

**GENETIC ABERRATIONS AND
THEIR CLINICAL
SIGNIFICANCE IN BREAST
AND OVARIAN CANCER**

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Department of Clinical Genetics, University
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OULU 1999



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AND OVARIAN CANCER**

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Abstract

In tumourigenesis, genetic alterations accumulate in the genes responsible for cell growth, proliferation and DNA repair: proto-oncogenes, tumour suppressor and DNA repair genes. Inactivation of tumour suppressor gene function is commonly recognised as a deletion of one of the two alleles; LOH, loss of heterozygosity. In the present study, LOH of several chromosomal regions was studied in both breast and ovarian cancer.

LOH for chromosome region 11q was examined in a large breast cancer consortium cohort (N=988) and in a Finnish ovarian cancer cohort (N=78), and the clinical significance of these alterations was evaluated. In breast cancer, LOH of the studied markers at 11q23.1, harbouring approximately 2 Mb of DNA, was seen to be associated with shortened cancer-specific survival. Two candidate genes, *ATM* (the ataxia telangiectasia disorder gene) and *DDX10* (a putative RNA helicase gene) map to this chromosomal region.

In ovarian cancer, LOH at 11q23.1-q24 was assigned mainly to two chromosomal regions, A and B, which are proximal and distal to 11q23.2-q23.3, respectively. Only the distal B region was seen to be associated with an aggressive disease course. Therefore, it appears that inactivation of the *ATM* or *DDX10* genes is not crucial for determining the outcome of ovarian cancer. The *CHK1* gene at 11q24, encoding a protein kinase required for DNA damage checkpoint function, is a putative target gene at the B region. On the basis of the present results, the main TSG in the studied region involved in the progression of breast cancer maps to 11q23.1 and the corresponding gene for ovarian cancer more distally to 11q23.3-q24.

In addition, LOH at 3p, 6q, 8p, 11p, 16q and 17p was examined and their role in the genetic evolution of ovarian cancer was evaluated. Of the studied chromosomal regions, LOH at 17p appeared to be an early event and LOH at 16q24.3, 11q23.3/q24 and 11p appear to occur later in the progression of ovarian cancer.

Keywords: cancer prognosis, chromosome 11, loss of heterozygosity

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Oulu, March 1999

Virpi Launonen

Abbreviations

ALL1 (MLL)	lymphoid/myeloid or mixed-lineage leukaemia gene
AMP	amplification
APOC3	apolipoprotein complement 3 gene
AT/ATM	ataxia telangiectasia/gene
BBC1	breast basic conserved gene
B-CLL	B-cell chronic lymphocytic leukaemia
BRCA1	breast cancer 1 gene
BRCA2	breast cancer 2 gene
BWS	Beckwith-Wiedemann syndrome
CDH1	E-cadherin gene
CGH	comparative genomic hybridisation
CHK1	DNA damage checkpoint gene
cM	centiMorgan
CMAR	cell matrix adhesion regulator gene
CYP1A1	cytochrome P450 (subfamily I, member 1) gene
DDX6	DEAD box-6 (RNA helicase) gene
DDX10	DEAD box-10 (RNA helicase) gene
DNA	deoxyribonucleic acid
E14 (CAND3/NPAT)	E14 gene
EOC	epithelial ovarian cancer
ERBB2	avian erythroblastic leukaemia viral (v-erb-b2) oncogene homologue 2
FHIT	fragile histidine triad gene
FRA3B	common fragile site in the human genome
GSTM1	glutathione S-transferase M1 gene
H19	adult skeletal muscle gene
HIAP1	apoptosis inhibitor 1 gene
HIAP2	apoptosis inhibitor 2 gene
HIC1	hypermethylated in cancer gene
HNPCC	hereditary non-polyposis colon cancer
HRAS	Harvey rat sarcoma viral (v-Ha-ras) oncogene homologue
IGF2	insulin-like growth factor 2 gene

kb	kilobase
KIP2/p57	cyclin-dependent kinase inhibitor 1C gene
KRAS	Kirsten rat sarcoma-1 viral (v-Ki-ras1) oncogene homologue
LOH	loss of heterozygosity
LOH11CR2A	potential tumour suppressor gene at 11q23
LOI	loss of imprinting
Mb	megabase
MCL	mantle cell lymphoma
MLH1	mutL (E. coli) homologue 1 gene
MMR	mismatch repair
MSH2	mutS (E. coli) homologue 2 gene
MSH6	G/T mismatch-binding protein
MYC	avian myelocytomatosis viral (v-myc) related oncogene
NCAM	neural cell adhesion molecule 1 gene
NUP98	nucleoporin 98 kD
OVCA1	ovarian cancer gene 1
OVCA2	ovarian cancer gene 2
p	short arm of the chromosome
PCR	polymerase chain reaction
PGL1	paranglioma or familial glomus tumours locus
PMS1	post-meiotic segregation increased (S. cerevisiae)-like 1 gene
PMS2	post-meiotic segregation increased (S. cerevisiae)-like 2 gene
PPP2R1B	gene encoding a β isoform of the subunit of serine/threonine protein phosphatase 2A
q	long arm of the chromosome
RB/RB1	retinoblastoma/gene
RER	replication error
SEN6A	cellular senescence gene
TP53	gene for tumour protein p53
T-PLL	T-cell prolymphocytic leukaemia
TSG	tumour suppressor gene
TSG101	potential tumour suppressor gene at 11p15.1-p15.2
VACM1	vasopressin-activated calcium-mobilizing receptor 1 gene
WT/WT1	Wilms' tumour/gene
WS/WRN	Werner's syndrome/gene

List of original articles

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

- I Laake K, Launonen V, Niederacher D, Gudlaugsdottir S, Seitz S, Rio P, Champème M-H, Bièche I, Birnbaum D, White G, Sztan M, Sever N, Plummer S, Osorio A, Broeks A, Huusko P, Spurr N, Borg Å, Cleton-Jansen A-M, van't Veer L, Benitez J, Casey G, Peterlin B, Olah E, Varley J, Bignon Y-J, Scherneck S, Sigurdardottir V, Lidereau R, Eyfjord J, Beckmann MW, Winqvist R, Skovlund E & Børresen-Dale A-L & the Breast Cancer Somatic Genetics Consortium (1999) Loss of heterozygosity at 11q23.1 and survival in breast cancer; results of a large European study. *Genes Chromosomes & Cancer*. In press.
- II Launonen V, Laake K, Huusko P, Niederacher D, Beckmann MW, Barkardottir RB, Geirsdottir EK, Gudmundsson J, Rio P, Bignon Y-J, Seitz S, Scherneck S, Bièche I, Champème M-H, Birnbaum D, White G, Varley J, Sztan M, Olah E, Osorio A, Benitez J, Spurr N, Velikonja N, Peterlin B, Borg Å, Cleton-Jansen A-M, Devilee P, Bloigu R, Lidereau R, Børresen-Dale A-L & Winqvist R & the Breast Cancer Somatic Genetics Consortium (1999) European multicentre study on LOH of APOC3 at 11q23 in 766 breast cancer patients; relation to clinical variables. *British Journal of Cancer*. In press.
- III Launonen V, Stenbäck F, Puistola U, Bloigu R, Huusko P, Kytölä S, Kauppila A & Winqvist R (1998) Chromosome 11q22.3-q25 LOH in ovarian cancer: association with a more aggressive disease course and involved subregions. *Gynecologic Oncology* 71: 299-304.
- IV Launonen V, Mannermaa A, Stenbäck F, Kosma V-M, Puistola U, Huusko P, Anttila M, Bloigu R, Saarikoski S, Kauppila A & Winqvist R (1999) Loss of heterozygosity on chromosomes 3, 6, 8, 11, 16 and 17 in ovarian cancer; correlation to clinicopathological variables. Submitted.

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1. Introduction

Breast cancer is the most common cancer in women worldwide (except in Japan), occurring in 29.8 per 100 000 women on average. The incidence is geographically variable, being highest in North-Western Europe (between 89.6-99.0 per 100 000) and in North America (113.3 per 100 000) (Parkin *et al.* 1993). The aetiology of breast cancer is complex. The risk factors can be divided into exogenous (socio-economic status, diet, breast irradiation, oral contraception and geography) and endogenous factors (hormonal imbalance, age at menarche, reproductive history, mastopathies and family history of breast cancer) (Bièche & Lidereau 1995, Offit 1998). In addition, old age increases the risk of breast cancer. So far, two main breast cancer predisposing genes, *BRCA1* and *BRCA2*, have been identified (Miki *et al.* 1994, Wooster *et al.* 1995). Linkage and mutation analysis of these genes have shown that the lifetime risk of cancer in gene carriers is elevated (Ford *et al.* 1998).

Ovarian cancer is the second most common gynaecological cancer and the incidence is 6.7 per 100 000. As in breast cancer, the highest incidence rates have been observed in North-Western Europe (between 17.4-18.9 per 100 000) and in North America (15.8 per 100 000) (Parkin *et al.* 1993). The survival of ovarian cancer patients is poor and it is the leading cause of death from gynaecological cancers (Parker *et al.* 1997). A reduced risk of ovarian cancer is associated with factors that decrease ovulation, including pregnancy, lactation and use of oral contraceptives (Offit 1998). Environmental risk factors and overall dietary fat are known to increase the risk of ovarian cancer. Breast/ovarian and site-specific ovarian cancer syndromes have been linked to mutations in the *BRCA1* and *BRCA2* genes (Miki *et al.* 1994, Wooster *et al.* 1995). In addition, ovarian cancer is associated with the hereditary non-polyposis colon cancer syndrome (HNPCC) genes, especially with *MLH1* and *MSH2* (Boyd & Rubin 1997, Boyd 1998).

The development of various cytogenetic and molecular genetic techniques has increased our basic understanding of tumourigenesis. However, at present there is only limited knowledge about the overall genetic evolution of cancer. Hence, recognition of the most crucial genetic aberrations affecting cancer prognosis should lead to improved diagnosis and treatment.

The aim of the present study was mainly focused on loss of heterozygosity (LOH) analysis on chromosome 11q. LOH analysis was used to localise putative tumour suppressor genes on this chromosome arm (TSGs). In total, 988 breast and 78 ovarian cancer cases were studied. The correlation between LOH findings and clinical variables

was also examined. Furthermore, in ovarian cancer, LOH at chromosome regions 3p, 6q, 8p, 11p, 16q and 17p was studied and the role of these losses in tumour progression was evaluated.

2. Review of the literature

2.1. Clinicopathological features of breast and ovarian cancer

Breast cancer is a malignant epithelial tumour originating in the terminal ducts or lobules in the mammary gland and is classified either as non-invasive (carcinoma *in situ*) or invasive (infiltrating). A majority of invasive breast carcinomas (70%) are histologically ductal. Lobular carcinoma is the second largest group (6%) followed by less common subtypes including medullar, mucinous, comedocarcinoma, Paget's disease, papillary, tubular and inflammatory carcinoma. (Berg & Hutter 1995)

One of the most important prognostic factors is the tumour staging [TNM-staging classification, UICC (International Union Against Cancer, 1987)] at the time of diagnosis. TMN-staging is based on tumour size, regional lymph node involvement and distant metastasis. Approximately half of the breast carcinoma patients are diagnosed with stage 0 (carcinoma *in situ*) or I and have the most favourable 10-year survival (95% and 88%, respectively). The 10-year survival in patients with a stage II tumour is 66%, and only 36% and 7% with a stage III and IV tumours, respectively. (DeVita *et al.* 1997, Bland *et al.* 1998)

Positive oestrogen receptor (ER) and progesterone receptor (PgR) expression is widely used for treatment selection. The presence of these receptors suggests a high likelihood of response to hormone therapy, and they seem to be associated with a favourable prognosis. Biomarkers including *ERBB2*, *EGF*, *TP53*, *BCL2*, *Ki-67* and S-phase fraction are other indicators for defining the prognosis and selection of treatment (Dowsett 1998).

Ovarian cancer is classified as epithelial (90% of all cases), germ-cell and sex cord-stromal cell tumours. Histologically, epithelial ovarian cancer consists of several different subtypes; serous, mucinous, endometrioid, Müllerian mixed, clear cell, Brenner, undifferentiated and unclassified tumours. (DeVita *et al.* 1997)

FIGO-staging is the most important prognostic factor in ovarian cancer. According to FIGO (International Federation of Gynaecological and Obstetrics) staging, stage I tumours are limited to the ovaries, stage II to ovaries with pelvic extension, stage III to ovaries with peritoneal implants and stage IV to ovaries with distant metastasis (DeVita *et al.* 1997). The 5-year survival of patients with stage I, II, III and IV is 70%, 45%, 17% and 5%, respectively (Shelling *et al.* 1995). In addition to staging, histologic subtype and

tumour grade have an impact on prognosis. Serum CA 125 level has also been widely used as a marker for the presence of ovarian cancer. Still more information is needed for using the other biomarkers, such as DNA ploidy, S-phase fraction, *ERBB2*, *TP53*, *Ki-67* and *BCL2*. (DeVita *et al.* 1997, Silvestrini *et al.* 1998)

2.2. Multistep carcinogenesis

The stepwise progression model of tumour development is widely accepted. It has been suggested that usually about three to seven genetic alterations are needed for development of malignancy. In tumour progression, aberrations accumulate in genes responsible for cell growth, proliferation and DNA repair: proto-oncogenes, tumour suppressor genes and DNA repair genes. (reviewed in Weinberg 1989, Vogelstein & Kinzler 1993, Macdonald & Ford 1997) The genetic model for the multistep nature of carcinogenesis is best studied in colorectal cancer (for a review see Fearon & Vogelstein 1990, Vogelstein & Kinzler 1993). In this model, normal epithelium gradually transforms to adenoma and eventually to invasive carcinoma through the accumulation of genetic changes. In the hereditary form of cancer, a susceptibility gene mutation is inherited and the additional mutations needed for cancer development arise in the specific target tissue. Several genes involved in hereditary cancer syndromes have been identified (Table 1). Interestingly, the risk of cancer varies among these syndromes. In addition, some allelic variants in the genes involved in carcinogen metabolism (e.g. *CYP1A1* and *GSTM1*) have been suggested to have a modifying effect on the development of cancer (Hussain & Harris 1998, Nedelcheva Kristensen *et al.* 1998, Krajinovic *et al.* 1999). Interestingly, association between rare alleles in the highly polymorphic *HRAS* microsatellite locus and common types of cancer, including breast cancer, has been observed (Krontiris *et al.* 1993). The rare alleles of this locus have also been found to modify the risk of ovarian cancer in BRCA1 mutation carriers (Phelan *et al.* 1996).

2.3. On the genetics of breast and ovarian cancer

During the past few years, new molecular techniques have offered an opportunity to localise and characterise frequently altered chromosomal regions and to identify the involved genes. Most breast and ovarian cancer cases appear to be sporadic and are seen in patients with no family history of cancer. However, there are a few known hereditary syndromes conferring predisposition to breast and ovarian cancer (Table 1).

Table 1. Three classes of cancer predisposition genes

Gene (locus)	Clinical syndrome	Cancer type
Proto-oncogenes		
MET (7q31)	Familial papillary renal carcinoma syndrome	Renal
RET (10q11.2)	Multiple endocrine neoplasia, type 2	Medullary thyroid, pheochromocytoma
CDK4 (12q13)	Familial melanoma	Melanoma, pancreatic
Tumour suppressor genes		
APC (5q21)	Familial adenomatous polyposis	Colon
BRCA1 (17q21)	Familial breast/ovarian cancer 1	Breast, ovarian, prostate, colon
BRCA2 (13q12)	Familial breast/ovarian cancer 2	Breast, ovarian, prostate, pancreatic
CDKN2 (9p21)	Familial melanoma	Melanoma, pancreatic
DPC4	Juvenile polyposis	Colon, pancreatic
LKB1 (19p)	Peutz-Jeghers syndrome	Colon, breast, testis, ovarian
MEN1 (11q13)	Multiple endocrine neoplasia type 1	Parathyroid, pancreatic, pituitary
NF1 (17q11.2)	Neurofibromatosis type 1	Neurofibroma, AML, brain
NF2 (22q12.2)	Neurofibromatosis type 2	Schwannoma, meningioma
PTCH (9q22.3)	Nevoid basal cell carcinoma syndrome	Basal cell skin
PTEN (10q23)	Cowden syndrome	Breast, thyroid, skin
RB1 (13q14.3)	Familial retinoblastoma	Retinoblastoma, osteosarcoma
TP53 (17p13.1)	Li-Fraumeni syndrome	Sarcoma, breast, brain, leukaemia
VHL (3p25)	Von Hippel-Lindau disease	Haemangioblastoma, basal cell, renal
DNA repair genes		
ATM (11q23.1)	Ataxia telangiectasia	Leukaemia, lymphoma, breast
BLM (15q26.1)	Bloom's syndrome	Leukaemia, lymphoma
FANCA (16q24)	Fanconi's anaemia type A	Leukaemia, lymphoma
MLH1 (3p21)	Hereditary non-polyposis colon cancer (HNPCC)	Colon, colorectal, ovarian, endometrium, bladder
MSH2 (2p16)		
MSH6 (2p16)		
PMS1 (2q32)		
PMS2 (7q22)		
XPA (9q22)	Xeroderma pigmentosum	Skin
XPC (3p25)		
XPD (19q13)		
XPF (16p13)		

Modified from Eng *et al.* (1994), Sancar (1995), Skuse & Lundlow (1995), Fearon (1997), Offit (1998).

2.3.1. DNA content and cytogenetics

The DNA content (ploidy) of tumours can be estimated using flow cytometry. An abnormal DNA content has been observed in about 60-70% of breast and ovarian tumours. An aneuploid DNA content has also been seen to be connected with poor prognosis. However, the prognostic value of DNA dosage measurements is still controversial (Devilee & Cornelisse 1994, Bièche & Lidereau 1995, Link *et al.* 1996).

Chromosomal aberrations have been revealed in almost 27 000 human neoplasms (reviewed in Mitelman *et al.* 1997). When comparing cytogenetic information from a total of 3185 cases representing 11 different types of solid tumours, deletions were more frequently seen than gains in all tumour types (Mertens *et al.* 1997). In breast cancer, the most common cytogenetic aberrations have been seen in chromosomes 1, 2q, 3p, 5, 6q, 8, 11, 12, 13q, 14q, 16, 17 and 18 and the most common numeric changes (trisomy) in chromosomes 7, 8, 18 and 20 (Heim & Mitelman 1995). In ovarian cancer, the most common structural aberrations have been observed in chromosomes 1, 3, 6q, 7p, 9q, 11, 17q and 19 and numeric changes in chromosomes 8, 13, 14, 17, 20, 22 and X (Heim & Mitelman 1995).

2.3.2. Proto-oncogenes

Proto-oncogenes are involved in controlling normal cell proliferation and differentiation. The proteins encoded by these genes are found in all cellular compartments and they can be classified according to function into growth factors, growth factor receptors, signal transducers and transcription factors (Macdonald & Ford 1997). To date, approximately 100 proto-oncogenes have been identified. Proto-oncogenes can be activated to oncogenes through point mutations, other structural alterations or by amplification (Macdonald & Ford 1997). So far, only three hereditary cancer syndromes have been found to result from germ-line mutations in proto-oncogenes (Table 1).

The most commonly amplified chromosomal regions containing putative oncogenes in breast and ovarian cancer are shown in Figs. 1 and 2, respectively. In breast cancer, amplification is frequently seen at *MYC* (8q24) and *ERBB2* (17q12) and also at the chromosomal region 11q13, including several genes (Bièche & Lidereau 1995). In addition, frequent chromosomal gains harbouring putative proto-oncogenes have been reported in chromosome regions 1q, 8q, 13q, 16p, 17q and 20q (Fig. 1) (Kallioniemi *et al.* 1994, Bièche & Lidereau 1995, Kuukasjärvi *et al.* 1997a, Tirkkonen *et al.* 1998).

Amplifications of *MYC* and *ERBB2* are also common in ovarian cancer. Other commonly amplified chromosome regions in ovarian cancer are 1q, 3q, 5p, 6p, 8q, 13q, 16p, 17q and 20q (Fig. 2) (Arnold *et al.* 1996, Tapper *et al.* 1997, 1998). In mucinous ovarian tumours, mutations in the *KRAS* gene have been detected in 45% of the studied cases (Macdonald & Ford 1997). Genetic alterations appear to differ among histological subtypes (Tapper *et al.* 1997).



Fig. 1. Frequently altered chromosomal regions in breast cancer. Gains and putative proto-oncogenes are represented on the right side and losses and putative TSGs on the left side.

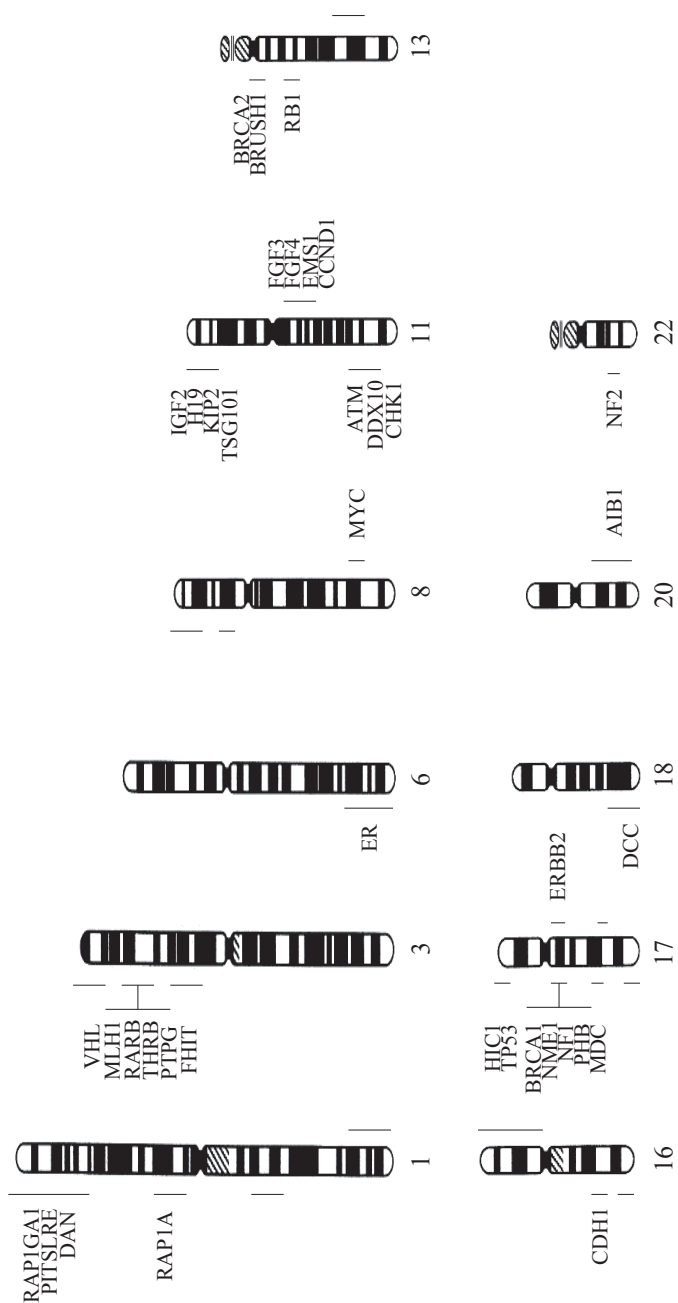


Fig. 2. Frequently altered chromosomal regions in breast cancer. Gains and putative proto-oncogenes are represented on the right side and losses and putative TSGs on the left side.

2.3.3. Tumour suppressor genes

Tumour suppressor genes (TSGs) are negative regulators of cell growth and have therefore also been called anti-oncogenes or gatekeepers. The concept of the function of TSGs in tumorigenesis was initially developed by Alfred Knudson in 1971 and was based on epidemiological studies of the age of onset for retinoblastoma (RB). In his 'two-hit' hypothesis, he suggested that inactivation of both alleles of the responsible gene (RB1) was needed for cancer formation. The 'two-hit' model has later been applied to explain the occurrence of cancer in other forms of familial cancer as well. In the hereditary form of cancer, a person has inherited the mutated predisposing allele from the affected parent and the wild-type allele derived from the unaffected parent is mutated somatically. The dominantly inherited cancer predisposition gene is actually behaving recessively at the cellular level. Table 1 shows TSGs known to increase the risk of breast and ovarian cancer. In the sporadic form of cancer, two somatic mutations are required for loss of TSG function (for reviews see Marshall 1991, Kinzler & Vogelstein 1997). In addition to point mutations, the wild-type allele can be inactivated by various other mechanisms, such as loss of the whole or part of a chromosome, loss of the normal chromosome and reduplication of the mutated one, gene conversion, mitotic recombination or deletion (Knudson 1978, Cavenee *et al.* 1983). Hence, the most commonly detected chromosomal losses in a certain region might indicate the existence of TSGs. Figs. 1 and 2 demonstrate the most frequent losses detected by comparative genomic hybridisation (CGH) and LOH (loss of heterozygosity) analysis in breast and ovarian cancer, respectively. Section 2.4 contains a more detailed description of LOH in breast and ovarian cancer.

A high proportion of hereditary breast and/or ovarian cancer cases are due to mutations in the *BRCA1* and *BRCA2* genes (Miki *et al.* 1994, Wooster *et al.* 1995). *BRCA1* and *BRCA2* may function as DNA repair genes, based on their ability to bind to *Rad51*, a protein involved in resolving double-stranded DNA breaks and recombination linked DNA repair (Kinzler & Vogelstein 1997). So far, no somatic *BRCA1* mutations have been detected in sporadic breast cancer tumours (Futreal *et al.* 1994). However, Thompson and co-workers (1995) found reduced expression of *BRCA1*, and Dobrovic & Simpfendorfer (1997) observed hypermethylation of the *BRCA1* promoter region in some sporadic breast cancer cases. Somatic *BRCA1* mutations appear to occur in a proportion of sporadic ovarian tumours (Hosking *et al.* 1995, Merajver *et al.* 1995, Berchuck *et al.* 1998). In addition, somatic *BRCA2* mutations appear to be infrequent in both breast and ovarian cancer (Foster *et al.* 1996, Lancaster *et al.* 1996, Teng *et al.* 1996). It also seems unlikely that transcriptional repression by methylation could be the mechanism leading to silencing of *BRCA2* in sporadic breast and ovarian cancer (Collins *et al.* 1997).

Germ-line mutations in the *TP53* gene are also known to increase the risk of breast cancer. However, most of the cases actually belong to Li-Fraumeni or Li-Fraumeni-like families. In addition to early-onset breast cancer, many other cancer types such as acute leukaemia, soft tissue sarcomas and brain tumours are seen in this syndrome (reviewed in Varley *et al.* 1997).

2.3.4. DNA repair genes

DNA repair genes, also called caretakers (Kinzler & Vogelstein 1997), are responsible for recognition and repair of damaged DNA. Defects in the mismatch repair (MMR) system have recently been shown to occur during tumourigenesis, particularly in HNPCC (Aaltonen *et al.* 1993, Ionov *et al.* 1993, Thibodeau *et al.* 1993). Other syndromes resulting from deficient DNA repair include rare autosomal recessive disorders associated with cancer such as Ataxia telangiectasia, Bloom's syndrome, Fanconi's anaemia and Xeroderma pigmentosum (Sancar 1995). Cancer predisposition syndromes related to defective DNA repair genes are shown in Table 1.

Defects of the MMR system were discovered through microsatellite studies of colorectal tumours related to HNPCC (Aaltonen *et al.* 1993, Ionov *et al.* 1993, Thibodeau *et al.* 1993). In tumours, the length of the microsatellite repeat is altered, reflecting malfunction in DNA replication or repair. This microsatellite instability is also called the replication error (RER) phenotype. A defective MMR system causes genomic instability and increases the mutation rate, resulting in a mutator phenotype in the tumour cells (Loeb 1994, Eshleman & Markowitz 1996). Microsatellite instability has also been detected in sporadic cancer, including colon, breast and ovarian tumours (Wooster *et al.* 1994, Liu *et al.* 1995). MMR genes were first identified in bacteria and yeast (Eshleman & Markowitz 1996). Five of the identified human MMR genes are known to be involved in HNPCC: *MLH1*, *MSH2*, *MSH6*, *PMS1* and *PMS2* (Leach *et al.* 1993, Nicolaides *et al.* 1994, Papadopoulos *et al.* 1994, Miyaki *et al.* 1997, Edelmann *et al.* 1997).

A gene causing the autosomal recessive disorder ataxia telangiectasia (AT) has been of great interest as heterozygous AT carriers have been estimated to have a 4-7-fold increased risk of breast cancer compared with the normal population (Swift *et al.* 1987, Pippard *et al.* 1988, Børresen *et al.* 1990, Swift *et al.* 1991, Easton 1994, Athma *et al.* 1996). Patients with AT are also known to be at an increased risk of other cancer, especially lymphoid neoplasias. AT is diagnosed in 1/40 000 to 1/100 000 live births and it has been estimated that the carrier frequency could be about 1% of the population (Savitsky *et al.* 1995).

The AT gene (*ATM*) was cloned in 1995 and it is known to be involved in cell cycle control, meiotic recombination and DNA damage repair, and to interact with the abl, p53 and p34 proteins (Savitsky *et al.* 1995, Baskaran *et al.* 1997, Shafman *et al.* 1997, Brown *et al.* 1999). Results from several mutation analysis studies have now been published. Vorechovsky *et al.* (1996a) found germ-line mutations in 3.4% of studied breast cancer patients with a family history of cancer, that did not, however, co-segregate with the malignancies. Stankovic and co-workers (1998) reported an increased risk of breast cancer in both AT homozygotes and heterozygotes. However, controversial results were seen in two other familial cancer studies, by Bay *et al.* (1998) and Chen *et al.* (1998). No evidence of an increased breast cancer risk was seen in two studies of sporadic and early onset breast cancer (Vorechovsky *et al.* 1996b, FitzGerald *et al.* 1997). However, Waha *et al.* (1998) reported that reduced transcriptional expression of the *ATM* gene could contribute to development of sporadic invasive breast carcinomas. In ovarian cancer, no somatic alterations were found in patients with or without LOH in their tumours (Koike *et al.* 1999). Studies involving mutation analysis in T-cell leukaemia and B-cell chronic lymphocytic leukaemia of non-AT patients have shown that somatic *ATM* aberrations

occur in at least some of the sporadic leukaemia cases (Stilgenbauer *et al.* 1997, Vorechovsky *et al.* 1997, Yuille *et al.* 1998, Stankovic *et al.* 1999).

2.4. Loss of heterozygosity in breast and ovarian cancer

In breast and ovarian cancer, several chromosomal regions have been observed to demonstrate losses and therefore unmasking putative TSGs. In breast cancer, high frequencies of deletion have been found at chromosomes 1, 3p, 6q, 8p, 11, 13q, 16q, 17, 18q and 22q (Fig. 1) (Bièche & Lidereau *et al.* 1995, Kerangueven *et al.* 1997a, Kuukasjärvi *et al.* 1997a, Tirkkonen *et al.* 1998). Chromosomal regions 1, 3p, 5q, 6q, 8p, 9p, 11, 13q, 16q, 17, 18q and 22q are commonly deleted in ovarian cancer (Fig. 2) (Weitzel *et al.* 1994, Shelling *et al.* 1995, Arnold *et al.* 1996, Tapper *et al.* 1997, 1998).

LOH analysis has been used for the detection of losses and it is based on Knudson's 'two-hit' hypothesis (Fig. 3) (see also section 2.3.3). In this method, the loss of one of the two alleles is detected in a tumour tissue when compared to the normal tissue of the same individual.

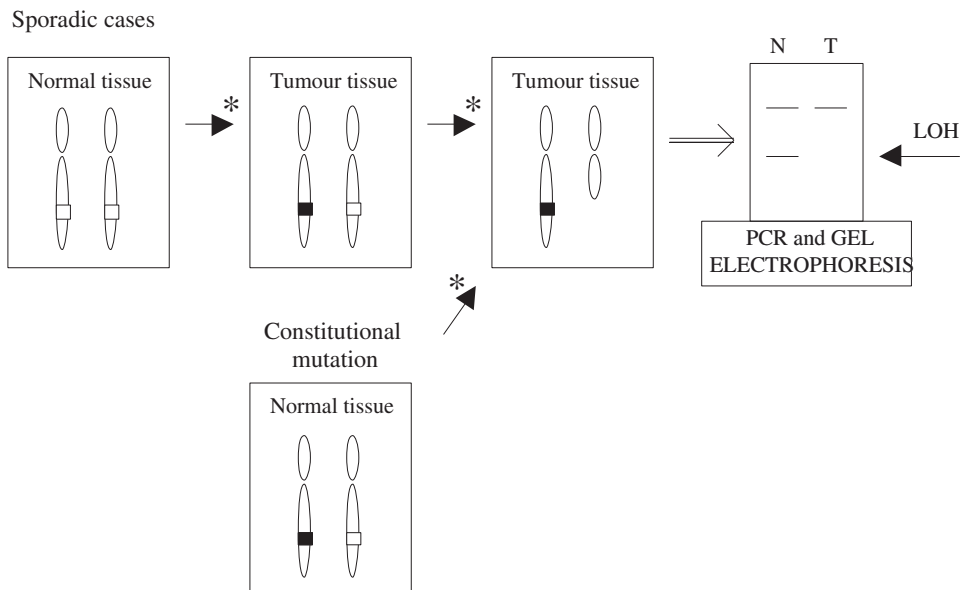


Fig. 3. Schematic presentation of loss of heterozygosity (LOH) analysis. Symbols: *, somatic mutation; N, normal DNA; T, tumour DNA.

2.4.1. Chromosome 11

2.4.1.1. Short arm of chromosome 11

Loss of heterozygosity at chromosome region 11p is frequently observed in breast and ovarian tumours (Viel *et al.* 1992, Winqvist *et al.* 1993, 1995, Gabra *et al.* 1995, Negrini *et al.* 1995, Lu *et al.* 1997, Karnik *et al.* 1998a, Bepler *et al.* 1999). Several other adulthood types of malignancy, e.g. stomach, bladder, testicular, prostate and non-small cell lung cancer, as well as embryonic tumours, such as rhabdomyosarcoma, hepatoblastoma and Wilms' tumour (WT), also show LOH at 11p (Albrecht *et al.* 1994, Al-Jehani *et al.* 1995, Shaw & Knowles 1995, Dahiya *et al.* 1997, Karnik *et al.* 1998a, b).

In breast cancer, LOH at 11p15 has been divided into two separate regions, of which one maps to 11p15.5 and the other to 11p15.5-p15.4, spanning approximately 0.5 Mb and 0.3 Mb of DNA, respectively (Karnik *et al.* 1998a). Karnik *et al.* (1998a) have also demonstrated that the distal region could include genes involved in early events of malignancy and that the proximal one could be associated with later events and a more aggressive disease course. A study by Winqvist *et al.* (1995) has indicated that LOH at 11q23 alone, or in combination with LOH at 11p15, is associated with poor survival after metastasis. On the other hand, Negrini and co-workers (1995) found no association between LOH at region 11p15.5 and the studied clinical variables.

In ovarian cancer, distinct LOH regions have been defined at 11p15.5-p15.3 and 11p15.1 (Gabra *et al.* 1995, Lu *et al.* 1997). LOH has also been seen at 11p13, a region harbouring the *WT1* gene (Viel *et al.* 1992). Although Gabra and co-workers (1995) found no correlation between LOH at 11p and studied clinical variables, associations with some clinical parameters have been reported by others. LOH at 11p15 has most often been seen in poorly differentiated and non-mucinous tumours, and in patients with liver and/or gallbladder metastases and poor survival (Eccles *et al.* 1992, Viel *et al.* 1992, Lu *et al.* 1997).

Interestingly, five chromosome rearrangement breakpoints associated with Beckwith-Wiedemann syndrome (BWS) have been mapped to a small chromosomal segment of 11p15.5 (Hoovers *et al.* 1995). Many putative candidate genes, e.g. three imprinted genes; the insulin-like growth factor 2 gene (*IGF2*), the *H19* gene and the cyclin-dependent kinase inhibitor IC gene (*KIP2/p57*), are located close to this breakpoint region (Hu *et al.* 1997). In addition, in both breast and ovarian cancer, loss of imprinting (LOI) has been detected in the *IGF2* gene resulting in biallelic expression (McCann *et al.* 1996, van Roozendaal *et al.* 1998, Wu *et al.* 1997, Kim *et al.* 1998). Recently, a new TSG (*TSG101*) at 11p15.1-15.2 was identified, but further investigations demonstrated that *TSG101* alterations are rare in breast cancer (Li *et al.* 1997, 1998, Steiner *et al.* 1997, Wang *et al.* 1998).

2.4.1.2. Long arm of chromosome 11

Several investigators have reported a high frequency of LOH on the long arm of chromosome 11 in breast and ovarian cancer (Foulkes *et al.* 1993, Hampton *et al.* 1994a, Gabra *et al.* 1995, 1996, Winqvist *et al.* 1995, Kerangueven *et al.* 1997a, b, Koreth *et al.* 1997, Negrini *et al.* 1995, Davis *et al.* 1996, Laake *et al.* 1997). In addition, 11q LOH is common in cervical, testicular, lung, bladder, stomach, nasopharyngeal, colorectal and prostate cancer, as well as in malignant melanoma (Baffa *et al.* 1996, Hampton *et al.* 1994b, Al-Jehani *et al.* 1995, Herbst *et al.* 1995, 1999, Rasio *et al.* 1995, Shaw & Knowles 1995, Hui *et al.* 1996, Tomlinson & Bodmer 1996, Dahiya *et al.* 1997). Many haematological malignancies, such as mantle cell lymphoma (MCL), B-cell chronic lymphocytic leukaemia (B-CLL) and T-cell prolymphocytic leukaemia (T-PLL), have also revealed chromosome 11q deletions (Stilgenbauer *et al.* 1996, 1997, Karhu *et al.* 1997, Monni *et al.* 1998).

In almost all studied tumour types, LOH at 11q appears to resemble a discontinuous 'zebra' pattern. By comparing data from different studies on LOH at 11q22-qter, three commonly deleted sub-regions can be found. The most proximal LOH region is at 11q23.1. The *ATM* gene is considered to be one of the main candidate target genes at this region (Savitsky *et al.* 1995). However, recent findings suggest that another putative TSG could reside at 11q23.1 (Vorechovsky *et al.* 1996b, Bay *et al.* 1998, FitzGerald *et al.* 1997, Chen *et al.* 1998, Koike *et al.* 1999).

The *E14* gene (also called *CAND3* or *NPAT*) is located immediately centromeric to *ATM* and is a housekeeping gene expressed in all tissues. *E14* and *ATM* are transcribed in opposite directions, sharing a common promoter region (Byrd *et al.* 1996). According to mutation analysis of AT patients and a methylation study of the promoter region in selected T-PLL cases without *ATM* mutations, it was suggested that *E14* is probably not a candidate gene (Byrd *et al.* 1996, Luo *et al.* 1998). In addition, *DDX10* (a putative RNA helicase gene) resides about 500 kb distally from *ATM* (Savitsky *et al.* 1996). The *VACMI* gene, which is a member of the cullin gene family involved in cell cycle regulation, is also located at 11q22-q23 (Byrd *et al.* 1997). Furthermore, the *PPP2R1B* gene (encoding a β isoform of the subunit of serine/threonine protein phosphatase 2A) was recently localised to 11q23. However, on the basis of mutation analysis of the *PPP2R1B* gene in lung, colon, breast and cervical cancer, it is possible that it is only involved in lung and colon cancer (Wang *et al.* 1998).

The second identified region of LOH maps to 11q23.3. *LOH11CR2A*, a putative TSG, is located in this chromosomal region. However, no mutations of the gene have been detected in breast, ovarian or lung tumours (Monaco *et al.* 1997). The *ALL1* gene (also called *MLL*), known to be rearranged, deleted, or fused to other genes in acute leukaemia and in solid tumours, also maps to 11q23.3 (Ziemin-van der Poel *et al.* 1991, Baffa *et al.* 1995). In addition, another gene of the DEAD-box family (*DDX6*) maps distally from *ALL1* on 11q23.3 (Lu & Yunis *et al.* 1992, Akao *et al.* 1996).

The most distal LOH region is situated at 11q24. The *CHK1* gene, encoding a protein kinase required for DNA damage checkpoint function, was recently mapped to 11q24 (Furnari *et al.* 1997, Sanchez *et al.* 1997). In addition, the genes *HIAP1* and *HIAP2* (inhibitor of apoptosis genes), *NCAM* (the gene encoding a neural adhesion molecule) and *PGL1* (the gene for hereditary non-chromaffin paraganglioma) have been localised to

this chromosomal region (Nguyen *et al.* 1986, Rajcan-Separovic *et al.* 1996, Baysal *et al.* 1997).

An association between LOH at 11q22.3-qter and prognosis has been described in both breast and ovarian cancer. In breast cancer, LOH at 11q23 has been reported to be associated with poor post-metastatic disease survival (Winqvist *et al.* 1995). A more distally located LOH region at 11q23.3-q24 has been seen to be associated with poor survival in ovarian cancer (Gabra *et al.* 1995, 1996). Interestingly, Lindblom *et al.* (1994) have observed that carriers of the constitutional t(11;22)(q23;q11) translocation appear to be at an increased risk of breast cancer.

2.4.1.3. Cell line studies on microcell-mediated transfer of chromosome 11

The existence of TSGs on chromosome 11 has been verified by several functional transfection studies. Chromosome regions 11p15 and 11q13-q23 have been observed to suppress tumourigenicity when transferred to the MCF-7 breast cancer cell line (Negrini *et al.* 1992, 1994). Phillips *et al.* (1996) have shown that one or more factors controlling metastasis development in breast cancer appear(s) to be contained in the long arm of this chromosome. Recently, Koreth *et al.* (1999) have defined two tumour suppressor loci at 11q23.1 and 11q25-qter. Similar observations of the ability of chromosome 11 to suppress tumourigenicity have also been reported in WT, rhabdomyosarcoma, cervical and lung cancer and melanoma cell lines (Weissman *et al.* 1987, Koi *et al.* 1989, Oshimura *et al.* 1990, Satoh *et al.* 1993, Robertson *et al.* 1996). Interestingly, transfer of human chromosome 11 does not suppress tumourigenicity in renal carcinoma cells, and displays only moderate suppression of tumourigenicity in endometrial carcinoma (Oshimura *et al.* 1990).

2.4.2. Chromosome 16q

Evidence for the existence of a TSG on chromosome 16q has been provided by LOH studies of breast, ovarian, prostate, hepatocellular and Wilms' tumours (Tsuda *et al.* 1990, Maw *et al.* 1992, Sato *et al.* 1991, Elo *et al.* 1997, Hansen *et al.* 1998, Withmore *et al.* 1998). In some of these studies, a correlation between LOH and clinical significance has been demonstrated. In particular, LOH at 16q22.2-q23.2 has been seen to be associated with breast cancer metastasis (Caligo *et al.* 1998, Driouch *et al.* 1997, 1998). In familial breast cancer, LOH at a more distal region (16q24) has been detected in connection with distant metastasis (Lindblom *et al.* 1993). On the other hand, in one breast cancer study, LOH at 16q23.2-q24.2 was found to be associated with good prognosis, and 16q LOH appeared to be an early event in tumour development and/or progression (Hansen *et al.* 1998).

Seven different cadherin genes have been mapped to the long arm of chromosome 16 (Kremmidiotis *et al.* 1998). They produce cellular adhesion molecules important for the invasion and metastasis of tumour cells. Alterations in the E-cadherin gene (*CDH1*) at 16q22.1 have been detected in both breast and ovarian cancer (Graff *et al.* 1995, Risinger *et al.* 1994, Berx *et al.* 1996). However, in breast cancer, several of the other identified candidate genes, including the cellular adhesion regulatory molecule gene (*CMAR*) and the breast basic conserved gene (*BBC1*), have been excluded, based on mutation analysis or their known function (Whitmore *et al.* and ref. therein 1998).

2.4.3. Chromosome 17

In addition to *BRCA1* (17q21) and *TP53* (17p13.1), chromosome 17 is suggested to contain many other genes involved in tumourigenesis of breast and ovarian tissues (Figs. 1 and 2). As mentioned previously, somatic *BRCA1* mutations appear to be rare in the sporadic forms of these malignancies (Futreal *et al.* 1994, Hosking *et al.* 1995, Merajver *et al.* 1995, Thompson *et al.* 1995, Dobrovic & Simpfendorfer 1997). *TP53* mutations have been found in about 30% and 50% of breast and ovarian cancer tumours, respectively (Devilee & Cornelisse 1994, Shelling *et al.* 1995). Distally from the *TP53* gene is located *HIC1* (hypermethylated in cancer) (at 17p13.3), a candidate target gene in breast cancer (Wales *et al.* 1995). Two other interesting genes, *OVCA1* and *OVCA2*, showing decreased expression in ovarian tumours, map to the same region (Schultz *et al.* 1996).

In a large consortium study on 1280 breast cancer patients, Phelan *et al.* (1998) have observed 8 distinct LOH regions, 2 at 17p and 6 at 17q. Similar discontinuous LOH regions on chromosome 17 have also been detected in breast and ovarian cancer studies by several other authors (Devilee & Cornelisse 1994 and ref. therein, Shelling *et al.* 1995 and ref. therein, Kerangueven *et al.* 1997b). In both cancer types, associations between LOH on chromosome 17 and various clinical parameters have been reported (Phillips *et al.* 1996, Pieretti *et al.* 1995, Niederacher *et al.* 1997, Plummer *et al.* 1997). In breast cancer, Niederacher and co-workers (1997) and Plummer and co-workers (1997) found an association between LOH on chromosome 17 and higher grade tumours. In ovarian cancer, Pieretti *et al.* (1995) found particularly high incidences of LOH on chromosome 17 in high grade and stage serous ovarian tumours. However, it has been suggested that LOH at 17p13.3 is particularly involved in early ovarian tumourigenesis, because high frequencies of such LOH have been detected in benign, borderline and low grade tumours (Gallion *et al.* 1992, Dodson *et al.* 1993, Phillips *et al.* 1996).

2.4.4. Other chromosomal regions

Loss of heterozygosity at chromosome region 3p has been revealed in several breast and ovarian cancer studies. Chen and co-workers (1994) detected two separate LOH regions (3p13-p14 and 3p24-p26) in their breast cancer study. In one ovarian cancer study, three sub-regions were identified; one was mapped to 3p21.1-p21.2 and two to 3p23-p24.2 (Rimessi *et al.* 1994). Recently, at least one additional LOH island at 3p14 has been described (Lounis *et al.* 1998). *FHIT* is a candidate gene at 3p14.2, as 30% of the studied breast tumours showed aberrant *FHIT* transcription (Negrini *et al.* 1996).

In ovarian cancer, Hendricks *et al.* (1997) and Buttitta *et al.* (1998) found only a few alterations in the studied ovarian cancer tumours or cell lines. On the other hand, Mandai *et al.* (1998) detected an abnormal *FHIT* transcript in 39% of ovarian carcinomas and 83% of borderline tumours. However, these findings were not seen to be in association with clinicopathological parameters or LOH findings.

High frequencies of LOH and at least five commonly deleted regions at 6q14-q27 have been identified, suggesting that this region harbours several TSGs (Orphanos *et al.* 1995, Saito *et al.* 1996, Sheng *et al.* 1996, Theile *et al.* 1996, Colitti *et al.* 1998). The *ER* gene is located at 6q25.1. Nevertheless, Bragadottir *et al.* (1995) detected no correlation between LOH at 6q and ER receptor status in a breast cancer study. Interestingly, in ovarian tumour cell studies, a cellular senescence gene (*SEN6A*) has been localised to chromosome region 6q14-q21 (Sandhu *et al.* 1996).

A third breast cancer susceptibility gene (*BRCA3*) has been suggested to reside at 8p12-p22 (Kerangueven *et al.* 1995, Seitz *et al.* 1997). Linkage has not yet been confirmed by other studies. This chromosomal region harbours the gene (*WRN*) responsible for Werner's syndrome (WS). Typical symptoms of this syndrome are premature ageing, age-related diseases and cancer (Yu *et al.* 1996). Interestingly, LOH at 8p appears to occur more frequently in breast than in ovarian tumours (Kerangueven *et al.* 1995, Shelling *et al.* 1995, Yaremko *et al.* 1996).

2.5. The genetic evolution of breast and ovarian cancer

Although the multistep nature of carcinogenesis is well established in colon cancer, much less is known about the gradual progression of breast and ovarian cancer. Breast and ovarian cancer are suggested to be monoclonal in origin (Fialkow *et al.* 1976, Noguchi *et al.* 1992, Tsao *et al.* 1993), but some evidence for polyclonality has also been reported (Teixeira *et al.* 1996, Lu *et al.* 1998). Furthermore, breast and ovarian cancer appear to be genetically very heterogeneous.

In the stepwise progression model for breast tumorigenesis, a normal cell is first transformed (Fig. 4), then proliferates and develops via atypical hyperplasia and *in situ* carcinoma to invasive carcinoma, and eventually to metastatic cancer (Devilee & Cornelisse 1994, Bièche & Lidereau 1995). In epidemiological studies, it has been estimated that patients with benign proliferative breast lesions or atypical hyperplasia are at an increased risk of breast cancer (Tavassoli *et al.* 1990). However, Kasami *et al.* (1997) found no such correlation in their genetic study. LOH analysis of premalignant

lesions supports the idea that benign lesions and atypical hyperplasia are precursors of invasive breast cancer (Radford *et al.* 1995, Fujii *et al.* 1996, O'Connell *et al.* 1998). Nevertheless, when comparing synchronous *in situ* carcinoma and invasive ductal carcinoma specimens from the same patient, genetic divergence has been observed (Radford *et al.* 1995, Fujii *et al.* 1996, Kuukasjärvi *et al.* 1997b). Genetic heterogeneity has also been observed in studies on primary and corresponding metastatic tumours (Kuukasjärvi *et al.* 1997a, Driouch *et al.* 1998). Kuukasjärvi and co-workers (1997a) concluded that breast cancer is monoclonal in origin and the genetic heterogeneity is most likely a result of increased genetic instability, clonal divergence and different selection pressures in different growth environments. It has also been suggested that primary tumours and relapses (local recurrence and distal metastasis) diverge early in tumourigenesis and evolve independently (Kuukasjärvi *et al.* 1997a, Driouch *et al.* 1998). Fig. 4 represents a model of the genetic evolution of breast cancer.

Knowledge of the genetic evolution of ovarian cancer is even more limited. Initially, in the multistep model of tumourigenesis, normal ovarian epithelial tissue first undergoes neoplastic transformation then progresses to benign tumour and through tumours of low malignant potential to invasive cancer and subsequently to metastatic disease. However, such a step-by-step progression does not appear to be an absolute model for tumourigenesis of ovarian tissue. Only a subset of borderline tumours, for instance, progresses to cancer (reviewed in Link *et al.* 1996, Chuaqui *et al.* 1997). In general, genetic alterations have more rarely been observed in benign and borderline ovarian tumours than in invasive ovarian carcinomas (Gallion *et al.* 1992, Dodson *et al.* 1993, Saretzki *et al.* 1997). Interestingly, Iwabuchi and co-workers (1995) found no DNA copy number abnormalities in their CGH study of benign ovarian tumours.

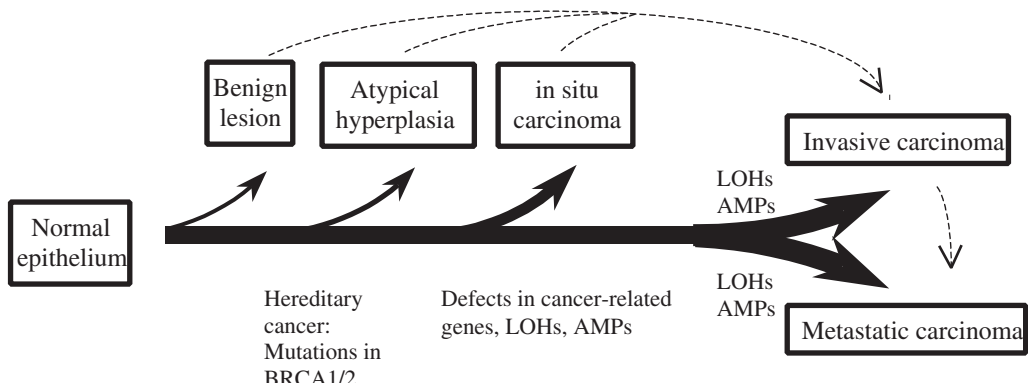


Fig. 4. Model for the genetic evolution of breast cancer [modified from Devilee & Cornelisse (1994), Bièche & Lidereau (1995), Kuukasjärvi *et al.* (1997a), Driouch *et al.* (1998)]. LOH, loss of heterozygosity; AMPs, amplifications.

3. Outlines of the present study

The identification of genetic aberrations and their relationship to clinicopathological features gives us information about the development and progression of cancer. In the present study, the specific aims were:

1. To define the minimal region of LOH at 11q23 in breast cancer and to evaluate the significance of 11q23 LOH in relation to the course of the disease.
2. To search for LOH and identify the most common and smallest LOH regions of chromosome 11q23-q24 in ovarian cancer and to compare the findings with clinical parameters.
3. To study LOH at different chromosomal regions (3p, 6q, 8p, 11p, 11q, 16q and 17p) in ovarian cancer and to evaluate their prognostic significance and involvement in the progression of the disease.

4. Materials and methods

Detailed descriptions of the materials and methods are presented in the original articles (I-IV).

4.1. Patients and tissue specimens

4.1.1. Breast cancer studies (I, II)

Altogether, 18 centres of the Breast Cancer Somatic Genetics Consortium from 12 countries participated in these two studies. Normal tissue/blood samples and corresponding primary tumour tissue specimens were collected from 988 breast cancer patients diagnosed between 1978 and 1996. Details of the studied cohort are summarised in Table 2. Most of the patients [70% (696/988)] were examined in both of the studies. The total numbers of patients analysed in studies I and II were 918 and 766, respectively.

Patients from four centres, altogether 118 cases [12%, (118/988)], were selected. Half of the patients from one of the Icelandic cohorts included patients with a familial breast cancer background, but they did not have mutations in the *BRCA1* or *BRCA2* genes. Both cohorts from the Netherlands were selected; one contained patients having Hodgkin's disease at a young age who subsequently developed breast cancer before the age of 45, and the other contained patients with metastatic disease during the follow-up period. Patients from the Swedish cohort were also selected on the basis of metastatic disease.

Clinical data was available from almost all of the cases. The age at disease onset and follow-up time of those patients still alive was recorded. Tumours were classified according to size (<2 cm, 2-5 cm or >5 cm in diameter), histology, tumour grade and ER receptor and PgR receptor status. In addition, information about node and metastasis status and possible adjuvant cancer therapy was obtained. Disease bilaterality was also recorded. Family history of cancer was considered positive when a patient had both breast and ovarian cancer, or a first or second degree relative with breast and/or ovarian cancer.

Table 2. Description of the breast cancer cohort used in studies I (LOH at 11q23.1) and II (LOH at 11q23.3).

Centre	Selection criteria	Study I No. of patients	Study II No. of patients
Finland	-	85	85
France I	-	46	46
France II	-	48	48
France III	-	61	61
Germany I	-	65	52
Germany II	-	137	76
Hungary	-	41	41
Iceland I	Sporadic and familial cases*	83	0
Iceland II	-	0	70
The Netherlands I	Hodgkin's disease and BC**	9	0
The Netherlands II	Metastasis***	11	11
Norway	-	164	160
Slovenia	-	40	17
Spain	-	21	21
Sweden	Metastasis***	15	15
U.K. I	-	19	19
U.K. II	-	44	44
U.S.A.	-	29	0
Total		918	766

Symbols: -, unselected cohort; *, Half of the patients were familial cases not having *BRCA1* or *BRCA2* mutations; **, Patients with Hodgkin's disease and subsequent breast cancer; ***, All patients had developed tumour metastases

4.1.2. Ovarian cancer studies (III, IV)

Seventy-eight randomly chosen patients with epithelial ovarian cancer (EOC) diagnosed between 1976-1994 were studied. Forty-nine of them were from Oulu University Hospital (III, IV) and twenty-nine from Kuopio University Hospital (IV). Primary tumour specimens were available from all but one patient. Altogether, 52 of the studied patients had developed cancer metastasis, of which tissue material was available in 19 cases.

Clinicopathological classification was carried out according to Serov *et al.* (1973) and Scully (1979). During the clinical follow-up time, nine of the EOC patients had also been diagnosed with another malignancy. The mean follow-up time of patients still alive was 43 months (variation: 1-189 months). Clinical data on tumour grade, histology and stage, as well as information about metastasis status, finding of residual tumour and second look findings/recurrent disease was collected.

Sections of paraffin-embedded normal tissue and corresponding tumour tissue specimens were obtained from all the studied patients. The suitability of the chosen samples for our analysis was verified from haematoxylin-eosin (HE) stained slides. To ensure that the tumour specimens were representative (containing at least 30% tumour cells), some of the specimens were prepared by micro-dissection.

4.2. DNA extraction

4.2.1. Fresh and frozen tissues (I, II)

DNA was extracted from normal tissue or blood leucocytes and corresponding primary tumour tissue (frozen or paraffin-embedded). Most of the tumour material was from fresh frozen tissue. Only the studied specimens from Slovenia were from paraffin-embedded tissues [4% (40/988)]. Every participating centre extracted their own samples using standard phenol/chloroform methods.

4.2.2. Paraffin-embedded tissues (III, IV)

Normal and tumour tissue DNA was extracted from paraffin-embedded tissue according to standard methods with slight modifications (Isola *et al.* 1994). For each of these specimens, 20 µm thick paraffin-embedded dissects were deparaffinised with 1 ml of xylene in an Eppendorf tube for 30 min. Subsequently, samples were centrifuged for 10 min and the xylene was discarded. The process was repeated until all the paraffin had been removed. The paraffin-free tissue materials were washed two times with 1 ml of 100% ethanol and centrifuged. After vacuum drying, the samples were suspended in 400 µl of DNA extraction buffer (50 mM Tris-HCl, 1 mM EDTA, 0.5% Tween 20) and 15 µl of proteinase K (10 mg/ml) and incubated overnight in a shaker at 55 °C. Twenty-four and forty-eight hours later 10-20 µl of proteinase K (10 mg/ml) was added. After 72

hours of incubation, DNA was extracted by using the standard phenol/chloroform procedure. DNA was precipitated with 200 μ l of 7.5 M ammonium acetate and 800 μ l of 100% ethanol at -20 °C for 4 hours and pelleted by centrifugation. After vacuum drying, DNA was resuspended in 50-100 μ l TE buffer (10 mM Tris, 1 mM EDTA) or sterile water.

4.3. LOH analysis

4.3.1. Microsatellite markers (I-IV)

LOH analysis (see Fig. 3) was performed using the highly informative microsatellite markers summarised in Table 3. References for the primer sequences used in breast cancer studies can be found in Laake *et al.* (1997) (I) and in Bhattacharya *et al.* (1991) (II). The primer sequences for ovarian cancer studies were obtained from the Genome Data Base (<http://gdbwww.gdb.org/>) and the Roswell Park Cancer Institute Data Base (<http://shows.med.buffalo.edu/>) (III-IV).

Table 3. Summary of microsatellite markers used in I-IV.

Chromosomal location	Locus	Cancer type (study)
3p14.2	D3S1300	Ovarian (IV)
6q27	D6S193	Ovarian (IV)
8p12	D8S2198	Ovarian (IV)
11p15.5	D11S1318	Ovarian (IV)
11q23.1	D11S1816	Breast (I)
	D11S1819	Breast and ovarian (I, III)
	D11S2179	Breast and ovarian (I, III)
	D11S1778	Breast and ovarian (I, III)
	D11S1294	Breast and ovarian (I, IV)
	D11S1818	Breast and ovarian (I, IV)
	D11S927	Breast (I)
11q23.2-q23.3	D11S1347	Ovarian (III)
	NCAM	Ovarian (III)
	D11S2077	Ovarian (III)
	D11S1885	Ovarian (III)
	D11S1340	Ovarian (III, IV)
	APOC3	Breast (II)
11q24	D11S912	Ovarian (III, IV)
16q24.3	D16S476	Ovarian (IV)
	D16S3028	Ovarian (IV)
17p13.1	D17S938	Ovarian (IV)

4.3.2. PCR methods (I-IV)

PCR was mainly carried out as described in Laake *et al.* (1997) (I), Bhattacharya *et al.* (1991) (II), <http://gdbwww.gdb.org/> (III-IV) and <http://shows.med.buffalo.edu/> (III-IV). In all methods used, the PCR products were subsequently resolved by electrophoresis on 6-7% denaturing polyacrylamide gels.

PCRs were alternatively carried out using radioactive (I-IV) or non-radioactive PCR methods (I, II). In the radioactive protocols, either direct incorporation of [α -³²P]-dCTP or [³²P]-end-labelled PCR primers were used. The PCR protocols based on non-radioactive chemistry were carried out using either silver staining after electrophoresis, or fluorescently labelled PCR primers and an automated DNA sequencer for fragment analysis (Pharmacia, A.L.F.; Perkin Elmer Applied Biosystems, model 373A).

4.3.3. Detection of LOH (I-IV)

In the radioactive methods, evaluation of LOH status was carried out by comparing the normal and tumour tissue allele intensity ratios in the autoradiograms (I-IV). Each studied case was evaluated by at least two investigators. In addition, quantitative laser densitometry scanning was used for the more difficult cases. In the silver staining method, LOH status was scored from the intensities seen in gels after electrophoresis. In microsatellite analysis using fluorescently labelled alleles, the results were analysed by using appropriate software (Pharmacia, Fragment Manager FM1.1; Perkin Elmer Applied Biosystems, Gene Scan), comparing normal and tumour tissue allele peak sizes, heights and area ratios. In all methods, intensity or signal ratio differences of at least 25% (depending on the proportion of tumour cells or the method used) were considered sufficient for LOH assignment.

4.4. Statistical analysis

The Chi-square test for linear trend (I), the Mann-Whitney test (II), Pearson's test (II) and Fisher's two-tailed exact test (III-IV) were used for the statistical evaluation of associations between LOH and the studied clinical variables. Logistic regression analysis was used for multivariate analysis (IV). Survival curves obtained by using Kaplan-Meier estimations were compared according to the log rank test (I-IV). Cox regression analysis was used for more detailed analysis of patients' survival time and connection with clinical factors (I, II, IV).

In the two large breast cancer cohort studies, P-values below 0.01 were considered significant (I, II). Because of the smaller number of cases in the ovarian cancer studies, P-values below 0.05 were accepted (III, IV), except when comparing LOH findings between different chromosomal regions, where only P<0.01 was accepted as statistically significant (IV). All analyses were carried out by using SPSS 6.1 for Windows.

5. Results

5.1. LOH at chromosome 11q23 in breast cancer (I, II)

Loss of heterozygosity at 11q23.1 was studied in 918 primary breast tumours using 7 DNA markers (11cen - D11S1816 - D11S1819 - D11S2179 - D11S1778 - D11S1294 - D11S1818 - D11S927 - 11qter) spanning approximately 6 Mb of DNA (I) (Fig. 5). Populations including less than 50 cases were analysed together. The LOH frequencies varied between 33% (D11S2179) and 42% (D11S1294). Fifty per cent of the studied cases exhibited LOH of at least one of the studied loci, and 26% showed LOH of all informative markers, indicating large deletions. In 24% of the cases, no LOH was detected among any of the studied markers.

Breakpoint mapping of this chromosomal region was carried out to determine the smallest shared sub-region of LOH. Unfortunately, there was great variation in the location of identified breakpoints in different cases, making it impossible to define one major narrow sub-region. However, separate LOH was most frequently seen at D11S1294 (in 7 cases) and at D11S1818 (in 10 cases).

In study II, a total of 766 patients were studied for LOH of the APOC3 marker at 11q23.3 (Fig. 5). LOH was detected in 42% of the tumours, and it appeared to be somewhat greater in patients with recurrent/metastatic disease (47%). In the larger populations (with at least 40 patients) LOH incidences frequencies varied between 32% and 56%. However, the highest LOH frequencies, 64% and 89%, were seen in two smaller selected populations (from Sweden and the Netherlands, respectively) representing a more aggressive metastatic disease course.

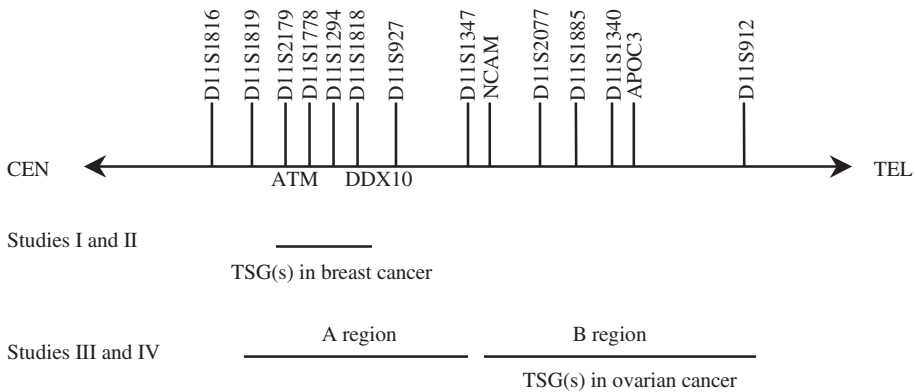


Fig. 5. Summary of LOH findings on 11q23-q24 (I-IV). DNA markers used are shown at the top of the figure.

5.1.1. Correlation with studied clinical variables

Of the studied 8 markers at 11q23, five (D11S1819, D11S2179, D11S1778, D11S1294 and D11S1818) at 11q23.1 were seen to be associated with an adverse disease course (Fig. 5). All of these markers are located close to the *ATM* gene. LOH at the proximal marker D11S1819 was seen to be associated with a greater tumour size ($P=0.01$) and distant metastasis at time of diagnosis ($P=0.0005$). LOH at the four more distal markers (D11S2179, D11S1778, D11S1294 and D11S1818) was associated with shortened survival time ($P=0.004$, $P=0.004$, $P=0.024$ and $P=0.0002$, respectively). In addition, LOH at D11S1778 and D11S1818 was found to correlate with later disease onset ($P=0.003$ and $P=0.00006$, respectively), and LOH at D11S1294 with a higher tumour grade ($P=0.002$). When LOH was detected among all informative markers on 11q23.1, no significant correlation was seen with any of the studied clinical variables. According to Cox regression analysis, the hazard ratios (HR) for LOH of the four markers associated with shortened cancer-specific survival time were found to be the highest (D11S2179, HR=1.8; D11S1778, HR=1.7; D11S1294, HR=1.6; D11S1818, HR=2.1).

LOH at *APOC3* (located at 11q23.3 and approximately 8 Mb distally from the *ATM* gene) was found to be slightly associated with disease onset at a greater age ($P=0.014$) (Fig. 5) (II). Data was also analysed separately for each of the larger sub-populations. In four populations, slight associations were observed between LOH at *APOC3* and the studied clinical variables; the Germany II cohort with ER receptor status ($P=0.029$), the France II cohort with greater tumour size ($P=0.026$) and a more advanced tumour grade ($P=0.024$), the Norwegian cohort (only sporadic cases) with a more advanced tumour grade ($P=0.025$) and the Finnish cohort with shortened survival time after disease recurrence/metastasis ($P=0.026$).

In studies I and II, no significant association was seen between LOH and the following variables: lymph node metastasis, histology, PgR status, clinical stage, adjuvant therapy, bilaterality vs. unilaterality and family history of breast cancer.

5.2. LOH at chromosome 11q23-q24 in ovarian cancer (III)

Loss of heterozygosity at 11q23-q24 was found in 61% of the patients. In primary tumours, the LOH frequency was 49%. All patients were studied for LOH of five markers [D11S1819 (q23.1) - D11S1347 (q23.2) - NCAM (q23.3) - D11S1340 (q23.3) - D11S912 (q24)] (Fig. 5). The LOH frequencies varied between 58% (D11S1819) and 42% (NCAM). Four additional markers were analysed in order to define the minimal deleted chromosomal sub-regions; D11S2179 and D11S1778 (located between D11S1819 and D11S1347), and D11S2077 and D11S1885 (located between NCAM and D11S1340).

In most of the cases, the LOH regions were large, spanning the whole of the long arm. Altogether, 13 cases were informative for determining the LOH islands. Six of them exhibited a breakpoint close to D11S1340/NCAM (11q23.2-q23.3). In both of the two identified main LOH sub-regions, A and B (proximal and distal from 11q23.2-q23.3, respectively) (Fig. 5), additional smaller sub-regions were observed.

In addition to LOH, extra allelic bands were detected in 12% (6/49) of the studied patients. Three cases displayed an extra band for one of the tested loci. One of these cases showed both an extra band and LOH at the same locus. Moreover, one of the studied tumours showed an extra band for 2 informative loci, another for 3, and a third for all informative loci.

5.2.1. Association with clinical variables

Loss of heterozygosity at 11q23.1 (sub-region A) was not seen to be associated with any of the studied clinical variables in ovarian cancer. On the other hand, LOH at both 11q23.3 and 11q24 appeared to correlate with an adverse disease course. In statistical analysis the results from primary and metastatic tumours were combined. The DNA marker D11S1340 at 11q23.3 was seen to be associated with shortened cancer-specific survival time ($P=0.005$) and serous histology ($P=0.036$), and D11S912 at 11q24 with a higher tumour stage ($P=0.003$), serous histology ($P=0.015$) and finding of residual tumour ($P=0.047$). Both D11S1340 and D11S912 are located in LOH sub-region B (Fig. 5).

Interestingly, LOH of any marker studied at 11q23-qter was found to be associated with a more advanced tumour grade ($P=0.030$). LOH was also seen in 70% of the tumours of patients that died from ovarian cancer during the follow-up time, but in only 47% of the patients still alive.

5.3. LOH at 3p, 6q, 8p, 11p, 11q, 16q and 17p in ovarian cancer (IV)

Loss of heterozygosity at 3p14.2 (D3S1300), 6q27 (D6S193), 8p12 (D8S2198), 11p15.5 (D11S1318), 11q23.1 (D11S1294 and D11S1818), 11q23.3 (D11S1340), 11q24 (D11S912), 16q24.3 (D16S476 and D16S3028) and 17p13.1 (D11S938) was analysed in 78 ovarian cancer patients. Of the 96 tumours studied 86% exhibited LOH of at least one of the studied loci. In the primary tumours, LOH frequencies varied between 27% (6q27) and 67% (17p13.1), and in the combined results from primary and metastatic tumours, between 31% (6q27) and 69% (17p13.1). In some of the tumour pairs studied, LOH at 11q23.1, 11q23.3, 11q24 and 16q24.3 was more frequently seen in the metastatic than in the primary tissue specimens.

When comparing the LOH findings among different loci, LOH of the studied markers on chromosome 11 appeared to occur simultaneously ($P < 0.005$). A similar result was obtained for the two markers at 16q24.3 ($P = 0.0001$). Interestingly, LOH at 17p13.1 appeared to occur independently of the other markers analysed. In addition, LOH at 11p15.5 was seen to be associated with LOH at 6q27 ($P = 0.01$), LOH at 11q23.1 with LOH at 6q27 ($P = 0.004$), 11q23.3 with 8p12 ($P = 0.005$), 11q24 with 16q24.3 ($P = 0.009$) and 6q27 with 8p12 ($P = 0.003$).

5.3.1. Association with clinical variables

Loss of heterozygosity at 6q27, 8p12 and 11q23.1 was not found to correlate with any of the studied clinical variables. LOH at 3p14.2, 11p15.5, 11q23.3, 11q24, 16q24.3 and 17p13.3 appeared to be associated with an unfavourable disease course. In primary tumours, LOH at 16q24.3 was found to be associated with tumour metastasis ($P = 0.002$) and a higher tumour stage ($P = 0.049$), and LOH at 17p13.1 with both serous and mucinous histology ($P = 0.0004$) and tumour metastasis ($P = 0.026$).

LOH at 3p14.2 was found to correlate only with tumour metastasis ($P = 0.040$). On chromosome 11, LOH at 11p15.5 was found to be associated with tumour metastasis ($P = 0.031$), finding of residual tumour ($P = 0.033$), shortened cancer-specific survival time ($P = 0.043$) and a higher tumour stage ($P = 0.050$). LOH at 11q23.3 was observed to be associated with shortened cancer-specific survival ($P = 0.019$) and serous histology ($P = 0.046$). LOH at 11q24 was seen to be associated with serous histology ($P = 0.0004$), a higher tumour grade ($P = 0.019$), an advanced tumour stage ($P = 0.029$), finding of residual tumour ($P = 0.038$) and metastatic tumour ($P = 0.047$).

At chromosome region 16q24.3 two loci were analysed. LOH at the more proximal marker D16S476 was found to be associated with metastatic tumours ($P = 0.0002$), a higher tumour stage ($P = 0.005$) and positive second look findings/recurrent tumour ($P = 0.011$). LOH of the distal marker D16S3028 was seen to correlate with a more advanced tumour stage ($P = 0.006$), metastatic tumour ($P = 0.010$), a higher tumour grade ($P = 0.016$) and serous histology ($P = 0.037$). LOH at 17p13.1 was found to be associated with serous and mucinous histology ($P = 0.0002$), tumour metastasis ($P = 0.009$) and a higher tumour stage ($P = 0.025$).

6. Discussion

6.1. LOH analysis

Studies of tumours using LOH analysis are methodologically challenging and demanding. The most critical aspect is the fact that normal cells always contaminate the tumour specimens, thus interfering the detection of loss. Therefore, scoring for LOH is almost always based on a quantitative analysis of the intensity of the two alleles in the autoradiogram (or fluorescence peaks, if fluorescent genotyping is used). In this study, microdissection was used if the proportion of tumour cells was not high enough (>30%) (studies III-IV).

Scoring of LOH is especially difficult with formalin-fixed tissues, in the presence of microsatellite instability or when the bands of the PCR products for some other reason are unclear. The average discordance rate of LOH scoring was 12% in one multicenter study by Devilee *et al.* (1997). In the present study, despite the carefully established scoring of LOH, variability in interpretation of LOH was also observed when blind scoring was performed (I). However, the results obtained in the present multicentre study seemed to be sufficiently homogeneous enabling us to pool data from all centres.

Finally, appearance of LOH might not always be caused by the loss of one of the alleles. Because of the PCR-based detection method, the term allelic imbalance has sometimes been used when loss of one allele is suspected to actually be the gain of the other allele.

6.2. LOH at 11q and its clinical significance

The long arm of chromosome 11 is one of the most common targets for LOH in human neoplasia (Seizinger *et al.* 1991). When summarising the previously published data, three main regions of LOH at 11q can be found; at 11q23.1, 11q23.3 and 11q24-qter (Fig. 6). Hence, it appears that chromosome 11q contains several genes that are either important in different types of malignancy or are cancer-specific.

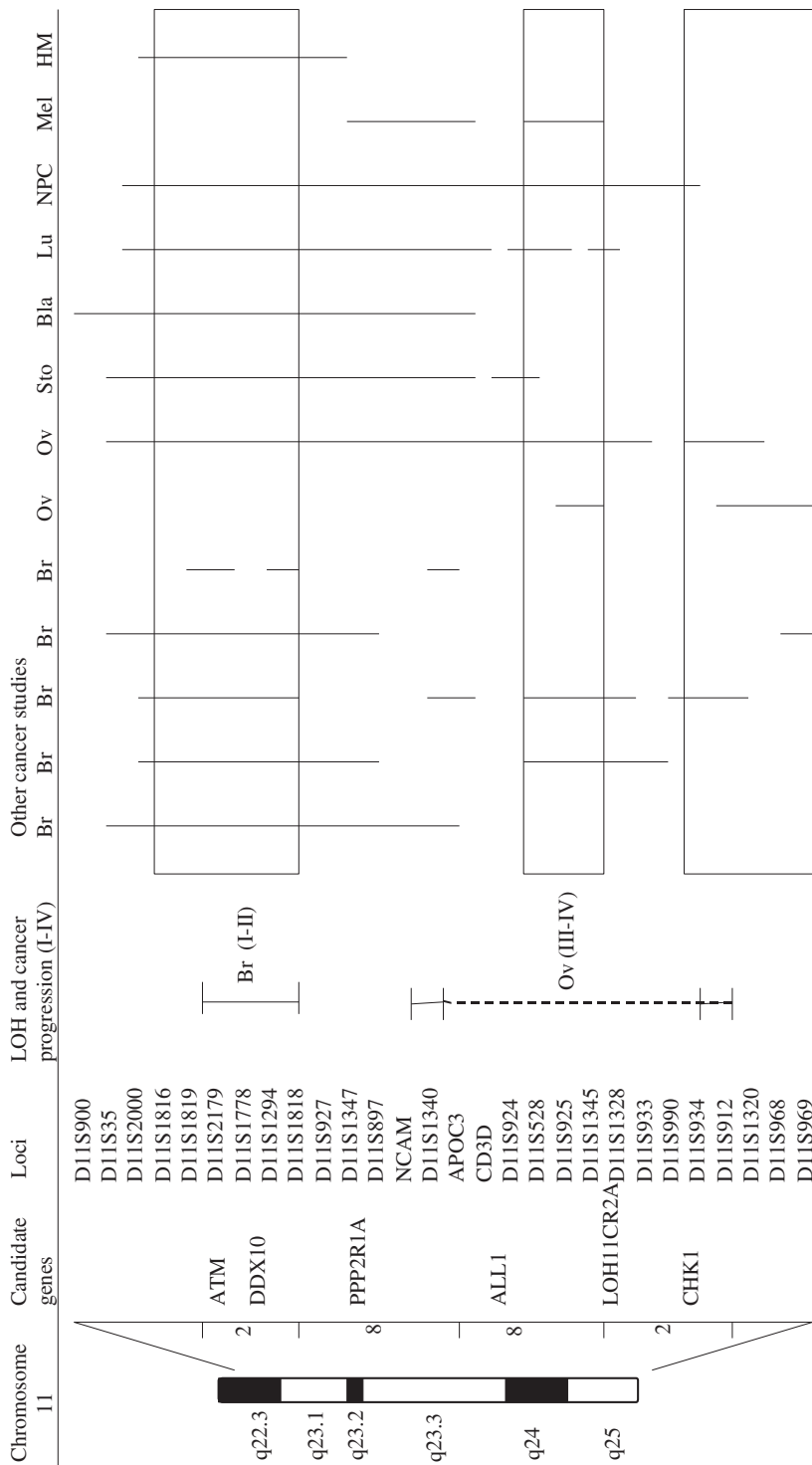


Fig. 6. Summary of LOH regions on 11q22.3-q25. Microsatellite markers and candidate genes have been positioned at appropriate chromosomal intervals (the distances are in Mb). Cancer types: Br = breast, Hampton *et al.* (1994a), Negrini *et al.* (1995), Kerangueven *et al.* (1997), Koreth *et al.* (1997), Laake *et al.* (1997); Ov = ovarian, Davis *et al.* (1996), Gabra *et al.* (1996); Sto = stomach, Baffa *et al.* (1996); Bla = bladder, Shaw & Knowles (1995); Lu = lung, Rasio *et al.* (1995); NPC, Nasopharyngeal, Hui *et al.* (1996); Mel = melanoma, Herbst *et al.* (1995, 1999); HM, haematological malignancies, Stiggenbauer *et al.* (1996, 1997), Monni *et al.* (1998).

6.2.1. Breast cancer studies (I, II)

In an initial study of a Finnish breast cancer cohort, the smallest common region of LOH at 11q appeared to be at 11q22-q23 (between loci D11S35 and APOC3) (Hampton *et al.* 1994a). Furthermore, Laake *et al.* (1997) defined three distinct LOH regions on the same chromosomal segment in their breast cancer study, suggesting further complexity in the mechanisms generating LOH. In the present work, two parallel studies of a large breast cancer consortium cohort were performed to further evaluate the clinical relevance of LOH at 11q23; one study was concentrated on the *ATM* gene region (11q23.1) (I) and the other on the APOC3 marker locus (11q23.3) (II).

In study I, on the basis of LOH frequencies and breakpoint mapping, we were not able to define the exact location of the main target TSG(s). On the other hand, the correlation between LOH findings and clinical parameters gave us further evidence of the existence of (a) putative TSG(s) at 11q23.1. In particular, the results obtained from survival analysis and Cox model studies (hazard ratios) suggest that one or more genes involved in breast cancer progression map to a 2 Mb region centered around markers D11S2179 and D11S1818 (Fig. 6). These results further confirm the previously reported deletion map (Laake *et al.* 1997). Altogether, four of the studied markers (cen-D11S2179-D11S1778-D11S1294-D11S1818-tel) map to 11q23.1. D11S2179, D11S1778 and D11S1294 are close to each other and within 500 kb of DNA. D11S2179 is an intragenic marker of *ATM* (Savitsky *et al.* 1995). The fourth marker, D11S1818, maps to less than 1 Mb distally from D11S1294. These two loci flank the *DDX10* gene (Savitsky *et al.* 1996). Interestingly, *DDX10* is fused in-frame to the *NUP98* gene in *de novo* and therapy-related myeloid malignancies associated with *inv(11)(p15q22)* (Arai *et al.* 1997). So far, no other putative target genes have been identified in this crucial LOH sub-region. However, our data indicate that the gene involved in breast cancer is associated with cancer-specific survival and could therefore be important in later stages of tumorigenesis. No strong correlation between LOH of any of these loci and tumour size, lymph node or distal metastasis, stage or tumour recurrence was observed. Only LOH of D11S1294 was seen to be associated with a higher tumour grade. Furthermore, it appears that the gene at 11q23 is not a main prognostic factor in familial breast cancer.

In our parallel study of LOH at 11q23 (II), APOC3 was selected on the basis of previous observations by Winqvist *et al.* (1995). This marker was highly informative, convenient to use and a great majority of the LOH-positive cases exhibited LOH at APOC3. In addition, LOH at 11q23 was seen to be associated with poor post-metastatic survival in breast cancer. In the present study, the overall LOH frequency was 42%, but we found no significant correlation between LOH of APOC3 and cancer-specific survival or survival after recurrence/metastasis. Neither were strong associations found for the other studied clinical parameters. When re-analysing the previously studied Finnish cohort, the clinical correlation appeared to be weaker than before (Winqvist *et al.* 1995). During the longer follow-up time (mean > 6 years) in the present analysis, 5 more patients had developed metastases. It therefore appears that the adverse effect of 11q23 LOH on survival is more dramatic within the first 2-3 years after relapse.

When comparing the LOH findings observed in studies I and II, only in 15% of the informative tumours was the LOH island found to be divided into two different sub-regions (only the closest informative marker at 11q23.1 and APOC3 at 11q23.3 were

considered). Interestingly, the breakpoint region of the constitutional translocation t(11;22)(q23;q11) is also proximal to the *APOC3* gene (Arai *et al.* 1996). In addition, Kerangueven *et al.* (1997b) and Laake *et al.* (1997) found one LOH sub-region harbouring the *APOC3* gene (Fig. 6). According to our statistical comparison of LOH and clinical parameters, it appears that the main factor is not located in the vicinity of the *APOC3* gene, but is more proximal and closer to the *ATM* and *DDX10* genes.

6.2.2. Ovarian cancer studies (III, IV)

In the ovarian cancer study on 11q23-q24 (III), two independent LOH regions (A and B) were found (Fig. 5). The breakpoint between these two regions appears to map to 11q23.2-q23.3 (flanked by loci D11S1347 and NCAM). Interestingly, the recently cloned *PPP2R1B* gene maps exactly to this breakpoint (Wang *et al.* 1998).

In contrast to the results obtained in the breast cancer study (I), LOH of the studied loci of the A region was not seen to be associated with any of the studied clinical factors in ovarian cancer. Therefore, most likely the crucial gene for progression of ovarian cancer is not located in the 11q23.1 region harbouring the *ATM* and *DDX10* genes. In addition, mutation analysis of the *ATM* showed that somatic alterations do not occur in ovarian tumours (Koike *et al.* 1999). In order to further investigate this suggestion, a larger cohort of ovarian cancer patients was analysed (IV). In this study, DNA markers D11S2179 and D11S1818 at 11q23.1 were examined. The results appeared to confirm our previous observation. However, the A region of LOH overlapped with a common LOH region detected in other cancers (Fig. 6), suggesting that this chromosomal segment could include another TSG whose function is not directly related to the progression of ovarian cancer.

LOH for two loci in region B (D11S1340 and D11S912) appeared to correlate with a more aggressive disease course (III, IV). Interestingly, no potential TSG has yet been identified very close to the marker D11S1340, and it also maps to the middle of two main LOH regions observed in other studies of cancer (Fig. 6). However, the significant correlation between LOH of D11S1340 and shortened cancer-specific survival time suggests the nearby location of an important gene for ovarian cancer. In addition, D11S1340 harbours one LOH sub-region defined in breast cancer by Kerangueven *et al.* (1997b) and Laake *et al.* (1997). D11S1340 is located approximately 3 Mb distally from *PPP2R1A*, and about 500 kb and 2 Mb proximally from *APOC3* and *ALL1*, respectively. Of the other candidate genes, *DDX6* and *LOH11CR2A* map distally from these two genes (Lu & Yunis *et al.* 1992, Akao & Matsuda 1996, Monaco *et al.* 1997).

The distal part of region B is of particular interest because of the strong association observed between LOH and several clinical factors, e.g. a higher tumour stage and grade and occurrence of tumour metastasis (III, IV). However, LOH was not associated with cancer-specific survival time. This result is in contrast to that obtained by Gabra *et al.* (1995, 1996). They found a strong correlation between LOH at 11q23.3-q24.3 and shortened survival time. The results of the present and previous studies show that the TSG(s) associated with aggressive disease behaviour in ovarian cancer maps to 11q23.3-q24. Interestingly, this region also includes two LOH regions shared among different types of malignancies (Fig. 6). One target gene of this region could be the recently identified *CHK1* gene at 11q24.

In summary, it appears that the main TSG of the studied region involved in the progression of breast cancer is located at 11q23.1 and that the corresponding gene for ovarian cancer maps more distally to 11q23.3-q24 (Fig. 6).

6.3. LOH at 3p, 6q, 8p, 11p, 11q, 16q and 17p in ovarian cancer (IV)

Loss of heterozygosity of the studied chromosomal regions appeared to be frequent in primary tumours. LOH findings were even more common in metastatic tumours, and when the results from primary and metastatic tumours were combined, 17p13.1 showed the highest LOH frequencies. LOH at 6q27, 8p12 and 11q23.1 was not seen to be associated with any of the studied clinical variables. Nevertheless, the possibility that these chromosomal segments contain TSGs important for ovarian cancer cannot be completely ruled out. Saito *et al.* (1996) observed a common LOH region at 6q27 close to the marker we studied. In addition, four other more proximal LOH regions have been reported, of which LOH at 6q16.3-q21 has been suggested to be involved in early stage tumours and LOH at 6q25.1-q25.2 in high grade invasive ovarian cancer tumours (Orphanos *et al.* 1995, Colitti *et al.* 1998). More precise studies are also needed regarding 8p, because it has been suggested that the gene at 8p12 responsible for Werner's syndrome may also play a role in tumourigenesis of non-WS patients (Yu *et al.* 1996).

LOH for 3p14.2, 11p15.5, 11q23.3-q24, 16q24.3 and 17p13.1 appeared to be clinically important in ovarian cancer. The studied locus at 3p14.2 is intragenic of *FHIT*, but is located more proximally and distally than the LOH regions reported by Rimessi *et al.* (1994) and Lounis *et al.* (1998), respectively. In the present study, LOH at 3p14.2 was found only to be associated with tumour metastasis. As the findings by others have been controversial, more studies are needed for evaluating the *FHIT* gene as a candidate gene at 3p14.2 in ovarian cancer (Hendricks *et al.* 1997, Buttitta *et al.* 1998, Mandai *et al.* 1998). However, the *FHIT* gene harbours FRA3B, the most common fragile site in the human genome. The connection between expression of instability at the FRA3B site and LOH would be worthwhile studying.

Chromosome region 11p has been shown to contain many genes involved in various types of cancer and syndromes related to neoplasms (Winqvist *et al.* 1993, 1995, Gabra *et al.* 1995, Albrecht *et al.* 1994, Al-Jehani *et al.* 1995, Hoovers *et al.* 1995, Shaw & Knowles 1995, Karnik *et al.* 1998a, b). The present results suggest that the chromosomal region 11p15.5 includes a gene important for progression of ovarian cancer, confirming the results of previous studies by Eccles *et al.* (1992), Viel *et al.* (1992) and Lu *et al.* (1997). Many putative TSGs also map to this chromosomal region (Hu *et al.* 1997). Interestingly, although LOH of all studied markers on chromosome 11 were statistically in association with each other, and therefore often occurring simultaneously, only LOH at 11p15.5 and 11q23.3-q24 were seen to be associated with an unfavourable disease course. However, we cannot be certain that the deletions had always occurred on the same homologue of chromosome 11 or what the mechanism behind this observation could be. In a breast cancer study by Winqvist and co-workers (1995), it was shown that LOH at 11q23 alone or in combination with LOH at 11p15 was associated with poor survival after metastasis. Because of the correlation between the different markers, it is also very difficult to obtain definitive clinical correlation.

In primary tumours, LOH of the two studied loci at 16q24.3 also appeared to occur simultaneously, and LOH was found to be associated with tumour metastasis and a higher tumour stage. Furthermore, when the results from primary and metastatic tumours were combined, LOH appeared to be associated with an unfavourable disease course. These results suggest that in addition to breast and prostate cancer, chromosome region 16q also contains an important gene related to the progression of ovarian cancer.

Chromosome 17 losses have been most frequently seen in different kinds of solid tumours (Seizinger *et al.* 1991). In study IV, LOH at 17p13.1 was seen in two-thirds of the tumours and it was found to be associated with an aggressive disease course. Most probably, *TP53* mutations explain at least 50% of these cases (Shelling *et al.* 1995). Pieretti *et al.* (1995) have also seen high incidences of LOH of chromosome 17 in high grade and stage serous ovarian tumours. On the other hand, Dodson *et al.* (1993) found a high frequency of LOH of chromosome 17 in low grade tumours. In the present study, high frequencies of LOH at 17p13.1 in grade I and stage I tumours indicate that it is most likely an early event in the progression of ovarian cancer. LOH at 16q24.3 and 11q23.3/11q24 appear to be later events, followed by LOH at 11p15.5.

Interestingly, LOH findings were not always shared by the primary and metastatic specimens from the same patient. In particular, LOH of chromosomal regions 11q and 16q appeared to occur more frequently in metastatic than in primary tumours. This confirms results reported by Kuukasjärvi and co-workers (1997a, b) and Driouch and co-workers (1998) in their studies of breast cancer. Primary and metastatic tumours appear to diverge early and progress independently as a result of increased genetic instability and different selection pressures in different growth environments.

7. Conclusions

In the present study, based on LOH findings and their clinical significance, we were able to localise putative chromosomal regions important for progression of breast and ovarian cancer. However, further studies are needed to identify and characterise the genes involved in these chromosomal regions. The following conclusions can be drawn from the present results:

1. LOH at 11q23 is a frequent event in breast cancer. The results obtained from comparison of LOH findings and clinical parameters show that a gene with an important role in breast cancer progression maps to 11q23.1, close to the *ATM* and *DDX10* genes, harbouring approximately 2 Mb of DNA.
2. The ovarian cancer studies demonstrated two distinct LOH regions on the long arm of chromosome 11 (A and B, distally and proximally from 11q23.2, respectively). This study further shows that the putative TSG(s) involved in progression of ovarian cancer is located in the B region (11q23.3-q24). Two candidate genes, *PPP2R1A* and *CHK1*, map to this interval.
3. Of the studied chromosomal regions (3p14, 6q27, 8p12, 11p15.5, 11q23.1-q24, 16q24.3 and 17p13.1), the most important TSGs involved in the evolution of ovarian cancer appear to be located on chromosomes 11, 16 and 17. LOH at 17p13.1 appears to be an early event, followed by LOH at 16q24.3, 11q23.3/11q24 and 11p15.5. In addition, the genetic heterogeneity observed in primary and metastatic specimens demonstrates that there are many different pathways involved in the progression of ovarian cancer.

8. References

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