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REVIEW

The *CHEK2* gene and inherited breast cancer susceptibility

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Checkpoint kinase 2 (CHEK2, Chk2) emerges as an important signal transducer of cellular responses to DNA damage and a candidate tumor suppressor whose defects contribute to molecular pathogenesis of diverse types of human malignancies, both sporadic and hereditary. Here, we briefly outline the molecular properties, regulation and physiological role of CHEK2, and review in more detail its defects that predispose to tumors, with particular emphasis on familial breast cancer. The frequency, penetrance and epidemiological as well as clinical significance of the two most studied breast cancer-predisposing variants of the *CHEK2* gene, 1100delC and I157T, are highlighted in more depth, and additional *CHEK2* mutations and their cancer relevance are discussed as well. These recent findings are considered also from a broader perspective of CHEK2 as the integral component of the ataxia telangiectasia-mutated-CHEK2-p53 pathway within the genome integrity maintenance system and a barrier against tumor progression. Finally, the potential value of information about the *CHEK2* status in family counseling and optimizition of individualized cancer treatment is discussed.
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CHEK2 gene, Chk2 kinase and its physiological role

Checkpoint kinase 2 (CHEK2, Chk2), the protein product of the *CHEK2* gene that localizes to human chromosome 22q12.1, represents a phylogenetically conserved signaling component within the cellular network that responds to DNA damage and protects genomic integrity (Bartek *et al*., 2001). The human gene spans approximately 50 kilobases (kb) of genomic DNA and consists of 14 exons. The CHEK2 protein structure shows three characteristic domains: an N-terminal SQ/TQ cluster domain (amino-acid residues 20–75), a fork head-associated (FHA) domain (residues 112–175), and a serine/threonine kinase domain (residues 225–490). The SQ/TQ cluster is a regulatory domain containing seven serine or threonine residues followed by glutamine (SQ or TQ motifs), putative phosphorylation sites preferred by the ataxia telangiectasia-mutated (ATM) protein kinase that activates CHEK2 in response to ionizing radiation and other genotoxic insults that elicit DNA double-strand breaks (Bartek *et al*., 2001; Kastan and Bartek, 2004). The FHA domain is involved in binding to other phosphorylated proteins, particularly through recognition of phosphothreonine residues. This domain participates in dynamic protein–phosphoprotein interactions of CHEK2 during the activation and signaling of DNA damage, and it may also affect other functional regions of the CHEK2 kinase itself (Li *et al*., 2002). The catalytic kinase domain occupies almost the entire carboxy-terminal half of CHEK2, and it shows structural homology, including an activation loop, with other serine/threonine kinases.

Activation of the CHEK2 kinase in response to DNA damage is a multistep dynamic process (Bartek and Lukas, 2003; Ahn *et al*., 2004), initiated by rapid, ATM-mediated phosphorylations of several SQ/TQ sites, particularly threonine 68, in the N-terminal regulatory domain of CHEK2. This promotes homodimerization and intermolecular phosphorylation of CHEK2 on threonines 383 and 387 within the autoinhibitory loop, and serine 516 of the kinase domain, events that collectively lead to kinase activation towards heterologous substrates. At least the initial, ATM-mediated phosphorylation of CHEK2 occurs exclusively at the sites of DNA damage, after which the activated CHEK2 rapidly moves throughout the nucleoplasm to spread the alert signal from damaged DNA and target its substrates (Lukas *et al*., 2003).

The spectrum of currently known substrates of the CHEK2 kinase includes proteins involved in cell cycle control, such as the Cdc25A and Cdc25C phosphatases, Plk3 kinase and the E2F1 transcription factor, as well as in DNA repair, such as BRCA1, and regulation of cell death, including the p53-mdm2 interplay and PML1 (Bartek and Lukas, 2003; Ahn *et al*., 2004; Kastan and Bartek, 2004). This reflects the wide, and constantly broadening mediator role of CHEK2 in the signaling pathways that respond to DNA damage, with direct impact on downstream effectors within the cell cycle checkpoints, DNA repair and apoptosis machineries (Ahn *et al*., 2004; Bartek *et al*., 2004; Lukas *et al*., 2004). This is perhaps best documented by cellular phenotypes

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of cells deficient in CHEK2 function. Such cells fail to delay their DNA replication when irradiated, and hence display the phenomenon known as radiation-resistant DNA synthesis (Falck *et al*., 2001b). Furthermore, defects in several cell cycle checkpoints, as well as in DNA repair and particularly in DNA-damage induced apoptosis are evident in such cells, while resistance to cell death in various tissues dominates the phenotype of *CHEK2* gene knockout mice (reviewed in Bartek and Lukas, 2003). Most relevant to the topic of this review, CHEK2 emerges as a candidate tumor suppressor (Bartek and Lukas, 2003; Kastan and Bartek, 2004), and germline defects of *CHEK2* predispose to familial breast cancer and some other types of malignancies, and somatic mutations of *CHEK2* have been implicated in pathogenesis of various types of sporadic tumors. The molecular basis, epidemiological aspects, biological as well as clinical significance of these cancer-related defects of *CHEK2* are highlighted in the following sections of this paper.

CHEK2 and inherited breast cancer susceptibility

The *CHEK2* genetic variation in inherited cancer susceptibility was first indicated in 1999 when Bell *et al*. (1999) discovered three *CHEK2* germline mutations among four classical Li-Fraumeni and 18 Li-Fraumeni-like families, suggesting that *CHEK2* gene could be a new predisposition gene to Li-Fraumeni syndrome. While one of the three identified variants was found to be a polymorphism residing on a homologous sequence on chromosome 15 (Sodha *et al*., 2000), two others, the 1100delC deletion in the kinase domain in exon 10 and the 470T > C (I157T) missense mutation in the FHA domain in exon 3, have since been widely studied for inherited susceptibility to breast as well as other cancers, and have turned a new page in our understanding and studies of the genetic background of familial breast cancer.

CHEK2 in Li-Fraumeni syndrome

The role of *CHEK2* as high-penetrance predisposition gene to Li-Fraumeni syndrome was questioned when the 1100delC and I157T *CHEK2* germline variants were identified among breast cancer patients and in the healthy population. The I157T variant was found among familial and unselected breast cancer patients as well as breast cancer patients from LFS/LSL families but also in 2.1–6.5% of healthy Finnish population controls (Allinen *et al*., 2001; Vahteristo *et al*., 2001). The 1100delC mutation was also discovered in two breast cancer patients with a cancer family history not typical of LFS or LFL (Vahteristo *et al*., 2001). Screening of LFS or LFL families (Bougeard *et al*., 2001; Lee *et al*., 2001; Vahteristo *et al*., 2001; Sodha *et al*., 2002b) has revealed no or very rare individual missense variants in the *CHEK2* gene and also the 1100delC variant has been found rare among LFS/LFL patients (Siddiqui *et al*., 2005). Although some of the variants identified in LFS/LFL families were also shown to have functional effect on CHEK2 (Lee *et al*., 2001) the evidence from different studies suggests that *CHEK2* is not a predisposition gene to Li-Fraumeni syndrome. Recently, by a genome-wide scan for linkage, Bachinski *et al*. (2005) identified a region of approximately 4 cM in chromosome 1q23 as a candidate locus in Li-Fraumeni kindreds. The possible role of *CHEK2* genetic variants as modifiers of cancer risk in Li-Fraumeni families is still an open question.

1100delC – a low-penetrance breast cancer predisposition allele

Based on segregation analysis of data from both a population-based series of breast cancer cases and high-risk families in the UK Antoniou *et al*. (2002) suggested that several common, low-penetrance genes with multiplicative effects on risk may account for the residual non-BRCA1/2 familial aggregation of breast cancer. Association analysis of the 11000delC allele in two large studies of familial breast cancer patients and populations controls found the 1100delC variant to be the first example of a population variant with association to familial breast cancer and a moderate risk of breast cancer. Based on linkage to chromosome 22, Meijers-Heijboer *et al*. (2002) identified the 1100delC variant in a large breast cancer pedigree and further found the variant in 5.1% of individuals with breast cancer from 718 Western-European and North-American families without *BRCA1* or *BRCA2* mutations and in 1.1% in healthy individuals. They estimated that the 1100delC variant results in an approximately twofold increase of breast cancer risk in women, accounting for about 1% of all female breast cancer. Carriers of the 11000delC allele from the populations studied shared the same allele at the polymorphic marker locus D22S275 within *CHEK2* gene suggesting a common ancient origin for the mutation. Vahteristo *et al*. (2001) previously found the 1100delC variant in two Finnish breast cancer patients with a cancer family history, and further analysis of 507 patients with familial breast cancer with no *BRCA1* and *BRCA2* mutations revealed a significantly elevated frequency of 1100delC, as compared with the 1.4% frequency in the Finnish population (Vahteristo *et al*., 2002). A high frequency was also seen in patients with only a single affected first-degree relative (6.2%). The variant did not segregate with cancer in the breast cancer pedigrees in either study, also suggesting a lower-penetrance effect and the presence of other breast cancer susceptibility alleles in the families.

A wide variation in the population frequency of 1100delC has been observed in different populations but the variant has been relatively rare in all populations studied. Highest population frequencies have been found in the Netherlands (1.3–1.6%) and in Finland (1.1–1.4%), and lower frequencies in the UK (0.35–0.5%), Germany (0.15–0.25%), Australia (0.14%) (The *CHEK2* Breast Cancer Case–Control Consortium, 2004), Sweden (0.6–1.0%, Einarsdottir *et al*., 2006; Wagenius *et al*., 2006), Poland (0.2–0.25%%, Cybulski

et al., 2004a, b), Czech Republic (0.3%, Kleibl *et al*., 2005), Italy (0.11%, Caligo *et al*., 2004), USA (0.3–0.4%, Offit *et al*., 2003; Friedrichsen *et al*., 2004) and Canada (0.2%, Bernstein *et al*., 2006); the variant has not been detected in the Spanish population (Osorio *et al*., 2004).

1100delC and breast cancer risk

The *CHEK2* Breast Cancer Case–Control Consortium (2004) studied the 1100delC allele in 10 860 breast cancer cases and 9065 controls from 10 case–control studies in five countries and found the variant in 1.9% of all cases and 0.7% of controls (OR 2.34; 95% CI 1.72–3.20). This large study confirmed that the 1100delC allele confers about twofold elevated breast cancer risk that is seen also in women unselected for family history. A trend for a higher breast cancer odds ratio was also seen for earlier ages of onset (The *CHEK2* Breast Cancer Case–Control Consortium, 2004) and carriers in breast cancer families develop breast cancer at an earlier age than noncarriers (Oldenburg *et al*., 2003). A higher frequency has also been observed among cases with an affected first-degree relative (Meijers-Heijboer *et al*., 2002; Vahteristo *et al*., 2002; Oldenburg *et al*., 2003; The *CHEK2* Breast Cancer Case–Control Consortium, 2004), with an increasing frequency with a higher number of affected first-degree relatives (Meijers-Heijboer *et al*., 2002; Oldenburg *et al*., 2003; The *CHEK2* Breast Cancer Case–Control Consortium, 2004; de Jong *et al*., 2005a). The results were consistent with the hypothesis of a multiplicative effect on breast cancer risk, 1100delC allele acting in concert with susceptibility allele(s) in other genes to increase the risk of breast cancer, in accordance with the model suggested by Antoniou *et al*. (2002). Based on population frequency in the UK (0.5%), the absolute cumulative risk of breast cancer was estimated to be 13.7% for carriers by the age of 70 years (as compared to 6.1% for noncarriers), with 0.7% of breast cancer and 0.5% of the excess familial risk being attributable to this variant in the UK population (*CHEK2* Breast Cancer Case–Control Consortium, 2004).

Johnson *et al*. (2005) estimated a 23.8% cumulative risk for female breast cancer by the age of 80 years for first-degree relatives of bilateral 1100delC carriers, with a higher risk estimated for those first-degree relatives also carrying the 1100delC allele (58.8% by age 80 years, compared with expected cumulative risk of 7.9%). They suggested that the 1100delC carriers with a bilaterally affected first-degree relative have a highly penetrant lifetime risk even of a similar order as that seen in *BRCA1* and *BRCA2* carriers, although larger patient series are needed to define the risk more precisely (Johnson *et al*., 2005).

The carriers of 1100delC allele also have an elevated risk for bilateral breast cancer. Vahteristo *et al*. (2002) found bilateral breast cancer patients to be sixfold more likely to be 1100delC carriers than patients with unilateral cancer among an unselected, population-based series of 1035 breast cancer patients. A similar excess among breast cancer cases with second primary contralateral breast cancer was reported by Broeks *et al*. (2004). However, no higher frequency of 1100delC was observed among familial breast cancer patients with family history of bilateral disease studied by Dufault *et al*. (2004). In their study, the carrier status for the bilateral cases was not known, however. de Bock *et al*. (2004) estimated that patients with the 1100delC variant had a more unfavorable prognosis regarding the occurrence of contralateral breast cancer (RR 5.74; 95% CI 1.67–19.65), distant metastasis-free survival (RR = 2.81; 95% CI 1.20–6.58), and disease-free survival (RR = 3.86; 95% CI 1.91–7.78) while not for overall survival by about 4 years follow-up time. In their analysis, Broeks *et al*. (2004) detected the highest percentage of carriers among those patients who had received radiation therapy for their first breast cancer, suggesting that ionizing radiation treatment might be a risk factor for breast cancer development among 1100delC carriers. The 1100delC carrier status was also associated with exposure to diagnostic ionizing radiation (excluding mammography) in a population-based study of 2311 female breast cancer cases and 496 general population controls enrolled in the Ontario and Northern California Breast Cancer Family Registries. The association was strongest among Caucasian women aged >45 years who were exposed >15 years before breast cancer diagnosis (OR, 4.28; 95% CI, 1.50–12.20) (Bernstein *et al*., 2006). The suggested association between ionizing radiation and breast cancer risk substantiates *CHEK2* gene's role as a checkpoint gene for IR-induced DNA damage. The association with radiation treatment of breast cancer patients, or other IR exposure, with breast cancer risk may thus have clinical significance for management and follow-up of breast cancer patients and the effect of ionizing radiation on breast cancer risk for *CHEK2* mutation carriers warrants further studies.

An elevated risk for male breast cancer for 1100delC carriers has also been suggested. Meijers-Heijboer *et al*. (2002) detected the 1100delC variant in 13.5% of individuals from families with male breast cancer, suggesting a 10-fold increase of risk in men and accounting for as much as 9% of all male breast cancer in the population. This association has not been replicated, however. In two population-based series of 109 US and 79 UK male breast cancer cases, none were found to carry the 1100delC allele while 1/138 US and 20/3749 UK controls were carriers (Neuhausen *et al*., 2004). In a population-based material of 114 Finnish male breast cancer patients, the mutation frequency among male breast cancer cases (1.8%) was similar to that seen in population controls (1.4%) (Syrjäkoski *et al*., 2004). Sodha *et al*. (2004) found the 1100delC in one out of 26 familial male breast cancer cases, and no other *CHEK2* mutations were identified in these cases either. Although the number of cases have been relatively small in these studies, it is likely that a risk for male breast cancer, if any, is substantially smaller

than initially suggested and the 1100delC mutation does not explain the familial aggregation of male breast cancer or male breast cancer risk in the population.

Other CHEK2 variants and breast cancer risk

Also other *CHEK2* variants have been associated with breast cancer risk. The I157T (470T > C) variant in the FHA domain in exon 3 has been found associated with breast cancer but appears to confer a lower risk than the 1100delC allele. Kilpivaara *et al*., 2004 reported 5.5% population frequency in Finland but a significantly higher frequency among unselected breast cancer patients (7.4%). They estimated that absolute risk of breast cancer in carriers would be 8.1% by age 70 years, compared with 5.5% for noncarriers, based on incidence rates in Finland. Similar frequencies have also been found in Poland (6.7% in cases vs 4.8% in controls) (Cybulski *et al*., 2004b) and among German (2.2% in cases vs 0.6% in controls) and Byelo-Russian patients (5.7% in cases vs 1.3% in controls) (Bogdanova *et al*., 2005). The estimated fraction of breast cancer attributable to this variant is also quite similar in these populations, with 2.2% in Finland, 1.9% in Poland and 1.6% in the German and 4.3% in the Byelorussian populations. In other populations studied, however, the I157T variant has been more rare. In the US, the I157T variant has been reported in 1.2% of (male) population (Dong *et al*., 2003). Among familial non-BRCA1/2 breast cancer patients in the UK, North America and the Netherlands, the variant was found only in 0.27% of patients and in 0.14% of controls, suggesting a negligible contribution in these populations (Schutte *et al*., 2003). Overall, lower risks have been observed for I157T than 1100delC and while I157T has been associated with breast cancer risk in the population, it has not exhibited a significant association with familial aggregation of breast cancer in the Finnish or German populations (Dufault *et al*., 2004; Kilpivaara *et al*., 2004; Bogdanova *et al*., 2005). However, among the Byelorussian population, a significantly elevated risk was observed among familial cases as well (Bogdanova *et al*., 2005), possibly suggesting the presence of other, perhaps population-specific susceptibility alleles, if confirmed in further studies.

Both the 1100delC as well as I157T allele show thus variation in population frequencies and even some population specificity, and also other, *CHEK2* founder alleles have been identified. The IVS2 + 1 G > A splicing mutation, found in a US patient with familial prostate cancer (Dong *et al*., 2003), has been found in the Polish population as a founder mutation with a 0.3% population frequency (Cybulski *et al*., 2004a), and has been detected also in German (0–0.4%, Dufault *et al*., 2004; Bogdanova *et al*., 2005) and Byelorussian populations (0.2%, Bogdanova *et al*., 2005). The allele associates possibly with a 2- to 4-fold elevated risk for breast cancer (Cybulski *et al*., 2004a; Bogdanova *et al*., 2005), while in another study on German familial breast cancer patients the variant was found only in 2/516 familial cases and 2/500 population controls (Dufault *et al*., 2004). Owing to the rarity of this allele, very large patient cohorts will be needed to evaluate the associated risk reliably.

In the Ashkenazi Jewish population, Shaag *et al*. (2005) identified *CHEK2* haplotypes in 30 high-risk families and discovered two novel amino-acid substitutions, S428F (1283C > T) in the kinase domain in exon 11 and P85L (254C > T) in the N-terminal region (exon 1). The S428 is a conserved amino acid and the human variant protein was found unable to complement the lethality of the yeast ortholog Rad53 deletion in *Saccharomyces cerevisiae*, while the wild type (wt)-CHEK2 and CHEK2-P85L did so. The S428F variant had a 1.37% frequency among 1673 controls and 2.88% among 1632 breast cancer patients. By also comparing the cancer incidence among mothers, sisters and daughters of the S428F carriers about twofold elevated risk was estimated for carriers of this CHEK2 allele. However, frequencies of P85L did not differ between cases (0.92%) and controls (0.85%). Neither variant was found among 768 American breast cancer patients of various non-Jewish ancestries, suggesting both are founder alleles specific for the Ashkenazi population.

Walsh *et al*. (2006) searched for large genomic rearrangements in *CHEK2* in a series of 300 high-risk breast cancer families with four or more cases of breast or ovarian cancer. They discovered a novel 5.6-kb genomic deletion, leading to loss of exons 9 and 10 and predicted protein truncation at codon 381 in two families of Czechoslovakian ancestry. This founder mutation was further found in 1.3% of 631 patients with breast cancer and in none of 367 healthy controls in the Czech and Slovak Republics. Further analyses for genomic rearrangements may reveal additional *CHEK2* mutations in other populations as well.

Other, rare variants have been found in other studies as well (Ingvarsson *et al*., 2002; Sodha *et al*., 2002a; Schutte *et al*., 2003; Dufault *et al*., 2004). However, without functional evidence for the missense variants and epidemiological analyses with sufficient statistical power the significance of such variants for breast cancer risk remains uncertain. Overall, they are likely to have small contribution to familial breast cancer predisposition or breast cancer risk in the population. Analyses of common SNPs and haplotype-tagging based approaches have found no increased risk for breast cancer or effect on survival associated with common genetic variation in the *CHEK2* gene (Goode *et al*., 2002; Kuschel *et al*., 2003; Einarsdottir *et al*., 2006).

Risk for other cancers

Unlike *BRCA1* and *BRCA2* mutations, *CHEK2* variants do not appear to cause elevated risk for ovarian cancer. The frequency of 1100delC in breast–ovarian cancer families has not been found elevated as compared to breast cancer families (Meijers-Heijboer *et al*., 2002;

Vahteristo *et al*., 2002), and the frequencies of 1100delC allele, I157T or IVS2 + 1 G > A among ovarian cancer patients do not differ from that among controls (Baysal *et al*., 2004; Cybulski *et al*., 2004b). The S428F variant was slightly more frequent among Ashkenazi Jewish breast cancer patients with family history of ovarian cancer than with family history of breast cancer but no significantly elevated risk was observed among ovarian cancer probands (Shaag *et al*., 2005).

CHEK2 variants associate with elevated prostate cancer risk, however (Cybulski *et al*., 2004a, b; Dong *et al*., 2003; Seppälä *et al*., 2003). Despite variation in the frequencies in different populations and lack of sufficient statistical power for such rare variants in different studies, the findings have been consistent with an elevated risk for familial as well as unselected prostate cancer associated with the truncating mutations (1100delC and IVS2 + 1 G > A) as well as the missense variant I157T. The observed risk has been highest for the truncating variants 1100delC and IVS2 + 1 G > A (with 8- to 9-fold elevated odds ratios for hereditary or familial prostate cancer, as compared to population controls) among the Polish and Finnish populations whereas the I157T allele seems to confer a more modest risk (2- to 4-fold elevated odds ratios for hereditary or familial prostate cancer and 1.5- to 1.7-fold elevated for unselected prostate cancer) (Cybulski *et al*., 2004a, b; Seppälä *et al*., 2003). However, rarity of the variants in different populations limits their contribution to prostate cancer as well (Wagenius *et al*., 2006). Also other, very rare variants have been identified in prostate cancer patient samples with a higher overall frequency among patients than among unaffected men (Dong *et al*., 2003). As in breast cancer, however, such rare, individual variants are likely to have small contribution to prostate cancer.

Colorectal cancer (CRC) risk associated with *CHEK2* variants has been more controversial. Among Dutch breast cancer families, the 1100delC variant was found more frequently in families with hereditary breast and colorectal cancer phenotype (HBCC) and Meijers-Heijboer *et al*. (2003) suggested that the 1100delC allele acts in synergy with another, unknown susceptibility gene for HBCC. However, no such association was observed among Finnish colorectal or breast cancer families (Kilpivaara *et al*., 2003) and the 1100delC allele was also found rare among patients with metachronous cancers of the breast and the colorectum (Isinger *et al*., 2006). The 1100delC allele (or truncating alleles 1100delC and IVS2 + 1G > A) has not been found significantly associated with CRC risk in population-based series of 662 Finnish CRC patients (Kilpivaara *et al*., 2003), 300 Polish colon cancer patients (Cybulski *et al*., 2004b) or 629 unselected Dutch CRC cases and 105 CRCs diagnosed before age 50 (de Jong *et al*., 2005b), or with colorectal disease (Lipton *et al*., 2003). A very low-risk effect cannot be excluded, however, and de Jong *et al*. (2005b) obtained also some evidence for an increasing frequency after stratifying patients according to age at diagnosis and family history of colorectal and endometrial cancer. Large case–control studies or meta-analyses are required to clarify the role of the *CHEK2* 1100delC variant for CRC risk. There is more consistent evidence for elevated CRC risk for the I157T variant, however, with a significant association found among both Polish colon cancer patients (Cybulski *et al*., 2004b) and Finnish CRC cases (Kilpivaara *et al*., 2006). Both the truncating variants and I157T have been also found to associate with thyroid cancer and I157T with kidney cancer as well (Cybulski *et al*., 2004b), suggesting that functional disturbance of CHEK2 predisposes cells from a wide distribution of organs for tumorigenic development. For many other cancer sites, the studies have been too small to estimate cancer risks, however, *CHEK2* variants have been infrequent or no significant association has been found so far with cancers of the bladder, larynx, lung, pancreas or stomach or with melanoma, Non-Hodgkin lymphoma, myelodysplastic syndromes and acute myeloid leukemias. (Hofmann *et al*., 2001; Cybulski *et al*., 2004b). The rarity of the variants in many populations limits the possibilities to reliably estimate cancer risks especially for more rare cancer types in large enough patient series.

Tumors from *CHEK2* mutation carriers – *CHEK2* in tumors

The CHEK2 protein expression has been found absent or grossly reduced in tumors from carriers of the 1100delC germline mutation and also in other familial or sporadic breast tumors (Sullivan *et al*., 2002; Vahteristo *et al*., 2002; Oldenburg *et al*., 2003; Kilpivaara *et al*., 2004; Honrado *et al*., 2005). Grossly reduced or absent CHEK2 protein expression has been observed in 80–100% of tumors from the 1100delC carriers (Vahteristo *et al*., 2002; Oldenburg *et al*., 2003) but in only 4–14% familial breast tumors from noncarriers (Vahteristo *et al*., 2002; Oldenburg *et al*., 2003). On the other hand, the 1100delC germline variant was seen in 19% (4/21) of cases with absent or reduced expression of the CHEK2 protein, whereas none of the 103 cases with normal CHEK2 staining pattern showed mutations (Vahteristo *et al*., 2002).

The CHEK2-1100delC protein is unstable (Vahteristo *et al*., 2002) and as indicated above, the 1100delC mutation is most often associated with complete loss of the protein expression (Vahteristo *et al*., 2002; Oldenburg *et al*., 2003). In cancers from the 1100delC carriers, as well as from carriers of some other *CHEK2* mutations, loss of heterozygosity (LOH) has been found in some tumors but the results have not been consistent and in some tumors, the mutant allele has been lost (Lee *et al*., 2001; Ingvarsson *et al*., 2002; Sodha *et al*., 2002a; Oldenburg *et al*., 2003). Oldenburg *et al*. (2003) detected LOH in *CHEK2* region in 11/88 familial breast tumors with all three 1100delC carrier tumors showing loss of the wt allele. However, while *CHEK2* LOH was associated with loss or reduction of protein expression in all the tumors studied, 1100delC was not significantly more frequent in tumors showing LOH at the *CHEK2* locus, which suggests that there may be also other mechanisms of inactivation. Methylation of CHEK2 has

not been observed in breast carcinomas with down-regulation of *CHEK2* mRNA expression (Sullivan *et al*., 2002), however, epigenetic silencing of *CHEK2* gene has been observed in Non-Hodgkin's lymphoma cell lines with drastic reduction of CHEK2 expression but no identified mutations (Kato *et al*., 2004). In breast cancer, Staalesen *et al*. (2004) has detected a very large number of tumor-specific splice variants, in addition to normal length mRNA, in all stage III breast tumors studied. They suggested that extensive splicing variation of *CHEK2* mRNA in breast tumors could be such a mechanisms, where splice variants may lack CHEK2 function or be mislocalized in cytoplasm.

Among unselected patients, tumors from 1100delC mutation carriers have been found more often to be hormone receptor positive (de Bock *et al*., 2004; Kilpivaara *et al*., 2005), and in one study, also of higher grade than those from noncarriers (Kilpivaara *et al*., 2005). As the 1100deC mutation is rare, very large materials would be needed to reliably evaluate specific phenotypic characteristics associated with the 1100delC mutation. Genome-level molecular analyses like gene expression profiling or array-CGH analyses may reveal more distinct tumor characteristics and functional downstream effects of the 1100delC allele or other *CHEK2* mutations. Such studies could also shed some light on the genes or pathways behind the postulated multiplicative effects on breast cancer risk with the *CHEK2* gene.

The I157T variant protein is stable and the mutation has a more subtle effect on CHEK2 function than the 1100delC variant. No difference in CHEK2 protein expression has been observed on breast tumors from I157T carriers vs noncarriers (Kilpivaara *et al*., 2004). Interestingly, Huzarski *et al*. (2005) suggested a strong association of I157T allele with lobular breast cancer among 482 breast cancer patients in Poland. However, no difference in the histological type (lobular or ductal breast cancer) has been observed among Finnish breast cancer patients and I157T variant was not found associated with other histopathological characteristics either (Kilpivaara *et al*., unpublished results).

Among unselected breast tumors, the reduction of CHEK2 protein expression has been observed in 21.1% (Kilpivaara *et al*., 2005). The reduced expression correlated with larger primary tumor size but not with other histopathologial characteristics. However, 94% of tumors with aberrant CHEK2 expression were ER positive, as compared to 79% among all tumors, similarly as seen among 1100delC mutation carrier tumors. Interestingly, Honrado *et al*. (2005) found reduced nuclear CHEK2 expression in 72–78% of sporadic or familial breast tumors but a significantly higher frequency of expression among *BRCA2* carriers, and suggested that CHEK2 immunohistochemical analysis, together with RAD51 expression analysis, could distinguish *BRCA2* carrier tumors from non-*BRCA1/2* tumors.

Loss of CHEK2 expression has been observed also in other malignancies. Five percent (29/564) of familial colorectal tumors demonstrated loss of expression for CHEK2, with germline 1100delC mutation found in three cases. No 1100delC mutations were found in patients whose tumors stained positive (van Puijenbroek *et al*., 2005). A low frequency (17.6%) of the loss of the wt allele has been observed in the CRC tumors of the 1100delC carriers, with also loss of the mutant allele observed (Kilpivaara *et al*., 2003).

Somatic mutations have been relatively rare in *CHEK2*, detected in various types of cancer, such as in some breast tumors (Sullivan *et al*., 2002), osteosarcomas, ovarian and lung cancers (Miller *et al*., 2002), bladder cancer (Bartkova *et al*., 2004, 2005), and vulval squamous cell carcinomas (Reddy *et al*., 2002) (reviewed in Bartek and Lukas, 2003).

Molecular and biological basis for cancer susceptibility due to *CHEK2* mutations

As an integral component of the cellular network that responds to DNA damage the CHEK2 kinase helps maintain genomic integrity and prevent fixation of potentially harmful mutations (Kastan and Bartek, 2004). Accumulating evidence strongly suggests that CHEK2 is a tumor suppressor and its defects can predispose to several types of cancer. At the molecular level, *CHEK2* defects lead to either loss of CHEK2 expression, or undermine the function of CHEK2 as a signaling molecule. Mutations in the SQ/TQ regulatory domain prevent proper activation of CHEK2 by the upstream kinase ATM, alterations of the FHA domain, including the I157T breast cancer predisposing variant subvert the protein–protein interactions and cancel proper interactions of such CHEK2 protein with its substrates including BRCA1, p53 and Cdc25A, (Li *et al*., 2002; Falck *et al*., 2001a,b) while defects in the kinase domain inhibit the catalytic activity of CHEK2. The latter category includes also the truncated protein resulting from the breast cancer susceptibility variant 1100delC. In addition, this truncated protein, as well as some other mutants of CHEK2, are labile proteins whose level in cancer cells is very low (Bartek and Lukas, 2003). Other types of CHEK2 defects lead to mislocalization of CHEK2 in the cytoplasm (Staalesen *et al*., 2004), or aberrant splicing of the *CHEK2* gene (Bartkova *et al*., 2005).

From the tissue biology point of view, the CHEK2 kinase, unlike its related kinase Chk1, is expressed in the vast majority of human normal tissues including many nonproliferating, terminally differentiated cells (Lukas *et al*, 2001; Latella *et al*., 2004). Thus, the ATM-CHEK2-p53 pathway operates in almost all cell types of the adult organism. Importantly, recent studies discovered constitutive activation of this pathway in a wide range of human tumors (DiTullio *et al*., 2002), including large subsets of premalignant lesions (Bartkova *et al*., 2005; Gorgoulis *et al*., 2005), suggesting that the process of oncogenic transformation leads to enhanced DNA damage and activates the checkpoint network as an inducible barrier against cancer progression

(Bartkova *et al*., 2005; Gorgoulis *et al*., 2005). It follows that individuals with germline mutations in genes involved in this anticancer barrier, such as *CHEK2*, *p53*, *BRCA1*, *BRCA2* or *ATM* for example, would be deficient in their natural protection against cancer, and therefore more susceptible to cancer development. This is also consistent with the fact that all familial breast cancer-predisposing genes identified to date are components of the genome maintenance machinery that responds to DNA damage. Furthermore, it makes a prediction that also other, so far unknown breast cancer-predisposing mutations likely target components of the DNA damage response network.

Finally, as the status of the DNA damage response machinery also dictates the cellular response to DNA damaging therapies such as ionizing radiation or many chemotherapeutic drugs, it seems plausible to speculate that knowledge about the status of this cellular network may help select appropriate, taylor-made combinations and doses of such anticancer treatment modalities in the future. This trend is further underscored by global efforts to develop small molecule modulators of various components of the DNA damage response, including the Chk1 and CHEK2 kinases (Zhou and Bartek, 2004), in an effort to predispose cancer cells selectively to such DNA damaging therapies.

Currently, the low risks associated with *CHEK2* variants should still be interpreted with caution in the context of clinical management of breast cancer families, and diagnostic testing and counseling for these variants is in general considered premature. Defining the interactive networks of genes and genetic variants behind the synergistic risk effects remains a major challenge for more accurate evaluation of breast cancer risks associated with *CHEK2* variants at individual level or with specific subgroups of patients, and perhaps even in different populations. Increased risk for contralateral breast cancer, and the possibility of ionizing radiation as a risk factor for breast cancer especially among *CHEK2* variant carriers, warrants also more extensive studies and may have more immediate implications for the clinical management of breast cancer patients.

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