Germline *CHEK2* Mutations in Jewish Ashkenazi Women at High Risk for Breast Cancer

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Abstract

Background: Germline mutations in *BRCA1* and *BRCA2* genes account for only 20–40% of familial breast cancer cases. The *CHEK2* gene encodes a checkpoint kinase, involved in response to DNA damage, and hence is a candidate gene for breast cancer susceptibility. Indeed, the *CHEK2**1100delC truncating mutation was reported in a subset of mostly North European breast cancer families. The rate of the *CHEK2**1100delC variant in the Ashkenazi Jewish population was reported to be 0.3%.

Objectives: To evaluate whether *CHEK2* germline mutations contribute to a breast cancer predisposition in Ashkenazi** Jewish high risk families.

Methods: High risk Ashkenazi Jewish women, none of whom was a carrier of the predominant Jewish mutations in *BRCA1/BRCA2*, were genotyped for germline mutations in the *CHEK2* gene by exon-specific polymerase chain reaction followed by denaturing gradient gel electrophoresis and sequencing of abnormally migrating fragments.

Results: Overall, 172 high risk women were genotyped: 75 (43.6%) with breast cancer (average age at diagnosis 49.6 ± 9.6 years, mean ± SD) and 97 asymptomatic individuals (age at counseling 48.3 ± 8.2 years). No truncating mutations were noted and four previously described missense mutations were detected (R3W 1.2%, I157T 1.2%, R180C 0.6% and S428F 5%), one silent polymorphism (E84E 20.5%) and one novel missense mutation (Y424H 1.2%). Segregation analysis of the I157T and S428F mutations (shown to affect protein function) with the cancer phenotype showed concordance for the *CHK2**I157T mutation, as did two of three families with the *CHK2**S428F mutation.

Conclusions: *CHEK2* missense mutations may contribute to breast cancer susceptibility in Ashkenazi Jews.

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Familial clustering of breast cancer is noted in about 5–10% of diagnosed breast cancer cases [1]. Mutations in the *BRCA1* (MIM # 113705) and *BRCA2* (MIM # 600185) genes account for a subset of these familial inherited cases [2]. Founder mutations in both genes have been described in various ethnically isolated populations, e.g., the *BRCA2**999del5, a recurrent Icelandic mutation [3]. In the Ashkenazi Jewish population, three predominant mutations in *BRCA1* (185delAG, 5382InsC) and *BRCA2* (6174delT) are present in 2.5% of the population [4]. These mutations are most commonly encountered in high risk families, yet they account for only a minority of inherited familial breast cancer cases [5] and in the Ashkenazi Jewish population approximately 12% of breast cancers are attributable to mutations in the *BRCA1* or *BRCA2* gene [6]. Thus, familial clustering with an autosomal mode of transmission of breast cancer can only be partially accounted for by *BRCA* gene germline mutations. It seems plausible to assume that mutations in other genes confer genetic susceptibility to breast cancer [7]. One of the strong candidates for a "breast cancer susceptibility gene" is the *CHEK2* (MIM# 604373) gene. The plausibility of the *CHEK2* gene as a candidate breast cancer gene stems from several sources of evidence. The *CHEK2* gene encodes a checkpoint serine/threonine kinase and is the human homolog of *Saccharomyces cerevisiae Rad53* and S. *pombe Cds*1 [8]. The *CHEK2* protein participates in many cellular responses to DNA damage. Following DNA damage, the CHEK2 protein is activated by ATM and subsequently activates P53 and *BRCA1*, which in turn play a pivotal role in DNA damage repair and apoptosis [9]. An additional line of support for the putative role of *CHEK2* as a breast cancer gene is provided by the finding of a germline mutation within this gene (the *CHEK2**1100delC mutation) that confers a moderate breast cancer risk [10]. The *CHEK2**1100delC truncating mutation was reported primarily among North European families with breast cancer [11]. The rate of the *CHEK2**1100delC variant among Ashkenazi Jews both in Israel and New York was reported to be 0.3% [12,13]. The contribution of *CHEK2* to breast cancer susceptibility in the Ashkenazi Jewish population was further supported by the 2005 report of Shaag et al. [14]. They found an ancient *CHEK2* allele associated with breast cancer in this ethnic group of high risk patients. This allele co-segregated with breast cancer phenotype in Ashkenazi high risk families, conferred a twofold increase in breast cancer risk and contained the *CHEK2**S428F missense substitution [14]. This specific mutation was shown to adversely affect *CHEK2* function in a yeast complementation assay and is

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hence considered a pathogenic mutation [14]. To further assess the putative contribution of *CHEK2* germline mutations to breast cancer susceptibility, we genotyped the *CHEK2* gene in Ashkenazi Jewish women at high risk for breast cancer.

Patients and Methods

Patients were recruited from two sources: consecutive breast cancer patients and high risk families. Patients with breast cancer diagnosed and treated at Sheba Medical Center, Israel from 1 January 2002 to 31 December 2004 were eligible for participation. The study was approved by the local and national Institutional Review Board and each patient signed a written informed consent. Breast cancer patients who met the criteria for "high risk" (see below) were included in the study. Ethnic origin was attributed by country of birth of the patient and her parents and both sets of grandparents. Data were collected from medical records and pathological reports and, when needed, were complemented by a telephone interview within 6 months of the diagnosis. High risk families were recruited from individuals who were counseled at the Oncogenetics Unit of the same medical center and for the same period (1 January 2002 to 31 December 2004). The clinical and demographic details of these individuals were gathered during the initial counseling session, using a detailed questionnaire. The "high risk" criteria were:

- Breast and/or ovarian cancer patients with at least one other first-degree family member affected with breast and/or ovarian cancer, at any age
- Breast and/or ovarian cancer patients with at least two additional first or second-degree family members affected with breast and/or ovarian cancer, at any age
- Asymptomatic individuals with at least one first-degree family member and an additional first or second-degree family member affected with breast and/or ovarian cancer, at any age
- Breast cancer patients with at least three additional first or second-degree relatives with cancer, one being breast cancer.

Genetic analyses of DNA extraction

DNA was extracted from peripheral venous leukocytes by using the PUREGene DNA extraction kit (Gentra Inc., Minneapolis, MN, USA), according to the manufacturer's recommended protocol.

Genotyping for the predominant Jewish mutations in *BRCA1/BRCA2*

All study participants were genotyped for the three predominant Jewish mutations: 185delAG and 5382insC in *BRCA1*, and 6174delT in *BRCA2*. Mutation analysis schemes were based on polymerase chain reaction and restriction enzyme digests that distinguish the wild type from the mutant allele, as previously described [15]. For each of these three mutations, a known mutation carrier was used as a positive control in each experiment.

PCR = polymerase chain reaction
DGGE = denaturing gradient gel electrophoresis

PCR, DGGE, and sequence analysis of the *CHEK2* gene

Genotyping the *CHEK2* gene was carried out using flanking intronic primers. Since there are several known *CHEK2* psuedogenes, especially with a sequence identity to exons 10–14, we used the long-range PCR approach to co-amplify this fragment and used that as a template for nested PCRs [16]. PCR was performed in a 50 µl reaction, containing 50–100 ng genomic DNA, PCR buffer (Fisher Biotec, Australia), 2.5 mM MgCl2, 200nM dNTPs, 10 pmol of each primer and 0.2 U FB1 DNA Polymerase (Fisher Biotec). Amplification was carried out as follows: an initial denaturation step of 5 minutes at 94°C, followed by 35 cycles of 94°C for 20 seconds annealing step, which was different for each fragment, 72°C for 20 seconds, and a final extension step at 72°C for 5 minutes. Primer sequences and amplification conditions for each fragment are available from the authors upon request. The resulting PCR products were subjected to DGGE using the suitable gradient and running at 30 volts for 17 hours. All abnormally migrating fragments were subject to sequence analysis using the big Dye terminator chemistry and kit (PE Biosystems, Foster City, CA), and using the ABI Prism 310 semiautomatic DNA sequencer (PE Biosystems). Sequences were manually read and compared with the wild-type sequence of the *CHEK2* gene (NT011520 GenBank accession number).

Results

The study group comprised 172 Jewish Ashkenazi high risk women; 43.6% (n=75) were breast and/or ovarian cancer patients. The age range at diagnosis for patients with unilateral breast cancer (n=62) was 30–70 years (49.65 ± 9.6 years, mean ± SD). Five women (7%) had bilateral breast cancer; their age range at diagnosis of the first tumor was 36–60 years (46.2 ± 9 years). Five women (7%) had ovarian cancer; their age at the time of diagnosis was 40, 50, 53, 63 and 64 years (54 ± 9.9 years). Three women (4%) had both breast and ovarian cancers and their age at the time of diagnosis (in all cases breast cancer was diagnosed prior to ovarian cancer) was 44, 50 and 51 years (48.3 ± 8.3 years). The remainder (56.4%, n=97) were asymptomatic high risk women with an age range at the time of counseling of 25–71 years (48.3 ± 8.2 years). Of these, 69 women (71.1%) had a first-degree family member affected with breast cancer, 42 (43.3%) had a second-degree relative with breast cancer and 30 (31%) of the unaffected woman also had a third-degree relative with breast cancer. In addition, 33 women had relatives affected with ovarian cancer: 16 had a first-degree relative, 10 a second-degree relative and 9 women a third-degree family member.

BRCA1/2 genotyping data

None of the study participants was a carrier of any of the predominant Jewish mutations in *BRCA1* (185delAG 5382InsC) or *BRCA2* (6174delT).

CHEK2 genotyping results

In total, seven sequence variants of *CHEK2* in 52 cases were identified [Table 1]. Four previously described missense mutations were detected, R3W, I157T, R180C and S428F [Table 2 and Figures

Table 1. The sequence alterations detected in *CHEK2* and their rate in the studied population

Exon	Mutation	Protein change	No. of carriers	% of carriers
1	c7t	R3W	2 (1 homozygous)	1.2%
1	a252g	E84E	35 (1 homozygous)	20.5%
2*	IVS2+19 C>T		7	4.1%
			10	5%
3	t470c	I157T	2	1.2%
3	c538t	R180C	1	0.6%
11	t1270c	Y424H	2	1.2%
11*	c1283t	S428F	7	4.1%
			10	5%

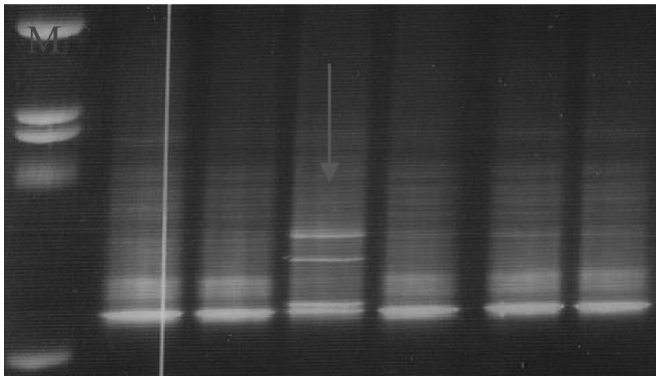

Figure 1. Detection of the *CHK2**S428F mutation by DGGE analysis. The mutant allele in marked by an arrow, M represents the DNA ladder marker.

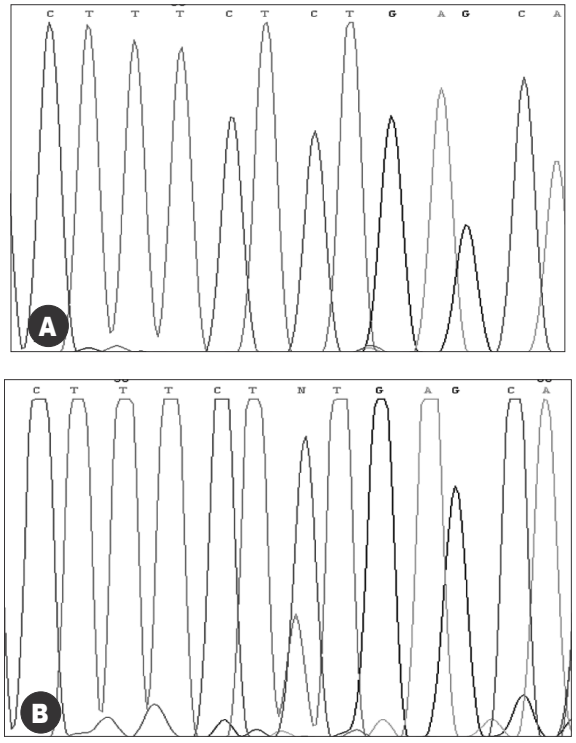

Figure 2. Identification of the *CHK2**S428F mutation by sequence analysis. **[A]** Wild-type allele, **[B]** Mutant allele.

1 and 2]. The missense variant, S428F, was identified initially in 7 women (4.1%). Twenty-eight additional unrelated high risk individuals were subsequently genotyped for that specific mutation (27 breast cancer patients, age range at diagnosis 33–79 years, 51.1 ± 9.2 years), and one ovarian cancer patient (diagnosed at age 61 years) and three additional mutation carriers were found, yielding a final carrier status of 5% (10/200).

All S428F carriers harbored another sequence alteration in intron 2, IVS2+19C>T. This substitution has no effect on the CHEK2 protein and therefore is probably a neutral polymorphism.

Co-segregation analyses of the *CHEK2**I157T and *CHEK2**S428F mutations [Figure 3]

- *CHEK2**I157T mutation: in pedigree 1 a mother and a daughter affected with breast cancer (42 and 30 years old, respectively) were both carriers. In pedigree 2, two half-sisters (same mother, different fathers) both carried the *CHEK2**I157T mutation; since both were carriers, their mother is an "obligate" carrier. One of the sisters had breast cancer (age 52 years) and the mother had colon cancer (age 62 years). Pedigrees 1 and 2 points toward *CHEK2**I157T involvement in breast cancer predisposition, in family number 2 there is a cluster of colon cancer that might also be attributed to *CHEK2**I157T.

- *CHEK2**S428F mutation: Of the 10 carriers, 2 were asymptomatic individuals at high risk for breast cancer and 8 were breast cancer patients (average age at diagnosis 50, range 41–57, median 51 years). In two of the three families (pedigrees 3 and 4) who were available for further examination, there was co-segregation of the variant with the cancer phenotype in all participating family members. However, in one family (pedigree 5), only one of two sisters who had breast cancer carried the *CHEK2**S428F variant. In family number 5 it is possible that the sisters' mother and aunt (both unavailable for testing) who had breast and ovarian cancer (age 63 and 60 years, respectively) were both carriers, and lack of co-segregation of *CHEK2**S428F with the cancer phenotype could be explained by another mutation in the family, originating from the paternal side.

Discussion

In the present study, none of the participants carried the *CHEK2**1100delC variant. The lack of *CHEK2**1100delC mutation carriers in this study and the paucity of this mutation in previous studies in Ashkenazi Jews [12,13] indicate minimal or no contribution of the *CHEK2**1100delC variant to inherited breast cancer in the Jewish Ashkenazi population. Overall, seven germline *CHEK2* sequence variants were noted among Jewish Ashkenazi individuals at high risk for breast cancer, but none of them leads to a truncated protein. Hence, defining the pathogenicty of these sequence variants and their putative contribution to breast cancer risk is not trivial as their effect on CHEK2 protein function has not been precisely determined. Therefore, inference to their pos-

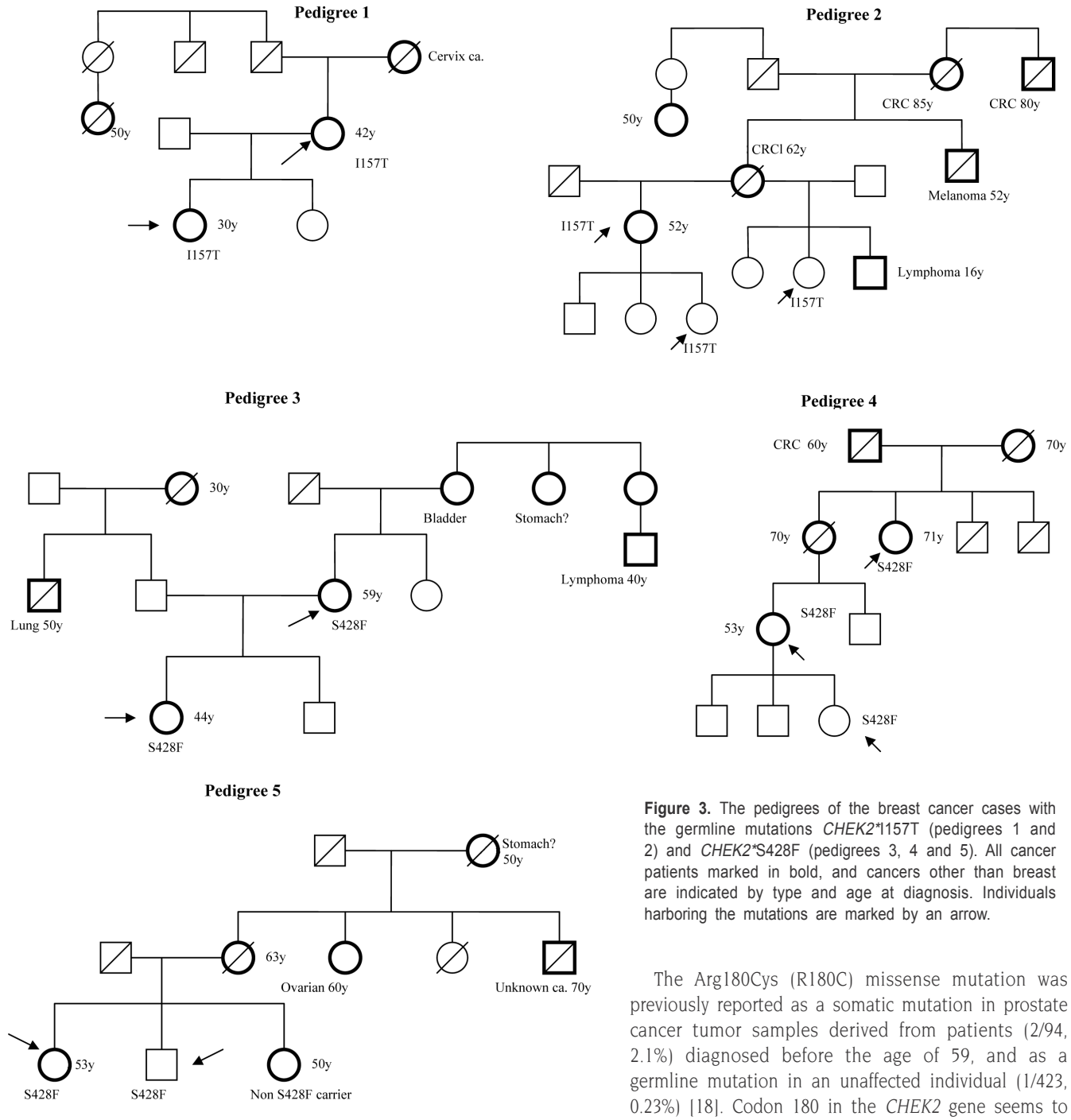

Figure 3. The pedigrees of the breast cancer cases with the germline mutations *CHEK2**I157T (pedigrees 1 and 2) and *CHEK2**S428F (pedigrees 3, 4 and 5). All cancer patients marked in bold, and cancers other than breast are indicated by type and age at diagnosis. Individuals harboring the mutations are marked by an arrow.

sible pathogenic effect is based on indirect lines of evidence and should be considered tentative at best.

The missense alteration Arg to Trp (*R3W*) was previously reported in a family with variant Li-Fraumeni syndrome [17]. Yet, functional studies failed to demonstrate any adverse effect of this mutation on kinase activity or protein turnover (t½) of this variant [17]. These functional analyses, together with the fact that this variant was noted in a homozygous state in one of the individuals in this study, in all likelihood indicate that this is a polymorphic variant.

The Arg180Cys (R180C) missense mutation was previously reported as a somatic mutation in prostate cancer tumor samples derived from patients (2/94, 2.1%) diagnosed before the age of 59, and as a germline mutation in an unaffected individual (1/423, 0.23%) [18]. Codon 180 in the *CHEK2* gene seems to be a mutational "hot spot," since another missense mutation was reported – Arg180His (R180H) – in an individual with sporadic prostate cancer (1/400, 0.25%) and in a bilateral breast cancer female patient who also carried the 1100delC variant [16]. Since no functional analyses evaluated the effects of this sequence variant on CHEK2 protein function, it is presently unclear whether this sequence variant is indeed pathogenic.

One novel missense mutation was identified in this study, Tyr424His (Y424H). This amino acid is located in the highly evolutionary conserved kinase domain of the CHEK2 protein. However, in the corresponding residue in the CHEK2 homo-

logue of S. *cerevisiae* (Rad53) histidine (and not tyrosine) is the amino acid. This fact alone suggests that *CHEK2**Y424H has no effect on the protein function and is merely a polymorphic variant.

Another missense mutation observed in the current study is the *CHEK2**I157T in the FHA domain, which is essential for CHEK2 activation [19]. This variant is common in North European countries and is detected in higher frequencies among breast cancer cases of north European descent, compared with unaffected ethnically matched controls: Poland (6.7% vs. 4.8%), Finland (7.4% vs.5.3%) and Germany (2.2% vs. 0.6%) [20,21]. In the present study the rate of *CHEK2**I157T was 1.2% (2/172) and seemed to segregate with the cancer phenotype in both families. Interestingly, in one family (pedigree 2, Figure 3) this variant appeared to be associated with colon cancer clustering diagnosed at a late age. Notably, among Polish non-Jews, this variant was detected in 9.3% (28/300) of colon cancer cases vs. 4.8% (193/4000) in the control population [20]. Several studies have demonstrated that the *CHEK2**I157T protein has a reduced kinase activity [22]; *CHEK2**I157T also fails to bind *BRCA1* [23] and P53 [24] and therefore does not phosphorylate them. Taken together, these observations suggest that this sequence variant is indeed pathogenic and is associated with breast and possibly colon cancer risk.

The most common *CHEK2* mutation identified in the current study was S428F. Functional evaluation of this variant also showed that it has an adverse effect on CHEK2 function, using yeast complementation assays [14]. This variant was first detected in a non-Hodgkin's aggressive lymphoma [25] and later reported as a mutation that confers a moderate risk (odds ratio 2.13, 95% confidence interval 1.26–3.69; $P = 0.004$) for developing breast cancer among Ashkenazi Jews [14]. In that study, the reported carrier frequencies of *CHEK2**S428F among sporadic Jewish Ashkenazi breast cancer patients was 2.88% (47/1632) compared with 1.37% (23/1673) in the control group [14]. In the present study the rate of *CHEK2**S428F was 5% (10/200) among familial high risk breast cancer patients. In two of the three families this variant co-segregated with the cancer phenotype, but in one it did not. This latter case could be an example of a phenocopy, and this lack of segregation does not rule out the *CHEK2**S428F mutation as a moderate breast cancer risk allele.

All *CHEK2**S428F carriers harbored another sequence alteration in intron 2, IVS2+19C>T. This substitution has no known effects on the CHEK2 protein and therefore is probably a neutral polymorphism. Co-segregation of *CHEK2**S428F with the intron 2 polymorphism suggests a shared haplotype in all mutation carriers, originating from a common ancestor. This finding further supports the notion of *CHEK2**S428F as an ancient mutation in the Ashkenazi Jewish population associated with conferring moderate breast cancer risk.

The limitations of the present study should be pointed out. This is a case-only study, focusing on an Ashkenazi Jewish high risk population from a single tertiary medical center in Israel, which may not be representative of the entire high risk population even in Israel. The evaluation of the putative pathogenicity of two of the missense variants detected herein (R180C and Y424H) is lacking and hence no assessment of these variants is presently feasible.

In conclusion, the *CHEK2**S428F is detected in about 5% of high risk Jewish Ashkenazi breast cancer families and confers an increased breast cancer risk in that population. The *CHEK2**I157T can also be detected in high risk families in this ethnic group and may be associated with a predisposition to breast and colon cancer. Further studies need to expand and elaborate on the putative contribution of these variants in Ashkenazi, non-Ashkenazi and ethnically diverse non-Jewish populations.

References

1. Antoniou A, Pharoah PDP, Narod S, et al. Average risks of breast and ovarian cancer associated with *BRCA1* or *BRCA2* mutations detected in case series unselected for family history: a combined analysis of 22 studies. *Am J Hum Genet* 2003;72:1117–30.
2. Anglian Breast Cancer Study Group. Prevalence and penetrance of *BRCA1* and *BRCA2* mutations in a population-based series of breast cancer cases. *Br J Cancer* 2000;83:1301–8.
3. Thorlacius S, Olafsdottir G, Tryggvadottir L, et al. A single *BRCA2* mutation in male and female breast cancer families from Iceland with varied cancer phenotypes. *Nat Genet* 1996;13:117–19.
4. Roa BB, Boyd AA, Volcik K, Richards CS. Ashkenazi Jewish population frequencies for common mutations in *BRCA1* and *BRCA2*. *Nat Genet* 1996;14:185–7.
5. Irving M, Elmslie F, Berg J. Genetics of breast cancer [Review]. *Int J Clin Pract* 2002;56:677–82.
6. Warner E, Foulkes W, Goodwin P, et al. Prevalence and penetrance of *BRCA1* and *BRCA2* gene mutations in unselected Ashkenazi Jewish women with breast cancer. *J Natl Cancer Inst* 1999;91:1241–7.
7. Thompson D, Easton D. The genetic epidemiology of breast cancer genes [Review]. *J Mammary Gland Biol Neoplasia* 2004;9:221–36.
8. Brown AL, Lee CH, Schwarz JK, Mitiku N, Piwnica-Worms H, Chung JH. A human Cds1-related kinase that functions downstream of ATM protein in the cellular response to DNA damage. *Cell Biol* 1999;96:3745–50.
9. Lee JS, Collins KM, Brown AL, Lee CH, Chung JH. hCds1-mediated phosphorylation of *BRCA1* regulates the DNA damage response. *Nature* 2000;404:201–4.
10. Oldenburg RA, Kroeze-Jansema K, Kraan J, et al. The *CHEK2**1100delC variant acts as a breast cancer risk modifier in non-*BRCA1/BRCA2* multiple-case families. *Cancer Res* 2003;63:8153–7.
11. Meijers-Heijboer H, van den Ouweland A, Klijn J, et al. *CHEK2* breast cancer consortium. Low penetrance susceptibility of breast cancer due to *CHEK2**1100delC in non-carriers of *BRCA1* or *BRCA2* mutations. *Nat Genet* 2002;31:55–9.
12. Offit K, Pierce H, Kirchhoff T, et al. Frequency of *CHEK2**1100delC in New York breast cancer cases and controls. BMC *Med Genet* 2003;4:1–4.
13. Ohayon T, Gal I, Baruch RG, Szabo C, Friedman E. *CHEK2** 1100delC and male breast cancer risk in Israel. *Int J Cancer* 2004; 108:479–81.
14. Shaag A, Walsh T, Renbaum P, et al. Functional and genomic approaches reveal an ancient *CHEK2* allele associated with breast cancer in the Ashkenazi Jewish population. *Hum Mol Genet* 2005;14:555–63.
15. Rohlfs EM, Learning WG, Friedman KJ, Couch FJ, Weber BL, Silverman LM. Direct detection of mutations in the breast and

ovarian cancer susceptibility gene *BRCA1* by PCR-mediated site-directed mutagenesis. *Clin Chem* 1997;43:24–9.
16. Sodha N, Houlston RS, Williams R, Yuille MA, Mangion J, Eeles RA. A robust method for detecting CHK2/RAD53 mutations in genomic DNA. *Hum Mutat* 2002;19:173–7.
17. Lee SB, Kim SH, Bell DW, et al. Destabilization of CHK2 by a missense mutation associated with Li-Fraumeni syndrome. *Cancer Res* 2001;61:8062–7.
18. Dong X, Wang L, Taniguchi K, et al. Mutations in *CHEK2* associated with prostate cancer risk. *Am J Hum Genet* 2003;72:270–80.
19. Lee CH, Chung JH. The hCds1 [Chk2]-FHA domain is essential for a chain of phosphorylation events on hCds1 that is induced by ionizing radiation. *Biol Chem* 2001;276:30537–41.
20. Cybulski C, Górski B, Huzarski T, et al. *CHEK2* is a multiorgan cancer susceptibility gene. *Am J Hum Genet* 2004;75:1131–5.
21. Allinen M, Huusko P, Mantyniemi S, Launonen V, Winqvist R. Mutation analysis of the *CHK2* gene in families with hereditary breast cancer. *Br J Cancer* 2001;85:209–12.
22. Falck J, Mailand N, Syljuasen RG, Bartek J, Lukas J. The ATM-Chk2-Cdc25A checkpoint pathway guards against radioresistant DNA synthesis. *Nature* 2001;410:842–7.
23. Li J, Williams BL, Haire LF, et al. Structural and functional versatility of the FHA domain in DNA-damage signaling by the tumor suppressor kinase Chk2. *Mol Cell* 2002;9:1045–4.
24. Falck J, Lukas C, Protopopova M, Lukas J, Selivanova G, Bartek J. Functional impact of concomitant versus alternative defects in the Chk2-p53 tumour suppressor pathway. *Oncogene* 2001;20:5503–10.
25. Tavor S, Takeuchi S, Tsukasaki K, et al. Analysis of the *CHK2* gene in lymphoid malignancies. *Leuk Lymphoma* 2001;42:517–20.

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