ATM, RAD50 and p53 in Breast Cancer

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Academic Dissertation

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Syöpiä on monenlaisia
Selkeitä ja salaisia

Syöpä se tuhoaa solut.
Katsoo elimistön herkät kolut.

Kun syöpä soluun osuu
Sen komentokeskus lähinnä hosuu.

Se leviää ain,
Lymfosyytit tuhota sen voi vain.

Jos se kasvaa kauan vois'
Mukanaan se kuolon tois'

Atte Tommiska 2007

To Atte and Aura, my major achievements in genetics

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LIST OF ORIGINAL PUBLICATIONS

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

I Pylkäs K*, **Tommiska J***, Syrjäkoski K, Kere J, Gatei M, Waddell N, Allinen M, Karppinen SM, Rapakko K, Kääriäinen H, Aittomäki K, Blomqvist C, Mustonen A, Holli K, Khanna KK, Kallioniemi OP, Nevanlinna H, Winqvist R. Evaluation of the role of Finnish ataxia-telangiectasia mutations in hereditary predisposition to breast cancer. Carcinogenesis 2007;28:1040-5.

II **Tommiska J**, Jansen L, Kilpivaara O, Edvardsen H, Kristensen V, Tamminen A, Aittomäki K, Blomqvist C, Børresen-Dale AL, Nevanlinna H. *ATM* variants and cancer risk in breast cancer patients from Southern Finland. BMC Cancer 2006;6:209.

III **Tommiska J***, Bartkova J*, Heinonen M, Hautala L, Kilpivaara O, Eerola H, Aittomäki K, Hofstetter B, Lukas J, von Smitten K, Blomqvist C, Ristimäki A, Heikkilä P, Bartek J, Nevanlinna H. The DNA damage signalling kinase ATM is aberrantly reduced or lost in BRCA1/BRCA2-deficient and ER/PR/ERBB2-triple-negative breast cancer. Oncogene (In press.).

IV **Tommiska J**, Seal S, Renwick A, Barfoot R, Baskcomb L, Jayatilake H, Bartkova J, Tallila J, Kaare M, Tamminen A, Heikkilä P, Evans DG, Eccles D, Aittomäki K, Blomqvist C, Bartek J, Stratton MR, Nevanlinna H, Rahman N. Evaluation of *RAD50* in familial breast cancer predisposition. Int J Cancer 2006;118:2911-6.

V **Tommiska J**, Eerola H, Heinonen M, Salonen L, Kaare M, Tallila J, Ristimäki A, von Smitten K, Aittomäki K, Heikkilä P, Blomqvist C, Nevanlinna H. Breast cancer patients with p53 Pro72 homozygous genotype have a poorer survival. Clin Cancer Res 2005;11:5098-103.

*These authors contributed equally to the study. Original publications have been reprinted with the permission of their copyright holders. In addition, some unpublished material is presented. Publication I is also included in the thesis of Katri Pylkäs (*ATM*, *ATR* and MRE11 complex genes in hereditary susceptibility to breast cancer, Acta Universitatis Ouluensis D Medica 914, University of Oulu, Oulu, 2007).

ABBREVIATIONS

Names of the genes are in *italics*.

A	adenine
A, Ala	alanine
aa	amino acid(s)
ABC	ATP-binding cassette
AI	allelic imbalance
Apaf-1	apoptotic protease activating factor 1
A-T	ataxia telangiectasia
ATLD	ataxia-telangiectasia –like disorder
ATM	*ataxia-telangiectasia mutated*
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
ATR	ataxia-telangiectasia and Rad3 –related
Bax	Bcl-2 –associated x protein
bp	base pair(s)
53BP1	p53 binding protein
Bcl-2	B-cell lymphoma protein 2
BLM	Bloom's syndrome protein
BRCA1	*breast cancer gene 1*
BRCA2	*breast cancer gene 2*
BRCT	BRCA1 carboxy terminal repeat
BRIP1 (*BACH1*)	*BRCA1-interacting 1* (*BRCA1-associated C-terminal helicase*)
C	cytosine
C, Cys	cysteine
C-terminus	carboxyterminus
CASP8	caspase 8
cDNA	complementary deoxyribonucleic acid
CDC2	cell division cycle 2 protein
CDC25A/C	cell division cycle 25A/C protein
CDK2	cyclin-dependent kinase 2
CHEK1	checkpoint kinase 1
CHEK2	checkpoint kinase 2
CI	confidence interval
CISH	chromogenic *in situ* hybridization
CS	Cowden syndrome
CSGE	conformation sensitive gel electrophoresis
D, Asp	aspartic acid
del	deletion
DFS	disease-free survival
dHPLC	denaturating high-performance liquid chromatography
DNA	deoxyribonucleic acid
DDR	DNA damage response

DSB	double-strand break
E6	human papillomavirus oncoprotein early 6
ER	estrogen receptor
ERBB2 (*HER2*)	*avian erythroblastic leukemia viral oncogene homolog 2*
ex	exon
F, Phe	phenylalanine
FA	Fanconi anemia
FANCD2	Fanconi anemia complementation group D2 protein
FAT	FRAP-ATM-TRRAP domain
FATC	C-terminal FAT-domain
FHA	fork-head associated
FRAP (mTOR)	FK506-binding protein 1-rapamycin associated protein
G	guanine
G, Gly	glycine
G1	gap 1, cell cycle phase before S-phase
G2	gap 2, cell cycle phase before mitosis
H, His	histidine
H2AX	histone 2A family member X
HPV	human papilloma virus
HR	homologous recombination
I, Ile	isoleucine
iASPP	inhibitory member of the apoptosis-stimulating protein of p53 family
IHC	immunohistochemistry
ins	insertion
IR	ionizing radiation
ivs	intervening sequence, intron
kb	kilobase(s)
kD	kiloDalton
L, Leu	leucine
LFS	Li-Fraumeni syndrome
LKB1 (*STK11*)	serine/threonine kinase gene defective in Peutz Jeghers syndrome
LOH	loss of heterozygosity
M	distant metastases
M	mitosis
MDC1	mediator of DNA damage checkpoint protein 1
MDM2	*mouse double minute homolog 2*
MDMX	mouse double minute homolog 4
MLH1	*homolog of Escherichia coli mutator L 1*
MOMP	mitochondrial outer membrane permeabilization
MRE11	*homolog of Saccharomyces cerevisiae meiotic recombination 11*
MRN	MRE11/RAD50/NBS1 complex
mRNA	messenger ribonucleic acid
MSH2	*homolog of Escherichia coli mutator S 2*
N	lymph node metastases
N, Asn	asparagine

N-terminus	aminoterminus
NBS	Nijmegen breakage syndrome
NBS1	*Nijmegen breakage syndrome 1*
NHEJ	non-homologous end-joining
NMD	nonsense-mediated mRNA decay
Noxa	phorbol-12-myristate-13-acetate-induced protein, PMAIP1
OR	odds ratio
p	short arm of a chromosome
P, Pro	proline
p21/WAF1	cyclin-dependent kinase inhibitor 1A, CDKN1A
p53	tumor protein 53
p53AIP1	p53-regulated apoptosis-inducing protein 1
PALB2	*partner and localizer of BRCA2*
PARP1	poly(ADP-ribose) polymerase 1
PCR	polymerase chain reaction
PI3K	phosphoinositide 3-kinase
PIKK	phosphoinositide 3-kinase-like protein kinase
PJS	Peutz Jeghers syndrome
PR	progesterone receptor
PTEN	*phosphatase and tensin homolog*
Puma	p53-upregulated modulator of apoptosis
q	long arm of a chromosome
Q, Gln	glutamine
R, Arg	arginine
RAD50	*homolog of Saccharomyces cerevisiae Rad50*
RAD51	*homolog of Saccharomyces cerevisiae Rad51*
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
RT	room temperature
R-T-PCR	reverse-transcriptase polymerase chain reaction
S, Ser	serine
S-phase	DNA synthesis and replication phase of cell cycle
SCCHN	squamous cell carcinoma of head and neck
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SMC1	structural maintenance of chromosomes protein 1
SNP	single nucleotide polymorphism
T	thymine
T	tumor size
T, Thr	threonine
TGFB1	transforming growth factor beta 1
TMA	tissue microarray
TRRAP	transformation/transcription-domain associated protein
5’UTR	5’ untranslated region
V, Val	valine
W, Trp	tryptophan
Y, Tyr	tyrosine

ABSTRACT

All the currently known breast cancer susceptibility genes are estimated to account for 20-30% of familial breast cancer and only 5% of the total breast cancer incidence, even though it is suggested that the proportion of breast cancer that can be attributed to genetic factors may be as high as 30%. It is thus likely that there are still other breast cancer susceptibility genes to be found. The aim of this study was to evaluate the role of three DNA damage response –associated genes, *ATM*, *RAD50*, and *p53*, in breast cancer.

ATM, a gene causative for ataxia telangiectasia (A-T), has long been a strong candidate for a breast cancer susceptibility gene, because of the increased breast cancer risk observed in obligate female heterozygous *ATM* mutation carriers in A-T families, and because of the function of the ATM kinase as a key DNA damage signal transducer within the genome maintenance machinery. We evaluated the role of known Finnish A-T –related *ATM* germline mutations as possible breast cancer predisposing alleles by analyzing their prevalence in large series of familial and unselected breast cancer cases from different geographical regions in Finland. Of the seven different alterations, two were observed in the studied familial breast cancer patients. Additionally, a third mutation previously associated with breast cancer susceptibility was also detected. Altogether, heterozygous *ATM* mutations were found in 6/541 familial and 7/1124 unselected cases, compared to 1/1107 in controls, suggesting an apparent, yet overall limited contribution to predisposition to cancer. The results also provided evidence for founder effects in the geographical distribution of these mutations. Furthermore, functional analyses of the mutations suggested that cancer susceptibility is not restricted to mutations with dominant-negative effect on ATM kinase activity; haploinsufficiency also seems to contribute to cancer in *ATM* mutation carriers.

As the A-T –related *ATM* mutations were not prevalent in Southern Finland, we also screened the entire coding region of the *ATM* gene in 47 familial breast cancer patients from Southern Finland and constructed haplotypes of the patients. The identified variants

were evaluated in additional breast cancer cases and controls. Four rare alterations were each found in only one patient of over 250 familial patients studied and not among controls. The fifth alteration studied further was found with closely similar frequencies in over 600 familial cases and controls. All the variants were too rare to significantly contribute to breast cancer susceptibility. In addition, two common *ATM* variants, 5557G>A and ivs38-8T>C, previously suggested to associate with bilateral breast cancer, were genotyped in an extensive set of 786 familial and 884 unselected breast cancer cases as well as 708 healthy controls. Neither of the variants, nor any haplotype containing them, was significantly associated with breast cancer risk, bilateral breast cancer or multiple primary cancers in any of the patient groups or subgoups. Altogether, our results suggest only a minor effect, if any, of *ATM* genetic variants on familial breast cancer in Southern Finland.

We also examined ATM expression by immunohistochemistry in breast carcinomas of *BRCA1/2* mutation carriers as well as in familial and sporadic non*BRCA1/2* tumors to evaluate its role in breast tumorigenesis. ATM protein expression was aberrantly reduced more frequently among *BRCA1*- (33%) and *BRCA2*- (30%) tumors than in non*BRCA1/2* tumors (10.7%). Furthermore, the non*BRCA1/2* tumors with reduced ATM expression were more often estrogen receptor (ER) negative, progesterone receptor (PR) negative, and of higher grade, and ATM was more commonly deficient in the difficult-to-treat ER/PR/ERBB2-triple-negative tumors than in other non*BRCA1/2* tumors.

RAD50 is part of a complex important in recognizing, signaling and repairing DNA double-strand breaks. To evaluate the contribution of *RAD50* to familial breast cancer we screened the whole coding region of the gene for mutations in 435 UK and 46 Finnish familial breast cancer cases. We identified one truncating mutation, Q350X, in one UK family. In addition, we screened 544 Southern Finnish familial breast cancer cases and 560 controls for the 687delT mutation, previously identified in two Northern Finnish breast cancer families. It was present in 3 cases (0.5%) and 1 control (0.2%). Protein expression analyses suggested that RAD50 687delT is a null allele and may contribute to cancer through haploinsufficiency. Altogether, our results suggest that *RAD50* can only

be making a minor contribution to familial breast cancer predisposition in UK and Southern Finland.

The p53 R72P polymorphism has been suggested to play a role in many cancers, including breast cancer. We genotyped altogether 1551 Finnish familial and unselected breast cancer patients and 733 healthy population controls for R72P, and evaluated the association with breast cancer risk as well as histopathologic features of the breast tumors and survival of the patients. The distribution of the genotypes was similar in all groups studied, suggesting no association with breast cancer risk. However, unselected breast cancer patients with 72P homozygous genotype seemed to present more often with lobular carcinoma whereas R72 allele carriers had a higher frequency of ductal carcinomas. Survival analysis showed that unselected breast cancer patients with 72P homozygous genotype had significantly poorer survival than patients with other genotypes. This effect on survival was independent of p53 expression in the tumors, and multivariate analysis showed that 72P homozygous genotype was overall an independent prognostic factor with approximately two-fold risk of death.

1 INTRODUCTION

Breast cancer is the most commonly occurring cancer among women, and its incidence is increasing worldwide. Positive family history of breast cancer is a well established risk factor for breast cancer, and it is suggested that the proportion of breast cancer that can be attributed to genetic factors may be as high as 30%. However, all the currently known breast cancer susceptibility genes are estimated to account for 20-30% of familial breast cancer, and only 5% of the total breast cancer incidence. It is thus likely that there are still other breast cancer susceptibility genes to be found. In addition, the model of polygenic susceptibility to breast cancer suggests that susceptibility to breast cancer is mediated through variants in many genes, each conferring a moderate risk of the disease, and that several common, low-penetrance susceptibility genes with multiplicative effects on risk may account for the residual familial aggregation of breast cancer. In addition to influencing breast cancer risk, variants in these genes may modify the cancer risk caused by other genes with which they interact, or have an impact on the progression and outcome of the disease, thus effecting the prognosis of the patient.

Cellular responses to DNA damage are crucial for maintaining homeostasis and preventing the development of cancer. In addition to directly repairing DNA breaks, cells respond to DNA damage by halting cell-cycle progression or undergoing programmed cell death, apoptosis. To ensure the maintenance of the genomic integrity, cells have developed a highly branched DNA damage response signaling network. Defects in this signaling network may result in tumorigenesis. The genes operating in DNA damage response are thus good candidates for breast cancer susceptibility genes. This study focuses on the role of three DNA damage response –associated genes, *ATM*, *RAD50* and *p53*, in breast cancer.

2 REVIEW OF THE LITERATURE

2.1 Cancer

Cancer is one of the leading causes of death in the developed countries. One-fourth of the Finnish people will be affected with cancer at some point in their lives. The risk of cancer increases with age. Every year about 24 000 people in Finland are diagnosed with cancer (Finnish Cancer Registry, www.cancerregistry.fi); half of them will survive.

Cancer is a genetic disease of somatic cells, which means that it occurs as a consequence of several somatic mutations in the cell. These mutations activate oncogenes (gain-of-function) or inactivate tumor suppressor genes (loss-of-function). Proto-oncogenes encode proteins that are activated during cell growth, such as growth factors, membrane-associated signaling proteins or transcription factors. When a proto-oncogene is turned on at an inappropriate time, it becomes an oncogene. The classic tumor suppressor genes are so-called gatekeeper genes: they limit cell growth by regulating basic cell functions and controlling cell cycle, proliferation, differentiation and apoptosis (programmed cell death). In addition to gatekeeper genes, two other classes of tumor suppressor genes have been described: caretaker genes that repair and correct errors in DNA, and landscaper genes that regulate the cellular microenvironment (Kinzler & Vogelstein 1997, 1998). When a mutation activates an oncogene or inactivates a tumor suppressor gene, it leads to a tendency towards uncontrolled cell growth and proliferation, which may ultimately lead to cancer.

Most cancers have a multifactorial etiology and are attributable to a varying blend of genetic and environmental factors; only about 5% of common cancers are due to a strong inherited susceptibility (Li 1995). A minority of cancers is due to monogenic cancer predisposition syndromes in which there is Mendelian inheritance with incomplete penetrance, conferring an increased risk to a characteristic spectrum of cancers. Environment has the principal role in causing sporadic cancer, and inherited genetic

factors make only a minor contribution to susceptibility to most types of cancer. Significant effects of heritable factors have been observed for prostate cancer, where approximately 40% of the risk may be explained by heritable factors, colorectal cancer (35%) and breast cancer (30%) (Lichtenstein et al. 2000). In most of the known cases so far, inherited cancer predisposition occurs because of the germline alterations in tumor suppressor genes. This inherited cancer predisposition usually shows dominant inheritance at the family level, but is recessive on the cellular level. One mutated allele is inherited; however, according to the classic two-hit hypothesis (Knudson 1971), tumor development requires two mutated alleles. The second hit is a somatic mutation in the wild-type allele resulting in two mutated alleles and loss of function of the tumor suppressor. Loss of heterozygosity (LOH) is a common way to lose the wild-type allele and has been a hallmark of a tumor suppressor gene. In addition to the classic two-hit hypothesis, other mechanisms, with which mutated tumor suppressor genes can cause cancer, are emerging: dominant-negative effect (Brachmann et al. 1996, Chenevix-Trench et al. 2002) and haploinsufficiency (Fero et al. 1998, Venkatachalam et al. 1998, Kwabi-Addo et al. 2001, reviewed in Fodde & Smits 2002). In the former, the mutated allele disturbs the function of the normal allele; in the latter, one normal allele is not sufficient for the proper function of the gene. In both cases, losing both normal alleles is not necessary for tumor progression.

2.2 Breast cancer

2.2.1 Epidemiology

Breast cancer is the most commonly occurring cancer among women, and its incidence is increasing worldwide. The estimated annual incidence of breast cancer worldwide is over one million cases, and there is a significant regional difference in the incidence rates: the high-risk areas North America and Northern Europe account for 16% of the worldwide population but 60% of the worldwide incidence of breast cancer (Parkin 2004, Parkin et al. 2005). In Finland, over 4000 women were diagnosed with breast cancer in 2006 (estimate based on 2005 incidence, Finnish Cancer Registry, www.cancerregistry.fi). Incidence rates also correlate with gender, ethnicity, and age. Approximately only one

out of every 150 breast cancers occurs in males (20 men were diagnosed in Finland in 2005.). Breast cancer incidence in women increases up to 10-fold from age 25 to age 40. In addition to demographic factors, numerous other risk factors have been identified. These include among other things older age at menopause, early age of menarche, nulliparity and older age at first child birth, no breast feeding, low physical activity, use of oral contraceptives or hormone replacement therapy, obesity in postmenopausal women, alcohol use, and high intake of unsaturated fat and well-done meat (reviewed in Oldenburg et al. 2007). Positive family history of breast cancer is a well established risk factor for breast cancer, with first-degree relatives of patients having approximately two-fold elevated risk. In Western countries, the overall lifetime risk for women who have no affected relative is 7.8%, for those who have one, the risk is 13.3%, and for those who have two, the risk is 21.1% (Collaborative Group on Hormonal Factors in Breast Cancer 2001).

Although breast cancer incidence is increasing, the prognosis has improved, partly because of earlier diagnosis and partly as a result of the use of adjuvant therapies. Four types of standard treatment are used for breast cancer: surgery, radiation therapy, chemotherapy and hormone therapy. Radiation therapy, chemotherapy, and hormone therapy are often used as adjuvant therapy with surgery.

2.2.2 Clinical and molecular features

Virtually all breast tumors are carcinomas, tumors derived from epithelial tissue. Carcinomas are the most common type of cancer. Breast cancers are typically adenocarcinomas, derived from glandular tissue, and can be classified into histopathologic subtypes with distinct biological and prognostic characteristics. Histologically the most common breast tumors are infiltrating ductal (70%) and lobular (6%) carcinomas. Both are derived from the terminal duct lobular unit (TDLU) (Sainsbury et al. 2000). Medullary carcinomas represent a minor (about 3% of breast cancer) morphologically and biologically distinct group characterized by lymphocyte infiltrates in the tumor periphery. In the clinical practice breast cancer patients are classified in stages based on the clinical and pathologic extent of the disease according to

the TNM system, where T refers to tumor size, N to the presence of metastases in the local regional lymph nodes, and M to distant metastases. Breast tumors are graded and classified histologically based on an assessment of tubule/gland formation, nuclear pleomorphism and mitotic counts as well differentiated (grade 1), moderately differentiated (grade 2), and poorly differentiated (grade 3). Both the TNM classification and histological grade are significantly associated with survival and are thus powerful prognostic factors (Sainsbury et al. 2000). Approximately 30% of breast tumors have mutations in the p53 tumor suppressor gene, often accompanied by loss of the wild-type allele (Børresen-Dale 2003). As most p53 missense mutations cause accumulation of the p53 protein in the tumor cells, immunohistochemisty is widely used to detect p53 mutations in breast tumors. Hormone receptor (estrogen receptor, ER and progesterone receptor, PR) positive breast cancers account for 75-80% of all breast cancers, and hormone receptor expression is associated with better prognosis due to good response to targeted hormone treatments/adjuvant hormone therapy (Duffy 2005). Overexpression or amplification of ERBB2 (HER2), a member of a receptor tyrosine kinase superfamily, is associated with aggressiveness and a poor prognosis in breast cancer (Menard et al. 2000, Yarden 2001). However, ERBB2-positive breast cancers can be treated with targeted therapy with trastuzumab (Herceptin), an antibody directed against the ERBB2 ectodomain, specifically inhibiting the growth of cells expressing ERBB2 (Duffy 2005). ERBB2-positive cases account for 15-20% of breast cancer cases. Ten to fifteen per cent of breast cancers are so-called triple-negative breast cancers, lacking expression of all these three receptors (Cleator et al. 2007).

Until recently, the subclassification of breast carcinomas has been based on cellular morphology and the presence of receptors such as ER, PR and ERBB2, identified by immunohistochemical staining. Very recently, however, cDNA microarray-based gene expression profiling has become a new classification tool, and five distinct types of breast cancers have been defined according to similarities in gene expression patterns (Perou et al. 2000, Sorlie et al. 2001). These include two ER-positive groups, luminal subtypes A and B, with features shared with luminal epithelial cells arising from the inner layer of the duct lining. The third group overexpresses ERBB2, and the fourth group is a normal

breast gene expression group, characterized by high expression of basal epithelial genes and low expression of luminal epithelial genes. The fifth group is the basal-like subgroup sharing features with normal breast basal epithelial cells, characterized by expression of basal (myoepithelial) cytokeratins 5/6 and 17, and absence of ER, PR and ERBB2 expression. These tumors are assumed to arise from the outer (basal) layer of breast duct (myoepithelial cells).

2.3 Breast cancer genetics

2.3.1 High-penetrance breast cancer susceptibility genes

2.3.1.1 p53, *PTEN* and *LKB1*

Breast cancer is a characteristic in certain rare autosomal dominant cancer predisposition syndromes. Li-Fraumeni syndrome (LFS) (Li & Fraumeni 1969) is characterized by childhood soft tissue sarcomas and osteosarcomas, brain tumors, breast cancer, and adrenocortical as well as pancreatic carcinomas. Mutations in the *p53* tumor suppressor gene account for approximately 70% of families fulfilling the classical criteria for LFS (Malkin et al. 1990, Birch et al. 1994, Frebourg et al. 1995 Varley et al. 1997). Altogether, nearly 400 families with germline *p53* mutations have been reported worldwide. It is estimated that 28-56% of *p53* mutation carrier women will develop breast cancer by the age of 45 years (Garber et al. 1991, Chompret et al. 2000).

Cowden syndrome (CS) is caused by germline mutations in the tumor suppressor gene *PTEN*: mutations in *PTEN* are present in about 80% of CS families (Liaw et al. 1997). CS is associated with benign and malignant tumors of the breast, thyroid and endometrium as well as multiple hamartomas of the skin, gastrointestinal tract, and central nervous system. Women with CS have a 20-50% lifetime risk of breast cancer (Eng 2003).

Germline mutations in *LKB1* (*STK11*) tumor suppressor gene predispose to Peutz-Jeghers syndrome (PJS) (Hemminki et al. 1998) characterized by hamartomatous polyps of the gastrointestinal tract and melanine pigmentation of the lips as well as increased risk of various cancers, particularly of gastrointestinal, breast, gynecologic, and pancreatic

tumors. The risk of breast cancer in PJS by the age of 70 is up to 50% (Hearle et al. 2006).

2.3.1.2 *BRCA1* and *BRCA2*

BRCA1 and *BRCA2* are caretaker genes that act as sensors of DNA damage and participate in the DNA repair processes. BRCA1 functions in DNA repair, protein ubiquitylation, chromatin remodeling, and cell-cycle checkpoint control; BRCA2 is involved in DNA double-strand break (DSB) repair through homologous recombination (HR) (Powell & Kachnic 2003, Yoshida & Miki 2004, Boulton 2006). Their inactivation thus allows other genetic defects to accumulate and leads to genetic instability. Both *BRCA1* (Miki et al. 1994) and *BRCA2* (Wooster et al. 1995) are high-penetrance breast cancer susceptibility genes. Approximately 85% of breast cancer mutations in *BRCA1* and *BRCA2* are frameshift or nonsense mutations leading to truncated protein products. LOH is a common phenomenon in both *BRCA1* and *BRCA2* tumors (reviewed in Honrado et al. 2005a and 2006), supporting the roles of *BRCA1* and *BRCA2* as classical tumor suppressor genes. Germline mutations in *BRCA1* and *BRCA2* confer strong lifetime risks of breast and ovarian cancer. The average cumulative risk of breast cancer at the age of 70 years for *BRCA1* and *BRCA2* mutation carriers has been estimated to be up to 80%, and the risk of ovarian cancer up to 60% for *BRCA1* and 40% for *BRCA2* mutation carriers (Ford et al. 1994, 1998, Easton et al. 1995, The Breast Cancer Linkage Consortium 1999, Risch et al. 2001, Antoniou et al. 2003, Marroni et al. 2004). Dependence of the risk on ethnicity, family history, specific mutation, and other modifying genes has been suggested. *BRCA1* and *BRCA2* mutations are found in about 45% of families with breast and ovarian cancer, while the mutation rate in families with only breast cancer ranges from 10-15% (for families with three breast cancers) to 25–35% (for families with more than five breast tumors) (Vahteristo et al. 2001, Honrado et al. 2005a). *BRCA2* mutations are also associated with male breast cancer. In addition, biallelic *BRCA2* mutations cause Fanconi anemia subtype FA-D1 and predispose to childhood malignancies (Howlett et al. 2002). Fanconi anemia is a rare, recessive, chromosomal instability disorder characterized by growth retardation, congenital

malformations, progressive bone marrow failure, cancer predisposition, and cellular hypersensitivity to DNA cross-linking agents.

Breast cancer tumors arising in *BRCA1* mutation carriers have been shown to associate with distinct histopathologic features (reviewed in Honrado et al. 2005a and 2006). *BRCA1* –associated tumors are generally of higher grade and more often ER and PR negative than sporadic or familial non*BRCA1/2* tumors, and ERBB2 overexpression is very infrequent in *BRCA1* carcinomas. The majority of *BRCA1* tumors are infiltrating ductal carcinomas, but there is a significantly higher frequency of medullary tumors among *BRCA1* tumors than in non-carrier tumors. *BRCA1* tumors are also associated with positive p53 immunostaining and somatic p53 mutations more frequently than non*BRCA1/2* tumors. Furthermore, most *BRCA1* tumors have a basal-like gene expression profile, characterized by expression of basal cytokeratins, overexpression of epidermal growth factor receptor (EGFR), and absence of ER, PR and ERBB2 expression (Sorlie et al. 2003, Foulkes et al. 2003, Palacios et al. 2004). On the contrary to *BRCA1* tumors, no clear histopathologic subtype specifically differentiating *BRCA2* tumors has been found, although studies on somatic genetic changes, gene expression profiles, and immunohistochemistry have revealed some differences in characteristics of *BRCA2* tumors compared to tumors of non-carriers (Tirkkonen et al. 1997, Hedenfalk et al. 2001, Honrado et al. 2006a, 2006b).

2.3.2 Low-penetrance breast cancer susceptibility genes

2.3.2.1 CHEK2

Checkpoint kinase 2 (CHEK2) is an important signal transducer within the cellular network that responds to DNA damage and protects genomic integrity (Bartek et al. 2001). *CHEK2* 1100delC variant was the first low-penetrance variant associated with familial breast cancer, and the first variant identified conferring a moderate risk of breast cancer (Meijers-Heijboer et al. 2002, Vahteristo et al. 2002). The frequency of 1100delC varies between different populations, but it is relatively low in all populations studied (highest frequencies in the Netherlands, 1.3-1.6%, and in Finland, 1.1-1.4%; not detected at all in the Spanish population). It is estimated to confer an approximately two-fold

increased risk of breast cancer, and the carriers of 1100delC also have an elevated risk of bilateral breast cancer (reviewed in Nevanlinna & Bartek 2006). Other variants in *CHEK2* have also been considered to be involved in causing breast cancer risk, and at least the I157T variant seems to be associated with breast cancer risk, but the risk is probably lower than that associated with 1100delC (Cybulski et al. 2004, Kilpivaara et al. 2004). Variants in *CHEK2* have also been associated with increased risk of prostate and colorectal cancer (Seppälä et al. 2003, Cybulski et al. 2004a, 2004b, 2006, Kilpivaara et al. 2006).

2.3.2.2 PALB2 and *BRIP1*

A new BRCA2 binding protein, PALB2, was recently identified. PALB2 colocalizes with BRCA2 in nuclear foci, promotes its localization and stability in key nuclear structures, such as chromatin and nuclear matrix, and enables its recombinational repair and checkpoint functions (Xia et al. 2006). More recently, truncating germline mutations in *PALB2* were found to be associated with familial breast cancer, conferring a two- to four-fould increased breast cancer risk (Rahman et al. 2007, Erkko et al. 2007). Pathogenic mutations in *PALB2* were also identified in families affected with Fanconi anemia and cancer in early childhood, demonstrating that biallelic *PALB2* mutations cause a new subtype of Fanconi anemia, FA-N, and, similar to biallelic *BRCA2* mutations, confer a high risk of childhood cancer (Reid et al. 2007, Xia et al. 2007).

The BRCA1 associated C-terminal helicase, BACH1 (also known as BRIP1), a protein binding directly to the BRCA1 BRCT domains and participating in DNA double-strand break repair with BRCA1 (Cantor et al. 2001), has been shown to be the protein defective in another Fanconi anemia subtype, FA-J (Levran et al. 2005, Litman et al. 2005). Recently, truncating mutations in *BRIP1* were also identified as breast cancer susceptibility alleles, conferring a two-fold increased risk of breast cancer (Seal et al. 2006).

2.3.3 Common polymorphisms associated with breast cancer risk

All the currently known breast cancer susceptibility genes are estimated to account for 20-30% of familial breast cancer (Figure 1) and only 5% of the total breast cancer incidence, even though it is suggested that the proportion of breast cancer that can be attributed to genetic factors may be as high as 30%. It is thus likely that there are still other breast cancer susceptibility genes to be found (Oldenburg et al. 2007). In addition, the model of polygenic susceptibility to breast cancer suggests that susceptibility to breast cancer is mediated through variants in many genes, each conferring a moderate risk of the disease, and that several common, low-penetrance genes with multiplicative effects on risk may account for the residual non*BRCA1/2* familial aggregation of breast cancer (Pharoah et al. 2002, Antoniou et al. 2002, Antoniou & Easton 2006). Linkage studies have also suggested putative other breast cancer susceptibility loci but the genes have not been identified so far, and attempts to identify new breast cancer susceptibility genes through candidate gene approach have not, despite the few exceptions, been very successful. As linkage studies lack power to detect alleles with moderate effects on risk, large case-control association studies are required (Easton et al. 2007).

During the past 10 years, case-control association studies have been used widely in the search for breast cancer susceptibility alleles, and most of these studies have focused on putative functional variants, including single nucleotide polymorphisms (SNPs), in genes that are candidates for breast cancer susceptibility genes because of their known biological functions, such as genes involved in DNA repair. However, most associations reported have not been confirmed by subsequent studies. This situation probably reflects the fact that most initial findings are false positives. On the other hand, as many studies lack the power to detect genetic variants that are associated with modest increases in risk, nonreplication may also occur because of a lack of adequate statistical power in the replication study, resulting in false negatives (Breast Cancer Association Consortium 2006).

Recently, the Breast Cancer Association Consortium (BCAC) found evidence of an association with breast cancer for D302H variant in caspase 8 (CASP8) and L10P variant

in transforming growth factor beta (TGFB1) (Cox A et al. 2007). They estimated that the variants in CASP8 and TGFB1 may account for approximately 0.3% and 0.2% of the excess familial risk of breast cancer, respectively, in populations of European ancestry. These data are the strongest evidence to date for common breast cancer susceptibility alleles. In CASP8, the histidine allele of D302H is associated with a moderate reduction in breast cancer risk. This SNP was initially identified through a candidate gene approach, as CASP8 is an important initiator of apoptosis and is activated by external death signals and in response to DNA damage (Chipuk & Green 2006). However, further experiments are required to establish whether D302H itself, or another variant in strong linkage disequilibrium with it, is causative, as the functional consequences of the aspartic acid-to-histidine substitution are not yet known (Cox A et al. 2007).

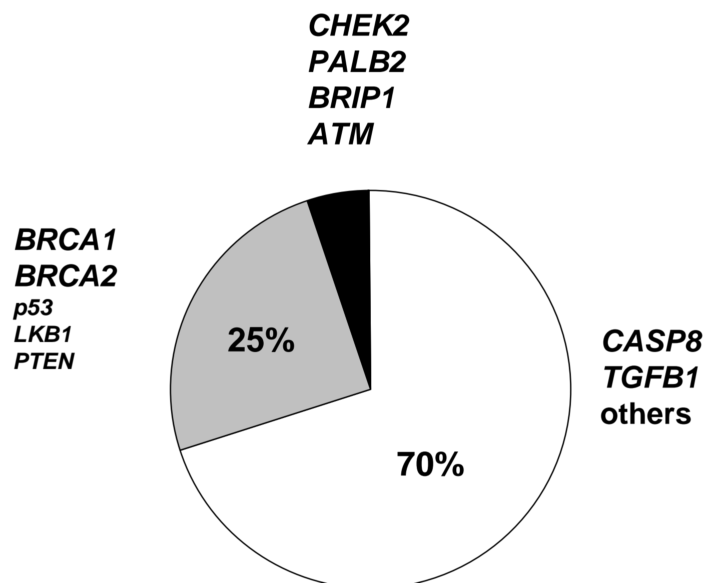


Figure 1. Contribution of known breast cancer susceptibility genes to the overall genetic factors involved in breast cancer (Adapted from Willems 2007).

In addition to attempts to identify common risk variants through candidate gene approach, SNPs are used in genome-wide association studies. Recently, BCAC conducted a large two-stage genome-wide association study followed by a third stage in which 30 SNPs were tested for confirmation, and five novel independent loci with strong and consistent evidence of association with breast cancer were found (Easton et al. 2007).

Further studies are needed to identify which genes and variants are the actual causative ones for breast cancer risk.

Common polymorphisms may also modify the cancer risk caused by mutations in the genes with which they interact. The penetrance of *BRCA1* and *BRCA2* mutations, for example, seems to depend on the family history, and the risks have been found to vary by age at diagnosis and the type of cancer of the index patient. These observations would be consistent with the hypothesis that the breast cancer risk of the mutation carriers is modified by other genetic factors (Antoniou & Easton 2006). The modifying effect would explain the differences between population-based estimates for *BRCA1/2* penetrance and estimates based on high-risk families (Antoniou et al. 2002). So far, the attempts to identify the genetic modifiers of cancer risk in *BRCA1* and *BRCA2* mutation carriers have yielded modest results: the studies have been small and the subsequent studies have failed to replicate the results (Chenevix-Trench et al. 2007). A single nucleotide polymorphism in the 5'UTR of *RAD51*, 135G>C, is the most convincing candidate as a modifier of *BRCA1/2*. RAD51 is an important component of DNA double-strand break repair mechanisms that interacts with both BRCA1 and BRCA2. The 135G>C SNP was initially suggested as a possible modifier of breast cancer risk in *BRCA1* and *BRCA2* mutation carriers (Wang et al. 2001). Subsequent studies have confirmed that 135G>C modifies the risk of breast cancer in *BRCA2* mutation carriers, but not in *BRCA1* mutation carriers (Levy-Lahad et al. 2001, Kadouri et al. 2004, Antoniou et al. 2007). To generate sufficient statistical power to reliably identify modifier genes of *BRCA1* and *BRCA2*, the Consortium of Investigators of Modifiers of *BRCA1* and *BRCA2* (CIMBA) has recently been established (Chenevix-Trench et al. 2007).

In addition to breast cancer susceptibility, genetic variation may play a role in the progression of the disease, affecting its aggressiveness, response to treatments, and overall outcome. The overall impact of genetic factors, including those yet to be discovered, on breast cancer outcome remains unclear, but given the implications to treatment, follow-up, and patient counseling, this is a field that deserves extensive studying.

2.4 ATM and the MRN complex

2.4.1 ATM structure

The *ATM* (*Ataxia Telangiectasia Mutated*) gene extends over 160 kb of genomic DNA on chromosome 11q22.3 and produces an approximately 13 kb transcript. It comprises of 66 exons, 62 of which encode a 350 kDa protein expressed in multiple embryonic and adult tissues (Savitsky et al. 1995, Chen & Lee 1996). ATM is a serine/threonine kinase belonging to the phosphoinositide 3-kinase (PI3-kinase) –like family present in organisms from mammals to yeast. ATM is included in a subgroup of these kinases termed PI3-kinase-like kinases (PIKK) (Abraham 2004). The conserved PI3-kinase domain is located towards the C-terminus of the ATM protein (Figure 2). In addition to a leucine zipper of an unknown function and an ATP binding site, the ATM protein contains two other domains, FAT and FATC (Bosotti et al. 2000). The FAT domain is named after three groups of proteins sharing this domain, **F**RAP (mTOR), **A**TM and **T**RRAP. The most C-terminal domain is called FATC, since this domain is found only in combination with FAT. It is suggested that these domains fold together in a configuration that ensures efficient function of the protein kinase domain (Lavin et al. 2004). In addition to the kinase domain, a second substrate-binding site, suggested to bind p53, BRCA1 and BLM, has been mapped to the N-terminus of ATM.

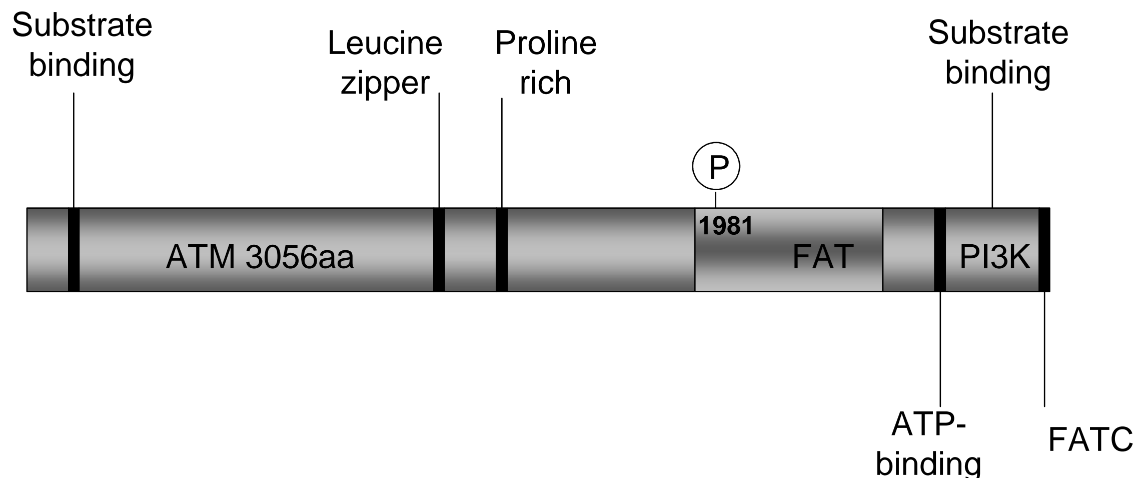


Figure 2. Structure of ATM (Adapted from Lavin et al. 2004.).

In proliferating cells ATM is largely a nuclear protein, which is in line with its function in DNA damage recognition and cell-cycle checkpoint signaling, but approximately 10% of the protein is extranuclear, present in cytoplasmic vesicles (Lavin et al. 2004).

2.4.2 Structure of the MRN complex

The highly conserved MRE11/RAD50/NBS1 (MRN) complex has a central role in many cellular responses to DNA double-strand breaks (DSBs), including homologous recombination (HR), non-homologous end-joining (NHEJ), telomere maintenance, and DNA damage checkpoint activation (Assenmacher & Hopfner 2004). The complex consists of the large coiled-coil ATP-binding cassette (ABC) ATPase RAD50, the exonuclease MRE11, and the checkpoint mediator NBS1. The exonuclease MRE11 forms the core of the complex directly binding NBS1, DNA, and RAD50. The core MRE11-RAD50 (MR) complex exists as a heterotetrameric assembly (M_2R_2) with two globular DNA binding heads (Hopfner et al. 2001) (Figure 3a). RAD50 employs its ABC ATPase, zinc-hook, and coiled coils to bridge DSBs and facilitate DNA end processing by MRE11. Contributing to the regulatory roles of the MRN complex, NBS1 harbors N-terminal phosphopeptide-interacting FHA and BRCT domains, as well as C-terminal ATM and MRE11 interaction domains (Williams et al. 2007).

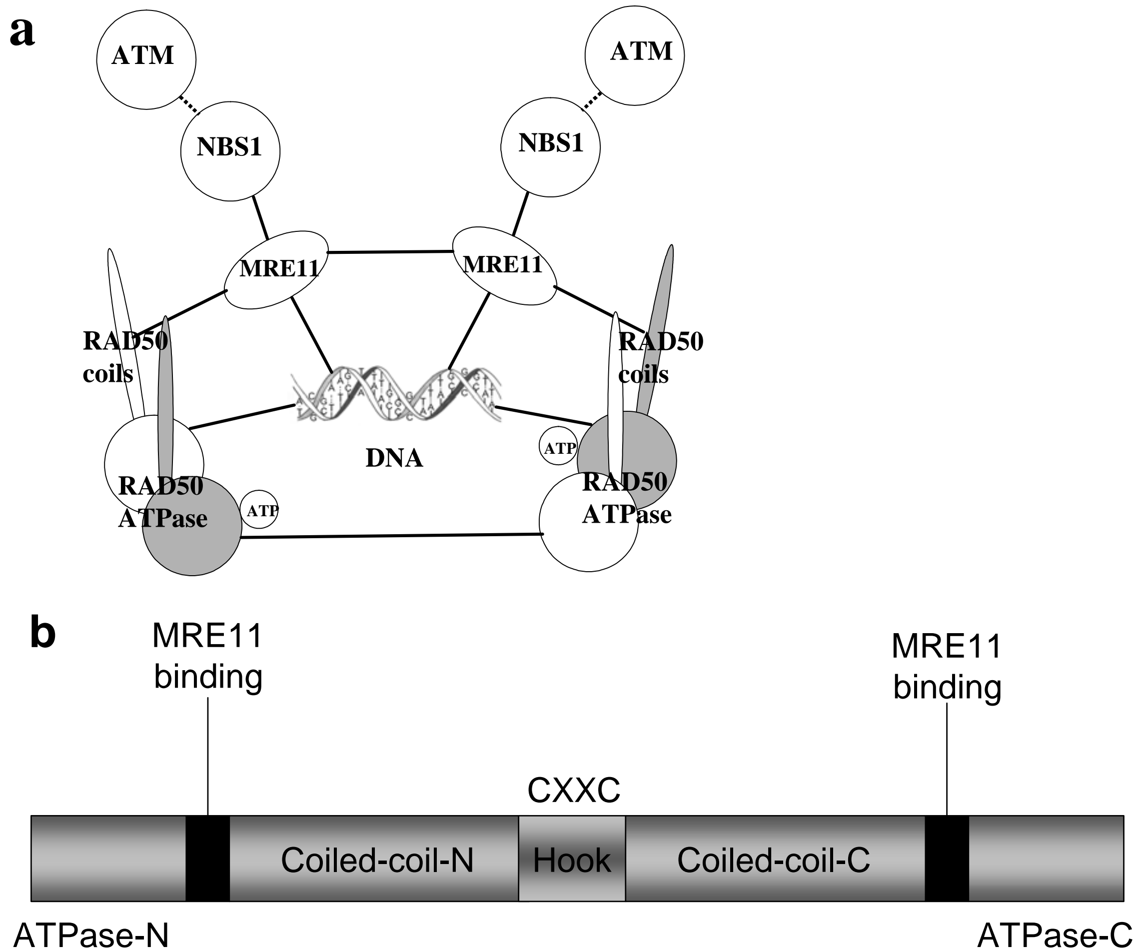


Figure 3. MRN complex and RAD50 (Adapted from Williams et al. 2007).
a) Structure and protein-protein and protein-nucleic acid interactions of the MRN complex.
b) Structure of RAD50.

RAD50 is a 1312 amino acid protein that contains two ABC-ATPase domains separated by two coiled-coil regions required for intramolecular interactions. A Cys-X-X-Cys motif of the zinc-hook structure located in the middle of the coiled-coil domain functions as a dimerization domain between two RAD50 arms (Hopfner et al. 2002, van den Bosch et al. 2003) (Figure 3b).

2.4.3 Function of ATM and the MRN complex in DNA damage response

Cellular responses to DNA damage are crucial for maintaining homeostasis and preventing the development of cancer. DNA damage response (DDR) is a complex, highly branched signaling network protecting the stability and integrity of the cellular genome (Shiloh 2006). Of the many kinds of DNA lesions, double-strand breaks (DSBs) are particularly effective in triggering DDR. DSBs are induced by ionizing radiation (IR) as well as by oxygen radicals formed in the normal metabolism. They are also a part of normal genomic transactions such as DNA replication and meiosis (Khanna & Jackson 2001). Failure to repair DSBs can lead to gross chromosomal rearrangements and ultimately to tumorigenesis. An ongoing DSB response has been observed in precancerous cells and tumor tissues (Gorgoulis et al. 2005, Bartkova et al. 2005a, 2005b). Two mechanisms are used in eukaryotic cells to repair DSBs: non-homologous end-joining (NHEJ) ligating free DNA ends and homologous recombination (HR) between sister chromatids. NHEJ acts throughout the cell cycle (Lieber et al. 2004, 2006), but HR is a high-fidelity process functioning in the late S and G2 phases of the cell cycle (Wyman et al. 2004).

In addition to directly repairing DNA breaks, cells respond to DNA damage by halting cell-cycle progression or undergoing programmed cell death, apoptosis (Kastan & Bartek 2004). To ensure that an earlier process, such as DNA replication or mitosis, is complete, and that the genomic integrity is maintained before cell-cycle progression, cells have developed a surveillance mechanism based on an intricate network of protein kinase signaling pathways that lead to cell-cycle delay or arrest in response to DNA damage at G1/S, intra-S, and G2/M checkpoints (Hartwell & Weinert 1989, Zhou & Elledge 2000). These cell-cycle checkpoints are central to the maintenance of the genomic integrity and basic viability of the cells, and defects in these pathways may result in tumorigenesis.

ATM is the initiator of the signaling cascades responding to DSBs. (Shiloh 2003, 2006). ATM is normally present in cells as an inactive dimer or multimer complex. After DNA damage, ATM undergoes autophosphorylation on Ser1981 and the inactive complex

dissociates into active monomers (Bakkenist & Kastan 2003). Once activated, ATM directly or indirectly phoshorylates over 30 substrates (Lavin et al. 2006), leading to activation of cell-cycle checkpoints and initiation of DNA repair. These substrates include DNA damage sensors, checkpoint mediators, signal transducers as well as the effectors directly executing the DDR and DNA repair functions (Kastan & Bartek 2004, Lavin & Kozlov 2007) (Figure 4). Many components of the signaling network downstream of ATM are themselves capable of activating several other downstream effectors, which amplifies the signal in the cascade. A remarkable feature of ATM function is its ability to approach the same endpoint from many directions by using different pathways (Shiloh 2003, 2006). In addition, the same effectors can be targeted by several different ATM-dependent mechanisms. A prominent example is the G1/S checkpoint, just before the entry into the S-phase, where p53 is in central role. ATM directly phosphorylates p53 on Ser15 and other sites, thus contributing to its stabilization and transcriptional activation. ATM also phosphorylates three other proteins, CHEK2, MDM2 and MDMX, all of which also influence the stability and transcriptional activity of p53 (Kastan & Lim 2000, Lavin & Kozlov 2007). CHEK2 activation by ATM leads to phosphorylation of p53 by CHEK2. This and direct phosphorylation of p53 as well as phosphorylation of MDM2 by ATM interferes the binding of p53 to MDM2, leading to stabilization and transcriptional activation of p53. Transcriptionally activated p53 then induces p21/WAF1, an inhibitor of cyclin-dependent kinases, to inhibit cyclin E/CDK2, leading to inability of cells to progress from G1 to S-phase (Kastan & Bartek 2004, Lavin & Kozlov 2007). This p53-dependent pathway is the dominant checkpoint response to DNA damage in mammalian cells in G1 phase and may lead to sustained G1 arrest (Bartek & Lukas 2001, Kastan & Bartek 2004). The other pathway activated by ATM leading to the G1 checkpoint is a rapid transient pathway that is p53-independent. In this pathway the phosphorylation of CHEK2 by ATM leads to upregulation of CHEK2. The phosphorylation of CDC25A by CHEK2 then stimulates the degradation of CDC25A preventing CDC25A-dependent dephoshorylation and activation of CDK2 (Falck et al. 2001), leading to G1 arrest. This pathway is not only involved in the rapid prevention of S-phase entry in the G1 checkpoint but also in the transient intra-S-phase response to DNA damage (Bartek & Lukas 2001, Falck et al. 2002).

ATM also phosphorylates various other proteins involved in the control of S-phase, such as NBS1, BRCA1, SMC1 and FANCD2 (Kastan & Bartek 2004, Lavin & Kozlov 2007 and the refs. therein). In addition to the CDC25A-pathway, another ATM-dependent pathway, NBS1/BRCA1/SMC1 pathway, is also involved in the control of the intra-S-phase checkpoint (Falck et al. 2002, Kitagawa et al. 2004). SMC1 controls DNA replication forks and assists in DNA repair, and the phosphorylation of SMC1 by ATM requires the checkpoint mediators NBS1 and BRCA1 (Kitagawa et al. 2004). Phosphorylation of FANCD2 by ATM is also involved in the control of this checkpoint (Taniguchi et al. 2002).

ATM is also involved in the regulation of the G2/M checkpoint before mitosis, which is controlled by the interacting ATM/CHEK2 and ATR/CHEK1 pathways. Under normal conditions, the dephosphorylation of CDC2 by CDC25C allows cyclin B/CDC2 to promote mitotic entry. ATM-dependent phosphorylation of CDC25C by CHEK2 thus inhibits mitotis-promoting activity of cyclin B/CDC2. The checkpoint mediators BRCA1 and 53BP1 are also needed in the G2/M checkpoint. BRCA1 is phosphorylated by ATM and plays a role in CDC25C regulation. It also activates CHEK1, which in turn blocks mitotic entry through phosphorylation of CDC25C (reviewed in Kastan & Bartek 2004 and Lavin & Kozlov 2007). In addition, phoshorylated BRCA1, together with BRCA2, induces DSB repair using HR, in part through the activation of the DNA-repair enzyme RAD51 (Cortez et al. 1999).

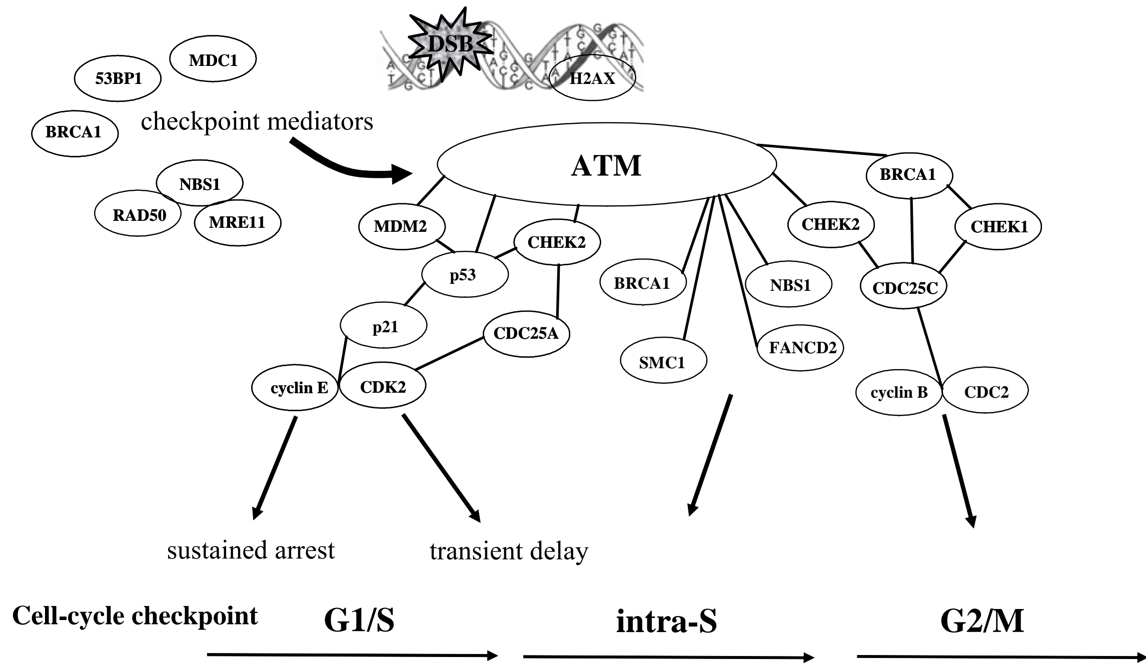


Figure 4. A simplified overview of the central players in ATM-regulated cell-cycle checkpoint pathways.

The MRN complex has a critical role in DSB recognition, stabilization and signaling. It acts as a sensor of DSBs and regulates chromatin remodeling near them. The MRN complex also initiates cell-cycle checkpoint signaling cascades through ATM activation. This is a two-way functional interaction, as the members of the complex are also downstream of ATM in these pathways (Lavin 2004). The early stages of DSB response are characterized by the rapid formation of nuclear foci at the damaged sites of the chromatin, representing huge conglomerates of recruited DDR proteins that gather together through repeated protein-protein interactions (Shiloh 2006). ATM is present in the nuclear foci at DSB sites, together with other DDR proteins MDC1, 53BP1, BRCA1 and the MRN complex (Bekker-Jensen et al. 2006). The MRN complex is the first to bind to DSB sites. It acts as a DSB sensor for ATM and recruits it to the damaged sites. The activation of ATM requires a physical interaction between ATM and the MRN complex and DNA (Uziel et al. 2003, Lee & Paull 2004, 2005, Paull & Lee 2005, Dupré et al. 2006). The MRN component binding ATM is NBS1, which has a role in chromatin structure modulation (You et al. 2005, Berkovich et al. 2007). MRN complex is followed by MDC1, whose binding to chromatin requires phosphorylation of histone H2AX by

ATM. Phosphorylated H2AX (γH2AX) has an important role in anchoring DDR proteins to the damaged sites (Fernandez-Capetillo et al. 2004). MDC1 is required for ATM recruitment and also for sustained interaction with damaged DNA (Mochan et al. 2003, 2004, Lou et al. 2006), as it is required for sustained binding of MRN and 53BP1 (Lukas et al. 2004, Bekker-Jensen et al. 2005), coming next after MDC1, to the damaged chromatin. 53BP1 is also needed for ATM activation (Mochan et al. 2003, 2004). MDC1, 53BP1 and NBS1 are all targets of ATM-dependent phosphorylation and were thus initially placed downstream of ATM. However, recent data have shown that these proteins function also upstream of ATM, being also activators and not only sensors upstream of transducers. The initiation of DDR is now viewed as a cyclic process amplifying the signal, and the signal amplification process depends on the interaction of the sensors and activators with damaged chromatin and ATM (Shiloh 2006).

2.4.4 Ataxia-telangiectasia

Biallelic mutations in the *ATM* gene cause ataxia-telangiectasia (A-T) (Savitsky et al. 1995). A-T is an autosomal recessive disease with an estimated frequency of 1:40 000-300 000. It is an early-onset progressive neurologic disorder characterized by progressive cerebellar ataxia, ocular apraxia, telangiectasias in eyes, ears and cheeks, radiosensitivity, immunodeficiency, and increased risk of cancer, particularly of lymphoma and leukemia (Lavin et al. 1997). A-T patients have a lifetime cancer risk of 30-40%. The most common malignancies are tumors of the immune system that occur in the first 15 years of life; and epithelial cancers, including breast cancer, are seen in adults. A-T patients usually die from respiratory failure or cancer; median life expectancy is approximately 30 years. Genetic instability is a hallmark of the A-T phenotype. Typical cellular features of A-T patients include increased radiosensitivity, cell-cycle checkpoint defects, and an increased level of spontaneously occurring chromosome aberrations in both lymphoid and non-lymphoid cells (Meyn 1999).

2.4.5 ATM and breast cancer

ATM has long been considered as a good candidate gene for breast cancer susceptibility. It is a major activator of cellular responses to DNA double-strand breaks, interacting with

other central players in the DNA damage response pathways, including also known breast cancer susceptibility genes, such as *BRCA1*, p53, and *CHEK2*. Furthermore, the function of the ATM protein makes it a good candidate for a role in breast cancer predisposition, as radiation exposure is associated with an increased risk of breast cancer (Khanna & Chenevix-Trench 2004). The first suggestion that *ATM* might be a breast cancer susceptibility gene came from studies reporting an increased breast cancer risk among obligate heterozygous mutation carriers in A-T families (Swift et al. 1987, 1991). An increased risk for malignancy, in particular, female breast cancer, among individuals heterozygous for germline *ATM* mutations in A-T families has been reported in many studies (Olsen et al. 2001, Thompson et al. 2005), but in a recent study, the increased breast cancer risk of the heterozygous mutation carriers in A-T families was seen only in the mothers of the A-T patients (Olsen et al. 2005).

On the basis of estimated A-T carrier frequency of 1% and an increased risk of breast cancer of about three-fold in obligate heterozygous mutation carriers in A-T families, it was initially suggested that A-T heterozygosity could account for as much as 5% of all breast cancer (Easton 1994). However, the role of *ATM* as a breast cancer susceptibility gene outside the A-T families has remained controversial, as many of the case-control studies have failed to show an elevated frequency of *ATM* mutations in breast cancer patients. A number of studies have searched for germline *ATM* mutations in breast cancer cases and compared the frequencies between breast cancer cases and population controls (reviewed in Khanna & Chenevix-Trench 2004 and Ahmed & Rahman 2006, see also 6.1.1), but the evidence regarding the role of *ATM* as a breast cancer susceptibility gene has been contradictory. One suggested reason for the discrepancy is that analysis of breast cancer cases unselected for family history would be an inefficient way to detect *ATM* mutations if they did confer the high increased risk of breast cancer initially suggested; analysis of multiple-case breast cancer families could be more successful (Khanna & Chenevix-Trench 2004). On the other hand, as the frequency of *ATM* mutations found in breast cancer patients in the general population has been low, many of the mutations are too rare to be easily evaluated in case-control studies. In addition, recent epidemiologic analyses suggest approximately two-fold increase of breast cancer

risk for heterozygous *ATM* mutation carriers in A-T families (Thompson et al. 2005), and most of the case-control studies have been too small to detect modest increases of risk associated with *ATM* variants. Furthermore, very few studies have screened the whole *ATM* gene in both cases and controls, thereby limiting the ability to directly compare the frequency and type of identified variants (Ahmed & Rahman 2006).

It has also been suggested that only *ATM* mutations with specific functional consequences would predispose to breast cancer (Khanna & Chenevix-Trench 2004) and that dominant-negative mutations, missense changes in particular, which give rise to stable kinase-inactive or non-phosphorylable proteins, are the ones mainly responsible for the increased cancer risk in *ATM* mutation carriers (Gatti et al. 1999). The majority of *ATM* mutations found in A-T patients are truncating mutations, and this and the large size of the gene led to the fact that most of the first studies on *ATM* mutations in breast cancer patients used methods that are biased towards detecting protein truncating mutations. Yet, two recent studies in A-T families did not identify differences in cancer risk based on mutation type (Cavaciuti et al. 2004, Thompson et al. 2005), but it was suggested that the breast cancer risk in A-T families would be associated specifically with mutations located in the binding domains of the ATM protein (Cavaciuti et al. 2004).

Recent studies have reported a significant prevalence of *ATM* mutations, especially of those causative for A-T, in breast cancer families (Thorstenson et al. 2003, Renwick et al. 2006, Bernstein et al. 2006, see 6.1.1).

2.4.6 Diseases associated with the MRN complex genes

Hypomorphic biallelic *NBS1* mutations cause the autosomal recessive condition, Nijmegen breakage syndrome (NBS) (Varon et al. 1998), a chromosomal instability syndrome characterized by microcephaly, growth retardation, immunodeficiency, and predisposition to cancer, particularly lymphomas. The phenotype of NBS patients shows significant overlap with A-T, and the cells from NBS patients are hypersensitive to ionizing radiation and have cytogenetic features indistinguishable from those of A-T. Biallelic *MRE11* mutations cause ataxia-telangiectasia-like disorder (ATLD) which is

characterized by slowly progressive ataxia and ocular apraxia, the same clinical features as A-T but with later onset and slower progression (Stewart et al. 1999). Cells from ATLD patients show many of the features characteristic of both A-T and NBS, including chromosomal instability, increased sensitivity to ionizing radiation, defective induction of stress-activated signal transduction pathways, and radioresistant DNA synthesis. Mutations in *RAD50* have not been associated with a defined human phenotype. However, a hypomorphic mutation of *RAD50* has been identified in a patient suffering from a syndrome broadly reminiscent of NBS (Shiloh 2003).

NBS1, and especially the Slavic founder mutation 657del5, has been associated with increased risk of prostate cancer (Cybulski et al. 2004c) and breast cancer (Gorski et al. 2003, Steffen et al. 2004, 2006), but the reports have been inconsistent for both cancers (Hebbring et al. 2006, Buslov et al. 2005, Kanka et al. 2007). Other heterozygous *NBS1* alterations as well as certain *NBS1* haplotypes have also been suggested to associate with breast cancer (Heikkinen et al. 2003, 2006, Lu et al. 2006) and childhood acute lymphoblastic leukemia (ALL) (Varon et al. 2001). Recently, a truncating *RAD50* mutation, 687delT, was reported to contribute to breast cancer in Northern Finland (Heikkinen et al. 2003, 2006).

2.5 p53

2.5.1 Structure and function

p53 gene maps to chromosome 17p13 and comprises of 11 exons, first of which is non-coding (Lamb & Crawford 1986). It encodes a 53 kD nuclear phosphoprotein with cancer-inhibiting properties. p53 is a transcription factor constitutively expressed in most cell types. It consists of an N-terminal transactivation domain, a central DNA-binding domain, a tetramerization domain and a C-terminal regulatory domain (Figure 5). The N-terminal transactivation domain includes the proline-rich region suggested to be involved in the growth suppression and apoptosis inducing activities of p53 (Walker & Levine 1996, Sakamuro et al. 1997).

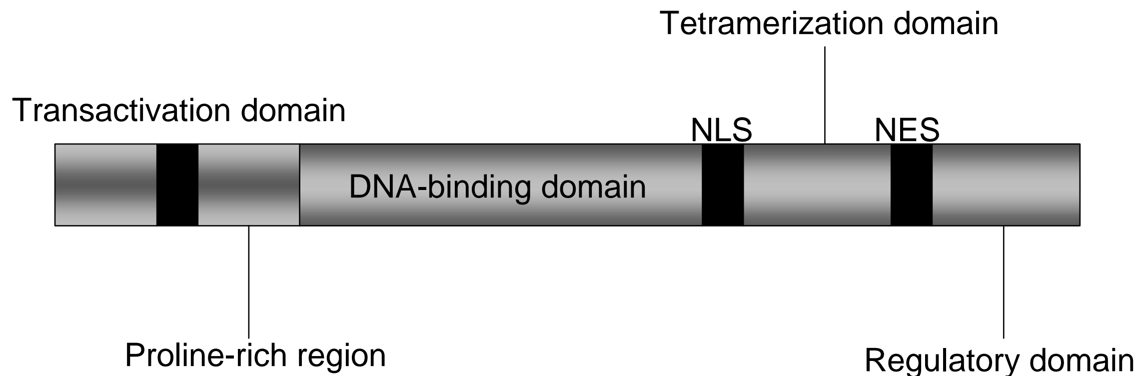


Figure 5. Structure of p53 (Adapted from Bai & Zhu 2006.). NLS, nuclear localization signal sequence; NES, nuclear export signal sequence.

The p53 protein is a multi-functional transcription factor involved in the control of cell-cycle progression, DNA integrity and apoptosis in cells exposed to DNA-damaging agents. The function of the p53 protein is regulated by several different biochemical modifications such as phosphorylation, acetylation and ubiquitination (reviewed in Lacroix et al. 2006).

Many functions of p53, including its primary role in tumor suppression, can be attributed to its ability to act as a sequence-specific transcription factor which regulates expression of different cellular genes to modulate various cellular processes (Farmer et al. 1992). As a sequence-specific transcription factor it regulates expression of over hundred different target genes. Genes activated by p53 are functionally diverse; they are downstream effectors of signaling pathways that elicit diverse responses such as cell-cycle checkpoints, cell-cycle arrest, DNA repair, cell survival, apoptosis, and senescence (Vogelstein et al. 2000, Vousden & Lu 2002). In response to various types of stress, p53 is accumulated in the nucleus and binds to specific sites in the regulatory regions of p53-responsive genes strongly promoting the transcription of these genes (Kern et al. 1991).

As a tumor suppressor, p53 is essential for preventing inappropriate cell proliferation and maintaining genome integrity following genotoxic stress (Vogelstein et al. 2000, Vousden & Lu 2002). Among various cellular stress responses induced by p53, most notable are the induction of cell-cycle arrest and apoptosis. p53 is involved in inducing

cell-cycle arrest in the G1/S, intra-S-phase, and G2/M checkpoints (see also 2.4.3). The induction of cell-cycle arrest at G1 and G2 by p53 provides additional time for the cell to repair genomic damage before entering the critical stages of DNA synthesis and mitosis. However, DNA-repair failure may result in the activation of apoptosis. As a cellular gatekeeper, one of the most important roles of p53 is to monitor cellular stress and to induce apoptosis when necessary (Levine 1997).

p53 controls apoptosis both through inducing transcription of pro-apoptotic genes and repressing transcription of anti-apoptotic genes. The genes controlled by p53 can induce apoptosis through two main pathways, the intrinsic, mitochondrial pathway and the extrinsic, death receptor pathway (Chipuk & Green 2006). The mitochondrial pathway is induced by cellular stresses, such as DNA damage or hypoxia, and operates through the Bcl-2 family of pro- and anti-apoptotic proteins. Pro-apoptotic proteins of the Bcl-2 family, such as Bax, Noxa, Puma, and p53AIP1 (Miyashita & Reed 1995, Oda E et al. 2000, Nakano & Vousden 2001, Oda K et al. 2000) localize to the mitochondria and elicit the mitochondrial outer-membrane permeabilization (MOMP) (Spierings et al. 2005) and release of cytochrome *c*, resulting in the formation of the apoptosome complex with Apaf-1 (Moroni et al. 2001) and in the Apaf-1 dependent activation of caspase 9. Caspase 9 in turn activates the executioner caspases 3 and 7. Caspases are cysteine proteases taking care of the apoptotic hallmarks, such as chromatin condensation, plasma membrane asymmetry, and clearance of the apoptotic cell. In the death receptor pathway, binding of death ligands to their death receptors on the plasma membrane, such as binding of the tumor necrosis factor (TNF) to its death receptor TNFR1, causes the recruitment of adaptor molecules (Muppidi et al. 2004) and activation of caspase 8, which again activates caspases 3 and 7, leading to cell death. p53 plays a role in death receptor induction and transport. It also directly binds anti-apoptotic and activates pro-apoptotic Bcl-2 proteins to regulate MOMP (Chipuk & Green 2006).

Due to its many functions in maintaining the genomic integrity, p53 has been named as “the guardian of the genome” (Lane 1992). Loss of p53 activity predisposes cells to the acquisition of oncogenic mutations and may favor genetic instability. *p53* has been

considered as a classic tumor suppressor gene. The development of human cancer often involves inactivation of this suppressor function through various mechanisms. Most p53 mutations disturb the transcriptional activity of p53, resulting in loss-of-function of its antiproliferative and apoptosis-inducing properties either through the complete loss of the wild-type p53 or through the dominant-negative effect of the mutated allele on the wild-type allele. However, evidence is piling up that some mutations are gain-of-function mutations, leading to oncogenic functions, such as activation of other oncogenes or attenuation of other tumor suppressor genes (Ko & Prives 1996, Kastan & Berkovich 2007). Recent data suggest that certain p53 mutants gain the ability to inhibit the MRN-ATM signaling pathway involved in controlling cellular responses to DNA double-strand breaks and may this way contribute to tumor development through enhanced genomic instability (Song & Hollstein 2007).

2.5.2 p53 in breast cancer

Inherited mutations in *p53* result in Li-Fraumeni syndrome and predispose to a wide spectrum of early-onset cancers, including breast cancer (see 2.3.1.1). In addition, somatic *p53* alterations are frequent in most human cancers; *p53* is the most frequently inactivated tumor suppressor gene in human cancer. The types of mutations observed in the germline and in sporadic cancer cases are in general similar. Unlike for instance *BRCA1* and *BRCA2*, where most mutations are truncating, most of the mutations (over 75%) in *p53* are missense mutations, and 97% of them cluster in exons encoding the DNA-binding domain (Olivier et al. 2002).

p53 mutations are found in 20-40% of all breast cancer tumors, depending on tumor size and stage of the disease, and mutation of *p53* seems to be an early event in breast tumorigenesis (Børresen-Dale 2003). *p53* mutations have been observed with higher frequency in breast cancer tumors of *BRCA1* mutation carriers than in sporadic tumors; for *BRCA2* the results have been less conclusive (Honrado et al. 2005). Some *p53* mutations found in the breast tumors of *BRCA1/2* mutation carriers are infrequently reported in sporadic cancers, which may reflect the distinct selective pressures operating during tumorigenesis in *BRCA1/2*-associated breast cancers (Gasco et al. 2003).

Many studies have confirmed that mutated *p53* is a strong independent prognostic factor in breast cancer. The risk of dying of breast cancer for patients with a *p53* mutation in their tumor has been estimated to be two to five-fold compared to patients with wild-type *p53* tumors (Pharoah et al. 1999 Olivier et al. 2006, Petitjean et al. 2007). The prognostic value of p53 accumulation in the tumor detected by immunohistochemistry has remained weaker in many studies, as correlation between *p53* mutation status and p53 staining in the tumor is not very strong. Positive immunostaining for p53 is in general considered to indicate somatic *p53* mutation and impaired p53 pathway, as most *p53* alterations found in breast carcinomas are point mutations leading to the synthesis of a stable, malfunctional, and nondegradable protein that accumulates in tumor cells, and thus can be detected by immunohistochemistry. It is, however, shown that not even all the *p53* missense mutations cause increased expression of a p53 protein detectable by immunostaining, and mutations resulting in protein truncation are not detected by immunohistochemistry (reviewed in Børresen-Dale 2003). Approximately 30% of the *p53* mutations detected by sequencing do not cause positive staining in the tumor (Sjögren et al. 1996, Norberg et al. 1998). *p53* mutation status as a predictor of therapy response in breast cancer has also been under extensive investigation recently. Due to different clinical and methodological settings used, the results have been heterogeneous and even contradictory (Petitjean et al. 2007). Association of *p53* mutation status with treatment outcome has been reported in a number of studies, and resistance to radiotherapy and different chemotherapeutic agents associated with certain specific types of *p53* mutations has also been suggested (reviewed in Børresen-Dale 2003 and Lacroix et al. 2006). The predictive value of *p53* mutations in breast cancer warrants further studies with larger numbers of cases.

2.5.3 p53 R72P polymorphism

Exon 4 of *p53* harbors a common polymorphism, 215G>C. This base change results in an arginine to proline change in the protein sequence. There is a correlation between the frequency of 72P and latitude (from 17% in Swedish Saamis to 63% in African Nigerians), suggesting that 72P might have been selected for because of its protective

effect against high levels of ultraviolet radiation (Beckman et al. 1994). However, the human R72 allele is new on the evolutionary scale: the wild-type allele in mouse and chimpanzee is 72P. The R72P polymorphism is located in a proline-rich region of p53 suggested to be required for the growth suppression activity of p53 (Walker & Levine 1996) and for its ability to induce apoptosis (Sakamuro et al. 1997). These two variant protein forms, R72 and 72P, have been shown to differ in their biological functions. The R72 variant is a stronger and faster inducer of apoptosis than the 72P variant (Thomas et al. 1999, Pim & Banks 2004), which may at least in part be due to the enhanced localization of R72 variant to the mitochondria (Dumont et al. 2003). The 72P allele also enhances binding of iASPP, an inhibitor of pro-apoptotic function of p53 (Bergamaschi et al. 2003), to p53, which may be another reason for the inferiority in apoptosis induction of this allele (Bergamaschi et al. 2006). On the other hand, the 72P variant has been found to be more efficient in inducing cell-cycle arrest (Pim & Banks 2004) and DNA repair (Siddique & Sabapathy 2006) than the R72 variant.

Because of the differences of these two alleles in important biological functions, such as induction of transcription and apoptosis, the role of R72P polymorphism in cancer has been investigated in a number of studies. The R72 variant is more susceptible than the 72P variant to degradation induced by human papillomavirus (HPV) E6 protein, and this was suggested to result in an increased susceptibility to HPV-induced tumors in R72 homozygous individuals (Storey et al. 1998). Some studies have reported an overrepresentation of the R72 variant in cervical cancers compared to control samples (Zehbe et al. 1991, 2002), whereas a larger meta-analysis study did not find such an association (Koushik et al. 2004).

The studies on R72P and risk of other types of cancer, including breast cancer, have also yielded inconsistent, even contradictory results. Most studies have been small and the results have not been replicated in subsequent studies. The association of the 72P allele with lung cancer has been suggested in several studies (Wang et al. 1999, Fan et al. 2000, Mechanic et al. 2007). On the contrary, initially suggested association of 72P with prostate cancer has not been seen in more recent studies (Huang et al. 2004). The 72P

allele has also been suggested to play a role in colorectal cancer development (Koushik et al. 2006) and pathogenesis: HNPCC (Lynch syndrome) patients with *MSH2* or *MLH1* germline mutations and carrying 72P were reported to develop colorectal cancer at a younger age than R72 homozygotes (Jones et al. 2004), while another study reported poorer survival among colorectal cancer patients with the 72P allele (Starinsky et al. 2004), but at least the association with the age at onset has been challenged by other studies (Sotamaa et al. 2005, Talseth et al. 2006, 2007).

After an initial report of increased breast cancer risk in women homozygous for 72P (Själander et al. 1996) the result was replicated in at least one study (Huang et al. 2003), but some other studies have reported increased risk in women homozygous for R72 (Buyry et al. 2003, Ohayon et al. 2005). Studies that argue against the contribution of the R72P polymorphism in breast cancer predisposition have also been reported (Wang-Gohrke et al. 2002, Suspitsin et al. 2003). Association of R72 homozygous genotype with multiple cancers, especially in *BRCA1/2* mutation carrier breast cancer families, has also been suggested (Martin et al. 2003).

3 AIMS OF THE STUDY

The aims of this study were

1. to evaluate the contribution of the Finnish ataxia-telangiectasia (A-T) –mutations on breast cancer risk in Finnish breast cancer families (I) and to investigate whether other *ATM* sequence variants are contributing to breast cancer in Southern Finland (II)
2. to study by immunohistochemistry the expression of the *ATM* gene in breast tumors (III) to explore its role in breast carcinogenesis
3. to examine the contribution of *RAD50* on familial breast cancer predisposition in Southern Finland and UK (IV)
4. to evaluate the effect of p53 R72P polymorphism on breast cancer risk, histopathologic features of the tumors and survival of the patients (V)

4 MATERIALS AND METHODS

4.1 Subjects

4.1.1 Breast cancer families (I, II, IV, V)

In study V, the series of familial breast cancer patients was genotyped for p53 R72P polymorphism to evaluate its association with breast cancer risk. The series included altogether 939 familial breast cancer patients collected at the Helsinki University Central Hospital Departments of Oncology, Clinical Genetics and Surgery (as described in Eerola et al. 2000). This series included 407 breast cancer patients with a stronger family history (three or more first- or second- degree relatives with breast or ovarian cancer in the family, including the proband), and 532 unrelated breast cancer cases reporting only a single affected first-degree relative. Among these 939 familial patients, 804 had a family history of breast cancer only and 135 patients had a relative affected with ovarian cancer. All cancer diagnoses were verified through the Finnish Cancer Registry or hospital records. For 627 of these familial patients, *BRCA1* and *BRCA2* mutations had been excluded. *BRCA1/2* mutation screening was done either by screening of the entire coding regions and exon-intron boundaries using protein truncation test (PTT) and denaturing gradient gel electrophoresis (DGGE), or as previously described in Vehmanen at al. 1997, Vahteristo et al. 2001 and Vahteristo et al. 2002. For 312 patients, the *BRCA1/2* mutation status was unknown.

In study I, index cases of altogether 541 *BRCA1* and *BRCA2* mutation negative families from Helsinki, Tampere, and Oulu areas were screened for eight Finnish A-T-related *ATM* mutations. Inclusion criteria for the families were 1) three or more affected in the family (285 cases), 2) two affected first-degree relatives (251 cases) or 3) two affected second-degree relatives (5 cases). Two hundred and two of the cases from families with three or more affected and 172 of the cases from families with two affected first-degree relatives belonged to the familial series from Helsinki described above.

In study II, forty-seven *BRCA1/2* mutation negative breast cancer patients with the above-mentioned stronger family history from the familial series were screened for germline alterations in the *ATM* gene. The five rare *ATM* variants found in the first screen were then screened in additional 237 *BRCA1/2* mutation negative breast cancer patients with the stronger family history. The rare missense variant *ATMex10* 998C>T (S333F) was also screened in additional 368 breast cancer cases with moderate family history.

In study IV, 590 Finnish breast cancer patients were screened for *RAD50* 687delT mutation. The patients belong to the series of familial breast cancer patients described above and are described in detail in IV. Of these patients, 281 were also screened for *RAD50* Q350X. Altogether 46 Finnish breast cancer patients were screened for the whole *RAD50* gene. They were from *BRCA1/2* negative families with three or more affected cases. The 702 *BRCA1/2* mutation negative UK patients screened for *RAD50* mutations in study IV were collected through the Breast Cancer Susceptibility Collaboration (UK) and are described in detail in IV.

In study V, the p53 R72P polymorphism was evaluated also in 49 *BRCA1* and 48 *BRCA2* mutation carriers affected with breast cancer to investigate whether this polymorphism modifies cancer risk in *BRCA1* or *BRCA2* mutation carriers.

4.1.2 Unselected breast cancer patients (I, II, V)

Unselected breast cancer patients were genotyped for the common polymorphisms *ATMivs38* -8T>C, *ATMex39* 5557G>A (D1853N) (II) and p53 R72P (IV) to evaluate the association of the polymorphisms with breast cancer risk as well as histopathologic features of the breast tumors and survival of the patients. The series of 888 unselected breast cancer patients included 626 consecutive newly diagnosed breast cancer patients collected 1997 - 1998 at the Helsinki University Central Hospital covering 87% of all breast cancer patients treated at the Department of Oncology during the collection period (described in detail in Syrjäkoski et al. 2000). Additionally, the series included samples collected from 262 consecutive newly diagnosed breast cancer patients at the Department of Oncology, Helsinki University Central Hospital, in 2000, covering 65% of all breast

cancer patients treated at the Department of Oncology at that time period (Kilpivaara et al. 2005).

Pathologic data were collected from pathology reports for all the primary breast tumors available among the 888 unselected breast cancer patients. Altogether, 46 bilateral breast cancer cases had been diagnosed among these 888 patients with histopathologic data available for a total of 932 tumors (932/934, 99.8% of all). The data set included information on tumor histology, grade, estrogen receptor (ER) and progesterone receptor (PR) status, p53 immunohistochemical expression and tumor diameter (T), nodal status (N), and distant metastases (M). The data set for the unselected patients collected 1997 - 1998 also included the age at the time of (first) breast cancer diagnosis and survival (follow-up in months).

In study I, the frequencies of the Finnish A-T-related *ATM* mutations observed in familial breast cancer patients were compared to those in geographically matched 1124 unselected breast cancer cases and 1107 healthy controls; 450 unselected breast cancer patients belonged to the series of 626 unselected patients from Helsinki collected 1997 - 1998.

4.1.3 Population controls (I, II, IV, V)

In study I, DNA from the 1107 healthy blood donors from matched geographical regions was obtained from the Finnish Red Cross Blood Service. The samples belong to the series collected at eight regional centers around the country covering all the geographical regions in Finland.

In studies II, IV and V, peripheral blood from the healthy blood donors from Southern Finland was obtained from the Finnish Red Cross Blood Service. The samples belong to the series of 1344 samples collected in 2003.

Samples from the 786 UK controls in study IV were obtained from Human Random Control DNA panels from the European Collection of Cell Cultures (Salisbury, UK).

4.1.4 Tumor arrays (III, V)

Paraffin-embedded tissue blocks of the available primary breast cancer tumors from unselected breast cancer patients (III, V) and familial breast cancer patients (III) as well as pathologic data from pathology reports including information on tumor histology, grade, estrogen receptor (ER) and progesterone receptor (PR) status and tumor diameter, nodal status, and distant metastases were collected. All tumors were additionally re-reviewed for histological grade and histological tumor type by one pathologist (P.H.). Grading was performed according to Scarff-Bloom-Richardson modified by Elston and Ellis (1991). The most representative area of the tumors was punched to produce breast cancer tissue microarrays (TMA) including four cores (diameter 0.6mm) from each of the original blocks.

The TMAs included altogether 1335 invasive breast cancer tumors: 921 of these were from familial patients (577 from families with three or more first- or second- degree relatives with breast or ovarian cancer, including the proband, and 344 from families with two affected first-degree relatives) and 414 from sporadic patients (patients without the above defined family history of breast or ovarian cancer). Fifty-two of the tumors from familial patients were from patients from *BRCA1* mutation positive families and 56 tumors were from patients from *BRCA2* mutation positive families. Altogether 655 familial tumors were from families for which *BRCA1* and *BRCA2* mutations had been excluded (including all cases with strong family history) and 158 tumors were from families for which the *BRCA1* and *BRCA2* mutation status was unknown (families with two affected cases). Altogether 134 patients had bilateral breast cancer; both tumors were available of 38 patients.

4.1.5 Cell lines (I, IV)

The cell lines used in the functional studies of the Finnish A-T-mutations (I) are described in I.

In study IV, lymphoblast cell lines were established from three heterozygous carriers of the *RAD50* 687delT mutation, and from one non-carrier, by Epstein-Barr virus (EBV) transformation.

4.2 DNA and RNA extraction, cDNA synthesis (I, II, IV, V)

Genomic DNA from peripheral blood lymphocytes was extracted by a standard phenol-chloroform method (I, II, IV, V). Total cellular RNA from lymphoblast cell lines was extracted by a standard Trizol (Invitrogen) method followed by purification with RNeasy mini-kit (Qiagen), and cDNA was synthesized using R-T-PCR System with random hexamer primers (Promega) (unpublished). Genomic DNA from frozen tumor tissue was obtained when RNA from the tissue was extracted by Trizol method (IV).

4.3 Mutation analysis and genotyping (I, II, IV, V)

4.3.1 Search for novel mutations (II, IV)

4.3.1.1 Denaturing high-performance liquid chromatography (dHPLC) (II)

Genomic DNA from the index patients from forty-seven *BRCA1/2* mutation negative breast cancer or breast-ovarian cancer families (three or more first or second degree relatives with breast or ovarian cancer in the family, including the proband) was screened for germline alterations in all coding exons and exon-intron boundaries of the *ATM* gene by denaturating high-performance liquid chromatography (dHPLC, WAVE, Transgenomic Inc., Omaha, NE, USA). The WECARE primers (described in Bernstein et al. 2003) were used for PCR amplification of genomic DNA. dHPLC was also used to screen the additional 237 familial non*BRCA1/2* breast cancer patients and 237 healthy population controls for the five rare *ATM* variants found in the first screen.

4.3.1.2 Conformation sensitive gel electrophoresis (CSGE) (IV)

All coding exons and exon-intron boundaries of the *RAD50* gene (IV) from index patients from forty-six *BRCA1/2* mutation negative Finnish breast cancer families were screened for mutations by conformation sensitive gel electrophoresis (CSGE) (Ganguly 2002). Briefly, genomic DNA was amplified by PCR and the PCR products denatured at +95°C

for 10 min, after which the PCR machine was switched off to allow the products to slowly cool to RT (45 min) and form heteroduplexes. Samples were run on mildly denaturing gels (10% acrylamide, 10% ethylene glycol, 15% formamide) at 3 W over night. The result was visualized by silver staining. CSGE was also used to screen the rare Finnish A-T-mutations in the breast cancer patients and population controls from Northern Finland in study I.

4.3.2 Screening of the known mutations and genotyping common polymorphisms (I, II, IV, V)

4.3.2.1 Agarose gel electrophoresis (I)

ATMex62 8710-8715delGAGACA mutation in patients from Southern Finland was screened by running the PCR products in 3.5% ethidium bromide –stained MetaPhor® agarose gel (Cambrex Biosciences) and visualizing them under UV light.

4.3.2.2 Minisequencing (primer extension) (I, II, IV)

Minisequencing (primer extension, Syvänen et al. 1993) was used to screen the Finnish A-T-mutations in familial breast cancer patients from Southern Finland (except for *ATMex62* 8710-8715delGAGACA mutation screened by direct agarose gel electrophoresis, I) and the two truncating *RAD50* mutations in Finnish familial breast cancer patients and healthy controls (IV). Genotyping the additional 237 familial non*BRCA1/2* breast cancer patients for the *ATMex39* 5557G>A (D1853N) polymorphism (II) was also done by minisequencing.

4.3.2.3 Restriction fragment length polymorphism (RFLP) analysis (II)

The *ATMivs38* -8T>C polymorphism was genotyped by RFLP. RFLP was also used to screen the rare missense variant *ATMex10* 998C>T (S333F) in additional 368 breast cancer cases with moderate family history and 367 healthy controls. The *ATMivs38* -8T>C change creates a restriction site for *Rsa*I; for *ATMex10* a mutagenesis PCR-primer was designed that creates a restriction site for *Eco*RI, which is abolished by the 998C>T change. In brief, genomic DNA was amplified by PCR and carriers of the *ATMivs38* -8T>C or *ATMex10* 998C>T change could be detected in a 2% or 3% ethidium bromide –

stained agarose gel after *Rsa*I or *Eco*RI (New England BioLabs, Beverly, MA, USA) digestion of the PCR-products, respectively.

4.3.2.4 Amplifluor™ fluorescent genotyping (II, V)

ATMex39 5557G>A (D1853N) polymorphism (II, unselected breast cancer patients, breast cancer patients with moderate family history and population controls) and p53 R72P polymorphism (V) were genotyped by using Amplifluor™ fluorescent genotyping (K-Biosciences, Cambridge, UK, http://www.kbioscience.co.uk).

4.3.2.5 Direct sequencing (I, II, IV)

Direct sequencing was used to characterize the new sequence alterations found with dHPLC (II) and CSGE (IV), and to confirm the positive results obtained when screening known rare alterations by dHPLC (II), CSGE (I), minisequencing (I, IV), and RFLP (II). Genomic DNA was first re-amplified and the PCR products purified with the QIAquick PCR purification kit (Qiagen) or with ExoSAP-IT treatment (Amersham Biosciences, Piscataway, NJ, USA). The purified PCR products were then bidirectionally sequenced using the ABI BigDyeTerminator Cycle Sequencing Kit (v3.0) and ABI Prism 310 Genetic Analyzer automated sequencer (Applied Biosystems, Foster City, CA, USA). The *RAD50* Q350X mutation was analysed in 267 additional UK familial breast cancer patient samples and 786 population controls by bidirectional sequencing of *RAD50* exon 7 (IV).

Expression of the mutant and wild-type *RAD50* alleles (unpublished) was evaluated by direct sequencing of cDNA generated from three lymphoblast cell lines established from heterozygous carriers of the 687delT mutation. PCR primers were designed in exons 5 and 7 to avoid amplification of residual genomic DNA. The presence of *ATM* 6903insA transcript in the mRNA pool of mutation carrier cell lines was also evaluated by direct sequencing with cDNA-specific primers as described in I.

Genomic DNA from tumor tissue from one *RAD50* 687delT mutation carrier was sequenced and compared to a non-carrier in order to study possible allelic imbalance (AI) (IV).

4.3.2.6 Microsatellite marker analysis (I)

D11S1819, D11S2179, D11S1778, D11S1294 and D11S1818 markers were used to determine the haplotypes of observed *ATM* mutation alleles in study I, and to study possible allelic imbalance (AI) in the tumors of one 6903insA and two 7570G>C carriers. The PCR products were analyzed with the Li-Cor IR^2 4200-S DNA Analysis system (Li-Cor Inc., Lincoln, NE) using an IRD800-labelled forward primer. Allele intensity ratios were quantified with the Gene Profiler 4.05 analysis program (Scanalytics, Inc., Fairfax, VA). AI was calculated from the formula AI = (T2 x N1)/(T1 x N2), where T1/2 represents tumor and N1/2 the corresponding normal alleles. A value >1.67 or <0.60 was considered to indicate AI, meaning that the intensity of one allele had decreased >40%.

4.4 Functional studies and protein expression studies (I, III, IV, V)

4.4.1 Cell survival studies (I)

In order to study the effect of the three *ATM* mutations on radiosensitivity, the cell survival of mutation carrier and control cell lines after exposure to ionizing radiation was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, as described in detail in I. Cell survival fraction was calculated relative to the number of viable cells in the non-irradiated culture at 96 hours.

4.4.2 Protein expression and kinase activity studies by immunoblotting (I, IV)

The effects of the three *ATM* mutations on ATM expression and kinase activity (I) were evaluated by western blotting analysis as described in detail in I. Briefly, ATM was immunoprecipitated from cellular extracts of the carrier and control cell lines with anti-ATM polyclonal antibody and the immunoprecipitates were resolved on SDS-PAGE gels and immunoblotted with the same antibody. To study ATM kinase activity, the phosphorylation of two ATM substrates, p53 (Ser15) and CHEK1 (Ser317), as well as ATM autophosphorylation on Ser1981 was evaluated before and after ionizing radiation:

extracts from mock or irradiated cells were analyzed by SDS-PAGE and immunoblotted with appropriate antibody.

The RAD50 protein expression (IV) was evaluated by immunoblotting on cell lysates from three lymphoblast cell lines established from patients heterozygous for 687delT mutation, compared to a control lymphoblast cell line homozygous for wild-type RAD50. For immunoblotting analysis, proteins from total cell lysates were separated using denaturing polyacrylamide gel electrophoresis (10% SDS-PAGE) and blotted onto nitrocellulose membrane. The RAD50 protein was visualized on parallel blots after incubation with three distinct primary antibodies (ab3622 from Abcam, Cambridge, UK, clone 13 from Transduction Laboratories, Lexington, KY, USA and 13B3 from GeneTex, Inc., San Antonio, TX, USA) using the ECL visualization reagents (Amersham Biosciences, Piscataway, NJ, USA).

4.4.3 Immunohistochemical analyses (III, IV, V)

4.4.3.1 p53 (III, V)

p53 protein expression on breast tumors (III, V) was studied by immunohistochemical staining of tumor tissue microarrays (TMA). The TMA slides were stained with a mouse monoclonal anti-human p53-antibody (Dako, Glostrup, Denmark) in a dilution of 1:300. Briefly, five-micron sections were cut from paraffin-embedded blocks, deparaffinized in xylene, and dehydrated in a series of graded alcohols. The sections were pre-treated in a microwave oven and incubated with antibody overnight. Samples were considered positive when 20% of the cancer cells were positive for the p53 staining.

4.4.3.2 ATM (III)

For ATM immunohistochemistry (III), the TMA slides were deparaffinized and processed for sensitive immunoperoxidase staining with the primary mouse monoclonal antibody against human ATM (ATML2p, recognizing an epitope between amino acids 2581 and 2599 of ATM, obtained from Dr. Yosef Shiloh, described in Angèle et al. 2000, 1:1000 dilution), incubated overnight, followed by detection using the Vectastain Elite kit (Vector Laboratories, Burlingame, CA, USA), as previously described (Lukas et al. 2001,

Vahteristo et al. 2002, Angèle et al. 2003, Bartkova et al. 2005a, 2005b). As more than 90% of epithelial cells in the normal breast tissues (n=15) were positive for the ATM protein, ATM expression was considered aberrantly reduced when fewer than 70% of breast cancer cells were ATM-positive in a given tumor. ATM was also regarded as aberrantly reduced when the staining intensity of the breast cancer cells was clearly lower compared with the ATM staining signal in adjacent normal stromal, epithelial and/or lymphocytic infiltrating cells on the same section. The results were scored as follows: 1 = reduced intensity of staining of carcinoma cells, 2 = reduced number of carcinoma cells with positive staining, 3 = reduced intensity of staining of carcinoma cells and reduced number of carcinoma cells with positive staining, 4 = normal staining of carcinoma cells.

4.4.3.3 ERBB2 (III)

ERBB2 (HER2) protein expression (III) on tumors on TMAs was analyzed by immunohistochemical (IHC) staining and *ERBB2* gene amplification with chromogenic *in situ* hybridization (CISH), as previously described (Tanner et al. 2000, Lassus et al. 2004). The *ERBB2* amplification was considered negative with 2-5 copies of *ERBB2* in single nucleus and positive with >6-10 copies. When CISH result was not available, unambiguous IHC result (weak/negative staining or strong positivity >90% of cells) was used.

4.4.3.4 RAD50, MRE11, and NBS1 (IV)

The RAD50 protein expression was studied by immunohistochemical staining in three archival breast tumors from three patients heterozygous for 687delT mutation, compared to a series of normal breast tissues (n=15), familial breast carcinomas with apparently wild-type RAD50 (n=25) and sporadic breast tumors (n=27). The tumor sections were deparaffinized and processed for sensitive immunoperoxidase staining with the primary mouse monoclonal antibody against human RAD50 (clone 2C6, Abcam, Cambridge, UK, 1:500 dilution), incubated overnight, followed by detection using the Vectastain Elite kit (Vector Laboratories, Burlingame, CA, USA), as previously described (Lukas et al. 2001). Given that around 95% of epithelial cells were reproducibly and strongly positive on sections of normal breast, expression was regarded aberrantly decreased when the RAD50 protein was detectable in fewer than 70% of cancer cells in a particular lesion,

and the staining intensity of the remaining positive cancer cells was reduced compared to that in normal cells present on the same section. For analyses of the MRE11 and NBS1 proteins on parallel sections, rabbit antibodies #4895 against MRE11 (Cell Signaling, diluted 1:1500) and #3002 against NBS1 (Cell Signaling, diluted 1:100) were used, respectively.

4.5 Statistical and bioinformatic analyses (I, II, III, IV, V)

4.5.1 Tests for statistical significance (I, II, III, IV, V)

Fisher's exact test or chi-square test was used to determine statistical significance (SPSS v12.0.1 for Windows, SPSS Inc., Chicago, IL, USA or Simple Interactive Statistical Analysis, SISA, http://home.clara.net/sisa/). All p-values are two-sided, and $p<0.05$ or $p<0.01$ (due to multiple testing) was considered significant.

4.5.2 Odds ratios (I, II, III, IV, V)

Odds ratios with 95% confidence intervals were calculated using Simple Interactive Statistical Analysis, SISA (http://home.clara.net/sisa/).

4.5.3 Survival analyses (V)

Univariate analyses of survival were performed by calculating Kaplan-Meier survival curves and comparing subsets of patients using log-rank test. To explore the effects of several variables on survival, Cox's proportional-hazards regression model was used. The data were analyzed using SPSS v12.0.1 for Windows (SPSS Inc., Chicago, IL, USA).

4.5.4 SIFT and PolyPhen analyses (II, IV)

SIFT analysis (Ng & Henikoff 2002) (http://blocks.fhcrc.org/sift/SIFT) and PolyPhen (Ramensky et al. 2002) (http://genetics.bwh.harvard.edu/pph/) were used to evaluate functional significance of the missense variants found. SIFT program calculates tolerance scores for amino acid changes based on sequence alignment and conservation across protein family or across evolutionary history. PolyPhen (=Polymorphism Phenotyping)

predicts possible impact of an amino acid substitution on the structure and function of a human protein using straightforward physical and comparative considerations.

4.5.5 Haplotype reconstruction (I, II)

In study I, haplotypes were reconstructed manually. In II, haplotypes were reconstructed using PHASE (version 2.1.1) (http://www.stat.washington.edu/stephens/software.html). The software implements a Bayesian statistical method for reconstructing haplotypes based on population genotype data (Stephens et al. 2001, Stephens & Donnelly 2003).

4.6 Ethical issues

This study was performed with informed consents from the patients as well as with permissions from the Ethics Committee (E8) of the Helsinki University Central Hospital and from the Ministry of Social Affairs and Health in Finland.

5 RESULTS

5.1 *ATM* and breast cancer (I, II, III)

5.1.1 *ATM* sequence variants and breast cancer risk Finland (I, II)

5.1.1.1 Finnish A-T mutations in breast cancer families (I)

The presence of seven Finnish A-T –related *ATM* mutations was analyzed in 541 breast cancer families: only two mutations, 6903insA and 7570G>C, were observed in these families (Table 1). Additionally, another mutation, 8734A>G in exon 62, was identified. *ATMex49* 6903insA was observed in the index patients of three families. However, it showed incomplete segregation with cancer in the family where additional family members were available for analysis. *ATMex49* 6903insA was also found in 5/1124 unselected breast cancer cases. All the eight mutation carriers originated from the Tampere region and shared the same haplotype (data not shown). *ATMex53* 7570G>C was found in one familial breast cancer patient from the Helsinki series (maternally originating from central Finland and paternally from Eastern Finland), also a carrier of *CHEK2* 1100delC mutation, and in 2/1124 unselected breast cancer patients from the Oulu region. This mutation was also observed in one healthy control (1/1107). All carriers of the mutation shared the same haplotype (data not shown). When screening the Finnish A-T-mutation, 8710-8715delGAGACA in *ATM* exon 62, with CSGE, another alteration, 8734A>G, previously related with breast cancer and A-T (Teraoka et al. 2001, Thorstenson et al. 2003), was observed and included in the study. *ATMex62* 8734A>G was found in index patients of two breast cancer families originating from the Tampere region. Both patients shared the same haplotype (data not shown). However, the segregation of the mutation with cancer was incomplete. *ATMex62* 8734A>G was not found in unselected breast cancer cases or in controls.

Table 1. Heterozygous *ATM* germline mutations in Finnish breast cancer families, unselected breast cancer cases and controls.

ATM mutation	Carrier Frequency (%)		
	Familial cases	Unselected cases	Controls
6903insA	0.6% (3/541)	0.4% (5/1124)	- (0/1107)
7570G>C	0.2% (1/541)	0.2% (2/1124)	0.1% (1/1107)
8734A>G	0.4% (2/541)	- (0/1124)	- (0/1107)
altogether	1.1% (6/541), *p*=0.006	0.6% (7/1124), *p*=0.07	0.1% (1/1107)

In addition, the following Finnish A-T mutations were screened in familial cases: IVS14+3-4delAT (exon 14 skipped), IVS37+9A>G (insertion Val, Ser, Stop), 6779-6780delTA (truncation), 8710-8715delGAGACA (deletion of Glu and Thr) and 9139C>T (Arg3047Stop). None of these mutations was found.

5.1.1.2 Functional analyses of breast cancer –related A-T mutations (I)

AI analysis was performed on available tumor samples of one 6903insA, and two 7570G>C carriers. None of the tumors showed loss of the wild-type allele (data not shown). ATM expression analysis by western blotting showed that the amount of ATM protein from the 7570G>G and 8734A>G missense mutation carrier cell lines was normal. For 6903insA, leading to premature translation stop at codon 2372, no truncated protein was observed, but the level of full-length ATM protein expression was reduced to half. However, direct sequencing of the cDNA generated from the carrier cell lines showed that 6903insA transcripts were still present in the mRNA pool, indicating that they were not eliminated from the cells by nonsense-mediated decay (NMD). ATM kinase activity analysis by evaluating the phosphorylation of two ATM substrates, p53 on Ser15 and CHEK1 on Ser317, and ATM autophosphorylation on Ser1981 before and after IR revealed that DNA damage –induced phosphorylation of p53 and CHEK1 as well as ATM autophosphorylation was dramatically lower in the 7570G>C carrier cell line; the 8734A>G carrier cell line was defective only in CHEK1 phosphorylation. For 6903insA, no difference between the carrier cell lines and controls was found, which indicates that the checkpoint signaling downstream of ATM is not compromised in 6903insA carrier cell lines. Radiosensitivity analysis evaluating cell survival after exposure to IR showed that the survival of the 7570G>C and 8734A>G carrier cell lines

was similar to controls, whereas the survival of the 6903insA carrier cell lines was indistinguishable from that of A-T cell lines.

5.1.1.3 Other *ATM* sequence variants in breast cancer patients from Southern Finland (II)

As only one of the Finnish A-T mutations, *ATMex53* 7570G>C, was found in one familial breast cancer patient from the Helsinki series, we screened the whole coding region and exon-intron boundaries of the *ATM* gene in 47 familial breast cancer patients to see whether there are any other *ATM* sequence variants contributing to breast cancer in Southern Finland. *ATM* sequence variants found in the full gene screen from 47 Finnish familial breast cancer patients as well as haplotypes constructed with PHASE software are presented in Table 2.

Table 2. *ATM* sequence variants and haplotypes found in 47 Finnish familial breast cancer patients.

	variant	**ex5**	**ex9**	**ex10**	**ex11**	**ex14**	**ex15**	**ex20**	**ex31**	**ex32**	**ex39a**	**ex39b**	**ex39c**	**ex40**	**ex41**	**ex46**	**ex47**	**ex62**
	freq.	1/47	5/47	1/47	1/47	1/47	1/47	1/47	1/47	1/47	1/47	4/47	18/47*	1/47	1/47	2/47	1/47	2/47
freq.	**haplotype**																	
0.65	**1**	T	C	C	T	A	T	-	A	C	G	T	G	G	T	T	G	A
0.01	**2**	T	C	C	T	A	T	-	A	C	G	T	G	G	T	T	G	**C**
0.01	**3**	T	C	C	T	A	T	-	A	C	G	T	G	G	T	T	**T**	A
0.02	**4**	T	C	C	T	A	T	-	A	C	G	T	G	G	T	**C**	G	A
0.01	**5**	T	C	C	T	A	T	-	A	C	G	T	G	**A**	T	T	G	**C**
0.01	**6**	T	C	C	T	A	T	-	A	C	**C**	T	G	G	T	T	G	A
0.15	**7**	T	C	C	T	A	T	-	A	C	G	T	**A**	G	T	T	G	A
0.02	**8**	T	C	C	T	A	T	-	A	C	G	**C**	**A**	G	T	T	G	A
0.01	**9**	T	C	C	T	A	T	-	A	**T**	G	**C**	**A**	G	T	T	G	A
0.01	**10**	T	C	C	T	A	T	-	**G**	C	G	T	**A**	G	T	T	G	A
0.01	**11**	T	C	C	T	**G**	T	-	A	C	G	T	G	G	T	T	G	A
0.01	**12**	T	C	C	**G**	A	T	-	A	C	G	T	**A**	G	T	T	G	A
0.01	**13**	T	C	**T**	T	A	T	-	A	C	G	**C**	**A**	G	T	T	G	A
0.01	**14**	T	**T**	C	T	A	T	**A**	A	C	G	T	G	G	**C**	T	G	A
0.03	**15**	T	**T**	C	T	A	T	-	A	C	G	T	G	G	T	T	G	A
0.01	**16**	T	**T**	C	T	A	**C**	-	A	C	G	T	G	G	T	T	G	A
0.01	**17**	**C**	C	C	T	A	T	-	A	C	G	T	G	G	T	T	G	A

* two homozygotes

variant	nucleotide change	amino acid change
ex5	162T>C	Y54Y
ex9	735C>T	V245V
ex10	998C>T	S333F
ex11	ivs10(-6)T>G	-
ex14	1814A>G	H605R
ex15	2119T>C	S707P
ex20	ivs20(+28)insA	-
ex31	4424A>G	Y1475C
ex32	4578C>T	P1526P
ex39a	ivs38(-15)G>C	-
ex39b	ivs38(-8)T>C	-
ex39c	5557G>A	D1853N
ex40	ivs40(+27)G>A	-
ex41	5793T>C	A1931A
ex46	ivs45(-54)T>C	-
ex47	6539G>T	G2180V
ex62	ivs 62(+8)A>C	-

Altogether 17 different sequence variants forming 17 different haplotypes were found. Seven of the variants were intronic, four were silent nucleotide substitutions and six were missense changes. Four rare missense alterations and one of the intronic changes (Table 3), *ATMivs10* -6T>G, a known A-T-mutation leading to skipping of the exon eleven in the transcript (Dörk et al. 2001), were then screened in additional series of familial 237 breast cancer cases and 237 healthy controls. As *ATMex10* 998C>T (S333F) missense variant was in this screen found more frequently in cases than in controls, it was further studied in additional 368 breast cancer cases with a moderate family history, and in 367 healthy controls. The frequencies of these five variants in breast cancer cases and healthy controls are presented in Table 3.

Table 3. Frequencies of five *ATM* variants in Finnish breast cancer cases and controls.

***ATM* variant**	**cases**	**controls**	**SIFT**	**PolyPhen**
ex10	6/652	5/604		
998C>T (S333F)	0.92%	0.83%	0.00	benign
ex11	1/265	0/228		
ivs10-6T>G	0.38%	0.00%		
ex14	1/253	0/232		
1814A>G (H605R)	0.40%	0.00%	0.00	possibly damaging
ex31	1/273	0/234		
4424A>G (Y1475C)	0.37%	0.00%	0.08	probably damaging
ex47	1/264	0/234		
6539G>T (G2180V)	0.38%	0.00%	0.03	probably damaging

None of the variants segregated with cancer in the families studied. Only the index case with 998C>T (S333F) carried the variant in one family studied, with three other affected sisters being non-carriers; for two other families no additional samples were available. Similarly, only the index cases carried the *ATMex14* 1814A>G (H605R) and ivs10-6T>G variants. For 1814A>G, three other affected relatives (sister, cousin and her daughter) were not carriers. For the ivs10-6T>G variant, the two other affected in the family were not carriers whereas two of the three healthy sisters (aged 63 and 73 years) of the index case carried the variant. Unfortunately, no samples of affected relatives were available for the *ATMex31* 4424A>G (Y1457C) and *ATMex47* 6539G>T (G2180V) variant carrier

cases. The fifth rare missense alteration, *ATMex15* 2119T>C (S707P), was not studied further as it has been extensively studied previously, and the results do not support its association with breast cancer (Dörk et al. 2001, Spurdle et al. 2002, Sommer et al. 2003, Bretsky et al. 2003).

Unselected and familial breast cancer patients as well as healthy controls were genotyped for the common polymorphisms *ATMivs38* -8T>C and *ATMex39* 5557G>A (D1853N) to evaluate the association of the polymorphisms with breast cancer risk, bilateral breast cancer, multiple cancers, and the age of breast cancer diagnosis as well as with histopathologic features of the breast tumors and survival of the patients. The genotype distribution of the common *ATMex39* 5557G>A (D1853N) polymorphism was closely similar in breast cancer cases and healthy controls, suggesting no effect on breast cancer risk. No association of this polymorphism with bilateral breast cancer or multiple cancers (breast cancer and at least one other non-breast cancer) was seen among the familial or unselected breast cancer cases. No association with histopathologic features (tumor histology, grade, hormone receptor status, TNM stage) of the breast tumors or survival among the unselected breast cancer patients was seen, either (data not shown). This alteration also appeared well tolerated in SIFT (score 0.17) and PolyPhen (benign) analysis.

No significant effect on breast cancer risk of the *ATMivs38* -8T>C polymorphism (or the combined variant, as all the carriers of ivs38-8T>C also carried 5557G>A) was observed. It was not associated with any histopathologic features of the breast tumors or survival among the unselected breast cancer patients (data not shown). The ivs38-8T>C polymorphism was not significantly associated with the risk of bilateral breast cancer among unselected or familial breast cancer patients. Among the unselected breast cancer patients, the ivs38-8T>C polymorphism tended to be associated with an increased risk of multiple primary cancers (breast cancer and at least one other non-breast cancer) (OR 2.56, 95% CI 1.23-5.35, p=0.02, Fisher's exact test), but the result was only of borderline significance and was not seen among the familial cases.

5.1.2 ATM expression in breast cancer tumors (III)

ATM staining was successful in altogether 1182/1335 (88.5%) tumors on TMAs. Altogether, the ATM protein expression was reduced in 10.7% of all non*BRCA1/2* breast cancer tumors. Proportion of tumors with reduced expression was similar among the tumors from familial and sporadic patients (Table 4). However, tumors from patients from *BRCA1* and *BRCA2* mutation positive families showed significantly more often reduced ATM expression than tumors from non*BRCA1/2* patients; 33.3% of *BRCA1* tumors and 30.0% of *BRCA2* tumors showed reduced expression, p=0.0003 and p=0.0009 as compared to non*BRCA1/2* tumors, respectively.

Table 4. ATM expression in breast cancer tumors.

		ATM expression			
	total (%)	**normal**	**1***	**2****	**3*****
all non*BRCA1/2* tumors	1106 (100)	988 (89.3)	72 (6.5)	21 (1.9)	25 (2.3)
sporadic	366 (100)	326 (89.1)	22 (6.0)	12 (3.3)	6 (1.6)
familial	740 (100)	662 (89.5)	50 (6.8)	9 (1.2)	19 (2.6)
three or more affected in the family	428 (100)	382 (89.3)	29 (6.8)	6 (1.4)	11 (2.6)
two affected first-degree relatives in the family	312 (100)	280 (89.7)	21 (6.7)	3 (1.0)	8 (2.6)
***BRCA1* tumors**	36 (100)	24 (66.7)	9 (25.0)	1 (2.8)	2 (5.6)
***BRCA2* tumors**	40 (100)	28 (70.0)	11 (27.5)	0 (0.0)	1 (2.5)

*reduced intensity of staining of carcinoma cells
**reduced number of carcinoma cells with positive staining
***reduced intensity of staining of carcinoma cells and reduced number of carcinoma cells with positive staining

Histopathologic features of non*BRCA1/2* tumors with normal and aberrant ATM protein expression are shown in Table 5. The tumors with reduced ATM expression were significantly more often ER negative (p=0.0002), PR negative (p=0.004) and of higher grade (p=0.0004, grade 3 vs. 1+2) than the tumors with normal ATM expression. The tumors with reduced ATM expression were also more often of medullary histology than the tumors with normal ATM expression (p=0.01). For other parameters studied (TNM stage, p53 expression) tumors with aberrant or normal ATM expression did not differ significantly.

Table 5. Histopathologic features of non*BRCA1/2* breast cancer tumors with normal and aberrant ATM expression.

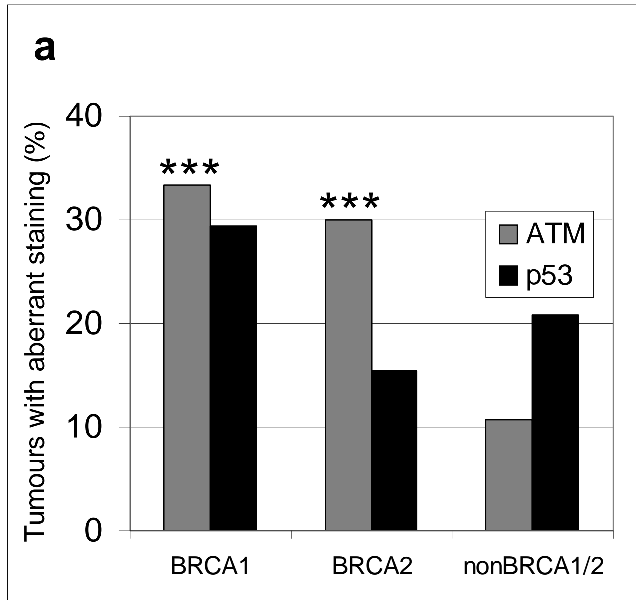
	Total (%)	**normal**	**aberrant**	***p****
Tumor histology (n=1106)				
Ductal carcinoma	798 (72.2)	705 (71.4)	93 (78.8)	
Lobular carcinoma	188 (17.0)	173 (17.5)	15 (12.7)	
Medullary carcinoma	14 (1.3)	9 (0.9)	5 (4.2)	0.01
Other	106 (9.6)	101 (10.2)	5 (4.2)	
Grade (n=1097)				
1	251 (22.9)	238 (24.3)	13 (11.1)	
2	509 (46.4)	458 (46.7)	51 (43.6)	
3	337 (30.7)	284 (29.0)	53 (45.3)	0.0004
T (n=1092)				
1	641 (58.7)	582 (59.7)	59 (50.4)	
2+3+4	451 (41.3)	393 (40.3)	58 (49.6)	0.06
N (n=1089)				
negative (0)	597 (54.8)	539 (55.3)	58 (50.4)	
positive (1+2)	492 (45.2)	435 (44.7)	57 (49.6)	0.3
M (n=1068)				
negative	1032 (96.6)	927 (97.0)	105 (93.8)	
positive	36 (3.4)	29 (3.0)	7 (6.3)	0.09
ER status (n=1055)				
negative	217 (20.6)	177 (18.8)	40 (34.8)	0.0002
positive	838 (79.4)	763 (81.2)	75 (65.2)	
PR status (n=1052)				
negative	349 (33.2)	297 (31.7)	52 (45.2)	0.004
positive	703 (66.8)	640 (68.3)	63 (54.8)	
ERBB2 (n=1066)				
negative	926 (86.9)	826 (87.0)	100 (85.5)	0.7
positive	140 (13.1)	123 (13.0)	17 (14.5)	
ER/PR/ERBB2 (n=1013)				
negative for all three	142 (14.0)	113 (12.6)	29 (25.2)	0.0006
positive for at least one	871 (86.0)	785 (87.4)	86 (74.8)	
p53 IHC (n=1065)				
negative	843 (79.2)	754 (79.5)	89 (76.1)	
positive	222 (20.8)	194 (20.5)	28 (23.9)	0.4

*Fisher's exact test

There was no association between ATM and ERBB2 expression alone, but ATM aberrant tumors were more often ER/PR/ERBB2-triple-negative than ATM normal tumors (Table 5). The subset of ER/PR/ERBB2-triple-negative non*BRCA1/2* breast tumors showed a very tight correlation with aberrantly reduced ATM expression ($p=0.0006$) as well as with positive p53 immunostaining ($p<0.00000001$) compared to tumors expressing at least one of these receptors (Figure 6).

No specific histopathologic characteristics differentiating ATM aberrant *BRCA1* or *BRCA2* tumors were seen (data not shown), but the number of tumors is probably too small to reveal any statistically significant difference.

No difference was noted in the age of breast cancer diagnosis between patients with ATM aberrant and normal tumors; the mean age of diagnosis in all groups was 55 years among non*BRCA1/2* patients and 47 years among *BRCA1* and *BRCA2* mutation carrier patients. There was no significant difference in survival of the patients between normal and reduced ATM groups (data not shown).



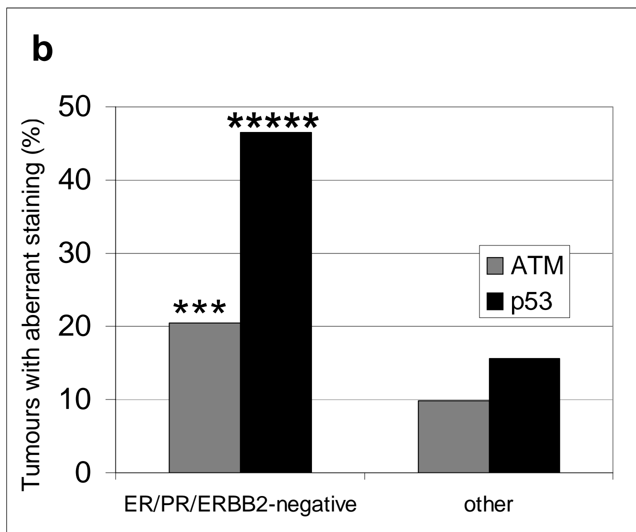


Figure 6. ATM and p53 expression in relation to *BRCA1/2* status in breast carcinomas.
a) ATM protein expression is aberrantly reduced or lost more frequently among *BRCA1* (*p*=0.0003) and *BRCA2* (*p*=0.0009) than in non*BRCA1/2* tumors.
b) ATM is more commonly deficient among the ER/PR/ERBB2-triple-negative subset of non*BRCA1/2* tumors (20%, 29/142) than in cases which express at least one of these markers (10%, 86/871) (*p*=0.0006). The triple-negative tumors are also more frequently p53 immunopositive than the other non*BRCA1/2* tumors (p<0.00000001).

5.2 *RAD50* and breast cancer (IV)

5.2.1 *RAD50* mutation analysis

Altogether eighteen sequence changes were found when the coding region and exon–intron boundaries of the *RAD50* gene were screened in 435 familial breast cancer cases from UK (Table 6). Six of the changes were intronic. Of the twelve exonic sequence changes, two were synonymous, nine were missense changes and one was a nonsense mutation, Q350X. In the 46 Finnish familial breast cancer patients screened, only one of the intronic changes, ivs20+35C>T, was found in one case. The exonic missense variants were evaluated for possible functional effect by SIFT and PolyPhen analysis which suggested that R193W, which was found in one UK breast cancer case and alters a key residue in the MRE11 binding segment, likely affects RAD50 function. R224H and R327H may affect RAD50 function but the other variants appear well tolerated by SIFT and PolyPhen analysis.

Table 6. *RAD50* variants in UK and Finnish familial breast cancer cases and controls.

***RAD50* variants**		**UK cases**	**Finnish cases**
Truncating mutations			
1048C>T	Q350X	1/702	0/281*
687delT		0/435	3/590**
Missense variants			
280A>C	I94L	6/435	0/46
373G>A	V127I	1/435	0/46
577C>T	R193W	1/435	0/46
671G>A	R224H	1/435	0/46
695C>A	A232D	1/435	0/46
943G>T	V315L	2/435	0/46
980G>A	R327H	1/435	0/46
2177G>A	R726H	1/435	0/46
2525T>C	V842A	1/435	0/46
Synonymous variants			
204C>T	H68H	1/435	0/46
3879C>T	I1293I	5/435	0/46
Intronic variants			
ivs1+12G>A		1/435	0/46
ivs9+73C>T		2/435	0/46
ivs0+35C>T		2/435	1/46
ivs20+49G>C		3/435	0/46
ivs22-15delTTC		1/435	0/46
ivs22+24A>G		10/435	0/46

*0/786 UK controls and 0/319 Finnish controls
**1/560 Finnish controls

The Q350X mutation was found in a UK woman who developed bilateral breast cancer at 43 years. The mutation was not present in her sister, who developed breast cancer at 73 years. There was a history of breast cancer on the paternal side and ovarian cancer in the mother, but we were not able to examine whether or not either of these cases carried the mutation. To further evaluate the Q350X mutation, 267 additional UK familial breast cancer cases, 786 UK controls, 235 Finnish breast cancer cases and 319 Finnish controls were screened for it by direct sequencing or minisequencing, respectively. No further case or control with Q350X was identified.

The 687delT mutation found in the Finnish population previously (Heikkinen et al. 2003) was evaluated among index cases from 590 Finnish breast cancer families and 560 healthy population controls. The mutation was found in three familial patients (0.5%) and

one control sample (0.2%). It showed incomplete segregation with cancer in the families. In one family, the mutation was found in the index case diagnosed with breast cancer at 48 years, and both of her sisters were also carriers; one was diagnosed with breast cancer at 52 years and the other was unaffected at 58 years. In another family, the index case carrying the mutation was diagnosed with breast cancer at 35 years and her affected mother (breast cancer at 48 years) was also a carrier. However, the maternal aunt of the index was an affected (breast cancer at 45 years) non-carrier. No other family member was available for testing from the third family.

5.2.2 Protein expression analysis of RAD50 687delT

Immunoblotting analysis of RAD50 protein in three 687delT carrier lymphoblast cell lines showed a decreased overall abundance of the full-length protein product, compared to a non-carrier lymphoblast cell line. In addition, the truncated protein form was not detected in lysates from the cell lines heterozygous for 687delT with any of the three antibodies, including an antibody prepared specifically against the N-terminus of RAD50, predicted to be preserved in the mutant protein. Analysis of *RAD50* cDNA from the 687delT carrier lymphoblast cell lines by direct sequencing showed that both alleles were expressed. However, the mutant allele was only barely detectable indicating a much lower expression level as compared to the wild-type allele (Figure 7, Tommiska et al., unpublished).

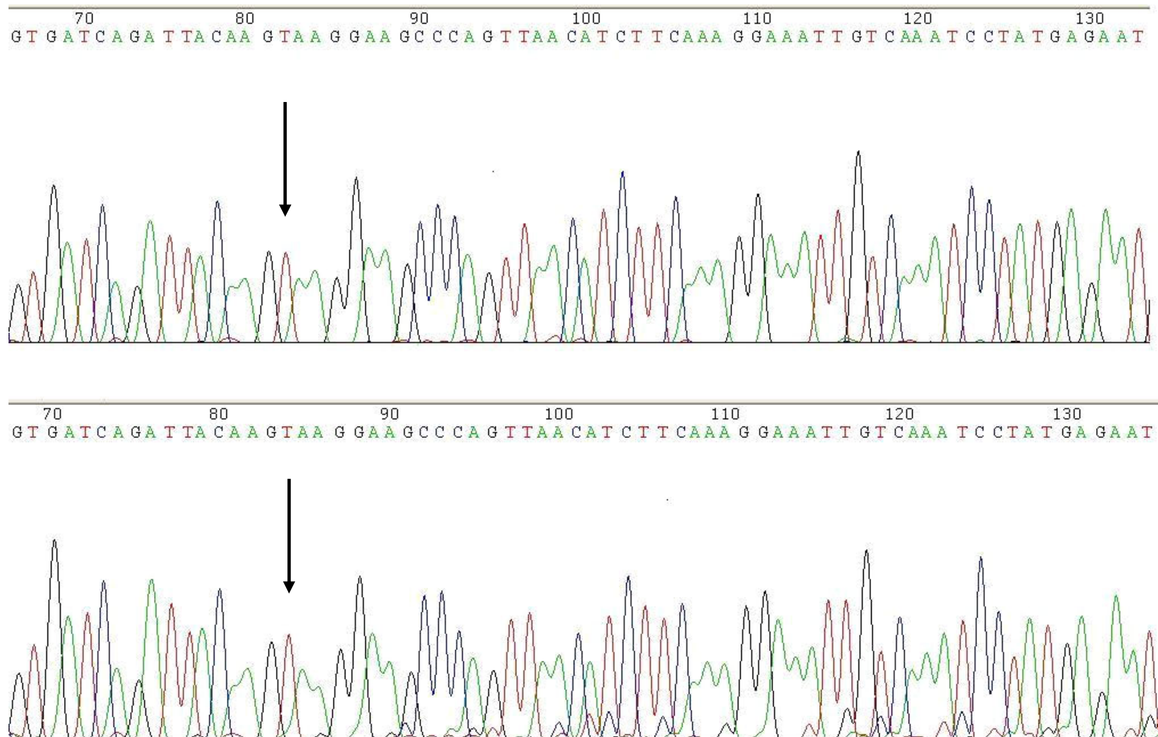


Figure 7. cDNA sequence from a normal lymphoblast cell line (top) and a cell line heterozygous for *RAD50* 687delT (bottom). The *RAD50* 687delT mRNA transcript is present in the heterozygous cell line at a very low level compared to the wild-type *RAD50* allele. Arrow shows the position of nucleotide 687T (Tommiska et al., unpublished).

Immunohistochemical analysis of three breast tumors from 687delT mutation carriers showed normal subcellular localization of the RAD50 protein. The overall staining pattern was nuclear and the signal detected by this semi-quantitative immunohistochemistry approach seemed comparable in the three mutation carrier tumors, in a set of additional 25 familial breast carcinomas with apparently wild-type RAD50, in the control normal breast tissues (n=15, with both luminal epithelial and myoepithelial cells positive), and in most of the 27 sporadic breast tumors examined. On the other hand, a small subset of sporadic carcinomas (3 out of 27) showed grossly reduced abundance of the RAD50 protein. No obvious defects of either the MRE11 or NBS1 protein expression levels were detected among the 28 familial breast tumors examined for RAD50, in contrast to the three sporadic carcinomas that showed concomitant reduction in staining

of all three proteins of the MRN complex. No evidence of AI was seen in the *RAD50* 687delT mutation carrier tumor studied (data not shown).

5.3 p53 R72P polymorphism and breast cancer (V)

5.3.1 R72P and breast cancer risk

The genotype frequencies of p53 R72P in the unselected and familial breast cancer patient series and population controls are shown in Table 7. The familial breast cancer patients were further divided in subgroups by strength of family history and inclusion of ovarian cancer. The distribution of the genotypes was closely similar in all the groups studied, with no deviation from the Hardy-Weinberg equilibrium among the 733 population controls or among the total of 1551 breast cancer patients studied (p=0.91), or among any subgroups.

Table 7. p53 R72P genotype frequencies among population controls, and unselected and familial breast cancer patients, by family history.

Study subjects	total	RR		RP		PP	
population controls	733	403	55.0%	278	37.9%	52	7.1%
all breast cancer patients	1551	825	53.2%	617	39.8%	109	7.0%
unselected breast cancer patients	858	459	53.5%	336	39.2%	63	7.3%
familial breast cancer patients*	923	478	51.8%	385	41.7%	60	6.5%
breast cancer only	793	401	50.6%	337	42.5%	55	6.9%
including also ovarian cancer	130	77	59.2%	48	36.9%	5	3.8%
index with only one affected first-degree relative	526	263	50.0%	231	43.9%	32	6.1%
three or more affected in the family	397	215	54.2%	154	38.8%	28	7.1%

*230 familial patients also belong to the series of unselected breast cancer patients

The mean age at diagnosis for the RR homozygotes was 56.5 years, for RP heterozygotes 56.6 years, and for PP homozygotes 56.8 years among the unselected patients, and 54.5, 54.9, and 56.4 among the familial patients, respectively. No association of any of the genotypes with bilateral breast cancer or multiple cancers (breast cancer and at least one other non-breast cancer) was seen.

Among the *BRCA1* and *BRCA2* mutation carriers, the mean age at diagnosis for *BRCA1* carriers with RR genotype (n=25) was 42.6 years, for RP heterozygotes 44.9 years, and for PP homozygotes 48.7, and for patients carrying a P allele (RP or PP) (n=24) 45.8 years. The mean ages at diagnosis for *BRCA2* carriers were 50.1, 42.9, and 45.1, respectively. *BRCA2* mutation carrier patients with a P allele (n=23) tended to be diagnosed at a younger age than RR homozygotes (n=25): mean age at diagnosis was 43.3 years and 50.1 years, respectively (p=0.03, t-test for equality of means). No association of any genotype with bilateral breast cancer or multiple cancers among the *BRCA1/*2 mutation carriers was seen.

5.3.2 R72P and histopathologic features of breast tumors

Analysis of the histopathologic features of the breast tumors from unselected breast cancer patients with different R72P genotypes showed that the different alleles were associated with specific histologic features of the tumors. Tumor histology of the PP homozygotes was significantly more often lobular than tumor histology of the other genotypes; the tumors of the RR homozygotes and heterozygotes had more often ductal histology (p=0.004) (Table 8). Carriers of the R allele also tended to have more often grade 3 tumors than PP homozygotes while PP homozygotes had more frequently grade 1 tumors (p=0.029). No association with hormone receptor status (ER and PR) or TNM stage was observed. The R72P genotype did not correlate with p53 expression as evaluated by immunohistochemistry. All these results were similar also among patients diagnosed below or at or over 50 years of age (data not shown).

Table 8. Tumor characteristics of unselected breast cancer patients with different p53 R72P genotypes.

	Total (%)	RR	RP	PP	*p*
Tumor histology (n=852)					
Ductal carcinoma	664 (77.9)	361 (79.0)	263 (79.9)	40 (60.6)	0.004
Lobular carcinoma	138 (16.2)	65 (14.2)	55 (16.7)	18 (27.3)	0.004
Medullary carcinoma	13 (1.5)	8 (1.8)	3 (0.9)	2 (3.0)	ns
Other	37 (4.3)	23 (5.0)	8 (2.4)	6 (9.1)	ns
Grade (n=809)					
1	221 (27.3)	110 (25.2)	88 (28.4)	23 (37.1)	0.029
2	349 (43.1)	198 (45.3)	124 (40.0)	27 (43.5)	ns
3	239 (29.5)	129 (29.5)	98 (31.6)	12 (19.4)	0.029
T (n=878)					
1	536 (61.0)	283 (60.6)	210 (61.0)	43 (64.2)	ns
2+3+4	342 (39.0)	184 (39.4)	134 (39.0)	24 (35.8)	ns
N (n=868)					
negative (0)	470 (54.1)	246 (52.7)	185 (55.1)	39 (60.0)	ns
positive (1+2+3)	398 (45.9)	221 (47.4)	151 (45.0)	26 (40.0)	ns
M (n=862)					
negative	823 (95.5)	441 (95.2)	321 (96.1)	61 (93.8)	ns
positive	39 (4.5)	22 (4.8)	13 (3.9)	4 (6.2)	ns
ER status (n=852)					
positive	671 (78.8)	347 (76.9)	276 (81.7)	48 (76.2)	ns
negative	181 (21.2)	104 (23.1)	62 (18.3)	15 (23.8)	ns
PR status (n=853)					
positive	580 (68.0)	293 (65.0)	244 (72.0)	43 (68.3)	ns
negative	273 (32.0)	158 (35.0)	95 (28.0)	20 (31.7)	ns
p53 IHC (n=650)					
positive	131 (20.2)	70 (20.2)	53 (20.8)	8 (16.7)	ns
negative	519 (79.8)	277 (79.8)	202 (79.2)	40 (83.3)	ns

5.3.3 R72P and survival of breast cancer patients

Kaplan-Meier survival analysis showed that unselected breast cancer patients with PP homozygous genotype had poorer survival than patients with other genotypes: cumulative survival at 80 months follow-up was 74% and 88%, respectively (p=0.003, log-rank test, n=621) (Figure 8). This was more pronounced when only patients with p53-negative

tumors were compared (p=0.001, n=356). For comparison, cumulative survival at 80 months follow-up was 74% among patients with p53-positive tumors and 92% among patients with p53-negative tumors (p<0.001, n=457).

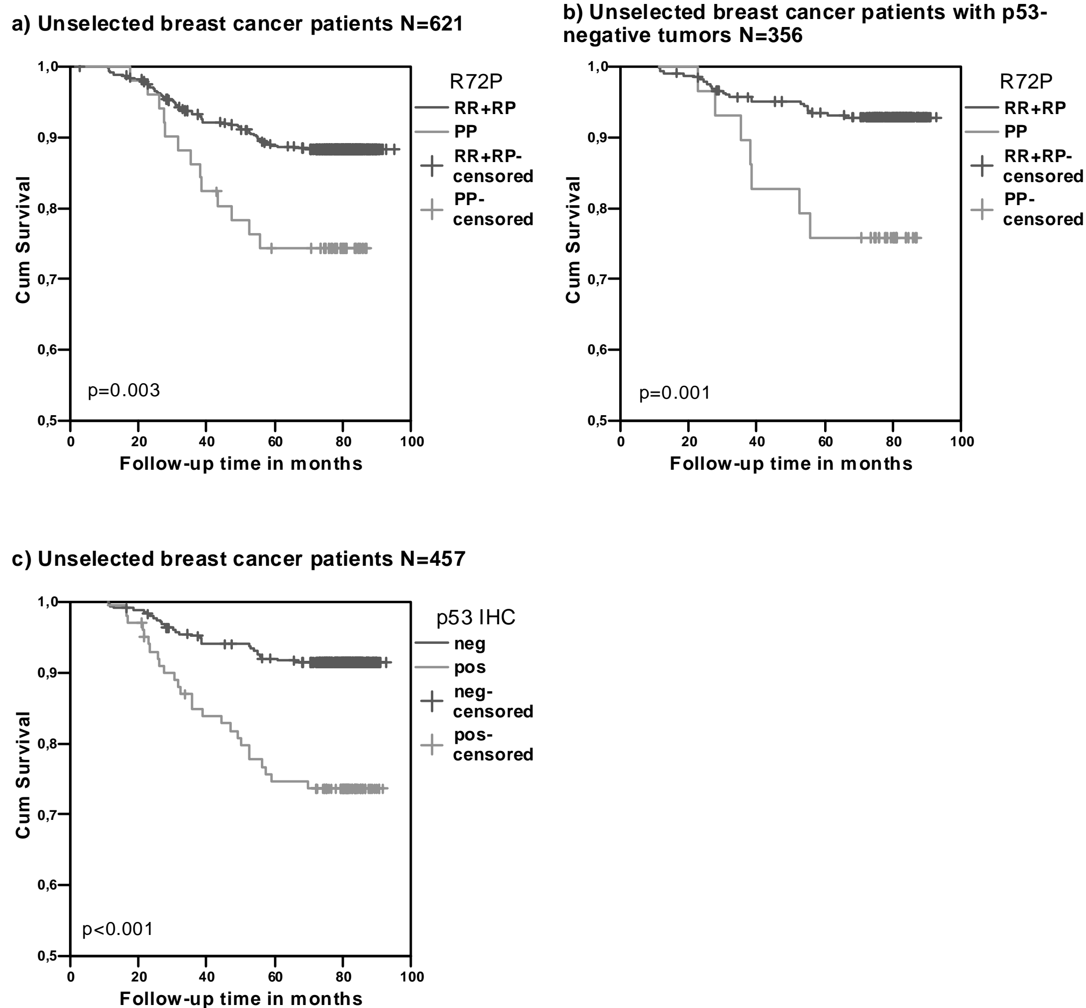


Figure 8. Kaplan-Meier survival curves of unselected breast cancer patients.
a) survival among all patients by R72P genotype (PP homozygotes vs. other genotypes)
b) survival among patients with p53 immunonegative tumors by R72P genotype
c) for comparison, survival among all patients by p53 expression

Multivariate analysis by Cox's proportional-hazards regression model indicated tumor diameter (T), lymph node status (N), progesterone receptor expression (PR), p53 expression, and PP homozygous genotype as independent prognostic factors among the breast cancer patients with information of these parameters available (n=373) (Table 9.).

Table 9. Multivariable analysis (Cox's proportional-hazards model) of prognostic factors in unselected breast cancer patients (n=373).

Variables	Risk ratio (95% CI)	*p*
Age at diagnosis	1.0 (1.0-1.0)	0.5
T		
2 vs. 1	1.1 (0.5-2.3)	0.8
3 vs. 1	2.7 (0.7-10.0)	0.1
4 vs. 1	8.5 (2.5-29.2)	0.001
N		
1 vs. 0	6.5 (2.7-16.0)	<0.001
2 vs. 0	21.3 (4.7-97.1)	<0.001
M (pos. vs. neg.)	4.9 (1.8-13.2)	0.002
Grade		
2 vs. 1	3.1 (0.4-24.6)	0.3
3 vs. 1	8.8 (1.1-72.4)	0.04
ER status (pos. vs. neg.)	1.9 (0.7-5.2)	0.2
PR status (pos. vs. neg.)	0.2 (0.1-0.5)	<0.001
p53 IHC (pos. vs. neg.)	3.4 (1.7-6.9)	0.001
p53 R72P (PP vs. RP and RR)	2.1 (1.4-3.3)	0.001

6 DISCUSSION

6.1 *ATM* and breast cancer (I, II, III)

6.1.1 *ATM* variants in breast cancer

Of the seven *ATM* mutations originally identified in Finnish A-T patients (Laake et al. 2000, Allinen et al. 2002), only two, 6903insA and 7570G>C, seem to be associated with familial breast cancer susceptibility in Finland. Besides these, another *ATM* mutation, 8734A>G, previously associated with breast cancer susceptibility (Thorstenson et al. 2003, Teraoka et al. 2001), was observed in two families. In Finland, 6903insA, 7570G>C and 8734A>G have been observed altogether in 9/630 breast cancer families (p=0.0003, OR 18.9, 95% CI 2.4-149.7, compared to healthy controls) (Allinen et al. 2002, I). However, incomplete segregation of the mutations in the families (both unaffected mutation carriers and mutation-negative breast cancer patients were observed) and the fact that 6903insA and 7570G>C have also been observed in breast cancer patients without known family history of the disease (7/1209, p=0.03, OR 7.6, 95% CI 0.9-61.9), and 7570G>C also in one healthy control, suggest incomplete penetrance for these mutations. Overall, the observed *ATM* mutations seem to explain only a small fraction of hereditary susceptibility to breast cancer in Finland, as they have been observed in 1.4% of the familial and 0.6% of the unselected cases studied so far. However, due to geographical clustering, their contribution to familial breast cancer in certain regions might be significant. In particular, the 6903insA and 8734A>G mutations cluster to the area of Tampere, and together their frequency in the breast cancer families studied from this region is 3.0% (5/168). In contrast, 7570G>C mainly concentrates to the Oulu region. In the Helsinki series, 7570G>C was the only mutation observed, in one familial case.

Consistent with previous findings (Broeks et al. 2000), none of the mutation carrier tumors tested showed loss of the wild-type allele, indicating that LOH is not involved in development of breast cancer in *ATM* mutation carriers. Instead of the complete loss of

normal protein, it has been suggested that mutations with dominant-negative effect are the ones mainly responsible for the increased risk of breast cancer in *ATM* mutation carriers (Chenevix-Trench et al. 2002). Analysis of the 7570G>C heterozygous cell line showed that substitution of the evolutionarily conserved Ala2524 residue with proline in the FAT domain (Bosotti et al. 2000) leads to a stable protein with defective kinase activity. Failure in correct folding due to this substitution could inactivate kinase functions (Lavin et al. 2004), which would lead to the defective phosphorylation of ATM Ser1981 and the two ATM downstream targets p53 Ser15 and CHEK1 Ser317, and explain the pathogenicity of 7570G>C. The other observed A-T mutation, 6903insA, causes a frameshift. No truncated protein was present in the carrier cell lines, indicating that if translated, the mutant protein is unstable, and the total amount of endogenous ATM was reduced to about half. This seems sufficient for normal function of the ATM checkpoint signaling pathway, but not to ensure normal level of cell survival after IR-induced damage. Different biological endpoints and functions could have different threshold requirements for ATM, which also has been reported previously (Delia et al. 2000, Fernet et al. 2004). Thus, even though one cellular pathway that might promote tumorigenesis is altered, others may function apparently normally. Accordingly, instead of the dominant-negative effect, haploinsufficiency might be a more plausible explanation for the cancer susceptibility associated with 6903insA. The third potentially pathogenic *ATM* mutation, 8734A>G, leads to Arg2912Gly substitution in the kinase domain. The carrier cell lines showed no defects in the phosphorylation of ATM Ser1981 or p53 Ser15, whereas CHEK1 Ser317 phosphorylation was impaired. Thus, Arg2912Gly substitution does not impair the phosphorylation of all ATM substrates, and the cancer predisposing effect of this mutation is not a dominant-negative one. Nevertheless, Arg2912Gly may impair some other protein-protein interactions required for optimal ATM kinase activity.

Screening of the whole coding region of the *ATM* gene in 47 familial breast cancer patients from Southern Finland revealed altogether 17 different sequence variants, forming 17 different haplotypes. Of the 17 variants identified, seven were intronic, four were silent, and six were missense changes. All the intronic and silent changes have been

reported previously. One of the intronic changes, ivs10-6T>G, a known A-T mutation leading to incorrect splicing of the exon 11 and premature truncation of the protein (Dörk et al. 2001), has been suggested to associate with breast cancer in different populations (Thorstenson et al. 2003, Broeks et al. 2000, Chenevix-Trench et al. 2002), but larger case-control studies have not found a significant difference in frequencies between breast cancer cases and healthy controls (Szabo et al. 2004, Lindeman et al. 2004, Thompson et al. 2005). In our study, ivs10-6T>G was found in one of 265 familial breast cancer cases, and not in 228 controls, but the mutation did not segregate with cancer in the family. One of the five rare missense variants identified, 2119T>C (S707P), has been very extensively evaluated for breast cancer risk previously (Dörk et al. 2001, Spurdle et al. 2002, Sommer et al. 2003, Bretsky et al. 2003), and the results do not support its association with breast cancer. All the four rare missense variants studied further, 1814A>G (H605R), 4424A>G (Y1457C), 6539G>T (G2180V), and 998C>T (S333F), may affect ATM function as suggested by bioinformatic analysis. Three of these variants were very rare, each identified in only one out of over 250 cases while not in population controls. The 1814A>G variant has previously been found also in a Danish breast cancer patient (Børresen-Dale, unpublished), whereas the 4424A>G variant has been found in a healthy population control, but not among breast cancer patients (Sommer et al. 2003). To our knowledge, the 6539G>T variant has not been reported previously. Even if these variants have a functional effect on the ATM protein, and are possibly pathogenic, their rarity limits the potential contribution to breast cancer susceptibility. The 998C>T was found in 0.92% of the cases and in 0.83% of the controls, not supporting an association with breast cancer.

The common polymorphism, *ATMex39* 5557G>A (D1853N), has been reported in the homozygous state to associate with enhanced clinical radiosensitivity in breast cancer patients (Angèle et al. 2003a), suggesting that it might be considered as a risk factor predisposing to adverse reactions after radiotherapy and supporting a possible functional effect for this variant. Heikkinen et al. (2005) recently reported that the *ATMivs38* -8T>C polymorphism occurring in *cis* position with 5557G>A was associated with bilateral breast cancer among altogether 176 familial breast cancer patients studied. The combined

variant was found to associate also with reduction of ATM protein level in lymphoblast cells. No aberrant transcripts were detected (Heikkinen et al. 2005), although it was hypothesized that the 5557G>A variant previously suggested to affect an exonic splicing enhancer element (Thorstenson et al. 2003), together with the ivs38-8T>C change, could have some effect on the correct splicing of the exon 39. Most recently, Langholz et al. (2006) reported that the association of ivs38-8T>C with bilateral breast cancer could not be replicated in the WECARE Study population of 708 asynchronous bilateral and 1397 unilateral breast cancer patients. While this does not support the previously suggested association of the ivs38-8T>C variant with bilateral breast cancer, Langholz et al. (2006) discussed the possibility that the variants studied may not be the causative alleles but may be contained in the same risk haplotype with other, possible risk alleles unique to the Finnish population. The WECARE cases were also unselected for family history, while those in the study by Heikkinen et al. (2005) belonged to breast cancer families. In our study, including about 800 unselected as well as almost 800 familial Finnish breast cancer cases, neither 5557G>A nor ivs38-8T>C, or any haplotype containing these variants in the Finnish population, was associated with breast cancer risk or bilateral breast cancer in any of the patient groups or subgoups studied. Among our familial breast cancer patients screened for the whole *ATM* gene, both of the variants 5557G>A and ivs38-8T>C were present in altogether three haplotypes. Our results suggest that the haplotype containing only the combined variant 5557G>A/ivs38-8T>C is not associated with bilateral breast cancer, and the two other haplotypes are far too rare to underlie the suggested association with bilateral breast cancer. The carrier frequency of the ivs38-8T>C variant (or the combined variant, as all the carriers of ivs38-8T>C also carried 5557G>A) was marginally higher in familial breast cancer patients, especially in those patients with only a moderate family history of breast cancer (8.1%), than in healthy controls (5.6%), but the difference did not reach statistical significance. Our results do not support an association of the variants with increased breast cancer risk in any of the patient groups studied, although small increases in risk cannot be excluded. However, our results are consistent with those by Langholz et al. (2006), who also found no association of the 5557G>A/ivs38-8T>C variant with bilateral breast cancer (OR=1, 95% CI 0.6-

1.5). The variants were not significantly associated (together or alone) with multiple primary tumors, either.

In conclusion, our results support the association of two A-T-related *ATM* mutations, 6903insA and 7570G>C, in addition to 8734A>G, with breast cancer susceptibility. This is consistent with the study of Renwick et al. (2006), reporting an approximately two-fold increase in risk of breast cancer associated with *ATM* mutations causing A-T. This risk appears to be similar to that of low-penetrance susceptibility allele *CHEK2* 1100delC. No evidence that other classes of *ATM* variants confer a risk of breast cancer was found in the UK (Renwick et al. 2006), Southern Finnish (II), or Northern Finnish patients (Heikkinen et al. 2005). Another A-T causing mutation, 7271T>G (Val2424Gly), was also recently evaluated in a large study of breast cancer patients from Northern America and Australia, and it was suggested to be associated with high risk (up to 14-fold increase) of breast cancer (Bernstein et al. 2006). In addition, our results do not support an association of 5557G>A or ivs38-8T>C variant, or any haplotype containing these in the Finnish population, with bilateral breast cancer or with increased breast cancer risk. Our results also provide evidence for founder effects in the geographical distribution of A-T-related breast cancer susceptibility alleles. They cluster to specific regions in Northern and Central Finland, but in Southern Finland *ATM* mutations seem to have a minor effect, if any, on familial breast cancer risk. The incomplete penetrance of the mutations implies that the breast cancer risk associated with them is likely to depend on environmental factors and/or susceptibility alleles in other genes, as suggested by the polygenic model for breast cancer susceptibility (Pharoah et al. 2002). Of these mutations, 7570G>C and 8734A>G lead to amino acid substitutions, but only 7570G>C showed dominant-negative effect on kinase activity. For *ATM* 6903insA carriers, haploinsufficiency might be a more plausible explanation for the predisposition to cancer. Consequently, breast cancer susceptibility is not restricted to *ATM* mutations with dominant-negative effect on the kinase activity.

6.1.2 ATM expression in breast cancer

ATM protein expression was reduced in 10.7% of all non*BRCA1/2* breast cancer tumors, and no difference was noted between sporadic (10.9%) and familial non*BRCA1/2* tumors (10.5%). Reduced ATM expression has been reported in 25-85% of sporadic breast cancer tumors previously. However, these studies have been small (including 17-106 cases), and used various immunohistochemical methods and different interpretation and cut-off levels (Kairous et al. 1999, Angèle et al. 2000, Ding et al. 2004, Angèle et al. 2003, Honrado et al. 2005b, Cuatrecasas et al. 2006). We employed an established immunohistochemical protocol (Lukas et al. 2001, Vahteristo et al. 2002) using the monoclonal ATM antibody previously validated for immunostaining on archival specimens of formalin-fixed, paraffin-embedded human tissues (Angèle et al. 2003, Bartkova et al. 2005a, 2005b). ATM protein expression in familial breast cancer tumors has previously been evaluated in only one study with a small number of cases. Honrado et al. (2005b) studied 46 familial non*BRCA1/2* tumors and found no significant difference in ATM expression between familial (non*BRCA1/2* or *BRCA1/2*) and sporadic tumors.

Whereas the familial and the sporadic non*BRCA1/2* tumors showed similar ATM expression, there was a clear, approximately 3-fold increase of the ATM aberrant cases among both the *BRCA1* and *BRCA2* tumors, as compared to the non*BRCA1/2* tumors. ATM expression was significantly more often reduced in tumors from *BRCA1* mutation carriers (33.3%) and in tumors from *BRCA2* mutation carriers (30.0%), than in non-*BRCA1/2* tumors (10.7%). In only one of the previous studies (Honrado et al. 2005b) also *BRCA1* tumors (n=33), *BRCA2* tumors (n=24), and familial non*BRCA1/2* tumors (n=46) have been studied for ATM expression: in that study there was no significant difference in ATM expression between *BRCA1/2* and non*BRCA1/2* tumors. The number of studied tumors was again smaller and the interpretation of the ATM expression very different.

Aberrant ATM expression in non*BRCA1/2* breast cancer tumors was associated with negative hormone receptor (ER and PR) status and high grade of the tumor. Increased frequency of abnormal ATM expression in high-grade breast cancer tumors has been reported previously (Ding et al. 2004, Cuatrecasas et al. 2006). Cuatrecasas et al. (2006)

studied ATM expression in 52 breast carcinomas and reported that it associated with differentiation and angiogenesis, but they did not detect correlation between ATM expression and ER or PR status. We also examined the immunohistochemical expression of p53. There was no significant association of p53 overexpression with normal or aberrantly reduced ATM among the non*BRCA1/2* tumors. In *BRCA1* tumors, the frequency of p53 immunopositivity was in general higher than in either *BRCA2* cancers or in non*BRCA1/2* tumors, but these differences did not reach statistical significance, probably due to small sample size, as several studies have reported higher incidence of positive p53 immunostaining in *BRCA1* tumors than in sporadic tumors; for *BRCA2* the results have been less conclusive (Honrado et al. 2005). However, the subset of ER/PR/ERBB2-triple-negative non*BRCA1/2* breast tumors showed a very tight correlation with p53 immunopositivity compared to tumors expressing at least one of these receptors. p53 overabundance has been reported in the triple-negative subset of breast tumors previously (Cleator et al. 2007). Interestingly, also the aberrant reduction of ATM occurred significantly more commonly among the ER/PR/ERBB2-triple-negative tumors.

Correlation of reduced ATM expression in breast carcinomas with tumor differentiation and hormone receptor status supports its role in cancer development and progression. The association of the aberrant ATM expression with these parameters was observed similarly both among the sporadic as well as familial breast tumors. These results suggest that ATM has a similar role in the progression of sporadic as well as familial tumors without *BRCA1* or *BRCA2* mutations. However, *BRCA1/2* tumors showed more often reduced ATM expression, and so did the triple-negative subset of non*BRCA1/2* tumors. The triple-negative tumors are typically highly proliferative, poorly differentiated, and display extensive genetic instability. Interestingly, the triple-negative cancers share these, and multiple additional features with the *BRCA1* breast tumors including high incidence of p53 mutations, broadly similar mRNA expression profiles, and poor prognosis (Cleator et al. 2007). Our results indicate that the increased frequency of aberrant ATM reduction may represent another feature shared by the triple-negative carcinomas and the tumors of the *BRCA1* mutation carriers.

ATM acts upstream of BRCA1 in the same pathway, since it directly phosphorylates BRCA1 on serine residues S1423 and S1524, thereby modulating the function of BRCA1 (Cortez et al. 1999). The p53 tumor suppressor protein is also involved in the DNA damage repair and, like BRCA1, it is also a direct substrate of ATM (Kastan & Bartek 2004, see also 2.4.3). Furthermore, both ATM and p53 have been found activated in early human pre-cancerous lesions as part of the suggested anti-cancer barrier (Bartkova et al. 2005a). According to the concept of the DNA damage response (DDR) as an anti-cancer barrier (Bartkova et al. 2005a), there is constitutive activation of DNA damage checkpoints in pre-invasive human cancerous lesions, including breast lesions, and the subsequent selection for defects of various components of the DNA damage network is proposed to be a way to overcome this anti-cancer barrier during tumor progression. The subset of ATM-deficient tumors may reflect this. On the other hand, our results are consistent with the scenario where the initial cancer-predisposing defect, such as a *BRCA1* or *BRCA2* mutation, would impair the genome integrity control and lead to increased number of unrepaired DSBs, which in turn would activate the ATM-regulated cell-cycle checkpoints and cell-death pathways, ultimately leading to selection of ATM inactivation to overcome the anti-cancer barrier and progress towards malignancy. In contrast to normal tissue, the early cancerous lesions are exposed to oncogene-induced chronic replication stress and DNA breakage (Bartkova et al. 2005a, Gorgoulis et al. 2005, Bartkova et al. 2006, Di Micco et al. 2006), and thus there is an increased demand for BRCA1/2 function in DNA damage signaling and repair. As a result, endogenous DNA damage in such pre-malignant lesions may no more be manageable by the DDR machinery with only one functional allele of *BRCA1* or *BRCA2*. In other words, such conditions may unmask "conditional haploinsufficiency" for *BRCA1/2* in the early lesions (Bartek et al. 2007). Under such conditions, the DDR machinery including the ATM-CHEK2-p53 cascade is activated, and this environment selects for inactivation of the activated DDR barrier, including ATM and p53. Thus, the BRCA1/2 defects would eventually lead to increased frequency of ATM inactivation, which is seen in the increased number of ATM aberrant carcinomas among the *BRCA1/2* tumors. The actual molecular mechanisms behind low ATM expression in breast carcinomas are currently

unknown. Epigenetic silencing by promoter hypermethylation is one of the possible causes, but in a recent study, no methylation of the ATM promoter was found in 74 breast carcinomas studied (Treilleux et al. 2007). Genomic alterations of the promoter region could be another explanation, but have not been extensively studied. In addition, a reduction in the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) has been suggested to cause down-regulation of ATM, and a subset of tumors showed low levels of both DNA-PKcs and ATM, indicating that cross-regulation between DNA-PKcs and ATM could explain the reduced ATM levels seen in breast carcinomas (Treilleux et al. 2007).

The ER/PR/ERBB2-triple-negative tumors are a challenge to treat (Cleator et al. 2007). The most exciting implication of identification of phenotypic and genetic similarities between *BRCA1* and triple-negative breast tumors would be the emerging possibility to design novel targeted therapies to which both of these classes of breast cancer might be exceptionally sensitive. One promising treatment of *BRCA1* tumors is small molecule-mediated inhibition of PARP1, the key element of the pathways that repair DNA single strand breaks (SSBs). PARP1 inhibition renders cells particularly dependent on homologous recombination and therefore on BRCA1/2 function, resulting in high sensitivity of *BRCA1/2* tumors (Bryant et al. 2005, Farmer et al. 2005), and enhanced sensitivity of cells with impaired DSB signaling (McCabe et al. 2006) to such treatment. This therapy may target not only the advanced cancers with complete lack of BRCA1/2 function upon loss of the second allele of *BRCA1/2*, but also the conditionally deficient *BRCA1/2*-heterozygous cells in the precursor lesions with supra-threshold levels of endogenous DNA damage, while the normal tissues of the patients (including the *BRCA1/2* mutation carriers) with sub-threshold levels of endogenous DNA damage would be resistant to such treatments.

6.2 *RAD50* and breast cancer (IV)

The possible role of *RAD50* in familial breast cancer predisposition is intriguing as RAD50 is part of the MRN complex and participates in critical cellular functions of DSB repair, functionally interacting with other breast cancer predisposition genes (van den

Bosch et al. 2003, Kastan & Bartek 2004). NBS1, another part of the MRN complex, has also been associated with breast cancer: *NBS1* 657del5 founder mutation has been reported to contribute significantly to the incidence of breast cancer in Poland (Gorski et al. 2003, Steffen et al. 2004, 2006), and *NBS1* mutations have also been suggested to contribute to breast cancer in Northern Finland (Heikkinen et al. 2003, 2006). Our *RAD50* mutation analysis in Southern Finnish and UK breast cancer families revealed only a single protein truncating mutation, Q350X, in one of the 481 breast cancer families studied. The mutation leads to truncation of 962 amino acids of the RAD50 protein, including most of the coiled-coil domain, the zinc-hook structure, and the C-terminal ATP-binding domain (Hopfner et al. 2001, Hopfner et al. 2002, Moncalian et al. 2004). No other case or control with the mutation was identified in the altogether 1488 UK samples and 600 Finnish samples analyzed. Therefore, even if *RAD50* Q350X is contributing to cancer predisposition in the UK family in which it was identified, due to its rarity it cannot be making a major contribution to familial breast cancer overall.

We identified altogether nine missense variants, two synonymous changes, and six intronic *RAD50* variants. Only one intronic variant (ivs20+35C>T) was found in one of the 46 Finnish patients screened. Two of the missense variants (I94L and R224H) and one of the synonymous changes (H68H) found in UK patients had been reported previously in breast cancer cases from Northern Finland; both variants were also present in healthy controls, although at lower frequency (Heikkinen et al. 2003). The I94L and R224H variants affect conserved residues; I94L in the aminoterminal ATPase domain and R224H in the coiled-coil domain (Hopfner et al. 2000, Hopfner et al. 2001). However, the I94L and R224H variants have more recently been studied in additional breast cancer cases from Northern Finland, and the frequency of R224H in unselected breast cancer patients (2/317, 0.6%) did not differ from the frequency in controls (9/1000, 0.9%), and I94L was not detected in those 317 cases (Heikkinen et al. 2006). R224H was not present among 80 families studied from Southern Finland (Tommiska et al., unpublished). In addition to R224H, two other variants, R913W, located in the N-terminal MRE11 binding coiled-coil segment of the protein (Hopfner et al. 2001), and R327H may also affect RAD50 function according to SIFT and PolyPhen analysis.

However, there is no strong evidence to suggest that any of these variants are breast cancer susceptibility alleles. All are rare, limiting any potential contribution to breast cancer susceptibility, even if they are pathogenic, and due to the rarity of these variants it is not possible to evaluate their significance for familial breast cancer risk by a case-control analysis.

The protein truncating mutation 687delT was detected in 3/590 (0.5%) Southern Finnish familial breast cancer cases and 1/560 controls (0.2%), but was not present in the 435 UK families studied. The mutation showed incomplete segregation with cancer in the families, which is consistent with previous data showing that *RAD50* 687delT did not segregate with cancer in one of the two Northern Finnish families in which it was identified; the family also carried a pathogenic *BRCA1* mutation (Heikkinen et al. 2003). *RAD50* 687delT was recently observed significantly more often in unselected breast cancer cases from Northern Finland (8/317, 2.5%) than in controls (6/1000, 0.6%, p=0.008). The frequency of carriers among cases was four-fold compared to that in controls. No difference was noted in frequencies between patients with or without family history of breast and/or ovarian cancer (Heikkinen et al. 2006). This and the incomplete segregation of the mutation with cancer in the families suggest that *RAD50* 687delT may be a low-penetrance breast cancer susceptibility allele (Heikkinen et al. 2006, IV). No *RAD50* 687delT carrier was found in altogether 512 breast cancer cases from Sweden, Norway and Iceland screened by Heikkinen et al. (2006). In our study, the mutation was not detected in the UK patients. Together these data suggest that *RAD50* 687delT is a Finnish founder mutation, and more prevalent in Northern Finland than in Southern Finland. Differences in prevalence of breast cancer susceptibility alleles between Northern and Southern Finland have been observed also with *BRCA1*, *BRCA2*, and *ATM* (Sarantaus et al. 2000, I). Common origin of the *RAD50* 687delT founder mutation was also supported by haplotype analysis in the Northern Finnish carriers (Heikkinen et al. 2006).

To investigate the functional effect of *RAD50* 687delT we studied RAD50 expression in lymphoblast cell lines from mutation carriers. This showed that the 687delT allele was

present in the mRNA pool, but at a very low level compared to the wild-type allele (Tommiska et al., unpublished), possibly due to partial degradation of the mutant mRNA by nonsense-mediated mRNA decay (NMD). The truncated protein was not detected on immunoblotting suggesting that, if translated, the residual truncated form is unstable. *RAD50* 687delT thus appears to be a null allele with no detectable expression of the mutant protein. This would enable formation of normal RAD50/MRE11 heterotetramers, suggesting the mutation is unlikely to have a dominant-negative effect on RAD50 function.

RAD50 protein expression and nuclear localization appeared normal in breast tumors from 687delT mutation carriers, suggesting that the wild-type allele was retained and expressed. In addition, no evidence of allelic imbalance was seen in the 687delT mutation carrier tumor studied by direct sequencing of the DNA. Our data are consistent with LOH analyses in breast tumors from previously reported 687delT carriers, which demonstrated that the wild-type allele was not lost (Heikkinen at al. 2003). This was also confirmed in the recent study by Heikkinen et al. (2006). Our semi-quantitative immunostaining results cannot exclude a moderate deficit of some 20-50% of the overall RAD50 protein in the breast tumors from 687delT mutation carriers. In addition, the overall amount of RAD50 in lymphoblast cells heterozygous for 687delT was reduced compared to that in non-carrier cells. Together these data suggest that *RAD50* is not acting as a classical tumor suppressor gene in breast cancer, but it is possible that RAD50 haploinsufficiency is contributing to cancer. Haploinsufficiency has been suggested by mouse models for also other important DDR proteins whose defects contribute to tumorigenesis, for example the tumor suppressor Chk1 kinase (Lam et al. 2004). The *CHEK2* 1100delC low-penetrance breast cancer susceptibility allele has also been suggested to act by haploinsufficiency (Jekimovs et al. 2005, Sodha et al. 2006), and the tumorigenesis associated with A-T –related *ATM* mutation 6903insA is also likely to be due to haploinsufficiency (I). Work using genetically modified mice imply that even subtle hypomorphism in the Rad50 function is sufficient to cause severe pathological consequences including predisposition to cancer (Bender et al. 2002). The tumor-prone phenotype of mice with hypomorphic *Rad50* mutations indicates that RAD50 haploinsufficiency might contribute to human

breast tumorigenesis, although with low penetrance due to the remaining functional allele.

Cytogenetic analysis of peripheral blood T-lymphocytes of Northern Finnish 687delT carriers revealed more chromosomal rearrangements, both simple and complex, than in healthy non-carrier controls. The increased genomic instability of mutation carriers suggests an effect for RAD50 haploinsufficiency on genomic integrity (Heikkinen at al. 2006). Another *RAD50* mutation, ivs3-1G>A, leading to incorrect splicing and thus to a premature stop codon, and also associated with genomic instability, was also found in Northern Finland (Heikkinen et al 2006). In conclusion, germline variants in the *RAD50* gene do occur in familial breast cancer patients, yet they are very rare. *RAD50* 687delT is a Finnish founder mutation with low penetrance, and more prevalent in Northern Finland than in Southern Finland where *RAD50* can only be making a minor contribution to familial breast cancer predisposition. Among reasons for the overall low frequency of *RAD50* germline mutations might be the essential role of RAD50 in embryogenesis: homozygous *Rad50* gene deletions in mouse models result in early embryonic lethality (Luo et al. 1999), suggesting that RAD50 plays an essential role in genome maintenance and defects in its function are not tolerated.

The abundance and localization of RAD50 appeared normal in all the 28 familial breast tumors examined by immunohistochemistry, and this was the case also for the MRE11 and NBS1 proteins. The fact that we identified a small subset of sporadic breast tumors with a simultaneous gross reduction of all three proteins of the MRN complex is consistent with a previous study of sporadic carcinomas (Angèle et al. 2003). Furthermore, the latter cases also document that the antibodies and our assay conditions were appropriate to detect such aberrations at the protein level when they existed. The subset of MRN-deficient carcinomas may again (as the subset of ATM-deficient carcinomas in III) reflect the selection for defects of the components of the activated DDR network as a way to overcome the anti-cancer barrier during tumor progression (Bartkova et al. 2005a).

6.3 p53 R72P and breast cancer (V)

The studies on p53 R72P polymorphism and breast cancer risk have yielded inconsistent, even contradictory results (see 2.6.3). We aimed to evaluate whether R72P associates with increased risk for breast cancer among extensive sets of 923 familial and 858 unselected breast cancer patients and 733 population controls. The genotype frequencies among all patient series, as well as in subgroups defined by different family history, bilateral breast cancer or multiple cancers, were closely similar. Similarly, no difference in the age at diagnosis was seen by R72P genotype. Our results thus indicate that the p53 R72P genotypes are not associated with increased breast cancer risk among unselected or familial breast cancer patients. Our results have recently been confirmed in a large study of the Breast Cancer Association Consortium (BCAC), in which altogether 8,743 breast cancer cases and 10,618 controls from Europe, the USA, Australia, and Asia, genotyped for R72P, were analyzed, and none of the genotypes was associated with increased breast cancer risk (Breast Cancer Association Consortium 2006).

The R72P polymorphism has also been studied together with other polymorphisms in genes interacting with p53, as in addition to gene-environment interactions, also gene-gene interactions may have an effect on cancer risk. The most extensively studied polymorphism possibly interacting with p53 R72P is *MDM2* SNP309, a T to G change in the promoter region of the inhibitor of p53, causing attenuation of the p53 pathway and accelerated tumor formation in individuals carrying a germline p53 mutation (Bond et al. 2004, Bond et al 2005, Bougeard et al. 2006, Ruijs et al. 2007). The p53 72Pro/Pro and *MDM2* SNP309 G/G genotypes have been reported to associate, for example, with increased risk of esophageal squamous cell carcinoma (Hong et al. 2005) and lung cancer, where the interaction between the polymorphisms seemed to increase the risk in a multiplicative manner (Zhang et al. 2006). Bougeard et al. (2006) also suggested that the impact of the *MDM2* SNP309 G allele on the age of tumor onset in germline p53 mutation carriers could be amplified by the p53 R72 allele. Some indication of gene-gene interaction was observed in breast cancer, too, but no consistent direction of interaction was apparent (Cox DG et al. 2007). In the pooled data set of BCAC, no indication of

either an increase in risk or an earlier age at onset of breast cancer in carriers of either *MDM2* SNP309 or p53 R72P or both was observed (Schmidt et al. 2007).

We also evaluated R72P genotype frequencies and possible modifying effect on breast cancer risk among *BRCA1* and *BRCA2* mutation carriers. *BRCA2* mutation carrier patients carrying a 72P allele (72P homozygotes and heterozygotes) tended to be diagnosed younger than the R72 homozygotes; Martin et al. (2003) found a similar trend among *BRCA2* mutation carriers, but the difference was not statistically significant in either study. They also found that presence of a 72P allele was associated with an earlier age of breast cancer diagnosis among *BRCA1* mutation carriers, which was not the case in our material. Association of R72 homozygous genotype with multiple primary cancers or family history of multiple primary cancers among *BRCA1/2* mutation carrier women was also suggested by Martin et al. (2003), but no association of any of the genotypes with multiple cancers was seen in our study. As the numbers of *BRCA1/2* mutation carrier patients in either study were quite small, larger studies will be needed to evaluate the possible modifying effect of R72P genotype on cancer risk among *BRCA1* and *BRCA2* mutation carriers. In the study including 447 Spanish *BRCA1/2* mutation carriers, genotype and haplotype analyses revealed that the presence of a specific haplotype carrying the 72P allele and not carring the 16-bp insertion (c.97-147ins16bp) in p53 intron 3, was associated with an earlier age of breast and/or ovarian cancer onset in *BRCA2* mutation carriers. This result was also supported by functional studies, as cells carrying the haplotype with 72P and without the 16-bp insertion showed a decrease in p53 apoptotic rate (Osorio et al. 2006).

Interestingly, analysis of histopathologic features of breast tumors suggested association of the R72P genotypes with different histologic types. The 72P homozygous breast cancer patients presented significantly more often with lobular carcinoma than patients carrying an R72 allele, and had often grade 1 tumors, whereas R72 allele carriers had more frequently ductal carcinomas and often grade 3 tumors. Association of 72P homozygosity with lower grade was only of borderline significance, but it would be consistent with a higher frequency of lobular carcinomas among 72P homozygous

patients, as lobular carcinomas have been found to be more often of lower grade (Molland et al. 2004). Other studies have examined the association of R72P polymorphism with histopathologic characteristics of breast tumors, too. Noma et al. (2004) found no significant association of different R72P genotypes with tumor histology, tumor size, or lymph node status in 191 Japanese breast cancer patients. In their study, too, the 72P homozygous patients tended to have tumors of lower grade, but the difference was not statistically significant. However, they observed that the tumors of the 72P homozygotes were significantly more often ER positive than the tumors of the R72 homozygotes, which was not seen in our 852 tumors. In an analysis of 664 Korean patients, no association with ER status, or histologic grade was seen, but carriers of the 72P allele had significantly more often negative axillary lymph node status than the R72 homozygotes (Han et al. 2004), which was not the case in Japanese or Finnish patients (Noma et al. 2004, V). In another Japanese study, no association of any of the genotypes with tumor size, node status, histology, grade, ER status or p53 expression was observed among 557 breast cancer patients (Toyama et al. 2007). Differences in patient selection criteria as well as in allele frequencies between different populations may have an impact on these results. Apparently the possible association of R72P with different histopathologic features of breast cancer tumors warrants further studies.

Analysis of survival among unselected breast cancer patients revealed association of the genotypes with differential survival. No difference in survival has been found between patients with lobular and ductal infiltrating carcinomas (Molland et al. 2004), but p53 72P homozygotes were found here to have a significantly poorer survival than R72 homozygotes or heterozygotes ($p=0.003$). Because in our material the patients with p53 immunopositive tumors did have significantly poorer survival, we excluded the effect of p53 positivity on survival by including only patients with p53 immunonegative tumors in the analysis. The effect of R72P on survival seemed to be independent of somatic p53 mutations in the tumors, as the effect was similar when only patients with negative immunostaining for p53 on the tumors were included in the analysis ($p=0.001$). Multivariate analysis showed that 72P homozygous genotype was overall an independent prognostic factor, with a two-fold increased risk of death. As 8.2% of all patients were

homozygous for the 72P allele (8.1% among p53 negative cases), codon 72 genotype could be a useful additional prognostic marker among this subgroup of patients. The effect of 72P homozygous genotype on survival was similar both among patients with ductal and lobular carcinoma (data not shown). The fact that the effect of 72P homozygous genotype on survival is independent of somatic p53 mutations is also supported by findings that breast tumors of 72P homozygotes have a lower frequency of somatic p53 mutations than tumors of R72 homozygotes and heterozygotes (Langerød et al. 2002, Noma et al. 2004). These data suggest that the p53 Pro/Pro is functionally impaired *per se*, independently of somatic p53 mutations. Our finding is also consistent with the R72 variant of wild-type p53 being a more potent inducer of apoptosis than the wild-type 72P variant. It has been suggested that R72 homozygotes may respond more favorably to radiation or chemotherapy (Dumont et al. 2003), and the superior activity of wild-type R72 in inducing apoptosis is reflected *in vivo* in more favorable outcome in patients whose cancers express the wild-type R72 variant, compared to those with the wild-type 72P, and receiving chemo-radiotherapy for advanced squamous cell carcinomas of head and neck (SCCHN) (Sullivan et al. 2004). These favorable effects of R72 allele may, however, be reversed by a somatic p53 mutation on this allele, as has been reported in SCCHN (Bergamaschi et al. 2003, Schneider-Stock et al. 2004), and retention of the R72 allele with loss of the 72P allele in the tumor tissue has been associated with reduced survival in heterozygous breast cancer patients (Bonafè et al. 2003). In one breast cancer study, the 72P allele has been suggested to have a protective effect against death, with borderline significance, but this effect was reduced by inclusion of known prognostic variables in the analysis (Goode et al. 2002). The effect of the 72P allele on poorer survival is supported, however, also by poorer survival of 72P allele carriers reported among colorectal and lung cancer patients (Starinsky et al. 2004, Mechanic et al. 2007). Also in ovarian cancer, the patients with the 72P tumor genotype have significantly poorer survival, but in association with non-missense or certain missense mutations in p53. On the contrary to breast cancer, in ovarian cancer the 72P allele is associated with higher frequency of p53 mutations, and p53 codon 72 genotype has an impact on the prognostic value of somatic p53 mutations (Wang et al. 2004, 2007).

Recently, supporting our results, in the study of 557 Japanese breast cancer patients, the 72P homozygous genotype was associated with poorer disease-free survival (DSF). This association was especially significant in patients who had received adjuvant chemotherapy (Toyama et al. 2007). In addition, the 72P homozygous Chinese breast cancer patients were observed to be less sensitive to anthracycline-based chemotherapy than other genotypes (Xu et al. 2005). On the contrary, in the very recent study of 204 Danish breast cancer patients, no association of the different R72P genotypes with DSF or the number of p53 mutations was found (Kyndi et al. 2006). Actually, Kyndi et al. (2006) proposed that LOH rather than R72P genotype would be associated with survival, as they observed a poorer survival among patients with retention of the 72P allele, a result completely opposite to the finding of Bonafè et al. (2003). However, both studies are based on a very limited number of patients, and larger studies are needed to clarify the role of R72P genotype, LOH, and p53 mutations and their interdependence on breast cancer survival. Unfortunately, survival studies are often difficult to compare with each other as different follow-up times and criteria (use of incident vs. prevalent cases or use of disease-specific vs. overall vs. disease-free survival), or stratification of the study population may be used, and all this may have a profound impact on the results. Also treatment regimens as well as allele frequencies vary between populations; 38% of Japanese breast cancer patients were homozygous for 72P (Toyama et al. 2007), compared to 8% of Finnish patients, for example, and genetic effects may only apply to a specific subgroup of patients, or in combination with other genetic polymorphisms. Furthermore, there is evidence of selective expression of the different alleles in R72P heterozygotes, and that this selective expression is dependent on ethnicity: healthy Caucasians were found to preferentially express the R72 allele whereas Asians preferentially expressed the 72P allele, and the situation was reversed in Asian heterozygous breast cancer patients, 75% of whom preferentially expressed the R72 allele (Siddique et al. 2005). The finding of codon 72 genotype as a prognostic factor in breast cancer warrants further studies, in different populations and especially taking into consideration also variations in other interacting genes.

7 SUMMARY AND CONCLUSIONS

The role of *ATM* gene in breast cancer susceptibility has been under an extensive investigation and debate for over a decade. Recent data have clarified the picture to some extent and confirmed that the *ATM* mutations associated with A-T are also breast cancer susceptibility alleles. In Finland, too, only few A-T –related *ATM* mutations contribute to familial breast cancer. These founder mutations may be responsible for excess familial breast cancer regionally in Northern and Central Finland, but in Southern Finland our results suggest only a minor effect, if any, of any rare *ATM* genetic variants on familial breast cancer. Two common *ATM* polymorphisms studied were not associated with cancer risk, either. Functional studies of the founder mutations suggested that dominant-negative effect is not the only mechanism with which *ATM* mutations are contributing to cancer; haploinsufficiency also seems to play a role in tumorigenesis associated with *ATM* mutations. The role of ATM in cancer development and progression was also supported by the results of the immunohistochemical studies of ATM expression, as reduced ATM expression in breast carcinomas was found to correlate with tumor differentiation and hormone receptor status. Aberrant ATM expression was also a feature shared by *BRCA1/2* and ER/PR/ERBB2-triple-negative breast carcinomas, indicating that different selective pressures may operate in these carcinomas during tumor progression, compared to other non*BRCA1/2* tumors. This may also have an implication for treatment of these ATM aberrant carcinomas. From the clinical point of view, the DNA-damage-threshold-related differences might be beneficial in response to therapy. The ER/PR/ERBB2-triple-negative breast carcinomas are a challenge to treat, and identification of phenotypic and genetic similarities between the *BRCA1/2* and the triple-negative breast tumors could have an implication in designing novel targeted therapies to which both of these classes of breast cancer might be exceptionally sensitive.

Mutations of another plausible breast cancer susceptibility gene, *RAD50*, were found to be very rare, and *RAD50* can only be making a minor contribution to familial breast cancer predisposition in UK and Southern Finland. The Finnish founder mutation *RAD50*

687delT seems to be a null allele and may carry a small increased risk of familial breast cancer. *RAD50* is not acting as a classical tumor suppressor gene, but it is possible that RAD50 haploinsufficiency is contributing to cancer.

In addition to relatively rare high- and low-penetrance breast cancer susceptibility alleles, common polymorphisms may also be associated with increased breast cancer risk. Furthermore, these polymorphisms may modify the cancer risk caused by the genes with which they interact, or have an impact on the progression and outcome of the disease, thus effecting the prognosis of the patient. Our results here suggest no effect of either allele of the common p53 R72P polymorphism on familial breast cancer risk or breast cancer risk in the population, but R72P seems to be associated with histopathologic features of the tumors and survival of the patients. Multivariate analysis showed that 72P homozygous genotype was overall an independent prognostic factor, with a two-fold increased risk of death. These results present important novel findings also with clinical significance, as codon 72 genotype could be a useful additional prognostic factor in breast cancer, especially among the subgroup of patients with wild-type p53 in their tumors. The role of 72P homozygous genotype as a prognostic and predictive marker in breast cancer warrants further studies.

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9 REFERENCES

Abraham RT. PI 3-kinase related kinases: 'big' players in stress-induced signaling pathways. DNA Repair (Amst) 2004;3:883-7.

Ahmed M, Rahman N. *ATM* and breast cancer susceptibility. Oncogene 2006;25:5906-11.

Allinen M, Launonen V, Laake K, Jansen L, Huusko P, Kääriäinen H, Børresen-Dale AL, Winqvist R. *ATM* mutations in Finnish breast cancer patients. J Med Genet 2002;39:192-6.

Angèle S, Romestaing P, Moullan N, Vuillaume M, Chapot B, Friesen M, Jongmans W, Cox DG, Pisani P, Gerard JP, Hall J. *ATM* haplotypes and cellular response to DNA damage: association with breast cancer risk and clinical radiosensitivity. Cancer Res 2003;63:8717-25.

Angèle S, Treilleux I, Bremond A, Taniere P and Hall J. Altered expression of DNA double-strand break detection and repair proteins in breast carcinomas. Histopathology 2003;43:347-53.

Angèle S, Treilleux I, Taniere P, Martel-Planche G, Vuillaume M, Bailly C, Bremond A, Montesano R, Hall J. Abnormal expression of the *ATM* and *TP53* genes in sporadic breast carcinomas. Clin Cancer Res 2000;6:3536-44.

Antoniou AC, Easton DF. Models of genetic susceptibility to breast cancer. Oncogene 2006;25:5898-905.

Antoniou AC, Pharoah PD, McMullan G, Day NE, Stratton MR, Peto J, Ponder BJ, Easton DF. A comprehensive model for familial breast cancer incorporating *BRCA1*, *BRCA2* and other genes. Br J Cancer 2002;86:76-83.

Antoniou A, Pharoah PD, Narod S, Risch HA, Eyfjord JE, Hopper JL, Loman N, Olsson H, Johannsson O, Borg A, Pasini B, Radice P, Manoukian S, Eccles DM, 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 OP, Thompson D, Evans C, Peto J, Lalloo F, Evans DG, Easton DF. Average risks of breast and ovarian cancer associated with *BRCA1* or *BRCA2* mutations detected in case series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 2003;72:1117-30.

Antoniou AC, Sinilnikova OM, Simard J, Léoné M, Dumont M, Neuhausen SL, Struewing JP, Stoppa-Lyonnet D, Barjhoux L, Hughes DJ, Coupier I, Belotti M, Lasset C, Bonadona V, Bignon YJ; Genetic Modifiers of Cancer Risk in BRCA1/2 Mutation Carriers Study (GEMO), Rebbeck TR, Wagner T, Lynch HT, Domchek SM, Nathanson KL, Garber JE, Weitzel J, Narod SA, Tomlinson G, Olopade OI, Godwin A, Isaacs C, Jakubowska A, Lubinski J, Gronwald J, Górski B, Byrski T, Huzarski T, Peock S, Cook M, Baynes C, Murray A, Rogers M, Daly PA, Dorkins H; Epidemiological Study of BRCA1 and BRCA2 Mutation Carriers (EMBRACE), Schmutzler RK, Versmold B, Engel C, Meindl A, Arnold N, Niederacher D, Deissler H; German Consortium for Hereditary Breast and Ovarian Cancer (GCHBOC), Spurdle AB, Chen X, Waddell N, Cloonan N; Kathleen Cuningham Consortium for Research into Familial Breast Cancer (kConFab), Kirchhoff T, Offit K, Friedman E, Kaufmann B, Laitman Y, Galore G, Rennert G, Lejbkowicz F, Raskin L, Andrulis IL, Ilyushik E, Ozcelik H, Devilee P, Vreeswijk MP, Greene MH, Prindiville SA, Osorio A, Benitez J, Zikan M, Szabo CI, Kilpivaara O, Nevanlinna H, Hamann U, Durocher F, Arason A, Couch FJ, Easton DF, Chenevix-Trench G; Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA). *RAD51* 135G-->C modifies breast cancer risk among *BRCA2* mutation carriers: results from a combined analysis of 19 studies. Am J Hum Genet 2007;81:1186-200.

Assenmacher N, Hopfner KP. MRE11/RAD50/NBS1: complex activities. Chromosoma 2004;113:157-66.

Bai L, Zhu WG. p53: structure, function and therapeutic applications. J Cancer Mol 2006;2:141-53.

Bakkenist CJ, Kastan MB. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. Nature 2003;421:499-506.

Bartek J, Falck J, Lukas J. CHK2 kinase--a busy messenger. Nat Rev Mol Cell Biol 2001;2:877-86.

Bartek J, Lukas J. Mammalian G1- and S-phase checkpoints in response to DNA damage. Curr Opin Cell Biol 2001;13:738-47.

Bartek J, Lukas J, Bartkova J. DNA Damage Response as an Anti-Cancer Barrier: Damage Threshold and the Concept of 'Conditional Haploinsufficiency'. Cell Cycle 2007 Jul 18;6(19) [Epub ahead of print]

Bartkova J, Horejsi Z, Koed K, Krämer A, Tort F, Zieger K, Guldberg P, Sehested M, Nesland JM, Lukas C, Ørntoft T, Lukas J, Bartek J. DNA damage response as a candidate anti-cancer barrier in early human tumorigenesis. Nature 2005a;434:864-70.

Bartkova J, Bakkenist CJ, Rajpert-De Meyts E, Skakkebaek NE, Sehested M, Lukas J, Kastan MB, Bartek J. ATM activation in normal human tissues and testicular cancer. Cell Cycle 2005b;4:838-45.

Bartkova J, Rezaei N, Liontos M, Karakaidos P, Kletsas D, Issaeva N, Vassiliou LV, Kolettas E, Niforou K, Zoumpourlis VC, Takaoka M, Nakagawa H, Tort F, Fugger K, Johansson F, Sehested M, Andersen CL, Dyrskjot L, Orntoft T, Lukas J, Kittas C, Helleday T, Halazonetis TD, Bartek J, Gorgoulis VG. Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. Nature 2006;444:633-7.

Beckman G, Birgander R, Själander A, Saha N, Holmberg PA, Kivelä A, Beckman L. Is p53 polymorphism maintained by natural selection? Hum Hered 1994;44:266-70.

Bekker-Jensen S, Lukas C, Kitagawa R, Melander F, Kastan MB, Bartek J, Lukas J. Spatial organization of the mammalian genome surveillance machinery in response to DNA strand breaks. J Cell Biol 2006;173:195-206.

Bekker-Jensen S, Lukas C, Melander F, Bartek J, Lukas J. Dynamic assembly and sustained retention of 53BP1 at the sites of DNA damage are controlled by Mdc1/NFBD1. J Cell Biol 2005;170:201-11.

Bender CF, Sikes ML, Sullivan R, Huye LE, Le Beau MM, Roth DB, Mirzoeva OK, Oltz EM, Petrini JHJ. Cancer predisposition and hematopoietic failure in Rad$^{s/s}$ mice. Genes & Dev 2002;16:2237-51.

Bergamaschi D, Gasco M, Hiller L, Sullivan A, Syed N, Trigiante G, Yulug I, Merlano M, Numico G, Comino A, Attard M, Reelfs O, Gusterson B, Bell AK, Heath V, Tavassoli M, Farrell PJ, Smith P, Lu X, Crook T. p53 polymorphism influences response in cancer chemotherapy via modulation of p73-dependent apoptosis. Cancer Cell 2003;3:387-402.

Bergamaschi D, Samuels Y, O'Neil NJ, Trigiante G, Crook T, Hsieh JK, O'Connor DJ, Zhong S, Campargue I, Tomlinson ML, Kuwabara PE, Lu X. iASPP oncoprotein is a key inhibitor of p53 conserved from worm to human. Nat Genet 2003;33:162-7.

Bergamaschi D, Samuels Y, Sullivan A, Zvelebil M, Breyssens H, Bisso A, Del Sal G, Syed N, Smith P, Gasco M, Crook T, Lu X. iASPP preferentially binds p53 proline-rich region and modulates apoptotic function of codon 72-polymorphic p53. Nat Genet 2006;38:1133-41.

Berkovich E, Monnat RJ Jr, Kastan MB. Roles of ATM and NBS1 in chromatin structure modulation and DNA double-strand break repair. Nat Cell Biol 2007;9:683-90.

Bernstein JL, Teraoka S, Haile RW, Børresen-Dale AL, Rosenstein BS, Gatti RA, Diep AT, Jansen L, Atencio DP, Olsen JH, Bernstein L, Teitelbaum SL, Thompson WD, Concannon P; WECARE Study Collaborative Group. Designing and implementing quality control for multi-center screening of mutations in the *ATM* gene among women with breast cancer. Hum Mutat 2003;21:542-50.

Bernstein JL, Teraoka S, Southey MC, Jenkins MA, Andrulis IL, Knight JA, John EM, Lapinski R, Wolitzer AL, Whittemore AS, West D, Seminara D, Olson ER, Spurdle AB, Chenevix-Trench G, Giles GG, Hopper JL, Concannon P. Population-based estimates of breast cancer risks associated with *ATM* gene variants c.7271T>G and c.1066-6T>G (IVS10-6T>G) from the Breast Cancer Family Registry. Hum Mutat 2006;27:1122-8.

Birch JM, Hartley AL, Tricker KJ, Prosser J, Condie A, Kelsey AM, Harris M, Jones PH, Binchy A, Crowther D, et al. Prevalence and diversity of constitutional mutations in the p53 gene among 21 Li-Fraumeni families. Cancer Res 1994;54:1298-304.

Bonafè M, Ceccarelli C, Farabegoli F, Santini D, Taffurelli M, Barbi C, Marzi E, Trapassi C, Storci G, Olivieri F, Franceschi C. Retention of the p53 codon 72 arginine allele is associated with a reduction of disease-free and overall survival in arginine/proline heterozygous breast cancer patients. Clin Cancer Res 2003;9:4860-4.

Bond GL, Hu W, Bond EE, Robins H, Lutzker SG, Arva NC, Bargonetti J, Bartel F, Taubert H, Wuerl P, Onel K, Yip L, Hwang SJ, Strong LC, Lozano G, Levine AJ. A single nucleotide polymorphism in the *MDM2* promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. Cell 2004;119:591-602.

Bond GL, Hu W, Levine AJ. MDM2 is a central node in the p53 pathway: 12 years and counting. Curr Cancer Drug Targets 2005;5:3-8.

van den Bosch M, Bree RT, Lowndes NF. The MRN complex: coordinating and mediating the response to broken chromosomes. EMBO Rep 2003;4:844-9.

Bosotti R, Isacchi A, Sonnhammer EL. FAT: a novel domain in PIK-related kinases. Trends Biochem Sci 2000;25:225-7.

Bougeard G, Baert-Desurmont S, Tournier I, Vasseur S, Martin C, Brugieres L, Chompret A, Bressac-de Paillerets B, Stoppa-Lyonnet D, Bonaiti-Pellie C, Frebourg T. Impact of the *MDM2* SNP309 and p53 Arg72Pro polymorphism on age of tumour onset in Li-Fraumeni syndrome. J Med Genet 2006;43:531-3.

Boulton SJ. Cellular functions of the BRCA tumour-suppressor proteins. Biochem Soc Trans 2006;34:633-45.

Brachmann RK, Vidal M, Boeke JD. Dominant-negative p53 mutations selected in yeast hit cancer hot spots. Proc Natl Acad Sci U S A 1996;93:4091-5.

Breast Cancer Association Consortium. Commonly studied single-nucleotide polymorphisms and breast cancer: results from the Breast Cancer Association Consortium. J Natl Cancer Inst 2006;98:1382-96.

The Breast Cancer Linkage Consortium. Cancer risks in *BRCA2* mutation carriers. J Natl Cancer Inst 1999;91:1310-6.

Bretsky P, Haiman CA, Gilad S, Yahalom J, Grossman A, Paglin S, Van Den Berg D, Kolonel LN, Skaliter R, Henderson BE. The relationship between twenty missense *ATM* variants and breast cancer risk: the Multiethnic Cohort. Cancer Epidemiol Biomarkers Prev 2003;12:733-8.

Broeks A, Urbanus JH, Floore AN, Dahler EC, Klijn JG, Rutgers EJ, Devilee P, Russell NS, van Leeuwen FE, van 't Veer LJ. *ATM*-heterozygous germline mutations contribute to breast cancer-susceptibility. Am J Hum Genet 2000;66:494-500.

Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, Kyle S, Meuth M, Curtin NJ, Helleday T. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature 2005;434:913-7.

Buslov KG, Iyevleva AG, Chekmariova EV, Suspitsin EN, Togo AV, Kuligina ESh, Sokolenko AP, Matsko DE, Turkevich EA, Lazareva YR, Chagunava OL, Bit-Sava EM, Semiglazov VF, Devilee P, Cornelisse C, Hanson KP, Imyanitov EN. *NBS1* 657del5 mutation may contribute only to a limited fraction of breast cancer cases in Russia. Int J Cancer 2005;114:585-9.

Buyru N, Tigli H, Dalay N. P53 codon 72 polymorphism in breast cancer. Oncol Rep 2003;10:711-4.

Børresen-Dale AL. *TP53* and breast cancer. Hum Mutat. 2003;21:292-300.

van den Bosch M, Bree RT, Lowndes NF. The MRN complex: coordinating and mediating the response to broken chromosomes. EMBO Rep 2003;4:844-9.

Cantor SB, Bell DW, Ganesan S, Kass EM, Drapkin R, Grossman S, Wahrer DC, Sgroi DC, Lane WS, Haber DA, Livingston DM. BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. Cell 2001;105:149-60.

Cavaciuti E, Lauge A, Janin N, Ossian K, Hall J, Stoppa-Lyonnet D, Andrieu N. Cancer risk according to type and location of *ATM* mutation in ataxia-telangiectasia families. Genes Chromosomes Cancer 2005;42:1-9.

Chen G, Lee EYHP. The product of the *ATM* gene is a 370-kDa nuclear phosphoprotein. J Biol Chem 1996;271:33693-7.

Chenevix-Trench G, Milne RL, Antoniou AC, Couch FJ, Easton DF, Goldgar DE; CIMBA. An international initiative to identify genetic modifiers of cancer risk in *BRCA1* and *BRCA2* mutation carriers: the Consortium of Investigators of Modifiers of *BRCA1* and *BRCA2* (CIMBA). Breast Cancer Res 2007;9:104.

Chenevix-Trench G, Spurdle AB, Gatei M, Kelly H, Marsh A, Chen X, Donn K, Cummings M, Nyholt D, Jenkins MA, Scott C, Pupo GM, Dork T, Bendix R, Kirk J, Tucker K, McCredie MR, Hopper JL, Sambrook J, Mann GJ, Khanna KK. Dominant negative *ATM* mutations in breast cancer families. J Natl Cancer Inst 2002;94:205-15.

Chipuk JE, Green DR. Dissecting p53-dependent apoptosis. Cell Death Differ 2006;13:994-1002.

Chompret A, Brugieres L, Ronsin M, Gardes M, Dessarps-Freichey F, Abel A, Hua D, Ligot L, Dondon MG, Bressac-de Paillerets B, Frebourg T, Lemerle J, Bonaiti-Pellie C, Feunteun J. P53 germline mutations in childhood cancers and cancer risk for carrier individuals. Br J Cancer 2000;82:1932-7.

Cleator S, Heller W, Coombes RC. Triple-negative breast cancer: therapeutic options. Lancet Oncol 2007;8:235-44.

Collaborative Group on Hormonal Factors in Breast Cancer. Familial breast cancer: collaborative reanalysis of individual data from 52 epidemiological studies including 58,209 women with breast cancer and 101,986 women without the disease. Lancet 2001;358:1389-99.

Cortez D, Wang Y, Qin J, Elledge SJ. Requirement of ATM-dependent phosphorylation of brca1 in the DNA damage response to double-strand breaks. Science 1999;286:1162-6.

Cox A, Dunning AM, Garcia-Closas M, Balasubramanian S, Reed MW, Pooley KA, Scollen S, Baynes C, Ponder BA, Chanock S, Lissowska J, Brinton L, Peplonska B, Southey MC, Hopper JL, McCredie MR, Giles GG, Fletcher O, Johnson N, dos Santos Silva I, Gibson L, Bojesen SE, Nordestgaard BG, Axelsson CK, Torres D, Hamann U, Justenhoven C, Brauch H, Chang-Claude J, Kropp S, Risch A, Wang-Gohrke S, Schurmann P, Bogdanova N, Dörk T, Fagerholm R, Aaltonen K, Blomqvist C, Nevanlinna H, Seal S, Renwick A, Stratton MR, Rahman N, Sangrajrang S, Hughes D, Odefrey F, Brennan P, Spurdle AB, Chenevix-Trench G; Kathleen Cunningham Foundation Consortium for Research into Familial Breast Cancer, Beesley J, Mannermaa A, Hartikainen J, Kataja V, Kosma VM, Couch FJ, Olson JE, Goode EL, Broeks A, Schmidt MK, Hogervorst FB, Van't Veer LJ, Kang D, Yoo KY, Noh DY, Ahn SH, Wedren S, Hall P, Low YL, Liu J, Milne RL, Ribas G, Gonzalez-Neira A, Benitez J, Sigurdson AJ, Stredrick DL, Alexander BH, Struewing JP, Pharoah PD, Easton DF; Breast Cancer Association Consortium. A common coding variant in *CASP8* is associated with breast cancer risk. Nat Genet 2007;39:352-8.

Cox DG, Deer D, Guo Q, Tworoger SS, Hankinson SE, Hunter DJ, De Vivo I. The p53 Arg72Pro and *MDM2* -309 polymorphisms and risk of breast cancer in the nurses' health studies. Cancer Causes Control 2007;18:621-5.

Cuatrecasas M, Santamaria G, Velasco M, Camacho E, Hernandez L, Sanchez M, Orrit C, Murcia C, Cardesa A, Campo E, Fernandez PL. *ATM* gene expression is associated with differentiation and angiogenesis in infiltrating breast carcinomas. Histol Histopathol 2006;21:149-56.

Cybulski C, Gorski B, Debniak T, Gliniewicz B, Mierzejewski M, Masojc B, Jakubowska A, Matyjasik J, Zlowocka E, Sikorski A, Narod SA, Lubinski J. *NBS1* is a prostate cancer susceptibility gene. Cancer Res 2004c;64:1215-9.

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 SA, Lubinski J. *CHEK2* is a multiorgan cancer susceptibility gene. Am J Hum Genet 2004a;75:1131-5.

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 SA, Lubinski J. A novel founder *CHEK2* mutation is associated with increased prostate cancer risk. Cancer Res 2004b;64:2677-9.

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 SA, Lubinski J. A large germline deletion in the Chek2 kinase gene is associated with an increased risk of prostate cancer. J Med Genet 2006;43:863-6.

Delia D, Mizutani S, Panigone S, Tagliabue E, Fontanella E, Asada M, Yamada T, Taya Y, Prudente S, Saviozzi S, Frati L, Pierotti MA, Chessa L. ATM protein and p53-serine 15 phosphorylation in ataxia-telangiectasia (AT) patients and at heterozygotes. Br J Cancer 2000;82:1938-45.

Di Micco R, Fumagalli M, Cicalese A, Piccinin S, Gasparini P, Luise C, Schurra C, Garre' M, Nuciforo PG, Bensimon A, Maestro R, Pelicci PG, d'Adda di Fagagna F. Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. Nature 2006;444:638-42.

Ding SL, Sheu LF, Yu JC, Yang TL, Chen BF, Leu FJ, Shen CY. Abnormality of the DNA double-strand-break checkpoint/repair genes, *ATM*, *BRCA1* and *TP53*, in breast cancer is related to tumour grade. Br J Cancer 2004;90:1995-2001.

Dörk T, Bendix R, Bremer M, Rades D, Klopper K, Nicke M, Skawran B, Hector A, Yamini P, Steinmann D, Weise S, Stuhrmann M, Karstens JH. Spectrum of *ATM* gene mutations in a hospital-based series of unselected breast cancer patients. Cancer Res 2001;61:7608-15.

Duffy MJ. Predictive markers in breast and other cancers: a review. Clin Chem 2005;51:494-503.

Dumont P, Leu JI, Della Pietra AC 3rd, George DL, Murphy M. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nat Genet 2003;33:357-365.

Dupré A, Boyer-Chatenet L, Gautier J. Two-step activation of ATM by DNA and the Mre11-Rad50-Nbs1 complex. Nat Struct Mol Biol 2006;13:451-7.

Easton DF. Cancer risks in A-T heterozygotes. Int J Radiat Biol 1994;66:S177-82.

Easton DF, Ford D, Bishop DT. Breast and ovarian cancer incidence in *BRCA1*-mutation carriers. Breast Cancer Linkage Consortium. Am J Hum Genet 1995;56:265–271.

Easton DF, Pooley KA, Dunning AM, Pharoah PD, Thompson D, Ballinger DG, Struewing JP, Morrison J, Field H, Luben R, Wareham N, Ahmed S, Healey CS, Bowman R; SEARCH collaborators, Meyer KB, Haiman CA, Kolonel LK, Henderson BE, Le Marchand L, Brennan P, Sangrajrang S, Gaborieau V, Odefrey F, Shen CY, Wu PE, Wang HC, Eccles D, Evans DG, Peto J, Fletcher O, Johnson N, Seal S, Stratton MR, Rahman N, Chenevix-Trench G, Bojesen SE, Nordestgaard BG, Axelsson CK, Garcia-Closas M, Brinton L, Chanock S, Lissowska J, Peplonska B, Nevanlinna H, Fagerholm R, Eerola H, Kang D, Yoo KY, Noh DY, Ahn SH, Hunter DJ, Hankinson SE, Cox DG, Hall P, Wedren S, Liu J, Low YL, Bogdanova N, Schurmann P, Dork T, Tollenaar RA, Jacobi CE, Devilee P, Klijn JG, Sigurdson AJ, Doody MM, Alexander BH, Zhang J, Cox A, Brock IW, MacPherson G, Reed MW, Couch FJ, Goode EL, Olson JE, Meijers-Heijboer H, van den Ouweland A, Uitterlinden A, Rivadeneira F, Milne RL, Ribas G, Gonzalez-Neira A, Benitez J, Hopper JL, McCredie M, Southey M, Giles GG, Schroen C, Justenhoven C, Brauch H, Hamann U, Ko YD, Spurdle AB, Beesley J, Chen X; kConFab; AOCS Management Group, Mannermaa A, Kosma VM, Kataja V, Hartikainen J, Day NE, Cox DR, Ponder BA. Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 2007;447:1087-93.

Eerola H, Blomqvist C, Pukkala E, Pyrhönen S, Nevanlinna H. Familial breast cancer in Southern Finland: How prevalent are breast cancer families and can we trust the family history reported by the patients? Eur J Cancer 2000;36:1143-8.

Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 1991;19:403-10.

Eng C. Constipation, polyps, or cancer? Let *PTEN* predict your future. Am J Med Genet A 2003;122:315-22.

Erkko H, Xia B, Nikkilä J, Schleutker J, Syrjäkoski K, Mannermaa A, Kallioniemi A, Pylkäs K, Karppinen SM, Rapakko K, Miron A, Sheng Q, Li G, Mattila H, Bell DW, Haber DA, Grip M, Reiman M, Jukkola-Vuorinen A, Mustonen A, Kere J, Aaltonen LA, Kosma VM, Kataja V, Soini Y, Drapkin RI, Livingston DM, Winqvist R. A recurrent mutation in *PALB2* in Finnish cancer families. Nature 2007;446:316-9.

Falck J, Mailand N, Syljuasen RG, Bartek J, Lukas J. The ATM-Chk2-Cdc25A checkpoint pathway guards against radioresistant DNA synthesis. Nature 2001;410:842-7.

Falck J, Petrini JH, Williams BR, Lukas J, Bartek J. The DNA damage-dependent intra-S phase checkpoint is regulated by parallel pathways. Nat Genet 2002;30:290-4.

Fan R, Wu MT, Miller D, Wain JC, Kelsey KT, Wiencke JK, Christiani DC. The p53 codon 72 polymorphism and lung cancer risk. Cancer Epidemiol Biomarkers Prev 2000;9:1037-42.

Farmer G, Bargonetti J, Zhu H, Friedman P, Prywes R, Prives C. Wild-type p53 activates transcription in vitro. Nature 1992;358:83-6.

Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, Santarosa M, Dillon KJ, Hickson I, Knights C, Martin NM, Jackson SP, Smith GC, Ashworth A. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 2005;434:917-21.

Fernandez-Capetillo O, Lee A, Nussenzweig M, Nussenzweig A. H2AX: the histone guardian of the genome. DNA Repair (Amst) 2004;3:959-67.

Fernet M, Moullan N, Lauge A, Stoppa-Lyonnet D, Hall J. Cellular responses to ionising radiation of AT heterozygotes: differences between missense and truncating mutation carriers. Br J Cancer 2004;90:866-73.

Fero ML, Randel E, Gurley KE, Roberts JM, Kemp CJ. The murine gene *p27Kip1* is haplo-insufficient for tumour suppression. Nature 1998;396:177-80.

Fodde R, Smits R. Cancer biology. A matter of dosage. Science 2002;298:761-3.

Ford D, Easton DF, Bishop DT, Narod SA, Goldgar DE. Risks of cancer in *BRCA1*-mutation carriers. Breast Cancer Linkage Consortium. Lancet. 1994;343:692-5.

Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, Bishop DT, Weber B, Lenoir G, Chang-Claude J, Sobol H, Teare MD, Struewing J, Arason A, Scherneck S, Peto J, Rebbeck TR, Tonin P, Neuhausen S, Barkardottir R, Eyfjord J, Lynch H, Ponder BA, Gayther SA, Zelada-Hedman M, and The Breast Cancer Linkage Consortium. Genetic heterogeneity and penetrance analysis of the *BRCA1* and *BRCA2* genes in breast cancer families. Am J Hum Genet 1998;62:676–689.

Foulkes WD, Stefansson IM, Chappuis PO, Begin LR, Goffin JR, Wong N, Trudel M, Akslen LA. Germline *BRCA1* mutations and a basal epithelial phenotype in breast cancer. J Natl Cancer Inst 2003;95:1482–5.

Frebourg T, Barbier N, Yan YX, Garber JE, Dreyfus M, Fraumeni J Jr, Li FP, Friend SH. Germ-line p53 mutations in 15 families with Li-Fraumeni syndrome. Am J Hum Genet 1995;56:608-15.

Ganguly A. An update on conformation sensitive gel electrophoresis. Hum Mutat 2002;19:334-42.

Garber JE, Goldstein AM, Kantor AF, Dreyfus MG, Fraumeni JF Jr, Li FP. Follow-up study of twenty-four families with Li-Fraumeni syndrome. Cancer Res 1991;51:6094-7.

Gasco M, Yulug IG, Crook T. *TP53* mutations in familial breast cancer: functional aspects. Hum Mutat 2003;21:301-6.

Gatti RA, Tward A, Concannon P. Cancer risk in *ATM* heterozygotes: a model of phenotypic and mechanistic differences between missense and truncating mutations. Mol Genet Metab 1999;68:419-23.

Goode EL, Dunning AM, Kuschel B, Healey CS, Day NE, Ponder BA, Easton DF, Pharoah PP. Effect of germ-line genetic variation on breast cancer survival in a population-based study. Cancer Res 2002;62:3052-7.

Gorgoulis VG, Vassiliou LV, Karakaidos P, Zacharatos P, Kotsinas A, Liloglou T, Venere M, Ditullio RA Jr, Kastrinakis NG, Levy B, Kletsas D, Yoneta A, Herlyn M, Kittas C, Halazonetis TD. Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature 2005;434:907-13.

Gorski B, Debniak T, Masojc B, Mierzejewski M, Medrek K, Cybulski C, Jakubowska A, Kurzawski G, Chosia M, Scott R, Lubinski J. Germline 657del5 mutation in the *NBS1* gene in breast cancer patients. Int J Cancer 2003;106:379-81.

Han W, Kang D, Park IA, Kim SW, Bae JY, Chung KW, Noh DY. Associations between breast cancer susceptibility gene polymorphisms and clinicopathological features. Clin Cancer Res 2004;10:124-30.

Hartwell LH, Weinert TA. Checkpoints: controls that ensure the order of cell cycle events. Science. 1989;246:629-34.

Hearle N, Schumacher V, Menko FH, Olschwang S, Boardman LA, Gille JJ, Keller JJ, Westerman AM, Scott RJ, Lim W, Trimbath JD, Giardiello FM, Gruber SB, Offerhaus GJ, de Rooij FW, Wilson JH, Hansmann A, Moslein G, Royer-Pokora B, Vogel T, Phillips RK, Spigelman AD, Houlston RS. Frequency and spectrum of cancers in the Peutz-Jeghers syndrome. Clin Cancer Res 2006;12:3209-15.

Hebbring SJ, Fredriksson H, White KA, Maier C, Ewing C, McDonnell SK, Jacobsen SJ, Cerhan J, Schaid DJ, Ikonen T, Autio V, Tammela TL, Herkommer K, Paiss T, Vogel W, Gielzak M, Sauvageot J, Schleutker J, Cooney KA, Isaacs W, Thibodeau SN. Role of the Nijmegen breakage syndrome 1 gene in familial and sporadic prostate cancer. Cancer Epidemiol Biomarkers Prev 2006;15:935-8.

Hedenfalk I, Duggan D, Chen Y, Radmacher M, Bittner M, Simon R, Meltzer P, Gusterson B, Esteller M, Kallioniemi OP, Wilfond B, Borg A, Trent J, Raffeld M, Yakhini Z, Ben-Dor A, Dougherty E, Kononen J, Bubendorf L, Fehrle W, Pittaluga S, Gruvberger S, Loman N, Johannsson O, Olsson H, Sauter G. Gene-expression profiles in hereditary breast cancer. N Engl J Med 2001;344:539-48.

Heikkinen K, Karppinen SM, Soini Y, Mäkinen M, Winqvist R. Mutation screening of Mre11 complex genes: indication of *RAD50* involvement in breast and ovarian cancer susceptibility. J Med Genet 2003;40:e131.

Heikkinen K, Rapakko K, Karppinen SM, Erkko H, Knuutila S, Lundan T, Mannermaa A, Børresen-Dale AL, Borg A, Barkardottir RB, Petrini J, Winqvist R. *RAD50* and *NBS1* are breast cancer susceptibility genes associated with genomic instability. Carcinogenesis 2006;27:1593-9.

Heikkinen K, Rapakko K, Karppinen SM, Erkko H, Nieminen P, Winqvist R. Association of common *ATM* polymorphism with bilateral breast cancer. Int J Cancer 2005;116:69-72.

Hemminki A, Markie D, Tomlinson I, Avizienyte E, Roth S, Loukola A, Bignell G, Warren W, Aminoff M, Höglund P, Järvinen H, Kristo P, Pelin K, Ridanpää M, Salovaara R, Toro T, Bodmer W, Olschwang S, Olsen AS, Stratton MR, de la Chapelle A, Aaltonen LA. A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. Nature 1998;391:184-7.

Hong Y, Miao X, Zhang X, Ding F, Luo A, Guo Y, Tan W, Liu Z, Lin D. The role of P53 and *MDM2* polymorphisms in the risk of esophageal squamous cell carcinoma. Cancer Res 2005;65:9582-7.

Honrado E, Benitez J, Palacios J. The molecular pathology of hereditary breast cancer: genetic testing and therapeutic implications. Mod Pathol 2005a;18:1305-20.

Honrado E, Benitez J, Palacios J. Histopathology of *BRCA1*- and *BRCA2*-associated breast cancer. Crit Rev Oncol Hematol 2006a;59:27-39.

Honrado E, Osorio A, Palacios J, Benitez J. Pathology and gene expression of hereditary breast tumors associated with *BRCA1*, *BRCA2* and *CHEK2* gene mutations. Oncogene 2006b;25:5837-45.

Honrado E, Osorio A, Palacios J, Milne RL, Sanchez L, Diez O, Cazorla A, Syrjäkoski K, Huntsman D, Heikkilä P, Lerma E, Kallioniemi A, Rivas C, Foulkes WD, Nevanlinna H, Benitez J. Immunohistochemical expression of DNA repair proteins in familial breast cancer differentiate BRCA2-associated tumors. J Clin Oncol 2005b;23:7503-11.

Hopfner KP, Craig L, Moncalian G, Zinkel RA, Usui T, Owen BA, Karcher A, Henderson B, Bodmer JL, McMurray CT, Carney JP, Petrini JH, Tainer JA. The Rad50 zinc-hook is a structure joining Mre11 complexes in DNA recombination and repair. Nature 2002;418:562-6.

Hopfner KP, Karcher A, Craig L, Woo TT, Carney JP, Tainer JA. Structural biochemistry and interaction architecture of the DNA double-strand break repair Mre11 nuclease and Rad50-ATPase. Cell 2001;105:473-85.

Hopfner KP, Karcher A, Shin DS, Craig L, Arthur LM, Carney JP, Tainer JA. Structural biology of Rad50 ATPase: ATP-driven conformational control in DNA double-strand break repair and the ABC-ATPase superfamily. Cell 2000;101:789-800.

Howlett NG, Taniguchi T, Olson S, Cox B, Waisfisz Q, De Die-Smulders C, Persky N, Grompe M, Joenje H, Pals G, Ikeda H, Fox EA, D'Andrea AD. Biallelic inactivation of *BRCA2* in Fanconi anemia. Science 2002;297:606-9.

Huang XE, Hamajima N, Katsuda N, Matsuo K, Hirose K, Mizutani M, Iwata H, Miura S, Xiang J, Tokudome S, Tajima K. Association of p53 codon Arg72Pro and p73 G4C14-to-A4T14 at exon 2 genetic polymorphisms with the risk of Japanese breast cancer. Breast Cancer 2003;10:307-11.

Huang SP, Wu WJ, Chang WS, Wu MT, Chen YY, Chen YJ, Yu CC, Wu TT, Lee YH, Huang JK, Huang CH. p53 Codon 72 and p21 codon 31 polymorphisms in prostate cancer. Cancer Epidemiol Biomarkers Prev 2004;13:2217-24.

Jekimovs CR, Chen X, Arnold J, Gatei M, Richard DJ, Spurdle AB, Khanna KK, Chenevix-Trench G; kConFab Investigators. Low frequency of *CHEK2* 1100delC allele in Australian multiple-case breast cancer families: functional analysis in heterozygous individuals. Br J Cancer 2005;92:784-90.

Jones JS, Chi X, Gu X, Lynch PM, Amos CI, Frazier ML. p53 polymorphism and age of onset of hereditary nonpolyposis colorectal cancer in a Caucasian population. Clin Cancer Res 2004;10:5845-9.

Kadouri L, Kote-Jarai Z, Hubert A, Durocher F, Abeliovich D, Glaser B, Hamburger T, Eeles RA, Peretz T. A single-nucleotide polymorphism in the *RAD51* gene modifies breast cancer risk in *BRCA2* carriers, but not in *BRCA1* carriers or noncarriers. Br J Cancer 2004;90:2002-5.

Kairouz R, Clarke RA, Marr PJ, Watters D, Lavin MF, Kearsley JH, Lee CS. ATM protein synthesis patterns in sporadic breast cancer. Mol Pathol 1999;52:252-6.

Kanka C, Brozek I, Skalska B, Siemiatkowska A, Limon J. Germline *NBS1* mutations in families with aggregation of Breast and/or ovarian cancer from north-east Poland. Anticancer Res 2007;27:3015-8.

Kastan MB, Bartek J. Cell-cycle checkpoints and cancer. Nature 2004;432:316-23.

Kastan MB, Berkovich E. p53: a two-faced cancer gene. Nat Cell Biol 2007;9:489-91.

Kastan MB, Lim DS. The many substrates and functions of ATM. Nat Rev Mol Cell Biol 2000;1:179-86.

Kern SE, Kinzler KW, Bruskin A, Jarosz D, Friedman P, Prives C, Vogelstein B. Identification of p53 as a sequence-specific DNA-binding protein. Science 1991;252:1708-11.

Khanna KK, Chenevix-Trench G. *ATM* and genome maintenance: defining its role in breast cancer susceptibility. J Mammary Gland Biol Neoplasia 2004;9:247-62.

Khanna KK, Jackson SP. DNA double-strand breaks: signaling, repair and the cancer connection. Nat Genet 2001;27:247-54.

Kilpivaara O, Alhopuro P, Vahteristo P, Aaltonen LA, Nevanlinna H. *CHEK2* I157T associates with familial and sporadic colorectal cancer. J Med Genet 2006;43:e34.

Kilpivaara O, Bartkova J, Eerola H, Syrjäkoski K, Vahteristo P, Lukas J, Blomqvist C, Holli K, Heikkilä P, Sauter G, Kallioniemi OP, Bartek J, Nevanlinna H. Correlation of CHEK2 protein expression and c.1100delC mutation status with tumor characteristics among unselected breast cancer patients. Int J Cancer 2005;113:575-80.

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 OP, Bartek J, Nevanlinna H. *CHEK2* variant I157T may be associated with increased breast cancer risk. Int J Cancer 2004;111:543-7.

Kinzler KW, Vogelstein B. Cancer-susceptibility genes. Gatekeepers and caretakers. Nature 1997;386:761, 763.

Kinzler KW, Vogelstein B. Landscaping the cancer terrain. Science 1998;280:1036-7.

Kitagawa R, Bakkenist CJ, McKinnon PJ, Kastan MB. Phosphorylation of SMC1 is a critical downstream event in the ATM-NBS1-BRCA1 pathway. Genes Dev 2004;18:1423-38.

Knudson AG Jr. Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci U S A 1971;68:820-3.

Ko LJ, Prives C. p53: puzzle and paradigm. Genes Dev 1996;10:1054-72.

Koushik A, Platt RW, Franco EL. p53 codon 72 polymorphism and cervical neoplasia: a meta-analysis review. Cancer Epidemiol Biomarkers Prev 2004;13:11-22.

Koushik A, Tranah GJ, Ma J, Stampfer MJ, Sesso HD, Fuchs CS, Giovannucci EL, Hunter DJ. p53 Arg72Pro polymorphism and risk of colorectal adenoma and cancer. Int J Cancer 2006;119:1863-8.

Kwabi-Addo B, Giri D, Schmidt K, Podsypanina K, Parsons R, Greenberg N, Ittmann M. Haploinsufficiency of the Pten tumor suppressor gene promotes prostate cancer progression. Proc Natl Acad Sci U S A 2001;98:11563-8.

Kyndi M, Alsner J, Hansen LL, Sorensen FB, Overgaard J. LOH rather than genotypes of TP53 codon 72 is associated with disease-free survival in primary breast cancer. Acta Oncol 2006;45:602-9.

Laake K, Jansen L, Hahnemann JM, Brøndum-Nielsen K, Lönnqvist T, Kääriäinen H, Sankila R, Lähdesmäki A, Hammarström L, Yuen J, Tretli S, Heiberg A, Olsen JH, Tucker M, Kleinerman R, Børresen-Dale AL. Characterization of *ATM* mutations in 41 Nordic families with ataxia telangiectasia. Hum Mutat 2000;16:232-46.

Lacroix M, Toillon RA, Leclercq G. p53 and breast cancer, an update. Endocr Relat Cancer 2006;13:293-325.

Lam MH, Liu Q, Elledge SJ, Rosen JM. Chk1 is haploinsufficient for multiple functions critical to tumor suppression. Cancer Cell 2004;6:45-59.

Lamb P, Crawford L. Characterization of the human p53 gene. Mol Cell Biol 1986;6:1379-85.

Lane DP. Cancer. p53, guardian of the genome. Nature 1992;358:15-6.

Langerød A, Bukholm IR, Bregård A, Lønning PE, Andersen TI, Rognum TO, Meling GI, Lothe RA, Børresen-Dale AL. The TP53 codon 72 polymorphism may affect the function of *TP53* mutations in breast carcinomas but not in colorectal carcinomas. Cancer Epidemiol Biomarkers Prev 2002;11:1684-8.

Langholz B, Bernstein JL, Bernstein L, Olsen JH, Børresen-Dale AL, Rosenstein BS, Gatti RA, Concannon P. On the proposed association of the *ATM* variants 5557G>A and IVS38-8T>C and bilateral breast cancer. Int J Cancer 2006;119:724-5.

Lassus H, Leminen A, Väyrynen A, Cheng G, Gustafsson JA, Isola J, Bützow R. ERBB2 amplification is superior to protein expression status in predicting patient outcome in serous ovarian carcinoma. Gynecol Oncol 2004;92:31-9.

Lavin MF. The Mre11 complex and ATM: a two-way functional interaction in recognising and signaling DNA double strand breaks. DNA Repair (Amst) 2004;3:1515-20.

Lavin MF, Delia D, Chessa L. ATM and the DNA damage response. Workshop on ataxia-telangiectasia and related syndromes. EMBO Rep 2006;7:154-60.

Lavin MF, Kozlov S. ATM activation and DNA damage response. Cell Cycle 2007;6:931-42.

Lavin MF, Scott S, Gueven N, Kozlov S, Peng C, Chen P. Functional consequences of sequence alterations in the *ATM* gene. DNA Repair (Amst) 2004;3:1197-205.

Lavin MF, Shiloh Y. The genetic defect in ataxia-telangiectasia. Annu Rev Immunol 1997;15:177-202.

Lee JH, Paull TT. Direct activation of the ATM protein kinase by the Mre11/Rad50/Nbs1 complex. Science 2004;304:93-6.

Lee JH, Paull TT. ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex. Science 2005;308:551-4.

Levine AJ. p53, the cellular gatekeeper for growth and division. Cell 1997;88:323-31.

Levran O, Attwooll C, Henry RT, Milton KL, Neveling K, Rio P, Batish SD, Kalb R, Velleuer E, Barral S, Ott J, Petrini J, Schindler D, Hanenberg H, Auerbach AD. The BRCA1-interacting helicase BRIP1 is deficient in Fanconi anemia. Nat Genet 2005;37:931-3.

Levy-Lahad E, Lahad A, Eisenberg S, Dagan E, Paperna T, Kasinetz L, Catane R, Kaufman B, Beller U, Renbaum P, Gershoni-Baruch R. A single nucleotide polymorphism in the *RAD51* gene modifies cancer risk in *BRCA2* but not *BRCA1* carriers. Proc Natl Acad Sci U S A 2001;98:3232-6.

Li FP. The 4th American Cancer Society Award for Research Excellence in Cancer Epidemiology and Prevention. Phenotypes, Genotypes, and Interventions for Hereditary Cancers. Cancer Epidemiol Biomarkers Prev 1995;4:579-82.

Li FP, Fraumeni JF Jr. Rhabdomyosarcoma in children: epidemiologic study and identification of a familial cancer syndrome. J Natl Cancer Inst 1969;43:1365-73.

Liaw D, Marsh DJ, Li J, Dahia PL, Wang SI, Zheng Z, Bose S, Call KM, Tsou HC, Peacocke M, Eng C, Parsons R. Germline mutations of the *PTEN* gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nat Genet 1997;16:64-7.

Lieber MR, Ma Y, Pannicke U, Schwarz K. The mechanism of vertebrate nonhomologous DNA end joining and its role in V(D)J recombination. DNA Repair (Amst) 2004;3:817-26.

Lieber MR, Yu K, Raghavan SC. Roles of nonhomologous DNA end joining, V(D)J recombination, and class switch recombination in chromosomal translocations. DNA Repair (Amst) 2006;5:1234-45.

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

Lindeman GJ, Hiew M, Visvader JE, Leary J, Field M, Gaff CL, Gardner RJ, Trainor K, Cheetham G, Suthers G, Kirk J. Frequency of the *ATM* IVS10-6T-->G variant in Australian multiple-case breast cancer families. Breast Cancer Res 2004;6:R401-7.

Litman R, Peng M, Jin Z, Zhang F, Zhang J, Powell S, Andreassen PR, Cantor SB. BACH1 is critical for homologous recombination and appears to be the Fanconi anemia gene product FANCJ. Cancer Cell 2005;8:255-65.

Lou Z, Minter-Dykhouse K, Franco S, Gostissa M, Rivera MA, Celeste A, Manis JP, van Deursen J, Nussenzweig A, Paull TT, Alt FW, Chen J. MDC1 maintains genomic stability by participating in the amplification of ATM-dependent DNA damage signals. Mol Cell 2006;21:187-200.

Lu J, Wei Q, Bondy ML, Li D, Brewster A, Shete S, Yu TK, Sahin A, Meric-Bernstam F, Hunt KK, Singletary SE, Ross MI, Wang LE. Polymorphisms and haplotypes of the *NBS1* gene are associated with risk of sporadic breast cancer in non-Hispanic white women <or=55 years. Carcinogenesis 2006;27:2209-16.

Lukas C, Bartkova J, Latella L, Falck J, Mailand N, Schroeder T, Sehested M, Lukas J, Bartek J. DNA damage-activated kinase Chk2 is independent of proliferation or differentiation yet correlates with tissue biology. Cancer Res 2001;61:4990-3.

Lukas C, Melander F, Stucki M, Falck J, Bekker-Jensen S, Goldberg M, Lerenthal Y, Jackson SP, Bartek J, Lukas J. Mdc1 couples DNA double-strand break recognition by Nbs1 with its H2AX-dependent chromatin retention. EMBO J 2004;23:2674-83.

Luo G, Yao MS, Bender CF, Bladl AR, Bradley A, Petrini JHJ. Disruption of mRad50 causes ES cell lethality, abnormal embryonic development and sensitivity to ionizing radiation. Proc Natl Acad Sci USA 1999;96:7376-81.

Malkin D, Li FP, Strong LC, Fraumeni JF Jr, Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 1990;250:1233-8.

Marroni F, Aretini P, D'Andrea E, Caligo MA, Cortesi L, Viel A, Ricevuto E, Montagna M, Cipollini G, Federico M, Santarosa M, Marchetti P, Bailey-Wilson JE, Bevilacqua G, Parmigiani G, Presciuttini S. Penetrances of breast and ovarian cancer in a large series of families tested for *BRCA1/2* mutations. Eur J Hum Genet 2004;12:899–906.

Martin AM, Kanetsky PA, Amirimani B, Colligon TA, Athanasiadis G, Shih HA, Gerrero MR, Calzone K, Rebbeck TR, Weber BL. Germline TP53 mutations in breast cancer families with multiple primary cancers: is *TP53* a modifier of *BRCA1*? J Med Genet 2003;40:e34.

McCabe N, Turner NC, Lord CJ, Kluzek K, Bialkowska A, Swift S, Giavara S, O'connor MJ, Tutt AN, Zdzienicka MZ, Smith GC, Ashworth A. Deficiency in the Repair of DNA Damage by Homologous Recombination and Sensitivity to Poly(ADP-Ribose) Polymerase Inhibition. Cancer Res 2006;66:8109-15.

Mechanic LE, Bowman ED, Welsh JA, Khan MA, Hagiwara N, Enewold L, Shields PG, Burdette L, Chanock S, Harris CC. Common genetic variation in *TP53* is associated with lung cancer risk and prognosis in African Americans and somatic mutations in lung tumors. Cancer Epidemiol Biomarkers Prev 2007;16:214-22.

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 DG, Houlston R, Murday V, Narod S, Peretz T, Peto J, Phelan C, Zhang HX, Szabo C, Devilee P, Goldgar D, Futreal PA, Nathanson KL, Weber B, Rahman N, Stratton MR; *CHEK2*-Breast Cancer Consortium. Low-penetrance susceptibility to breast cancer due to *CHEK2*(*)1100delC in noncarriers of *BRCA1* or *BRCA2* mutations. Nat Genet 2002;31:55-9.

Menard S, Tagliabue E, Campiglio M, Pupa SM. Role of *HER2* gene overexpression in breast carcinoma. J Cell Physiol 2000;182:150-62.

Meyn MS. Ataxia-telangiectasia, cancer and the pathobiology of the *ATM* gene. Clin Genet 1999;55:289-304.

Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, et al. A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*. Science 1994;266:66-71.

Miyashita T, Reed JC. Tumor suppressor p53 is a direct transcriptional activator of the human *bax* gene. Cell 1995;80:293-9.

Mochan TA, Venere M, DiTullio RA Jr, Halazonetis TD. 53BP1 and NFBD1/MDC1-Nbs1 function in parallel interacting pathways activating ataxia-telangiectasia mutated (ATM) in response to DNA damage. Cancer Res 2003;63:8586-91.

Mochan TA, Venere M, DiTullio RA Jr, Halazonetis TD. 53BP1, an activator of ATM in response to DNA damage. DNA Repair (Amst) 2004;3:945-52.

Molland JG, Donnellan M, Janu NC, Carmalt HL, Kennedy CW, Gillett DJ. Infiltrating lobular carcinoma –a comparison of diagnosis, management and outcome with infiltrating duct carcinoma. Breast 2004;13:389-96.

Moncalian G, Lengsfeld B, Bhaskara V, Hopfner KP, Karcher A, Alden E, Tainer JA, Paull TT. The rad50 signature motif: essential to ATP binding and biological function. J Mol Biol 2004;335:937-51.

Moroni MC, Hickman ES, Lazzerini Denchi E, Caprara G, Colli E, Cecconi F, Müller H, Helin K. Apaf-1 is a transcriptional target for E2F and p53. Nat Cell Biol 2001;3:552-8.

Muppidi JR, Tschopp J, Siegel RM. Life and death decisions: secondary complexes and lipid rafts in TNF receptor family signal transduction. Immunity 2004;21:461-5.

Nakano K, Vousden KH. PUMA, a novel proapoptotic gene, is induced by p53. Mol Cell 2001;7:683-94.

Nevanlinna H, Bartek J. The *CHEK2* gene and inherited breast cancer susceptibility. Oncogene 2006;25:5912-9.

Ng PC, Henikoff S. Accounting for human polymorphisms predicted to affect protein function. Genome Res 2002;12:436-46.

Noma C, Miyoshi Y, Taguchi T, Tamaki Y, Noguchi S. Association of p53 genetic polymorphism (Arg72Pro) with estrogen receptor positive breast cancer risk in Japanese women. Cancer Lett 2004;210:197-203.

Norberg T, Lennerstrand J, Inganäs M, Bergh J. Comparison between p53 protein measurements using the luminometric immunoassay and immunohistochemistry with detection of p53 gene mutations using cDNA sequencing in human breast tumors. Int J Cancer 1998;79:376-83.

Oda E, Ohki R, Murasawa H, Nemoto J, Shibue T, Yamashita T, Tokino T, Taniguchi T, Tanaka N. Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. Science 2000;288:1053-8.

Oda K, Arakawa H, Tanaka T, Matsuda K, Tanikawa C, Mori T, Nishimori H, Tamai K, Tokino T, Nakamura Y, Taya Y. p53AIP1, a potential mediator of p53-dependent apoptosis, and its regulation by Ser-46-phosphorylated p53. Cell 2000;102:849-62.

Ohayon T, Gershoni-Baruch R, Papa MZ, Distelman Menachem T, Eisenberg Barzilai S, Friedman E. The R72P P53 mutation is associated with familial breast cancer in Jewish women. Br J Cancer 2005;92:1144-8.

Oldenburg RA, Meijers-Heijboer H, Cornelisse CJ, Devilee P. Genetic susceptibility for breast cancer: how many more genes to be found? Crit Rev Oncol Hematol 2007;63:125-49.

Olivier M, Eeles R, Hollstein M, Khan MA, Harris CC, Hainaut P. The IARC *TP53* database: new online mutation analysis and recommendations to users. Hum Mutat 2002;19:607-14.

Olivier M, Langerød A, Carrieri P, Bergh J, Klaar S, Eyfjord J, Theillet C, Rodriguez C, Lidereau R, Bieche I, Varley J, Bignon Y, Uhrhammer N, Winqvist R, Jukkola-Vuorinen A, Niederacher D, Kato S, Ishioka C, Hainaut P, Børresen-Dale AL. The clinical value of somatic *TP53* gene mutations in 1,794 patients with breast cancer. Clin Cancer Res 2006;12:1157-67.

Olsen JH, Hahnemann JM, Børresen-Dale AL, Brøndum-Nielsen K, Hammarström L, Kleinerman R, Kääriäinen H, Lönnqvist T, Sankila R, Seersholm N, Tretli S, Yuen J, Boice JD Jr, Tucker M. Cancer in patients with ataxia-telangiectasia and in their relatives in the nordic countries. J Natl Cancer Inst 2001;93:121-7.

Olsen JH, Hahnemann JM, Børresen-Dale AL, Tretli S, Kleinerman R, Sankila R, Hammarström L, Robsahm TE, Kääriäinen H, Bregard A, Brøndum-Nielsen K, Yuen J, Tucker M. Breast and other cancers in 1445 blood relatives of 75 Nordic patients with ataxia telangiectasia. Br J Cancer 2005;93:260-5.

Osorio A, Martinez-Delgado B, Pollan M, Cuadros M, Urioste M, Torrenteras C, Melchor L, Diez O, De La Hoya M, Velasco E, Gonzalez-Sarmiento R, Caldes T, Alonso C, Benitez J. A haplotype containing the p53 polymorphisms Ins16bp and Arg72Pro modifies cancer risk in *BRCA2* mutation carriers. Hum Mutat 2006;27:242-8.

Palacios J, Honrado E, Osorio A, Diez O, Rivas C, Benitez J. Re: germline *BRCA1* mutations and a basal epithelial phenotype in breast cancer. J Natl Cancer Inst 2004;96:712–4.

Parkin DM. International variation. Oncogene 2004;23:6329-40.

Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74-108.

Paull TT, Lee JH. The Mre11/Rad50/Nbs1 complex and its role as a DNA double-strand break sensor for ATM. Cell Cycle 2005;4:737-40.

Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lønning PE, Børresen-Dale AL, Brown PO, Botstein D. Molecular portraits of human breast tumours. Nature 2000;406:747-52.

Petitjean A, Achatz MI, Børresen-Dale AL, Hainaut P, Olivier M. *TP53* mutations in human cancers: functional selection and impact on cancer prognosis and outcomes. 1: Oncogene 2007;26:2157-65.

Pharoah PD, Antoniou A, Bobrow M, Zimmern RL, Easton DF, Ponder BA. Polygenic susceptibility to breast cancer and implications for prevention. Nat Genet 2002;31:33-6.

Pharoah PD, Day NE, Caldas C. Somatic mutations in the p53 gene and prognosis in breast cancer: a meta-analysis. Br J Cancer 1999;80:1968-73.

Pim D, Banks L. p53 polymorphic variants at codon 72 exert different effects on cell cycle progression. Int J Cancer 2004;108:196-9.

Powell SN, Kachnic LA. Roles of BRCA1 and BRCA2 in homologous recombination, DNA replication fidelity and the cellular response to ionizing radiation. Oncogene 2003;22:5784-91.

Rahman N, Seal S, Thompson D, Kelly P, Renwick A, Elliott A, Reid S, Spanova K, Barfoot R, Chagtai T, Jayatilake H, McGuffog L, Hanks S, Evans DG, Eccles D; Breast Cancer Susceptibility Collaboration (UK), Easton DF, Stratton MR. *PALB2*, which encodes a *BRCA2*-interacting protein, is a breast cancer susceptibility gene. Nat Genet 2007;39:165-7.

Ramensky V, Bork P, Sunyaev S. Human non-synonymous SNPs: server and survey. Nucleic Acids Res 2002;30:3894-900.

Reid S, Schindler D, Hanenberg H, Barker K, Hanks S, Kalb R, Neveling K, Kelly P, Seal S, Freund M, Wurm M, Batish SD, Lach FP, Yetgin S, Neitzel H, Ariffin H, Tischkowitz M, Mathew CG, Auerbach AD, Rahman N. Biallelic mutations in *PALB2* cause Fanconi anemia subtype FA-N and predispose to childhood cancer. Nat Genet 2007;39:162-4.

Renwick A, Thompson D, Seal S, Kelly P, Chagtai T, Ahmed M, North B, Jayatilake H, Barfoot R, Spanova K, McGuffog L, Evans DG, Eccles D; Breast Cancer Susceptibility Collaboration (UK); Easton DF, Stratton MR, Rahman N. *ATM* mutations that cause ataxia-telangiectasia are breast cancer susceptibility alleles. Nat Genet 2006;38:873-5.

Risch HA, McLaughlin JR, Cole DE, Rosen B, Bradley L, Kwan E, Jack E, Vesprini DJ, Kuperstein G, Abrahamson JL, Fan I, Wong B, Narod SA. Prevalence and penetrance of germline *BRCA1* and *BRCA2* mutations in a population series of 649 women with ovarian cancer. Am J Hum Genet 2001;68:700-10.

Ruijs MW, Schmidt MK, Nevanlinna H, Tommiska J, Aittomäki K, Pruntel R, Verhoef S, Van't Veer LJ. The single-nucleotide polymorphism 309 in the *MDM2* gene contributes to the Li-Fraumeni syndrome and related phenotypes. Eur J Hum Genet 2007;15:110-4.

Sainsbury JR, Anderson TJ, Morgan DA. ABC of breast diseases: breast cancer. BMJ 2000;321:745-50.

Sakamuro D, Sabbatini P, White E, Prendergast GC. The polyproline region of p53 is required to activate apoptosis but not growth arrest. Oncogene 1997;15:887-98.

Sarantaus L, Huusko P, Eerola H, Launonen V, Vehmanen P, Rapakko K, Gillanders E, Syrjäkoski K, Kainu T, Vahteristo P, Krahe R, Pääkkönen K, Hartikainen J, Blomqvist C, Löppönen T, Holli K, Ryynänen M, Bützow R, Borg Å, Wasteson Arver B, Holmberg E, Mannermaa A, Kere J, Kallioniemi OP, Winqvist R, Nevanlinna H. Multiple founder effects and geographical clustering of *BRCA1* and *BRCA2* families in Finland. Eur J Hum Genet 2000;8:757-63.

Savitsky K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaite L, Tagle DA, Smith S, Uziel T, Sfez S. A single ataxia telangiectasia gene with a product similar to PI-3 kinase. Science 1995;268:1749-53.

Schmidt MK, Reincke S, Broeks A, Braaf LM, Hogervorst FB, Tollenaar RAEM, Johnson N, Fletcher O, Peto J, Tommiska J, Blomqvist C, Nevanlinna H, Gregory K, Dunning A, Pharoah P, Easton D, Dörk T, Van 't Veer LJ on behalf of the Breast Cancer Association Consortium. Do MDM2 SNP309 and TP53 R72P, two functional single nucleotide polymorphisms, interact in breast cancer development? Analyses in a large pooled series of cases and controls from the Breast Cancer Association Consortium. Cancer Res. 2007;67:9584-90.

Schneider-Stock R, Mawrin C, Motsch C, Boltze C, Peters B, Hartig R, Buhtz P, Giers A, Rohrbeck A, Freigang B, Roessner A. Retention of the arginine allele in codon 72 of the p53 gene correlates with poor apoptosis in head and neck cancer. Am J Pathol 2004;164:1233-41.

Seal S, Thompson D, Renwick A, Elliott A, Kelly P, Barfoot R, Chagtai T, Jayatilake H, Ahmed M, Spanova K, North B, McGuffog L, Evans DG, Eccles D; Breast Cancer Susceptibility Collaboration (UK), Easton DF, Stratton MR, Rahman N. Truncating mutations in the Fanconi anemia J gene *BRIP1* are low-penetrance breast cancer susceptibility alleles. Nat Genet 2006;38:1239-41.

Seppälä EH, Ikonen T, Mononen N, Autio V, Rokman A, Matikainen MP, Tammela TL, Schleutker J. *CHEK2* variants associate with hereditary prostate cancer. Br J Cancer 2003;89:1966-70.

Shiloh Y. ATM and related protein kinases: safeguarding genome integrity. Nature Rev Cancer 2003;3:155-69.

Shiloh Y. The ATM-mediated DNA-damage response: taking shape. Trends Biochem Sci 2006;31:402-10.

Siddique MM, Balram C, Fiszer-Maliszewska L, Aggarwal A, Tan A, Tan P, Soo KC, Sabapathy K. Evidence for selective expression of the p53 codon 72 polymorphs: implications in cancer development. Cancer Epidemiol Biomarkers Prev 2005;14:2245-52.

Siddique M, Sabapathy K. Trp53-dependent DNA-repair is affected by the codon 72 polymorphism. Oncogene 2006;25:3489-500.

Själander A, Birgander R, Hallmans G, Cajander S, Lenner P, Athlin L, Beckman G, Beckman L. p53 polymorphisms and haplotypes in breast cancer. Carcinogenesis 1996;17:1313-6.

Sjögren S, Inganäs M, Norberg T, Lindgren A, Nordgren H, Holmberg L, Bergh J. The p53 gene in breast cancer: prognostic value of complementary DNA sequencing versus immunohistochemistry. J Natl Cancer Inst 1996;88:173-82.

Sodha N, Mantoni TS, Tavtigian SV, Eeles R, Garrett MD. Rare germ line CHEK2 variants identified in breast cancer families encode proteins that show impaired activation. Cancer Res 2006;66:8966-70.

Sommer SS, Jiang Z, Feng J, Buzin CH, Zheng J, Longmate J, Jung M, Moulds J, Dritschilo A. ATM missense mutations are frequent in patients with breast cancer. Cancer Genet Cytogenet 2003;145:115-20.

Song H, Hollstein M, Xu Y. p53 gain-of-function cancer mutants induce genetic instability by inactivating ATM. Nat Cell Biol 2007;9:573-80.

Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Eystein Lønning P, Børresen-Dale AL. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001;98:10869-74.

Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, Deng S, Johnsen H, Pesich R, Geisler S, Demeter J, Perou CM, Lønning PE, Brown PO, Børresen-Dale AL, Botstein D. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci USA 2003;100:8418–23.

Sotamaa K, Liyanarachchi S, Mecklin JP, Järvinen H, Aaltonen LA, Peltomäki P, de la Chapelle A. p53 codon 72 and *MDM2* SNP309 polymorphisms and age of colorectal cancer onset in Lynch syndrome. Clin Cancer Res 2005;11:6840-4.

Spierings D, McStay G, Saleh M, Bender C, Chipuk J, Maurer U, Green DR. Connected to death: the (unexpurgated) mitochondrial pathway of apoptosis. Science 2005;310:66-7.

Spurdle AB, Hopper JL, Chen X, McCredie MR, Giles GG, Newman B, Chenevix-Trench G, Khanna K. No evidence for association of ataxia-telangiectasia mutated gene T2119C and C3161G amino acid substitution variants with risk of breast cancer. Breast Cancer Res 2002;4:R15.

Starinsky S, Figer A, Ben-Asher E, Geva R, Flex D, Fidder HH, Zidan J, Lancet D, Friedman E. Genotype phenotype correlations in Israeli colorectal cancer patients. Int J Cancer 2004;114:58-73.

Steffen J, Nowakowska D, Niwinska A, Czapczak D, Kluska A, Piatkowska M, Wisniewska A, Paszko Z. Germline mutations 657del5 of the *NBS1* gene contribute significantly to the incidence of breast cancer in Central Poland. Int J Cancer 2006;119:472-5.

Steffen J, Varon R, Mosor M, Maneva G, Maurer M, Stumm M, Nowakowska D, Rubach M, Kosakowska E, Ruka W, Nowecki Z, Rutkowski P, Demkow T, Sadowska M, Bidzinski M, Gawrychowski K, Sperling K. Increased cancer risk of heterozygotes with *NBS1* germline mutations in Poland. Int J Cancer 2004;111:67-71.

Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet 2003;73:1162-9.

Stephens M, Smith N, Donnelly P. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978-89.

Stewart GS, Maser RS, Stankovic T, Bressan DA, Kaplan MI, Jaspers NG, Raams A, Byrd PJ, Petrini JH, Taylor AM. The DNA double-strand break repair gene *hMRE11* is mutated in individuals with an ataxia-telangiectasia-like disorder. Cell 1999;99:577-87.

Storey A, Thomas M, Kalita A, Harwood C, Gardiol D, Mantovani F, Breuer J, Leigh IM, Matlashewski G, Banks L. Role of a p53 polymorphism in the development of human papillomavirus-associated cancer. Nature 1998;393:229-34.

Sullivan A, Syed N, Gasco M, Bergamaschi D, Trigiante G, Attard M, Hiller L, Farrell PJ, Smith P, Lu X, Crook T. Polymorphism in wild-type p53 modulates response to chemotherapy *in vitro* and *in vivo*. Oncogene 2004;23:3328-37.

Suspitsin EN, Buslov KG, Grigoriev MY et al. Evidence against involvement of p53 polymorphism in breast cancer predisposition. Int J Cancer 2003;103:431-3.

Swift M, Morrell D, Massey RB, Chase CL. Incidence of cancer in 161 families affected by ataxia-telangiectasia. N Engl J Med 1991;325:1831-6.

Swift M, Reitnauer PJ, Morrell D, Chase CL. Breast and other cancers in families with ataxia-telangiectasia. N Engl J Med 1987;316:1289-94.

Syrjäkoski K, Vahteristo P, Eerola H, Tamminen A, Kivinummi K, Sarantaus L, Holli K, Blomqvist C, Kallioniemi O-P, Kainu T, Nevanlinna H. Population-based study of *BRCA1* and *BRCA2* mutations in 1035 unselected Finnish breast cancer patients. J Natl Cancer Inst 2000;92:1529-31.

Syvänen AC, Sajantila A, Lukka M. Identification of individuals by analysis of biallelic DNA markers, using PCR and solid-phase minisequencing. Am J Hum Genet 1993;52:46-59.

Szabo CI, Schutte M, Broeks A, Houwing-Duistermaat JJ, Thorstenson YR, Durocher F, Oldenburg RA, Wasielewski M, Odefrey F, Thompson D, Floore AN, Kraan J, Klijn JG, van den Ouweland AM, Wagner TM, Devilee P, Simard J, van 't Veer LJ, Goldgar DE, Meijers-Heijboer H. Are *ATM* mutations 7271T-->G and IVS10-6T-->G really high-risk breast cancer-susceptibility alleles? Cancer Res 2004;64:840-3.

Talseth BA, Meldrum C, Suchy J, Kurzawski G, Lubinski J, Scott RJ. Age of diagnosis of colorectal cancer in HNPCC patients is more complex than that predicted by R72P polymorphism in *TP53*. Int J Cancer 2006;118:2479-84.

Talseth BA, Meldrum C, Suchy J, Kurzawski G, Lubinski J, Scott RJ. *MDM2* SNP309 T>G alone or in combination with the *TP53* R72P polymorphism does not appear to influence disease expression and age of diagnosis of colorectal cancer in HNPCC patients. Int J Cancer 2007;120:563-5.

Taniguchi T, Garcia-Higuera I, Xu B, Andreassen PR, Gregory RC, Kim ST, Lane WS, Kastan MB, D'Andrea AD. Convergence of the fanconi anemia and ataxia telangiectasia signaling pathways. Cell 2002;109:459-72.

Tanner M, Gancberg D, Di Leo A, Larsimont D, Rouas G, Piccart MJ, Isola J. Chromogenic *in situ* hybridization: a practical alternative for fluorescence in situ hybridization to detect *HER-2/neu* oncogene amplification in archival breast cancer samples. Am J Pathol 2000;157:1467-72.

Teraoka SN, Malone KE, Doody DR, Suter NM, Ostrander EA, Daling JR, Concannon P. Increased frequency of *ATM* mutations in breast carcinoma patients with early onset disease and positive family history. Cancer 2001;92:479-87.

Thomas M, Kalita A, Labrecque S, Pim D, Banks L, Matlashewski G. Two polymorphic variants of wild-type p53 differ biochemically and biologically. Mol Cell Biol 1999;19:1092-1100.

Thompson D, Antoniou AC, Jenkins M, Marsh A, Chen X, Wayne T, Tesoriero A, Milne R, Spurdle A, Thorstenson Y, Southey M, Giles GG, Khanna KK, Sambrook J, Oefner P, Goldgar D, Hopper JL, Easton D, Chenevix-Trench G; KConFab Investigators. Two *ATM* variants and breast cancer risk. Hum Mutat 2005;25:594-5.

Thompson D, Duedal S, Kirner J, McGuffog L, Last J, Reiman A, Byrd P, Taylor M, Easton DF. Cancer risks and mortality in heterozygous *ATM* mutation carriers. J Natl Cancer Inst 2005;97:813-22.

Thorstenson YR, Roxas A, Kroiss R, Jenkins MA, Yu KM, Bachrich T, Muhr D, Wayne TL, Chu G, Davis RW, Wagner TM, Oefner PJ. Contributions of *ATM* mutations to familial breast and ovarian cancer. Cancer Res 2003;63:3325-33.

Tirkkonen M, Johannsson O, Agnarsson BA, Olsson H, Ingvarsson S, Karhu R, Tanner M, Isola J, Barkardottir RB, Borg A, Kallioniemi OP. Distinct somatic genetic changes associated with tumor progression in carriers of *BRCA1* and *BRCA2* germ-line mutations. Cancer Res 1997;57:1222-7.

Toyama T, Zhang Z, Nishio M, Hamaguchi M, Kondo N, Iwase H, Iwata H, Takahashi S, Yamashita H, Fujii Y. Association of *TP53* codon 72 polymorphism and the outcome of adjuvant therapy in breast cancer patients. Breast Cancer Res 2007;9:R34.

Treilleux I, Chapot B, Goddard S, Pisani P, Angèle S, Hall J. The molecular causes of low ATM protein expression in breast carcinoma; promoter methylation and levels of the catalytic subunit of DNA-dependent protein kinase. Histopathology 2007;51:63-9.

Uziel T, Lerenthal Y, Moyal L, Andegeko Y, Mittelman L, Shiloh Y. Requirement of the MRN complex for ATM activation by DNA damage. EMBO J 2003;22:5612-21.

Vahteristo P, Eerola H, Tamminen A, Blomqvist C, Nevanlinna H. A probability model for predicting *BRCA1* and *BRCA2* mutations in breast and breast-ovarian cancer families. Br J Cancer 2001;84:704-8.

Vahteristo P, Bartkova J, Eerola H, Syrjäkoski K, Ojala S, Kilpivaara O, Tamminen A, Kononen J, Aittomäki K, Heikkilä P, Holli K, Blomqvist C, Bartek J, Kallioniemi OP, Nevanlinna H. A *CHEK2* genetic variant contributing to a substantial fraction of familial breast cancer. Am J Hum Genet 2002;71:432-8.

Varley JM, McGown G, Thorncroft M, Santibanez-Koref MF, Kelsey AM, Tricker KJ, Evans DG, Birch JM. Germ-line mutations of *TP53* in Li-Fraumeni families: an extended study of 39 families. Cancer Res 1997;57:3245-52.

Varon R, Reis A, Henze G, von Einsiedel HG, Sperling K, Seeger K. Mutations in the Nijmegen Breakage Syndrome gene (*NBS1*) in childhood acute lymphoblastic leukemia (ALL). Cancer Res 2001;61:3570-2.

Varon R, Vissinga C, Platzer M, Cerosaletti KM, Chrzanowska KH, Saar K, Beckmann G, Seemanová E, Cooper PR, Nowak NJ, Stumm M, Weemaes CM, Gatti RA, Wilson RK, Digweed M, Rosenthal A, Sperling K, Concannon P, Reis A. Nibrin, a novel DNA double-strand break repair protein, is mutated in Nijmegen breakage syndrome. Cell 1998;93:467-76.

Vehmanen P, Friedman L, Eerola H, McClure M, Ward B, Sarantaus L, Kainu T, Syrjäkoski K, Pyrhönen S, Kallioniemi O, Muhonen T, Luce M, Frank T, Nevanlinna H. Low proportion of *BRCA1* and *BRCA2* mutations in Finnish breast cancer families: strong evidence for additional susceptibility genes. Hum Mol Genet 1997;6:2309-15.

Venkatachalam S, Shi YP, Jones SN, Vogel H, Bradley A, Pinkel D, Donehower LA. Retention of wild-type p53 in tumors from p53 heterozygous mice: reduction of p53 dosage can promote cancer formation. EMBO J 1998;17:4657-67.

Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature 2000;408:307-10.

Vousden KH, Lu X. Live or let die: the cell's response to p53. Nat Rev Cancer 2002;2:594-604.

Walker KK, Levine AJ. Identification of a novel p53 functional domain that is necessary for efficient growth suppression. Proc Natl Acad Sci USA 1996;93:15335-40.

Wang WW, Spurdle AB, Kolachana P, Bove B, Modan B, Ebbers SM, Suthers G, Tucker MA, Kaufman DJ, Doody MM, Tarone RE, Daly M, Levavi H, Pierce H, Chetrit A, Yechezkel GH, Chenevix-Trench G, Offit K, Godwin AK, Struewing JP. A single nucleotide polymorphism in the 5' untranslated region of *RAD51* and risk of cancer among *BRCA1/2* mutation carriers. Cancer Epidemiol Biomarkers Prev 2001;10:955-60.

Wang Y, Kringen P, Kristensen GB, Holm R, Baekelandt MM, Olivier M, Skomedal H, Hainaut P, Trope CG, Abeler VM, Nesland JM, Børresen-Dale AL, Helland A. Effect of the codon 72 polymorphism (c.215G>C, p.Arg72Pro) in combination with somatic sequence variants in the *TP53* gene on survival in patients with advanced ovarian carcinoma. Hum Mutat 2004;24:21-34.

Wang Y, Kristensen GB, Børresen-Dale AL, Helland A. *TP53* mutations and codon 72 genotype –impact on survival among ovarian cancer patients. Ann Oncol 2007;18:964-6.

Wang YC, Chen CY, Chen SK, Chang YY, Lin P. p53 codon 72 polymorphism in Taiwanese lung cancer patients: association with lung cancer susceptibility and prognosis. Clin Cancer Res 1999;5:129-34.

Wang-Gohrke S, Becher H, Kreienberg R, Runnebaum IB, Chang-Claude J. Intron 3 16 bp duplication polymorphism of p53 is associated with an increased risk for breast cancer by the age of 50 years. Pharmacogenetics 2002;12:269-72.

Willems PJ. Susceptibility genes in breast cancer: more is less? Clin Genet 2007;72:493-6.

Williams RS, Williams JS, Tainer JA. Mre11-Rad50-Nbs1 is a keystone complex connecting DNA repair machinery, double-strand break signaling, and the chromatin template. Biochem Cell Biol 2007;85:509-20.

Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G. Identification of the breast cancer susceptibility gene *BRCA2*. Nature 1995;378:789-92.

Wyman C, Ristic D, Kanaar R. Homologous recombination-mediated double-strand break repair. DNA Repair (Amst) 2004;3:827-33.

Xia B, Dorsman JC, Ameziane N, de Vries Y, Rooimans MA, Sheng Q, Pals G, Errami A, Gluckman E, Llera J, Wang W, Livingston DM, Joenje H, de Winter JP. Fanconi anemia is associated with a defect in the *BRCA2* partner *PALB2*. Nat Genet 2007;39:159-61.

Xia B, Sheng Q, Nakanishi K, Ohashi A, Wu J, Christ N, Liu X, Jasin M, Couch FJ, Livingston DM. Control of BRCA2 cellular and clinical functions by a nuclear partner, PALB2. Mol Cell 2006;22:719-29.

Xu Y, Yao L, Ouyang T, Li J, Wang T, Fan Z, Lin B, Lu Y, Xie Y. p53 Codon 72 polymorphism predicts the pathologic response to neoadjuvant chemotherapy in patients with breast cancer. Clin Cancer Res 2005;11:7328-33.

Yarden Y. Biology of HER2 and its importance in breast cancer. Oncology 2001;61 Suppl 2:1-13.

Yoshida K, Miki Y. Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage. Cancer Sci 2004;95:866-71.

You Z, Chahwan C, Bailis J, Hunter T, Russell P. ATM activation and its recruitment to damaged DNA require binding to the C terminus of Nbs1. Mol Cell Biol 2005;25:5363-79.

Zehbe I, Voglino G, Wilander E, Delius H, Marongiu A, Edler L, Klimek F, Andersson S, Tommasino M. p53 codon 72 polymorphism and various human papillomavirus 16 E6 genotypes are risk factors for cervical cancer development. Cancer Res 2001;61:608-11.

Zehbe I, Voglino G, Wilander E, Genta F, Tommasino M. Codon 72 polymorphism of p53 and its association with cervical cancer. Lancet 1999;354:218-9.

Zhang X, Miao X, Guo Y, Tan W, Zhou Y, Sun T, Wang Y, Lin D. Genetic polymorphisms in cell cycle regulatory genes MDM2 and TP53 are associated with susceptibility to lung cancer. Hum Mutat 2006;27:110-7.

Zhou BB, Elledge SJ. The DNA damage response: putting checkpoints in perspective. Nature 2000;408:433-9.