From the Department of Medicine, Huddinge, Karolinska Institutet and the Department of Oncology, Karolinska University Hospital, Södersjukhuset, Stockholm, Sweden

FAMILY HISTORY AND BREAST CANCER SUSCEPTIBILITY

Clinical and Molecular studies

Sara Margolin

Karolinska Institutet

Stockholm 2006

Published and printed by
REPROPRINT AB
Stockholm 2006
www.reproprint.se
Gårdsvägen 4 169 70 Solna
ISBN 91-7140-868-1

ABSTRACT

Family history and breast cancer susceptibility – clinical and molecular studies

Sara Margolin MD

Department of Medicine, Huddinge, Karolinska Institutet and
Department of Oncology, Karolinska University Hospital, Södersjukhuset, Stockholm, Sweden

Apart from gender, family history is the most important risk factor for breast cancer. In 5-10 % of the cases there is a family history pattern of an autosomal dominant disease and there is also a familial clustering of breast cancer associated with a more modest increased risk of the disease. Mutations in the known high risk genes BRCA1, BRCA2 and p53 account for less than 25% of the familial risk for breast cancer, while the remainder remain genetically unexplained despite a large effort in research. A polygenic model has been proposed to best explain the residual familial breast cancer risk and also to contribute to sporadic breast cancer susceptibility in interaction with environmental factors.

In order to further elucidate the impact of genetic susceptibility for familial and sporadic breast cancer, a population-based cohort of 489 breast cancer patients from southern Stockholm was collected. For all patients, information on family history, clinical data and 5 years follow-up was retrieved. In addition, a risk-cohort of 350 non-BRCA1/2 familial patients from the Stockholm region was used for these studies.
In total 32% of the patients in the population-based cohort reported a family history of breast cancer and 10% was defined a high-risk familial group. There was no relation between family history and age of onset, hormonal background, tumour characteristics, treatment or prognosis.

The population-based cohort was screened for mutations in BRCA1 (exon 11). Two mutations (<1%) were detected, both in cases with a family history of both breast and ovarian cancer.

Sporadic (n=313) and familial (n=387) breast cancer cases and controls (n=760) were screened for the rare truncating variant, CHEK2 1100delC, which has previously been shown to be associated with familial and unselected breast cancer. Of the familial patients 2.3% carried the variant compared to 0.7% of the controls. The prevalence was not increased in sporadic patients (0.3%). The variant seemed to influence age at onset, with a lower mean age in carriers than in non-carriers.

Analysis of a common single nucleotide polymorphism, C975G, in the *estrogen receptor α (ESR1)* gene in 288 sporadic, 197 low risk and 191 high risk non BRCA1/2 familial breast cancer suggested a protective effect of the variant allele in high-risk familial breast cancer compared to controls. No association was seen in low risk familial or sporadic cases.

Three polymorphisms in the *estrogen receptor β (ESR2*) gene were analysed for association with familial and sporadic breast cancer. In total 723 breast cancer cases were genotyped, 323 sporadic cases and 400 non-BRCA1/2 familial cases. There was no overall significant difference in genotype distribution but one common haplotype, G-A-G, was associated with an increased risk of sporadic breast cancer indicating a role for *ERβ* in breast cancer susceptibility.

Keywords: breast cancer, family history, sporadic, familial, BRCA1, CHEK2, polymorphism, association, estrogen receptor alpha, estrogen receptor beta

ISBN 91-7140-868-1

PAPERS INCLUDED IN THE THESIS

The thesis is based on the following papers, which will be referred to in the text by their Roman Numerals:

I. Margolin S, Johansson H, Rutqvist LE, Lindblom A, Fornander T. Family history, and impact on clinical presentation and prognosis, in a population based breast cancer cohort from the Stockholm county.
Familial Cancer 2006; in press (e-pub ahead of print)

II. Margolin S, Werelius B, Fornander T, Lindblom A. BRCA1 Mutations in a Population-based Study of Breast cancer in Stockholm County
Genetic Testing, 2004; 8, 127-132

III. Margolin S, Eiberg H, Lindblom A, Bisgaard ML. CHEK2 1100delC in Swedish familial and sporadic breast cancer
Submitted

IV. Skoglund J*, Margolin S*, Zhou XL, Maguire P, Werelius B, Lindblom A. The Estrogen receptor alpha C975G variant in familial and sporadic breast cancer - a case-control study
Anticancer research, 2006; 26, 3077-3082

V. Maguire P*, Margolin S*, Skoglund J, Sun XF, Gustafsson JÅ, Børresen-Dale AL, Lindblom A. Estrogen receptor beta (ESR2) polymorphisms in familial and sporadic breast cancer
Breast Cancer Research and Treatment, 2005; 94, 145-152

*These authors contributed equally to this work

ABBREVIATIONS

3´UTR	3´ untranslated region
aa	Amino acid
AR	Androgen receptor
Arg	Arginine
AT(M)	Ataxia teleangiectasia (mutated gene)
ATP	Adenosin triphosphate
BMD	Bone Mineral Density
BRCA	Breast cancer gene
CGH	Comparative genome hybridisation
CHEK (CHK)	Check point kinase
CI	Confidence Interval
COMT	Catechol-O-Methyltransferase
CYP	Cytochrome P450
Del	Deletion
DNA	Deoxyribonucleic acid
DSB	Double strand break
ER, ESR	Estrogen receptor
GST	Glutathion S-transferases
HNPCC	Hereditary non polyposis colon cancer
HR	Hazard ratio
HRT	Hormone replacement therapy
HWE	Hardy Weinberg equilibrium
Ins	Insertion
LD	Linkage disequilibrium
LFS	Li Fraumeini syndrome
LOH	Loss of heterozygosity
LOD	Logarithm of the odds
NAT	N-acetyl tranferases
OCCR	Ovarian cancer cluster region
OR	Odds ratio
PCR	Polymerase chain reaction
PgR	Progesterone receptor
Ppi	Inorganic pyrophosphate
PTEN	Phosphatase and tensin homologue
PTT	Protein truncation test
Rb1	Retinoblastoma gene
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
RR	Relative risk
SNP	Single nucleotide polymorphism
STK11	Serine/threonine kinase 11
TP53	Tumour protein 53 gene
TNF	Tumour necrosis factor

CONTENTS

INTRODUCTION

Apart from gender, family history is the most important risk factor for breast cancer. Other identified risk factors are mainly hormonal and life style factors and in rare cases irradiation in young age (Table 1). In most individual cases there is however no definite answer to the crucial question occupying many affected women "why me?" In the 18th century it was common, also for the medical profession, to blame the disease on damage done to the breast by blows or the accumulation of black bile after menopause (a notion going back to antiquity) which could be prevented by regular bleeding [139]. The hereditary component, which was observed in up to one in three affected women, was explained by an inherited constitution e.g. bilious or sanguineous. In Victorian times, breast cancer was often blamed on chronic irritation by the tight habit of dressing and another common notion was that women's inborn, but varying, emotional fragility was predisposing them to cancer, breast cancer in particular [139].

The modern research on hereditary breast cancer started with epidemiological studies and moved on to studies of loss of heterozygosity (LOH) and linkage studies in the nineties leading to the discovery of the breast cancer genes BRCA1 and BRCA2. Thereafter has followed an intense search for more high and low risk genes. The clinical research field of family history and BRCA1/2 include studies on prevalence and penetrance of these genes, preventive strategies and if and how family history and mutation carrier status influence clinical presentation and prognosis.

The aim of this thesis has been to study the proportion and effect of family history in breast cancer and to identify underlying inherited alleles that contribute to the genetic susceptibility to the disease. Apart from women with a strong family history of the disease, also cases with a more moderate or no family history have been the focus of these studies, groups where the cause of the disease probably is multifactorial and involves a complex interaction between genetic and environmental factors. Identification of causative biological factors may have impact on prevention and clinical management of the disease.

1.1 EPIDEMIOLOGICAL AND PEDIGREE STUDIES

In the early 1980s several systematically epidemiological studies of familial susceptibility was conducted, almost all showing an elevated risk for breast cancer in female relatives to breast cancer patients [26,174]. One of the largest population-based studies was conducted in Sweden including 2660 women. Within this cohort, the relative breast cancer risk was 1.7 for women with an affected relative [9].

A metaanalysis of 74 studies from these years quantifying the risk of breast cancer associated with a family history of the disease showed that the pooled estimate of relative risk was 1.9 with any relative and 2.1 with a first-degree relative. If both mother and sister were affected the RR was 3.6 and in general risks were increased in younger subjects and if the relative had been diagnosed before age 50 [213]. In the same period the heterogeneous nature of familiality was described by studying pedigrees. In some families, there seemed to be a strong hereditary factor leading to multiple cases of breast cancer, with cases younger than average at diagnosis and with more bilateral cases [15,175]. Segregation analyses of Danish breast cancer families provided evidence for an autosomal dominant inheritance, which was later supported by American segregation studies, and a connection between premenpopausal breast cancer and ovarian

	Factor	Risk group	Effect on risk	References
Demographic factors	Gender	Female vs. male	+++	Socialstyrelsen [245]
	Age	Old vs. young	+++	Socialstyrelsen [245]
	Geographic area	US and Northern Europe vs. Asia	++(+)	Parkin [208,209]
Family history	High risk family history	Yes vs. no	+++	Claus; Ford [59,89]
	One close relative with breast cancer	Yes vs. no	+	Claus ; Pharaoh [59,213]
Reproductive factors	Age at menarche	Early vs. late	+	Kelsey; Hsieh [130,148]
	Age at menopause	Late vs. early	+	Kelsey; Hsieh [130,148]
	Pregnancies	0 vs. ≥ 1	+	Kelsey [148]
	Age at first birth	≥ 35 vs. ≤ 20	++	Kelsey [148]
	Breast-feeding	>1 yr vs. none	+	Collab.[7]
	Abortion	Yes vs. no	+/-	Beral[34]
Other hormonal factors	HRT	Current use vs. none	+(+)	Beral, Olsson, Collab.[33,203,227]
	Oral contraceptives	Current use vs. none	+	Collab.[1]
	BMI postmenopausal	High vs. low	+	van den Brandt [285]
	BMI premenopausal	Low vs. high	+	van den Brandt [285]
	Serum estradiol	High vs. low	++	Hankinsson; Thomas [115,264]
Breast properties	Mammographic pattern	Dense vs. not	++	Saftlas; Byrne [47,230]
	Atypical ductal hyperplasia	Yes vs. no	++	Byrne; Page [47,205]
Other	Ionizing radiation in young age	Yes vs. no	+	Hancock [114]
	Previous breast cancer	Yes vs. no	+(+)	Harvey; Bernstein [35,116]
	Alcohol	Daily intake vs. not	+	Hamajima [112]
	Diet	Dairy products, fat intake	+/-	Al Sarakbi; Parodi; Holmes [12,127,210]

Table 1. *Risk factors for / associated with breast cancer (Adapted from [301]; +/-: no effect on risk, + = Relative Risk (RR) 1-2, ++ =RR 2-5, +++ RR >5). Collab. = no author listed*

cancer in some families was suggested [59,102,196,302]. The lifetime risk of breast cancer in carriers was estimated to be 0,82 compared to 0.08 without the susceptibility allele [196]. In another Swedish study, a cohort of 1975 live patients in Stockholm was studied with regard to family history and 6.7% were classified as hereditary breast cancer with an autosomal dominant inheritance pattern. In this study, there was no association to bilaterality although the familial patients tended to be younger at diagnosis than sporadic patients [170]. In a cohort of 489

population-based breast cancer cases from the Stockholm region, almost 10% were defined as having high-risk family history of breast or breast-ovarian cancer (Paper I).

1.2 HIGH-RISK SUSCEPTIBILITY GENES

With information from segregation studies and with progress in molecular techniques an intense search for high-risk genes started in the early nineties. Linkage analysis and tumour studies (LOH-studies) were used to pinpoint regions and a candidate gene approach was sometimes used to test putative predisposing genes in selected cases.

For linkage analysis large families are needed, preferably with many affected relatives. Linkage is based on the fact that if two genetic loci lie in very close physical proximity, they are likely to segregate together during the process of meiosis. The further apart the loci are the less the chance of them being inherited together. When doing genetic mapping, linkage analysis uses known polymorphic markers scattered throughout the genome. If a certain segment of a chromosome is always shared in affected family members but not in the healthy family members, the gene involved in the disease might be localised in this area of the genome. The usual statistic measure of linkage is LOD score, which is the "logarithm of odds". A LOD score of +3 or more is considered to be strong evidence of linkage (1000:1 odds for linkage).

Tumour studies involve screening for genetic aberrations in paired blood and tumour samples to identify genes showing LOH. LOH areas are hypothesized to harbour potential tumour suppressor genes based on Knudsons two-hit hypothesis [154].

1.2.1 TP53

The rare Li Fraumeni syndrome (LFS), containing soft tissue sarcomas, brain tumours, premenopausal breast cancer and leukaemia occurring at an unusually young age, was first described in 1969 [165]. LFS is an autosomal dominant syndrome estimated to confer a cancer risk to carriers, up to the age of 45, of nearly 100% for females and about 75% for males, the difference because of the female breast cancer risk [57]. Because of the rarity and high mortality of the syndrome, linkage analysis had been impossible and the predisposing gene, TP53, was discovered in 1990 through the candidate gene approach [178,250]. The tumour suppressor gene TP53 was studied because of the growing knowledge that TP53 was inactivated in the sporadic forms of most of the cancers associated with LFS. Further studies on TP53 in young breast cancer patients and in familial breast cancer have revealed very few or no germ line mutations of the gene outside the syndrome [38,240,312].

1.2.2 BRCA1

In 1990 familial breast cancer was linked, with a LOD-score of almost 6, to a marker on chromosome 17q21 [111]. The study was based on 23 extended families with 146 affected members and 40% of the families showed linkage, mainly early-onset families. In 1993 Narod et al. showed that also breast-ovarian cancer families were linked to the same marker [193]. In 1994 BRCA1 was identified, by positional cloning, by a group in Utah [183]. The function of the protein was initially unclear although in tumours from mutation carriers, loss of the wild-type allele occurred in >90% indicating a tumor-suppressing function [61,244].

1.2.3 BRCA2

Shortly after the identification of BRCA1 the second major breast cancer susceptibility gene, BRCA2, was localized to chromosome 13q12-13 by linkage in large, non-BRCA1 families by a British group [306]. A year later, the gene was cloned and was also suggested to be a tumour suppressor gene by LOH studies in tumours of carriers [60,305]. BRCA2 was found to be involved also in male breast cancer, a fact that made it possible to use linkage analysis also to find this gene [60,260,273,305].

Both BRCA1 and BRCA2 have been shown to be involved in maintaining genomic integrity. Together with several proteins including RAD 51, CHEK2, ATM and TP53 they participate in DNA repair (especially double-strand breaks) and transcriptional regulation in response to DNA damage and cell cycle control (figure 1) [310]. BRCA1 has also been suggested to function as a stem-cell regulator [92]. Almost all protein-truncating mutations (frame shift, nonsense and splice site mutations) in BRCA1 and BRCA2 known so far have shown association to disease. A missense variation, resulting in an altered but not shortened protein, is more difficult to evaluate. Large rearrangements such as deletions or insertions may be important in some populations and are not detected with routinely used screening methods [97,126,189]. A deletion of exon 13 in BRCA1 has been shown to have a founder effect and can be detected by using MLPA (multiplex ligation-dependent probe amplification), a method currently starting to be routine in many laboratories in addition to conventionally used screening methods [5,126,157]. More than one thousand different mutations in each gene have been reported and 60% of these have been reported just once (http://research.nhgri.nih.gov/bic/). The mutations are spread throughout the genes and there are no obvious hot spots.

1.2.4 Other rare high risk genes

1.2.4.1 PTEN

Cowden's disease is a rare syndrome with an autosomal dominant pattern of inheritance, named after the first described patient in 1963 [171]. Multiple hamartomas and benign tumours of the skin, mucous membranes, breast and thyroid characterize it [82]. The susceptibility gene has been identified as the tumour suppressor gene PTEN, also known as MMAC1 and TEP1 [164,166,251]. Women with Cowden disease are at increased risk of breast cancer but in breast cancer patients outside the syndrome mutations are rare or absent [45,53,86,94,278].

1.2.4.2 STK11

The Peutz-Jegher syndrome is a rare autosomal dominant disorder, caused by germ-line mutations in the STK1/LKB1 gene. The gene was mapped to 19p13.3 by linkage and is considered to be a tumor suppressor gene [121]. Multiple gastrointestinal hamartomatous polyps, melanocytic maculae of the lips and mucous membranes and an increased risk of malignancies including breast cancer characterize the syndrome [140,168,275]. In order to establish whether STK11/LKB1 is associated with an increased risk of breast cancer also outside this syndrome, familial breast cancer patients with LOH on 19p have been screened for germ line mutations but no mutations were identified [55].

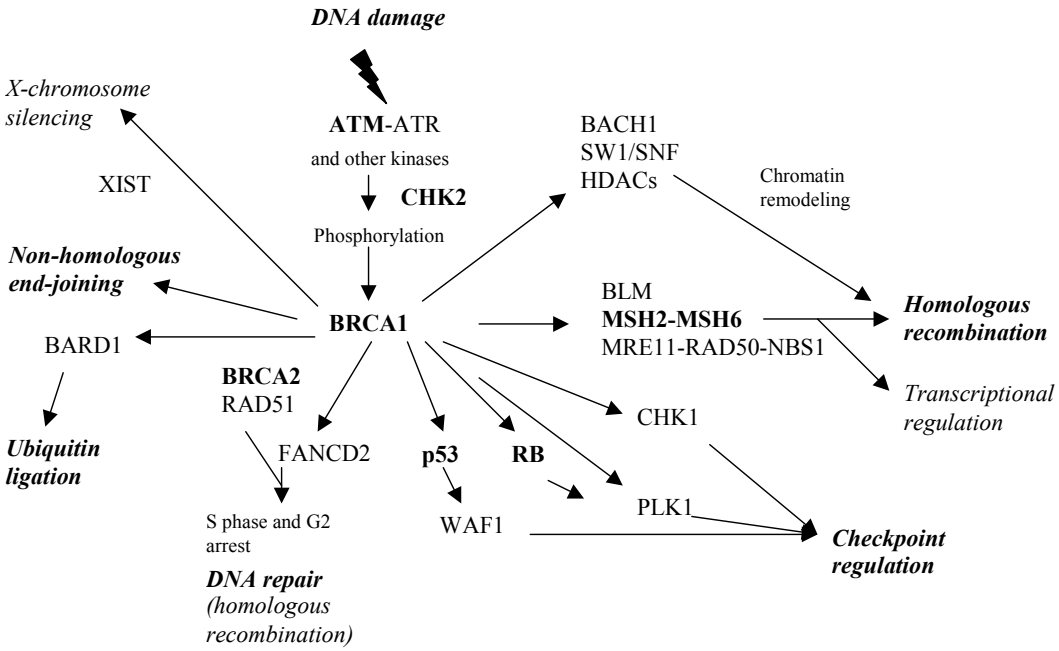

Figure 1*: The BRCA1 network, adapted from Narod and Foulkes [194]. RB=retinoblastoma protein, MSH2-MSH6 are DNA mismatch repair (MMR) genes. BRCA1, BRCA2, p53, RB, MSH2-MSH6, CHK2 and ATM are all cancer susceptibility genes.*

1.2.4.3 ATM

Ataxia teleangiectasia (AT) is an autosomal recessive disease characterized clinically by cerebellar ataxia, oculocutaneous telangiectasias, radio-sensitivity and immuno-deficiency. Homozygotes have an increased risk of malignancy particularly leukaemias and lymphomas. The ATM-gene was cloned in 1995 and appears to function as a checkpoint in response to DNA damage [234]. ATM heterozygotes were already in the 80s reported to have an elevated risk of breast cancer in studies emanating from AT-pedigrees [39,255]. In a study on 88 breast cancer families which also contained cases with other AT-related cancers such as lymphoma or leukemia, 4,3% ATM heterozygotes were found, which was more than the expected carrier frequency in the population of 1% [307]. However, whether ATM heterozygotes have an elevated breast cancer risk or not remains controversial. A Swedish study on familial breast cancer and another study on breast cancer with young-onset, could not find an increased prevalence of protein-truncating ATM-mutations while a Dutch study found 8.5% mutations in breast cancer patients selected for young age at onset, long-term survival and bilaterality[43,53,85]. Some recent studies indicate that non-truncating missense mutations in the ATM gene may predispose to breast cancer [247,261].

1.2.4.4 E-cadherin

E-cadherin or CDH1 encodes a protein located on the cell surface with important function in cell-cell adhesion and inactivation of E-cadherin has been suggested to contribute to a tumour's metastastic potential [58]. Its inactivation, which may occur through LOH, point mutations or

methylation, is commonly a late event in cancer and associated with a worse prognosis [68,311]. Somatic mutations in E-cadherin are common in diffuse gastric cancer and germ line mutations are known to cause hereditary gastric cancer of diffuse subtype, the families often also containing multiple cases with breast cancer [44,107]. The estimated cumulative risk of breast cancer in female carriers from these families is 39% [215]. Somatic mutations are also common in invasive lobular breast cancer, the majority of mutations found in combination with LOH of the wild type E-cadherin-locus, the behaviour of a classical tumour suppressor gene [36]. In order to elucidate the possible role of E-cadherin in hereditary breast cancer, familial breast cancer cases and families with cases of breast, gastric and colon cancer, whose tumours demonstrated LOH at the E-cadherin locus, were screened for germ line mutations but no mutations were found [232]. In conclusion, E-cadherin germ line mutations are important in families with hereditary diffuse gastric cancer and do in these families confer a substantial risk also for breast cancer, but does not seem to play an important role for breast cancer susceptibility outside this rare syndrome.

1.3 LOW RISK GENES

In 5-10% of breast cancer cases, more often if early onset, there is a pattern in the family of a highly penetrant trait [196]. The known high-risk genes, including BRCA1 and BRCA2 and the even rarer TP53, ATM and PTEN account for less than 25% of the familial risk for breast cancer [74]. Besides this dominant inheritance of breast cancer in families, there is also the less obvious clustering of breast cancer described in epidemiological studies [213]. Familial clustering outside the high risk families could be due to shared life style factors or environmental factors but recent twin studies suggest that a larger proportion of breast cancer are due to heritable factors than earlier estimates, particularly if the genetic factors are modulated by environmental risk factors [167,211]. In a study on twins from Sweden, Denmark and Finland, there was a statistically significant effect of heritable factors for breast cancer in 27% [167]. It is likely that most of the genetic susceptibility for breast cancer, as well as other common cancers, result from the combined effect of common genetic variants, each of which have a modest effect individually, the "common variant: common disease" hypothesis [222]. A polygenic model with variations in several loci, each contributing a modest independent risk has been shown to best explain the residual non-BRCA1/2 familial aggregation of breast cancer [20,21]. The effect of low-penetrant genes might also explain sporadic breast cancer [212].

The main approach to identify common low-penetrance genes is association studies, where the frequency of a genetic variant is compared in large series of affected cases and unaffected controls (figure 2). Association studies are, in contrast to linkage studies, based on a candidate gene approach and thus limited by our current biological knowledge concerning proteins involved in carcinogenesis. A systematic review of genetic polymorphisms and breast cancer risk showed conflicting or non-conclusive results for most of the variants and few associations reported in the literature have been confirmed by subsequent studies [73]. In order to overcome problems of power, detecting variants conferring modest risks, large sample sizes are needed. In general, unselected breast cancer cases have been used for association studies, but the study size can be reduced by selecting cases enriched for genetic susceptibility such as patients with family history or with bilateral breast cancer [19]. In addition to association studies on single polymorphisms, haplotype studies are increasingly used [214]. The following list does not claim to be complete but is an example of investigated candidate genes:

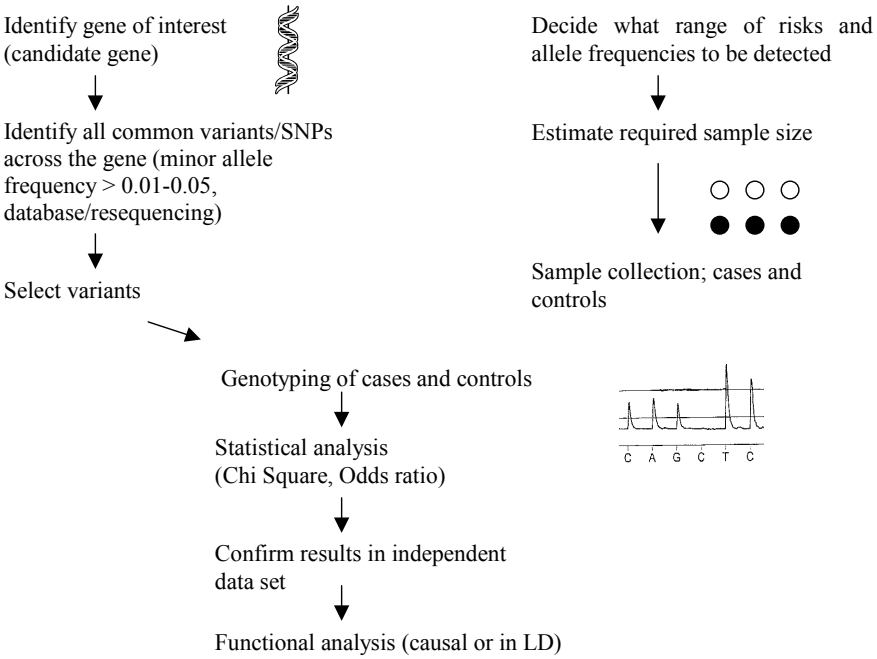

***Figure 2**. The stages in the design of an association analysis, using cases and controls (adapted from Pharaoh et al [214]).*

1.3.1 DNA repair genes

During the last years numerous association studies on different genes involved in base excision repair, homologous recombination of double strand break (DSB) repair, non homologous end-joining and other aspects of maintaining DNA stability have been published, including studies of variants in BRCA1, BRCA2 and RAD51. There is, so far, no evidence of true association except for one variant in the CHEK2 gene. However some studies of other genes have shown association that need to be confirmed by larger studies.

1.3.1.1 CHEK2

CHEK2 (also CHK2) is a cell cycle check-point kinas that is involved in a complicated network of proteins such as BRCA1, TP53 and ATM, regulating the cell cycle and DNA-repair [29]. A protein-truncating mutation, 1100delC, previously found in rare high risk families with Li-Fraumeni syndrome, has been shown to be associated with an elevated risk for familial, non-*BRCA1/BRCA2* breast cancer, however not acting as a high risk gene but rather as a modifier of so far unknown gene/s [31,180,202,281,282]. A subsequent large study has also demonstrated a doubled risk in unselected cases carrying the variant [8]. The frequency of the variant has been around 1% in controls, 2,0% in unselected breast cancer and 5% in familial non-*BRCA1/BRCA2* breast cancer although in some populations the variant is even more rare or almost non-existent [8,141,153,180,281]. In a Swedish population based breast cancer cohort, there was an increased CHEK2 1100delC prevalence in familial (2.3%) but not in sporadic breast cancer compared to controls (0.7%) and the variant seemed to influence age at onset (Paper III). The 1100delC mutation has also been shown to associate with bilateral breast cancer and was initially reported

to increase the risk for male breast cancer by a ten-fold, however a subsequent Finnish study did not confirm this, at least not at the population level [42,180,256,281]. In another study, the carriers were reported to have more receptor-positive tumours, had more often family history and had an unfavorable prognosis both regarding contra lateral breast cancer and disease-free survival [67].

Other variants in the *CHEK2* gene have been reported, but they seem to confer a lower or no risk for breast cancer [37,72,95,151,236]. A missense mutation, I157T, has been shown to associate to lobular breast cancer [133]. Apart from breast cancer, *CHEK2* also have been reported to confer a moderately increased risk of thyroid, prostate, kidney and colon cancer [66]. The highest prevalence of *CHEK2* 1100delC was seen in familial non- *BRCA 1/2* breast cancer also harboring colon cancer cases (18%) [181]. A subsequent Swedish study on patients with metachronous breast and colorectal cancer did not however show a statistically significant difference compared to controls (2.5% vs. 1%). In a Swedish case-control study on prostate cancer, the *CHEK2* 1100delC variant was found in 1.2% of the cases (sporadic: 0.7%; familial: 1.6%; hereditary: 1.4%) and in 1.0% of the controls and was concluded to be not clinically important high-risk gene for hereditary prostate cancer susceptibility in the population of southern Sweden [280].

1.3.2 Steroid hormone metabolism genes

Besides family history, the majorities of risk factors for breast cancer are related to reproductive factors and are thought to reflect longer lifetime exposures to endogenous steroid hormones [122]. Both genes involved in the sex hormone biosynthesis (*CYP17, CYP19* and *17 beta hydroxysteroid dehydrogenase type 2*) and the catabolism of estrogens (*COMT, CYP1A1, CYP1B1*) as well as the steroid hormone receptors (*ESR1, ESR2, PgR, AR, vitamin D receptor*) are strong candidate breast cancer susceptibility genes and have been investigated in several studies, often with inconsistent results [73,185,269]. In the metaanalysis by Dunning et al in 1999, only the (TTTA)10 polymorphism in *CYP19* was found to increase the risk of breast cancer with an OR of 2.3 (95% CI 1.4-4.2) [73]. The results on two restriction fragment length polymorphisms (RFLPs) in the *vitamin D receptor* gene (BsmI and Apa1) are also consistent, showing an association in several studies [41,65,108,109,128,172,241]. 13 SNPs in the *PgR* gene was analysed in a study of >1000 breast cancer cases with no association with any variant or haplotype [103].

1.3.2.1 ERα (ESR1)

The estrogen receptor (ER), denoted ERα or ESR1 after the identification of ERβ, was cloned and sequenced in 1986 although it was identified much earlier by its affinity for 17β estradiol [104,274]. It is located on chromosome 6q25.1, has eight exons and spans over 140kb. Its cDNA defines a sequence of 6322 nucleotides of which 1785 are coding while the remainder are untranslated regions at the 5´and 3´ ends [104,217]. ERα belongs to the nuclear receptor superfamily and has six conserved domains with different function (figure 3a) [217]. On estrogen binding the ER forms homo-dimers and binds to DNA at specific sites called estrogen-responsive elements (EREs) [161]. The gene is expressed in various tissues including CNS, bone, endometrium and the mammary gland [27]. ERα is also expressed in a majority of human breast cancers and is requisite for response to endocrine treatment [3,83]. Severe retardation of the mammary gland and infertility is seen in ERα knockout mice [173].

ERα was initially screened as a potential high-risk gene in familial breast cancer and one study indicated a possible clinical significance of the Gly160Cys variant [16].

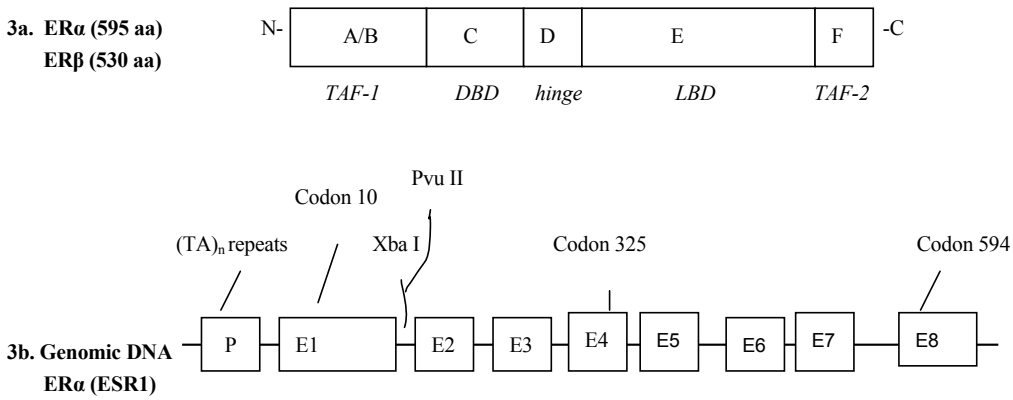

Figure 3a. *Functional domains of ERα and ERβ. A/B domain=an amino-terminal transcription activation domain (TAF1), C domain=a central DNA binding domain (DBD) that contains two zinc-binding fingers, D-domain=a hinge region, E-domain=a hormone-binding domain (ligand binding domain, LBD) required for stable dimerization of the receptor and F region= transcriptional activating factor 2 (TAF-2).*

Figure 3b*. Localisation of common polymorphisms in the ERα gene, E= exon, P=promoter.*

However 230 Stockholm breast cancer families were screened for this mutation and none were detected [313]. More than 30 SNPs have been reported in or upstream the *ERα* gene, mostly rare variants (figure 3). Several positive association studies on two RFLPs in intron 1, XbaI and PvuII, have been published on both breast and endometrial cancer, however there are also negative publications [14,48,239,295,297]. The most studied exonic variant is the common codon 325, CCC (pro) to CCG (pro), which has shown diverging results in association studies in both familial and sporadic breast cancer (Table 2) [64,113,147,248,294,295]. In a Swedish case-control study there was an association of the codon 325 variant and high risk familial breast cancer but no effect in sporadic or low risk familial breast cancer (Paper IV).

Variants in *ERα* have also been studied in association with other potentially hormone related characters like bone mineral density and mammographic density. Carriers of the XbaI and PvuII polymorphisms had denser breast tissue in one study [289]. Studies on bone mineral density (BMD) have also mainly focused on these two RFLPs in *ERα* and have demonstrated an association with BMD and the XbaI polymorphism, the results on PvuII are more contradictory [100,134,219,309]. In a small study of the codon 325 polymorphism in postmenopausal women, GG homozygotes had lower femoral neck BMD compared to CC homozygotes (p=0.03).

The functional effect of the common variants in *ERα* is unclear. Both XbaI and PvuII are located in an apparently unfunctional area of the gene (intron I) and separated by only 50 base pairs. These polymorphisms are, not surprisingly, in strong linkage disequilibrium with each other but also with a TA repeat polymorphism upstream in the promoter region of the ER alpha, a region that might be more interesting in relation to function [30]. Recently, it was noted that loss of the

PvuII site results in a potential binding site for *myb* transcription factors and can result in a higher *ERα* transcription [123]. The polymorphism in codon 325 as well as other common SNPs in *ERα* (codon 10, 594) are silent since they do not cause any amino acid change. An association of these SNPs with disease might be due to linkage with other functional variant/s or a true effect on gene expression e.g on binding of transcriptional factors or splicing [204].

Reference	**Study size cases / controls**	**Population**	**Effect on risk**	**Other results**	**G allele frequency cases/controls**
Roodi [225]	118 ER+, 70ER- no controls	North-American	NA	G assoc family history (p=0.0005)	Spor br ca 0.14 / not done Fam br ca 0.56 / not done
Iwase [138]	70 / 30	British	↑ risk G vs. C (p=0.06)	No assoc with ER or PgR in tumor	0.28 / 0.13
Southey [248]	388 / 294 (121 familial cases)	Australian early onset	None		Spor br ca 0.25 / 0.21 Fam br ca 0.17
Schubert [235]	133 high risk families no controls	North-American	NA	Did not segregate with disease	0.21 / not done
Curran [64]	125 / 125	Australian	None		0.17 / 0.23
Vasconselos[294]	70 / 69	Portugese	↑ risk G vs. C	G-allele assoc N+	0.43 / 0.24
Kang [147]	110 / 45	Korean	None	G assoc ER+, PgR+, p53- in tumor	0.50 / no information
Han [113]	100 / 100	Korean	↓ risk for GG homozygotes	G assoc p53- in tumor	0.46 / 0.57
Wedrén [295]	1296/1349	Swedish postmeno-pausal	↓ risk for GC heterozygotes for ductal but not lobular br ca	Assoc two haplotypes containing codon 325 pm, esp. if high BMI	0.20 / 0.23
Hsiao [129]	189 / 177	Taiwanese	↓ risk w G allele	G assoc N+	0.52 / 0.58 Fam br ca 0.44

Table 2 : *Published studies on ERα codon 325 polymorphism (925C/G, rs1801132) and breast cancer. NA=not applicable*

1.3.2.2 ERβ (ESR2)

A second estrogen receptor, ERβ or ESR2 was discovered in 1996 [160,191]. As well as ERα, ERβ belongs to the nuclear receptor super family and is activated in the nucleus by dimerization [191]. The DNA binding domains are almost homologous in ERα and ERβ (96%) and they are therefore expected to bind to Estrogen Response Elements (EREs) with similar affinity and specificity. The ligand binding (LBD) and activating function regions (TAF-1 and TAF-2) are less (60% and 30% respectively) conserved indicating a different function (figure 3a) [191]. There are several splicing variants of *ERβ*, one of them *ERβcx,* which is identical to *ERβ* except for the last exon (exon 8) which is replaced by another unique sequence [200]. ERβcx lacks the ligand binding properties of ERβ and does not bind estradiol. ERβcx prefers to heterodimerise with ERα rather than ERβ, thereby inhibiting ERα DNA binding (dominant negative effect on ERα function) [200].

ERβ is expressed in many different tissues in humans, including the female reproductive system and the normal breast where ERβ is the predominant estrogen receptor [27]. There is an ERβ knockout mouse (BERKO), which develops normally, but has fewer and smaller litters than wild type mice as a result of reduced ovarian efficiency, and they lack cyclical growth in the mammary gland [56,156]. In breast cancer, ERβ is thought to counteract ERα, being anti-proliferative and pro-apoptotic [231]. About 50% of breast cancers express both ERα and ERβ whereas 10-20% don't express any of the oestrogen receptors [231]. There are several reports demonstrating a positive correlation between ERβ expression and prognosis but also studies with neutral effect [231]. The roles of both ERβ and ERβcx as predictors of hormone treatment response in breast cancer are controversial [231].

As well as *ERα, ERβ* is an obvious candidate low risk breast cancer susceptibility gene but, compared to *ERα*, there are fewer association studies on *ERβ* and so far the results are controversial [91,103,117,135,315] (Table 3). In a Swedish population-based breast cancer, a common *ERβ* haplotype was associated with an increased risk of sporadic but not familial breast cancer, indicating a possible role for *ERβ* in breast cancer susceptibility (Paper V).

There are several studies on *ERβ* variants and other diseases or characteristics (e.g. androgen levels, BMD, blood pressure, Alzheimer, eating and ovulatory disorders), and recently a publication on prostate cancer showed an association with a common SNP in the promoter region [90,100,197,199,226,253,263,299]. There is also a study of a CA repeat polymorphism in *ERβ* that demonstrated an association of colon and rectal cancer risk in women, but not in men[243].

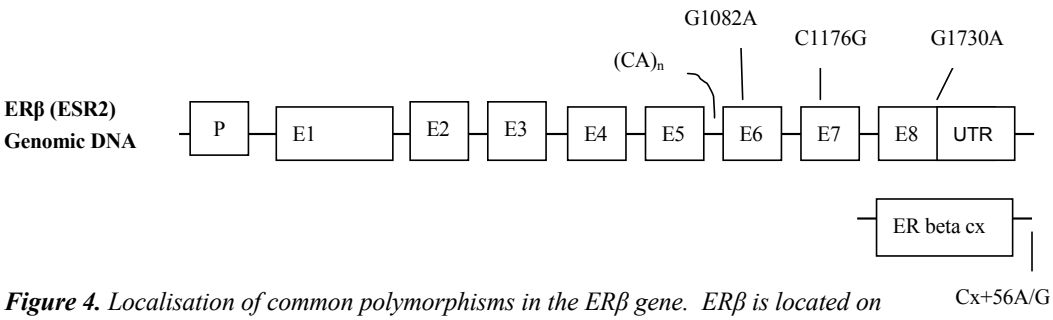

Figure 4. *Localisation of common polymorphisms in the ERβ gene. ERβ is located on chromosome 14q22-24 and consists of 8 exons. Exon 1-7 of ERβcx is identical to ERβ. Through alternative splicing, exon 8 is replaced by the cx sequence. P=promoter, E=exon, UTR=untranslated region*

Reference	Study size cases / controls	Population	Polymorphism	Effect on risk	Other results
Hasegawa [117]	93 / 91	Japanese	4 SNPs	None	
Forsti [91]	219 / 248	Finnish	6 SNPs including G1082A G1730A	None	
Zheng [315]	1134 / 1235	Chinese	7 SNPs including G1082A	Assoc with C1176G (exon 6) and intron 5 pm and postmenop. breast cancer	A potential synergy with C1176G and steroid sex hormones on breast cancer risk
Gold [103]	1011 / 615	North-American (388 Ashkenazi Jews)	8 SNPs including Cx+56A/G (rs928554) G1730A (rs4986938) G1082A (rs1256049)	No overall assoc.	One haplotype (7 SNPs) assoc. with increased risk among Ashkenazi Jews.
Iobagiu [135]	139 / 145	French	$(CA)_n$ in intron 5 cut-off 22repeats	None	Association with a combination of long $(CA)_n$ repeats and microsatellite markers in ESR1 and AR (gene-gene interaction)

Table 3. *Published studies on ERβ polymorphisms and breast cancer*

1.3.3 Carcinogen metabolism genes

Genes coding for enzymes involved in detoxification/metabolism of environmental carcinogens are obvious candidate genes. This group of genes includes members of the cytochrome P450 family such as *CYP1A1* and *CYP1B1*, which are both, involved in metabolism of polycyclic aromatic hydrocarbons (PAHs) and estrogen, and *CYP2D6*, which is induced by cigarette smoking. In a metaanalysis of 17 association studies on the four variants that have been studied in the *CYP1A1* gene, there was no consistent association between breast cancer and any of the genotypes [179].

The Glutathione S-transferases, GSTs, are a family of enzymes that detoxifies carcinogens including estrogen quinines, reactive intermediates of estrogen metabolism that can bind to DNA, by facilitating their conjugation to glutathione and subsequent excretion. There are a number of association studies of variants in members of this family, e.g. *GSTM1, GSTP1* and *GSTT1* and in the metaanalysis from 1999 the *GSTT1* polymorphism Ile105Val was associated with a modest increase in breast cancer risk (OR 1.6, p=0.02) [73]. There are a number of more recent studies of members of this family, most of them, including a study of >2000 cases and controls, however negative for association [80,186,233,287,304].

Finally, the N-acetyl-transferases, NAT1 and NAT2, are also phase II enzymes involved in the detoxification of acryl amines from tobacco smoke and also amines produced during cooking of meat. Polymorphisms in both genes result in two main phenotypes, slow acetylators (homozygous for low-activity alleles) and fast acetylators. There are several association studies on the risk of breast cancer in slow acetylators, sometimes in combination with smoking and meat consumption, mostly on *NAT2*. In the metaanalysis there was no association between the two *NAT*s and breast cancer [73]. Some recent studies have however shown an association with NAT slow acetylators, smoking and breast cancer, however not consistently, and sometimes in interaction with other gene variants e.g. *GSTs* [50,101,163,242,286,287].

1.3.4 Other candidate genes

In addition to these categories, there are also several other candidate genes, which have been studied in relation to breast cancer. The *HRAS1* minisatellite has been described as a low-penetrance breast cancer susceptibility locus with a relative risk for breast cancer of 1.9 for the so-called rare alleles in a metaanalysis [300]. Two subsequent studies, with more than 700 cases and controls each have however not shown any association [84,259]. Other candidates are genes in the tumour necrosis factor *(TNF*) family e.g. *TNF alfa, TNF beta* and the *TNF receptor type II*. Most studied is TNF alpha where the results are inconsistent, the largest study however negative [24].

Somatic mutations in the *TP53* gene are a common event in breast cancer as well as in other malignancies making it an obvious candidate gene. An association between the codon Arg72Pro variant has been shown in several studies on both sporadic and familial breast cancer, but like for many of the associations discussed, the results are controversial and negative studies exist [46,73,131,182,198,201,254,291,300].

The *HER2* polymorphism I665V has been investigated in several relatively large studies and the Val allele has been associated to an increased risk of breast cancer especially in women with early onset of and a family history of breast cancer [184,190,229,292,308]. There are however also negative studies, the largest by Benusiglio et al on more than 2000 cases and controls [32,62,118,150,195]. Skewed X-inactivation is also a suggested risk factor for both sporadic and non-*BRCA1/BRCA2* familial breast cancer [158].

1.4 THE SEARCH FOR ADDITIONAL PREDISPOSING BREAST CANCER GENES – PRESENT AND FUTURE

After the identification of *BRCA1* and *BRCA2* more than a decade ago, there has been no major break-through ("BRCA3") in the search for high-risk genes leaving a substantial part of the breast cancer families genetically unexplained for. Linkage analysis was used to find both *BRCA1* and *BRCA2* and two factors were of importance for their successful mapping [111,306]. First, the high penetrance associated with these genes made it possible to find families with many affected to use for studies. Second, a typical phenotype could be used to define families for linkage, for *BRCA1* early age of onset and for *BRCA2* the prevalence of male breast cancer. In fact, it has been shown that the majority of families with more than 6 breast cancer cases have mutations in one of the two known genes suggesting that other genes are likely to have a lower penetrance [89]. This fact in addition to of the lack of an identified unique phenotypic character to be used for defining families has made it difficult to find additional genes. One genome-wide

study on non-*BRCA1/2* families have been published which suggested a putative locus on chromosome 2q [132]. No other whole genome-wide screen have been published so far, however studies have focused on separate regions such as 6q, 8p and 13q [146,149,220,237,268,316].

It has been shown that tumours of *BRCA1/2* mutation carriers show different phenotypic characteristics compared to unselected tumours also with molecular techniques [119,284]. Similar approaches including gene expression profiling have been used to determine phenotypic characteristics of "BRCA3" tumours, as identification of homogenous subgroups could potentially increase the power of conventional genetic analysis [78,120,206]. In a study using micro array expression, 17 familial non-BRCA1/2 tumour clustered in two groups suggesting two predisposing loci in this cohort [120]. Comparative genome Hybridization (CGH) was used in a study on tumours from non-*BRCA1/2* breast cancer families and LOH and linkage analysis in the same families suggested a putative breast cancer locus on 13q [146]. This region was however not confirmed in a linkage study using families from the same region or in another study on families with different ethnicity [70,268]. Other studies using CGH have suggested chromosomes 6, 8, 17, 19 and 20 to be of interest [106,177].

The candidate gene approach to find new low risk breast cancer genes using single polymorphisms in association studies have also yielded limited success despite large effort. The association studies could be improved by choosing candidate genes not only from known biological function but also from linkage peaks generated from family based studies, animal models or by selecting subgroups of patients defined by a trait, (e.g. dense breast tissue) that is hereditary [214]. Instead of studying a single or a few polymorphisms in the candidate gene, all polymorphisms in the gene could be identified, either by resequencing the gene or by using data from the dbSNP database (http://www.ncbi.nlm.nh.gov/projects/SNP) which now contains nearly 9 or the around 11 million SNPs with a minor allele frequency of 1% or more that are estimated to exist in the human genome [159]. It has been shown that many SNPs are in strong linkage disequilibrium (LD) with closely located SNPs and a considerably lower number of so called tagSNPs can be chosen, either individually or in multimarker combinations, so called haplotypes, to capture most of the allelic variation in a region [96,145]. These tagSNPs can then be studied in larger case-control studies thus focusing on the whole gene rather than on a single SNP [214].

Genome-wide association studies have until now been impossible but the technical development of high throughput SNP analytical platforms and the completion of a genome-wide high density SNP map, will make it feasible [214,290]. Two main approaches to genome wide association studies have been proposed. The sequence based approach advocates screening for functional SNPs in coding regions of the genome meaning that 50 000 -100 000 SNPs would have to be genotyped [40]. This method would be able to detect lower frequency alleles (1-20%) but misses' functional noncoding SNPs [40]. With the haplotype approach, 200 000-500 000 tagSNPs are needed to detect disease alleles with a frequency of >5%, but there is no prejudice about types of SNPs which might be important [124]. For the purpose of design and analysis of genetic studies the international HapMap Consortium has created a haplotype map of the whole genome [13]. In addition to the common variant: common disease model there is also the possibility of multiple rare variants, exemplified by the rare *CHEK2* 1100delC variant in breast cancer [218,290]. For these variants, the genome wide approach might not work, and the candidate gene approach will still be valid [214].

In conclusion, the search for additional predisposing high or low-risk breast cancer genes is ongoing but is complicated by a heterogeneous disease and a complex multifactorial inheritance.

1.5 CLINICAL IMPLICATIONS OF FAMILY HISTORY AND BRCA1/2 MUTATIONS

1.5.1 Prevalence of family history and BRCA1 and BRCA2 mutations

1.5.1.1 Family history

In 5-10 % of breast cancer cases there is a pattern in the family of a highly penetrant dominant trait [196]. These families are often defined as hereditary breast cancer, however there is no international consensus on an exact criteria for this definition (cp. Amsterdam criteria for HNPCC). In general, three 1st or 2nd degree relatives with breast cancer in at least two generations, often with an age criteria, are required, but in some publications only 1st degree relatives with breast cancer are taken into account [9,69,216]. In further 18-23% of the breast cancer patients, there is a 1st or 2nd degree relative with the disease, this group sometimes defined as familial breast cancer or two close relatives [9,69,77,176]. Familial breast cancer may also be used to include all patients with a family history of the disease (sometimes also 3rd degree relatives), 10-31% in previous studies [9,69,77,137,176,187,228,279,303].

1.5.1.2 BRCA1/2 mutations in high risk families

It was estimated that mutations in BRCA1 and BRCA2 explained about two thirds of hereditary breast cancer [306]. Subsequent studies have however showed lower mutation frequencies and there are large variations in prevalence of mutations due to ethnicity of the population or to selection based on family history of breast and/or ovarian cancer, male breast cancer or young age at onset.

The highest prevalence is found in populations harbouring ***founder mutations***. These are mutations that have occurred in specific ethnic or isolated populations. Founder mutations exist in several populations and are most well characterised in the Icelandic and Ashkenazi Jewish populations [258]. In Iceland a single mutation, *BRCA2* 999del 5, is found in a majority of the breast cancer families [270]. In Ashkenazim, there are three founder mutations, two in *BRCA1*, 185delAG and 5382insC, and one in *BRCA2*, 6174delT accounting for >90 % of mutations in this ethnic group [276].

In families with both breast and ovarian cancer the mutation frequency is higher than in families with breast cancer only. In a large collaboration study of 237 families with at least four cases of breast cancer, 81% of the breast-ovarian cancer families were due to *BRCA1* and 14% to *BRCA2*. In families with breast cancer only 33% mutations in both genes were detected [89].

1.5.1.3 BRCA1/2 prevalence in Nordic countries

Scandinavian studies on *BRCA1* and *BRCA2* frequency in selected high-risk families vary between countries and also within Sweden probably due to different founder mutations and fairly stable populations with little migration. In Finnish breast and breast-ovarian cancer families 21% mutations in the high risk genes were detected, 10% in *BRCA1* and 11% in *BRCA2* [296]. In Norway four local mutations account for 68% of *BRCA1* mutation carriers and *BRCA2* mutations are very rare [188].

In a study of breast and ovarian cancer families in southern Sweden there was a *BRCA1* mutation frequency of 23%, and 11% mutations in *BRCA2* [110]. On the west coast of Sweden, there is a strong effect of a single founder, 3171ins5 in *BRCA1,* and 40% of high-risk families

are *BRCA1* carriers while only 3% are detected in *BRCA2* [81]. In 106 high-risk breast cancer families from the Stockholm region only one *BRCA1* mutation was found. In the same study 20 breast-ovarian cancer families were screened and 7 were *BRCA1* mutation carriers (35%) [314]. In *BRCA2*, only 2 mutations in 162 Stockholm families (1%) were detected [54]. In another study from Stockholm, where more strict criteria were used for screening, there were 7% *BRCA1* mutations in familial breast cancer and 34% in families with breast and ovarian cancer. Again, only 1% of the families showed *BRCA2* mutations [22]. In previous studies on *BRCA1* in patients from the Stockholm region, 70% of the mutations, including the main founders, have been detected in exon 11 [22,23,314].

1.5.1.4 BRCA1/2 prevalence in unselected breast cancer and in the normal population

The mutation frequency in breast cancer patients, not selected for family history or age varies. Several of these studies focus on founder mutations previously detected in high risk families in their own population, while a few have screened the whole gene/s. In the ethnically selected populations of Ashkenazi Jews and Icelanders, founder mutations are found in 7-12% [87,271,293]. In other studies, the reported mutation frequencies vary between 2% and 6% [17,51,207,257,288]. The lowest prevalence in unselected breast cancer, <1% BRCA1 mutations, was found in a population-based breast cancer cohort from the Stockholm region (Paper II).

In the general population the prevalence of BRCA1 mutations is estimated to be less than 0.1%[6]. In the Icelandic population 0.6% are carriers of the BRCA2 founder mutation and in slightly more than 2% of the non-cancer Ashkenazim one of the three founders are detected [87,271].

1.5.2 Penetrance of BRCA1 and BRCA2 mutations

The early estimates of the penetrance of BRCA1 and 2 were based on families showing linkage to either of the genes [75,88]. Families suitable for linkage studies are extreme high-risk families with multiple cases of breast and ovarian cancer and the estimates are therefore relevant to that type of families but might overestimate the risk for carriers with more modest family history. Another approach has been to use breast cancer patients, not selected for family history, in order to get less biased data. The most used group for these studies have been Ashkenazim as they have a higher prevalence of mutations [87,152,293]. In the largest of these studies, based on 1008 index cases, the lifetime risk of breast cancer among female mutation carriers was 82%, similar to risks in families with many cases. Lifetime risks for ovarian cancer were 54% and 23% for *BRCA1* and *BRCA2* mutation carriers respectively. The penetrance seemed to be higher in later birth cohorts [152].

In 1997, Struewing et al. presented a penetrance study, not based on cases but on >5000 Jewish non-cancer individuals, and in those identified 120 mutation carriers. The estimated risk of breast cancer, by the age of 70, was 56% and for ovarian and prostate cancer 16% each [252]. All the penetrance studies on Ashkenazim are based on the three founder mutations in this population. Another study of the penetrance of a founder mutation is from Iceland and population based. The estimated risk of breast cancer for female carriers of the 999del5 mutation in *BRCA2* was 37% by the age of 70 [272]. Interestingly, a recent publication on the Icelandic founder mutation showed a four-fold increase in penetrance over time (1920-2000) [277].

A metaanalysis of 22 population-based studies including 500 mutation carriers estimated a risk of breast cancer, by age 70 of 65% in *BRCA1* mutation carriers. The corresponding figure for

BRCA2 was 45%. The risk of ovarian cancer was 39% and 11% respectively [18]. Also this study showed a reduction in risk in women from earlier birth cohorts.

These penetrance studies assume that all truncating mutations confer the same cancer risk. However, there is some evidence that the mutation position in the gene influences the risk. For BRCA1, mutations in the central region of the gene seem to lead to a lower risk for breast cancer than other mutations, whereas mutations toward the 3' end have a lower proportion of ovarian cancer [99,266]. In *BRCA2*, there is an "Ovarian Cancer Cluster region" (OCCR) in the central region, where mutations are associated with a higher ovarian cancer risk and lower risk for breast cancer than other mutations in the gene [98,265].

The interindividual variability in cancer risk among BRCA1/2 carriers may also be explained by risk modifying genes either in the DNA repair pathway (figure 1) or other low risk candidate genes, individual characteristics (e.g. reproductive history) and exogenous exposure (oral contraceptives, HRT, smoking) [221]. There are only a few association studies on potential genetic modifiers (e.g.*RAD51, HRAS1, AR*) but no validated data as yet [221]. Studies on reproductive factors suggest an influence on breast cancer risk also in *BRCA1/2* carriers [221]. Early age at menarche has been associated with an increased risk of breast cancer among women with *BRCA1* (but not *BRCA2*) mutations and parity seems to be have an opposed effect on risk in *BRCA1* vs. *BRCA2* carriers [63,105,155]. Long term breast feeding has been shown to decrease breast cancer risk in *BRCA1* carriers while the effect of oral contraceptives on breast cancer risk in *BRCA1/2* carriers is still unclear [105,142,143,192,221].

1.5.3 Phenotypic characteristics of familial breast cancer

1.5.3.1 Family history of breast cancer

Most studies on family history and phenotypic characteristics of breast cancer have focused on frequency of bilateral tumours and age at diagnosis. Bilaterality seems to be slightly more frequent in patients with family history, the difference in several studies however often not large enough to reach statistical significance [10,15,69,137,170,228]. There is no convincing data that a family history of breast cancer is more prevalent in cases with young age of onset, even though this might be a common clinical apprehension [9,15,137 ,170,228].

1.5.3.2 BRCA1 mutation carriers

Mutations in BRCA1 mainly confer a considerably elevated risk for female breast and ovarian cancer but the risk for cancer of the cervix and corpus uteri, the fallopian tubes and the peritoneum is also higher than in non-carriers. The risk of cancer in male carriers does not seem to be increased [267].

The breast tumours from *BRCA1* carriers differ from those in non-carriers. The majority of the tumours are histologically ductal and of grade III with mainly a higher mitotic count reflecting in a high S-phase. Medullar or atypical medullar tumours are more often found in *BRCA1* carriers and the tumours often have a heavy lymphocyte infiltration or so called pushing margin [2,144]. Additionally, the *BRCA1* positive patients generally have hormone-receptor negative as well as HER-2 negative tumours [144,162]. There are also more somatic p53 mutations in tumours from BRCA1 patients [162]. Recently, it was also shown that *BRCA1* related tumours, much more often are of a basal epithelial type than other breast cancers, a feature that can be recognised with immunohistochemistry, and might be useful in the clinic in recognising potential mutation carriers [93].

1.5.3.3 BRCA2 mutation carriers

Germ line mutations in *BRCA2* are associated with breast cancer in both female and male carriers and ovarian cancer. In addition, there is a two-fold relative risk for other cancers in both men and women, the risk most pronounced for cancer of the prostate, pancreas and the gallbladder/bile duct [4]. In contrast to BRCA1 induced tumours, breast tumours of BRCA2 carriers show no significant differences compared to non-carriers regarding histological features and hormone-receptor expression [2,162].

1.5.3.4 Prognosis

Just as for phenotypic characteristics and familial breast cancer it is hard to compare prognostic data on familial breast cancer in studies with a different classification of family history. However, in most studies there is no statistically significant prognostic difference related to a family history of the disease, even though in several studies familial patients tend to do slightly better than sporadic patients [52,228,262,279].

Tumours of *BRCA1* carriers are usually characterized by several negative prognostic factors (high grade, lack of hormone receptor expression). In a review of 10 studies on mutation carriers and breast cancer prognosis published in 1999, there was no statistically significant difference in survival in 8 studies, and in two small studies carriers did worse than non-carriers [52]. Other, more recent studies have shown a higher risk for contra lateral recurrence but other outcomes were not significantly different in carriers compare to patients without family history [76,79,298]. In a study on Ashkenazi women undergoing breast-conserving treatment for invasive breast cancer, *BRCA1* mutations, but not *BRCA2* mutations, were associated with reduced survival, but the poor prognosis was mitigated by adjuvant chemotherapy. The risk for metachronous ipsilateral disease did not appear to be increased for either *BRCA1* or *BRCA2* mutation carriers, at least up to 10 years of follow up [223].

.

AIMS

Paper I: Define the proportion of different levels of family history in a cohort of population-based breast cancer patients, and to determine whether familial breast cancer have phenotypic characteristics, including prognosis, different from those of sporadic patients

Paper II: Investigate the prevalence of *BRCA1* mutations in a population-based material of breast cancer patients from the Stockholm region, and determine whether there is a reason to screen breast cancer patients, not selected for family history, for *BRCA1* mutations.

Paper III: Evaluate the prevalence of the rare truncating variant *CHEK2* 1100delC in Swedish sporadic and familial breast cancer and if possible, clarify its role as a modifier or a low risk gene.

Paper IV: Clarify the role of the C975G variant in *ER alpha (ESR1*) as a low penetrance susceptibility allele in sporadic and familial breast cancer.

Paper V: Investigate the role of three polymorphisms in *ER beta (ESR2)* as low penetrance susceptibility alleles in sporadic and familial breast cancer.

MATERIAL AND METHODS

1.6 MATERIAL

1.6.1 Patient cohort Södersjukhuset / Huddinge (studies I-V)

From October 1998 to May 2000 all new patients with a surgically treated primary invasive breast cancer admitted to the Department of Oncology at Huddinge University Hospital and Söder Hospital (Södersjukhuset/SÖS) (covering the population of 850 000 people in southern Stockholm) were asked to take part in a research study on BRCA1/2 and other genetic risk factors in unselected breast cancer. The inclusion of the patient was done after oral and written consent and took place at the postoperative visit or, if more appropriate, at a subsequent visit. A blood sample was then obtained.

A questionnaire was given to all patients to be completed on their first visit to the clinic. Information was obtained regarding breast- and ovarian cancers and other cancers including age of diagnosis in other family members (parents, siblings, maternal/paternal grandparents, maternal/paternal aunts and uncles and others). There was no confirmation of the diagnoses of the relatives from pathology reports. Patient data on hormonal factors (parity, age at first delivery, age at menarche and menopause, use of contraceptives or HRT) and former cancer diagnoses was also obtained, and sometimes supplemented with information from the patients' records.

If the patient had a previous breast cancer, the patient was considered eligible if the present cancer was contralateral, or ipsilateral with a different histopathological and/or receptor pattern and localized in another sector of the breast. If the two cancers had similar properties, the latter was regarded as a local recurrence and the patient was excluded from the study.

All patients included in the study were offered the possibility of later knowing the result of the *BRCA1* screening and if appropriate at the time were referred to genetic counselling.

1.6.1.1 Classification according to family history (Paper I and II)

Patients were classified according to their level of family history on either maternal or paternal side in the following groups. If affected relatives were reported on both maternal and paternal side, the patient was classified according to the most prominent family history.

- Breast-ovarian cancer family: Patients with both breast and ovarian cancer or ovarian cancer in any 1^{st} – 3^{rd} degree relative
- Familial breast cancer: Patients with two or more 1^{st} or 2^{nd} degree relatives with breast cancer.
- Two close relatives: Patients with one 1^{st} or 2^{nd} degree relative with breast cancer.
- Cancer family: Patients with three or more 1^{st} or 2^{nd} degree relatives with cancer, other than breast or ovarian cancer, or two cancer cases in 1^{st} degree relatives.
- Sporadic: If the patient did not fit into any of the groups mentioned, the patient was classified as sporadic and then sub-grouped according to age at onset: 35 years or younger, 36 to 50 years and over 50 years, with the exception of those not knowing their family cancer history.

For the comparison of different levels of family history and clinical parameters in paper I the breast-ovarian cancer families and familial breast cancers were put together in a ”high risk familial breast cancer” group. The group of two close relatives and cancer families, who hypothetically can harbor low risk genes formed a ”low risk familial breast cancer ” group and the sporadic cases were presented as 50 years or younger, or older than 50 years. As a comparable group to the ”young sporadic cases”, the familial high and low risk patients, 50 years or younger were united in a ”young familial group”.

1.6.1.2 Classification according to family history (Paper III-V)

In association studies of candidate low risk genes there is less evidence to use ovarian cancer to define genetic susceptibility for breast cancer. The breast-ovarian cancer families were therefore reclassified according to breast cancer family history only. The cancer families were classified as sporadic and those with unknown family history are also included in the sporadic cases. Three groups were formed:

- High-risk familial breast cancer: Three 1st or 2nd degree relatives with breast cancer.
- Low risk familial breast cancer: Two 1st or 2nd degree relatives with breast cancer
- Sporadic: No family history of breast cancer

1.6.1.3 Clinical parameters (Paper I-IV)

Information on clinical parameters (age at onset, age at menarche and menopause, parity, age at first delivery, oral contraceptives, HRT, tumour characteristics including bilaterality, stage, grade (Elston-Ellis) and hormone receptors (ER, PgR), method of surgery, adjuvant therapy, recurrences and deaths from breast cancer and new breast primaries) was obtained from the patient’s questionnaire, medical record and pathology reports.

1.6.2 Patients from Department of Clinical Genetics (Paper III-V)

In paper III-V, the population-based cohort collected at SÖS/HS was supplemented with breast cancer patients collected at the Department of Clinical Genetics at Karolinska University Hospital. These patients were selected because of their family history and had been collected either as part of a previous research project or in the clinic (after informed consent) [169]. Altogether 350 cases were used, 248 cases in paper III, 265 cases in paper IV and 259 cases in paper V.

These patients were defined according to their family history as described for the SÖS/Huddinge cohort (Paper III-V) and 224 had high risk and 126 low risk family history. All cases had proceeded through genetic counseling and those fulfilling the criteria for BRCA1/2 screening (Table 4) had been screened negative for mutations. For these cases family history was known and for most cases age at diagnosis (missing in 9 patients). The mean age at diagnosis was 54 years (24-92 years).

1. Two or more 1st or 2nd degree relatives with at least one case of breast cancer and one case of ovarian cancer or a single individual with both breast and ovarian cancer.
2. Three or more 1st or 2nd degree relatives with breast cancer, at least one with onset before 51 years of age
3. Families with only two 1st or 2nd degree relatives with breast cancer, at least one with onset before 41 years of age
4. One single individual with breast cancer before 36 years of age
5. Two or more 1st or 2nd degree relatives with ovarian cancer
6. One single woman with ovarian cancer before 51 years of age.

Table 4: *The Stockholm criteria for BRCA1/2 screening* [22].

1.6.3 Controls (Paper III-V)

As controls for paper III-V, we used DNA from blood-donors collected as control material for association studies at Karolinska University Hospital, Stockholm, Sweden.760 controls were used in paper III, 665 in paper IV and 480 in paper V. The material is anonymised, but information on gender of the individuals is available.

1.7 METHODS

DNA was obtained from the collected blood samples, using standard phenol/chloroform extraction.

1.7.1 PTT (Paper II)

The Protein Truncation Test (PTT) is an in vitro coupled transcription and translation method for detecting protein truncating mutations (Figure 5) [125].
Advantages of PTT are that it is a relatively quick, robust and simple method to screen large exons for disease causing mutations. Mutations close to the start or the end of a transcript might however escape detection (can be avoided by using overlapping DNA fragments) and large deletions, rearrangements or insertions are not identified. Mutations detected by PTT are confirmed, and the exact position identified, by direct sequencing.
PTT is used on DNA and is suitable for mutation screening of the large exon 11 in BRCA1 and exon 10 and 11 in BRCA2. If smaller exons are screened with PTT, cDNA is required.

1.7.2 Multiplex PCR (Paper III)

Multiplex Polymerase Chain Reaction (PCR) is a variant of PCR, which enables simultaneous amplification of several targets in one reaction by using more than one pair of primers [49]. Several CHEK2 exons, including exon 10 where 1100delC is located, are duplicated across the genome [246]. These homologous duplications or amplicons result in similar, non-functional copies, which have to be avoided when examining the functional CHEK2 gene. In paper III, two separate pairs of primers were used, one pair specific for the mutation 1100delC and one pair specific for the wild-type. The two reactions were run separately, each in multiplex with a control PCR. The products were separated on Agarose gels.

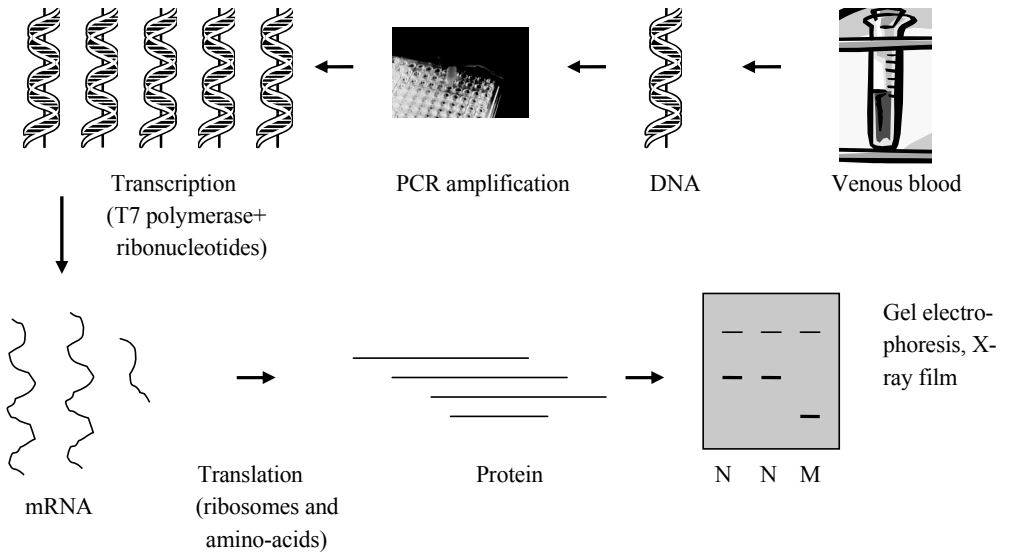

Figure 5. *PTT: Sequences for T7 RNA polymerase and a translation initiation site are incorporated in the PCR product in a first PCR reaction (amplification of the DNA segment of interest) and in a second step the PCR product is incubated with T7 polymerase, ribonucleotides, rabbit ribosomes and a mixture of amino acids, one of which is radio-labelled. Under suitable conditions, transcription of the PCR product to mRNA and a subsequent translation to a protein occur. The synthesized peptides are then separated by gel electrophoresis and the bands visualized on an X-ray film. If the protein product is shortened by nonsense or a frame shift mutation, an extra band will be detected on the film.*

1.7.3 Pyrosequencing (Paper IV and V)

Pyrosequencing is an approach for real-time DNA sequencing and is widely used for SNP detection [224]. The pyrosequencing enzymatic cascade starts with the release of pyrophosphate (PPi) as a result of nucleotide incorporation by DNA polymerase. The PPi formed in the DNA polymerase reaction is converted to ATP by ATP sulfurylase and the ATP production is used to generate light by the firefly luciferase, seen as a peak in a pyrogram (Figure 6). Unincorporated nucleotides are degraded by apyrase before adding the next nucleotide, allowing a repeated addition of nucleotides. The sequence can be determined since the added nucleotide in each step is known.

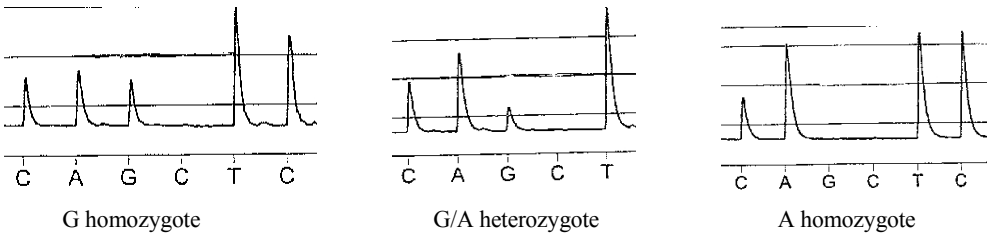

Figure 6: *Pyrogram readouts from genotyping of polymorphism rs 928554 in ERβ.*

1.7.4 Restriction fragment length polymorphism, RFLP (Paper V)

Inherited polymorphisms might be located in a cleavage site for restriction endonucleases. If a single base change (SNP or mutation) is present in a particular cleavage site in one of the DNA molecules the site will no longer be recognized by the restriction endonuclease and the different sized DNA fragments can be detected by Southern blot.
RFLPs are used as genetic markers in whole genome linkage studies but also in association studies of SNPs (Figure 7).

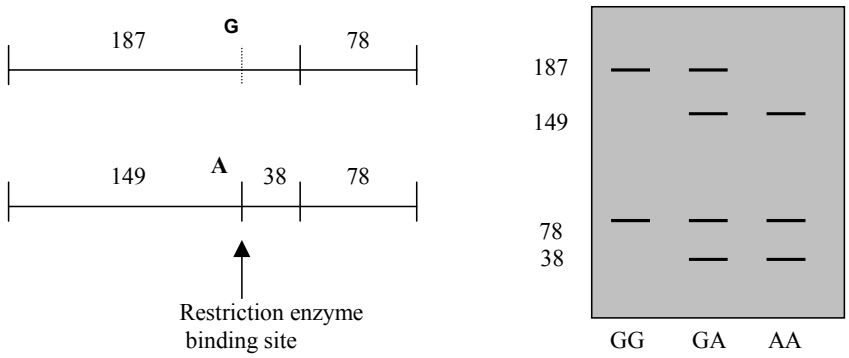

Figure 7. *In paper V, RFLP was used to evaluate the rs928554 SNP instead of pyrosequencing, due to the suboptimal sequence surrounding this variant. Samples homozygous for the G allele demonstrated 2 bands upon digestion, one187 bp band and a second of 78bp. Heterozygote samples produced 4 bands of size 187, 149, 78 and 38 bp each. The A allele homozygous variant samples produced 3 bands of size 149 bp, 78 bp and 38 bp each.*

1.7.5 Association analysis (Paper III-V)

Genotypic and allelic data was compared between cases and controls using the chi-square test or if more appropriate the Fisher´s exact test. Odds ratios were calculated with 95% confidence intervals with wt/wt as reference genotype. The genotype frequencies in controls were found to be in Hardy-Weinberg equilibrium (HWE).

In the *ER beta* (Paper V) we evaluated three polymorphisms both separately in cases and controls and as haplotypes. A haplotype is the physical arrangement of loci along a chromosome or in a single gene. Linkage disequilibrium (LD) refers to the fact that alleles at neighboring sites can co-occur on the same haplotype more often than is expected by chance and can be measured by |D´| ranging from 0 (no disequilibrium) to 1 (complete disequilibrium) [283]. Haplotype testing, instead of testing separate SNPs, may increase the power in association studies [25]. In a candidate gene, there may be tens or more SNPs, and a negative association does not rule out another important variant in the gene. With the haplotype approach, it is possible to investigate the whole gene, using only a few markers so called tagging SNPs, since closely located SNPs often are in LD with each other [214]. It may also be that a specific haplotype harbor two or more variants that are functionally important only if they occur together, and the effect is not detected if the single polymorphisms are analyzed separately [214].

There are several algorithms used to define haplotypes blocks from unphased genotype data on single SNPs, one of them developed by Gabriel et al [96]. This method is based on pairwise estimates of |D´| and each comparison is called strong LD, inconclusive or strong recombination. A block is constructed if 95% of informative comparisons are in strong LD. In paper V we used the computer programs Haploview v 3.1.1, which is based on this algorithm and the UNPHASED program (including COCAPHASE) to estimate LD in the region and association of inferred haplotypes [28,71].

RESULTS AND DISCUSSION

A total of 785 patients underwent surgery for breast cancer and received postoperative treatment / follow-up at the Department of Oncology at Huddinge University Hospital and Södersjukhuset (SÖS) during the period. 89 of these were identified through the registration at the Oncologic Center (OC) in Stockholm after the study was completed and were never registered in the study due to logistic problems. Of the remaining 696 patients, 489 patients, all women, were willing to take part in the study (70%). In total 207 patients, including 1 man, were registered in the study but did not participate, mainly because they were not invited but also because of co-morbidity like dementia or in some cases declined participation. In total, 62 % of the eligble patients were included in the study (489/785) (Figure 8).

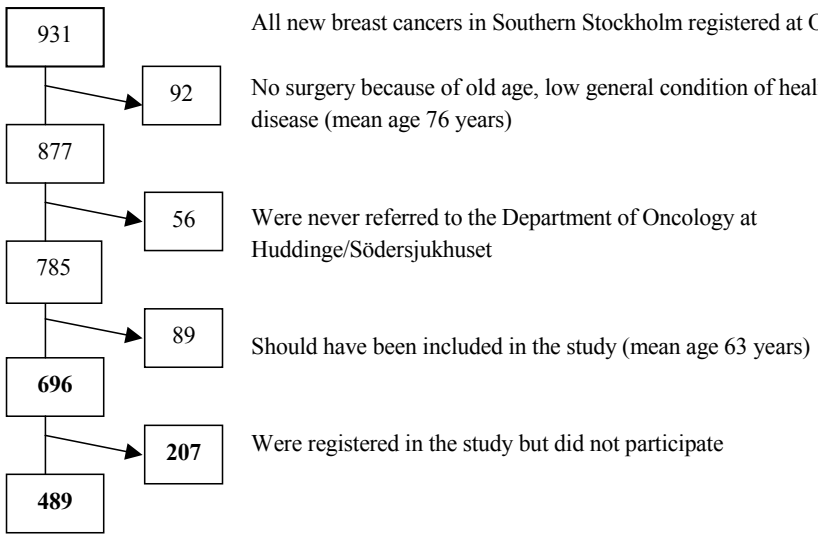

Figure 8. *Patients in the population-based cohort from SÖS/Huddinge.*

The non-participating cases (n=207) were slightly older than the participating group (p<0, 01), but there was no significant difference in stage (p=0.36) or in the rate of self-reported family history between groups (p=0.70) (Table 4).

In the southern part of Stockholm there is a relatively high rate of immigrants and information on ethnicity was obtained from the patient's record. In 46 cases there was a non-Swedish background (in 3 cases only assessed from the name only) and 30 of these had European descent (12 from Nordic countries), 7 were from South America, 4 from the Middle East, 1 from the East Asia and one from Africa.

	Participating (n=489)	Non-participating (n=207)
Mean age at diagnosis (years)	60	64
Minimum-maximum age	27-88	25-95
Family history		
Ovarian cancer in patient or close relative	3%	2%
Familial breast cancer	6%	5%
One 1st or 2nd degree relative w. breast ca.	22%	22%
Sporadic (no breast or ovarian cancer)	66%	66%
Not known (e.g. adopted)	2%	5%
Stage		
I	56%	49%
II	37%	43%
III	6%	7%
IV	0%	1%

Table 4: *Comparison participating vs. not participating patients*

1.8 PAPER I

Family history is an important risk factor for breast cancer and a dominant inheritance pattern is seen in 5-10% of the cases [196]. This high-risk group has been the focus of an intense research for high-risk susceptibility genes during the past two decades. However, the possibility that low-penetrance genes may play an important part in breast cancer susceptibility and the result of recent twin studies suggesting that a more substantial share of the breast cancer susceptibility is due to genetic factors than only the high risk families, has created a renewed interest in the more modest familiality described by earlier epidemiological studies [11,167,211-213].

In this study we wanted to define the proportion of different levels of family history in a population based cohort of breast cancer and to identify possible differences between familial and sporadic patients concerning age at onset, hormonal background, tumor characteristics and prognosis.

In total 174 (35%) of the 489 patients in the study reported a family history. Almost 10% had either high-risk familial breast cancer or familial breast-ovarian cancer, while the remainder was classified as low risk familial breast cancer (Figure 9). Among the patients 50 years and younger at onset (n=100), 40% reported a family history compared to 35% of those, older than 50 years at onset (n=389). 25% had solely paternal family history, a group that often is missing in studies on family history and breast cancer because only 1st degree relatives are included. Many of the high-risk families were small and most were defined as late-onset according to age at onset of the major part of affected family members (Figure 10).

Age at onset was similar in the familial and sporadic cases (59 vs. 61 years, p=0.14). Regarding hormonal factors, there was no major difference either, and a family history of breast cancer did not influence women in a deterrent way on parity, use of oral contraceptives or HRT.

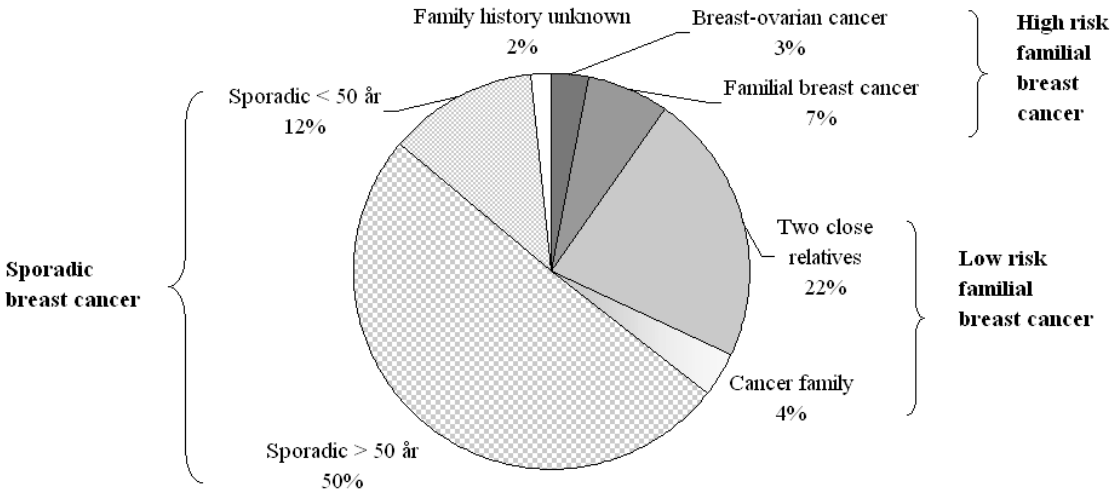

Figure 9. *Family history in unselected breast cancer patients (n=489).*

Tumor size did not differ according to family history but younger women (≤ 50) tended to have larger tumors, at least in part due to the mammography screening program in Stockholm starting at the age of 50 at the time for the study. There were more node-positive patients in the young sporadic group compared to the familial patients of the same age (52% vs. 32%, p=0.06), without obvious explanation in other tumor characteristics like grade and hormone receptor positivity.

As anticipated because of the regional and national guide-lines concerning breast cancer treatment, family history had no major impact on the method of surgery or adjuvant treatment although interestingly, more young node-negative patients with a family history received chemotherapy, compared to sporadic patients (44% vs. 31%).

Regarding prognosis, there was no statistically significant difference in recurrence free survival between patients with a family history of breast cancer and sporadic patients after a median follow-up of 4.7 years. If the high-risk familial group was analyzed separately, there was a non-significant advantage in the multivariate analysis (controlling for age at diagnosis, tumor size and nodal status) with a HR of 0.73 (95% CI 0.3-1.8) compared to the sporadic group. Due to few events (n=76), we chose to analyze all recurrences together (loco regional recurrence, distant recurrence and contra-lateral breast cancer). Recently there was a Swedish publication on the risk of contra lateral breast cancer in patients with a family history of breast cancer [238]. In this study, there was a 27% risk of developing a contralateral breast cancer after 20 years follow up compared to the expected risk of 5% among breast cancer patients in general. In our study cohort, where we currently have a follow up of 5.5 years, 5% (8/164) of the familial patients have been diagnosed with a new contralateral breast cancer compared with 3% (10/307) of the sporadic patients. Altogether18 new malignancies other than breast cancer, 8 in sporadic patients (3%) and 10 in familial patients (6%) have been diagnosed during follow-up (among them 6 endometrial tumors, 4 lung cancers and 2 ovarian cancers).

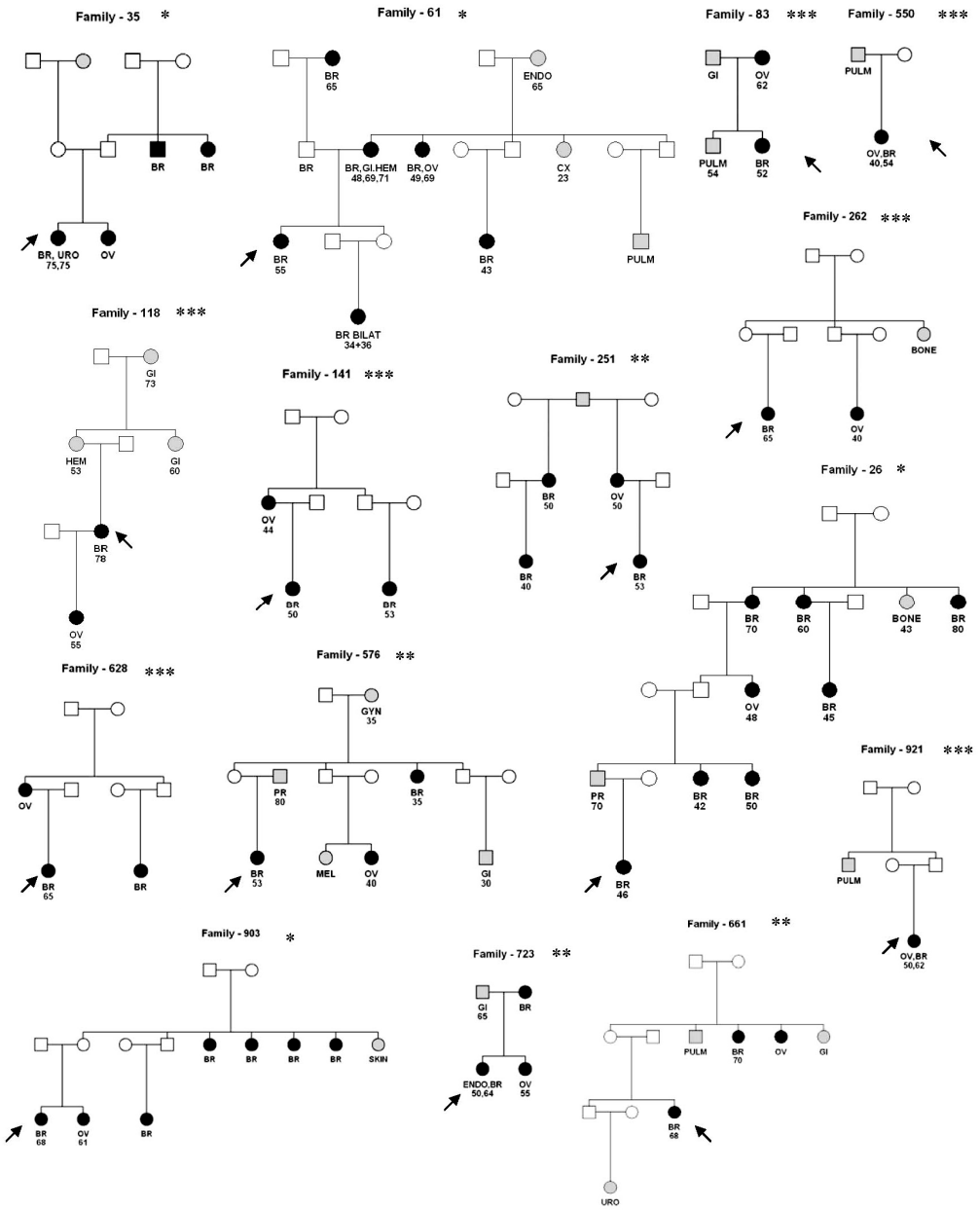

Figure 10: *Pedigrees of patients classified as breast-ovarian cancer families in paper I and II. In paper III-V these families were reclassified to * high familial breast cancer, ** low risk familial breast cancer and *** sporadic breast cancer. Black symbols are breast or ovarian cancer cases, grey symbol indicates another malignancy, number under the symbol is age at diagnosis, if known. BR=breast cancer, OV=ovarian cancer, ENDO= endometrial cancer, PR=prostate cancer, HEM=hematological malignancy, MEL=malignant melanoma, URO= urological tumour other than prostate, PULM=lung cancer, GI=gastrointestinal malignancy, GYN=gynecological malignancy .*

1.9 PAPER II

The mutation frequency of BRCA1 and BRCA2 in women with breast cancer varies according to family history, age at diagnosis and ethnicity. Previous studies on the contribution of mutations in these genes in breast cancer unselected for age and family history has reported mutation frequencies between 1% and 12%, however screening methods, ethnicity, study size and to what extent the gene/s have been screened have differed. In familial breast cancer from the Stockholm region, previous studies had shown a comparatively low frequency of BRCA1 and almost none in BRCA2 but less was known of the mutation frequency in patients without family history or very young age. We therefore wanted to clarify the proportion of BRCA1 mutations in unselected breast cancer cases from the Stockholm region.

Two BRCA1 mutations were identified in the population-based study cohort of 489 individuals with a new diagnosis of breast cancer (0.4%). Both mutations were found in families with both breast and ovarian cancer.

Since the screening was limited to exon 11, 0.4% is an underestimation, however in previous studies from Stockholm more than 70% of the mutations in BRCA1 were detected in exon 11, including the Stockholm founders [22,23,314]. There is a possibility that the mutation spectrum might be different in unselected patients but this is not supported by the literature [18]. Large genomic rearrangements are not detected with PTT or other common screening methods including sequencing, but PTT has otherwise proven a reliable method for exon 11 screening with high sensitivity and specificity of disease causing mutations [125]. Our estimate of the mutation frequency in unselected patients from the Stockholm region is ≤1%, which internationally is a low figure, probably reflecting the relatively low rates detected also in high risk families in our region.

In conclusion, BRCA1 mutations are rare in unselected breast cancer cases in the Stockholm region and are likely to be found only in high-risk patients, especially in families with both breast and ovarian cancer. There is no need to screen breast cancer patients in general for BRCA1 mutations. Family history should be used to define those new breast cancer patients who would benefit from genetic counseling.

All patients included in the study were offered the possibility of knowing the result of the BRCA1 screening. 105 patients have shown interest, and 38 were informed and could discuss their family history and risk on a medical visit, free of charge, while the remainder chose a telephone call from the principal investigator. 14 patients have been referred for proper genetic counseling. Two more mutation carriers have been detected in this cohort through clinical mutation screening, one patient from a breast-ovarian cancer family had a mutation in BRCA1 and one patient from a premenopausal breast cancer family had a mutation in BRCA2.

1.10 PAPER III

The protein truncating variant 1100delC in CHEK2 is almost the only low risk variant which has, with certainty, been shown to associate with breast cancer. The variant was first shown to associate with familial non-BRCA1/2 breast cancer and a large pooled study has also reported an effect in unselected breast cancer with a RR of 2 [8]. However, if the variant acts a modifier of still unknown high-risk gene/s or is a low risk variant on its own is unclear. The variant is rare, and its frequency in the normal population varies between countries.

In our control material consisting of 760 blood-donors from the Stockholm region, 0.7% carried the variant which is a lower prevalence than seen in the "high prevalence" countries Finland and the Netherlands but higher than in Central or Southern Europe, where the variant is non-existing. There are two other recent Swedish studies on CHEK2 1100delC, one in prostate cancer and one in breast-colon cancer families, and their control group carrier frequencies were 1%, both studies however smaller than the present study [136,280].

Among the familial patients, 2.3% carried the variant, similar in high and low risk cases but only one of the sporadic patients was a variant carrier (0.3%). In this study, as well as in paper IV and V, the study material consisted of patients recruited from two sources, the population based material from the Department of Oncology at SÖS/Huddinge and the patients from the Department of Clinical genetics, selected on family history. If separately analyzing the population based cohort, the variant frequency was 1.1% (5/452) which is slightly higher than in the controls (p=0.41)(Table 5).

Interestingly, the mean age was 10 years lower in variant carriers than in non-carriers and this was found in both materials constituting the study population indicating a true difference despite small sample size ($p<0.01$ in the combined material). As anticipated, the variant did not segregate with disease in the families where DNA was available from more family members. Our data, with an association of the variant only in patients with a family history of breast cancer and a possible influence of the variant on age at onset, rather support the role of the CHEK2 1100delC as a modifier than being a low risk gene of its own.

Although there is an association of CHEK2 with breast cancer, the effect is modest and the variant rare. In the clinical setting, there is no need for CHEK2 1100delC screening at present but the variant might prove interesting in combination with other genetic/non-genetic factors in the future.

	CHEK2 1100delC+ /total tested	**p-value[1]**
Familial Risk cohort	**5/247 (2.0%)**	0.07
Population-based cohort	**5/452 (1.1%)**	0.51
Sporadic breast cancer	1/313 (0.3%)	0.68
Familial breast cancer	4/139 (2.9%)	0.04
Controls	**5/760 (0.7%)**	

Table 6. *Prevalence of CHEK2 1100delC in familial risk cohort from Department of Clinical Genetics and in Population based cohort from SÖS/Huddinge compared to controls.* [1] *p-values were calculated with Fisher´s test for association.*

1.11 PAPER IV

Estrogen plays a central role in both normal mammary development and breast carcinogenesis and the estrogen receptors ERα and ERβ are obvious candidate breast cancer susceptibility genes. We chose to study a common synonymous SNP (pro/pro) in exon 4 of ERα (C975G, rs18011132) a variant previously studied with non-consistent results in breast cancer, the studies however small and the material selected in different ways.

In our material of 388 familial cases subdivided in high and low risk, 288 sporadic cases and 653 controls, the frequency of the C975G variant in ERα was lower in high risk familial cases compared to controls (18 versus 22%, p=0.046). The odds ratio for GG homozygotes compared to CC homozygotes was 0.2 (95% CI 0.06-0.8). There was no association of the variant with sporadic or low risk familial breast cancer. If the population based material from SÖS/Huddinge was analyzed separately, there was no difference in variant frequency in breast cancer cases (22%) compared to controls. Interestingly, in the group of bilateral cases (n=39), a group hypothetically enriched for genetic susceptibility, none were GG homozygotes and the G allele frequency was 19%, thus similar to the high-risk familial group.

Several studies, all however small, contradict our result (Table 2) [138,225,294]. There is some support in studies presented as negative [64,248,295]. In a recent large Swedish study on postmenopausal breast cancer, there was a slightly lower G allele frequency in cases compared to controls (20% vs. 23%, NS) [295]. In this study there was an association with the C975 variant and ductal, but not lobular cancer. Two studies have shown a lower G allele frequency in familial cases only and one small study suggested a protective effect of the GG genotype in the Korean population where the G allele is more common than in Caucasians [113,129,249].

In the last few years there has been a rapid technical development of SNP analysis and molecular knowledge including the dbSNP database and the completion of the HapMap project [13,159]. Two recent large studies have focused on several polymorphisms in the ERα gene, one Swedish study on postmenopausal breast cancer and an American study made by Gold et al. [103,295]. The Swedish study focused on 5 variants including the two RFLPs in intron 1, the C975G variant in our study, the promoter $(TA)_n$ repeat and a rare synonymous SNP in exon 3. Two haplotypes, both including the C975G SNP and either of the RFLPs in intron 1 were shown to associate with ductal, but not lobular cancer especially for women with high BMI [295]. Unfortunately the potentially interesting promoter variant $(TA)_n$ was not possible to evaluate due to problems with HWE in the controls. The American study is the most thorough and focused on 17 variants in the ER alpha gene, and observed three common haplotypes, composed of 8 SNPs including the C975G variant, that were associated with a decreased risk of breast cancer, and another haplotype that was associated with an increased risk [103]. When testing the SNPs separately there was no overall statistically significant association, however in a subgroup of Ashkenazi Jewish cases, three SNPs were associated with disease, one in the promoter and the other two in exon 1 and intron 1 respectively. There was no information on family history in this study.

In the Huddinge/SÖS patient cohort there was a possible association with the C975G variant and hormone receptor status of the breast tumour. In patients with ER negative tumours, 20% were CG heterozygotes compared to 35% of ER positive patients (p=0.022). If this was a true association, a lower frequency of GG homozygotes, among ER negative patients, would be expected, which was not seen. The group of GG homozygotes with information on ER in the tumour was however small (n=15) and a previous Korean study support a correlation with the G-allele and hormone receptor expression, although there are also negative studies [138,147,225]. There

was no difference in genotype distribution in the SÖS/Huddinge cohort of patients regarding histology (ductal vs. lobular), PgR status of the tumour or age at diagnosis (≤50 years vs. > 50 years).

In conclusion, our data indicate an association of the common exonic synonymous (Pro/Pro) variant C975G in ERα with a protective effect on familial high-risk non-BRCA1/2 breast cancer susceptibility. The high-risk families are believed to segregate high-penetrance alleles, which may be modified by this variant or another variant in linkage disequilibrium with this. Further studies should use strategies in which a comprehensive set of SNPs, in and upstream ERα, are identified either through resequencing or using data from the dbSNP database. These should be validated and a smaller set used to tag common haplotypes in the gene in a larger independent case/control material with thorough knowledge of family history and clinical information.

1.12 PAPER V

The second estrogen receptor, ERβ, was identified 1996 and there are only a few reports on its possible association with breast cancer susceptibility (none when our study was initiated). We chose to study three common polymorphisms in the ERβ gene; G1082A in exon 5 (rs1256049), G1730A in the 3´untranslated region (3´UTR) of exon 8 (rs4986938) and a G>A polymorphism located 56 bases 3´of the ERβ alternative transcript cx (exon 9) for association with breast cancer (Figure 4). These SNPs were identified through publications where the whole gene had been screened for mutations and were found to have a minor allele frequency above 1% [197,226]. Our material consisted of 723 breast cancer cases, divided accorded to family history in sporadic (n=323) and familial breast cancer (n=400), and 480 controls. The familial patients were further subdivided into 212 familial high-risk cases and 188 familial low risk cases.

The SNPs were genotyped by pyrosequencing (G1082A and G1730A) or RFLP (cx+56A/G). The genotype and allele frequencies of each variant was tested in each of the three breast cancer groups (high risk familial, low risk familial and sporadic) and compared to controls. There was no overall statistically significant difference in genotype distribution in cases and controls. In the low risk group, there was a suggestively protective effect for heterozygotes of the G1730A variant (p=0.09, OR 0.73, 95% CI 0.50-1.05) and a similar non-signficant elevated risk was seen with the G allele of the G1082A SNP in sporadic cases (p=0.08, OR 1.6, 95%CI 0.98-2.97). If the population-based cohort from SÖS/Huddinge (n=474) was tested separately compared to controls there was no overall difference for any of the genotypes either.

There is a large amount of linkage disequilibrium in the ERβ gene[103] and all three SNPs were shown to be in strong LD, with |D´|values ranging from 0.86-1.00. We then included our SNPs in haplotype construction and these inferred haplotypes were tested for association in each of the breast cancer groups and the controls. We identified seven different haplotypes in our material, two of which we considered rare (frequency<0.005%). There was an increased risk of breast cancer for one haplotype 1-2-2 (G-A-G) in sporadic breast cancer compared to controls (p=0.03) and the contrary haplotype 2-1-1 (A-G-A) was associated with a decreased risk of breast cancer (p=0.03). In the high-risk familial group the haplotype frequencies were similar as in the controls.

Our results indicate possible effect of inherited ERβ haplotypes and breast cancer susceptibility in patients without an extensive family history. Only one other study has used the haplotype approach when examining ERβ for association with breast cancer. Gold et al identified a haplotype consisting of 7 SNPs including our three, which was associated with an increased risk

of breast cancer in an Ashkenazi Jewish population and also could narrow the region further to identify a related haplotype (5 SNPs including ours) which was also associated with breast cancer risk (p=0.001). Three other studies on individual SNPs in ER beta have been negative in relation to breast cancer, these however smaller in study size (Table 3) [91,117,135]. Even though the SNPs in our study are probably not causative (synonymous or untranslated) they may be in LD with an as yet unidentified causative variant/s within or close to the ERβ gene. Further larger studies, preferably in materials like ours with a well-defined family history, of the ERβ locus are required to fully understand its role in breast cancer susceptibility.

.

CONCLUSIONS

Around 35% of breast cancer patients reported a family history of some degree, and 10% constituted a high-risk group. There was no relation between family history and age at onset, hormonal background, tumor characteristics, treatment or prognosis in our material, which was population-based, and prospectively collected and also included paternal inheritance.

Screening for BRCA1 mutations in a population-based cohort of breast cancer patients from our region revealed only two mutation carriers, both in cases with a family history of breast and ovarian cancer. There is no need for general screening of BRCA1 in breast cancer patients, however family history should be used to select patients who would benefit from genetic counseling.

The rare truncating variant, 1100delC, in checkpoint kinase CHEK2 exists in the Swedish population. In our material the prevalence was increased in both high and low risk familial breast cancer compared to controls. The variant seems to influence age at onset and this together with lack of association in sporadic patients may indicate a role as a modifier of still unknown high-risk gene/s rather than being a true low penetrance gene.

Analysis of a common single nucleotide polymorphism, C975G, in the ERα gene in sporadic and familial breast cancer suggested a protective effect of the variant allele in high-risk familial breast cancer compared to controls. No association was seen in low risk familial or sporadic cases. There was also a possible association between genotype and ER expression in the breast tumours.

Three polymorphisms in the ERβ gene were analyzed for association with familial and sporadic breast cancer, both separately and as haplotype constructions. There was no overall significant difference in genotype distribution but one common haplotype was associated with an increased risk of sporadic breast cancer indicating a role for ERβ in breast cancer susceptibility.

This thesis aimed to illuminate the contribution of low risk alleles in breast cancer susceptibility. The design used selected candidate gene variants to be studied in cohorts of breast cancer patients selected by family history into different risk groups. The compiled results support an effect of low risk alleles and suggest that the effect may vary from acting as low risk factors on their own to acting with an additive or modifying effect with other genetic or non-genetic risk-factors. Further studies of breast cancer as a complex disease need larger breast cancer cohorts to elucidate the genetic susceptibility to breast cancer.

ACKNOWLEDGEMENTS

I would like to express my gratitude to those who contributed to this study, in particular:

Annika Lindblom, my main supervisor, for your constant and never failing support and encouragement. You have a deep knowledge and unique devotion in the cancer genetics research field, which you have generously shared with me. Thank you also for mentorship in other areas of life.

Tommy Fornander, my supervisor and mentor in the clinic for many years, for sincere support and for your unique ability to elucidate complicated issues both in research and in the clinic.

Lars-Erik Rutqvist, former head of the Department of Oncology at Huddinge University Hospital / Södersjukhuset, for support and for sharing your great experience in breast cancer research. .

Jonas Bergh, present head of the Breast and Sarcoma section at the Department of Oncology at Karolinska University Hospital, for your profound interest in breast cancer and support of my present and future research projects.

Barbro Werelius, technician at Annika Lindblom´s lab, for skilful and kind support.

Johanna Skoglund, fellow PhD student at the lab, for excellent collaboration and good spirits.

Hemming Johansson for invaluable statistical assistance.

Former and present members of Annikas lab and the Department of Clinical genetics: **Paula Maguire** for fruitful collaboration, **Norma Lundberg** for assistance with patient administration, **Xiao-Lei Zhou, Tatjana Djureinovic, Jana Vandrovcova, Heléne Fischer, Tao Liu, Liping Luo, Quan Du, Johanna Rantala** and **Berith Wejderot** for contribution to the scientific athmosophere.

Marie Luise Bisgaard for pleasant and rewarding collaboration and support.

The dedicated colleagues, working with hereditary cancer in the clinic and also present or former PhD students of Annika´s: **Anna von Wachenfeldt** at Södersjukhuset and **Brita Arver** and **Annelie Liljegren** at Radiumhemmet, for interesting discussions and good company.

Christoffer Lageroos, for valuable computer assistance

Inga-Britt Pettersson, former research secretary for your professionalism, support and concern in great as in little things.

Ulla Glas, former head of the Department of Oncology at Södersjukhuset, for generous support during the first years of my career in Oncology.

Asgerdur Sverrisdottir, former colleague, fellow PhD student and roommate at Södersjukhuset for many years, for support and friendship.

All colleagues belonging to the breast-section at the Department of Oncology at Karolinska University Hospital, Södersjukhuset for continuous encouragement and for creating a wonderful atmosphere in spite of heavy work load : **Mariann Iiristo** for generous support in administrative issues, **Ann-Marie Billgren, Ingveldur Björnsdottir, Ulla Blom-Goldman, Tomas Jansson, Reza Khoshnoud, Else Svensson** and **Gerard Winblad.**

All other **present** and **former colleagues** at the Department of Oncology, Karolinska University Hospital at Södersjukhuset/Huddinge, especially all those who have assisted in informing the patients and collecting the blood samples for these studies.

All present and previous **nurses** at the Oncologic Out-patient Department, breast section and Oncologic Day-Care Unit at Södersjukhuset/Huddinge for help with research blood samples and support in the daily work, especially **Tina Bondesson, Lotta Bodell** and **Lena Ljungkvist Brodin**

All **breast cancer patients** who have participated in the trials.

Tova Hannegård-Hamrin for long-time friendship and linguistic advice.

Family and friends, especially **Gregory, Dan** and **Rebecka**, for encouragement, love and perspective in life.

These studies were supported by **grants** from the the Nilsson-Ehle foundation, the Swedish Cancer Society, the Gustav V Jubileé Foundation, the FoU Foundation, the Swedish Doctoral Society, the Swedish Society of Medicine, Huddinge University Hospital, Karolinska University Hospital, Bert von Kantzows foundation and the Swedish EU-R&D Foundation.

REFERENCES

1. Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies. Collaborative Group on Hormonal Factors in Breast Cancer. Lancet 1996;347(9017)**:**1713-27.

2. Pathology of familial breast cancer: differences between breast cancers in carriers of BRCA1 or BRCA2 mutations and sporadic cases. Breast Cancer Linkage Consortium. Lancet 1997;349(9064)**:**1505-10.

3. Tamoxifen for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. Lancet 1998;351(9114)**:**1451-67.

4. Cancer risks in BRCA2 mutation carriers.The Breast Cancer Linkage Consortium. J Natl Cancer Inst 1999;91(15)**:**1310-6.

5. The exon 13 duplication in the BRCA1 gene is a founder mutation present in geographically diverse populations. The BRCA1 Exon 13 Duplication Screening Group. Am J Hum Genet 2000;67(1)**:**207-12.

6. Prevalence and penetrance of BRCA1 and BRCA2 mutations in a population-based series of breast cancer cases. Anglian Breast Cancer Study Group. Br J Cancer 2000;83(10)**:**1301-8.

7. Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast cancer and 96973 women without the disease. Lancet 2002;360(9328)**:**187-95.

8. CHEK2*1100delC and susceptibility to breast cancer: a collaborative analysis involving 10,860 breast cancer cases and 9,065 controls from 10 studies. Am J Hum Genet 2004;74(6)**:**1175-82.

9. Adami HO, Hansen J, Jung B, Rimsten A. Familiality in breast cancer: a case-control study in a Sweden population. Br J Cancer 1980;42(1)**:**71-7.

10. Adami HO, Hansen J, Jung B, Rimsten A. Characteristics of familial breast cancer in Sweden: absence of relation to age and unilateral versus bilateral disease. Cancer 1981;48(7)**:**1688-95.

11. Ahlbom A, Lichtenstein P, Malmstrom H, Feychting M, Hemminki K, Pedersen NL. Cancer in twins: genetic and nongenetic familial risk factors. J Natl Cancer Inst 1997;89(4)**:**287-93.

12. Al Sarakbi W, Salhab M, Mokbel K. Dairy products and breast cancer risk: a review of the literature. Int J Fertil Womens Med 2005;50(6)**:**244-9.

13. Altshuler D, Brooks LD, Chakravarti A, Collins FS, Daly MJ, Donnelly P. A haplotype map of the human genome. Nature 2005;437(7063)**:**1299-320.

14. Andersen TI, Heimdal KR, Skrede M, Tveit K, Berg K, Borresen AL. Oestrogen receptor (ESR) polymorphisms and breast cancer susceptibility. Hum Genet 1994;94(6)**:**665-70.

15. Anderson DE. Breast cancer in families. Cancer 1977;40(4 Suppl)**:**1855-60.

16. Anderson TI, Wooster R, Laake K, et al. Screening for ESR mutations in breast and ovarian cancer patients. Hum Mutat 1997;9(6)**:**531-6.

17. Anton-Culver H, Cohen PF, Gildea ME, Ziogas A. Characteristics of BRCA1 mutations in a population-based case series of breast and ovarian cancer. Eur J Cancer 2000;36(10):1200-8.

18. Antoniou A, Pharoah PD, Narod S, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 2003;72(5):1117-30.

19. Antoniou AC, Easton DF. Polygenic inheritance of breast cancer: Implications for design of association studies. Genet Epidemiol 2003;25(3):190-202.

20. Antoniou AC, Pharoah PD, McMullan G, Day NE, Ponder BA, Easton D. Evidence for further breast cancer susceptibility genes in addition to BRCA1 and BRCA2 in a population-based study. Genet Epidemiol 2001;21(1):1-18.

21. Antoniou AC, Pharoah PD, McMullan G, et al. A comprehensive model for familial breast cancer incorporating BRCA1, BRCA2 and other genes. Br J Cancer 2002;86(1):76-83.

22. Arver B, Borg A, Lindblom A. First BRCA1 and BRCA2 gene testing implemented in the health care system of Stockholm. Genet Test 2001;5(1):1-8.

23. Arver B, Claro A, Langerod A, Borresen-Dale AL, Lindblom A. BRCA1 screening in patients with a family history of breast or ovarian cancer. Genet Test 1999;3(2):223-6.

24. Azmy IA, Balasubramanian SP, Wilson AG, et al. Role of tumour necrosis factor gene polymorphisms (-308 and -238) in breast cancer susceptibility and severity. Breast Cancer Res 2004;6(4):R395-400.

25. Bader JS. The relative power of SNPs and haplotype as genetic markers for association tests. Pharmacogenomics 2001;2(1):11-24.

26. Bain C, Speizer FE, Rosner B, Belanger C, Hennekens CH. Family history of breast cancer as a risk indicator for the disease. Am J Epidemiol 1980;111(3):301-8.

27. Balfe PJ, McCann AH, Welch HM, Kerin MJ. Estrogen receptor beta and breast cancer. Eur J Surg Oncol 2004;30(10):1043-50.

28. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21(2):263-5.

29. Bartek J, Lukas J. Chk1 and Chk2 kinases in checkpoint control and cancer. Cancer Cell 2003;3(5):421-9.

30. Becherini L, Gennari L, Masi L, et al. Evidence of a linkage disequilibrium between polymorphisms in the human estrogen receptor alpha gene and their relationship to bone mass variation in postmenopausal Italian women. Hum Mol Genet 2000;9(13):2043-50.

31. Bell DW, Varley JM, Szydlo TE, et al. Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome. Science 1999;286(5449):2528-31.

32. Benusiglio PR, Lesueur F, Luccarini C, et al. Common ERBB2 polymorphisms and risk of breast cancer in a white British population: a case-control study. Breast Cancer Res 2005;7(2):R204-9.

33. Beral V. Breast cancer and hormone-replacement therapy in the Million Women Study. Lancet 2003;362(9382):419-27.

34. Beral V, Bull D, Doll R, Peto R, Reeves G. Breast cancer and abortion: collaborative reanalysis of data from 53 epidemiological studies, including 83?000 women with breast cancer from 16 countries. Lancet 2004;363(9414):1007-16.

35. Bernstein JL, Thompson WD, Risch N, Holford TR. Risk factors predicting the incidence of second primary breast cancer among women diagnosed with a first primary breast cancer. Am J Epidemiol 1992;136(8):925-36.

36. Berx G, Cleton-Jansen AM, Strumane K, et al. E-cadherin is inactivated in a majority of invasive human lobular breast cancers by truncation mutations throughout its extracellular domain. Oncogene 1996;13(9):1919-25.

37. Bogdanova N, Enssen-Dubrowinskaja N, Feshchenko S, et al. Association of two mutations in the CHEK2 gene with breast cancer. Int J Cancer 2005;116(2):263-6.

38. Borresen AL, Andersen TI, Garber J, et al. Screening for germ line TP53 mutations in breast cancer patients. Cancer Res 1992;52(11):3234-6.

39. Borresen AL, Andersen TI, Tretli S, Heiberg A, Moller P. Breast cancer and other cancers in Norwegian families with ataxia-telangiectasia. Genes Chromosomes Cancer 1990;2(4):339-40.

40. Botstein D, Risch N. Discovering genotypes underlying human phenotypes: past successes for mendelian disease, future approaches for complex disease. Nat Genet 2003;33 Suppl:228-37.

41. Bretherton-Watt D, Given-Wilson R, Mansi JL, Thomas V, Carter N, Colston KW. Vitamin D receptor gene polymorphisms are associated with breast cancer risk in a UK Caucasian population. Br J Cancer 2001;85(2):171-5.

42. Broeks A, de Witte L, Nooijen A, et al. Excess risk for contralateral breast cancer in CHEK2*1100delC germline mutation carriers. Breast Cancer Res Treat 2004;83(1):91-3.

43. Broeks A, Urbanus JH, Floore AN, et al. ATM-heterozygous germline mutations contribute to breast cancer-susceptibility. Am J Hum Genet 2000;66(2):494-500.

44. Brooks-Wilson AR, Kaurah P, Suriano G, et al. Germline E-cadherin mutations in hereditary diffuse gastric cancer: assessment of 42 new families and review of genetic screening criteria. J Med Genet 2004;41(7):508-17.

45. Brownstein MH, Wolf M, Bikowski JB. Cowden's disease: a cutaneous marker of breast cancer. Cancer 1978;41(6):2393-8.

46. Buyru N, Tigli H, Dalay N. P53 codon 72 polymorphism in breast cancer. Oncol Rep 2003;10(3):711-4.

47. Byrne C, Schairer C, Brinton LA, et al. Effects of mammographic density and benign breast disease on breast cancer risk (United States). Cancer Causes Control 2001;12(2):103-10.

48. Cai Q, Shu XO, Jin F, et al. Genetic polymorphisms in the estrogen receptor alpha gene and risk of breast cancer: results from the Shanghai Breast Cancer Study. Cancer Epidemiol Biomarkers Prev 2003;12(9):853-9.

49. Chamberlain JS, Gibbs RA, Ranier JE, Nguyen PN, Caskey CT. Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification. Nucleic Acids Res 1988;16(23):11141-56.

50. Chang-Claude J, Kropp S, Jager B, Bartsch H, Risch A. Differential effect of NAT2 on the association between active and passive smoke exposure and breast cancer risk. Cancer Epidemiol Biomarkers Prev 2002;11(8):698-704.

51. Chappuis PO, Hamel N, Paradis AJ, et al. Prevalence of founder BRCA1 and BRCA2 mutations in unselected French Canadian women with breast cancer. Clin Genet 2001;59(6):418-23.

52. Chappuis PO, Rosenblatt J, Foulkes WD. The influence of familial and hereditary factors on the prognosis of breast cancer. Ann Oncol 1999;10(10):1163-70.

53. Chen J, Birkholtz GG, Lindblom P, Rubio C, Lindblom A. The role of ataxia-telangiectasia heterozygotes in familial breast cancer. Cancer Res 1998;58(7):1376-9.

54. Chen J, Hedman MZ, Arver BW, Sigurdsson S, Eyfjord JE, Lindblom A. BRCA2 germline mutations in Swedish breast cancer families. Eur J Hum Genet 1998;6(2):134-9.

55. Chen J, Lindblom A. Germline mutation screening of the STK11/LKB1 gene in familial breast cancer with LOH on 19p. Clin Genet 2000;57(5):394-7.

56. Cheng G, Weihua Z, Makinen S, et al. A role for the androgen receptor in follicular atresia of estrogen receptor beta knockout mouse ovary. Biol Reprod 2002;66(1):77-84.

57. Chompret A, Brugieres L, Ronsin M, et al. P53 germline mutations in childhood cancers and cancer risk for carrier individuals. Br J Cancer 2000;82(12):1932-7.

58. Christofori G, Semb H. The role of the cell-adhesion molecule E-cadherin as a tumour-suppressor gene. Trends Biochem Sci 1999;24(2):73-6.

59. Claus EB, Risch N, Thompson WD. Autosomal dominant inheritance of early-onset breast cancer. Implications for risk prediction. Cancer 1994;73(3):643-51.

60. Collins N, McManus R, Wooster R, et al. Consistent loss of the wild type allele in breast cancers from a family linked to the BRCA2 gene on chromosome 13q12-13. Oncogene 1995;10(8):1673-5.

61. Cornelis RS, Neuhausen SL, Johansson O, et al. High allele loss rates at 17q12-q21 in breast and ovarian tumors from BRCAl-linked families. The Breast Cancer Linkage Consortium. Genes Chromosomes Cancer 1995;13(3):203-10.

62. Cox DG, Hankinson SE, Hunter DJ. The erbB2/HER2/neu receptor polymorphism Ile655Val and breast cancer risk. Pharmacogenet Genomics 2005;15(7):447-50.

63. Cullinane CA, Lubinski J, Neuhausen SL, et al. Effect of pregnancy as a risk factor for breast cancer in BRCA1/BRCA2 mutation carriers. Int J Cancer 2005;117(6):988-91.

64. Curran JE, Lea RA, Rutherford S, Weinstein SR, Griffiths LR. Association of estrogen receptor and glucocorticoid receptor gene polymorphisms with sporadic breast cancer. Int J Cancer 2001;95(4):271-5.

65. Curran JE, Vaughan T, Lea RA, Weinstein SR, Morrison NA, Griffiths LR. Association of A vitamin D receptor polymorphism with sporadic breast cancer development. Int J Cancer 1999;83(6):723-6.

66. Cybulski C, Gorski B, Huzarski T, et al. CHEK2 is a multiorgan cancer susceptibility gene. Am J Hum Genet 2004;75(6):1131-5.

67. de Bock GH, Schutte M, Krol-Warmerdam EM, et al. Tumour characteristics and prognosis of breast cancer patients carrying the germline CHEK2*1100delC variant. J Med Genet 2004;41(10):731-5.

68. Debies MT, Welch DR. Genetic basis of human breast cancer metastasis. J Mammary Gland Biol Neoplasia 2001;6(4):441-51.

69. Doepel M, Kellokumpu IH, v Smitten KA. Hereditary breast cancer in Finnish women. Eur J Surg 1995;161(11):805-9.

70. Du Q, Luo L, von Wachenfeldt A, Kockum I, Luthman H, Lindblom A. No evidence for a familial breast cancer susceptibility gene at chromosome 13q21 in Swedish breast cancer families. Int J Cancer 2002;98(5)**:**799-800.

71. Dudbridge F. Pedigree disequilibrium tests for multilocus haplotypes. Genet Epidemiol 2003;25(2)**:**115-21.

72. Dufault MR, Betz B, Wappenschmidt B, et al. Limited relevance of the CHEK2 gene in hereditary breast cancer. Int J Cancer 2004;110(3)**:**320-5.

73. Dunning AM, Healey CS, Pharoah PD, Teare MD, Ponder BA, Easton DF. A systematic review of genetic polymorphisms and breast cancer risk. Cancer Epidemiol Biomarkers Prev 1999;8(10)**:**843-54.

74. Easton DF. How many more breast cancer predisposition genes are there? Breast Cancer Res 1999;1(1)**:**14-7.

75. 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(1)**:**265-71.

76. Eccles D, Simmonds P, Goddard J, et al. Familial breast cancer: an investigation into the outcome of treatment for early stage disease. Fam Cancer 2001;1(2)**:**65-72.

77. Eerola H, Blomqvist C, Pukkala E, Pyrhonen S, Nevanlinna H. Familial breast cancer in southern Finland: how prevalent are breast cancer families and can we trust the family history reported by patients? Eur J Cancer 2000;36(9)**:**1143-8.

78. Eerola H, Heikkila P, Tamminen A, Aittomaki K, Blomqvist C, Nevanlinna H. Histopathological features of breast tumours in BRCA1, BRCA2 and mutation-negative breast cancer families. Breast Cancer Res 2005;7(1)**:**R93-100.

79. Eerola H, Vahteristo P, Sarantaus L, et al. Survival of breast cancer patients in BRCA1, BRCA2, and non-BRCA1/2 breast cancer families: a relative survival analysis from Finland. Int J Cancer 2001;93(3)**:**368-72.

80. Egan KM, Cai Q, Shu XO, et al. Genetic polymorphisms in GSTM1, GSTP1, and GSTT1 and the risk for breast cancer: results from the Shanghai Breast Cancer Study and meta-analysis. Cancer Epidemiol Biomarkers Prev 2004;13(2)**:**197-204.

81. Einbeigi Z, Bergman A, Kindblom LG, et al. A founder mutation of the BRCA1 gene in Western Sweden associated with a high incidence of breast and ovarian cancer. Eur J Cancer 2001;37(15)**:**1904-9.

82. Eng C. Genetics of Cowden syndrome: through the looking glass of oncology. Int J Oncol 1998;12(3)**:**701-10.

83. Ferno M, Borg A, Johansson U, et al. Estrogen and progesterone receptor analyses in more than 4,000 human breast cancer samples. A study with special reference to age at diagnosis and stability of analyses. Southern Swedish Breast Cancer Study Group. Acta Oncol 1990;29(2)**:**129-35.

84. Firgaira FA, Seshadri R, McEvoy CR, et al. HRAS1 rare minisatellite alleles and breast cancer in Australian women under age forty years. J Natl Cancer Inst 1999;91(24)**:**2107-11.

85. FitzGerald MG, Bean JM, Hegde SR, et al. Heterozygous ATM mutations do not contribute to early onset of breast cancer. Nat Genet 1997;15(3)**:**307-10.

86. FitzGerald MG, Marsh DJ, Wahrer D, et al. Germline mutations in PTEN are an infrequent cause of genetic predisposition to breast cancer. Oncogene 1998;17(6)**:**727-31.

87. Fodor FH, Weston A, Bleiweiss IJ, et al. Frequency and carrier risk associated with common BRCA1 and BRCA2 mutations in Ashkenazi Jewish breast cancer patients. Am J Hum Genet 1998;63(1):45-51.

88. Ford D, Easton DF, Bishop DT, Narod SA, Goldgar DE. Risks of cancer in BRCA1-mutation carriers. Breast Cancer Linkage Consortium. Lancet 1994;343(8899):692-5.

89. Ford D, Easton DF, Stratton M, et al. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. Am J Hum Genet 1998;62(3):676-89.

90. Forsell C, Enmark E, Axelman K, et al. Investigations of a CA repeat in the oestrogen receptor beta gene in patients with Alzheimer's disease. Eur J Hum Genet 2001;9(10):802-4.

91. Forsti A, Zhao C, Israelsson E, Dahlman-Wright K, Gustafsson JA, Hemminki K. Polymorphisms in the estrogen receptor beta gene and risk of breast cancer: no association. Breast Cancer Res Treat 2003;79(3):409-13.

92. Foulkes WD. BRCA1 functions as a breast stem cell regulator. J Med Genet 2004;41(1):1-5.

93. Foulkes WD, Stefansson IM, Chappuis PO, et al. Germline BRCA1 mutations and a basal epithelial phenotype in breast cancer. J Natl Cancer Inst 2003;95(19):1482-5.

94. Freihoff D, Kempe A, Beste B, et al. Exclusion of a major role for the PTEN tumour-suppressor gene in breast carcinomas. Br J Cancer 1999;79(5-6):754-8.

95. Friedrichsen DM, Malone KE, Doody DR, Daling JR, Ostrander EA. Frequency of CHEK2 mutations in a population based, case-control study of breast cancer in young women. Breast Cancer Res 2004;6(6):R629-35.

96. Gabriel SB, Schaffner SF, Nguyen H, et al. The structure of haplotype blocks in the human genome. Science 2002;296(5576):2225-9.

97. Gad S, Caux-Moncoutier V, Pages-Berhouet S, et al. Significant contribution of large BRCA1 gene rearrangements in 120 French breast and ovarian cancer families. Oncogene 2002;21(44):6841-7.

98. Gayther SA, Mangion J, Russell P, et al. Variation of risks of breast and ovarian cancer associated with different germline mutations of the BRCA2 gene. Nat Genet 1997;15(1):103-5.

99. Gayther SA, Warren W, Mazoyer S, et al. Germline mutations of the BRCA1 gene in breast and ovarian cancer families provide evidence for a genotype-phenotype correlation. Nat Genet 1995;11(4):428-33.

100. Gennari L, Merlotti D, De Paola V, et al. Estrogen receptor gene polymorphisms and the genetics of osteoporosis: a HuGE review. Am J Epidemiol 2005;161(4):307-20.

101. Gertig DM, Hankinson SE, Hough H, et al. N-acetyl transferase 2 genotypes, meat intake and breast cancer risk. Int J Cancer 1999;80(1):13-7.

102. Go RC, King MC, Bailey-Wilson J, Elston RC, Lynch HT. Genetic epidemiology of breast cancer and associated cancers in high-risk families. I. Segregation analysis. J Natl Cancer Inst 1983;71(3):455-61.

103. Gold B, Kalush F, Bergeron J, et al. Estrogen receptor genotypes and haplotypes associated with breast cancer risk. Cancer Res 2004;64(24):8891-900.

104. Greene GL, Gilna P, Waterfield M, Baker A, Hort Y, Shine J. Sequence and expression of human estrogen receptor complementary DNA. Science 1986;231(4742):1150-4.

105. Gronwald J, Byrski T, Huzarski T, et al. Influence of selected lifestyle factors on breast and ovarian cancer risk in BRCA1 mutation carriers from Poland. Breast Cancer Res Treat 2006;95(2):105-9.

106. Gronwald J, Jauch A, Cybulski C, et al. Comparison of genomic abnormalities between BRCAX and sporadic breast cancers studied by comparative genomic hybridization. Int J Cancer 2005;114(2):230-6.

107. Guilford PJ, Hopkins JB, Grady WM, et al. E-cadherin germline mutations define an inherited cancer syndrome dominated by diffuse gastric cancer. Hum Mutat 1999;14(3):249-55.

108. Guy M, Lowe LC, Bretherton-Watt D, Mansi JL, Colston KW. Approaches to evaluating the association of vitamin D receptor gene polymorphisms with breast cancer risk. Recent Results Cancer Res 2003;164:43-54.

109. Guy M, Lowe LC, Bretherton-Watt D, et al. Vitamin D receptor gene polymorphisms and breast cancer risk. Clin Cancer Res 2004;10(16):5472-81.

110. Hakansson S, Johannsson O, Johansson U, et al. Moderate frequency of BRCA1 and BRCA2 germ-line mutations in Scandinavian familial breast cancer. Am J Hum Genet 1997;60(5):1068-78.

111. Hall JM, Lee MK, Newman B, et al. Linkage of early-onset familial breast cancer to chromosome 17q21. Science 1990;250(4988):1684-9.

112. Hamajima N, Hirose K, Tajima K, et al. Alcohol, tobacco and breast cancer--collaborative reanalysis of individual data from 53 epidemiological studies, including 58,515 women with breast cancer and 95,067 women without the disease. Br J Cancer 2002;87(11):1234-45.

113. Han W, Kang D, Lee KM, et al. Full sequencing analysis of estrogen receptor-alpha gene polymorphism and its association with breast cancer risk. Anticancer Res 2003;23(6C):4703-7.

114. Hancock SL, Tucker MA, Hoppe RT. Breast cancer after treatment of Hodgkin's disease. J Natl Cancer Inst 1993;85(1):25-31.

115. Hankinson SE, Willett WC, Manson JE, et al. Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women. J Natl Cancer Inst 1998;90(17):1292-9.

116. Harvey EB, Brinton LA. Second cancer following cancer of the breast in Connecticut, 1935-82. Natl Cancer Inst Monogr 1985;68:99-112.

117. Hasegawa S, Miyoshi Y, Ikeda N, et al. Mutational analysis of estrogen receptor-beta gene in human breast cancers. Breast Cancer Res Treat 2003;78(1):133-4.

118. Hauptmann M, Sigurdson AJ, Chatterjee N, et al. Re: Population-based, case-control study of HER2 genetic polymorphism and breast cancer risk. J Natl Cancer Inst 2003;95(16):1251-2.

119. Hedenfalk I, Duggan D, Chen Y, et al. Gene-expression profiles in hereditary breast cancer. N Engl J Med 2001;344(8):539-48.

120. Hedenfalk I, Ringner M, Ben-Dor A, et al. Molecular classification of familial non-BRCA1/BRCA2 breast cancer. Proc Natl Acad Sci U S A 2003;100(5):2532-7.

121. Hemminki A, Tomlinson I, Markie D, et al. Localization of a susceptibility locus for Peutz-Jeghers syndrome to 19p using comparative genomic hybridization and targeted linkage analysis. Nat Genet 1997;15(1):87-90.

122. Henderson BE, Feigelson HS. Hormonal carcinogenesis. Carcinogenesis 2000;21(3):427-33.

123. Herrington DM, Howard TD, Brosnihan KB, et al. Common estrogen receptor polymorphism augments effects of hormone replacement therapy on E-selectin but not C-reactive protein. Circulation 2002;105(16):1879-82.

124. Hirschhorn JN, Daly MJ. Genome-wide association studies for common diseases and complex traits. Nat Rev Genet 2005;6(2):95-108.

125. Hogervorst FB, Cornelis RS, Bout M, et al. Rapid detection of BRCA1 mutations by the protein truncation test. Nat Genet 1995;10(2):208-12.

126. Hogervorst FB, Nederlof PM, Gille JJ, et al. Large genomic deletions and duplications in the BRCA1 gene identified by a novel quantitative method. Cancer Res 2003;63(7):1449-53.

127. Holmes MD, Willett WC. Does diet affect breast cancer risk? Breast Cancer Res 2004;6(4):170-8.

128. Hou MF, Tien YC, Lin GT, et al. Association of vitamin D receptor gene polymorphism with sporadic breast cancer in Taiwanese patients. Breast Cancer Res Treat 2002;74(1):1-7.

129. Hsiao WC, Young KC, Lin SL, Lin PW. Estrogen receptor-alpha polymorphism in a Taiwanese clinical breast cancer population: a case-control study. Breast Cancer Res 2004;6(3):R180-6.

130. Hsieh CC, Trichopoulos D, Katsouyanni K, Yuasa S. Age at menarche, age at menopause, height and obesity as risk factors for breast cancer: associations and interactions in an international case-control study. Int J Cancer 1990;46(5):796-800.

131. Huang XE, Hamajima N, Katsuda N, et al. 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(4):307-11.

132. Huusko P, Juo SH, Gillanders E, et al. Genome-wide scanning for linkage in Finnish breast cancer families. Eur J Hum Genet 2004;12(2):98-104.

133. Huzarski T, Cybulski C, Domagala W, et al. Pathology of breast cancer in women with constitutional CHEK2 mutations. Breast Cancer Res Treat 2005;90(2):187-9.

134. Ioannidis JP, Stavrou I, Trikalinos TA, et al. Association of polymorphisms of the estrogen receptor alpha gene with bone mineral density and fracture risk in women: a meta-analysis. J Bone Miner Res 2002;17(11):2048-60.

135. Iobagiu C, Lambert C, Normand M, Genin C. Microsatellite profile in hormonal receptor genes associated with breast cancer. Breast Cancer Res Treat 2006;95(2):153-9.

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

137. Israeli D, Tartter PI, Brower ST, Mizrachy B, Bratton J. The significance of family history for patients with carcinoma of the breast. J Am Coll Surg 1994;179(1):29-32.

138. Iwase H, Greenman JM, Barnes DM, Hodgson S, Bobrow L, Mathew CG. Sequence variants of the estrogen receptor (ER) gene found in breast cancer patients with ER negative and progesterone receptor positive tumors. Cancer Lett 1996;108(2):179-84.

139. Jasen P. Breast cancer and the language of risk, 1750-1950. Soc Hist Med 2002;15(1):17-43.

140. Jeghers H, Mc KV, Katz KH. Generalized intestinal polyposis and melanin spots of the oral mucosa, lips and digits; a syndrome of diagnostic significance. N Engl J Med 1949;241(26):1031-6.

141. Jekimovs CR, Chen X, Arnold J, et al. Low frequency of CHEK2 1100delC allele in Australian multiple-case breast cancer families: functional analysis in heterozygous individuals. Br J Cancer 2005;92(4):784-90.

142. Jernstrom H, Loman N, Johannsson OT, Borg A, Olsson H. Impact of teenage oral contraceptive use in a population-based series of early-onset breast cancer cases who have undergone BRCA mutation testing. Eur J Cancer 2005;41(15):2312-20.

143. Jernstrom H, Lubinski J, Lynch HT, et al. Breast-feeding and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. J Natl Cancer Inst 2004;96(14):1094-8.

144. Johannsson OT, Idvall I, Anderson C, et al. Tumour biological features of BRCA1-induced breast and ovarian cancer. Eur J Cancer 1997;33(3):362-71.

145. Johnson GC, Esposito L, Barratt BJ, et al. Haplotype tagging for the identification of common disease genes. Nat Genet 2001;29(2):233-7.

146. Kainu T, Juo SH, Desper R, et al. Somatic deletions in hereditary breast cancers implicate 13q21 as a putative novel breast cancer susceptibility locus. Proc Natl Acad Sci U S A 2000;97(17):9603-8.

147. Kang HJ, Kim SW, Kim HJ, et al. Polymorphisms in the estrogen receptor-alpha gene and breast cancer risk. Cancer Lett 2002;178(2):175-80.

148. Kelsey JL, Gammon MD, John EM. Reproductive factors and breast cancer. Epidemiol Rev 1993;15(1):36-47.

149. Kerangueven F, Essioux L, Dib A, et al. Loss of heterozygosity and linkage analysis in breast carcinoma: indication for a putative third susceptibility gene on the short arm of chromosome 8. Oncogene 1995;10(5):1023-6.

150. Keshava C, McCanlies EC, Keshava N, Wolff MS, Weston A. Distribution of HER2(V655) genotypes in breast cancer cases and controls in the United States. Cancer Lett 2001;173(1):37-41.

151. Kilpivaara O, Vahteristo P, Falck J, et al. CHEK2 variant I157T may be associated with increased breast cancer risk. Int J Cancer 2004;111(4):543-7.

152. King MC, Marks JH, Mandell JB. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science 2003;302(5645):643-6.

153. Kleibl Z, Novotny J, Bezdickova D, et al. The CHEK2 c.1100delC germline mutation rarely contributes to breast cancer development in the Czech Republic. Breast Cancer Res Treat 2005;90(2):165-7.

154. Knudson AG, Jr. Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci U S A 1971;68(4):820-3.

155. Kotsopoulos J, Lubinski J, Lynch HT, et al. Age at menarche and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. Cancer Causes Control 2005;16(6):667-74.

156. Krege JH, Hodgin JB, Couse JF, et al. Generation and reproductive phenotypes of mice lacking estrogen receptor beta. Proc Natl Acad Sci U S A 1998;95(26):15677-82.

157. Kremeyer B, Soller M, Lagerstedt K, et al. The BRCA1 exon 13 duplication in the Swedish population. Fam Cancer 2005;4(2):191-4.

158. Kristiansen M, Knudsen GP, Maguire P, et al. High incidence of skewed X chromosome inactivation in young patients with familial non-BRCA1/BRCA2 breast cancer. J Med Genet 2005.

159. Kruglyak L, Nickerson DA. Variation is the spice of life. Nat Genet 2001;27(3):234-6.

160. Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel receptor expressed in rat prostate and ovary. Proc Natl Acad Sci U S A 1996;93(12):5925-30.

161. Kumar V, Chambon P. The estrogen receptor binds tightly to its responsive element as a ligand-induced homodimer. Cell 1988;55(1):145-56.

162. Lakhani SR, Van De Vijver MJ, Jacquemier J, et al. The pathology of familial breast cancer: predictive value of immunohistochemical markers estrogen receptor, progesterone receptor, HER-2, and p53 in patients with mutations in BRCA1 and BRCA2. J Clin Oncol 2002;20(9):2310-8.

163. Lee KM, Park SK, Kim SU, et al. N-acetyltransferase (NAT1, NAT2) and glutathione S-transferase (GSTM1, GSTT1) polymorphisms in breast cancer. Cancer Lett 2003;196(2):179-86.

164. Li DM, Sun H. TEP1, encoded by a candidate tumor suppressor locus, is a novel protein tyrosine phosphatase regulated by transforming growth factor beta. Cancer Res 1997;57(11):2124-9.

165. Li FP, Fraumeni JF, Jr. Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome? Ann Intern Med 1969;71(4):747-52.

166. Liaw D, Marsh DJ, Li J, et al. Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nat Genet 1997;16(1):64-7.

167. Lichtenstein P, Holm NV, Verkasalo PK, et al. 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(2):78-85.

168. Lim W, Hearle N, Shah B, et al. Further observations on LKB1/STK11 status and cancer risk in Peutz-Jeghers syndrome. Br J Cancer 2003;89(2):308-13.

169. Lindblom A. A molecular study on familial breast cancer: Karolinska Institute, 1993.

170. Lindblom A, Rotstein S, Larsson C, Nordenskjold M, Iselius L. Hereditary breast cancer in Sweden: a predominance of maternally inherited cases. Breast Cancer Res Treat 1993;24(2):159-65.

171. Lloyd KM, 2nd, Dennis M. Cowden's disease. A possible new symptom complex with multiple system involvement. Ann Intern Med 1963;58:136-42.

172. Lowe LC, Guy M, Mansi JL, et al. Plasma 25-hydroxy vitamin D concentrations, vitamin D receptor genotype and breast cancer risk in a UK Caucasian population. Eur J Cancer 2005;41(8):1164-9.

173. Lubahn DB, Moyer JS, Golding TS, Couse JF, Korach KS, Smithies O. Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. Proc Natl Acad Sci U S A 1993;90(23):11162-6.

174. Lubin JH, Burns PE, Blot WJ, et al. Risk factors for breast cancer in women in northern Alberta, Canada, as related to age at diagnosis. J Natl Cancer Inst 1982;68(2):211-7.

175. Lynch HT, Harris RE, Guirgis HA, et al. Early age of onset and familial breast cancer. Lancet 1976;2(7986):626-7.

176. Lynch HT, Lynch JF. Breast cancer genetics in an oncology clinic: 328 consecutive patients. Cancer Genet Cytogenet 1986;22(4):369-71.

177. Maguire P, Holmberg K, Kost-Alimova M, Imreh S, Skoog L, Lindblom A. CGH analysis of familial non-BRCA1/BRCA2 breast tumors and mutation screening of a candidate locus on chromosome 17q11.2-12. Int J Mol Med 2005;16(1):135-41.

178. Malkin D, Li FP, Strong LC, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. Science 1990;250(4985):1233-8.

179. Masson LF, Sharp L, Cotton SC, Little J. Cytochrome P-450 1A1 gene polymorphisms and risk of breast cancer: a HuGE review. Am J Epidemiol 2005;161(10):901-15.

180. Meijers-Heijboer H, van den Ouweland A, Klijn J, et al. Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in noncarriers of BRCA1 or BRCA2 mutations. Nat Genet 2002;31(1):55-9.

181. Meijers-Heijboer H, Wijnen J, Vasen H, et al. The CHEK2 1100delC mutation identifies families with a hereditary breast and colorectal cancer phenotype. Am J Hum Genet 2003;72(5):1308-14.

182. Menzel HJ, Sarmanova J, Soucek P, et al. Association of NQO1 polymorphism with spontaneous breast cancer in two independent populations. Br J Cancer 2004;90(10):1989-94.

183. Miki Y, Swensen J, Shattuck-Eidens D, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 1994;266(5182):66-71.

184. Millikan R, Eaton A, Worley K, et al. HER2 codon 655 polymorphism and risk of breast cancer in African Americans and whites. Breast Cancer Res Treat 2003;79(3):355-64.

185. Mitrunen K, Hirvonen A. Molecular epidemiology of sporadic breast cancer. The role of polymorphic genes involved in oestrogen biosynthesis and metabolism. Mutat Res 2003;544(1):9-41.

186. Mitrunen K, Jourenkova N, Kataja V, et al. Glutathione S-transferase M1, M3, P1, and T1 genetic polymorphisms and susceptibility to breast cancer. Cancer Epidemiol Biomarkers Prev 2001;10(3):229-36.

187. Molino A, Giovannini M, Pedersini R, et al. Correlations between family history and cancer characteristics in 2256 breast cancer patients. Br J Cancer 2004;91(1):96-8.

188. Moller P, Borg A, Heimdal K, et al. The BRCA1 syndrome and other inherited breast or breast-ovarian cancers in a Norwegian prospective series. Eur J Cancer 2001;37(8):1027-32.

189. Montagna M, Dalla Palma M, Menin C, et al. Genomic rearrangements account for more than one-third of the BRCA1 mutations in northern Italian breast/ovarian cancer families. Hum Mol Genet 2003;12(9):1055-61.

190. Montgomery KG, Gertig DM, Baxter SW, et al. The HER2 I655V polymorphism and risk of breast cancer in women < age 40 years. Cancer Epidemiol Biomarkers Prev 2003;12(10):1109-11.

191. Mosselman S, Polman J, Dijkema R. ER beta: identification and characterization of a novel human estrogen receptor. FEBS Lett 1996;392(1):49-53.

192. Narod SA, Dube MP, Klijn J, et al. Oral contraceptives and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. J Natl Cancer Inst 2002;94(23):1773-9.

193. Narod SA, Feunteun J, Lynch HT, et al. Familial breast-ovarian cancer locus on chromosome 17q12-q23. Lancet 1991;338(8759):82-3.

194. Narod SA, Foulkes WD. BRCA1 and BRCA2: 1994 and beyond. Nat Rev Cancer 2004;4(9):665-76.

195. Nelson SE, Gould MN, Hampton JM, Trentham-Dietz A. A case-control study of the HER2 Ile655Val polymorphism in relation to risk of invasive breast cancer. Breast Cancer Res 2005;7(3):R357-64.

196. Newman B, Austin MA, Lee M, King MC. Inheritance of human breast cancer: evidence for autosomal dominant transmission in high-risk families. Proc Natl Acad Sci U S A 1988;85(9):3044-8.

197. Nilsson M, Naessen S, Dahlman I, Linden Hirschberg A, Gustafsson JA, Dahlman-Wright K. Association of estrogen receptor beta gene polymorphisms with bulimic disease in women. Mol Psychiatry 2004;9(1):28-34.

198. 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(2):197-203.

199. Ogawa S, Emi M, Shiraki M, Hosoi T, Ouchi Y, Inoue S. Association of estrogen receptor beta (ESR2) gene polymorphism with blood pressure. J Hum Genet 2000;45(6):327-30.

200. Ogawa S, Inoue S, Watanabe T, et al. Molecular cloning and characterization of human estrogen receptor betacx: a potential inhibitor ofestrogen action in human. Nucleic Acids Res 1998;26(15):3505-12.

201. 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(6):1144-8.

202. Oldenburg RA, Kroeze-Jansema K, Kraan J, et al. The CHEK2*1100delC variant acts as a breast cancer risk modifier in non-BRCA1/BRCA2 multiple-case families. Cancer Res 2003;63(23):8153-7.

203. Olsson HL, Ingvar C, Bladstrom A. Hormone replacement therapy containing progestins and given continuously increases breast carcinoma risk in Sweden. Cancer 2003;97(6):1387-92.

204. Pagani F, Baralle FE. Genomic variants in exons and introns: identifying the splicing spoilers. Nat Rev Genet 2004;5(5):389-96.

205. Page DL, Schuyler PA, Dupont WD, Jensen RA, Plummer WD, Jr., Simpson JF. Atypical lobular hyperplasia as a unilateral predictor of breast cancer risk: a retrospective cohort study. Lancet 2003;361(9352):125-9.

206. Palacios J, Honrado E, Osorio A, et al. Immunohistochemical characteristics defined by tissue microarray of hereditary breast cancer not attributable to BRCA1 or BRCA2 mutations: differences from breast carcinomas arising in BRCA1 and BRCA2 mutation carriers. Clin Cancer Res 2003;9(10 Pt 1):3606-14.

207. Papelard H, de Bock GH, van Eijk R, et al. Prevalence of BRCA1 in a hospital-based population of Dutch breast cancer patients. Br J Cancer 2000;83(6):719-24.

208. Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of 25 major cancers in 1990. Int J Cancer 1999;80(6):827-41.

209. Parkin DM, Pisani P, Ferlay J. Global cancer statistics. CA Cancer J Clin 1999;49(1):33-64, 1.

210. Parodi PW. Dairy product consumption and the risk of breast cancer. J Am Coll Nutr 2005;24(6 Suppl):556S-68S.

211. Peto J, Mack TM. High constant incidence in twins and other relatives of women with breast cancer. Nat Genet 2000;26(4):411-4.

212. 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(1):33-6.

213. Pharoah PD, Day NE, Duffy S, Easton DF, Ponder BA. Family history and the risk of breast cancer: a systematic review and meta-analysis. Int J Cancer 1997;71(5):800-9.

214. Pharoah PD, Dunning AM, Ponder BA, Easton DF. Association studies for finding cancer-susceptibility genetic variants. Nat Rev Cancer 2004;4(11):850-60.

215. Pharoah PD, Guilford P, Caldas C. Incidence of gastric cancer and breast cancer in CDH1 (E-cadherin) mutation carriers from hereditary diffuse gastric cancer families. Gastroenterology 2001;121(6):1348-53.

216. Pharoah PD, Lipscombe JM, Redman KL, Day NE, Easton DF, Ponder BA. Familial predisposition to breast cancer in a British population: implications for prevention. Eur J Cancer 2000;36(6):773-9.

217. Ponglikitmongkol M, Green S, Chambon P. Genomic organization of the human oestrogen receptor gene. Embo J 1988;7(11):3385-8.

218. Pritchard JK. Are rare variants responsible for susceptibility to complex diseases? Am J Hum Genet 2001;69(1):124-37.

219. Qin YJ, Shen H, Huang QR, et al. Estrogen receptor alpha gene polymorphisms and peak bone density in Chinese nuclear families. J Bone Miner Res 2003;18(6):1028-35.

220. Rahman N, Teare MD, Seal S, et al. Absence of evidence for a familial breast cancer susceptibility gene at chromosome 8p12-p22. Oncogene 2000;19(36):4170-3.

221. Rebbeck TR. Inherited predisposition and breast cancer: modifiers of BRCA1/2-associated breast cancer risk. Environ Mol Mutagen 2002;39(2-3):228-34.

222. Reich DE, Lander ES. On the allelic spectrum of human disease. Trends Genet 2001;17(9):502-10.

223. Robson ME, Chappuis PO, Satagopan J, et al. A combined analysis of outcome following breast cancer: differences in survival based on BRCA1/BRCA2 mutation status and administration of adjuvant treatment. Breast Cancer Res 2004;6(1):R8-R17.

224. Ronaghi M, Karamohamed S, Pettersson B, Uhlen M, Nyren P. Real-time DNA sequencing using detection of pyrophosphate release. Anal Biochem 1996;242(1):84-9.

225. Roodi N, Bailey LR, Kao WY, et al. Estrogen receptor gene analysis in estrogen receptor-positive and receptor-negative primary breast cancer. J Natl Cancer Inst 1995;87(6):446-51.

226. Rosenkranz K, Hinney A, Ziegler A, et al. Systematic mutation screening of the estrogen receptor beta gene in probands of different weight extremes: identification of several genetic variants. J Clin Endocrinol Metab 1998;83(12):4524-7.

227. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. Jama 2002;288(3):321-33.

228. Russo A, Herd-Smith A, Gestri D, et al. Does family history influence survival in breast cancer cases? Int J Cancer 2002;99(3):427-30.

229. Rutter JL, Chatterjee N, Wacholder S, Struewing J. The HER2 I655V polymorphism and breast cancer risk in Ashkenazim. Epidemiology 2003;14(6):694-700.

230. Saftlas AF, Hoover RN, Brinton LA, et al. Mammographic densities and risk of breast cancer. Cancer 1991;67(11):2833-8.

231. Saji S, Hirose M, Toi M. Clinical significance of estrogen receptor beta in breast cancer. Cancer Chemother Pharmacol 2005;56 Suppl 1:21-6.

232. Salahshor S, Haixin L, Huo H, et al. Low frequency of E-cadherin alterations in familial breast cancer. Breast Cancer Res 2001;3(3):199-207.

233. Sarmanova J, Susova S, Gut I, et al. Breast cancer: role of polymorphisms in biotransformation enzymes. Eur J Hum Genet 2004;12(10):848-54.

234. Savitsky K, Sfez S, Tagle DA, et al. The complete sequence of the coding region of the ATM gene reveals similarity to cell cycle regulators in different species. Hum Mol Genet 1995;4(11):2025-32.

235. Schubert EL, Lee MK, Newman B, King MC. Single nucleotide polymorphisms (SNPs) in the estrogen receptor gene and breast cancer susceptibility. J Steroid Biochem Mol Biol 1999;71(1-2):21-7.

236. Schutte M, Seal S, Barfoot R, et al. Variants in CHEK2 other than 1100delC do not make a major contribution to breast cancer susceptibility. Am J Hum Genet 2003;72(4):1023-8.

237. Seitz S, Rohde K, Bender E, et al. Strong indication for a breast cancer susceptibility gene on chromosome 8p12-p22: linkage analysis in German breast cancer families. Oncogene 1997;14(6):741-3.

238. Shahedi K, Emanuelsson M, Wiklund F, Gronberg H. High risk of contralateral breast carcinoma in women with hereditary/familial non-BRCA1/BRCA2 breast carcinoma. Cancer 2006;106(6):1237-42.

239. Shin A, Kang D, Nishio H, et al. Estrogen receptor alpha gene polymorphisms and breast cancer risk. Breast Cancer Res Treat 2003;80(1):127-31.

240. Sidransky D, Tokino T, Helzlsouer K, et al. Inherited p53 gene mutations in breast cancer. Cancer Res 1992;52(10):2984-6.

241. Sillanpaa P, Hirvonen A, Kataja V, et al. Vitamin D receptor gene polymorphism as an important modifier of positive family history related breast cancer risk. Pharmacogenetics 2004;14(4):239-45.

242. Sillanpaa P, Hirvonen A, Kataja V, et al. NAT2 slow acetylator genotype as an important modifier of breast cancer risk. Int J Cancer 2005;114(4):579-84.

243. Slattery ML, Sweeney C, Murtaugh M, et al. Associations between ERalpha, ERbeta, and AR genotypes and colon and rectal cancer. Cancer Epidemiol Biomarkers Prev 2005;14(12):2936-42.

244. Smith SA, Easton DF, Evans DG, Ponder BA. Allele losses in the region 17q12-21 in familial breast and ovarian cancer involve the wild-type chromosome. Nat Genet 1992;2(2):128-31.

245. Socialstyrelsen TNBoHaW. Cancer Incidence in Sweden 2004, 2005.

246. Sodha N, Williams R, Mangion J, Bullock SL, Yuille MR, Eeles RA. Screening hCHK2 for mutations. Science 2000;289(5478):359.

247. Sommer SS, Jiang Z, Feng J, et al. ATM missense mutations are frequent in patients with breast cancer. Cancer Genet Cytogenet 2003;145(2):115-20.

248. Southey MC, Batten LE, McCredie MR, et al. Estrogen receptor polymorphism at codon 325 and risk of breast cancer in women before age forty. J Natl Cancer Inst 1998;90(7)**:**532-6.

249. Southey MC, Tesoriero AA, Andersen CR, et al. BRCA1 mutations and other sequence variants in a population-based sample of Australian women with breast cancer. Br J Cancer 1999;79(1)**:**34-9.

250. Srivastava S, Zou ZQ, Pirollo K, Blattner W, Chang EH. Germ-line transmission of a mutated p53 gene in a cancer-prone family with Li-Fraumeni syndrome. Nature 1990;348(6303)**:**747-9.

251. Steck PA, Pershouse MA, Jasser SA, et al. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 1997;15(4)**:**356-62.

252. Struewing JP, Hartge P, Wacholder S, et al. The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. N Engl J Med 1997;336(20)**:**1401-8.

253. Sundarrajan C, Liao WX, Roy AC, Ng SC. Association between estrogen receptor-beta gene polymorphisms and ovulatory dysfunctions in patients with menstrual disorders. J Clin Endocrinol Metab 2001;86(1)**:**135-9.

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

255. Swift M, Reitnauer PJ, Morrell D, Chase CL. Breast and other cancers in families with ataxia-telangiectasia. N Engl J Med 1987;316(21)**:**1289-94.

256. Syrjakoski K, Kuukasjarvi T, Auvinen A, Kallioniemi OP. CHEK2 1100delC is not a risk factor for male breast cancer population. Int J Cancer 2004;108(3)**:**475-6.

257. Syrjakoski K, Vahteristo P, Eerola H, et al. Population-based study of BRCA1 and BRCA2 mutations in 1035 unselected Finnish breast cancer patients. J Natl Cancer Inst 2000;92(18)**:**1529-31.

258. Szabo CI, King MC. Population genetics of BRCA1 and BRCA2. Am J Hum Genet 1997;60(5)**:**1013-20.

259. Tamimi RM, Hankinson SE, Ding S, et al. The HRAS1 variable number of tandem repeats and risk of breast cancer. Cancer Epidemiol Biomarkers Prev 2003;12(12)**:**1528-30.

260. Tavtigian SV, Simard J, Rommens J, et al. The complete BRCA2 gene and mutations in chromosome 13q-linked kindreds. Nat Genet 1996;12(3)**:**333-7.

261. Teraoka SN, Malone KE, Doody DR, et al. Increased frequency of ATM mutations in breast carcinoma patients with early onset disease and positive family history. Cancer 2001;92(3)**:**479-87.

262. Thalib L, Wedren S, Granath F, et al. Breast cancer prognosis in relation to family history of breast and ovarian cancer. Br J Cancer 2004;90(7)**:**1378-81.

263. Thellenberg-Karlsson C, Lindstrom S, Malmer B, et al. Estrogen receptor beta polymorphism is associated with prostate cancer risk. Clin Cancer Res 2006;12(6)**:**1936-41.

264. Thomas HV, Reeves GK, Key TJ. Endogenous estrogen and postmenopausal breast cancer: a quantitative review. Cancer Causes Control 1997;8(6)**:**922-8.

265. Thompson D, Easton D. Variation in cancer risks, by mutation position, in BRCA2 mutation carriers. Am J Hum Genet 2001;68(2)**:**410-9.

266. Thompson D, Easton D. Variation in BRCA1 cancer risks by mutation position. Cancer Epidemiol Biomarkers Prev 2002;11(4):329-36.

267. Thompson D, Easton DF. Cancer Incidence in BRCA1 mutation carriers. J Natl Cancer Inst 2002;94(18):1358-65.

268. Thompson D, Szabo CI, Mangion J, et al. Evaluation of linkage of breast cancer to the putative BRCA3 locus on chromosome 13q21 in 128 multiple case families from the Breast Cancer Linkage Consortium. Proc Natl Acad Sci U S A 2002;99(2):827-31.

269. Thompson PA, Ambrosone C. Molecular epidemiology of genetic polymorphisms in estrogen metabolizing enzymes in human breast cancer. J Natl Cancer Inst Monogr 2000(27):125-34.

270. Thorlacius S, Olafsdottir G, Tryggvadottir L, et al. A single BRCA2 mutation in male and female breast cancer families from Iceland with varied cancer phenotypes. Nat Genet 1996;13(1):117-9.

271. Thorlacius S, Sigurdsson S, Bjarnadottir H, et al. Study of a single BRCA2 mutation with high carrier frequency in a small population. Am J Hum Genet 1997;60(5):1079-84.

272. Thorlacius S, Struewing JP, Hartge P, et al. Population-based study of risk of breast cancer in carriers of BRCA2 mutation. Lancet 1998;352(9137):1337-9.

273. Thorlacius S, Tryggvadottir L, Olafsdottir GH, et al. Linkage to BRCA2 region in hereditary male breast cancer. Lancet 1995;346(8974):544-5.

274. Toft D, Shyamala G, Gorski J. A receptor molecule for estrogens: studies using a cell-free system. Proc Natl Acad Sci U S A 1967;57(6):1740-3.

275. Tomlinson IP, Houlston RS. Peutz-Jeghers syndrome. J Med Genet 1997;34(12):1007-11.

276. Tonin P, Weber B, Offit K, et al. Frequency of recurrent BRCA1 and BRCA2 mutations in Ashkenazi Jewish breast cancer families. Nat Med 1996;2(11):1179-83.

277. Tryggvadottir L, Sigvaldason H, Olafsdottir GH, et al. Population-based study of changing breast cancer risk in Icelandic BRCA2 mutation carriers, 1920-2000. J Natl Cancer Inst 2006;98(2):116-22.

278. Tsou HC, Teng DH, Ping XL, et al. The role of MMAC1 mutations in early-onset breast cancer: causative in association with Cowden syndrome and excluded in BRCA1-negative cases. Am J Hum Genet 1997;61(5):1036-43.

279. Tsuchiya A, Kanno M, Nomizu T, Hatakeyama Y, Kimijima I, Abe R. Clinical characteristics of breast cancer patients with family history. Fukushima J Med Sci 1998;44(1):35-41.

280. Wagenius M, Borg A, Johansson L, Giwercman A, Bratt O. CHEK2*1100delC is not an important high-risk gene in families with hereditary prostate cancer in southern Sweden. Scand J Urol Nephrol 2006;40(1):23-5.

281. Vahteristo P, Bartkova J, Eerola H, et al. A CHEK2 genetic variant contributing to a substantial fraction of familial breast cancer. Am J Hum Genet 2002;71(2):432-8.

282. Vahteristo P, Tamminen A, Karvinen P, et al. p53, CHK2, and CHK1 genes in Finnish families with Li-Fraumeni syndrome: further evidence of CHK2 in inherited cancer predisposition. Cancer Res 2001;61(15):5718-22.

283. Wall JD, Pritchard JK. Haplotype blocks and linkage disequilibrium in the human genome. Nat Rev Genet 2003;4(8):587-97.

284. van Beers EH, van Welsem T, Wessels LF, et al. Comparative genomic hybridization profiles in human BRCA1 and BRCA2 breast tumors highlight differential sets of genomic aberrations. Cancer Res 2005;65(3):822-7.

285. van den Brandt PA, Spiegelman D, Yaun SS, et al. Pooled analysis of prospective cohort studies on height, weight, and breast cancer risk. Am J Epidemiol 2000;152(6):514-27.

286. van der Hel OL, Peeters PH, Hein DW, et al. NAT2 slow acetylation and GSTM1 null genotypes may increase postmenopausal breast cancer risk in long-term smoking women. Pharmacogenetics 2003;13(7):399-407.

287. van der Hel OL, Peeters PH, Hein DW, et al. GSTM1 null genotype, red meat consumption and breast cancer risk (The Netherlands). Cancer Causes Control 2004;15(3):295-303.

288. Van Der Looij M, Szabo C, Besznyak I, et al. Prevalence of founder BRCA1 and BRCA2 mutations among breast and ovarian cancer patients in Hungary. Int J Cancer 2000;86(5):737-40.

289. van Duijnhoven FJ, Bezemer ID, Peeters PH, et al. Polymorphisms in the estrogen receptor alpha gene and mammographic density. Cancer Epidemiol Biomarkers Prev 2005;14(11 Pt 1):2655-60.

290. Wang WY, Barratt BJ, Clayton DG, Todd JA. Genome-wide association studies: theoretical and practical concerns. Nat Rev Genet 2005;6(2):109-18.

291. 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(3):269-72.

292. Wang-Gohrke S, Chang-Claude J. Re: Population-based, case-control study of HER2 genetic polymorphism and breast cancer risk. J Natl Cancer Inst 2001;93(21):1657-9.

293. Warner E, Foulkes W, Goodwin P, et al. Prevalence and penetrance of BRCA1 and BRCA2 gene mutations in unselected Ashkenazi Jewish women with breast cancer. J Natl Cancer Inst 1999;91(14):1241-7.

294. Vasconcelos A, Medeiros R, Veiga I, et al. Analysis of estrogen receptor polymorphism in codon 325 by PCR-SSCP in breast cancer: association with lymph node metastasis. Breast J 2002;8(4):226-9.

295. Wedren S, Lovmar L, Humphreys K, et al. Oestrogen receptor alpha gene haplotype and postmenopausal breast cancer risk: a case control study. Breast Cancer Res 2004;6(4):R437-49.

296. Vehmanen P, Friedman LS, Eerola H, et al. Low proportion of BRCA1 and BRCA2 mutations in Finnish breast cancer families: evidence for additional susceptibility genes. Hum Mol Genet 1997;6(13):2309-15.

297. Weiderpass E, Persson I, Melhus H, Wedren S, Kindmark A, Baron JA. Estrogen receptor alpha gene polymorphisms and endometrial cancer risk. Carcinogenesis 2000;21(4):623-7.

298. Veronesi A, de Giacomi C, Magri MD, et al. Familial breast cancer: characteristics and outcome of BRCA 1-2 positive and negative cases. BMC Cancer 2005;5:70.

299. Westberg L, Baghaei F, Rosmond R, et al. Polymorphisms of the androgen receptor gene and the estrogen receptor beta gene are associated with androgen levels in women. J Clin Endocrinol Metab 2001;86(6):2562-8.

300. Weston A, Godbold JH. Polymorphisms of H-ras-1 and p53 in breast cancer and lung cancer: a meta-analysis. Environ Health Perspect 1997;105 Suppl 4:919-26.

301. Willett WC, .Rockhill B., Hankinsson S.E., Hunter D.J., Colditz G.A. Epidemiology and nongenetic risk factors for breast cancer. In: J.R.. H, ed. Diseases of the Breast. 2nd ed: Lippincott Williams and Wilkins, 2000: 175-220.

302. Williams WR, Anderson DE. Genetic epidemiology of breast cancer: segregation analysis of 200 Danish pedigrees. Genet Epidemiol 1984;1(1)**:**7-20.

303. Wobbes T, van de Wiel MP, van der Sluis RF, Theeuwes AG. The effect of familiality on clinical presentation and survival in mammary carcinoma. Eur J Surg Oncol 1987;13(2)**:**119-21.

304. Vogl FD, Taioli E, Maugard C, et al. Glutathione S-transferases M1, T1, and P1 and breast cancer: a pooled analysis. Cancer Epidemiol Biomarkers Prev 2004;13(9)**:**1473-9.

305. Wooster R, Bignell G, Lancaster J, et al. Identification of the breast cancer susceptibility gene BRCA2. Nature 1995;378(6559)**:**789-92.

306. Wooster R, Neuhausen SL, Mangion J, et al. Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. Science 1994;265(5181)**:**2088-90.

307. Vorechovsky I, Luo L, Lindblom A, et al. ATM mutations in cancer families. Cancer Res 1996;56(18)**:**4130-3.

308. Xie D, Shu XO, Deng Z, et al. Population-based, case-control study of HER2 genetic polymorphism and breast cancer risk. J Natl Cancer Inst 2000;92(5)**:**412-7.

309. Yamada Y, Ando F, Niino N, Ohta S, Shimokata H. Association of polymorphisms of the estrogen receptor alpha gene with bone mineral density of the femoral neck in elderly Japanese women. J Mol Med 2002;80(7)**:**452-60.

310. 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(11)**:**866-71.

311. Yoshida R, Kimura N, Harada Y, Ohuchi N. The loss of E-cadherin, alpha- and beta-catenin expression is associated with metastasis and poor prognosis in invasive breast cancer. Int J Oncol 2001;18(3)**:**513-20.

312. Zelada-Hedman M, Borresen-Dale AL, Claro A, Chen J, Skoog L, Lindblom A. Screening for TP53 mutations in patients and tumours from 109 Swedish breast cancer families. Br J Cancer 1997;75(8)**:**1201-4.

313. Zelada-Hedman M, Borresen-Dale AL, Lindblom A. Screening of 229 family cancer patients for a germline estrogen receptor gene (ESR) base mutation. Hum Mutat 1997;9(3)**:**289.

314. Zelada-Hedman M, Wasteson Arver B, Claro A, et al. A screening for BRCA1 mutations in breast and breast-ovarian cancer families from the Stockholm region. Cancer Res 1997;57(12)**:**2474-7.

315. Zheng SL, Zheng W, Chang BL, et al. Joint effect of estrogen receptor beta sequence variants and endogenous estrogen exposure on breast cancer risk in Chinese women. Cancer Res 2003;63(22)**:**7624-9.

316. Zuppan P, Hall JM, Lee MK, Ponglikitmongkol M, King MC. Possible linkage of the estrogen receptor gene to breast cancer in a family with late-onset disease. Am J Hum Genet 1991;48(6)**:**1065-8.