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Report

The *CHEK2* 1100delC Mutation Identifies Families with a Hereditary Breast and Colorectal Cancer Phenotype

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Because of genetic heterogeneity, the identification of breast cancer–susceptibility genes has proven to be exceedingly difficult. Here, we define a new subset of families with breast cancer characterized by the presence of colorectal cancer cases. The 1100delC variant of the cell cycle checkpoint kinase *CHEK2* gene was present in 18% of 55 families with hereditary breast *and* colorectal cancer (HBCC) as compared with 4% of 380 families with non-HBCC ($P < .001$), thus providing genetic evidence for the HBCC phenotype. The *CHEK2* 1100delC mutation was, however, not the major predisposing factor for the HBCC phenotype but appeared to act in synergy with another, as-yet-unknown susceptibility gene(s). The unequivocal definition of the HBCC phenotype opens new avenues to search for this putative HBCC-susceptibility gene.

Cell cycle checkpoint kinase 2 (CHEK2, also known as "CHK2" [MIM 604373] and as "Cds1" in *Schizosaccharomyces pombe* and "RAD53" in *Saccharomyces cerevisiae*) is a key mediator in DNA damage–response pathways (Zhou and Elledge 2000; Bartek et al. 2001; Myung and Kolodner 2002; Rouse and Jackson 2002). In the course of our search for new breast cancer genes, we recently identified the kinase-deficient 1100delC variant of *CHEK2* as a low-penetrance breast cancer–susceptibility allele (Meijers-Heijboer et al. 2002). The prevalence of the *CHEK2* 1100delC mutation among families with non-BRCA1/BRCA2 breast cancer was 4.2% as compared with 1.1% among healthy individuals, implying an estimated twofold increased risk to develop breast cancer for women carrying the mutant allele. Similar results were then reported for Finnish families with breast cancer, thus independently confirming our observations (Vahteristo et al. 2002). We had noted that several of the families with *CHEK2* 1100delC breast cancer also included colorectal cancer cases, but its significance had been unclear (H.M.-H. and M.S., unpublished observations). Family EUR60, for example, encompassed six colorectal cancer cases, four of which had been diagnosed before age 50 years, and none could be explained by mutations of the *APC* (MIM 175100), *MLH1* (MIM 120436), *MSH2* (MIM 120435), or *MSH6* (MIM 600678) genes (fig. 1). A subtype of familial breast cancer that includes colorectal cancer had already been recognized by one of us in the early 1970s (Lynch et al. 1972), but evidence for such a phenotype has never been provided. Here, we have evaluated the involvement of the *CHEK2* 1100delC mutation in colorectal-cancer susceptibility.

Families with colorectal cancer were collected through the International Concerted Action Polyp Prevention (CAPP) and the Dutch Foundation for Detection of Hereditary Tumors (STOET). Families with colorectal can-

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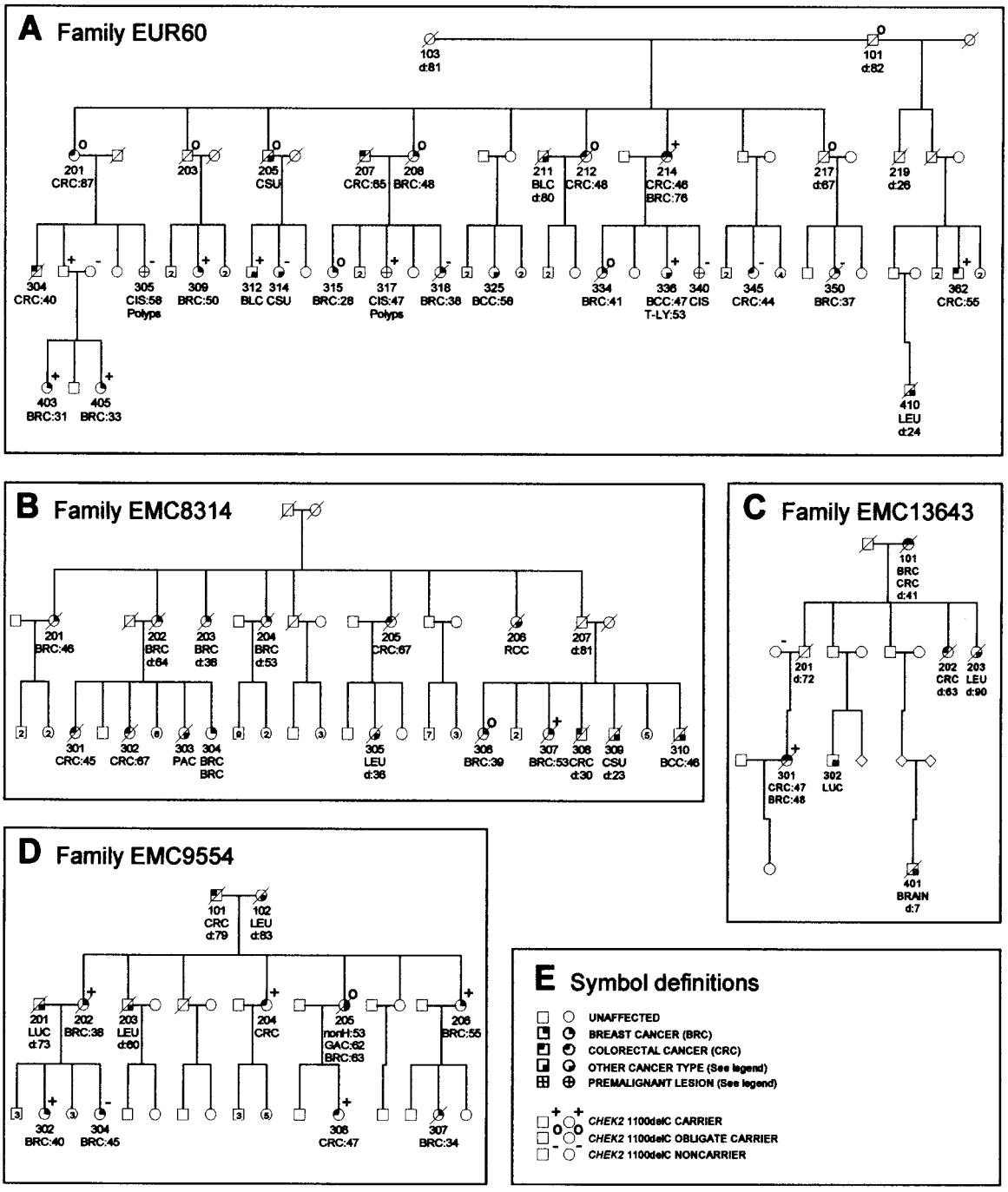

Figure 1 Abridged pedigrees of families with HBCC breast cancer who carry the *CHEK2* 1100delC mutation. Tumor type and age at diagnosis of the tumors are indicated below the individual identifiers. When known, the age of death (d:) is indicated below the tumor type for those cases where the age at diagnosis was unknown. Data from unaffected individuals who are not obligate *CHEK2* 1100delC mutation carriers are omitted to preserve confidentiality. For simplicity, unaffected family members of the youngest generations are also omitted. Abbreviations for the various tumor types: BCC = basal cell carcinoma; BLC = bladder cancer; BRAIN = brain cancer, BRC = breast cancer; CIS = carcinoma in situ of the breast; CRC = colorectal cancer; CSU = cancer site unknown; GAC = gastric cancer; LEU = leukemia; LUC = lung cancer; nonH = non-Hodgkin lymphoma; PAC = pancreatic cancer; Polyps = adenomatous polyps in the colorectum; RCC = renal cell carcinoma; and T-LY = T-cell lymphoma. Note that several individuals affected with early-onset cancer are noncarriers of the *CHEK2* 1100delC mutation, illustrating incomplete cosegregation (*A* and *C*). The high-penetrant cancer–predisposition pattern among all four families contrasts the estimated twofold breast cancer risk associated with the *CHEK2* 1100delC mutation, supporting synergism of *CHEK2* with the putative HBCC-susceptibility gene(s).

cer were classified by clinical and genetic criteria, resulting in two main groups of (*i*) families with familial adenomatous polyposis (FAP [MIM 175100]), characterized by >100 adenomatous polyps in the colorectum ($n = 91$) or >20 polyps in the case of attenuated FAP ($n = 4$), and (*ii*) families with hereditary nonpolyposis colorectal cancer (HNPCC [MIM 114500]) or with a phenotype reminiscent of HNPCC ($n = 234$), defined by at least two patients with colorectal cancer who were first-degree relatives, of whom at least one had been diagnosed before age 50 years. Pathogenic mutations of the *APC* gene were identified in 61 of 95 families with FAP and of the *MLH1, MSH2,* or *MSH6* genes in 127 families with HNPCC (table 1). Extensive mutational analyses had failed to identify mutations of these genes in the index cases of the remaining 34 families with FAP and 107 families with HNPCC and HNPCC-like disease. Of the 107 mutation-negative families with HNPCC and HNPCC-like disease, 70 met the Amsterdam criteria for HNPCC. Mutational analyses included the complete coding sequences of the *APC, MLH1, MSH2,* and *MSH6* genes, as well as all known Dutch founder mutations and deletions, as described elsewhere (van der Luijt et al. 1997; Wijnen et al. 1997, 1998, 1999). All families with breast cancer had been clinically ascertained through the Rotterdam family cancer clinic. Families with breast cancer were defined by at least two patients with breast cancer who were first- or second-degree relatives, of whom at least one had been diagnosed before age 60 years. A first cohort of families with non-BRCA1/BRCA2 breast cancer ($n = 188$) was described elsewhere, as part of a study by the International *CHEK2*-Breast Cancer Consortium (Meijers-Heijboer et al. 2002). Note that we used more stringent inclusion criteria for the current study, resulting in minor differences between the data sets. A second cohort of families with non-BRCA1/BRCA2 breast cancer ($n = 247$) did not overlap with the first cohort, and the *CHEK2* 1100delC mutation status was unknown prior to this study. Both cohorts of families with breast cancer were excluded for pathogenic mutations of the *BRCA1* (MIM 113705) or *BRCA2* (MIM 600185) genes by mutational analyses of the complete coding sequences of both genes, as well as screening for all known Dutch founder mutations and deletions, as described elsewhere (Petrij-Bosch et al. 1997; Meijers-Heijboer et al. 2002). Informed consents to search for the cancer-susceptibility genes have been obtained for all families, and all studies have been approved by local medical ethical committees.

We determined the prevalence of the *CHEK2* 1100delC mutation in a cohort of 329 families with colorectal cancer (table 1). DNA from a blood sample of the index case of each family was screened for the *CHEK2* 1100delC mutation by an allele-specific oligohybridization assay (Meijers-Heijboer et al. 2002), and all positive samples were confirmed by direct sequencing of independently amplified templates (Sodha et al. 2002). The *CHEK2* 1100delC mutation was not identified in any of 95 families with FAP. Of the 234 families with HNPCC or HNPCC-like disease, 6 (2.6%) carried the *CHEK2* 1100delC mutation. Mutational analysis of the three main mismatch-repair genes had previously detected a germline mutation of *MLH1, MSH2,* or *MSH6* in three of the six families with *CHEK2* 1100delC colorectal cancer but had failed to identify pathogenic sequence variants in the other three families (table 1). Although the prevalence of the *CHEK2* 1100delC mutation among the families with HNPCC and HNPCC-like disease was somewhat higher than among control subjects, this difference was not significant (2.6% vs. 1.1%; odds ratio [OR] 2.34; 95% CI 0.95–5.79; $P = .07$).

The presence of colorectal cancer cases in some of the families with *CHEK2* 1100delC breast cancer prompted

Table 1

Prevalence of the *CHEK2* 1100delC Mutation among Families with Colorectal Cancer and Families with Breast Cancer

Cohorts and Subgroups	*CHEK2* 1100delC+/Total Tested
Controls (Meijers-Heijboer et al. 2002)	18/1620 (1.1%)
Families with colorectal cancer:	
Families with FAP	0/95 (0.0%)
Families with HNPCC and HNPCC-like disease:	6/234 (2.6%)
MLH1-positive	1/61
MSH2-positive	1/58
MSH6-positive	1/8
Non-*MLH1/MSH2/MSH6*	3/107
Families with non-*BRCA1/BRCA2* breast cancer:	25/435 (5.8%)
Families with HBCC:	10/55 (18.2%)
Cohort 1	4/30
Cohort 2	6/25
Families with non-HBCC:	15/380 (4.0%)
Cohort 1	8/158
Cohort 2	7/222

Table 2

Particulars of the Families with Non-*BRCA1/BRCA2* Breast Cancer

	Finding in Families with Breast Cancer						
	With *CHEK2* 1100delC (%)			Without *CHEK2* 1100delC (%)			
	HBCC	Non-HBCC	All	HBCC	Non-HBCC	All	*P*
Average age at diagnosis of index cases	43.8	46.6	45.5	46.7	45.8	45.8	.87[a]
Families including patients with bilateral BRC	3 (30)	2 (13)	5 (20)	13 (29)	96 (26)	109 (27)	.45[a]
Number of patients with BRC in the family:							
One patient diagnosed at age <60 years	1 (10)	5 (33)	6 (24)	14 (31)	84 (23)	98 (24)	
Two patients diagnosed at age <60 years	3 (30)	5 (33)	8 (32)	17 (38)	151 (41)	168 (41)	
Three patients diagnosed at age <60 years	2 (20)	2 (13)	4 (16)	6 (13)	80 (22)	86 (21)	
More than three patients diagnosed at age <60 years	4 (40)	3 (20)	7 (28)	8 (18)	50 (14)	58 (14)	.29[a]
Total number of families with breast cancer	10 (40)	15	25	45 (11)	365	410	<.001[b]

[a] *P* value for the difference between all families with *CHEK2* 1100delC-positive and *CHEK2* 1100delC-negative breast cancer.
[b] *P* value for the difference between families with *CHEK2* 1100delC-positive and *CHEK2* 1100delC-negative HBCC.

us to further analyze our original cohort of families with breast cancer from the Rotterdam family cancer clinic (Meijers-Heijboer et al. 2002). In this cohort, the prevalence of the *CHEK2* 1100delC mutation was 6.4% among the families with non-BRCA1/BRCA2 breast cancer (12 of 188 families; table 1). We then set to classify the families with breast cancer within this cohort by more stringent clinical criteria that defined a putative hereditary breast *and* colorectal cancer phenotype (HBCC). We define a "family with the HBCC phenotype" as a family with breast cancer characterized by the presence of at least two patients with breast cancer who were first- or second-degree relatives and of whom at least one is diagnosed before age 60 years *and*

1. at least one patient with breast cancer and colorectal cancer diagnosed at any age; *or*
2. at least one individual with colorectal cancer diagnosed before age 50 years who was a first- or second-degree relative of a patient with breast cancer; *or*
3. at least two patients with colorectal cancer diagnosed at any age of whom at least one was a first- or second-degree relative of a patient with breast cancer.

(An anamnestic report of colorectal cancer was considered reliable only when the diagnosis had been made after 1960.) Of the 188 families with breast cancer, 30 met our clinical criteria for HBCC (table 1). Four of these 30 (13.3%) families with HBCC carried the *CHEK2* 1100delC mutation, suggesting that the mutant allele indeed identified an HBCC phenotype. Such retrospectively defined criteria are, however, inherently subjective. We therefore applied the HBCC criteria prospectively to another cohort of 247 families with non-BRCA1/BRCA2 breast cancer from the Rotterdam family cancer clinic. This second cohort of families with breast cancer did not overlap with the first cohort, and the *CHEK2* 1100delC mutation status of the families was unknown. Of the 247 families with breast cancer from this second cohort, 25 met our clinical criteria for HBCC (table 1). Of these 25 families with HBCC, 6 (24.0%) carried the *CHEK2* 1100delC mutation, as compared with 7 of 222 (3.2%) families with non-HBCC from this cohort, thereby confirming the strong association of the HBCC phenotype with the *CHEK2* 1100delC mutation.

Identification of a similar phenotype with an increased risk of breast cancer among families with colorectal cancer was not unequivocal. When "mirror" HBCC criteria were applied to our cohort of families with colorectal cancer, comparable with the HBCC criteria for families with breast cancer (see list above), 44 of the 234 families with HNPCC and HNPCC-like disease met these criteria. Of these 44 families with HBCC-like colorectal cancer, 2 (4.5%) carried the *CHEK2* 1100delC mutation, as compared with 4 of the 190 (2.1%) remaining families with HNPCC and HNPCC-like disease. Although these data may suggest an "HBCC-like" phenotype for families with colorectal cancer similar to that of families with HBCC breast cancer, the evidence is circumstantial and awaits further evaluation. We anticipate that the *CHEK2* 1100delC mutation does confer a colorectal cancer risk but that this risk is even lower than its rather modest breast cancer risk of twofold. Substantially larger series of families with HNPCC and HNPCC-like disease would thus be required to reach sufficient statistical power to identify such a low-penetrance colorectal-cancer risk.

Altogether, we identified the *CHEK2* 1100delC mutation in 10 of 55 (18.2%) families with HBCC, compared with 15 of 380 (4.0%) families with non-HBCC breast cancer (OR 5.41; 95% CI 2.29–12.8; $P < .001$). To evaluate the influence of other parameters thought to associate with the *CHEK2* 1100delC mutation, we performed univariate and multivariate analyses on all 435 families with non-BRCA1/BRCA2 breast cancer from the two Rotterdam family cohorts (table 2). Consistent with

our report elsewhere (Meijers-Heijboer et al. 2002), but in contrast with the Finnish report (Vahteristo et al. 2002), the prevalence of the *CHEK2* 1100delC mutation was increased among families with more than three members with breast cancer diagnosed before age 60 years (11% vs. 5%; OR 2.36; 95% CI 0.94–5.90; $P = .07$). Consistent with both reports (Meijers-Heijboer et al. 2002; Vahteristo et al. 2002), there was no difference in the age at breast cancer diagnosis for the index cases of the families with *CHEK2* 1100delC breast cancer, as compared with the index cases of families without the mutant allele (45.5 vs. 45.8 years). The prevalence of the *CHEK2* 1100delC mutation was similar among families with and without patients with bilateral breast cancer (4.4% versus 6.2%). Cases of male breast cancer were not observed in any of the families with *CHEK2* 1100delC breast cancer (Meijers-Heijboer et al. 2002). No significant differences between the families with HBCC and non-HBCC breast cancer were observed for any of the parameters, except for the prevalence of the *CHEK2* 1100delC mutation (table 2). The association of the *CHEK2* 1100delC mutation with the HBCC phenotype remained strong after correction for the number of breast cancer cases diagnosed before age 60 years and the presence of bilateral breast cancer cases in the family (multivariate OR 5.19; 95% CI 2.17–12.4; $P < .001$). The *CHEK2* 1100delC mutation thus provided conclusive genetic evidence for the existence of an HBCC subtype of familial breast cancer.

We identified 55 families with HBCC in a series of 435 families with non-BRCA1/BRCA2 breast cancer from the Rotterdam family cancer clinic, representing 13% of the total (table 1). Examples of pedigrees with HBCC are shown in figure 1. Of the 55 families with HBCC, 17 (31%) had been included by the first HBCC criterion, 7 (13%) by the second criterion, and 21 (38%) by the third criterion (see list above). Ten (18%) families met multiple HBCC criteria, and five of these carried the *CHEK2* 1100delC mutation. Forty-five families with HBCC also included cancers from anatomical sites other than the mammary glands or colorectum, with an average of almost three cases per family (altogether 129 other cancers, excluding basal cell carcinomas). None of these 45 families with HBCC had a cancer pattern reminiscent of the Li-Fraumeni syndrome (LFS [MIM 151623]) (Bell et al. 1999).

We assessed cosegregation of the *CHEK2* 1100delC genotype with the disease phenotype for nine informative families with breast cancer from the two Rotterdam family cohorts. Cosegregation was incomplete for five of these nine families. Among first- and second-degree relatives of the index patients, only 7 of 13 (54%) typed patients with breast cancer carried the *CHEK2* 1100delC mutation. The age at breast cancer diagnosis was similar for the mutation carriers and the noncarriers (52.7 vs. 56.8 years; $P = .63$), and double tumors were not observed among these 13 additionally typed patients with breast cancer. When colorectal cancer was considered to be part of the phenotype, 9 of 16 (56%) patients carried the mutant allele. For comparison, we observed cosegregation of the family-specific mutation with the disease phenotype for 86% of additionally typed patients with breast and ovarian cancer from families with *BRCA1* and *BRCA2* mutations (Meijers-Heijboer et al., in press), indicating that the incomplete cosegregation of the *CHEK2* 1100delC mutation could not be explained just by the presence of sporadic breast or colorectal cancer cases in the families. Cosegregation was also incomplete for all three informative families with *CHEK2* 1100delC colorectal cancer, where none of five additionally typed patients with colorectal cancer carried the mutant allele. Three of the six families with *CHEK2* 1100delC colorectal cancer also carried a pathogenic mutation of a mismatch-repair gene. In the family with *MLH1* HNPCC, three patients with colorectal cancer were carriers of the *MLH1* mutation, two were obligate carriers, and none were known to be a noncarrier of the *MLH1* mutation. Two patients with *MLH1* colorectal cancer were available for typing. One of these also carried the *CHEK2* 1100delC mutation and was diagnosed with colorectal cancer at age 34 years and with endometrial cancer at age 55 years. The other patient was a noncarrier of the *CHEK2* 1100delC mutation and was diagnosed with colorectal cancer at age 52 years. In the family with *MSH2* HNPCC, one patient with colorectal cancer was diagnosed at age 30 years and was carrier of both the *MSH2* mutation and the *CHEK2* 1100delC mutation. Another patient with colorectal cancer from this family was diagnosed at age 37 years and was a noncarrier of the *MSH2* mutation but was not available for *CHEK2* 1100delC typing. In the family with *MSH6* HNPCC, two patients with colorectal cancer were carriers of the *MSH6* mutation, one was an obligate carrier, and none were known to be noncarriers of the *MSH6* mutation. Of the two *MSH6* mutation carriers, one was diagnosed with colorectal cancer at age 65 years and also carried the *CHEK2* 1100delC mutation. The other *MSH6* mutation carrier was diagnosed with colorectal cancer at age 45 years and with endometrial cancer at age 54 years, but did not carry the *CHEK2* 1100delC mutation.

The *CHEK2* 1100delC mutation is an unusual cancer-susceptibility allele, in that not all patients with breast or colorectal cancer from the families with the *CHEK2* 1100delC mutation carry the mutant allele, even though the mutant allele was significantly associated with their familial clustering of breast and colorectal cancer (Meijers-Heijboer et al. 2002; Vahteristo et al. 2002; the present study). We hypothesize that the *CHEK2* 1100delC mutation acts in synergy with another, as-yet-unknown cancer-susceptibility gene or genes. Thus, the estimated

twofold increase in breast cancer risk for *CHEK2* 1100delC mutation carriers (Meijers-Heijboer et al. 2002) represents a surplus to the cancer risk among the families with CHEK2 1100delC that is due to the unknown susceptibility gene. Considering the generally high-penetrant cancer-predisposition pattern among the families with the *CHEK2* 1100delC mutation (fig. 1), the unknown susceptibility gene would appear to be at least moderately penetrant or low penetrant in a more complex polygenic model. If this is true, one may comprehend that the *CHEK2* 1100delC mutation tends to associate with the more severely affected families with breast cancer (Meijers-Heijboer et al. 2002; the present study), even though the increased breast cancer risk conferred by the *CHEK2* 1100delC mutation is estimated to be only a modest twofold. Also, the *CHEK2* 1100delC mutation would not completely cosegregate but merely associate with the cancer phenotype, since it confers only a surplus of cancer risk. Two recent reports suggested a synergistic role of CHEK2 at the intra-S phase checkpoint of the cell division cycle. Using a variety of human cells defective in DNA damage–response proteins, it was shown that ATM-dependent radio-sensitive DNA synthesis (RDS) diverges via the CHEK2-CDC25A-CDK2 pathway and the MRE11-RAD50-NBS1 pathway. Whereas each of these pathways induced a partial replication block upon ionizing radiation, complete inhibition of RDS was achieved only by concerted action of both pathways (Falck et al. 2002) (ATM [MIM 208900], CDC25A [MIM 116947], CDK2 [MIM 116953], MRE11 [MIM 600814], RAD50 [MIM 604040], and NBS1 [MIM 602667]). In *S. cerevisiae,* mutation of the *CHEK2* homologue *RAD53* caused a modest increase in the rate of spontaneous gross chromosomal rearrangements (GCR), whereas double mutants of *RAD53* and *TEL1* (*hATM*) had a highly synergistic effect on the GCR rate (Myung et al. 2001). Perhaps the putative HBCC-susceptibility gene should be looked for among candidates that are known to function in suppression of genome instability at the intra-S phase checkpoint of the cell cycle.

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Electronic-Database Information

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for APC, ATM, BRCA1, BRCA2, CDC25A, CDK2, CHEK2, FAP, HNPCC, LFS, MLH1, MRE11, MSH2, MSH6, NBS1, and RAD50)

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