Low-penetrance susceptibility to breast cancer due to *CHEK2**1100delC in noncarriers of *BRCA1* or *BRCA2* mutations

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Mutations in *BRCA1* and *BRCA2* confer a high risk of breast and ovarian cancer[1], but account for only a small fraction of breast cancer susceptibility[1,2]. To find additional genes conferring susceptibility to breast cancer, we analyzed *CHEK2* (also known as *CHK2*), which encodes a cell-cycle checkpoint kinase that is implicated in DNA repair processes involving BRCA1 and p53 (refs 3–5). We show that *CHEK21100delC, a truncating variant that abrogates the kinase activity[6], has a frequency of 1.1% in healthy individuals. However, this variant is present in 5.1% of individuals with breast cancer from 718 families that do not carry mutations in *BRCA1* or *BRCA2* (*P*=0.00000003), including 13.5% of individuals from families with male breast cancer (*P*=0.00015). We estimate that the *CHEK2**1100delC variant results in an approximately twofold increase of breast cancer risk in women and a tenfold increase of risk in men. By contrast, the variant confers no increased cancer risk in carriers of *BRCA1* or *BRCA2* mutations. This suggests that the biological mechanisms underlying the elevated risk of breast cancer in *CHEK2* mutation carriers are already subverted in carriers of *BRCA1* or *BRCA2* mutations, which is consistent with participation of the encoded proteins in the same pathway.**

To investigate breast cancer susceptibility that is not attributable to mutations in *BRCA1* or *BRCA2*, we carried out a genome-wide linkage search in family EUR60, our largest family in which breast cancer susceptibility is not due to either gene. The highest lod score we obtained was 1.2 (maximum possible lod score=4.7) on chromosome 22q between *D22S1150* and *D22S928*. The haplotype linked to chromosome 22 showed partial segregation with breast cancer (Fig. 1).

The gene *CHEK2* is located on chromosome 22q and encodes the human ortholog of yeast Cds1 and Rad53, which are G2 checkpoint kinases[7,8]. Activation of these proteins in response to DNA damage prevents cellular entry into mitosis. In mammalian cells, CHEK2 is activated, through phosphorylation by ATM[8–10], in response to DNA damage induced by ionizing radiation. CHEK2 phosphorylates p53, mediating activation and stabilization of p53 by ATM[3,4]. CHEK2 also phosphorylates Cdc25C, preventing entry into mitosis[7], and associates with, phosphorylates and activates functions of BRCA1 (ref. 5).

Germline *CHEK2* sequence variants have been reported in families with Li-Fraumeni syndrome that do not carry *TP53* mutations[11]. Screening for mutations in *CHEK2* is complicated by the presence of many partial copies throughout the genome[12]. However, the mutation 1100delC clearly occurs in the functional copy of *CHEK2* and abolishes the kinase activity of the protein[6,13]; thus *CHEK2**1100delC is a plausible candidate for causing cancer predisposition. Mutation screening of *CHEK2* in family EUR60 revealed the 1100delC mutation in seven individuals with breast cancer (Fig. 1).

To evaluate the significance of *CHEK2**1100delC in predisposition to breast cancer, we assessed its frequency in families with breast cancer, individuals with breast cancer unselected for family history, and controls. We detected *CHEK2**1100delC in 18 of 1,620 (1.1%) control individuals from the UK, the Netherlands and North America (including Canada) and found no significant frequency variation among control groups (Table 1). By contrast, 55 of 1,071 (5.1%) individuals with breast cancer from 718 families without *BRCA1* or *BRCA2* mutations carry *CHEK2**1100delC (Table 1; *P*=0.00000003).

The *CHEK2**1100delC variant is present in 7 of 52 (13.5%) individuals with breast cancer from families without *BRCA1* or *BRCA2* mutations who had one or more individuals with male breast cancer (*P*=0.00015 compared with all controls combined, *P*=0.032 compared with families without *BRCA1* or *BRCA2* mutations who did not have male breast cancer). The variant was found in 5 of 117 (4.3%) individuals from families without *BRCA1* or *BRCA2* mutations who had one or more individuals with ovarian cancer (*P*=0.016 compared with all

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controls combined, P=0.97 compared with families without *BRCA1* or *BRCA2* mutations who have female breast cancer only) and in 44 of 912 (4.8%) individuals from families without *BRCA1* or *BRCA2* mutations who have female breast cancer only (P=0.0000002 compared with controls). Within the latter group, there was evidence of increasing prevalence of the variant, as increasing numbers of individuals were diagnosed with breast cancer before 60 years of age (Table 1; P_{trend}=0.003). The mean age at diagnosis of individuals with breast cancer who harbored the *CHEK2**1100delC mutation (45.4 years) was not significantly different than that of affected individuals who did not carry the mutation (45.1 years).

We assessed linkage of *CHEK2**1100delC to breast cancer in 20 families without *BRCA1* or *BRCA2* mutations, in which the index case harbored the variant and at least one other individual with breast cancer had been typed. Of 27 additional individuals typed, 16 (59%) carried *CHEK2**1100delC, compared with the 41% that would be expected if the variant were unrelated to breast cancer (estimated relative risk 2.2, P=0.049).

We then assessed the frequency of *CHEK2**1100delC in a population-based series of individuals with breast cancer (Table 1). Of 636 cases, 9 (1.4%, 95% CI=0.6%–2.7%) carried *CHEK2**1100delC. This frequency did not differ significantly, either from the combined UK/Dutch control series (adjusted odds ratio 1.41, 95% CI=0.59–3.38) or from the control series directly matched to these individuals (odds ratio 2.52, 95% CI=0.78–8.18). Finally, we assessed the frequency of *CHEK2**1100delC in families with breast cancer that carry *BRCA1* or *BRCA2* mutations. The frequency of the variant in individuals from families with *BRCA1* or *BRCA2* mutations (5/520, 1.0%) did not differ from that of control individuals, but was lower than in the families without *BRCA1* or *BRCA2* mutations (Table 1; P=0.002).

We analyzed *D22S275*, a polymorphic marker within *CHEK2*, in individuals from 51 pedigrees containing *CHEK2**1100delC. All individuals harboring the variant carried the same allele of this marker, which we estimate has a frequency of 18% and 13%, respectively, in the UK and Dutch populations. This finding suggests that all *CHEK2**1100delC alleles are derived from a common founder.

That the *CHEK2**1100delC variant was found in 5.1% of individuals with breast cancer from families without *BRCA1* or *BRCA2* mutations, compared with its frequency of 1.1% in the healthy population, indicates that it confers an increased risk of breast cancer. However, the low frequency in the population-based series of individuals with breast cancer indicates that the risk of breast cancer conferred by *CHEK2**1100delC is modest (upper 95% confidence limit is 3.38). This is consistent with the limited segregation of the allele with breast cancer in families without *BRCA1* or *BRCA2* mutations. The high frequency of *CHEK2**1100delC in families without BRCA1 or BRCA2 mutations that include individuals with male breast cancer indicates that the variant confers a higher relative risk for male than female breast cancer. By contrast, there is no evidence that the frequency

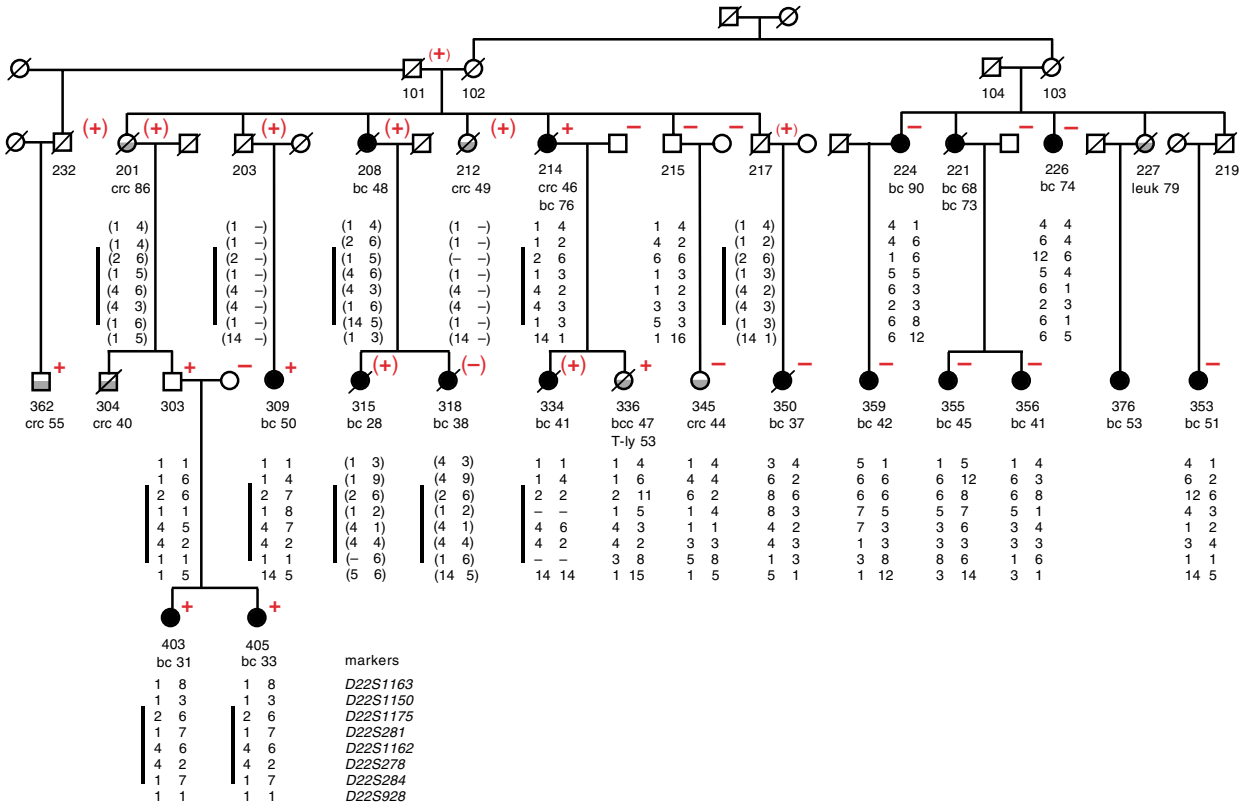

Fig. 1 Abridged pedigree of family EUR60. Filled symbols indicate individuals with invasive breast cancer (bc). Half-shaded symbols indicate individuals with cancer other than breast (crc, colorectal cancer; leuk, leukemia; bcc, basal-cell carcinoma; T-ly, T-cell lymphoma). The individual identifier is below each symbol, and the age at diagnosis of cancer is below the identifier. The marker haplotype linked to chromosome 22 representing maximal segregation with breast cancer in the genome-wide search is indicated by the vertical black bar. Haplotypes inferred from offspring are shown in parentheses. Data from unaffected individuals who are not obligate carriers are omitted to preserve confidentiality. *CHEK2* is located between *D22S1163* and *D22S1150*. Plus signs indicate individuals harboring *CHEK2**1100delC (in parenthesis where reconstructed by haplotype analysis). Minus signs indicate individuals without *CHEK2**1100delC. One individual with breast cancer (318) carrying the chromosome 22–linked haplotype has not inherited *CHEK2**1100delC owing to a recombination event between *D22S1150* and *D22S1175*.

of *CHEK2**1100delC is elevated in families with breast and ovarian cancer compared with families having female breast cancer only (although the number studied is small).

The markedly higher frequency of *CHEK2**1100delC in affected families without *BRCA1* or *BRCA2* mutations, as compared with healthy controls, must in part result from a clustering of cases that is due to the variant conferring an elevated risk of breast cancer. However, it may also reflect an interaction between *CHEK2**1100delC and other (as-yet unidentified) breast cancer predisposition genes in these families. To evaluate this, we used segregation analysis to estimate the relative risk of breast cancer associated with *CHEK2**1100delC. Under a simple model in which the risks conferred by *CHEK2**1100delC and other genes combine multiplicatively, the estimated breast cancer risk ratio associated with *CHEK2**1100delC in families without *BRCA1* or *BRCA2* mutations was 1.70 (95%CI=1.32–2.20) in females and 10.28 (95%CI=3.54–29.87) in males. Although we did not observe a significant risk associated with *CHEK2**1100delC in the combined UK/Dutch population-based case-control studies, the estimated risk (OR 1.41, 95%CI=0.59–3.38) was of the same magnitude as that found in the family-based analysis, in agreement with a multiplicative model. On the assumption that estimates derived from the affected families without *BRCA1* or *BRCA2* mutations are applicable at the population level, approximately 1% of female breast cancer incidence, 9% of male breast cancer incidence and 0.5% of the excess breast cancer risk in first-degree relatives of affected individuals is attributable to *CHEK2**1100delC.

In contrast to families with breast cancer that do not carry *BRCA1* or *BRCA2* mutations, the frequency of *CHEK2**1100delC in affected families with *BRCA1* or *BRCA2* mutations is not different from that of controls. Thus, although *CHEK2**1100delC seems to confer an increased risk of breast cancer on the background of some genotypes that show predisposition to breast cancer, the allele does not seem to confer an elevated breast cancer risk in carriers of *BRCA1* or *BRCA2* mutations. To our knowledge, this is the first example of genes that confer susceptibility to cancer interacting in a manner that is clearly demonstrable at an epidemiological level in humans. It is unlikely that this effect is simply attributable to the high risk of cancer in *BRCA1* or *BRCA2* mutation carriers leaving no potential for further increase. Most studies estimate the breast cancer risk by age 50 to be no more than 50% in *BRCA1* mutation carriers and 30% in *BRCA2* mutation carriers[1]. A twofold increase in risk conferred by *CHEK2**1100delC in *BRCA1/2* mutation carriers is therefore theoretically possible and would have been detectable in our analyses, if present.

Table 1 • *CHEK21100delC in families with breast cancer, individuals with breast cancer unselected for family history and controls**

	Positive for *CHEK2**1100delC (%)	
	Index cases	All cases
Controls		
UK (UKCCS)	1/292 (0.3%)	
UK (RMHT/ICR)	3/288 (1.0%)	
UK (NWCCGP)	4/230 (1.7%)	
Netherlands (A)	3/184 (1.6%)	
Netherlands (B - ERGO)	6/460 (1.2%)	
North America (Philadelphia)	1/166 (0.6%)	
Total	18/1620 (1.1%)	
Individuals with breast cancer unselected for family history		
UK (UKCCS)	7/557 (1.3%)	
Netherlands (ERGO)	2/79 (2.5%)	
Total	9/636 (1.4%)	
BRCA1/2-negative families with breast cancer[a]		
UK	12/211 (5.7%)	25/423 (5.9%)
Netherlands	11/226 (4.9%)	21/334 (6.3%)
North America	6/264 (2.3%)	8/273 (2.9%)
Germany	1/17 (5.9%)	1/41 (2.4%)
Total	30/718 (4.2%)	55/1071 (5.1%)
Families with at least one male with breast cancer[b]	4/33 (12.1%)	7/52 (13.5%)
Families with at least one female with ovarian cancer[c]	4/99 (4.0%)	5/117 (4.3%)
Families with 1 case <60	2/93 (2.2%)	2/109 (1.8%)
Families with 2 cases <60	7/192 (3.7%)	11/277 (4.0%)
Families with 3 cases <60	6/175 (3.4%)	12/294 (4.1%)
Families with 4 cases <60	5/84 (6.0%)	9/143 (6.3%)
Families with >4 cases <60	3/49 (6.1%)	10/89 (11.2%)
BRCA1/2-positive families with breast cancer[d]		
UK	0/52 (0.0%)	0/124 (0.0%)
Netherlands	1/141 (0.7%)	4/203 (2.0%)
North America	0/122 (0.0%)	1/187 (0.6%)
Germany	0/3 (0.0%)	0/6 (0.0%)
BRCA1 +ve	1/215 (0.5%)	5/352 (1.4%)
BRCA2 +ve	0/103 (0.0%)	0/168 (0.0%)
Total	1/318 (0.3%)	5/520 (1.0%)

[a]Refers to families with breast cancer that do not carry *BRCA1* or *BRCA2* mutations. [b]Eight males with breast cancer were tested, of whom two (from families harboring the *CHEK2**1100delC variant) carried the variant and six (from families without *CHEK2**1100delC) did not carry the variant. [c]Five women with ovarian cancer (from families without *CHEK2**1100delC) were tested in these families; none of these women harbored the variant allele. [d]Refers to families with breast cancer that carry *BRCA1* or *BRCA2* mutations.

The genetic interaction between *CHEK2* and *BRCA1* or *BRCA2* mutations probably reflects functional interactions among BRCA1, BRCA2 and CHEK2. CHEK2 is regulated by ATM (as is BRCA1) and itself phosphorylates and regulates BRCA1. It is thus plausible that CHEK2 and BRCA1 are components of the same biological pathway. If this pathway is already subverted by inactivating mutations in *BRCA1*, then abolition of CHEK2 function may confer no demonstrable additional risk of disease (an additive, rather than multiplicative, effect of *CHEK2**1100delC and *BRCA1* or *BRCA2* mutations, which might be predicted by this model, would not be excluded by our data because it would result in a very small relative risk). The low frequency of *CHEK2**1100delC in families with *BRCA2* mutations suggests that a similar functional interaction also exists between BRCA2 and CHEK2.

We have shown that *CHEK2**1100delC is a low-penetrance allele conferring susceptibility to breast cancer. Although many such alleles have previously been suggested[14], this is the first to be confirmed to a high degree of statistical significance. Moreover, our data indicate that *CHEK2**1100delC cannot be a high-penetrance allele for Li-Fraumeni susceptibility[11,15], as the population prevalence of the variant is approximately 1%, but Li-Fraumeni syndrome is very rare. Our results provide a scientific basis for management of breast cancer susceptibility related to *CHEK2**1100delC in clinical practice. However, the demand for clinical testing of an allele that confers an approximately twofold risk of female breast cancer is unknown. Moreover, the utility of such testing and the contexts in which it is undertaken are currently unclear and will require careful consideration.

Methods

Affected families and individuals, and controls. We ascertained families with breast cancer through several clinical genetics centers in the UK, the Netherlands, North America (including Canada) and Germany. All families include at least two cases of female breast cancer in first- or second-degree relatives, or at least one case of female breast cancer and a case of ovarian cancer or male breast cancer in first- or second-degree relatives. We tested two series of individuals with breast cancer unselected for family history: (i) a population-based series of 557 affected individuals diagnosed under age 45, ascertained through the UK Case Control Study of Breast Cancer (UKCCS) as described previously[2] and (ii) a population-based series of 79 affected individuals diagnosed at ages 55 and older, ascertained through the Erasmus Rotterdam Health and the Elderly Study (ERGO). We used six groups of healthy control individuals. Three of these were from the UK: (i) controls from the UKCCS, chosen as age-matched healthy women from the same general practice as the affected individual (n=292); (ii) spouses of siblings of individuals with cancer attending the Royal Marsden Hospital National Health Service Trust (n=288) and (iii) children from the North Cumbria Community Genetics Project from the northwest UK (n=230), from whom umbilical cord blood was obtained. Two series of control individuals were from the Netherlands: 184 (91 female, 93 male) spouses of individuals heterozygous with respect to cystic fibrosis from the southwest Netherlands; and 460 age-matched controls from the ERGO study. The North American control individuals (n=166) were individuals from the same neighborhood from a breast cancer case-control study in the Philadelphia area, or spouses marrying into families with breast cancer ascertained for linkage analysis from the same area. All studies were approved by local ethical committees or institutional review boards, and all individuals (or, in the case of the cord-blood samples from newborns, their parents) gave full informed consent.

Mutation screening of *BRCA1*, *BRCA2* and *CHEK2*. We screened the full coding sequence and splice junctions of *BRCA1* and *BRCA2* for mutations in at least one individual from every family, either by using heteroduplex analysis (conformation-sensitive gel electrophoresis) or the protein truncation test for exons 10 and 11 of *BRCA2* and exon 11 of *BRCA1* and heteroduplex analysis for the remainder of the coding sequence, or by direct sequencing. In addition, we screened families from the Netherlands for the large genomic rearrangements known to be present in this population[16]. We defined families as noncarriers of *BRCA1* or *BRCA2* mutations if they did not have a mutation clearly associated with breast cancer (such as a truncating mutation or one of the previously described pathogenic missense variants). One family without a detectable *BRCA1* or *BRCA2* mutation (CRC114) was classified as a *BRCA2* carrier family because we found evidence of linkage to chromosome 13q markers flanking the gene (lod score greater than 3). In EUR60, we fully screened individuals 214, 224, 226, 309, 336, 345, 353, 355, 356, 359, 403 and 405 and offspring of 315, 318, 334 and 350 (Fig. 1) for mutations in both genes and for the known Dutch genomic rearrangements. Moreover, analyses of microsatellite markers flanking *BRCA1* and *BRCA2* in this family provide evidence against linkage to both loci (lod scores: *BRCA1*, –1.75; *BRCA2*, –2.22). We also screened the full coding sequence of *CHEK2* for mutations using heteroduplex analysis, first amplifying exons 10–14 in a long-distance PCR to avoid genomic copies of *CHEK2* (ref. 17).

We detected the 1100delC mutation in *CHEK2* of family EUR60 by PCR amplification of exon 10, application of PCR products to nylon filters and hybridization under high stringency of [^{32}P]oligonucleotides complementary to *CHEK2**1100delC and the wildtype sequence. Oligonucleotides used for amplification of exon 10 were designed so that the reverse primer had a base mismatch in the most 3′ nucleotide compared with sequences from nonfunctional copies; the primers thus preferentially amplified the functional *CHEK2* on chromosome 22 rather than nonfunctional copies elsewhere in the genome[17]. PCR primers are available upon request. Every filter contained samples with (positive) and without (negative) *CHEK2**1100delC and was scored independently by at least three individuals. We confirmed all instances of the 1100delC mutation by PCR re-amplification from genomic DNA and direct forward and reverse sequencing of PCR products.

To validate the oligohybridization assay, we analyzed 209 samples by this assay and independently by heteroduplex analysis of a nested PCR product from a chromosome 22–specific template generated by long-distance PCR. Both methods identified 204 negatives and 5 positives (which were separately confirmed for each method by sequencing of newly amplified templates).

Analysis of microsatellite markers. For the genome-wide linkage search in EUR60, we amplified fluorescently labeled polymorphic microsatellite markers and electrophoresed the products on ABI377 DNA sequencers (Applied Biosystems). Gels were analyzed using the ABI Genescan and Genotyper software. In regions generating lod scores greater than –1, additional markers were end-labeled with [γ-^{32}P]ATP, electrophoresed on denaturing polyacrylamide gels and exposed to X-ray film. We analyzed more than 500 markers across the genome and calculated lod scores on the same basis as our previous breast cancer linkage analyses[1] using Vitesse. For analyses of *D22S275*, we typed individuals with *CHEK2**1100delC from families with and without *BRCA1* or *BRCA2* mutations, population-based breast cancer cases and controls. To assess the population frequency of the *D22S275* allele found in individuals with *CHEK2**del1100C, we typed 360 chromosomes of control individuals from the UK and 54 chromosomes from Dutch controls.

Statistical method. We evaluated differences in the prevalence of *CHEK2**1100delC in individuals with breast cancer by family type, adjusting for possible differences in population prevalence, using logistic regression with population-specific strata (UK, Netherlands, Germany, North America). As several affected individuals were tested in some families, we used a robust variance approach, implemented in Stata software (v. 7), to account for the dependence between individuals in the same family. We also carried out separate analyses of the prevalence among the index cases (one per family). For those families in whom several individuals had been tested, we defined the index case as the youngest individual with breast cancer who had been tested for both *CHEK2* and *BRCA1* or *BRCA2* mutations. In comparing families with and without *BRCA1* or *BRCA2* mutations, we excluded individuals in carrier families who did not have the disease-associated mutation. To assess the linkage of *CHEK2**1100delC with disease within families of variant-positive index cases, we computed the probability of each secondary case carrying the variant according to the formula $\psi/(\psi+2^r-1)$, where ψ is the risk ratio associated with the disease and r is the degree of relationship. We then constructed a test of the hypothesis that the segregation differed from chance (ψ=1) using a pseudo-likelihood approach, using a robust variance estimation to allow for dependence among relative pairs.

To estimate the risk of breast cancer associated with *CHEK2**1100delC, we carried out segregation analysis using the package MENDEL[18]. Parameters estimated were the *CHEK2* allele frequency in each population and the breast cancer risk ratio for *CHEK2* carriers relative to noncarriers. We computed risks to noncarriers of *CHEK2* mutations so that total risk averaged across all genotypes agreed with national age- and population-specific breast cancer incidence rates, as described in previous segregation analyses[19]. We carried out ascertainment correction by conditioning on the phenotypic and *BRCA1* or *BRCA2* genotypic data available for each pedigree. Because this model does not explicitly incorporate the effects of other susceptibility genes, it assumes implicitly that the effects of *CHEK2* and other genes conferring susceptibility can be regarded as independent, as in a multiplicative model. (For simplicity, we ignored the effect of *BRCA1* or *BRCA2* mutations in noncarrier families that were missed in the mutation screen. Under the assumption that *CHEK2**1100delC confers no risk in carriers of *BRCA1* or *BRCA2* mutations, this simplification would imply that our estimate of relative risk in noncarrier individuals is slightly biased towards one.) We evaluated goodness of fit of the models by computing the predicted *CHEK2* carrier probability for each tested affected individual, and thus comparing predicted frequency in different categories of family with the observed frequency. All analyses excluded family EUR60, in which the association was initially observed.

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Competing interests statement

The authors declare that they have no competing financial interests.

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