Helsinki University Biomedical Dissertations No. 89

CHEK2 in Breast and Colorectal Cancer

Outi Kilpivaara

Department of Obstetrics and Gynecology
Helsinki University Central Hospital
University of Helsinki
Helsinki, Finland

ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Medicine of the University of Helsinki, for public examination in lecture hall 3, Biomedicum Helsinki, Haartmaninkatu 8, Helsinki,
on June 1st, 2007, at 12 noon.

Helsinki 2007

Supervised by

Docent Heli Nevanlinna

Department of Obstetrics and Gynecology

Helsinki University Central Hospital

University of Helsinki

Reviewed by

Professor Seppo Pyrhönen

Department of Oncology and Radiotherapy

Turku University Hospital

University of Turku

and

Docent Johanna Schleutker

Institute of Medical Technology

Tampere University Hospital

University of Tampere

Official Opponent

Docent Virpi Launonen

Molecular and Cancer Biology Program

Department of Medical Genetics

University of Helsinki

ISSN 1457-8433

ISBN 978-952-10-3940-9 (paperback)

ISBN 978-952-10-3941-6 (PDF)

http://ethesis.helsinki.fi

Helsinki University Printing House

Helsinki 2007

If you worried about falling off the bike, you'd never get on.
Lance Armstrong

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Abstract

Breast and colorectal cancers, are common types of cancer, with over two million newly diagnosed cases annually worldwide. Cancer is a genetic disease and defects in DNA integrity restoring functions make a significant contribution to cancer risk. CHEK2 is a checkpoint kinase functioning as a regulator of cell cycle checkpoints, apoptosis, and DNA repair in response to DNA double-strand breaks.

The aim of this study was to evaluate the role of *CHEK2* in breast cancer predisposition in Finnish breast cancer families and in breast cancer risk at the population level. We were interested in the clinical and biological characteristics of the breast tumors associated with the *CHEK2* germline mutations or aberrant CHEK2 protein expression and the effect on survival of patients with these *CHEK2* defects. We also assessed the role of *CHEK2* mutations, namely 1100delC and I157T, in colorectal cancer susceptibility in Finland.

A total of 1383 breast cancer cases and 1885 healthy controls were screened for the *CHEK2* variant I157T. Seventy-seven carriers of I157T were identified among 1035 breast cancer cases unselected for family history of breast cancer (7.4%) and 100 carriers among 1885 healthy controls (5.3%). Altogether, *CHEK2* I157T is associated with breast cancer risk, conferring a 1.4-fold risk to variant carriers. Immunohistochemical studies showed that CHEK2 I157T, unlike 1100delC, does not affect protein expression in breast tumors. The features of CHEK2 I157T were compared with wild-type CHEK2 in functional studies, and the *CHEK2* I157T mutation was found not to affect CHEK2 stability or activation in response to ionizing radiation. CHEK2 I157T is defective in substrate binding, and we were able to show that CHEK2 I157T can dimerize with wt CHEK2, which may lead to a decreased number of functional CHEK2 in a cell.

Clinical and biological characteristics of the breast tumors associated with *CHEK2* 1100delC and aberrant CHEK2 protein expression were studied in 1297 and 611

unselected breast cancer cases, respectively. One-fifth of breast tumors showed loss or reduction in CHEK2 immunostaining. Generally, the tumors from 1100delC carriers or those with aberrant expression were similar to noncarrier tumors or tumors with normal expression, respectively. Tumors with reduced CHEK2 expression were, however, larger than normally staining ones, and the most aberrantly staining tumors were more often estrogen receptor (ER)-positive. Tumors from *CHEK2* 1100delC carriers were more often of higher grade than tumors from noncarriers and they also tend to be ER-positive more often.

Contribution of *CHEK2* 1100delC to colorectal cancer risk and to the hereditary breast and colorectal cancer (HBCC) phenotype was studied in a set of 662 CRC patients unselected for family history and in 507 familial breast cancer cases, respectively. 2.6% of colorectal cancer (CRC) cases (17/662) carried 1100delC, which is not significantly higher than the geographically adjusted population frequency of 1.9%. Neither was the frequency of 1100delC higher in HBCC families than in breast cancer families. Our results suggest that *CHEK2* 1100delC may not be a susceptibility allele for CRC, although a small effect cannot be excluded. The role of *CHEK2* missense variant I157T was also studied for colorectal cancer susceptibility and for association with clinical characteristics and family history of cancer. A population-based series of 1042 CRC cases was screened for *CHEK2* I157T and a significantly higher frequency of I157T was observed among both familial (10.4%) and sporadic (7.4%) CRC cases: 7.8% in all cases combined vs. 5.3% in population controls. Association of I157T with familial CRC has not been studied previously. *CHEK2* I157T seems to be a susceptibility allele for both familial and sporadic CRC, conferring a 1.5-fold risk for carriers of this variant. Furthermore, we observed a higher frequency of the variant among cases with multiple primary tumors or a family history of cancer, supporting the suggested role for *CHEK2* I157T as a multiple cancer susceptibility allele.

List of original publications

This thesis is based on the following original publications, referred to in the text by their Roman numerals:

I Kilpivaara O., Vahteristo P., Falck J., Syrjäkoski K., Eerola H., Easton D., Bartkova J., Lukas J., Heikkilä P., Aittomäki K., Holli K., Blomqvist C., Kallioniemi O.-P., Bartek J., and Nevanlinna H.: The *CHEK2* variant I157T may be associated with increased breast cancer risk. International Journal of Cancer, 111(4):543-7, 2004

II Kilpivaara O., Bartkova J., Eerola H., Syrjäkoski K., Vahteristo P., Lukas J., Blomqvist C., Holli K., Heikkilä P. Sauter G., Kallioniemi O.-P., Bartek J., and Nevanlinna H.: Correlation of CHEK2 protein expression and c.1100delC mutation status with tumor characteristics among unselected breast cancer patients. International Journal of Cancer, 113(4): 575-580, 2005

III Kilpivaara O., Laiho P., Aaltonen L. A., Nevanlinna H.: *CHEK2* 1100delC and colorectal cancer. Journal of Medical Genetics, 40(10): e110, 2003

IV Kilpivaara O., Alhopuro P., Vahteristo P., Aaltonen L. A., Nevanlinna H: *CHEK2* I157T associates with familial and sporadic colorectal cancer. Journal of Medical Genetics, 43(7):e34, 2006

Abbreviations

All gene names are in *italics*.

aa	amino acid(s)
APC	adenomatous polyposis coli
APL	acute promyelocytic leukemia
ATM	ataxia telangiectasia mutated
bp	base pair
cDNA	complementary DNA
Cds1	*Schizosaccharomyces pombe* (fission yeast) homolog of CHEK2
CHEK1	cell cycle checkpoint kinase 1, *CHK1*
CHEK2	cell cycle checkpoint kinase 2, *CHK2*
CI	confidence interval
CRC	colorectal cancer
CSGE	conformation-sensitive gel electrophoresis
DNA	deoxyribonucleic acid
DSB	double-strand break
ER	estrogen receptor
FHA	fork-head-associated
G1	cell cycle phase before S phase
G2	cell cycle phase after S phase and before mitosis
HBCC	hereditary breast and colorectal cancer phenotype
HNPCC	hereditary nonpolyposis colorectal cancer
HR	homologous recombination
I, Ile	isoleucine
IR	ionizing radiation
IVS	intervening sequence / intron
LFL	Li-Fraumeni-like syndrome
LFS	Li-Fraumeni syndrome
LOH	loss of heterozygosity
M	M phase, mitosis
MMR	mismatch repair
MSI	microsatellite instability
NHEJ	nonhomologous end joining
nt	nucleotide
OMIM	Online Mendelian Inheritance in Man™
OR	odds ratio
p	short arm of a chromosome
PCR	polymerase chain reaction
PML	promyelocytic leukemia
pN	lymph node status
PR	progesterone receptor
pT	tumor size
q	long arm of a chromosome

Q	glutamine
Rad53	*Saccharomyces cerevisiae* (budding yeast*)* homolog of CHEK2
RER	replication error
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
S	serine
S phase	synthesis phase of cell cycle when DNA synthesis and replication occur
SCD	SQ/TQ cluster domain
SNP	single nucleotide polymorphism
SSCP	single-strand conformation polymorphism
T, Thr	threonine
TGFβR1	transforming growth factor beta receptor 1
UTR	untranslated region
UV	ultraviolet
VHL	von Hippel-Lindau
wt	wild-type

1 Introduction

Breast and colorectal cancers, both belong to the most common types of cancer. Cancer is a genetic disease, and in a significant number of cases the susceptibility to cancer is inherited. Mutations in high-penetrance susceptibility genes are rare at the population level, but confer a high risk for cancer and result in familial clustering of cancer cases. Lower penetrance susceptibility genes are, however, more common in the population and may contribute to cancer risk through interactions with other susceptibility genes and environmental factors. The effect of low-penetrance susceptibility genes may be great at the population level, but the prediction of an individual's cancer risk is challenging if even possible. Information on cancer susceptibility alleles is increasing rapidly, and in the future it may become possible that information on several genetic factors and their interactions is utilized such that it is applicable at the individual level in clinical management of cancer.

Our DNA is continuously challenged by situations where the DNA strands may break. Cells have developed a refined machinery to assure the integrity of DNA, and defects in this network of protein interactions may eventually result in cancer. One of the key players in DNA double-strand break (DSB) responses is CHEK2, a checkpoint kinase functioning as a regulator of cell cycle checkpoints, apoptosis, and DNA repair. This study focuses on the role of CHEK2 and its variants in breast and colorectal cancer susceptibility, and further, on the clinical and biological characteristics of the disease.

2 Review of the literature

2.1 Cancer as a genetic disease

Cancer is one of the most common causes of death in the developed world. One-third of people will be diagnosed with cancer during their lifetime, and the diagnosis will touch most of us, either personally or via a loved one. Cancer is generally thought of being a disease of genes (Vogelstein and Kinzler, 2002). Cancer is characterized by several acquired qualities that differentiate cancerous tissue and cells from normal ones (Hanahan and Weinberg, 2000). Cancer cells can proliferate in the absence of growth signals, and they are insensitive to anti-growth stimuli. Cancer cells can, furthermore, evade apoptosis, and they have limitless replicative potential – they can live forever. All tissues require oxygen and nutrients, and in order to grow a tumor needs to develop angiogenic ability (Hanahan and Folkman, 1996). These capacities enable cancer cells to invade other tissues and form metastases, which in the majority of cases is the cause of cancer death. Cancers arise as a result of genetic changes that promote the above-mentioned qualities mentioned accumulating over time. Genes that have been implicated in tumorigenesis are traditionally classified as oncogenes and tumor suppressor genes. Oncogenes are an altered form of cellular proto-oncogenes that function in regulation of the cell cycle, cell division, and differentiation (Vogelstein and Kinzler, 2002). When appropriately activated by a mutation, a proto-oncogene becomes an oncogene and stimulates uncontrolled growth. At the cellular level, oncogenes are dominant, meaning that only one copy of the genes needs to be altered to promote oncogenesis. Tumor suppressors, as the name indicates, function in preventing inappropriate growth.

Several rare hereditary cancer syndromes have been identified to date. The great majority of these are caused by a mutation in a tumor suppressor gene e.g. mutation in *VHL* in von Hippel-Lindau syndrome, in *LKB1* in Peutz-Jeghers syndrome, and in *PTEN* in Cowden syndrome (Latif et al., 1993; Liaw et al., 1997; Hemminki et al., 1998). Because of the dominance at the celluar level, activated oncogenes are presumably lethal during development and are therefore very

rarely inherited. The oncogenes involved in hereditary cancers include *RET* in thyroid cancer, *CDK4* in cutaneous melanoma, and *MET* in papillary renal cell carcinoma (Mulligan et al., 1993; Zuo et al., 1996; Schmidt et al., 1997).

2.1.1 Tumor suppressor genetics

Over thirty years ago Knudson proposed his famous two-hit hypothesis suggesting that both alleles of a tumor suppressor gene need to be inactivated to promote tumorigenesis (Knudson, 1971; Knudson, 2001). Three different types of tumor suppressors are classically described: gatekeepers, caretakers, and landscapers (Kinzler and Vogelstein, 1997; Kinzler and Vogelstein, 1998). Gatekeepers are characterized by functions that directly inhibit cellular growth or promote death by apoptosis. These are the most classical tumor suppressors, like the *RB* gene in retinoblastoma, as described in Knudson's original work (Knudson, 1971). Caretaker genes are usually involved in the control of genomic integrity and inactivation of a caretaker may not initiate tumor formation in itself, but could promote transformation by making the cell genetically unstable and therefore more prone to other mutations. Typically, genes involved in DNA repair belong to caretakers such as the breast cancer susceptibility genes *BRCA1* and *BRCA2*. Landscapers play an indirect role in tumorigenesis by creating an abnormal microenvironment promoting tumorigenesis, which is known to happen in certain polyposis syndromes of the colon.

Classically, the mechanism of tumorigenesis in association with tumor suppressor genes in inherited cancers involves the loss of the wild-type (wt) allele by loss of heterozygosity (LOH), often caused by a loss of a whole chromosome or a chromosome arm. Mutation of one allele may also result in reduction of gene product dosage, a phenomenon called haploinsufficiency. Tumor suppressor mutations can, however, have qualitative differences and function by a dominant negative mode of action whereby the wt protein is prevented from carrying out its function by binding to the mutant protein, or the mutation can result in the gain of an appropriate function. In addition, tumor suppressor mutations may have a

different effect on function depending on the type of the mutation itself, tissue type, and environmental factors (Payne and Kemp, 2005). Several tumor suppressors have been shown to function through haploinsufficiency, e.g. $p27^{kip1}$ (Fero et al., 1998), p53 (Venkatachalam et al., 1998), and TGFβ1 (Tang et al., 1998) even though their principal method of function may be the traditional two-hit mechanism.

2.2 Breast cancer

2.2.1 Epidemiology of breast cancer

Breast cancer is the most common type of cancer in women worldwide, with an estimated 1.15 million new cases in 2002 (23% of all cancers in women). There are 4.4 million women living with breast cancer globally, nearly 17000 in Finland alone (diagnosis within 5 years); breast cancer is the most prevalent cancer in the world because of its high incidence and relatively good prognosis (Parkin et al., 2005). In Finland, over 4000 women were diagnosed with breast cancer in 2006 (estimate based on 2004 incidence; Finnish Cancer Registry, www.cancerregistry.fi). The majority of breast cancer cases are sporadic, while up to 10% of breast cancers are hereditary in nature and caused by dominantly inherited mutations (McPherson et al., 2000; Dapic et al., 2005; Lacroix and Leclercq, 2005). The major breast cancer predisposition genes are *BRCA1* and *BRCA2*, which confer a very high lifetime risk of breast and ovarian malignancy (Miki et al., 1994; Wooster et al., 1995; Antoniou et al., 2003). Breast cancer is also a characteristic in rare hereditary cancer syndromes, such as Li-Fraumeni syndrome (mutated gene *p53*) and Cowden syndrome (*PTEN*), appearing also in Peutz-Jeghers syndrome (*LKB1*).

Familial aggregation of breast cancer, when *BRCA1* and *BRCA2* mutations have been ruled out, may be a result of several low-penetrance genes with a multiplicative effect (Antoniou et al., 2002). Moreover, results from a population-based study and modeling of breast cancer risk indicate that breast cancer susceptibility is conferred by the combined effects of higher and lower risk

variants (Pharoah et al., 2002). Breast cancer risk can be described as a continuum between environmental factors and high-penetrance susceptibility genes, where several low-penetrance genetic variants interact with each other and the environment (Balmain et al., 2003). Altogether, it is estimated that about 30% of breast cancer is estimated to be caused by heritable factors (Lichtenstein et al., 2000).Family history remains the strongest risk factor, while other known risk factors for breast cancer include certain reproductive factors, body size, exogenous hormones, ionizing radiation, physical inactivity, and possibly diet (McPherson et al., 2000; Hulka and Moorman, 2001; Coyle, 2004; Parkin et al., 2005). The incidence rate has been constantly growing, and the greatest increase in incidence has been seen in areas where the incidence was formerly low, e.g. in China and other Eastern Asian countries. In addition, the development of effective screening programs in affluent countries has contributed to increased detection of early invasive breast tumors, which may otherwise have been diagnosed later or not at all (Parkin et al., 2005). The estimate for the number of new cases worldwide in 2010 is 1.4-1.5 million (Parkin et al., 2005). Breast cancer also occurs in males, but it is very rare, the greatest risk factor for male breast cancer being a mutation in *BRCA2* (Weiss et al., 2005).

2.2.2 Clinicopathologic features of breast cancer

Practically all breast tumors are carcinomas, with the tumor arising from epithelial cells (Berg and Hutter, 1995). Breast tumors are typically adenocarcinomas; the malignancy originates in the glandular epithelia. The most common histological types of breast carcinoma are infiltrating ductal carcinoma (~70%), lobular carcinoma (~6%), and medullary carcinoma (~3%). Breast carcinomas are classified according to the TNM staging system (T, extent of the primary tumor; N, absence or presence of the disease in the lymph nodes; M, absence or presence of distant metastasis). The numerical staging helps in planning treatment and evaluating treatment results; as it also indicates prognosis. The TNM staging system is continuously being updated and improved by the International Union Against Cancer (IUCC) (http://www.uicc.org/). Hormone receptor expression,

estrogen receptor (ER) and progesterone receptor (PR) in breast tumors, is considered to be an indicator of good response to hormone treatment and good prognosis (reviewed in Duffy, 2005).

2.3 Colorectal cancer

An estimated one million new colorectal cancer (CRC) cases occurred worldwide in 2002 (Parkin et al., 2005). In Finland, colorectal cancer is the second most common cancer in women (after breast cancer) and the third most common cancer in men (after prostate and lung cancers) (Finnish Cancer Registry, www.cancerregistry.fi). The great majority of colorectal cancer cases are sporadic, indicating that cancer occurs in individuals without a family history of cancer. Consistent evidence suggests that certain lifestyle-associated factors, such as physical inactivity, obesity, excess alcohol use, and meat consumption, are linked to an increased risk of colorectal cancer (Giovannucci, 2002). One of the most important risk factors for colorectal cancer is, however, family history of CRC, indicating that inherited susceptibility plays a significant role in colorectal cancer development; 35% of colorectal cancers are likely attributable to hereditary factors (Lichtenstein et al., 2000; Slattery et al., 2003).

2.3.1 Genetic risk factors for colorectal cancer

Familial colorectal cancers such as familial adenomatous polyposis (FAP) and Lynch syndrome (also known as hereditary nonpolyposis colorectal cancer, HNPCC), account for about 5% of the incidence of CRC (Burt and Neklason, 2005). These autosomally dominantly inherited cancer syndromes are, in the majority of cases, caused by mutations in the *APC* gene and DNA mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*), respectively, as reviewed in (Peltomaki, 2005; Lipton and Tomlinson, 2006). There is, however, evidence that approximately 20-30% of colorectal cancer have a heredity component (Lynch and de la Chapelle, 2003, Bodmer, 2006) and that a relative with CRC increases an individual's lifetime risk of CRC significantly (Johns and Houlston,

2001). The first identified genetic variant in CRC that doesn not result in familial clustering of the disease but predisposes to CRC was missense variant I1307K in the *APC* gene among Ashkenazi Jewish CRC families (Laken et al., 1997). This finding was subsequently confirmed by Frayling et al., (1998). They also identified another missense variant in *APC*, E1317Q, which associated with adenoma and early onset CRC cases among patients of Ashkenazi descent. Further confirmation of these results was presented by Lamlum et al. (2000). These findings have led to the so-called rare variant hypothesis (Frayling et al., 1998; Bodmer, 1999), which suggests that rare dominantly acting variants conferring a moderate risk may together define the inherited susceptibility to multifactorial diseases like cancer.

The association between colorectal cancer and several polymorphisms in genes involved in metabolic pathways, methylation, immune responses, and iron metabolism as well as colonic microenvironment-modifying genes, and oncogenes and tumor suppressor genes have been studied in meta-analyses (de Jong et al., 2002; Chen et al., 2005; Hubner and Houlston, 2007). Polymorphisms in several genes, including *GSTT1*, *NAT2*, *HRAS1*, and *ALDH2* have been associated with moderately increased risk for colorectal cancer (de Jong et al., 2002; Chen et al., 2005). A common functional polymorphism in methyl tetrahydrofolate reductase (MTHFR), 677C>T (A222V), has been associated with decreased risk of colorectal cancer (de Jong et al., 2002; Hubner and Houlston, 2007; Huang et al., 2007), as has 1298A>C (E429A) (Huang et al., 2007). MTHFR may represent a low-penetrance susceptibility gene for CRC, and the polymorphisms would specifically protect against a colorectal adenoma developing into cancer since no association with colorectal adenoma was observed for either of the variants (Huang et al., 2007). There is also convincing evidence that a tumor suppressor, transforming growth factor β receptor 1 (TGFβR1) polymorphism, a stretch of six alanines instead of the more common nine in the first coding exon, would increase risk of CRC with OR of 1.24, 95% CI=1.1-1.4 (Kaklamani et al., 2003; Pasche et al., 2004).

2.4 DNA double-strand break (DSB) responses

Humans, as well as other higher organisms, have evolved complicated signaling pathways for DNA damage repair and promotion of genomic stability. DNA in every cell is exposed to damaging agents that may result in DNA breakage. DNA damage may be caused by ultraviolet (UV) radiation, mutagenic chemicals, ionizing radiation (IR), cell oxidative metabolism, or mechanical stress on chromosomes, but may also occur normally during the processes of DNA replication, meiotic exchange, and V(D)J recombination of the immunoglobulin genes. The most serious type of DNA damage is DNA double-strand break (DSB). In DNA DSB, both strands are broken at corresponding sites and the ends of the chromatin may become physically dissociated from each other, which may in turn result in inappropriate recombination with other genomic sites. In addition, DNA DSBs are generated on purpose in the initiation of recombination in meiosis, and it also occurs in developmentally regulated rearrangements such as immunoglobulin class switch and V(D)J recombination. Generation of DNA DSBs may result in induction of mutations and chromosomal translocations (Lengauer et al., 1998; Richardson and Jasin, 2000; Ferguson and Alt, 2001; Khanna and Jackson, 2001). In a normally functioning cell, DNA DSBs initiate a signaltransduction cascade. DNA damage is first detected by sensors, which then activate the transducers (protein kinases). The kinase cascade amplifies the signal and targets it to downstream effectors. Defects in cellular processes that respond to DNA DSBs are fundamental to the etiology of most cancers (Khanna and Jackson, 2001)). DNA DSBs may induce gene mutations, translocations and cell transformations, thus contributing to oncogenesis (for review, see Hoeijmakers, 2001). Many of the proteins belonging to DNA DSB response pathways are associated with cancer (Thompson and Schild, 2002). The key breast cancer susceptibility gene products; BRCA1, BRCA2, and TP53, are all involved in DNA DSB repair and chromosomal stability (Jasin, 2002; Valerie and Povirk, 2003; Yoshida and Miki, 2004; Bertrand et al., 2004; Gatz and Wiesmuller, 2006).

There are two separate mechanisms of DNA DSB repair: the homologous recombination repair (HRR) pathway and the nonhomologous end-joining (NHEJ) pathway (Hoeijmakers, 2001; Valerie and Povirk, 2003). The choice of pathway may be determined by whether the DNA region has already replicated and the precise nature of the break. HRR is usually preferred when the identical DNA copy is available since NHEJ is more prone to errors. NHEJ functions at all stages of the cell cycle, but plays the predominant role in both the G1 phase and the S phase regions of DNA that have not yet replicated, while HRR functions primarily in repairing DSBs arising in S or G2 phase chromatid regions that have replicated (Rothkamm et al., 2003).

The importance of DNA DSB responses is highlighted by the fact that numerous cancer susceptibility syndromes are caused by defects in genes involved in DNA DSB responses (Hoeijmakers, 2001; Khanna and Jackson, 2001; Kastan and Bartek, 2004; O'Driscoll and Jeggo, 2006). These syndromes are presented in Table 1.

Table 1. *Cancer susceptibility linked to defects in genes involved in DNA DSB responses.*

Syndrome	Gene	Cancer Susceptibility	Other features
Ataxia telangiectasia (AT)	*ATM*	leukemia, lymphoma (stomach, liver, pancreas, ovary, breast, salivary gland)	cerebellar ataxia, telangiectases, immunological defects
AT-Like Disorder (ATLD)	*MRE11A*	like AT	milder clinical course than in AT
Nijmegen Breakage Syndrome (NBS)	*NBS*	leukemia, lymphoma	microcephaly, growth retardation, immunodeficiency
Werner Syndrome (WRN)	*WRN (RECQL2)*	sarcoma, (general susceptibility to malignancies)	scleroderma-like skin changes, cataract, subcutaneous calcification, premature arteriosclerosis, diabetes mellitus, a wizened and prematurely aged face
Bloom's Syndrome (BLM)	*BLM (RECQL3)*	general susceptibility to malignancies	pre- and postnatal growth deficiency; sun-sensitive, telangiectatic, hypo- and hyperpigmented skin
Rothmund-Thomson Syndrome (RTS)	*RECQL4*	sarcoma	skin atrophy and dyspigmentation,telangiectasia, juvenile cataract, congenital bone defects, hair growth disturbances, hypogonadism
Li-Fraumeni Syndrome	*TP53*	soft tissue sarcomas and osteosarcomas, breast, brain, leukemia, adrenocortex	typically early onset of tumors, multiple tumors within an individual
Hereditary Breast and Ovarian Cancer (HBOC)	BRCA1	breast, ovarian	
	BRCA2	breast (also in males), ovarian, prostate, pancreatic	

2.5 Cell cycle checkpoint kinase 2 (CHEK2)

Cell cycle checkpoint kinase 2 (CHEK2) has an important role in regulating cellular responses to DSBs. It participates in controlling the cell cycle at several checkpoints, committing to apoptosis, and regulating DNA DSB repair; see Figure 1 (Bartek and Lukas, 2003; O'Driscoll and Jeggo, 2006).

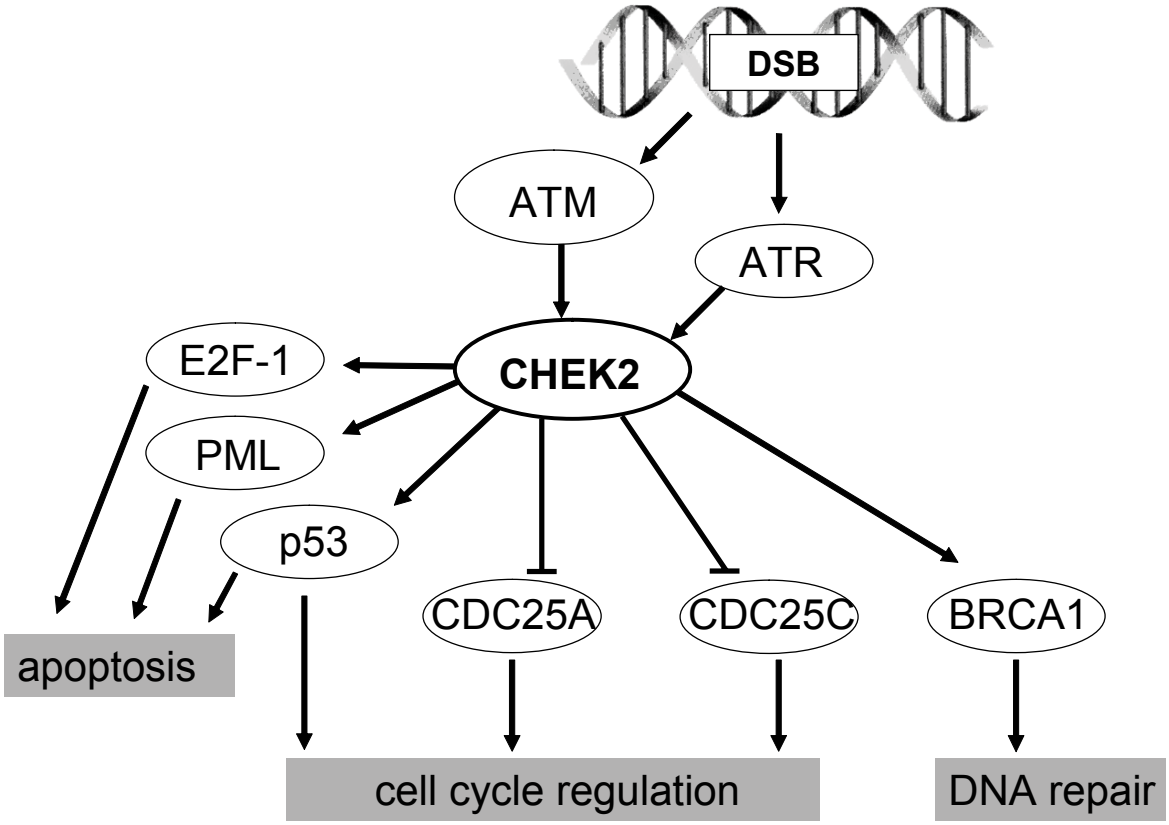

Figure 1 *Simplified presentation of the CHEK2 pathway: three major functions of CHEK2 and its important interaction molecules.*

2.5.1 CHEK2 gene and protein structure

The *CHEK2* gene (ENSG00000183765, OMIM +604373) consists of 14 protein coding exons located on chromosome 22q12.1. According to current knowledge, *CHEK2* has one untranslated exon at the 5' end of the gene located approximately 7 kb upstream of the first protein coding exon. *CHEK2* exons 10-14

have given rise to several pseudogenes that are present in several chromosomes, which has complicated the research of these *CHEK2* exons.

The *CHEK2* gene encodes a protein product of 543 amino acids (aa). The CHEK2 protein has three separate well-conserved protein domains: the N-terminal regulatory SQ/TQ cluster domain (SCD), the forkhead-associated (FHA) domain responsible for protein-protein interactions, and a large C-terminal kinase domain (Matsuoka et al., 1998). The SCD consists of five serine-glutamine (SQ) and two threonine-glutamine (TQ) pairs in the aminoterminus (aa residues 19-69). SCD has an important role in the (auto)activation and regulation of CHEK2 since this domain is a target of several phosphorylations. The FHA domain (aa residues 112-175) is responsible for phosphorylation-dependent protein-protein interactions of CHEK2 and defining the substrate specificity of CHEK2 (Durocher and Jackson, 2002). The kinase domain covers almost half of the whole protein (aa residues 220-486), defining CHEK2 as a serine-threonine kinase. The kinase domain also has two important (auto)phosphorylation sites (Thr 383 and Thr387), which are important for CHEK2 activation (Lee and Chung, 2001).

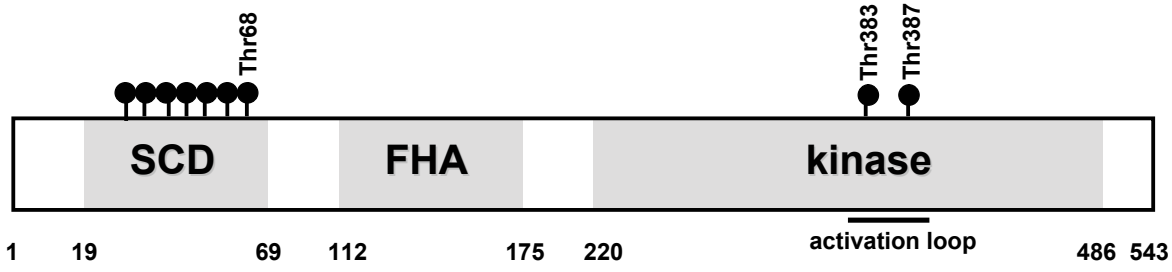

Figure 2 *Structure of CHEK2 protein with its domains and major phosphorylation sites.*

2.5.2 CHEK2 activation and function in DSB responses

CHEK2 is a serine-threonine kinase playing a central role in cell cycle regulation, apoptosis, and DNA repair mechanisms. CHEK2 is activated through a series of phosphorylations in response to DNA DSBs. Upon IR –induced DSBs, ATM is the major activator of CHEK2, and the activation is initiated by phosphorylation of

Thr68 in the SCD (Ahn et al., 2000; Matsuoka et al., 2000; Melchionna et al., 2000). When DSBs are caused by UV irradiation or hydroxyurea treatments, CHEK2 is likely to be activated by ATR kinase (ATM and Rad3 –related) instead (Tominaga et al., 1999; Matsuoka et al., 2000). After the initiating phosphorylation, dimerization of CHEK2 takes place through a FHA domain and a Thr68-phosphorylated SCD (Ahn and Prives, 2002; Ahn et al., 2002; Xu et al., 2002). CHEK2 becomes fully activated by a series of autophosphorylations, including phosphorylation of Thr383 and Thr387 in the activation loop and Ser516 C-terminal to the kinase domain (Lee and Chung, 2001; Wu and Chen, 2003). CHEK2 dimerization upon the initial phosphorylation may promote the *trans*-phosphorylation in the FHA domain and the subsequent release of active CHEK2 monomers (Ahn et al., 2002; Xu et al., 2002). There are several equally important steps in the activation of CHEK2, and it has been suggested that the CHEK2 pathway would become fully activated only when number of DNA DSBs is sufficiently high and that smaller injuries would be repaired without inducing cell cycle arrest (Buscemi et al., 2004), as has been observed to happen in yeast (Leroy et al., 2001).

CHEK2 relays the message of DNA damage forward to effectors that function in several pathways leading to cell cycle arrest in the G1/S, S, and G2/M phases, activation of DNA repair, and apoptosis. The most important and studied substrates of CHEK2 phosphorylation are p53, BRCA1, and CDC25 phosphatases. CDC25A and CDC25C phosphatases are important cell cycle checkpoint regulators that are in turn regulated by CHEK2; CDC25 phosphatases are reviewed in Donzelli and Draetta (2003). Phosphorylation of Ser123 in CDC25A directs it to proteasome-mediated degradation (Falck et al., 2001b) and prevents CDC25A from activating CDK2 and thus the cell cycle progression from G1 to S. CHEK2 also regulates cell cycle progression in G2/M, where phosphorylation of CDC25C on Ser216 leads to binding of CDC25C by 14-3-3 proteins, thus preventing CDC25C from activating CDC2, a kinase that regulates entry to mitosis (Peng et al., 1997; Matsuoka et al., 1998).

Phosphorylation of p53 on Ser20 by CHEK2 stabilizes it, and p53 in turn regulates downstream targets controlling the cell cycle checkpoints, apoptosis, and DNA repair (Chehab et al., 1999; Chehab et al., 2000; Hirao et al., 2000; Shieh et al., 2000). In nondamaged cells, p53 is quickly directed to proteasome-mediated degradation by binding of Mdm2 (Haupt et al., 1997; Kubbutat et al., 1997; Midgley and Lane, 1997). The number of CHEK2-p53 complexes has been observed to increase in response to DNA damage (Falck et al., 2001a). There are also reports that question the role of CHEK2 in p53 regulation (Jack et al., 2002; Ahn et al., 2003; Jallepalli et al., 2003). This controversy is understandable since the regulatory networks are complex and there is variation in response to different kinds and different amounts of cellular stress. There might also be differences in study designs and in cell or tissue types investigated. CHEK2 is considered a modifier or amplifier in several responses, and not be the primary kinase in the actions (Bartek and Lukas, 2003).

BRCA1 has a central role in breast cancer susceptibility, but its precise mechanism of function in DNA repair remains somewhat unclear, although well established (Zhang and Powell, 2005). CHEK2 phosphorylates BRCA1 on Ser988, and it leads to release of BRCA1 from CHEK2 itself (Lee et al., 2000) and promotion of less error-prone homologous recombination in DNA repair (Zhang et al., 2004).

Promyelocytic leukemia (PML) protein has been named after the *PML* gene, which is found to be translocated in the majority of acute promyelocytic leukemias (APLs) (de The et al., 1991). CHEK2 phosphorylates PML protein in response to DNA DSBs on Ser117 both *in vivo* and *in vitro*, and this phosphorylation by CHEK2 is also a prerequisite for the colocalization of PML and CHEK2 in nuclear bodies and their separation after IR (Yang et al., 2002). Thus, CHEK2 has an important role in regulating PML-mediated apoptosis after IR. Furthermore, PML is involved in p53-mediated DNA integrity-restoring functions (Bernardi et al., 2004; de Stanchina et al., 2004).

Transcription factor E2F1 functions in controlling apoptosis, DNA repair, and proliferation (DeGregori and Johnson, 2006). CHEK2 phosphorylates E2F1 on Ser364 *in vivo* and *in vitro*, which leads to stabilization and transcriptional activation of E2F1 and to changes in E2F1 nuclear localization (Stevens et al., 2003). This activation of E2F1 provides a signal for E2F1-mediated, p53-independent apoptosis and cell cycle arrest (Stevens et al., 2003; Rogoff et al., 2004). Activation of E2F1 may create an amplifying effect on DNA damage signaling since, as a transcription factor, E2F1 has been shown to induce CHEK2 expression (Rogoff et al., 2004). The targets of CHEK2 phosphorylation presented here are not the only CHEK2 substrates identified to date, but represent the ones most studied and perhaps also the most important.

CHEK2 is a predominantly nuclear protein expressed throughout the cell cycle (Matsuoka et al., 1998; Lukas et al., 2001). CHEK2 is also abundant in quiescent cells and is detectable regardless of the differentiation or proliferation state of cells (Lukas et al., 2001). CHEK2 is also a relatively stable protein with a half-life of over two hours (Lee et al., 2001) and the level of CHEK2 has been shown to remain practically unchanged even for six hours (Lukas et al., 2001). The nuclear localization is unaffected by DSB-induced activation (Tominaga et al., 1999). Activation is observed to be restricted to the DNA DSB sites, but once activated CHEK2 mediates the message of DNA damage throughout the nucleus (Lukas et al., 2003).

CHEK2 functions have been studied by producing Chk2 (CHEK2 homolog) knock-out mice. Chk2 is not an essential gene in mice since $Chk2^{-/-}$ mice are viable (Hirao et al., 2002). These knock-out mice appear normal, but they are significantly more resistant to ionizing radiation than wt mice (Takai et al., 2002). Cells lacking Chk2 are defective in p53 stabilization, induction of p53-dependent transcripts, maintaining G2 arrest, and resisting p53-mediated apoptosis in response to IR (Hirao et al., 2000; Hirao et al., 2002; Takai et al., 2002). A study in human cells with antisense inhibition of CHEK2 supports the model in which CHEK2 is required for the damage-induced G2 checkpoint (Yu et al., 2001). By

the age of one year, Chk2$^{-/-}$ mice did not develop tumors spontaneously, but it has been speculated that tumors are too rare to detect or that they may take a longer time to develop (Hirao et al., 2002). Chk2$^{-/-}$ mice did, however, develop more tumors and at an earlier age when exposed to chemical carcinogen compared with wt mice (Hirao et al., 2002).

2.5.3 *CHEK2* becomes a cancer susceptibility gene

The majority of LFS patients have a germline mutation in *p53* (Malkin et al., 1990; Srivastava et al., 1990). The characteristics of LFS include a predisposition for several tumors: breast cancer, brain tumors, leukemia, early onset sarcomas, and adenocortical carcinoma (Li and Fraumeni, 1969), as reviewed in Varley et al. (1997) and Varley (2003). *CHEK2* was first discovered as a tumor suppressor candidate by Bell et al. in 1999, when they identified germline mutations in CHEK2 in patients with Li-Fraumeni syndrome (LFS) or Li-Fraumeni-like syndrome (LFL) who did not have a mutation in *p53*. Further studies on CHEK2 in LFS, LFL, and breast cancer families with phenotypic features of LFS revealed two carriers of CHEK2 1100delC, and both were breast cancer patients with a family history only suggestive of LFS (Vahteristo et al., 2001b).

This observation and linkage studies on breast cancer families led to the identification of CHEK2 c.1100delC as a breast cancer susceptibility allele by two research groups almost simultaneously (Meijers-Heijboer et al., 2002; Vahteristo et al., 2002). CHEK2 c.1100delC (called 1100delC) was found to associate with hereditary non*BRCA1/2* breast cancer with similar frequencies in both studies, 4.2% and 5.5% vs. 1.1% and 1.4% in population controls, respectively (Meijers-Heijboer et al., 2002; Vahteristo et al., 2002). The frequency of 1100delC was not significantly elevated among breast cancer patients unselected for family history in these studies. Later, a large study involving 10860 cases and 9065 controls proved that there is, in fact, an association between *CHEK2* 1100delC and unselected breast cancer, with frequencies of 1.9% and 0.7% in cases and controls, respectively (p=0.0000001, estimated OR=2.34, 95% CI=1.72-3.20)

(CHEK2 Breast Cancer Case-Control Consortium, 2004). Thus CHEK2 1100delC doubles the risk for breast cancer. A very recent report with a large study group conclude that *CHEK2* 1100delC is associated with a threefold risk of breast cancer in women in the general population and may also increase the risk of other cancers (Weischer et al., 2007).

2.5.4 Cancer-associated mutations in *CHEK2*

2.5.4.1 *CHEK2* 1100delC

The *CHEK2* 1100delC mutation resides at the beginning of the tenth protein coding exon of *CHEK2*. Deletion of one cytosine residue results in a frameshift and finally a stop codon at aa position 381, in the middle of the kinase domain. Studies have shown that either the truncated protein product is not expressed or the expression is dramatically lowered (Dong et al., 2003; Jekimovs et al., 2005; Bahassi et al., 2007). Since 2002, when *CHEK2* 1100delC became acknowledged as the first low-penetrance breast cancer susceptibility allele (Meijers-Heijboer et al., 2002; Vahteristo et al., 2002), this mutation has been under extensive investigation.

Interestingly, *CHEK2* 1100delC seems to not be present in all populations, but the frequency of 1100delC varies from 0.0% to 1.4% in the general population in studied populations, being highest in Finland and the Netherlands; see Table 2 (Meijers-Heijboer et al., 2002; Vahteristo et al., 2002). *CHEK2* 1100delC predisposes to familial breast cancer as well as to breast cancer in general (CHEK2 Breast Cancer Case-Control Consortium, 2004). Prevalence of 1100delC among male breast cancer patients seems, however, to be similar to that of the general population, Table 2, although first suggested otherwise (Meijers-Heijboer et al., 2002). *CHEK2* 1100delC has also been studied among breast cancer families with *BRCA1* or *BRCA2* mutation, but none or very few 1100delC carriers have been identified; thus, no association has been shown, Table 2.

Very recently, a *CHEK2* 1100delC knock-in mouse was generated for investigating the effects of 1100delC mutation in cells. Embryonic cells from these mice show an increased number of DSBs and polyploidy, and their cell cycle profile is altered (Bahassi et al., 2007). Furthermore, these authors were able to show a dose-dependent relationship between Chk2 mRNA and *CHEK2* 1100delC status. Jekimovs et al. (2005) observed this same phenomenon in humans, when comparing 1100delC carriers to wt CHEK2. Interestingly, Bernstein et al. (2006) noted an increased risk for breast cancer in *CHEK2* 1100delC carrier women exposed to radiation (chest X-rays). These data support the biological relevance of functional CHEK2 in response to DNA DSBs and breast carcinogenesis.

Table 2. *Prevalence of CHEK2 1100delC among breast cancer cases unselected for family history, familial cases (both nonBRCA1/2 and BRCA1/2), and male breast cancer cases in different populations.*

	Cases		Controls					
Study group	**%**	**+ve/Total**	**%**	**+ve/Total**	**OR**	**95% CI**	***p***	**Reference**
Unselected cases								
Australian	0.7	10/1474	0.1	1/736	5.0	0.6-39.3	0.09	CHEK2 Consortium, 2004
British (East Anglia)	1.2	35/2886	0.5	20/3749	2.3	1.3-4.0	0.002	CHEK2 Consortium, 2004
British	1.3	7/564	0.3	1/288	3.6	0.4-29.5	0.20	CHEK2 Consortium, 2004
Dutch	3.8	65/1706	1.6	3/184	2.4	0.7-7.7	0.13	CHEK2 Consortium, 2004
Dutch	3.3	35/1066	0.0	0/265	-	-	-	CHEK2 Consortium, 2004
Finnish (Helsinki, Tampere)	2.0	21/1035	1.4	26/1885	1.5	0.8-2.7	0.18	Vahteristo et al., 2002
Finnish (Kuopio)	2.9	13/464	1.1	5/447	2.5	0.9-7.2	0.07	CHEK2 Consortium, 2004
German	0.3	2/601	0.2	1/650	2.2	0.2-24.0	0.52	CHEK2 Consortium, 2004
German	1.1	11/985	0.2	1/401	4.5	0.6-35.1	0.11	CHEK2 Consortium, 2004
Polish	0.5	11/2012	0.25	10/4000	2.2	0.9-5.2	0.1	Gorski et al., 2005
Russian	2.7	22/815	0.2	1/448	12.4	1.7-92.3	0.0016	Chekmariova et al., 2006
Spanish (Basque Country)	0.9	2/214	0.0	0/120	-	-	-	Martinez-Bouzas et al., 2007
Swedish (postmenopausal)	1.3	20*/1510	0.6	8/1334	2.2	0.9-5.1	0.05	Einarsdottir et al., 2006
US (Washington, dg <45yrs)	1.2	6/505	0.4	2/458	2.7	0.6-13.7	0.20	Friedrichsen et al., 2004
US and Canadian	1.3	30/2311	0.2	1/496	6.7	2.4-18.7	0.20	Bernstein et al., 2006
Familial cases (BRCA1/2 neg)								
British	5.7	12/211	1.0	8/810	6.0	2.4-15.0	0.000	Meijers-Heijboer et al., 2002
Dutch	4.9	11/226	1.4	9/644	3.6	1.5-8.8	0.003	Meijers-Heijboer et al., 2002
Finnish (Helsinki, Tampere)	5.5	28/505	1.4	26/1885	4.2	2.4-7.2	0.000	Vahteristo et al., 2002
German	1.6	8/516	0.5	6/1315	3.4	1.2-9.9	0.02	Dufault et al., 2004
Italian	0.1	1/696	0.0	0/334	-	-	-	Caligo et al., 2004
Spanish	0.0	0/400	0.0	0/400	-	-	-	Osorio et al., 2004
US and Canadian	2.3	6/264	0.6	1/166	3.8	0.5-32.1	0.18	Meijers-Heijboer et al., 2002
US (New York)	1.1	1/92	0.3	5/1665	3.6	0.4-31.6	0.21	Offit et al., 2003
Male breast cancer								
British	0.0	0/79	0.5	20/3749	-	-	-	Neuhausen et al., 2004
Finnish	1.8	2/114	1.4	26/1885	1.3	0.3-5.4	0.74	Syrjäkoski et al., 2004
Israeli	0.0	0/54	0.0	0/146	-	-	-	Ohayon et al., 2004
US (Colorado, Idaho, Utah, Wyoming)	0.0	0/109	0.7	1/138	-	-	-	Neuhausen et al., 2004
US (New York)	0.0	0/16	0.0	0/146	-	-	-	Offit et al., 2003
***BRCA1/2* mutation carriers**								
British	0.0	0/52	1.0	8/810	-	-	-	Meijers-Heijboer et al., 2002
Dutch	0.7	1/141	1.4	9/644	-	-	-	Meijers-Heijboer et al., 2002
Finnish	0.0	0/19	1.4	26/1885	-	-	-	Vahteristo et al., 2002
Israeli	0.5	1/219	0.0	1/146	0.7	0.04-10.7	0.77	Ohayon et al., 2004
Italian	0.0	0/183	0.0	0/334	-	-	-	Caligo et al., 2004
US and Canadian	0.0	0/122	0.6	1/166	-	-	-	Meijers-Heijboer et al., 2002

*one case was homozygous for 1100delC

2.5.4.2 *CHEK2* I157T

CHEK2 I157T was first described by Bell et al. in 1999 in a LFL family as an LFS mutation and subsequently also in a LFS family (Bell et al., 1999; Lee et al., 2001). *CHEK2* 470T>C leads to aa substitution of isoleucine by threonine at position 157 in the FHA domain of CHEK2. The variant is commonly known as I157T even when referring to the change at a DNA level. The nature of this missense variant was studied, and the mutation was observed to deleteriously affect binding of CHEK2's three notorious substrates p53, BRCA1, and CDC25A (Falck et al., 2001a; Falck et al., 2001b; Li et al., 2002), even though CHEK2 I157T becomes normally activated after γ-radiation (Wu et al., 2001). CHEK2 I157T has also been shown to impair the oligomerization and autophosphorylations of CHEK2 (Schwarz et al., 2003). *CHEK2* I157T was identified in Finland in the screening of *CHEK2* for mutations in LFS and breast cancer families (Allinen et al., 2001; Vahteristo et al., 2001b) and was also detected in normal controls. In this thesis, the contribution of this variant to cancer risk was further studied.

2.5.4.3 *CHEK2* IVS2+1G>A and a large deletion in *CHEK2*

Variation in *CHEK2* seems to be very population-specific, and several variants have been reported only in one population or in very few populations. One of these is a splice-site mutation *CHEK2* IVS2+1G>A in intron two, which results in a 4 bp insertion and a premature termination codon in exon 3 (154X). This variant was first described by Dong et al. (2003) in a prostate cancer case in the United States. Since this variant has a clear effect on CHEK2 protein function, it has been actively investigated. In all studies, the frequency of *CHEK2* IVS2+1G>A has been very low in controls, 0.48% being the highest reported in a larger sample set of Polish origin (Cybulski et al., 2004a). These authors reported a significant association of *CHEK2* IVS2+1G>A with both unselected and familial prostate cancer, and soon after, with breast, thyroid, and stomach cancers in Poland (Cybulski et al., 2004a; Cybulski et al., 2004b). A joint study with German and Byelorussian breast cancer cases reported this variant to be infrequent, but

observed a nonsignificantly higher number of IVS+1G>A carriers among breast cancer patients than among controls (Bogdanova et al., 2005). No difference in frequency of IVS2+1G>A was detected in screening of 516 German breast cancer families and 500 controls (two positive cases were identified in both groups) (Dufault et al., 2004). This mutation has also been screened in 345 Finnish familial breast cancer cases, but no mutation carriers were identified (Kilpivaara et al., unpublished). This mutation does not seem to exist in the Finnish population, or it is even rarer than in other studied populations, and thus, its contribution to breast cancer risk in Finland is unlikely.

Until very recently, all reported mutations in *CHEK2* have been point mutations or mutations involving only very few bases. Walsh et al. (2006) reported a large deletion (5.6 kb) in *CHEK2* in two high-risk breast cancer families of Czechoslovakian ancestry. This deletion was found in eight patients with breast cancer (n=631, 1.3%) and in none of the 367 healthy controls in the Czech Republic and Slovakia (Walsh et al., 2006). Soon after, the Polish group defined the mutation to be a deletion of 5395 bp (exons 9 and 10) and they observed it in 39/4454 unselected breast cancer cases (0.9%) and in 24/5496 controls (0.4%), p=0.009, OR=2.0, 95%CI=1.2-3.4 (Cybulski et al., 2006b). They also studied the prevalence among prostate cancer patients and identified the deletion in 15/1864 unselected cases (0.8%) and 4/249 familial prostate cancer cases (1.6%), where association with familial prostate cancer was statistically significant (p=0.03, OR=3.7, 95% CI=1.3-10.8) (Cybulski et al., 2006a). This large genomic deletion in CHEK2 has thus far been identified only in patients of Slavic origin, and it seems to exhibit similar frequencies as IVS2+1G>A. This *CHEK2* deletion is currently under investigation in Finland.

2.5.4.4 Other germline variants in *CHEK2*

Several rare variants in *CHEK2* have been identified in cancer patients. Missense variant R145W with a deleterious effect on *CHEK2* was first identified in a CRC cell line and subsequently in a variant LFS family (Bell et al., 1999; Lee et al.,

2001). Friendrichsen et al. (2004) observed one mutation carrier among 506 breast cancer cases and 459 controls in the USA, but no other observations of this mutation have been reported. This mutation is seemingly very rare, or restricted, like other *CHEK2* variants, to certain populations. Unique cases, *CHEK2* variants E161del (483delAGA), R117G, R137Q, R180H, have been observed in breast cancer families (Sodha et al., 2002a; Sodha et al., 2002b), and very recently, delE161 and R117G have been found to be pathogenic in bioinformatic as well as in biochemical studies (Sodha et al., 2006). Another missense variant with an unknown functional effect has been observed in Iceland. Variant T59K was detected in 8/1172 Icelandic cancer cases (breast, colorectal, stomach, ovarian), but in none of the 452 controls (Ingvarsson et al., 2002). This variant may represent a population-specific rare variant in *CHEK2* since this is, to my knowledge, the only report of this variant.

Two novel missense variants, S428F (1283C>T) and P85L (254C>T), were recently identified in an Ashkenazi Jewish population (Shaag et al., 2005). Variant P85L was found to be neutral, but variant S428F residing in the CHEK2 kinase domain abrogates the CHEK2 function and is associated with a twofold increase in breast cancer risk among Ashkenazi Jews, 2.88% (47/1632) carriers in cases and 1.37% (23/1673) in controls (p=0.004, OR=2.13, 95% CI=1.26-3.69) (Shaag et al., 2005).

The effect of common variation in *CHEK2* on breast cancer risk and survival has also been evaluated. Kuschel et al. (2003) studied two polymorphisms in *CHEK2* in British breast cancer patients, but observed no risk associated with the variation. The same material was used in another study, where the effect of two *CHEK2* SNPs on breast cancer patients' survival was assessed, and the result was again negative (Goode et al., 2002). Einarsdottir et al. (2006b) chose six SNPs in *CHEK2* and investigated the association between breast cancer risk and survival with regard to variation in *CHEK2*. They also found no association between *CHEK2* variation and breast cancer risk or survival (Einarsdottir et al., 2006a; Einarsdottir et al., 2006b). It was recently reported, however, that a SNP in

CHEK2 is associated with an adverse prognosis in glioblastoma multiforme (Simon et al., 2006)

2.5.4.5 Contribution of *CHEK2* mutations to various cancer types

The contribution of *CHEK2* mutations have been examined in cancers of several organs. Since the main focus of this study was in breast and colorectal cancers, they are discussed in more detail under specific headings. The contribution of 1100delC to cancer risk in several cancer types has been researched vigorously in recent years.

When *CHEK2* 1100delC was first found to be associated with hereditary breast cancer, the association was evaluated also in families with ovarian cancer cases. No increased risk for ovarian cancer was associated with *CHEK2* 1100delC when comparing breast cancer only with breast-ovarian cancer families (Meijers-Heijboer et al., 2002; Vahteristo et al., 2002) or with ovarian cancer cases (Baysal et al., 2004; Cybulski et al., 2004a). *CHEK2* 1100delC was identified with a high frequency in families with both breast and colorectal cancers, which has even led to the suggestion of a new hereditary cancer phenotype called hereditary breast and colorectal cancer (HBCC) (Meijers-Heijboer et al., 2003). Several studies have since challenged this proposal by investigating the association between colorectal cancer and 1100delC (III; de Jong et al., 2005; Brinkman et al., 2006; Naseem et al., 2006). Studies in prostate cancer have shown less straightforward results; 1100delC was associated with hereditary prostate cancer in Finland (Seppälä et al., 2003), but other reports failed to prove a statistically significant association with *CHEK2* 1100delC (Dong et al., 2003; Cybulski et al., 2004b; Wagenius et al., 2006). Results are consistent, although frequencies vary between populations. In Poland, the truncating mutations (1100delC and IVS2+1G>A) together are associated with an increased risk for both familial and unselected prostate cancers (Cybulski et al., 2004b). *CHEK2* variants have been infrequent or the contribution to cancer susceptibility has been nonexistent in melanoma (Cybulski et al., 2004a; Debniak et al., 2004), esophageal cancer

(Koppert et al., 2004), bladder cancer, laryngeal cancer, lung cancer, pancreatic cancer, and stomach cancer (Cybulski et al., 2004a). Variants in *CHEK2* in non-Hodgkin's lymphoma have been observed (Hangaishi et al., 2002; Tort et al., 2002), and *CHEK2* I157T has also been shown to be associated with an increased risk (p=0.05, OR=2.0, 95% CI=1.1-3.8) (Cybulski et al., 2004a). *CHEK2* 1100delC does not seem to be associated with multiple primary cancers (Huang et al., 2004), and in general, the cancer susceptibility conferred by 1100delC appears to be limited to breast cancer (Thompson et al., 2006), although a very recent report with large study material suggest that 1100delC may also increase risk of other cancers (Weischer et al., 2007).

2.5.5 *CHEK2* mutations in tumors

Somatic mutations in *CHEK2* are relatively rare (Ingvarsson et al., 2002; Bartek and Lukas, 2003; Williams et al., 2006). While no regularly occurring mutations exist, reports have been madeof single or a few cases in different cancer types. Somatic mutations in *CHEK2* have been observed in breast cancer (Sullivan et al., 2002), osteosarcomas, lung cancer, and ovarian cancer (Miller et al., 2002). Haruki et al. (2000) reported somatic *CHEK2* D311V in lung cancer, and this D311V was shown to exhibit impaired kinase activity and reduced expression (Matsuoka et al., 2001). Somatic mutations in *CHEK2* have also been identified in prostate cancer (R117G and E321K) (Wu et al., 2006) and in a case of myelodysplastic syndrome (A507G) (Hofmann et al., 2001). In addition, malignant gliomas have been studied, but no mutations have been identified (Ino et al., 2000). Also in glioblastomas the only variations observed in *CHEK2* were 1100delC and I157T, which were probably germline mutations, and they were present at frequencies similar to that in the normal population (Sallinen et al., 2005).

2.5.6 Loss of heterozygosity at *CHEK2*

Observed loss of heterozygosity (LOH) at a certain chromosomal location is considered an indication of a tumor suppressor gene location. This, however, is not always true the other way round; as discussed earlier, tumor suppressor mutations can affect function in various ways (Santarosa and Ashworth, 2004).

Several studies have searched for LOH at CHEK2 location 22q in tumors. Although comparing LOH studies is challenging because of different markers used, studies have generally come to the conclusion that tumorigenesis associated with *CHEK2* mutations may not involve LOH, or at least it may not be the only mechanism inactivating the wt allele (Oldenburg et al., 2003; Sodha et al., 2002a; Sodha et al., 2006). A functional study on cell lines carrying CHEK2 1100delC supports this view since the number of functional CHEK2 in these cells is half thst of wt cells, suggesting that 1100delC contributes to carcinogenesis by haploinsufficiency (Jekimovs et al., 2005).

3 Aims of the study

When this thesis work started, *CHEK2* 1100delC was just about to be established as the first low-penetrance susceptibility allele in breast cancer, and the missense variant *CHEK2* I157T had been recently identified in breast cancer cases.

The aims of this study were to evaluate:

1. the role of the *CHEK2* gene for breast cancer predisposition in Finnish breast cancer families and for breast cancer risk at the population level

2. the clinical and biological characteristics of the breast tumors associated with the *CHEK2* germline mutations or aberrant CHEK2 protein expression

3. the role of *CHEK2* mutations, namely 1100delC and I157T, in colorectal cancer susceptibility in Finland

4 Material and methods

4.1 Samples

4.1.1 Breast cancer patient samples

The series of 1035 unselected breast cancer cases has originally been described in Syrjäkoski et al. (2000), and it includes consecutive newly diagnosed breast cancer patients recruited between 1997 and 1998 at the Helsinki University Central Hospital, Department of Oncology (n=627), and between 1997 and 1999 at the Tampere University Hospital (n=408). It covers 82% (87% in Helsinki and 75% in Tampere) of all breast cancer patients treated at the respective hospitals during the study period. This series has been used in Studies I and II.

Another series of unselected breast cancer cases was used and also described for the first time in Study II. This series includes 262 consecutive newly diagnosed breast cancer patients recruited between January and June in 2000 at the Helsinki University Central Hospital, Department of Oncology. This series covers 65% of all breast cancer cases treated during the study period.

Familial breast cancer case series (n=507) used in Study I includes 216 index cases with a stronger family history of breast and/or ovarian cancer (three or more breast/ovarian cancer cases in first-, or second-degree relatives including the proband) and separately 291 index cases with only one affected first-degree relative. This series has been previously described in Vahteristo et al. (2001b). The screening for *BRCA1* and *BRCA2* mutations in this series has been described in Vahteristo et al. (2001a) and Vehmanen et al. (1997). Data concerning the characteristics of tumors and the clinical data were collected from patient files. All cancer diagnoses were confirmed through the Finnish Cancer Registry or hospital records.

4.1.2 Breast tumor arrays for CHEK2 immunohistochemistry

The construction of tumor arrays and the immunohistochemistry protocol for estrogen receptor (ER), progesterone receptor (PR), and p53 have been previously described in detail (Kononen et al., 1998; Torhorst et al., 2001). A breast cancer array of 124 tumors from 75 Finnish BRCA1/2-negative breast cancer families was used in Study I. In Study II, a breast tumor array of 611 unselected breast tumors was used. These samples were collected in 1985-1994 at the University Hospital in Basel (Basel, Switzerland), Women's Hospital Rheinfelden (Rheinfelden, Germany), and the Kreiskrankenhaus Lörrach (Lörrach, Germany). Formalin-fixed, paraffin-embedded tumor material was available from the Institute of Pathology, University of Basel. Information on pathologic stage, tumor diameter, and nodal status was collected from the pathology reports. All slides from all tumors were reviewed by one pathologist to define the histological grade and the histologic tumor type. Detailed information on samples is given in Poremba et al. (2002).

4.1.3 Colorectal cancer patient samples

A Finnish population-based series of 1042 colorectal cancer cases was collected at nine central hospitals in southeastern Finland between 1994 and 1998. This material was used in Studies III (partly) and IV. The patient series has been described in detail in Aaltonen et al. (1998) and Salovaara et al. (2000). Clinical data (used in Study IV) for the patients include age at diagnosis, family history of cancer, information from pathology reports, and tumor grade.In addition, other unselected CRC cases from Helsinki University Central Hospital and Central Finland Central Hospital (Jyväskylä) were used in Study III (n=44).

Familial colorectal cancer case was defined in these studies as a colorectal cancer proband with at least one first-degree relative affected with colorectal cancer.

4.1.4 Population samples

The series of 1885 healthy control individual peripheral blood samples was collected at the Finnish Red Cross Blood Transfusion Service's eight regional centers (Jyväskylä, Kuopio, Lappeenranta, Oulu, Pori, Tampere, Turku, and Vaasa), representing different geographical regions in Finland.

4.1.5 Criteria for Hereditary Breast and Colorectal Cancer Phenotype (HBCC)

The HBCC phenotype has been described by Meijers-Heijboer et al. (2003) and the criteria are as follows. A HBCC family includes at least two first- or second-degree relatives affected with breast cancer, at least one of whom had been diagnosed before 60 years of age and either 1) at least one breast cancer case with CRC, or 2) a first/second-degree relative of a breast cancer case diagnosed with CRC before 50 years of age, or 3) two or more CRC cases, at least of whomt one was a first/second-degree relative of a breast cancer case.

4.2 Methods

4.2.1 DNA extraction

Genomic DNA from breast cancer patients and controls was extracted from peripheral blood leukocytes by using a standard phenol-chloroform method. Colorectal cancer patient DNA was extracted from either colon mucosa or blood leukocytes by a standard nonenzymatic method (Lahiri and Nurnberger, 1991). The method was also applied for DNA extraction from colorectal cancer samples that had been evaluated by a pathologist prior to extraction to display more than 55% carcinoma tissue (typically 60-80%).

4.2.2 Minisequencing

Minisequencing (also known as primer extension) is a method developed by Syvänen et al. (1993) that utilizes the specificity of DNA polymerase to incorporate a single nucleotide at the 3'-end of a sequence specific primer. Solid-phase minisequencing is typically used for detecting (known) point mutations/SNPs in PCR-amplified products. One of the primers used in the PCR reaction is biotinylated, which enables the attachment of DNA to the streptavidin-coated solid phase. The optimal size for the PCR product is 100-250 nt. DNA is denaturated by NaOH. A mixture of DNA polymerase, mutation/SNP-specific primer, and ^{3}H-labeled nucleoside is applied. The same sample is pipetted into two wells of a plate with two different nucleosides in the mixture (one NTP for the wt and one for the mutant allele). Radiation is quantified by a scintillation counter, and the results of the two wells are compared.

4.2.3 Conformation-sensitive gel electrophoresis

Conformation-sensitive gel electrophoresis (CSGE), introduced by Ganguly et al. (1993), was used in Studies I, II, III, and IV. The CSGE method is based on heteroduplex formation between two PCR-amplified DNA strands harboring mismatched nucleotides, which results in a mobility shift in the gel compared with a homoduplex. PCR products were denatured for 10 min at 95°C, and heteroduplexes were formed when reactions were allowed to cool down to room temperature in 45 min. Reactions were run in a mildly denaturing CSGE gel (10% acrylamide, 15% formamide, and 10% ethylene glycol) at 3 W overnight and visualized by silver-nitrate ($AgNO_3$) staining or by labeling one primer with ^{33}P and exposing the dried gel on a BioMax film (Kodak, Rochester, NY, USA).

4.2.4 Restriction fragment length polymorphism analysis

RFLP analysis was utilized in Study II for screening CHEK2 I157T variant in colorectal cancer patients and controls. *CHEK2* exons 2-3 were amplified from genomic DNA with primers 2-3F (5'-GGCTATTTTCCTACAATTAGC) and 2-3R

(5'-CATATTCTGTAAGGACAGGAC), and the PCR product was digested with restriction endonuclease *Bts*I (New England Biolabs, Beverly, MA, USA). The resulting fragments were separated using 2% agarose gel electrophoresis. The *Bts*I enzyme digests a PCR product with the wt allele once and a PCR product with the T470C (I157T) allele twice.

The unpublished screening of *CHEK2* IVS2+1G>A was carried out using the aforementioned PCR primers for *CHEK2* exons 2-3. The PCR product was digested by restriction endonuclease *HPY*188III (New England Biolabs, Beverly, MA, USA), and the resulting fragments were separated in 2% agarose gel electrophoresis.

4.2.5 Direct DNA sequencing

DNA sequencing has been used in all studies for confirming detected variants by other methods, for identifying the mobility shiftcausing sequence variant in DNA, and in Study III also to detect LOH at *CHEK2* exon 10. Briefly, the genomic DNA region of interest was first amplified by PCR and purified using either a QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA) or ExoSAP-IT (USB, Cleveland, OH, USA) according to the manufacturer's instructions. Sequencing reactions were carried out using a BigDye Terminator kit v 3.0 / 3.1 (Applied Biosystems, Foster City, CA, USA), and reactions were purified by acetate-ethanol precipitation. Reactions were run and analyzed in an ABI310 Automated Sequencer (Applied Biosystems, Foster City, CA, USA).

4.2.6 Immunohistochemistry

Immunohistochemical staining of breast tumor microarray with CHEK2 monoclonal antibody was used in Studies I and II. The staining method for the monoclonal antibody (DCS-270 against the aminoterminal SQ/TQ-rich domain of human CHEK2) has been described in Bartkova et al. (2001) and Lukas et al.(2001). The staining pattern was assessed blind to the *CHEK2* genetic status.

4.2.7 Functional studies on CHEK2

The methodology for CHEK2 I157T functional investigations (transient transfections, cycloheximide treatments, and irradiation of cells) in Study I has been described in detail elsewhere (Bartkova et al., 2001; Falck et al., 2001a; Falck et al., 2001b).

4.2.8 Statistics

Statistical package SPSS for Windows (SPSS Inc., Chicago, IL, USA) or SISA (http://home.clara.net/sisa/) was used for all analyses. Statistical significance of associations was evaluated using chi-squared test or Fisher's exact test in all studies. Survival analyses were performed calculating Kaplan-Meier curves and comparing the subsets of cases using a log rank test. Two-sided *p*-values ≤0.05 were considered significant.

4.3 Ethical issues

All studies were carried out with the informed consent of patients and approval from the ethics committees of the respective hospitals as well as from the Ministry of Social Affairs and Health in Finland.

5 Results

5.1 Association of CHEK2 I157T with breast cancer (I)

Subsequently to establishment of *CHEK2* 1100delC as a low-penetrance breast cancer susceptibility allele (Meijers-Heijboer et al., 2002; Vahteristo et al., 2002), the I157T missense variant in *CHEK2* was studied for breast cancer predisposition.

A total of 1383 breast cancer cases and 1885 population controls were screened for *CHEK2* I157T, identifying 99 carriers among cases and 100 among controls (see Table 3). The population frequency of *CHEK2* I157T in Finland was thus defined as 5.3%, with little to no geographical variation. In the population-based series, the frequency of *CHEK2* I157T was 7.4% (77/1035), which is significantly higher than the frequency in controls (p=0.02, OR=1.43, 95% CI=1.06-1.95). The carrier frequency among familial breast cancer cases (5.5%, 28/507) was similar to population controls (p=0.85, OR=1.04, 95% CI=0.68-1.61). *CHEK2* I157T confers a 1.4-fold risk of breast cancer for carriers.

Table 3. *Frequency of CHEK2 I157T in breast cancer cases and controls.*

	+ve/Total	%	p [1]	OR[1]	95% CI
Controls	100/1885	5.3			
All breast cancer cases	99/1383	7.2	0.029	1.38	1.03-1.83
Unselected breast cancer cases	77/1035	7.4	0.021	1.43	1.06-1.95
Familial breast cancer cases	28/507	5.5	0.847	1.04	0.68-1.61
Breast cancer only	24/448	5.4	0.965	1.01	0.64-1.60
Breast and ovarian cancer families	4/59	6.8	0.554	1.30	0.46-3.65
Index with only one affected 1st-degree relative	14[2]/291	4.8	0.725	0.90	0.51-1.60
Three or more affected in the family (in 1st- or 2nd-degree relatives)	14/216	6.5	0.470	1.23	0.69-2.20

[1]compared with controls
[2]includes two homozygotes

5.2 Genetic variants in CHEK2 (I)

Screening of 14 *CHEK2* coding exons and exon-intron boundaries in 75 breast cancer families resulted in identification of nine different sequence variants. *CHEK2* 1100delC and I157T were both identified in four separate families. In

addition to these recognized *CHEK2* variants, a silent mutation 252A>G (E84E) and six intronic changes were observed. All intronic variants reside quite far from the exon-intron junctions. Variants and their frequencies are presented in Table 4.

Table 4. *Variants observed in CHEK2 in the screening of 75 breast cancer families.*

Variant	Observed number
252A>G (E84E)	1
319+44insA	23/25/2
444+24C>T	1
470T>C (I157T)	4
1100delC (381X)	4
1375+78C>G	12/38/26
1462-211A>G	3
1462-198C>T	6
1462-25A>G	3

5.3 Functional studies on CHEK2 I157T and CHEK2 protein expression in breast cancer (I)

The CHEK2 I157T protein was compared with the wt CHEK2 protein as well as with another FHA domain variant CHEK2 R145W, which is known to be unstable (Bartkova et al., 2001; Lee et al., 2001). CHEK2 I157T behaves like wt CHEK2 with respect to stability (protein level) and ionizing radiation (IR) -induced modification *in vivo* (phosphorylation). Dimerization of CHEK2 is an important step in CHEK2 activation after IR (Ahn et al., 2002; Xu et al., 2002). Differentially tagged inactive CHEK2 wt and CHEK2 I157T were coexpressed in cell lines, and formation of both homodimers (wt-wt and I157T-I157T) as well as heterodimers (wt-I157T) was observed. Given the inability of CHEK2 I157T to efficiently bind and phosphorylate its substrates, it may interfere with functional CHEK2 proteins, diminishing the functional CHEK2 pool in a cell.

Immunohistochemical staining of CHEK2 in a breast cancer array of 124 tumors confirms the results of functional studies on CHEK2-I157T stability. This tumor array included five tumors from CHEK2 I157T carriers. In four of five cases, tumors showed normal CHEK2 protein expression, and in one case the expression was only moderately reduced.

5.4 Characteristics of tumors with aberrant CHEK2 expression (II)

Immunohistochemical staining of CHEK2 in tumor array of 611 unselected breast tumors was successful for 440 tumors. The results were categorized into four groups: reduced intensity of staining in the carcinoma cells (5/440, 1.1%), reduced number of positive carcinoma cells (67/440, 15.2%), reduced intensity and number of positive carcinoma cells (21/440, 4.8%), and normal staining pattern (347/440, 78.9%) compared with staining of normal breast tissue. CHEK2 protein expression was reduced in 21.1% of breast tumors analyzed (93/440). Characteristics of tumors, grouped according to CHEK2 expression, are presented in Table 5. Generally, tumors with reduced CHEK2 expression do not differ from tumors where CHEK2 expression is intact. The mean age of diagnosis for both groups was 61 years, and no difference in overall survival of patients was observed. Tumors with aberrant CHEK2 expression, however, seem to be larger than other tumors (pT1-2 vs. pT3-4, p=0.002). 35.2% of pT3-4 tumors were CHEK2 aberrant compared with 18.6% of pT1-2 tumors. Especially, pT4 tumors are more common among those with aberrant CHEK2 expression than among those with normal expression (21/93, 22.6% and 31/344, 9.0%; respectively; p=0.0003, OR=2.9, 95% CI=1.6-5.4). Twenty-one of the 440 tumors showed aberrant staining with regard to both staining intensity and number of stained carcinoma cells. Since CHEK2 expression is the most aberrant in these tumors, this group was carefully studied for associations with any tumor characteristics; however, the small number of tumors limits statistically significant observations. The most interesting finding was that 94% (16/17) of the most aberrantly stained tumors were ER-positive, whereas only 79% of all tumors were ER-positive.

Table 5. *Tumor characteristics of 611 unselected breast tumors grouped according to CHEK2 expression status. (Adapted from Kilpivaara O. et al.: Correlation of CHEK2 protein expression and c.1100delC mutation status with tumor characteristics among unselected breast cancer patients. International Journal of Cancer, 113(4): 575-580, 2005.)*

	Total (%)	**CHEK2 expression**		***p***
		normal	**aberrant**	
	611	347/440	93/440	
Histology (n=611)				0.239
ductal	450 (73.6)	262 (75.5%)	77 (82.8%)	
lobular	81 (13.3)	41 (11.8%)	5 (5.4%)	
medullary	19 (3.1)	14 (4.0%)	2 (2.2%)	
other	61 (10.0)	30 (8.6%)	9 (9.7%)	
Tumor grade (n=611)				0.346
1	169 (27.7)	85 (24.5%)	26 (28.0%)	
2	260 (42.6)	141 (40.6%)	42 (45.2%)	
3	182 (29.8)	121 (34.9%)	25 (26.9%)	
Estrogen receptor status (n=483)				0.124
positive	360 (79.5)	209 (76.3%)	61 (84.7%)	
negative	93 (20.5)	65 (23.7%)	11 (15.3%)	
Progesterone receptor status (n=437)				0.352
positive	216 (49.4)	140 (40.3%)	31 (46.3%)	
negative	221 (50.6)	126 (36.3%)	36 (53.7%)	
Tumor size (pT) (n=605)				0.002
1-2	515 (84.3)	298 (86.6%)	68 (73.1%)	
3-4	90 (14.7)	46 (13.4%)	25 (26.9%)	
Lymph node status (pN) (n=573)				0.116
positive (1-2)	279 (48.7)	157 (48.6%)	50 (58.1%)	
negative	294 (51.3)	166 (51.4%)	36 (41.9%)	
p53 immunohistochemistry (n=434)				0.220
normal (negative)	348 (80.2)	204 (77.9%)	60 (84.5%)	
aberrant (posotive)	86 (19.8)	58 (22.1%)	11 (15.5%)	

5.5 Characteristics of *CHEK2* 1100delC carrier tumors (II, Kilpivaara et al., unpublished)

The set of 1297 unselected breast cancer cases had altogether 1365 primary breast tumors, and clinical and histopathologic information was available for 1338 tumors. An association was observed between *CHEK2* 1100delC carriers and higher tumor grade (p=0.021). Specifically, there were less grade one tumors among *CHEK2* 1100delC carriers than among noncarriers (p=0.008). A higher frequency of ER-positive tumors was noted among *CHEK2* 1100delC carriers (91.2% vs. 78.3%, p=0.071), and although not statistically significant, this finding is in line with the observation of high frequency of ER-positive tumors among crossly reduced CHEK2 immunohistochemical staining. Generally, the tumors from *CHEK2* 1100delC carriers and noncarriers were similar. The mean age at diagnosis was 57 years. The strongest association of *CHEK2* 1100delC was observed with bilaterality, as also reported previously (Vahteristo et al., 2002). The proportion of bilateral tumors among *CHEK2* 1100delC tumors was significantly higher than the proportion of bilateral tumors among noncarriers (18.8% vs. 4.9%, p=0.005). Detailed information of tumor characteristics and the association with *CHEK2* 1100delC is presented in Table 6.

We also analyzed survival among all our breast cancer cases who were evaluated for *CHEK2* 1100delC and were eligible for survival analysis (n=743), mean follow-up 1385 days, 95% CI=1345-1426, limited to 5 years (1826 days)) (Kilpivaara et al., unpublished). *CHEK2* 1100delC was not associated with overall (or breast cancer-specific) survival of patients in our material. The disease-free survival was poorer in 1100delC carriers (p=0.03) and was likely due to poorer survival with regard to diagnosis of contralateral breast cancer (p=0.001) (see Figure 3). The disease-free survival with regard to a recidive tumor, distant metastasis, or primary cancer in other organs was similar in *CHEK2* 1100delC carriers and noncarriers.

Table 6. *Characteristics of 1338 tumors from 1297 unselected breast cancer patients analyzed for CHEK2 1100delC. (Adapted from Kilpivaara O. et al., International Journal of Cancer, 113(4): 575-580, 2005.)*

		CHEK2		***p***
	Total (%)	**wt**	**1100delC**	
	1338	1301 (97.2)	37 (2.8)	
Histology (n=1328)				
ductal	966(72.2)	935 (71.9)	31 (83.8)	
lobular	190 (14.2)	188 (14.5)	2 (5.4)	
medullary	16 (1.2)	15 (1.2)	1 (2.7)	
other	156 (11.7)	154 (11.9)	2 (0.6)	
Tumor grade (n=1119)				0.021
1	300 (26.8)	298 (27.4)	2 (6.3)	
2	496 (44.3)	476 (43.8)	20 (62.5)	
3	323 (28.9)	313 (28.8)	10 (31.3)	
Estrogen receptor status (n=1242)				0.071
positive	977 (78.8)	946 (78.3)	31 (91.2)	
negative	265 (21.3)	262 (21.7)	3 (8.8)	
Progesterone receptor status (n=1243)				0.963
positive	809 (65.1)	787 (65.1)	22 (64.7)	
negative	434 (34.9)	422 (34.9)	12 (35.3)	
Tumor size (pT) (n=1278)				0.145*
1-2	1207 (94.4)	1174 (94.6)	33 (89.2)	
3-4	71 (5.6)	67 (5.4)	4 (10.8)	
Lymph node status (pN) (n=1224)				0.412
negative	712 (58.2)	694 (58.4)	18 (51.4)	
positive (1-2)	512 (41.8)	495 (41.6)	17 (48.6)	
Distant metastasis (n=1235)				0.813
negative	1188 (96.2)	1156 (96.2)	32 (97.0)	
positive	47 (3.8)	46 (3.8)	1 (3.0)	
Laterality (n=1297)				
bilateral breast carcinoma	68 (5.2)	62 (4.9)	6 (18.8)	0.005*
unilateral breast carcinoma	1229 (94.8)	1203 (95.1)	26 (81.3)	

* Fisher's exact test

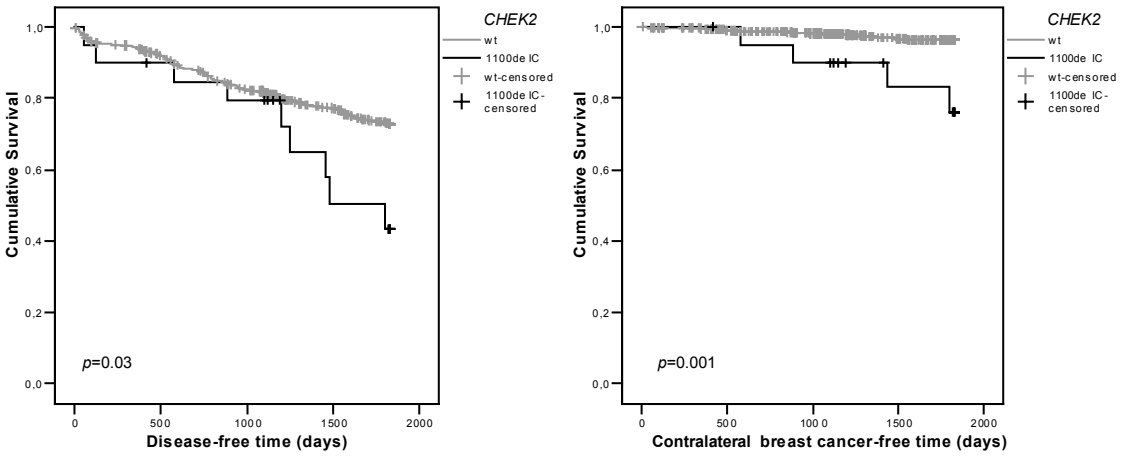

Figure 3 *Kaplan-Meier survival curves for disease-free and contralateral breast cancer-free survival with regard to CHEK2 1100delC carriership.*

5.6 Characteristics of *CHEK2* I157T – positive tumors (Kilpivaara et al., unpublished)

We analyzed the characteristics of 745 breast tumors from 697 unselected breast cancer cases, including 53 cases with bilateral disease (101 tumors) (Syrjäkoski et al., 2000; II). Tumors from *CHEK2* I157T carriers are of lower grade than those from noncarriers (p=0.01). (The proportions of *CHEK2* I157T carriers among grade 1, 2, and 3 tumors are 11.0%, 5.4%, and 3.7%, respectively.), Otherwise the tumors are very similar. Furthermore, there is no difference in either overall or disease-free survival (metastasis-free, cancer-free, contralateral breast cancer-free, recidive-free, and all combined) between *CHEK2* I157T carriers and noncarriers (data not shown). The details of tumors characteristics are presented in Table 7.

Table 7. *Characteristics of 745 breast tumors from 697 unselected breast cancer patients analyzed for CHEK2 I157T.*

		CHEK2		***p***
	Total (%)	**wt**	**I157T**	
	745(100.0)	699(93.8)	46(6.2)	
Histology (n=705)				
ductal	519(69.8)	487(65.5)	32(69.6)	
lobular	118(15.9)	110(15.8)	8(17.4)	
medullary	8(1.1)	8(1.1)	0(0.0)	
other	60(8.5)	56(8.5)	4(9.1)	
Tumor grade (n=675)				0.01
1	163(24.1)	145(22.9)	18(42.9)	
2	298(44.1)	282(44.5)	16(38.1)	
3	214(31.7)	206(32.5)	8(19.0)	
Estrogen receptor status (n=704)				0.93
positive	553(78.6)	519(78.5)	34(79.1)	
negative	151(21.4)	142(21.5)	9(20.9)	
Progesterone receptor status (n=704)				0.29
positive	489(69.5)	456(69.0)	33(76.7)	
negative	215(30.5)	205(31.0)	10(23.3)	
Tumor size (pT) (n=725)				1.00*
1-2	671(92.6)	629(92.5)	42(93.3)	
3-4	54(7.4)	51(7.5)	3(6.7)	
Lymph node status (pN) (n=721)				0.55
negative	387(53.7)	362(53.4)	25(58.1)	
positive (1-2)	334(46.3)	316(46.6)	18(41.9)	
Distant metastasis (n=709)				0.46*
negative	672(94.8)	635(94.9)	37(92.5)	
positive	37(5.2)	34(5.1)	3(7.6)	

*Fisher's exact test

5.7 *CHEK2* 1100delC in colorectal cancer susceptibility and HBCC (III)

Screening colorectal cancer cases for *CHEK2* 1100delC resulted in identification of 17 carriers among 662 cases (2.6%). The frequency was 1.3% (2/149) among cases with a family history of colorectal cancer, and nominally higher in cases without a family history of colorectal cancer (15/513, 2.9%). The frequency of *CHEK2* 1100delC is higher in Eastern Finland than in other parts of Finland. Since the majority of CRC cases originated from Eastern-Central Finland, we adjusted the population control frequency to match the geographical distribution of patients (matched frequency 1.9%). No significant difference was observed in frequencies between CRC cases and population controls, *p*-values and odds ratios with 95% confidence intervals for all cases, familial cases, and nonfamilial cases are *p*=0.266, OR=1.393, 95% CI=0.775-2.504; *p*=1.000 OR=0.720, 95% CI=0.172-3.020; and *p*=0.134 OR=1.592, 95% CI=0.863-2.939, respectively.

Eighty families (15.8%) of our set of 507 familial breast cancer cases previously analyzed for CHEK2 1100delC (Vahteristo et al., 2002) also contain CRC cases, and 19 of these (3.7% of all) fulfill the definition of HBCC. The frequency of *CHEK2* 1100delC was very similar in breast cancer families with or without CRC cases (4/80, 5.0% among breast and CRC families; 1/19, 5.3% among HBCC families; and 24/427, 5.6% among breast cancer families).

All 17 CRC cases (2 familial cases and 15 nonfamilial cases) who carried the *CHEK2* 1100delC mutation were analyzed for loss of heterozygosity (LOH) at *CHEK2* exon 10 in their colorectal tumors. LOH was observed in three cases (one familial, two nonfamilial). In two cases, the wt allele was lost, and in one nonfamilial case the mutated allele was lost.

5.8 *CHEK2* I157T is associated with familial and sporadic colorectal cancer (IV)

A total of 972 colorectal cancer patient (noncancerous tissue) samples were successfully analyzed for *CHEK2* I157T. Seventy-six carriers (7.8%) of this variant were identified. This frequency is significantly higher than in the normal Finnish population (5.3%, OR=1.5, 95% CI=1.1-2.1, *p*=0.008). The frequency of *CHEK2* I157T was even higher among CRC patients with a family history of colorectal cancer (10.4%). The details of frequencies in different groups are presented in Table 8. Furthermore, the relationships between characteristics of colorectal tumors and *CHEK2* I157T status were examined. None of the characteristics (tumor location, tumor grade, tumor stage (Dukes), RER status) was associated with *CHEK2* I157T. Age at diagnosis was also similar in variant carriers and non-carriers (67 years). *CHEK2* I157T was more frequent in cases with a family history of any type of cancer (three or more cancer cases in first-degree family members, including the index case); 29/290, 10.0% vs. 47/679, 6.9% (*p*=0.10). In addition, patients with multiple primary tumors were more likely to be carriers of *CHEK2* I157T (16/140, 11.4% vs. 60/832, 7.2%; p=0.09).

Table 8. *Frequency of CHEK2 I157T in colorectal cancer cases and controls.*

	+ve/Total	%	*p* *	OR*	95% CI
Controls	100/1885	5.3		1.0	
All CRC Cases	76/972	7.8	0.008	1.5	1.1-2.1
Familial CRC Cases	14/135	10.4	0.01	2.1	1.1-3.7
Non-familial CRC Cases	62/837	7.4	0.03	1.4	1.0-2.0

*vs. population controls

Family history of cancer is defined as three or more cancer cases in first-degree family members, including the index case.
Family history of CRC is defined as two or more CRC cases in first-degree family members, including the index case.

6 Discussion

6.1 *CHEK2* I157T in breast cancer predisposition (I)

CHEK2 germline variant I157T is associated with breast cancer risk. The associated risk is, however, smaller than the twofold risk conferred by *CHEK2* 1100delC (Vahteristo et al., 2002). Unlike 1100delC, this variant is not associated with a family history of breast cancer, although both identified homozygous carriers of I157T had a first-degree relative with breast cancer. The population frequency of I157T is relatively high in Finland. In addition to our observation (5.3%), another study reported a frequency of 6.5% (13/200) in Northern Finland (Allinen et al., 2001). A similar figure was reported from Poland, where the frequency of *CHEK2* I157T is 4.8% (193/4000) (Cybulski et al., 2004a). An even stronger association was observed in a study with combined Byelorussian and German breast cancer cases (OR=4.1, 95% CI= 1.8-9.2, p<0.001) (Bogdanova et al., 2005). *CHEK2* I157T seems to be a genetic variant specific to Northern/Eastern European populations. In other studied populations, the variant is apparently very rare or absent (Table 9) (Friedrichsen et al., 2004; Schutte et al., 2003). Based on breast cancer incidence in Finland (IARC, 1997), this variant would confer an estimated absolute risk of 8.1% by age 70, compared with 5.5% absolute risk in noncarriers, and 2.2% of all breast cancer cases in Finland would be attributable to *CHEK2* I157T. Studies on *CHEK2* in Finland and Poland have shown similar results on variant I157T frequencies in breast, colorectal, and prostate cancer (Table 9). The frequencies of other *CHEK2* variants vary between Finland and Poland; the frequency of 1100delC is higher in Finland, whereas the frequency of IVS2+1G>A is higher in Poland (Cybulski et al., 2004a; Vahteristo et al., 2002; Kilpivaara et al., unpublished). The large deletions in *CHEK2* observed in Poland are currently under investigation in Finland (unpublished data).

In screening of the *CHEK2* coding region for germline variants in breast cancer families, we identified nine different changes in the DNA sequence. There were

four cases of both 1100delC and I157T, which were the only alterations that would likely lead to a change in the CHEK2 protein.

The CHEK2 I157T protein seems to be stable compared with CHEK2 1100delC and R145W (Bartkova et al., 2001; Lee et al., 2001). Similar observations were also made when CHEK2 I157T was studied in functional assays; CHEK2 I157T behaves like wt CHEK2 when it comes to stability and modification after IR exposure. Previous studies have, however, proven that CHEK2 I157T is defective in phosphorylating and binding its substrates, including p53, Cdc25A, and BRCA1 (Falck et al., 2001a; Falck et al., 2001b; Li et al., 2002). Furthermore, CHEK2 I157T has been reported to undermine the normal functions of CHEK2 when coexpressed in human cell culture (Falck et al., 2001b). Since dimerization is an important step in DNA damage-induced CHEK2 activation, the observation that CHEK2 I157T can form a dimer with wt CHEK2 is of great importance. The defective characteristics of CHEK2 I157T may thus have an effect on the functional CHEK2 pool in a cell.

Immunohistochemical studies on breast tumors from *CHEK2* I157T carriers showed no marked difference in CHEK2 expression patterns or expression levels compared to tumors of wt *CHEK2* carriers.

6.1.1 Characteristics of *CHEK2* I157T – positive tumors (Kilpivaara et al., unpublished)

CHEK2 I157T has been suggested to be strongly associated with breast cancer of lobular histology (Huzarski et al., 2005). We studied the characteristics of breast tumors in our material, but we found no association with lobular histology. The frequency of I157T among lobular cancers was 6.8%, which is slightly higher than the 6.2% observed among all tumor histologies, but this is not a significant difference. The only significant finding was an association of *CHEK2* I157T with lower grade (grade 1) tumors. However, the number of tumors in the *CHEK2* I157T group was quite small (n=46), and thus one needs to be careful when drawing conclusions. Cybulski et al. (2006) recently reported characteristics of

CHEK2 mutation-positive breast tumors among younger women (age at diagnosis < 51 years). Interestingly, they combined all *CHEK2* mutation carriers into one group and compared them with noncarriers. The majority of *CHEK2* mutation carriers are, however, carriers of I157T (207/252), and thus, the characteristics of mutation carriers largely reflect the characteristics of I157T carriers. Cybulski et al. (2006) report an association with lobular histology and also characterize the *CHEK2* mutation carrier tumors as being larger and the mutation carriers as more often having a family history of breast cancer. Their conclusion is supported by our observation that tumors from *CHEK2* mutation-positive cases are similar to breast cancers in the population at large. We saw no difference in either disease-free survival or overall survival between *CHEK2* I157T carriers and noncarriers. In another study, however, metastasis-free survival of *CHEK2* I157T carriers was reported to be worse than that of noncarriers based on a very small number of cases (n=13) (Meyer et al., 2007).

6.2 Characteristics of breast tumors from *CHEK2* 1100delC carriers (II; Kilpivaara et al., unpublished)

Characteristics of breast tumors may give cues to predict prognosis and may help in determining the optimal treatment for a cancer type. CHEK2 protein expression has been found to be absent or grossly reduced in tumors from *CHEK2* 1100delC carriers, which is in accordance with the truncating nature of the mutation (Vahteristo et al., 2002; Oldenburg et al., 2003). *CHEK2* 1100delC has previously been shown to be strongly associated with family history of breast cancer (Meijers-Heijboer et al., 2002; Vahteristo et al., 2002) and with bilateral disease (Vahteristo et al., 2002; de Bock et al., 2004). In our analysis of 1297 unselected breast cancer cases exploring relationships of tumor characteristics or clinicopathologic features with *CHEK2* 1100delC, we observed *CHEK2* 1100delC to be associated with higher tumor grade (p=0.02). Nearly 94% of tumors from *CHEK2* 1100delC carriers were grade 2-3, when the corresponding number for tumors from wt *CHEK2* patients was 73%. Schmidt et al. (2007) studied younger breast cancer cases (diagnosed before 50 years) and they found no difference in

grades. *CHEK2* 1100delC tumors seem, however, to be estrogen receptor–positive, although the difference here is not statistically significant. An association with positive hormone receptor status was observed for both estrogen and progesterone receptors in a Dutch study (de Bock et al., 2004), and the 1100delC was shown to be more prevalent among ER-positive tumor carriers (de Bock et al., 2006; Schmidt et al., 2007). A report of 13 carrier tumors suggested a contradictory view, with *CHEK2* 1100delC being associated with breast carcinoma carrying characteristics of the basal phenotype (Dede et al., 2006) such as high histological grade, lack of hormone receptors, and HER-2 expression (Banerjee et al., 2006).

In our study, the strongest association of 1100delC was seen with bilaterality of the disease, thus strengthening the previous results. This same phenomenon was observed in another study series, where it was also suggested that IR treatment could be a risk factor for *CHEK2* 1100delC carriers for development of contralateral breast tumor (Broeks et al., 2004). In addition, similar findings were recently reported in Russia, where 1100delC was found to be associated with bilateral breast cancer, but also with early onset of the disease (Chekmariova et al., 2006). De Bock et al. (2004) also reported poorer disease-free survival for *CHEK2* 1100delC carriers with regard to survival without distant metastases and survival without contralateral breast cancer, but did not observe an effect on patients' overall survival. Further results for a similarity between *CHEK2* 1100delC carrier and noncarrier tumors were reported by Schmidt et al. (2007), who also observed worse recurrence-free survival that could not be explained by increased risk for contralateral breast cancer. We found no difference in overall survival with regard to *CHEK2* 1100delC. Disease-free survival, however, is poorer among *CHEK2* 1100delC carriers, largely due to an increased risk for contralateral breast cancer. A very recent study combined *CHEK2* mutation carriers (1100delC, I157T, and IVS2+1G>A) into one group and observed worse metastasis-free survival than in noncarriers of *CHEK2* mutations (Meyer et al., 2007). Although their material was quite small, their finding is interesting since the cases all received postoperative radiotherapy following breast-conservative surgery, which gives

room to speculate whether functional CHEK2 is especially needed in cells exposed to radiation-inducing DSBs.

6.2.1 CHEK2 protein expression in breast cancer (II)

We have previously shown that reduced CHEK2 protein expression is strongly associated with CHEK2 germline mutation 1100delC and that an overall reduction in expression was present in 21/124 breast tumors (16.9%) (Vahteristo et al., 2002). We then analyzed the CHEK2 expression in a separate series of 611 breast tumors unselected for family history. A similar proportion of tumors with reduced CHEK2 expression was identified (21.1%). Tumors with reduced CHEK2 expression were larger than normally expressing tumors, and especially pT=4 class tumors were prominent among aberrantly staining tumors (p=0.0003). A similar trend was observed among *CHEK2* 1100delC carriers, but the analysis is limited by the small number of 1100delC carriers. An interesting detail is that among tumors with the most aberrant CHEK2 expression (reduced number of stained cells and reduced intensity of staining) a great majority of tumors are ER-positive (94%), which is in line with enriched ER-positivity observed among *CHEK2* 1100delC carrier tumors, as well as with a recent report where an inverse correlation between CHEK2 and ER expressions was observed (Hinnis et al., 2007). In our material, CHEK2 expression status of a tumor did not have an effect on patients' overall survival. Honrado et al. (2005) investigated the expression of DNA repair proteins in breast tumors and noted that *BRCA* 1/2-positive tumors more often show CHEK2 expression than familial non*BRCA1/2* tumors or sporadic tumors. Furthermore, they suggested that by combining CHEK2 with the Rad51 protein expression profile, *BRCA2*-positive tumors could be distinguished from familial non*BRCA1/2* tumors (Honrado et al., 2005).

Overall, breast tumors with reduced CHEK2 expression and tumors from *CHEK2* 1100delC carriers are similar, which is in line with the association between the mutation and aberrant protein expression. However, the 1100delC mutation frequency is low compared with aberrant protein expression in tumors. Moreover,

inconsistent observations have been made about LOH, and there may be other factors that contribute to somatic *CHEK2* inactivation, e.g. through epigenetic silencing or variation in splicing (Sullivan et al., 2002; Kato et al., 2004; Staalesen et al., 2004).

6.3 *CHEK2* mutations in HBCC and colorectal cancer (III and IV)

Meijers-Heijboer et al. (2003) originally proposed that *CHEK2* 1100delC is associated with the HBCC (hereditary breast and colorectal cancer) phenotype. They observed that 1100delC was more common in families with breast and colorectal cancer (n=55) than in families with only breast cancer (18.2% vs. 4.0%) (Meijers-Heijboer et al., 2003). We studied the 1100delC mutation in Finnish colorectal cancer families and families with defined HBCC phenotype and observed no significantly higher frequency of *CHEK2* 1100delC in CRC patients or HBCC families (n=19) compared with population controls or breast cancer families. All of the identified CRC patient carriers of *CHEK2* 1100delC (n=17) were analyzed for an allelic imbalance at *CHEK2* exon 10, where 1100delC resides. Only three cases showed loss of the mutated allele and in one case the wt allele was lost in tumor tissue, which supports the observation that *CHEK2* 1100delC may not be a susceptibility allele for CRC. CHEK2 expression has been studied at the protein level in 564 colorectal cancer cases, where 29 (5%) had lost CHEK2 expression and only three of those were carriers of 1100delC, suggesting that other mechanisms are involved in inactivating CHEK2 expression (van Puijenbroek et al., 2005).

A recent study reports only one 1100delC carrier family among 113 tested British HBCC families, thus not supporting *CHEK2*'s role in HBCC (Naseem et al., 2006). In another study, British patients with breast and colorectal cancers were screened for 1100delC, but no carriers were identified among 97 cases (Brinkman et al., 2006). Isinger et al. (2006) investigated *CHEK2* 1100delC in tumors of 75 Swedish patients with metachronous cancer of the breast and colorectum, identifying two carriers of 1100delC, which was similar to the frequency observed

in controls, thus not supporting the idea of *CHEK2* 1100delC being a risk allele for combined breast and colorectal cancer. Also in Sweden, *CHEK2* 1100delC was observed at a similar frequency in CRC cases and controls (Djureinovic et al., 2006). Furthermore, this lack of *CHEK2* 1100delC has been reported in 34 Spanish HBCC families; however, this information is of questionable value since no 1100delC carriers have been reported in Spain in general (Collado et al., 2004; Osorio et al., 2004; Bellosillo et al., 2005; Sanchez de Abajo et al., 2005). Another study from the Netherlands showed similar results to our study, as they also observed no association between *CHEK2* 1100delC and CRC, with frequencies of 10/629 in unselected CRC cases (1.6%) and 1/230 in controls (0.4%) (de Jong et al., 2005). Further evidence that *CHEK2* 1100delC may not be a CRC susceptibility allele comes from a study with colorectal adenoma cases in the UK. Again, 1100delC was found at a similar frequency as in population controls (3/149, 2.0% vs. 18/1620, 1.1%) (Lipton et al., 2003). *CHEK2* 1100delC in colorectal cancer has been investigated in Poland in combination with other truncating mutations in *CHEK2* (Cybulski et al., 2007). These authors identified only 11 carriers of truncating CHEK2 mutation among 1085 unselected CRC cases (1.0%), which is a very similar proportion as among controls (58/5496, 1.1%). Together these studies show that *CHEK2* 1100delC is likely not a predisposing allele for colorectal cancer, and it also seems unlikely that it would be behind the familial aggregation of breast and colorectal cancers, observed in the HBCC phenotype. There may, however, be population-specific differences and other contributing alleles, making the evaluation of the role of 1100delC challenging.

In contrast to *CHEK2* 1100delC, the I157T variant shows a consistent association with colorectal cancer. Cybulski et al. (2004a) first suggested that *CHEK2* may be a multiorgan cancer susceptibility gene and that I157T was associated with cancer at several sites including colorectum. Given that results from Finnish and Polish studies on breast and prostate cancers and *CHEK2* I157T are fairly consistent, we studied the frequency of *CHEK2* I157T in Finnish colorectal cases (I; Seppälä et al., 2003; Cybulski et al., 2004b; Gorski et al., 2005;) (see Table 9).

Furthermore, we assessed the previously unstudied association of *CHEK2* I157T with familial CRC. Our results indicate that *CHEK2* I157T is associated with colorectal cancer risk in Finland, conferring a 1.5-fold risk for carriers (95% CI=1.1-2.1, *p*=0.008). These results are very similar to those of the Polish study (Cybulski et al., 2004a). The OR for association with familial CRC was 2.1 (95% CI=1.1-3.7, *p*=0.01).

We also observed a trend towards a higher frequency of I157T in CRC patients who have a family history of any cancer type or who themselves have multiple primary tumors, supporting the proposed multiorgan susceptibility allele function for *CHEK2* I157T. Similarly, the I157T variant was more common among our unselected breast cancer cases with multiple cancers (other than breast cancer) than among those with one primary breast cancer (8/68, 11.8% vs. 36/518, 6.5%) (IV).

Cybulski et al. (2007) raised the important and obvious question of wheter the truncating and missense mutations have different effects on colorectal cancer risk. This has been observed in VHL disease patients, whose risk of pheochromocytoma is increased when they have missense mutation in the *VHL* gene, whereas patients with truncating mutations do not develop pheochromocytoma (Crossey et al., 1994; Chen et al., 1995).

In addition to I157T, a few other missense mutations have been identified in colorectal cancer. Two of 119 Icelandic CRC patients were found to carry *CHEK2* T59K, a missense variant of unknown effect on function. This variant was also identified in other cancer cases, but not in controls (Ingvarsson et al., 2002). Brinkman et al. (2006) failed to find any 1100delC carriers among combined breast and CRC cases, but they did observe two novel missense variants in the kinase domain: N405K and Y390C.

Studies on *CHEK2* I157T have been limited by this variant seemingly only being present only in Eastern/Central European populations. However, the results on

cancer susceptibility in these populations show a consistent association with increased risk of breast, colorectal, and prostate cancers.

Table 9. *Frequency of CHEK2 I157T in breast, prostate, and colorectal cancers in different populations.*

	Cases		Controls					
Study group	**%**	**+ve/Total**	**%**	**+ve/Total**	**OR***	**95% CI**	***p***	**Reference**
Breast cancer								
Unselected cases								
Finnish	7.4	77/1035	5.3	100/1885	1.4	1.1-2.0	0.02	I (Kilpivaara et al., 2004)
Byelorussian	5.6	24/424	1.3	4/307	4.5	1.6-13.2	0.005	Bogdanova et al., 2005
German	2.2	22/996	0.6	3/486	3.6	1.1-12.2	0.044	Bogdanova et al., 2005
Polish	6.7	68/1017	4.8	193/4000	1.4	1.1-1.9	0.02	Cybulski et al., 2004b
US (Washington, dg<45yrs)	0.4	2/506	0.9	4/459	0.5	0.1-2.5	0.35	Friedrichsen et al., 2004
Familial cases (BRCA1/2 neg)								
British	0.0	0/193	0.0	0/448	-	-	-	Schutte et al., 2003
Byelorussian	6.3	6/96	1.3	4/307	5.1	1.4-18.3	0.019	Bogdanova et al., 2005
Dutch	0.0	0/225	0.0	0/181	-	-	-	Schutte et al., 2003
Finnish	8.9	7/79	6.5	13/200	1.4	0.5-3.7	0.49	Allinen et al., 2001
Finnish	5.5	28/507	5.3	100/1885	1.0	0.7-1.6	0.85	I (Kilpivaara et al., 2004)
German	1.9	10/516	1.6	8/500	1.2	0.5-3.1	0.68	Dufault et al., 2004
German	0.6	1/156	0.6	3/486	1.0	0.1-10.1	0.581	Bogdanova et al., 2005
US	0.7	2/272	1.1	1/94	-	-	-	Schutte et al., 2003
***BRCA1/2* carriers**								
British	0.0	0/47	0.0	0/448	-	-	-	Schutte et al., 2003
Dutch	0.0	0/141	0.0	0/181	-	-	-	Schutte et al., 2003
US	0.0	0/147	1.1	1/94	-	-	-	Schutte et al., 2003
Prostate cancer								
Unselected cases								
Finnish	7.8	42/537	5.4	26/480	1.5	0.9-2.5	0.13	Seppälä et al., 2003
Polish	7.8	54/690	4.8	29/600	1.7	1.1-2.7	0.03	Cybulski et al., 2004a
US (Minnesota), sporadic cases	1.5	6/400	1.2	5/423	0.6	0.2-2.7	0.53	Dong et al., 2003
Familial cases								
Finnish	10.8	13/120	5.4	26/480	2.1	1.1-4.3	0.04	Seppälä et al., 2003
Polish	16.3	16/98	4.8	29/600	3.8	2.0-7.4	0.00002	Cybulski et al., 2004a
US (Minnesota)	1.8	7/400	1.2	5/423	1.5	0.5-4.7	0.50	Dong et al., 2003
Colorectal cancer								
Unselected cases								
Polish	9.3	28/300	4.8	193/4000	2.0	1.3-3.1	0.001*	Cybulski et al., 2004b
Finnish	7.8	76/896	5.3	100/1885	1.5	1.1-2.1	0.008	IV (Kilpivaara et al., 2006)
Familial cases								
Finnish	10.4	14/135	5.3	100/1885	2.1	1.1-3.7	0.01	IV (Kilpivaara et al., 2006)

*Fisher's exact test

7 Concluding remarks

The role of CHEK2 in breast and colorectal cancer susceptibility in Finland was investigated. *CHEK2* I157T was found to be a low-penetrance breast cancer susceptibility allele, conferring a 1.4-fold risk for carriers. Reduced or absent CHEK2 protein expression was observed in one-fifth of breast tumors from patients unselected for family history, implying that defective CHEK2 signaling contributes to tumorigenesis. Reduction in CHEK2 expression was more common in tumors with larger diameter and ER expression, but with regard to other tumor characteristics and prognosis of a patient no association was observed. The evaluation of *CHEK2* 1100delC tumors is complicated by the low frequency of the variant. Results from comparison of *CHEK2* 1100delC carrier tumors with noncarrier tumors were in line with the findings from the CHEK2 expression study. Tumors from *CHEK2* 1100delC carriers were more often of higher grade than tumors from noncarriers, and they also tended to be ER-positive more often, although generally 1100delC status does not seem to radically affect the tumor characteristics.

Our results from a large set of CRC cases suggest that *CHEK2* 1100delC may not be a susceptibility allele for CRC, although a very small effect cannot be excluded. Furthermore, *CHEK2* 1100delC is equally frequent in HBCC phenotype families and in breast cancer families.

Over 1000 CRC cases were screened for *CHEK2* I157T, and a significantly higher frequency of I157T was observed among both familial and sporadic CRC cases. The relation of *CHEK2* I157T with familial CRC has not been studied previously. CHEK2 I157T seems to be a susceptibility allele for both familial and sporadic CRC, conferring a 1.5-fold risk for carriers of this variant. *CHEK2* I157T has been proposed to have a role as a multiple cancer susceptibility allele, which is supported by our results since we observed a higher frequency of the variant among cases with multiple primary tumors or those with a family history of cancer.

During the last five years *CHEK2* has established its role as an important cancer susceptibility gene. It has become apparent that *CHEK2* is a low-penetrance susceptibility gene for several cancer types, significantly contributing to familial cancer risk as well as to cancer risk at the population level. However, many challenges remain. Cellular processes are all about interactions and most certainly there are several other low-penetrance cancer susceptibility alleles and genes to be identified, and genes with a synergistic risk effect with *CHEK2* variants. Their identification is demanding since there are, as we have seen, differences in variant frequencies between populations, and the contribution to risk may also vary. While many pieces of the cancer puzzle have been identified, we still need to assemble the big picture, finding the interactions and how they are formed. This is the goal for the future, and I am humbled in being able to locate one piece of this puzzle.

8 Acknowledgments

This study was carried out at the Department of Obstetrics and Gynecology, Helsinki University Central Hospital, during 2002-2006. My sincere thanks go to all of the people who contributed to this project:

Professor Olavi Ylikorkala, director of the Institute of Clinical Medicine, Professor Maija Haukkamaa, the administrative head of our department, and Professor Jorma Paavonen, the director of the Department of Obstetrics and Gynecology for providing excellent research facilities.
My supervisor, Docent Heli Nevanlinna, for her guidance in the world of breast cancer genetics, her continuous enthusiasm, and support during these years.
Helsinki Biomedical Graduate School (HBGS) for help and advice and for travel and lab visit grants.
My excellent PhD Thesis Committee members, Professors Anne Kallioniemi and Seppo Pyrhönen, for scientific conversations and encouragement.
Official reviewers, Professor Seppo Pyrhönen and Docent Johanna Schleutker, for insightful and constructive comments on my thesis.
My author-editor, Carol Ann Pelli, for thesis language revision.

All coauthors for making it all go so smoothly: Professor Lauri Aaltonen; Professor Kristiina Aittomäki; Pia Alhopuro, MD; Professor Jiri Bartek; Jirina Bartkova, MD, PhD; Professor Carl Blomqvist; Hannaleena Eerola, MD, PhD; Jacob Falck, PhD; Päivi Heikkilä, MD, PhD; Professor Kaija Holli, Professor Olli-Pekka Kallioniemi, Päivi Laiho, PhD; Jiri Lukas Vet. MD, PhD; Professor Guido Sauter; Kirsi Syrjäkoski, PhD; and Pia Vahteristo, PhD.
Research nurse Sini Marttinen, for helpfully answering my questions concerning CRC samples.

My former boss and mentor ever since, Professor Jim Schröder, for his optimism and encouraging “You can do it!” attitude.

Colleagues and coworkers, former and present, at the Department of Obstetrics and Gynecology and the Clinical Chemistry research laboratory as well as the Biomedicum Biochip Center for creating such a nice work atmosphere, when being busy in the lab and while on a coffee break. Especially, Reetta Jalkanen and Sari Tuupanen for sharing the ups and downs in life and science, sharing the office, and traveling together. Anna-Kaarina Järvinen for companionship on trips to Aulanko and Anaheim, for attending HBGS occasions together and for stumbling along the pathway to the doctorate together.
Miina Ollikainen, for great pieces of advice during last phases of PhD project and for refreshing coffee break discussions of more or less scientific topics.
Laila Selkinen for helping with all possible practical matters during these years.

Members of the HN group, former and present: Kirsimari Aaltonen, Hannaleena Eerola, Rainer Fagerholm, Jenny Forsström, Laura Hautala, Tuomas Heikkinen Kati Kämpjärvi, Minna Merikivi, Nina Puolakka, Matias Rantanen, Laura Sarantaus, Sirpa Stick, Jonna Tallila, Anitta Tamminen, Johanna Tommiska, Pia Vahteristo, and others who visited the HN group during these years.

Pia Vahteristo for handing off the CHEK2 legacy and for showing me the way, for putting pressure on me (I needed that), and for friendship.
Johanna Tommiska and Rainer Fagerholm for sharing the path to the doctorate together. It has definitely been an extraordinary journey. Skål!
Anitta Tamminen for answering my never-ending questions, and for sharing the good and bad days (and milk for coffee).
Kati Kämpjärvi for keeping up the CHEK2 research and for friendship extending beyond science.

My dear friends: Anne for being my “sister”; Tapsa for just being Tapsa; goddaughter Elsa and Lassi for bringing joy and laughter to my life; Anna, Saija (also for invaluable help with ‘karonkka’ arrangements), and Terhi for all the experiences together; all friends and their families for numerous unforgettable moments and for putting up with me.

Friends overseas, especially Helene, who has encouraged and supported me more than one could ever ask for.
Jonna, “my little sister”, for following me to the world of genetics research (against my advice ☺) and for lifelong friendship.
My dear cousin, Laura, for the thesis cover design.
Sports for balancing my life, teaching me the power of mind over body and enabling me to meet good friends, especially Iina, who forced me to do another push-up when my body had already given up.

My family, my dear mother Leena and my late father Kauko, for always loving me, encouraging me, believing in me, and letting me choose my own path in life without questioning. The entire Hukka and Kilpivaara clans for being there whenever needed and for proving that blood is definitely thicker than water.

All of the patients, who by volunteering to participate, made these studies possible.

I am grateful for the following organizations for financially supporting my work:
The Academy of Finland, the Helsinki University Central Hospital Research Fund, the Sigrid Juselius Foundation, the Biomedicum Helsinki Foundation, the Ida Montin Foundation, the Finnish Cultural Foundation (Uusimaa), the Finnish Cultural Foundation, the Emil Aaltonen Foundation, the Maud Kuistila Memorial Foundation, the Finnish Cancer Organisations, the Oskar Öflund Foundation, the Paulo Foundation, the University of Helsinki Funds (Medicine), Research and Science Foundation of Farmos, and the K. Albin Johansson Foundation.

Helsinki, May 2007

Otto Kilpivaara

9 References

Aaltonen,L.A., Salovaara,R., Kristo,P., Canzian,F., Hemminki,A., Peltomaki,P., Chadwick,R.B., Kaariainen,H., Eskelinen,M., Jarvinen,H., Mecklin,J.P. and de la Chapelle,A. (1998) Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. *N.Engl.J.Med.,* **338,** 1481-1487.

Ahn,J. and Prives,C. (2002) Checkpoint kinase 2 (Chk2) monomers or dimers phosphorylate Cdc25C after DNA damage regardless of threonine 68 phosphorylation. *J.Biol.Chem.,* **277,** 48418-48426.

Ahn,J., Urist,M. and Prives,C. (2003) Questioning the role of checkpoint kinase 2 in the p53 DNA damage response. *J.Biol.Chem.,* **278,** 20480-20489.

Ahn,J.Y., Li,X., Davis,H.L. and Canman,C.E. (2002) Phosphorylation of threonine 68 promotes oligomerization and autophosphorylation of the Chk2 protein kinase via the forkhead-associated domain. *J.Biol.Chem.,* **277,** 19389-19395.

Ahn,J.Y., Schwarz,J.K., Piwnica-Worms,H. and Canman,C.E. (2000) Threonine 68 phosphorylation by ataxia telangiectasia mutated is required for efficient activation of Chk2 in response to ionizing radiation. *Cancer Res.,* **60,** 5934-5936.

Allinen,M., Huusko,P., Mantyniemi,S., Launonen,V. and Winqvist,R. (2001) Mutation analysis of the CHK2 gene in families with hereditary breast cancer. *Br.J.Cancer,* **85,** 209-212.

Antoniou,A., Pharoah,P.D., Narod,S., Risch,H.A., Eyfjord,J.E., Hopper,J.L., Loman,N., Olsson,H., Johannsson,O., Borg,A., Pasini,B., Radice,P., Manoukian,S., Eccles,D.M., Tang,N., Olah,E., Anton-Culver,H., Warner,E., Lubinski,J., Gronwald,J., Gorski,B., Tulinius,H., Thorlacius,S., Eerola,H., Nevanlinna,H., Syrjäkoski,K., Kallioniemi,O.P., Thompson,D., Evans,C., Peto,J., Lalloo,F., Evans,D.G. and Easton,D.F. (2003) 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.,* **72,** 1117-1130.

Antoniou,A.C., Pharoah,P.D., McMullan,G., Day,N.E., Stratton,M.R., Peto,J., Ponder,B.J. and Easton,D.F. (2002) A comprehensive model for familial breast cancer incorporating BRCA1, BRCA2 and other genes. *Br.J.Cancer,* **86,** 76-83.

Bahassi el,M., Penner,C.G., Robbins,S.B., Tichy,E., Feliciano,E., Yin,M., Liang,L., Deng,L., Tischfield,J.A. and Stambrook,P.J. (2007) The breast cancer susceptibility allele CHEK2*1100delC promotes genomic instability in a knock-in mouse model. *Mutat.Res.,* **616,** 201-209.

Balmain,A., Gray,J. and Ponder,B. (2003) The genetics and genomics of cancer. *Nat.Genet.,* **33 Suppl,** 238-244.

Banerjee,S., Reis-Filho,J.S., Ashley,S., Steele,D., Ashworth,A., Lakhani,S.R. and Smith,I.E. (2006) Basal-like breast carcinomas: clinical outcome and response to chemotherapy. *J.Clin.Pathol.,* **59,** 729-735.

Bartek,J. and Lukas,J. (2003) Chk1 and Chk2 kinases in checkpoint control and cancer. *Cancer.Cell.,* **3,** 421-429.

Bartkova,J., Falck,J., Rajpert-De Meyts,E., Skakkebaek,N.E., Lukas,J. and Bartek,J. (2001) Chk2 tumour suppressor protein in human spermatogenesis and testicular germ-cell tumours. *Oncogene,* **20,** 5897-5902.

Baysal,B.E., DeLoia,J.A., Willett-Brozick,J.E., Goodman,M.T., Brady,M.F., Modugno,F., Lynch,H.T., Conley,Y.P., Watson,P. and Gallion,H.H. (2004)

Analysis of CHEK2 gene for ovarian cancer susceptibility. *Gynecol.Oncol.,* **95,** 62-69.

Bell,D.W., Varley,J.M., Szydlo,T.E., Kang,D.H., Wahrer,D.C., Shannon,K.E., Lubratovich,M., Verselis,S.J., Isselbacher,K.J., Fraumeni,J.F., Birch,J.M., Li,F.P., Garber,J.E. and Haber,D.A. (1999) Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome. *Science,* **286,** 2528-2531.

Bellosillo,B., Tusquets,I., Longaron,R., Perez-Lezaun,A., Bellet,M., Fabregat,X., Serrano,S. and Sole,F. (2005) Absence of CHEK2 mutations in Spanish families with hereditary breast cancer. *Cancer Genet.Cytogenet.,* **161,** 93-95.

Berg,J.W. and Hutter,R.V. (1995) Breast cancer. *Cancer,* **75,** 257-269.

Bernardi,R., Scaglioni,P.P., Bergmann,S., Horn,H.F., Vousden,K.H. and Pandolfi,P.P. (2004) PML regulates p53 stability by sequestering Mdm2 to the nucleolus. *Nat.Cell Biol.,* **6,** 665-672.

Bernstein,J.L., Teraoka,S.N., John,E.M., Andrulis,I.L., Knight,J.A., Lapinski,R., Olson,E.R., Wolitzer,A.L., Seminara,D., Whittemore,A.S. and Concannon,P. (2006) The CHEK2*1100delC allelic variant and risk of breast cancer: screening results from the Breast Cancer Family Registry. *Cancer Epidemiol.Biomarkers Prev.,* **15,** 348-352.

Bertrand,P., Saintigny,Y. and Lopez,B.S. (2004) p53's double life: transactivation-independent repression of homologous recombination. *Trends Genet.,* **20,** 235-243.

Bodmer,W. (1999) Familial adenomatous polyposis (FAP) and its gene, APC. *Cytogenet.Cell Genet.,* **86,** 99-104.

Bodmer,W.F. (2006) Cancer genetics: colorectal cancer as a model. *J.Hum.Genet.,* **51,** 391-396.

Bogdanova,N., Enssen-Dubrowinskaja,N., Feshchenko,S., Lazjuk,G.I., Rogov,Y.I., Dammann,O., Bremer,M., Karstens,J.H., Sohn,C. and Dork,T. (2005) Association of two mutations in the CHEK2 gene with breast cancer. *Int.J.Cancer,* **116,** 263-266.

Brinkman,H., Barwell,J., Rose,S., Tinworth,L., Sodha,N., Langman,C., Brooks,L., Payne,S., Fisher,S., Rowan,A., Tomlinson,I. and Hodgson,S. (2006) Evidence against a major genetic basis for combined breast and colorectal cancer susceptibility. *Clin.Genet.,* **70,** 526-529.

Broeks,A., de Witte,L., Nooijen,A., Huseinovic,A., Klijn,J.G., van Leeuwen,F.E., Russell,N.S. and van't Veer,L.J. (2004) Excess risk for contralateral breast cancer in CHEK2*1100delC germline mutation carriers. *Breast Cancer Res.Treat.,* **83,** 91-93.

Burt,R. and Neklason,D.W. (2005) Genetic testing for inherited colon cancer. *Gastroenterology,* **128,** 1696-1716.

Buscemi,G., Perego,P., Carenini,N., Nakanishi,M., Chessa,L., Chen,J., Khanna,K. and Delia,D. (2004) Activation of ATM and Chk2 kinases in relation to the amount of DNA strand breaks. *Oncogene,* **23,** 7691-7700.

Caligo,M.A., Agata,S., Aceto,G., Crucianelli,R., Manoukian,S., Peissel,B., Scaini,M.C., Sensi,E., Veschi,S., Cama,A., Radice,P., Viel,A., D'Andrea,E. and Montagna,M. (2004) The CHEK2 c.1100delC mutation plays an irrelevant role in breast cancer predisposition in Italy. *Hum.Mutat.,* **24,** 100-101.

Chehab,N.H., Malikzay,A., Appel,M. and Halazonetis,T.D. (2000) Chk2/hCds1 functions as a DNA damage checkpoint in G(1) by stabilizing p53. *Genes Dev.,* **14,** 278-288.

Chehab,N.H., Malikzay,A., Stavridi,E.S. and Halazonetis,T.D. (1999) Phosphorylation of Ser-20 mediates stabilization of human p53 in response to DNA damage. *Proc.Natl.Acad.Sci.U.S.A.,* **96,** 13777-13782.

CHEK2 Breast Cancer Case-Control Consortium. (2004) CHEK2*1100delC and susceptibility to breast cancer: a collaborative analysis involving 10,860 breast cancer cases and 9,065 controls from 10 studies. *Am.J.Hum.Genet.,* **74,** 1175-1182.

Chekmariova,E.V., Sokolenko,A.P., Buslov,K.G., Iyevleva,A.G., Ulibina,Y.M., Rozanov,M.E., Mitiushkina,N.V., Togo,A.V., Matsko,D.E., Voskresenskiy,D.A., Chagunava,O.L., Devilee,P., Cornelisse,C., Semiglazov,V.F. and Imyanitov,E.N. (2006) CHEK2 1100delC mutation is frequent among Russian breast cancer patients. *Breast Cancer Res.Treat.,* **100,** 99-102.

Chen,F., Kishida,T., Yao,M., Hustad,T., Glavac,D., Dean,M., Gnarra,J.R., Orcutt,M.L., Duh,F.M. and Glenn,G. (1995) Germline mutations in the von Hippel-Lindau disease tumor suppressor gene: correlations with phenotype. *Hum.Mutat.,* **5,** 66-75.

Chen,K., Jiang,Q.T. and He,H.Q. (2005) Relationship between metabolic enzyme polymorphism and colorectal cancer. *World J.Gastroenterol.,* **11,** 331-335.

Collado,M., Landt,O., Barragan,E., Lass,U., Cervera,J., Sanz,M.A. and Bolufer,P. (2004) Locked nucleic acid-enhanced detection of 1100delC*CHEK2 germ-line mutation in Spanish patients with hematologic malignancies. *Clin.Chem.,* **50,** 2201-2204.

Coyle,Y.M. (2004) The effect of environment on breast cancer risk. *Breast Cancer Res.Treat.,* **84,** 273-288.

Crossey,P.A., Richards,F.M., Foster,K., Green,J.S., Prowse,A., Latif,F., Lerman,M.I., Zbar,B., Affara,N.A. and Ferguson-Smith,M.A. (1994) Identification of intragenic mutations in the von Hippel-Lindau disease tumour suppressor gene and correlation with disease phenotype. *Hum.Mol.Genet.,* **3,** 1303-1308.

Cybulski,C., Gorski,B., Huzarski,T., Byrski,T., Gronwald,J., Debniak,T., Wokolorczyk,D., Jakubowska,A., Kowalska,E., Oszurek,O., Narod,S.A. and Lubinski,J. (2006) CHEK2-positive breast cancers in young Polish women. *Clin.Cancer Res.,* **12,** 4832-4835.

Cybulski,C., Gorski,B., Huzarski,T., Masojc,B., Mierzejewski,M., Debniak,T., Teodorczyk,U., Byrski,T., Gronwald,J., Matyjasik,J., Zlowocka,E., Lenner,M., Grabowska,E., Nej,K., Castaneda,J., Medrek,K., Szymanska,A., Szymanska,J., Kurzawski,G., Suchy,J., Oszurek,O., Witek,A., Narod,S.A. and Lubinski,J. (2004a) CHEK2 is a multiorgan cancer susceptibility gene. *Am.J.Hum.Genet.,* **75,** 1131-1135.

Cybulski,C., Huzarski,T., Gorski,B., Masojc,B., Mierzejewski,M., Debniak,T., Gliniewicz,B., Matyjasik,J., Zlowocka,E., Kurzawski,G., Sikorski,A., Posmyk,M., Szwiec,M., Czajka,R., Narod,S.A. and Lubinski,J. (2004b) A novel founder CHEK2 mutation is associated with increased prostate cancer risk. *Cancer Res.,* **64,** 2677-2679.

Cybulski,C., Wokolorczyk,D., Huzarski,T., Byrski,T., Gronwald,J., Gorski,B., Debniak,T., Masojc,B., Jakubowska,A., Gliniewicz,B., Sikorski,A., Stawicka,M., Godlewski,D., Kwias,Z., Antczak,A., Krajka,K., Lauer,W., Sosnowski,M., Sikorska-Radek,P., Bar,K., Klijer,R., Zdrojowy,R., Malkiewicz,B., Borkowski,A., Borkowski,T., Szwiec,M., Narod,S.A. and Lubinski,J. (2006a) A large germline deletion in the Chek2 kinase gene is associated with an increased risk of prostate cancer. *J.Med.Genet.,* **43,** 863-866.

Cybulski,C., Wokolorczyk,D., Huzarski,T., Byrski,T., Gronwald,J., Gorski,B., Debniak,T., Masojc,B., Jakubowska,A., van de Wetering,T., Narod,S.A. and Lubinski,J. (2006b) A deletion in CHEK2 of 5,395 bp predisposes to breast cancer in Poland. *Breast Cancer Res.Treat.,* Aug 8; [Epub ahead of print]

Cybulski,C., Wokolorczyk,D., Kladny,J., Kurzawski,G., Suchy,J., Grabowska,E., Gronwald,J., Huzarski,T., Byrski,T., Gorski,B., D Ecedil Bniak,T., Narod,S.A. and Lubinski,J. (2007) Germline CHEK2 mutations and colorectal cancer risk: different effects of a missense and truncating mutations? *Eur.J.Hum.Genet.,* **15,** 237-241.

Dapic,V., Carvalho,M.A. and Monteiro,A.N. (2005) Breast cancer susceptibility and the DNA damage response. *Cancer Control,* **12,** 127-136.

de Bock,G.H., Mourits,M.J., Schutte,M., Krol-Warmerdam,E.M., Seynaeve,C., Blom,J., Brekelmans,C.T., Meijers-Heijboer,H., van Asperen,C.J., Cornelisse,C.J., Devilee,P., Tollenaar,R.A. and Klijn,J.G. (2006) Association between the CHEK2*1100delC germ line mutation and estrogen receptor status. *Int.J.Gynecol.Cancer,* **16 Suppl 2,** 552-555.

de Bock,G.H., Schutte,M., Krol-Warmerdam,E.M., Seynaeve,C., Blom,J., Brekelmans,C.T., Meijers-Heijboer,H., van Asperen,C.J., Cornelisse,C.J., Devilee,P., Tollenaar,R.A. and Klijn,J.G. (2004) Tumour characteristics and prognosis of breast cancer patients carrying the germline CHEK2*1100delC variant. *J.Med.Genet.,* **41,** 731-735.

de Jong,M.M., Nolte,I.M., te Meerman,G.J., van der Graaf,W.T., de Vries,E.G., Sijmons,R.H., Hofstra,R.M. and Kleibeuker,J.H. (2002) Low-penetrance genes and their involvement in colorectal cancer susceptibility. *Cancer Epidemiol.Biomarkers Prev.,* **11,** 1332-1352.

de Jong,M.M., Nolte,I.M., Te Meerman,G.J., van der Graaf,W.T., Mulder,M.J., van der Steege,G., Bruinenberg,M., Schaapveld,M., Niessen,R.C., Berends,M.J., Sijmons,R.H., Hofstra,R.M., de Vries,E.G. and Kleibeuker,J.H. (2005) Colorectal cancer and the CHEK2 1100delC mutation. *Genes Chromosomes Cancer,* **43,** 377-382.

de Stanchina,E., Querido,E., Narita,M., Davuluri,R.V., Pandolfi,P.P., Ferbeyre,G. and Lowe,S.W. (2004) PML is a direct p53 target that modulates p53 effector functions. *Mol.Cell,* **13,** 523-535.

de The,H., Lavau,C., Marchio,A., Chomienne,C., Degos,L. and Dejean,A. (1991) The PML-RAR alpha fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. *Cell,* **66,** 675-684.

Debniak,T., Gorski,B., Cybulski,C., Kurzawski,G., Zlowocka,E., Kladny,J., Chosia,M. and Lubinski,J. (2004) Rarity of germline 1100delC mutation in CHK2 in patients with malignant melanoma of the skin. *Melanoma Res.,* **14,** 121-124.

Dede,D.S., Sari,E., Bulut,N. and Altundag,K. (2006) Does breast cancer associated with CHEK2 1100delC carry characteristics of basal phenotype? *Breast Cancer Res.Treat.,* Oct 25; [Epub ahead of print]

DeGregori,J. and Johnson,D.G. (2006) Distinct and Overlapping Roles for E2F Family Members in Transcription, Proliferation and Apoptosis. *Curr.Mol.Med.,* **6,** 739-748.

Djureinovic,T., Lindblom,A., Dalen,J., Dedorson,S., Edler,D., Hjern,F., Holm,J., Lenander,C., Lindforss,U., Lundqvist,N., Olivecrona,H., Olsson,L., Pahlman,L., Rutegard,J., Smedh,K., Tornqvist,A., Eiberg,H. and Bisgaard,M.L. (2006) The CHEK2 1100delC variant in Swedish colorectal cancer. *Anticancer Res.,* **26,** 4885-4888.

Dong,X., Wang,L., Taniguchi,K., Wang,X., Cunningham,J.M., McDonnell,S.K., Qian,C., Marks,A.F., Slager,S.L., Peterson,B.J., Smith,D.I., Cheville,J.C., Blute,M.L., Jacobsen,S.J., Schaid,D.J., Tindall,D.J., Thibodeau,S.N. and Liu,W. (2003) Mutations in CHEK2 associated with prostate cancer risk. *Am.J.Hum.Genet.,* **72,** 270-280.

Donzelli,M. and Draetta,G.F. (2003) Regulating mammalian checkpoints through Cdc25 inactivation. *EMBO Rep.,* **4,** 671-677.

Dufault,M.R., Betz,B., Wappenschmidt,B., Hofmann,W., Bandick,K., Golla,A., Pietschmann,A., Nestle-Kramling,C., Rhiem,K., Huttner,C., von Lindern,C., Dall,P., Kiechle,M., Untch,M., Jonat,W., Meindl,A., Scherneck,S., Niederacher,D., Schmutzler,R.K. and Arnold,N. (2004) Limited relevance of the CHEK2 gene in hereditary breast cancer. *Int.J.Cancer,* **110,** 320-325.

Duffy,M.J. (2005) Predictive markers in breast and other cancers: a review. *Clin.Chem.,* **51,** 494-503.

Durocher,D. and Jackson,S.P. (2002) The FHA domain. *FEBS Lett.,* **513,** 58-66.

Einarsdottir,K., Humphreys,K., Bonnard,C., Palmgren,J., Iles,M.M., Sjolander,A., Li,Y., Chia,K.S., Liu,E.T., Hall,P., Liu,J. and Wedren,S. (2006a) Linkage disequilibrium mapping of CHEK2: common variation and breast cancer risk. *PLoS Med.,* **3,** e168.

Einarsdottir,K., Rosenberg,L.U., Humphreys,K., Bonnard,C., Palmgren,J., Li,Y., Li,Y., Chia,K.S., Liu,E.T., Hall,P., Liu,J. and Wedren,S. (2006b) Comprehensive analysis of the ATM, CHEK2 and ERBB2 genes in relation to breast tumour characteristics and survival: a population-based case-control and follow-up study. *Breast Cancer Res.,* **8,** R67.

Falck,J., Lukas,C., Protopopova,M., Lukas,J., Selivanova,G. and Bartek,J. (2001a) Functional impact of concomitant versus alternative defects in the Chk2-p53 tumour suppressor pathway. *Oncogene,* **20,** 5503-5510.

Falck,J., Mailand,N., Syljuasen,R.G., Bartek,J. and Lukas,J. (2001b) The ATM-Chk2-Cdc25A checkpoint pathway guards against radioresistant DNA synthesis. *Nature,* **410,** 842-847.

Ferguson,D.O. and Alt,F.W. (2001) DNA double-strand break repair and chromosomal translocation: lessons from animal models. *Oncogene,* **20,** 5572-5579.

Fero,M.L., Randel,E., Gurley,K.E., Roberts,J.M. and Kemp,C.J. (1998) The murine gene p27Kip1 is haplo-insufficient for tumour suppression. *Nature,* **396,** 177-180.

Frayling,I.M., Beck,N.E., Ilyas,M., Dove-Edwin,I., Goodman,P., Pack,K., Bell,J.A., Williams,C.B., Hodgson,S.V., Thomas,H.J., Talbot,I.C., Bodmer,W.F. and Tomlinson,I.P. (1998) The APC variants I1307K and E1317Q are associated with colorectal tumors, but not always with a family history. *Proc.Natl.Acad.Sci.U.S.A.,* **95,** 10722-10727.

Friedrichsen,D.M., Malone,K.E., Doody,D.R., Daling,J.R. and Ostrander,E.A. (2004) Frequency of CHEK2 mutations in a population based, case-control study of breast cancer in young women. *Breast Cancer Res.,* **6,** R629-35.

Ganguly,A., Rock,M.J. and Prockop,D.J. (1993) Conformation-sensitive gel electrophoresis for rapid detection of single-base differences in double-stranded PCR products and DNA fragments: evidence for solvent-induced bends in DNA heteroduplexes. *Proc.Natl.Acad.Sci.U.S.A.,* **90,** 10325-10329.

Gatz,S.A. and Wiesmuller,L. (2006) p53 in recombination and repair. *Cell Death Differ.,* **13,** 1003-1016.

Giovannucci,E. (2002) Modifiable risk factors for colon cancer. *Gastroenterol.Clin.North Am.,* **31,** 925-943.

Goode,E.L., Dunning,A.M., Kuschel,B., Healey,C.S., Day,N.E., Ponder,B.A., Easton,D.F. and Pharoah,P.P. (2002) Effect of germ-line genetic variation on breast cancer survival in a population-based study. *Cancer Res.,* **62,** 3052-3057.

Gorski,B., Cybulski,C., Huzarski,T., Byrski,T., Gronwald,J., Jakubowska,A., Stawicka,M., Gozdecka-Grodecka,S., Szwiec,M., Urbanski,K., Mitus,J., Marczyk,E., Dziuba,J., Wandzel,P., Surdyka,D., Haus,O., Janiszewska,H., Debniak,T., Toloczko-Grabarek,A., Medrek,K., Masojc,B., Mierzejewski,M., Kowalska,E., Narod,S.A. and Lubinski,J. (2005) Breast cancer predisposing alleles in Poland. *Breast Cancer Res.Treat.,* **92,** 19-24.

Hanahan,D. and Folkman,J. (1996) Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell,* **86,** 353-364.

Hanahan,D. and Weinberg,R.A. (2000) The hallmarks of cancer. *Cell,* **100,** 57-70.

Hangaishi,A., Ogawa,S., Qiao,Y., Wang,L., Hosoya,N., Yuji,K., Imai,Y., Takeuchi,K., Miyawaki,S. and Hirai,H. (2002) Mutations of Chk2 in primary hematopoietic neoplasms. *Blood,* **99,** 3075-3077.

Haruki,N., Saito,H., Tatematsu,Y., Konishi,H., Harano,T., Masuda,A., Osada,H., Fujii,Y. and Takahashi,T. (2000) Histological type-selective, tumor-predominant expression of a novel CHK1 isoform and infrequent in vivo somatic CHK2 mutation in small cell lung cancer. *Cancer Res.,* **60,** 4689-4692.

Haupt,Y., Maya,R., Kazaz,A. and Oren,M. (1997) Mdm2 promotes the rapid degradation of p53. *Nature,* **387,** 296-299.

Hemminki,A., Markie,D., Tomlinson,I., Avizienyte,E., Roth,S., Loukola,A., Bignell,G., Warren,W., Aminoff,M., Hoglund,P., Jarvinen,H., Kristo,P., Pelin,K., Ridanpaa,M., Salovaara,R., Toro,T., Bodmer,W., Olschwang,S., Olsen,A.S., Stratton,M.R., de la Chapelle,A. and Aaltonen,L.A. (1998) A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. *Nature,* **391,** 184-187.

Hinnis,A.R., Luckett,J.C. and Walker,R.A. (2007) Survivin is an independent predictor of short-term survival in poor prognostic breast cancer patients. *Br.J.Cancer,* **96,** 639-645.

Hirao,A., Cheung,A., Duncan,G., Girard,P.M., Elia,A.J., Wakeham,A., Okada,H., Sarkissian,T., Wong,J.A., Sakai,T., De Stanchina,E., Bristow,R.G., Suda,T., Lowe,S.W., Jeggo,P.A., Elledge,S.J. and Mak,T.W. (2002) Chk2 is a tumor suppressor that regulates apoptosis in both an ataxia telangiectasia mutated (ATM)-dependent and an ATM-independent manner. *Mol.Cell.Biol.,* **22,** 6521-6532.

Hirao,A., Kong,Y.Y., Matsuoka,S., Wakeham,A., Ruland,J., Yoshida,H., Liu,D., Elledge,S.J. and Mak,T.W. (2000) DNA damage-induced activation of p53 by the checkpoint kinase Chk2. *Science,* **287,** 1824-1827.

Hoeijmakers,J.H. (2001) Genome maintenance mechanisms for preventing cancer. *Nature,* **411,** 366-374.

Hofmann,W.K., Miller,C.W., Tsukasaki,K., Tavor,S., Ikezoe,T., Hoelzer,D., Takeuchi,S. and Koeffler,H.P. (2001) Mutation analysis of the DNA-damage checkpoint gene CHK2 in myelodysplastic syndromes and acute myeloid leukemias. *Leuk.Res.,* **25,** 333-338.

Honrado,E., Osorio,A., Palacios,J., Milne,R.L., Sanchez,L., Diez,O., Cazorla,A., Syrjäkoski,K., Huntsman,D., Heikkila,P., Lerma,E., Kallioniemi,A., Rivas,C., Foulkes,W.D., Nevanlinna,H. and Benitez,J. (2005) Immunohistochemical expression of DNA repair proteins in familial breast cancer differentiate BRCA2-associated tumors. *J.Clin.Oncol.,* **23,** 7503-7511.

Huang,J., Domchek,S.M., Brose,M.S., Rebbeck,T.R., Nathanson,K.L. and Weber,B.L. (2004) Germline CHEK2*1100delC mutations in breast cancer patients with multiple primary cancers. *J.Med.Genet.,* **41,** e120.

Huang,Y., Han,S., Li,Y., Mao,Y. and Xie,Y. (2007) Different roles of MTHFR C677T and A1298C polymorphisms in colorectal adenoma and colorectal cancer: a meta-analysis. *J.Hum.Genet.,* **52,** 73-85.

Hubner,R.A. and Houlston,R.S. (2007) MTHFR C677T and colorectal cancer risk: A meta-analysis of 25 populations. *Int.J.Cancer,* **120,** 1027-1035.

Hulka,B.S. and Moorman,P.G. (2001) Breast cancer: hormones and other risk factors. *Maturitas,* **38,** 103-13; discussion 113-6.

Huzarski,T., Cybulski,C., Domagala,W., Gronwald,J., Byrski,T., Szwiec,M., Woyke,S., Narod,S.A. and Lubinski,J. (2005) Pathology of breast cancer in women with constitutional CHEK2 mutations. *Breast Cancer Res.Treat.,* **90,** 187-189.

IARC (1997) Cancer incidence in five continents. Volume VII. *IARC Sci.Publ.,* **(143),** i-xxxiv, 1-1240.

Ingvarsson,S., Sigbjornsdottir,B.I., Huiping,C., Hafsteinsdottir,S.H., Ragnarsson,G., Barkardottir,R.B., Arason,A., Egilsson,V. and Bergthorsson,J.T. (2002) Mutation analysis of the CHK2 gene in breast carcinoma and other cancers. *Breast Cancer Res.,* **4,** R4.

Ino,Y., Wahrer,D.C., Bell,D.W., Haber,D.A. and Louis,D.N. (2000) Mutation analysis of the hCHK2 gene in primary human malignant gliomas. *Neurogenetics,* **3,** 45-46.

Isinger,A., Bhat,M., Borg,A. and Nilbert,M. (2006) CHEK2 1100delC in patients with metachronous cancers of the breast and the colorectum. *BMC Cancer,* **6,** 64.

Jack,M.T., Woo,R.A., Hirao,A., Cheung,A., Mak,T.W. and Lee,P.W. (2002) Chk2 is dispensable for p53-mediated G1 arrest but is required for a latent p53-mediated apoptotic response. *Proc.Natl.Acad.Sci.U.S.A.,* **99,** 9825-9829.

Jallepalli,P.V., Lengauer,C., Vogelstein,B. and Bunz,F. (2003) The Chk2 tumor suppressor is not required for p53 responses in human cancer cells. *J.Biol.Chem.,* **278,** 20475-20479.

Jasin,M. (2002) Homologous repair of DNA damage and tumorigenesis: the BRCA connection. *Oncogene,* **21,** 8981-8993.

Jekimovs,C.R., Chen,X., Arnold,J., Gatei,M., Richard,D.J., Spurdle,A.B., Khanna,K.K., Chenevix-Trench,G. and kConFab Investigators. (2005) Low frequency of CHEK2 1100delC allele in Australian multiple-case breast cancer families: functional analysis in heterozygous individuals. *Br.J.Cancer,* **92,** 784-790.

Johns,L.E. and Houlston,R.S. (2001) A systematic review and meta-analysis of familial colorectal cancer risk. *Am.J.Gastroenterol.,* **96,** 2992-3003.

Kaklamani,V.G., Hou,N., Bian,Y., Reich,J., Offit,K., Michel,L.S., Rubinstein,W.S., Rademaker,A. and Pasche,B. (2003) TGFBR1*6A and cancer risk: a meta-analysis of seven case-control studies. *J.Clin.Oncol.,* **21,** 3236-3243.

Kastan,M.B. and Bartek,J. (2004) Cell-cycle checkpoints and cancer. *Nature,* **432,** 316-323.

Kato,N., Fujimoto,H., Yoda,A., Oishi,I., Matsumura,N., Kondo,T., Tsukada,J., Tanaka,Y., Imamura,M. and Minami,Y. (2004) Regulation of Chk2 gene expression in lymphoid malignancies: involvement of epigenetic mechanisms in Hodgkin's lymphoma cell lines. *Cell Death Differ.,* **11 Suppl 2,** S153-61.

Khanna,K.K. and Jackson,S.P. (2001) DNA double-strand breaks: signaling, repair and the cancer connection. *Nat.Genet.,* **27,** 247-254.

Kilpivaara,O., Bartkova,J., Eerola,H., Syrjäkoski,K., Vahteristo,P., Lukas,J., Blomqvist,C., Holli,K., Heikkila,P., Sauter,G., Kallioniemi,O.P., Bartek,J. and Nevanlinna,H. (2005) Correlation of CHEK2 protein expression and c.1100delC mutation status with tumor characteristics among unselected breast cancer patients. *Int.J.Cancer,* **113,** 575-580.

Kinzler,K.W. and Vogelstein,B. (1998) Landscaping the cancer terrain. *Science,* **280,** 1036-1037.

Kinzler,K.W. and Vogelstein,B. (1997) Cancer-susceptibility genes. Gatekeepers and caretakers. *Nature,* **386,** 761, 763.

Knudson,A.G. (2001) Two genetic hits (more or less) to cancer. *Nat.Rev.Cancer.,* **1,** 157-162.

Knudson,A.G.,Jr. (1971) Mutation and cancer: statistical study of retinoblastoma. *Proc.Natl.Acad.Sci.U.S.A.,* **68,** 820-823.

Kononen,J., Bubendorf,L., Kallioniemi,A., Barlund,M., Schraml,P., Leighton,S., Torhorst,J., Mihatsch,M.J., Sauter,G. and Kallioniemi,O.P. (1998) Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat.Med.,* **4,** 844-847.

Koppert,L.B., Schutte,M., Abbou,M., Tilanus,H.W. and Dinjens,W.N. (2004) The CHEK2(*)1100delC mutation has no major contribution in oesophageal carcinogenesis. *Br.J.Cancer,* **90,** 888-891.

Kubbutat,M.H., Jones,S.N. and Vousden,K.H. (1997) Regulation of p53 stability by Mdm2. *Nature,* **387,** 299-303.

Kuschel,B., Auranen,A., Gregory,C.S., Day,N.E., Easton,D.F., Ponder,B.A., Dunning,A.M. and Pharoah,P.D. (2003) Common polymorphisms in checkpoint kinase 2 are not associated with breast cancer risk. *Cancer Epidemiol.Biomarkers Prev.,* **12,** 809-812.

Lacroix,M. and Leclercq,G. (2005) The "portrait" of hereditary breast cancer. *Breast Cancer Res.Treat.,* **89,** 297-304.

Lahiri,D.K. and Nurnberger,J.I.,Jr. (1991) A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res.,* **19,** 5444.

Laken,S.J., Petersen,G.M., Gruber,S.B., Oddoux,C., Ostrer,H., Giardiello,F.M., Hamilton,S.R., Hampel,H., Markowitz,A., Klimstra,D., Jhanwar,S., Winawer,S., Offit,K., Luce,M.C., Kinzler,K.W. and Vogelstein,B. (1997) Familial colorectal cancer in Ashkenazim due to a hypermutable tract in APC. *Nat.Genet.,* **17,** 79-83.

Lamlum,H., Al Tassan,N., Jaeger,E., Frayling,I., Sieber,O., Reza,F.B., Eckert,M., Rowan,A., Barclay,E., Atkin,W., Williams,C., Gilbert,J., Cheadle,J., Bell,J., Houlston,R., Bodmer,W., Sampson,J. and Tomlinson,I. (2000) Germline APC variants in patients with multiple colorectal adenomas, with evidence for the particular importance of E1317Q. *Hum.Mol.Genet.,* **9,** 2215-2221.

Latif,F., Tory,K., Gnarra,J., Yao,M., Duh,F.M., Orcutt,M.L., Stackhouse,T., Kuzmin,I., Modi,W. and Geil,L. (1993) Identification of the von Hippel-Lindau disease tumor suppressor gene. *Science,* **260,** 1317-1320.

Lee,C.H. and Chung,J.H. (2001) The hCds1 (Chk2)-FHA domain is essential for a chain of phosphorylation events on hCds1 that is induced by ionizing radiation. *J.Biol.Chem.,* **276,** 30537-30541.

Lee,J.S., Collins,K.M., Brown,A.L., Lee,C.H. and Chung,J.H. (2000) hCds1-mediated phosphorylation of BRCA1 regulates the DNA damage response. *Nature,* **404,** 201-204.

Lee,S.B., Kim,S.H., Bell,D.W., Wahrer,D.C., Schiripo,T.A., Jorczak,M.M., Sgroi,D.C., Garber,J.E., Li,F.P., Nichols,K.E., Varley,J.M., Godwin,A.K., Shannon,K.M., Harlow,E. and Haber,D.A. (2001) Destabilization of CHK2 by a missense mutation associated with Li-Fraumeni Syndrome. *Cancer Res.,* **61,** 8062-8067.

Lengauer,C., Kinzler,K.W. and Vogelstein,B. (1998) Genetic instabilities in human cancers. *Nature,* **396,** 643-649.

Leroy,C., Mann,C. and Marsolier,M.C. (2001) Silent repair accounts for cell cycle specificity in the signaling of oxidative DNA lesions. *EMBO J.,* **20,** 2896-2906.

Li,F.P. and Fraumeni,J.F.,Jr. (1969) Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome? *Ann.Intern.Med.,* **71,** 747-752.

Li,J., Williams,B.L., Haire,L.F., Goldberg,M., Wilker,E., Durocher,D., Yaffe,M.B., Jackson,S.P. and Smerdon,S.J. (2002) Structural and functional versatility of the FHA domain in DNA-damage signaling by the tumor suppressor kinase Chk2. *Mol.Cell,* **9,** 1045-1054.

Liaw,D., Marsh,D.J., Li,J., Dahia,P.L., Wang,S.I., Zheng,Z., Bose,S., Call,K.M., Tsou,H.C., Peacocke,M., Eng,C. and Parsons,R. (1997) Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat.Genet.,* **16,** 64-67.

Lichtenstein,P., Holm,N.V., Verkasalo,P.K., Iliadou,A., Kaprio,J., Koskenvuo,M., Pukkala,E., Skytthe,A. and Hemminki,K. (2000) Environmental and heritable factors in the causation of cancer--analyses of cohorts of twins from Sweden, Denmark, and Finland. *N.Engl.J.Med.,* **343,** 78-85.

Lipton,L., Fleischmann,C., Sieber,O.M., Thomas,H.J., Hodgson,S.V., Tomlinson,I.P. and Houlston,R.S. (2003) Contribution of the CHEK2 1100delC variant to risk of multiple colorectal adenoma and carcinoma. *Cancer Lett.,* **200,** 149-152.

Lipton,L. and Tomlinson,I. (2006) The genetics of FAP and FAP-like syndromes. *Fam.Cancer.,* **5,** 221-226.

Lukas,C., Bartkova,J., Latella,L., Falck,J., Mailand,N., Schroeder,T., Sehested,M., Lukas,J. and Bartek,J. (2001) DNA damage-activated kinase Chk2 is independent of proliferation or differentiation yet correlates with tissue biology. *Cancer Res.,* **61,** 4990-4993.

Lukas,C., Falck,J., Bartkova,J., Bartek,J. and Lukas,J. (2003) Distinct spatiotemporal dynamics of mammalian checkpoint regulators induced by DNA damage. *Nat.Cell Biol.,* **5,** 255-260.

Lynch,H.T. and de la Chapelle,A. (2003) Hereditary colorectal cancer. *N.Engl.J.Med.,* **348,** 919-932.

Malkin,D., Li,F.P., Strong,L.C., Fraumeni,J.F.,Jr, Nelson,C.E., Kim,D.H., Kassel,J., Gryka,M.A., Bischoff,F.Z. and Tainsky,M.A. (1990) Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science,* **250,** 1233-1238.

Martinez-Bouzas,C., Beristain,E., Guerra,I., Gorostiaga,J., Mendizabal,J.L., De-Pablo,J.L., Garcia-Alegria,E., Sanz-Parra,A. and Tejada,M.I. (2007) CHEK2 1100delC is present in familial breast cancer cases of the Basque Country. *Breast Cancer Res.Treat.,* **103,** 111-113.

Matsuoka,S., Huang,M. and Elledge,S.J. (1998) Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. *Science,* **282,** 1893-1897.

Matsuoka,S., Nakagawa,T., Masuda,A., Haruki,N., Elledge,S.J. and Takahashi,T. (2001) Reduced expression and impaired kinase activity of a Chk2 mutant identified in human lung cancer. *Cancer Res.,* **61,** 5362-5365.

Matsuoka,S., Rotman,G., Ogawa,A., Shiloh,Y., Tamai,K. and Elledge,S.J. (2000) Ataxia telangiectasia-mutated phosphorylates Chk2 in vivo and in vitro. *Proc.Natl.Acad.Sci.U.S.A.,* **97,** 10389-10394.

McPherson,K., Steel,C.M. and Dixon,J.M. (2000) ABC of breast diseases. Breast cancer-epidemiology, risk factors, and genetics. *BMJ,* **321,** 624-628.

Meijers-Heijboer,H., van den Ouweland,A., Klijn,J., Wasielewski,M., de Snoo,A., Oldenburg,R., Hollestelle,A., Houben,M., Crepin,E., van Veghel-Plandsoen,M., Elstrodt,F., van Duijn,C., Bartels,C., Meijers,C., Schutte,M., McGuffog,L., Thompson,D., Easton,D., Sodha,N., Seal,S., Barfoot,R., Mangion,J., Chang-Claude,J., Eccles,D., Eeles,R., Evans,D.G., Houlston,R., Murday,V., Narod,S., Peretz,T., Peto,J., Phelan,C., Zhang,H.X., Szabo,C., Devilee,P., Goldgar,D., Futreal,P.A., Nathanson,K.L., Weber,B., Rahman,N., Stratton,M.R. and CHEK2-Breast Cancer Consortium. (2002) Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in noncarriers of BRCA1 or BRCA2 mutations. *Nat.Genet.,* **31,** 55-59.

Meijers-Heijboer,H., Wijnen,J., Vasen,H., Wasielewski,M., Wagner,A., Hollestelle,A., Elstrodt,F., van den Bos,R., de Snoo,A., Fat,G.T., Brekelmans,C., Jagmohan,S., Franken,P., Verkuijlen,P., van den Ouweland,A., Chapman,P., Tops,C., Moslein,G., Burn,J., Lynch,H., Klijn,J., Fodde,R. and Schutte,M. (2003) The CHEK2 1100delC mutation identifies families with a hereditary breast and colorectal cancer phenotype. *Am.J.Hum.Genet.,* **72,** 1308-1314.

Melchionna,R., Chen,X.B., Blasina,A. and McGowan,C.H. (2000) Threonine 68 is required for radiation-induced phosphorylation and activation of Cds1. *Nat.Cell Biol.,* **2,** 762-765.

Meyer,A., Dork,T., Sohn,C., Karstens,J.H. and Bremer,M. (2007) Breast cancer in patients carrying a germ-line CHEK2 mutation: Outcome after breast conserving surgery and adjuvant radiotherapy. *Radiother.Oncol.,* **82,** 349-353.

Midgley,C.A. and Lane,D.P. (1997) p53 protein stability in tumour cells is not determined by mutation but is dependent on Mdm2 binding. *Oncogene,* **15,** 1179-1189.

Miki,Y., Swensen,J., Shattuck-Eidens,D., Futreal,P.A., Harshman,K., Tavtigian,S., Liu,Q., Cochran,C., Bennett,L.M. and Ding,W. (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science,* **266,** 66-71.

Miller,C.W., Ikezoe,T., Krug,U., Hofmann,W.K., Tavor,S., Vegesna,V., Tsukasaki,K., Takeuchi,S. and Koeffler,H.P. (2002) Mutations of the CHK2 gene are found in some osteosarcomas, but are rare in breast, lung, and ovarian tumors. *Genes Chromosomes Cancer,* **33,** 17-21.

Mulligan,L.M., Kwok,J.B., Healey,C.S., Elsdon,M.J., Eng,C., Gardner,E., Love,D.R., Mole,S.E., Moore,J.K. and Papi,L. (1993) Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. *Nature,* **363,** 458-460.

Naseem,H., Boylan,J., Speake,D., Leask,K., Shenton,A., Lalloo,F., Hill,J., Trump,D. and Evans,D.G. (2006) Inherited association of breast and colorectal cancer: limited role of CHEK2 compared with high-penetrance genes. *Clin.Genet.,* **70,** 388-395.

Neuhausen,S., Dunning,A., Steele,L., Yakumo,K., Hoffman,M., Szabo,C., Tee,L., Baines,C., Pharoah,P., Goldgar,D. and Easton,D. (2004) Role of

CHEK2*1100delC in unselected series of non-BRCA1/2 male breast cancers. *Int.J.Cancer,* **108,** 477-478.

O'Driscoll,M. and Jeggo,P.A. (2006) The role of double-strand break repair - insights from human genetics. *Nat.Rev.Genet.,* **7,** 45-54.

Offit,K., Pierce,H., Kirchhoff,T., Kolachana,P., Rapaport,B., Gregersen,P., Johnson,S., Yossepowitch,O., Huang,H., Satagopan,J., Robson,M., Scheuer,L., Nafa,K. and Ellis,N. (2003) Frequency of CHEK2*1100delC in New York breast cancer cases and controls. *BMC Med.Genet.,* **4,** 1.

Ohayon,T., Gal,I., Baruch,R.G., Szabo,C. and Friedman,E. (2004) CHEK2*1100delC and male breast cancer risk in Israel. *Int.J.Cancer,* **108,** 479-480.

Oldenburg,R.A., Kroeze-Jansema,K., Kraan,J., Morreau,H., Klijn,J.G., Hoogerbrugge,N., Ligtenberg,M.J., van Asperen,C.J., Vasen,H.F., Meijers,C., Meijers-Heijboer,H., de Bock,T.H., Cornelisse,C.J. and Devilee,P. (2003) The CHEK2*1100delC variant acts as a breast cancer risk modifier in non-BRCA1/BRCA2 multiple-case families. *Cancer Res.,* **63,** 8153-8157.

Osorio,A., Rodriguez-Lopez,R., Diez,O., de la Hoya,M., Ignacio Martinez,J., Vega,A., Esteban-Cardenosa,E., Alonso,C., Caldes,T. and Benitez,J. (2004) The breast cancer low-penetrance allele 1100delC in the CHEK2 gene is not present in Spanish familial breast cancer population. *Int.J.Cancer,* **108,** 54-56.

Parkin,D.M., Bray,F., Ferlay,J. and Pisani,P. (2005) Global cancer statistics, 2002. *CA Cancer.J.Clin.,* **55,** 74-108.

Pasche,B., Kaklamani,V., Hou,N., Young,T., Rademaker,A., Peterlongo,P., Ellis,N., Offit,K., Caldes,T., Reiss,M. and Zheng,T. (2004) TGFBR1*6A and cancer: a meta-analysis of 12 case-control studies. *J.Clin.Oncol.,* **22,** 756-758.

Payne,S.R. and Kemp,C.J. (2005) Tumor suppressor genetics. *Carcinogenesis,* **26,** 2031-2045.

Peltomaki,P. (2005) Lynch syndrome genes. *Fam.Cancer.,* **4,** 227-232.

Peng,C.Y., Graves,P.R., Thoma,R.S., Wu,Z., Shaw,A.S. and Piwnica-Worms,H. (1997) Mitotic and G2 checkpoint control: regulation of 14-3-3 protein binding by phosphorylation of Cdc25C on serine-216. *Science,* **277,** 1501-1505.

Pharoah,P.D., Antoniou,A., Bobrow,M., Zimmern,R.L., Easton,D.F. and Ponder,B.A. (2002) Polygenic susceptibility to breast cancer and implications for prevention. *Nat.Genet.,* **31,** 33-36.

Poremba,C., Heine,B., Diallo,R., Heinecke,A., Wai,D., Schaefer,K.L., Braun,Y., Schuck,A., Lanvers,C., Bankfalvi,A., Kneif,S., Torhorst,J., Zuber,M., Kochli,O.R., Mross,F., Dieterich,H., Sauter,G., Stein,H., Fogt,F. and Boecker,W. (2002) Telomerase as a prognostic marker in breast cancer: high-throughput tissue microarray analysis of hTERT and hTR. *J.Pathol.,* **198,** 181-189.

Richardson,C. and Jasin,M. (2000) Frequent chromosomal translocations induced by DNA double-strand breaks. *Nature,* **405,** 697-700.

Rogoff,H.A., Pickering,M.T., Frame,F.M., Debatis,M.E., Sanchez,Y., Jones,S. and Kowalik,T.F. (2004) Apoptosis associated with deregulated E2F activity is dependent on E2F1 and Atm/Nbs1/Chk2. *Mol.Cell.Biol.,* **24,** 2968-2977.

Rothkamm,K., Kruger,I., Thompson,L.H. and Lobrich,M. (2003) Pathways of DNA double-strand break repair during the mammalian cell cycle. *Mol.Cell.Biol.,* **23,** 5706-5715.

Sallinen,S.L., Ikonen,T., Haapasalo,H. and Schleutker,J. (2005) CHEK2 mutations in primary glioblastomas. *J.Neurooncol.,* **74,** 93-95.

Salovaara,R., Loukola,A., Kristo,P., Kaariainen,H., Ahtola,H., Eskelinen,M., Harkonen,N., Julkunen,R., Kangas,E., Ojala,S., Tulikoura,J., Valkamo,E., Jarvinen,H., Mecklin,J.P., Aaltonen,L.A. and de la Chapelle,A. (2000) Population-based molecular detection of hereditary nonpolyposis colorectal cancer. *J.Clin.Oncol.,* **18,** 2193-2200.

Sanchez de Abajo,A., de la Hoya,M., Godino,J., Furio,V., Tosar,A., Perez-Segura,P., Diaz-Rubio,E. and Caldes,T. (2005) The CHEK2 1100delC allele is not relevant for risk assessment in HNPCC and HBCC Spanish families. *Fam.Cancer.,* **4,** 183-186.

Santarosa,M. and Ashworth,A. (2004) Haploinsufficiency for tumour suppressor genes: when you don't need to go all the way. *Biochim.Biophys.Acta,* **1654,** 105-122.

Schmidt,L., Duh,F.M., Chen,F., Kishida,T., Glenn,G., Choyke,P., Scherer,S.W., Zhuang,Z., Lubensky,I., Dean,M., Allikmets,R., Chidambaram,A., Bergerheim,U.R., Feltis,J.T., Casadevall,C., Zamarron,A., Bernues,M., Richard,S., Lips,C.J., Walther,M.M., Tsui,L.C., Geil,L., Orcutt,M.L., Stackhouse,T., Lipan,J., Slife,L., Brauch,H., Decker,J., Niehans,G., Hughson,M.D., Moch,H., Storkel,S., Lerman,M.I., Linehan,W.M. and Zbar,B. (1997) Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. *Nat.Genet.,* **16,** 68-73.

Schmidt,M.K., Tollenaar,R.A., de Kemp,S.R., Broeks,A., Cornelisse,C.J., Smit,V.T., Peterse,J.L., van Leeuwen,F.E. and Van't Veer,L.J. (2007) Breast cancer survival and tumor characteristics in premenopausal women carrying the CHEK2*1100delC germline mutation. *J.Clin.Oncol.,* **25,** 64-69.

Schutte,M., Seal,S., Barfoot,R., Meijers-Heijboer,H., Wasielewski,M., Evans,D.G., Eccles,D., Meijers,C., Lohman,F., Klijn,J., van den Ouweland,A., Futreal,P.A., Nathanson,K.L., Weber,B.L., Easton,D.F., Stratton,M.R., Rahman,N. and Breast Cancer Linkage Consortium. (2003) Variants in CHEK2 other than 1100delC do not make a major contribution to breast cancer susceptibility. *Am.J.Hum.Genet.,* **72,** 1023-1028.

Schwarz,J.K., Lovly,C.M. and Piwnica-Worms,H. (2003) Regulation of the Chk2 protein kinase by oligomerization-mediated cis- and trans-phosphorylation. *Mol.Cancer.Res.,* **1,** 598-609.

Seppälä,E.H., Ikonen,T., Mononen,N., Autio,V., Rokman,A., Matikainen,M.P., Tammela,T.L. and Schleutker,J. (2003) CHEK2 variants associate with hereditary prostate cancer. *Br.J.Cancer,* **89,** 1966-1970.

Shaag,A., Walsh,T., Renbaum,P., Kirchhoff,T., Nafa,K., Shiovitz,S., Mandell,J.B., Welcsh,P., Lee,M.K., Ellis,N., Offit,K., Levy-Lahad,E. and King,M.C. (2005) Functional and genomic approaches reveal an ancient CHEK2 allele associated with breast cancer in the Ashkenazi Jewish population. *Hum.Mol.Genet.,* **14,** 555-563.

Shieh,S.Y., Ahn,J., Tamai,K., Taya,Y. and Prives,C. (2000) The human homologs of checkpoint kinases Chk1 and Cds1 (Chk2) phosphorylate p53 at multiple DNA damage-inducible sites. *Genes Dev.,* **14,** 289-300.

Simon,M., Ludwig,M., Fimmers,R., Mahlberg,R., Muller-Erkwoh,A., Koster,G. and Schramm,J. (2006) Variant of the CHEK2 gene as a prognostic marker in glioblastoma multiforme. *Neurosurgery,* **59,** 1078-85; discussion 1085.

Slattery,M.L., Levin,T.R., Ma,K., Goldgar,D., Holubkov,R. and Edwards,S. (2003) Family history and colorectal cancer: predictors of risk. *Cancer Causes Control,* **14,** 879-887.

Sodha,N., Bullock,S., Taylor,R., Mitchell,G., Guertl-Lackner,B., Williams,R.D., Bevan,S., Bishop,K., McGuire,S., Houlston,R.S. and Eeles,R.A. (2002a)

CHEK2 variants in susceptibility to breast cancer and evidence of retention of the wild type allele in tumours. *Br.J.Cancer,* **87,** 1445-1448.

Sodha,N., Houlston,R.S., Bullock,S., Yuille,M.A., Chu,C., Turner,G. and Eeles,R.A. (2002b) Increasing evidence that germline mutations in CHEK2 do not cause Li-Fraumeni syndrome. *Hum.Mutat.,* **20,** 460-462.

Sodha,N., Mantoni,T.S., Tavtigian,S.V., Eeles,R. and Garrett,M.D. (2006) Rare germ line CHEK2 variants identified in breast cancer families encode proteins that show impaired activation. *Cancer Res.,* **66,** 8966-8970.

Srivastava,S., Zou,Z.Q., Pirollo,K., Blattner,W. and Chang,E.H. (1990) Germ-line transmission of a mutated p53 gene in a cancer-prone family with Li-Fraumeni syndrome. *Nature,* **348,** 747-749.

Staalesen,V., Falck,J., Geisler,S., Bartkova,J., Borresen-Dale,A.L., Lukas,J., Lillehaug,J.R., Bartek,J. and Lonning,P.E. (2004) Alternative splicing and mutation status of CHEK2 in stage III breast cancer. *Oncogene,* **23,** 8535-8544.

Stevens,C., Smith,L. and La Thangue,N.B. (2003) Chk2 activates E2F-1 in response to DNA damage. *Nat.Cell Biol.,* **5,** 401-409.

Sullivan,A., Yuille,M., Repellin,C., Reddy,A., Reelfs,O., Bell,A., Dunne,B., Gusterson,B.A., Osin,P., Farrell,P.J., Yulug,I., Evans,A., Ozcelik,T., Gasco,M. and Crook,T. (2002) Concomitant inactivation of p53 and Chk2 in breast cancer. *Oncogene,* **21,** 1316-1324.

Syrjäkoski,K., Kuukasjarvi,T., Waltering,K., Haraldsson,K., Auvinen,A., Borg,A., Kainu,T., Kallioniemi,O.P. and Koivisto,P.A. (2004) BRCA2 mutations in 154 finnish male breast cancer patients. *Neoplasia,* **6,** 541-545.

Syrjäkoski,K., Vahteristo,P., Eerola,H., Tamminen,A., Kivinummi,K., Sarantaus,L., Holli,K., Blomqvist,C., Kallioniemi,O.P., Kainu,T. and Nevanlinna,H. (2000) Population-based study of BRCA1 and BRCA2 mutations in 1035 unselected Finnish breast cancer patients. *J.Natl.Cancer Inst.,* **92,** 1529-1531.

Syvanen,A.C., Sajantila,A. and Lukka,M. (1993) Identification of individuals by analysis of biallelic DNA markers, using PCR and solid-phase minisequencing. *Am.J.Hum.Genet.,* **52,** 46-59.

Takai,H., Naka,K., Okada,Y., Watanabe,M., Harada,N., Saito,S., Anderson,C.W., Appella,E., Nakanishi,M., Suzuki,H., Nagashima,K., Sawa,H., Ikeda,K. and Motoyama,N. (2002) Chk2-deficient mice exhibit radioresistance and defective p53-mediated transcription. *EMBO J.,* **21,** 5195-5205.

Tang,B., Bottinger,E.P., Jakowlew,S.B., Bagnall,K.M., Mariano,J., Anver,M.R., Letterio,J.J. and Wakefield,L.M. (1998) Transforming growth factor-beta1 is a new form of tumor suppressor with true haploid insufficiency. *Nat.Med.,* **4,** 802-807.

Thompson,D., Seal,S., Schutte,M., McGuffog,L., Barfoot,R., Renwick,A., Eeles,R., Sodha,N., Houlston,R., Shanley,S., Klijn,J., Wasielewski,M., Chang-Claude,J., Futreal,P.A., Weber,B.L., Nathanson,K.L., Stratton,M., Meijers-Heijboer,H., Rahman,N. and Easton,D.F. (2006) A multicenter study of cancer incidence in CHEK2 1100delC mutation carriers. *Cancer Epidemiol.Biomarkers Prev.,* **15,** 2542-2545.

Thompson,L.H. and Schild,D. (2002) Recombinational DNA repair and human disease. *Mutat.Res.,* **509,** 49-78.

Tominaga,K., Morisaki,H., Kaneko,Y., Fujimoto,A., Tanaka,T., Ohtsubo,M., Hirai,M., Okayama,H., Ikeda,K. and Nakanishi,M. (1999) Role of human Cds1 (Chk2) kinase in DNA damage checkpoint and its regulation by p53. *J.Biol.Chem.,* **274,** 31463-31467.

Torhorst,J., Bucher,C., Kononen,J., Haas,P., Zuber,M., Kochli,O.R., Mross,F., Dieterich,H., Moch,H., Mihatsch,M., Kallioniemi,O.P. and Sauter,G. (2001) Tissue microarrays for rapid linking of molecular changes to clinical endpoints. *Am.J.Pathol.,* **159,** 2249-2256.

Tort,F., Hernandez,S., Bea,S., Martinez,A., Esteller,M., Herman,J.G., Puig,X., Camacho,E., Sanchez,M., Nayach,I., Lopez-Guillermo,A., Fernandez,P.L., Colomer,D., Hernandez,L. and Campo,E. (2002) CHK2-decreased protein expression and infrequent genetic alterations mainly occur in aggressive types of non-Hodgkin lymphomas. *Blood,* **100,** 4602-4608.

Vahteristo,P., Bartkova,J., Eerola,H., Syrjäkoski,K., Ojala,S., Kilpivaara,O., Tamminen,A., Kononen,J., Aittomaki,K., Heikkila,P., Holli,K., Blomqvist,C., Bartek,J., Kallioniemi,O.P. and Nevanlinna,H. (2002) A CHEK2 genetic variant contributing to a substantial fraction of familial breast cancer. *Am.J.Hum.Genet.,* **71,** 432-438.

Vahteristo,P., Eerola,H., Tamminen,A., Blomqvist,C. and Nevanlinna,H. (2001a) A probability model for predicting BRCA1 and BRCA2 mutations in breast and breast-ovarian cancer families. *Br.J.Cancer,* **84,** 704-708.

Vahteristo,P., Tamminen,A., Karvinen,P., Eerola,H., Eklund,C., Aaltonen,L.A., Blomqvist,C., Aittomaki,K. and Nevanlinna,H. (2001b) p53, CHK2, and CHK1 genes in Finnish families with Li-Fraumeni syndrome: further evidence of CHK2 in inherited cancer predisposition. *Cancer Res.,* **61,** 5718-5722.

Valerie,K. and Povirk,L.F. (2003) Regulation and mechanisms of mammalian double-strand break repair. *Oncogene,* **22,** 5792-5812.

van Puijenbroek,M., van Asperen,C.J., van Mil,A., Devilee,P., van Wezel,T. and Morreau,H. (2005) Homozygosity for a CHEK2*1100delC mutation identified in familial colorectal cancer does not lead to a severe clinical phenotype. *J.Pathol.,* **206,** 198-204.

Varley,J.M. (2003) Germline TP53 mutations and Li-Fraumeni syndrome. *Hum.Mutat.,* **21,** 313-320.

Varley,J.M., Evans,D.G. and Birch,J.M. (1997) Li-Fraumeni syndrome--a molecular and clinical review. *Br.J.Cancer,* **76,** 1-14.

Vehmanen,P., Friedman,L.S., Eerola,H., McClure,M., Ward,B., Sarantaus,L., Kainu,T., Syrjäkoski,K., Pyrhonen,S., Kallioniemi,O.P., Muhonen,T., Luce,M., Frank,T.S. and Nevanlinna,H. (1997) Low proportion of BRCA1 and BRCA2 mutations in Finnish breast cancer families: evidence for additional susceptibility genes. *Hum.Mol.Genet.,* **6,** 2309-2315.

Venkatachalam,S., Shi,Y.P., Jones,S.N., Vogel,H., Bradley,A., Pinkel,D. and Donehower,L.A. (1998) Retention of wild-type p53 in tumors from p53 heterozygous mice: reduction of p53 dosage can promote cancer formation. *EMBO J.,* **17,** 4657-4667.

Vogelstein,B. and Kinzler,K.W. (2002) The Genetic Basis of Human Cancer. **2nd Edition,** 821.

Wagenius,M., Borg,A., Johansson,L., Giwercman,A. and Bratt,O. (2006) CHEK2*1100delC is not an important high-risk gene in families with hereditary prostate cancer in southern Sweden. *Scand.J.Urol.Nephrol.,* **40,** 23-25.

Walsh,T., Casadei,S., Coats,K.H., Swisher,E., Stray,S.M., Higgins,J., Roach,K.C., Mandell,J., Lee,M.K., Ciernikova,S., Foretova,L., Soucek,P. and King,M.C. (2006) Spectrum of mutations in BRCA1, BRCA2, CHEK2, and TP53 in families at high risk of breast cancer. *JAMA,* **295,** 1379-1388.

Weischer,M., Bojesen,S.E., Tybjaerg-Hansen,A., Axelsson,C.K. and Nordestgaard,B.G. (2007) Increased risk of breast cancer associated with CHEK2*1100delC. *J.Clin.Oncol.,* **25,** 57-63.

Weiss,J.R., Moysich,K.B. and Swede,H. (2005) Epidemiology of male breast cancer. *Cancer Epidemiol.Biomarkers Prev.,* **14,** 20-26.

Williams,L.H., Choong,D., Johnson,S.A. and Campbell,I.G. (2006) Genetic and epigenetic analysis of CHEK2 in sporadic breast, colon, and ovarian cancers. *Clin.Cancer Res.,* **12,** 6967-6972.

Wooster,R., Bignell,G., Lancaster,J., Swift,S., Seal,S., Mangion,J., Collins,N., Gregory,S., Gumbs,C. and Micklem,G. (1995) Identification of the breast cancer susceptibility gene BRCA2. *Nature,* **378,** 789-792.

Wu,X. and Chen,J. (2003) Autophosphorylation of checkpoint kinase 2 at serine 516 is required for radiation-induced apoptosis. *J.Biol.Chem.,* **278,** 36163-36168.

Wu,X., Dong,X., Liu,W. and Chen,J. (2006) Characterization of CHEK2 mutations in prostate cancer. *Hum.Mutat.,* **27,** 742-747.

Wu,X., Webster,S.R. and Chen,J. (2001) Characterization of tumor-associated Chk2 mutations. *J.Biol.Chem.,* **276,** 2971-2974.

Xu,X., Tsvetkov,L.M. and Stern,D.F. (2002) Chk2 activation and phosphorylation-dependent oligomerization. *Mol.Cell.Biol.,* **22,** 4419-4432.

Yang,S., Kuo,C., Bisi,J.E. and Kim,M.K. (2002) PML-dependent apoptosis after DNA damage is regulated by the checkpoint kinase hCds1/Chk2. *Nat.Cell Biol.,* **4,** 865-870.

Yoshida,K. and Miki,Y. (2004) Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage. *Cancer.Sci.,* **95,** 866-871.

Yu,Q., Rose,J.H., Zhang,H. and Pommier,Y. (2001) Antisense inhibition of Chk2/hCds1 expression attenuates DNA damage-induced S and G2 checkpoints and enhances apoptotic activity in HEK-293 cells. *FEBS Lett.,* **505,** 7-12.

Zhang,J. and Powell,S.N. (2005) The role of the BRCA1 tumor suppressor in DNA double-strand break repair. *Mol.Cancer.Res.,* **3,** 531-539.

Zhang,J., Willers,H., Feng,Z., Ghosh,J.C., Kim,S., Weaver,D.T., Chung,J.H., Powell,S.N. and Xia,F. (2004) Chk2 phosphorylation of BRCA1 regulates DNA double-strand break repair. *Mol.Cell.Biol.,* **24,** 708-718.

Zuo,L., Weger,J., Yang,Q., Goldstein,A.M., Tucker,M.A., Walker,G.J., Hayward,N. and Dracopoli,N.C. (1996) Germline mutations in the p16INK4a binding domain of CDK4 in familial melanoma. *Nat.Genet.,* **12,** 97-99.