRCA1/2*-negative familial case subjects and 1/723 control subjects and R145W in none of the case or control subjects (table 1).

Codon 117 is within the forkhead-associated (FHA) domain of CHEK2. FHA protein motifs are phospho-

Table 1

***CHEK2* Variants in *BRCA1/2*-Negative Breast Cancer Cases, *BRCA1/2*-Positive Breast Cancer Cases, and Controls**

	Number of				
			Subjects Positive for		
Set, Group, and Geographic Region	Subjects	Families	R117G	R145W	I157T
Variant Ascertainment Set[a]:					
Families negative for *BRCA1/BRCA2* in					
United Kingdom	72	72	1	0	0
North America	4	4	0	0	0
Netherlands	13	13	1	0	0
Total	89	89	2	0	0
Variant Evaluation Set[b]:					
Families negative for *BRCA1/BRCA2*[c] in					
United Kingdom	241	121	2[d]	0	0
North America	284	272	0	0	2
Netherlands	212	212	0	0	0
Total	737	605	2	0	2
Families positive for BRCA1/BRCA2 in					
United Kingdom	83	47	0	0	0
North America	235	147	0	0	0
Netherlands	141	141	0	0	0
Total	459	335	0	0	0
Control individuals in					
United Kingdom	448	…	0	0	0
North America	94	…	0	0	1
Netherlands	181	…	1	0	0
Total	723		1	0	1

[a] Full screen of *CHEK2* coding sequence.
[b] Allele-specific hybridization analysis of variant.
[c] The subjects negative for *BRCA1/2* in the Variant Evaluation Set do not include any cases from families that were screened in the Variant Ascertainment Set.
[d] Both individuals positive for R117G were from the same family.

peptide recognition domains (Durocher et al. 2000), and the CHEK2 FHA domain mediates ATM-dependent CHEK2 phosphorylation and targeting of CHEK2 to in vivo binding partners, such as BRCA1 (Li et al. 2002). The FHA domain is also implicated in CHEK2 oligomerization, which, in turn, is involved in regulation of CHEK2 activation, signal amplification, and transduction in DNA damage checkpoint pathways (Xu et al. 2002). Arg117 is a functionally important and highly conserved residue in the FHA domain (identical in both *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*). It anchors peptides via hydrogen bonding interactions with the phosphothreonine side chain, and disruption by an artificially introduced Arg^{117}→Ala substitution (R117A) severely reduces phosphopeptide binding (Li et al. 2002). Furthermore, there is no detectable binding of BRCA1 by R117A, in contrast to wild-type CHEK2 (Li et al. 2002). Thus, it is biologically plausible that the R117G variant, which is likely to have similar functional defects to R117A, confers susceptibility to breast cancer. However, only 2/737 *BRCA1/2*-negative case subjects in the Variant Evaluation Set carried R117G, and these were from the same family. One of 723 control subjects carried R117G (table 1). The three pedigrees afflicted with breast cancer in either set in which R117G was found are shown in figure 1. In one (EMC8138), all four tested individuals affected with breast cancer carried the variant. In the two other families (B546, B556), the variant was present in only two of three tested individuals with breast cancer. The probability that five out of the seven secondary cases of breast cancer in these families would carry the R117G variant, assuming that it is not associated with an increased risk of breast cancer, is 0.13.

Codon 157 is also in the FHA domain, though remote from the site of phosphopeptide binding (Li et al. 2002). I157T was reported elsewhere to be present in 8.9% (7/79) of Finnish pedigrees with hereditary breast cancer and 6.5% (13/200) of control individuals, suggesting that the breast cancer risk, if any, is weak (Allinen et al. 2001). Our data are consistent with this, as we identified I157T in 2/737 *BRCA1/2*-negative familial cases and in 1/723 control subjects. Samples were not available to assess segregation with the disease in either family positive for I157T.

Although none of the three variants we identified were associated with a statistically significantly increased risk,

Table 2

Germline *CHEK2* Variants Identified in Subjects with Breast Cancer and Probe Sequences Used for Their Detection in ASO Analyses

Protein Change	Nucleotide change	Wild-Type Sequence	Probe Sequence
R117G	349A→G	ggtttgggagggacaaaagct	ggtttgggggggacaaaagct
R145W	433C→T	aaacactttcggattttcag	aaacacttttggattttcag
I157T	470T→C	aaaaactcttacattgcatacat	aaaaactcttacactgcatacat

the CIs for the relative risk associated with these variants are wide. For all three variants combined, the crude 95% CIs, on the basis of the observed frequency in case and control subjects from the Variant Evaluation Set, are (0.21–21.3). The relative risks obtained from this data set are an overestimate of the "true" relative risk, because the frequency of a susceptibility allele will be higher in familial cases than unselected cases. As a comparison, in the analysis of *CHEK2* 1100delC, the estimated true relative risk was approximately twofold, but the crude relative risk on the basis of a similar series of familial cases was approximately fivefold (5% in case subjects vs. 1% in control subjects). Thus, the data on the three new *CHEK2* variants are consistent either with no effect or of a relative risk comparable with that conferred by *CHEK2* 1100delC.

Although there is considerable uncertainty about the level of risk associated with these three *CHEK2* variants, we can be more certain about their overall contribution to breast cancer incidence. The upper 95% CI on the combined frequency of these variants in breast cancer cases with a comparable family history is 1.5% (as compared with 5% for *CHEK2* 1100delC). We have estimated elsewhere that ~1% of breast cancer incidence and 1/2% of the familial aggregation of the excess breast cancer risk in first-degree relatives of case subjects is likelyto be due to *CHEK2* 1100delC. Even if the variants evaluated in this report are associated with a risk comparable with *CHEK2* 1100delC, much <1% of the familial aggregation of breast cancer or of breast cancer incidence overall is likely to be attributable to them.

At least two other nonsynonymous germline *CHEK2* variants have been reported in breast cancer since our analyses were performed: a R3W variant in an individual with breast cancer and a family history of LFL (Lee et al. 2001), and T59K, which was reported in four Icelandic individuals with breast cancer (Ingvarsson et al. 2002). It is possible that these or other as-yet-unidentified *CHEK2* variants may confer susceptibility to breast cancer, and additional studies in other familial breast cancer series will be of interest. However, we identified only two individuals with variant sequences in the 89 cases that were fully screened for mutations, compared with six occurrences of *CHEK2* 1100delC in the same family set. The low frequency of *CHEK2* variants other than 1100delC in familial breast cancer cases indicates that, even if some are associated with an increased risk, their overall contribution to breast cancer susceptibility is likely to be very low. The relatively high prevalence of *CHEK2* 1100delC in both the British and Dutch populations suggests that this variant is relatively old and subject to little selective pressure. The absence of any other protein-truncating alterations with significant frequencies could simply be due to chance but might reflect some functional constraints on the viability of individuals with alternative mutations.

Since we screened familial pedigrees with breast cancer from three specific populations, we cannot exclude the possibility that the contribution of *CHEK2* variants to breast cancer susceptibility differs in other populations. However, our results indicate that in North European populations, 1100delC is responsible for almost all of the contribution to breast cancer susceptibility made by sequence variants of *CHEK2*.

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Appendix

The following members of the Breast Cancer Linkage Consortium contributed samples for this study: A. Brady, T. Cole, A. Collins, H. Cox, A. Donaldson, D. F. Easton, D. Eccles, R. Eeles, G. Evans, H. Gregory, J. Gray, R. Houlston, J. Klijn, F. Lalloo, A. Lucassen, J. Mackay, H. Meijers-Heijboer, G. Mitchell, P. Morrison, V. Murday, S. Narod, K. L. Nathanson, J. Patterson, T. Peretz, C. M. Phelan, N. Rahman, M. Rogers, A. Schofield, M. R. Stratton, P. Tonin, B. Weber, and W. Weber.

Electronic-Database Information

URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM) http://www

.ncbi.nlm.nih.gov/Omim/ (for *CHEK2,* ATM, p53, Cdc25C, Cdc25A, BRCA1, BRCA2, and Li Fraumeni Syndrome)

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