Insights into breast cancer: New familial patterns and identification of a potential predictive marker

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Insights into breast cancer:
New familial patterns and identification of a potential predictive marker

Carolina Ellberg

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Faculty opponent
Per Karlsson
The last proportion of heredity in breast cancer has proven to be somewhat elusive despite massive attempts to identify the associated factors. Approximately 50 percent of breast cancer caused by familial factors is currently explained. The five-year survival for breast cancer patients is excellent; however, breast cancer is considered a chronic disease, and given enough time, new tumors can develop. Women age 40 and older are offered screening mammography. However, population screening is expensive, and being able to pinpoint those who are at high risk of breast cancer would be beneficial. Both genetic and environmental risk factors could be used to select women who need screening. A major aim of this thesis was to try to identify potential familial patterns as candidates for hereditary breast cancer.

In Paper I, we studied horizontal family history of breast cancer in relation to histology to discover a candidate phenotype for recessive inheritance. A horizontal pedigree pattern is characterized by two or more sisters diagnosed with breast cancer, without a family history of breast cancer in prior generations. A horizontal inheritance was more common in patients with tubular carcinoma compared with other histologic subtypes. Therefore, we propose that breast cancer patients with tubular carcinoma who have a sister or sisters diagnosed with breast cancer are candidates for genetic studies when searching for a recessively inherited predisposing gene.

In paper II, we studied the occurrence of cancer in first-degree relatives of breast cancer patients diagnosed with the lobular carcinoma histologic subtype compared with other histologic subtypes of breast cancer. We found a hereditary pattern involving breast cancer patients with lobular carcinoma and having a father diagnosed with cancer. The association was independent of a family history of breast cancer in sisters, the mother and grandmothers. Similarly, even though prostate cancer was prominent in the fathers, the association remained after removal of fathers diagnosed with prostate cancer.

In paper III, we confirmed a previously reported younger age at breast cancer diagnosis in carriers of a BRCA1 mutation of paternal origin compared with maternal origin. Additionally, we observed an older age at ovarian cancer diagnosis in carriers of a BRCA1 mutation of paternal origin compared with maternal origin. No such observations were observed for BRCA2 mutation carriers.

In paper IV, we studied the occurrence of spider telangiectasias at the time of breast cancer diagnosis in relation to hormonal risk factors. We reported that the occurrence of spider telangiectasias was associated with several hormonal risk factors such as weight, parity, history of oral contraceptive use, and menopausal hormone therapy use. A better overall survival was observed in older breast cancer patients who displayed spider telangiectasias at the time of breast cancer diagnosis.

Key words
Breast cancer, Familial, Recessive, Parental inheritance, Heredity, BRCA1, BRCA2, Spider telangiectasias

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Insights into breast cancer:

New familial patterns and identification of a potential predictive marker

Carolina Ellberg
Till mamma och mormor
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List of original papers

This thesis is based on the following papers, referred to in the text by their roman numerals

I. Can a phenotype for recessive inheritance in breast cancer be defined? Carolina Ellberg, Göran Jönsson and Håkan Olsson
   *Familial cancer, 9: 525-530, 2010*

II. Cancer in father predicts lobular breast cancer in daughter
    Carolina Ellberg and Håkan Olsson
    *BMC Cancer, 11:497, 2011*

III. Impact of a paternal origin of germline *BRCA1/2* mutations on the age at breast and ovarian cancer diagnosis in a southern Swedish cohort
     Carolina Ellberg, Helena Jernström, Per Broberg, Åke Borg, Håkan Olsson
     *Manuscript submitted*

IV. Breast cancer and spider telangiectasias at diagnosis and its relation to histopathology and prognosis: a population-based study
    *Breast Cancer Research and Treatment, 131:177–186, 2012*

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Abbreviations

BC - Breast cancer
OvC - Ovarian cancer
IDC - Invasive ductal carcinoma
ILC - Invasive lobular carcinoma
ER - Estrogen receptor
PR - Progesterone receptor
C - Cytosine
p - Phosphodiester
G - Guanine
OCP - Oral contraceptive pills
TC - Tubular carcinoma
MHT - Menopausal hormone therapy
WTH - Waist-to-hip
BMI - Body mass index
AI - Aromatase inhibitors
IHC - Immunohistochemistry
Ki-67 - Ki67 antigen
NOS - Not otherwise specified
NST - No special type
CMF - Cyclophosphamide, methotrexate and flurouracil
PARP - Poly (ADP-ribose) polymerase
OR - Odds ratio
CI - Confidence interval
HR - Hazard ratio
RVE - Robust variance estimation
MICE - Multivariate Imputation by Chained Equations
CGH - Comparative genomic hybridization

Gene names

IGF2 - Insulin growth factor 2
BRCA1 - Breast cancer, early onset 1
BRCA2 - Breast cancer, early onset 2
RAD51 - RAD51 recombinase
TP53 - Tumor protein 53
STK11 - Serine/threonine protein kinase 11
ATM - Ataxia telangiectasia mutated
CHEK2 - Chekpoint kinase 2
PALB2 - Partner and localizer of BRCA2
BRIP1 - BRCA1 interacting protein C-terminal helicase 1
PTEN - Phosphatase and tensin homolog
CDH1 - Cadherin 1, type 1, E-cadherin
CDKN2A - Cyclin-dependent kinase inhibitor 2A
HER2 - v-erb-b2 avian erythroblastic leukemia viral oncogene homolog 2
HIF-1α - Hypoxia inducible factor 1 α
VEGF-A - Vascular endothelial growth factor A
FGF - Fibroblast growth factor
MMP - Matrix metalloproteinases
TGF-β1 - Transforming growth factor, beta 1
Summary

The last proportion of heredity in breast cancer has proven to be somewhat elusive despite massive attempts to identify the associated factors. Approximately 50 percent of breast cancer caused by familial factors is currently explained. The five-year survival for breast cancer patients is excellent; however, breast cancer is considered a chronic disease, and given enough time, new tumors can develop. Women age 40 and older are offered screening mammography. However, population screening is expensive, and being able to pinpoint those who are at high risk of breast cancer would be beneficial. Both genetic and environmental risk factors could be used to select women who need screening. A major aim of this thesis was to try to identify potential familial patterns as candidates for hereditary breast cancer.

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Introduction

One third of the world’s population will be diagnosed with cancer at some point in their life-time. Although the 5-year survival has increased, there is an increase in the incidence of cancer in the entire population, and especially in the developing countries. In Sweden approximately 7500 women and 45 men were diagnosed with breast cancer (BC) in 2011, and although the 5-year survival is 90%, still 1400 women and 15 men died in 2011 due to their BC. One third of all cancer diagnoses in women was BC diagnoses in Sweden in 2011, and approximately one in every nine women will be diagnosed with BC before age 75.

Tumor development in the breast from the first change in a normal cell, until a malignant tumor has developed is estimated to be on average 20 to 40 years. In 2000, Hanahan and Weinberg suggested key elements called “The hallmarks of cancer”, which are necessary for tumor development: resistance to cell death, sustained proliferative signaling, evasion of growth suppressors, enabling replicative immortality, activation of the ability to invade tissues and form metastases, and the induction of tumor angiogenesis. In 2011, they published an updated version containing four new hallmarks in addition to the other six: induction of genome instability and generation of mutations, avoidance of immune destruction, deregulation of cellular energetics, and the ability to induce tumor-promoting inflammation.

Figure 1 My thesis in relation to the Hallmarks of cancer. Papers I, II, and III all touch upon heredity and familial breast cancer. In paper IV spider telangiectasias, indicators of high levels of hormonal influence are suggested as markers of angiogenesis. Reprinted from Cell, Vol. 144, Hanahan, D., Weinberg, RA., Hallmarks of cancer: the next generation, pages no. 646-674, Copyright © 2011 with permission from Elsevier.
The presence of breasts is one of the main aspects for distinguishing mammals from other living creatures, such as reptiles, birds and insects. With a support system of interlobular stroma and fibroadipose tissue, the female breast has 6-10 duct systems, which originate at the nipples. Similar to a tree branching into finer and finer branches, the ducts in the mature, lactating breast end in clusters of acini. The ductal-lobular system is layered with an inner epithelium, with a main secretory function, and an outer myoepithelium, with a contractile function. Additionally, the breast is also lined with adipose tissue.

Figure 2. Anatomy of the female breast. The nipple and areola are shown on the outside of the breast. The lymph nodes, lobes, lobules, ducts, muscle, ribs, chest wall, and fatty tissue of the breast are also shown. © 2011 Terese Winslow LLC, U.S. Govt. has certain rights
Development and life-cycle of the breast

In humans, the male and female breasts are anatomically similar from gestational age until pre-puberty. At puberty, the female breast starts to further develop, while the male breast does not further progress and develop. The development and life-cycle of the breast in females is schematically described in figures 3a, 3b, and 3c.

![Table of Breast Development Stages](image)

**Figure 3a.** Brief description of embryonic breast development.\(^7\)\(^11\)

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\(^7\)\(^11\) References and notes.
Breast development: Postpartum until the end of puberty

<table>
<thead>
<tr>
<th>Postnatal</th>
<th>0-7 to w3/4</th>
<th>Withdrawal of maternal hormones induces neonatal prolactin, resulting in secretion of colostral milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn-2 yrs.</td>
<td></td>
<td>Further canalization of end vesicles and ductal structure development, and menopause-like involution</td>
</tr>
<tr>
<td>2 yrs. - onset of puberty</td>
<td></td>
<td>Quiescence, same growth rate of the breasts as the rest of the body</td>
</tr>
<tr>
<td>Pre-puberty</td>
<td></td>
<td>Elevation of the nipple</td>
</tr>
<tr>
<td>Early puberty</td>
<td>Tanner stage 1</td>
<td>Development of the breast buds, formation of a single mound of tissue, enlargement of the areola</td>
</tr>
<tr>
<td></td>
<td>Tanner stage 2 - Thelarche</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Epithelium forms into a branching bilayered ductal structure with an outer layer of basal myoepithelial cells and an inner luminal cell layer. Luminal cells will differentiate into cells lining the ducts (ductal luminal cells) and secretory cells (alveolar luminal cells). Branching and ductal formation starts at the terminal end buds.</td>
</tr>
<tr>
<td></td>
<td>Tanner stage 3</td>
<td>Further increase of breast tissue and areola</td>
</tr>
<tr>
<td></td>
<td>Late puberty</td>
<td>Menarche</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Induction of menses, the first bleeding.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Formation of second mound of tissue, and increased nipple and areola, and recession of the areola</td>
</tr>
<tr>
<td></td>
<td>Late puberty</td>
<td>Stable menarche</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Regularity in monthly cycle</td>
</tr>
</tbody>
</table>

By the time the female reaches late adolescence, the nulliparous breast has reached ductal and stromal maturation and contains mainly lobules type 1. Type 1 lobules consists of a short terminal duct ending in a cluster of approximately 11 alveoli buds and are often referred to as terminal ductal lobular units (TDLU).

Figure 3b Breast development from birth until the end of puberty.⁷-¹¹
<table>
<thead>
<tr>
<th>Pregnancy</th>
<th>First half of pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proliferation resulting in acini formation, more well-differentiated lobules than primitive budding lobules. By the end of the first half the ductal tree is structurally set.</td>
</tr>
<tr>
<td></td>
<td>Type 1 lobules develops into type 2, further to type 3 and finally type 3 into preparation of type 4.</td>
</tr>
<tr>
<td></td>
<td>Second half of pregnancy</td>
</tr>
<tr>
<td></td>
<td>Acini further enlarges through cytoplasm enlargement. Proliferation rate of new acini is smaller than fully differentiated acini. Lipids are accumulating in the epithelium.</td>
</tr>
<tr>
<td></td>
<td>Mostly type 4 lobules present.</td>
</tr>
<tr>
<td>Partum</td>
<td>Lactation</td>
</tr>
<tr>
<td></td>
<td>Females that has gone through pregnancy and is lactating, differentiation of cells follows lactation and milk production.</td>
</tr>
<tr>
<td></td>
<td>Type 4 lobules are present.</td>
</tr>
<tr>
<td>Adult and mature breast</td>
<td>No more lactation in parous women</td>
</tr>
<tr>
<td></td>
<td>Mostly type 3 lobules present.</td>
</tr>
<tr>
<td>&gt; 40 yrs.</td>
<td>Regression</td>
</tr>
<tr>
<td></td>
<td>Type 3 lobules in parous females regress, probably due to involution, back to type 2 and finally to type 1.</td>
</tr>
<tr>
<td>Menopause</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Type 1 lobules present in parous and nulliparous women both. In parous females it's a regressed form of type 1 where most cells are in G0 phase, and in nulliparous women it's a proliferative type 1 where few cells are in G0 phase.</td>
</tr>
</tbody>
</table>

**Figure 3c** Breast development from first pregnancy until menopause. 7-11
Risk factors for breast cancer

Genetics and inheritance

In monogenic inheritance, the inherited genotype involves only one locus, whereas the polygenic inheritance, involves several loci. A genotype could be autosomal, which means that the locus inherited is situated on chromosomes one to 22. A genotype could also be gonosomal, which means that the locus is located on the X or Y chromosomes. A third type of genotype is mitochondrial, which means that the locus is located on the mitochondrial DNA, which is generally maternally inherited in humans.12

When discussing the correlation between genotype and phenotype, there are several inheritance patterns through which inheritance can occur. For a monogenic trait, there is the classical dominant inheritance, in which the dominant phenotype is visible when the carrier is either homozygous or heterozygous for the dominant allele. The recessive genotype only becomes the phenotype when the carrier is homozygous for the recessive allele. There is also the co-dominant genotype, which is phenotypically visible when the carriers are heterozygous for the alleles.12

The penetrance for a genotype in cancer genetics is what determines whether, and when an inherited genotype that predisposed the phenotype (cancer) manifests disease. Penetrance can be altered on different levels. One such example is maternal or paternal imprinting. When maternal imprinting occurs, it is only the paternal allele that is expressed, and vice versa. One example of a maternally imprinted gene is the insulin-like growth factor 2 (IGF2).13 Environmental modifiers influence the penetrance of the inherited phenotype, such as pregnancy in BRCA1 mutation carriers, which increases the risk of a breast cancer diagnosis. There are also genetic modifiers of an inherited genotype, such as low penetrance genes.
Breast cancer genetics

It has been estimated that approximately 25% of all BC cases are caused by familial factors\textsuperscript{14} and that approximately 5-10% are caused by a monogenic inheritance.\textsuperscript{15} Almost half of familial BC have a known cause, whereas the other half remains unexplained. Of the known causes of familial BC, the two rare, high-penetrance genes, \textit{BRCA1} and \textit{BRCA2}, account for the largest percentage (Figure 4).\textsuperscript{16, 17} However, it is likely that a large proportion of familial BC with unknown cause is due to combinations of low-penetrance genetic components, such as loci, genes, polymorphisms, and single nucleotide polymorphisms, independent of or in combination with environmental risk factors.\textsuperscript{18, 19} Some of these have been identified, while others will be more difficult to identify due to the interaction between genes and the environment.\textsuperscript{20}

\textbf{Figure 4} Approximated distribution of hereditary breast cancer: known and unknown causes.
Genes or loci that have been found to increase the risk of breast cancer generally belong to one of the following biological pathways: mammary gland development, DNA repair, cell cycle control, and estrogen receptor signaling (Figure 5)\textsuperscript{21}.

**Figure 5** Pathways to which the different genes increasing risk of breast cancer falls. Rare high- or moderate-penetrance genes are indicated in red, whereas blue genes are candidate genes implicated in breast cancer risk found in or adjacent to common low-penetrant breast cancer susceptibility loci.\textsuperscript{21} Reprinted from American journal of Pathology, Vol. 182, No.4, Ghoussaini, M., Pharaoh, P., and Easton, D., *Inherited Genetic Susceptibility to Breast Cancer: The beginning of the end or the end of the beginning?*, pages no. 1038-1051, Copyright © 2013 with Permission from the American Society for Investigative Pathology.
Rare high-penetration genes, lifetime risk >50%

Breast cancer 1, early onset (BRCA1) and breast cancer 2, early onset (BRCA2) are the two most important rare, high-penetration BC genes. BRCA1 and BRCA2 have partially different functions, which are indicated by their different tumor spectra. Female BRCA1 mutation carriers have, in addition to an increased BC and ovarian cancer (OvC) risk, an increased risk of fallopian tube carcinoma and primary papillary serous carcinoma of the peritoneum. Male BRCA1 mutation carriers have similar risks of cancer as the general population. Female BRCA2 mutation carriers have, in addition to the increased risk of BC and OvC, an increased risk of both fallopian tube carcinoma and primary papillary serous carcinoma of the peritoneum that is much higher than that of the general population. For male BRCA2 mutation carriers, the risk of prostate cancer and male breast cancer are substantially increased. For BRCA2 mutation carriers, additional malignancies could include pancreatic cancer and tumors in the gallbladder, bile duct and stomach, as well as melanoma.

Identified in 1994, BRCA1 is located on chromosome 17q21. BRCA1 consists of 24 coding exons and is transcribed into the BRCA1 protein consisting of 1863 amino acids. BRCA1 is involved in maintaining genomic integrity in multiple systems: homologous repair, checkpoint control, spindle regulation and transcriptional regulation, as well as in response to estrogentic proliferation and genotoxic stress. In 1995, BRCA2 was identified; it is located on chromosome 13q12.3 and consists of 26 coding exons that are transcribed into the BRCA2 protein consisting of 3418 amino acids. Less is known regarding all the functions of BRCA2 in cells, but it is known that it plays an important role in regulating a protein (RAD51), thereby being a crucial part of the homologous repair processes in response to double strand breaks.

For BRCA1 mutation carriers, the risk for developing BC by age 70 has been shown to vary between 40% and 87%, and the risk for OvC varies between 16% and 68%. For BRCA2 mutation carriers, the risk of a BC diagnosis ranges between 40% and 84% and the risk of an OvC diagnosis ranges between 11% and 27%. This variation in penetrance indicates involvement of other factors, which could include both genetic and lifestyle factors. Some of the observed differences in penetrance between studies may be due to differences in study design, such as high-risk cases only vs. population based studies. Regarding the genetic factors that influence penetrance, several modifiers of penetrance for BRCA1 and BRCA2 have been reported during the last couple of years.
BC tumors in *BRCA1* and *BRCA2* mutation carriers are most commonly of the histologic type invasive ductal carcinoma (IDC). However, compared to the distribution in sporadic BC cases, medullary carcinoma is more common in both *BRCA1* and *BRCA2* mutation carriers with a majority in *BRCA1* mutation carriers. Whereas invasive lobular carcinoma (ILC) is less frequent in *BRCA1* mutation carriers, the occurrence in percentage is similar in *BRCA2* mutation carriers compared with BC tumors in general population. Tumors in *BRCA1* mutation carriers are generally estrogen receptor (ER) negative, and progesterone receptor (PR) negative, while the opposite is true for tumors of *BRCA2* mutation carriers.\(^{36}\)

**Other rare high-penetrance genes**

The third rare high-penetrance gene is the tumor-suppressor gene Tumor protein 53 (*TP53*). *TP53* is mutated in the cancer predisposing syndrome Li-Fraumeni cancer syndrome.\(^{37}\) *TP53* was identified as the cause of Li-Fraumeni syndrome in the early 1990s.\(^{38,39}\) Li-Fraumeni syndrome causes a wide range of tumors, such as sarcomas, brain tumors, adrenocortical tumors, childhood cancers, and early-onset BC.\(^{37,40,41}\) Women carrying a mutation in this gene have an approximately 100% life-time risk of developing BC\(^ {42}\) and a 30% risk of developing BC by age 30.\(^ {16}\) The transcription factor p53, encoded by *TP53*, plays a major role in cell-cycle regulation. p53 can activate several anti-proliferative pathways, inducing apoptosis, cell-cycle arrest, and senescence. *TP53* is one of the most common altered genes in any type of sporadic tumor.\(^ {43}\)

The last of the rare high-penetrance genes known today is the Serine/threonine protein kinase 11 (*STK11*, formerly known as *LKB1*) mutated in the Peutz-Jeghers syndrome.\(^ {44}\) Heterozygous mutation carriers of *STK11* have an increased risk of breast cancer\(^ {45}\). *STK11* encoded by *STK11* is important in regulating cell-cycle arrest through various pathways, one of which includes p53.\(^ {46}\)

It is unlikely that there are many high-penetrance genes left to be discovered for BC. Those that may exist are most likely very rare or even family/region specific and will therefore most likely only be found accidentally.

**Moderate-penetrance genes, lifetime risk >20%**

By re-sequencing former candidate genes of high-penetrance BC found through family-based linkage analysis and positional cloning for genes with a moderate-penetrance of BC were discovered, for example, searching up-stream and down-stream in the DNA-repair signaling pathways in which *BRCA1* and *BRCA2* act.\(^ {21}\)
Such genes include mutations in the ATM, where heterozygous carriers have an approximately two-fold increased risk of developing BC\textsuperscript{47-49}. CHEK2,\textsuperscript{50, 51} PALB2,\textsuperscript{52} and BRIP1\textsuperscript{53} also interacts with or in the DNA-repair pathways through which BRCA1 and BRCA2 act. The two other moderate-penetrance genes are part of other rare syndromes and were found using linkage studies in families with either Cowden’s disease, PTEN,\textsuperscript{54} or Hereditary diffuse gastric cancer, CDH1.\textsuperscript{55} There are most likely more of these moderate-penetrance genes to be found. This would require re-sequencing families with clusters of tumors with today’s more in-depth methods in very large cohorts. Some of these genes may even be coupled with hormonal life-style factors that influence BC risk. Therefore, there is a need for studies combining sequencing information with known endogenous and environmental BC risk factors.

**Low-penetrance genes, lifetime risk >10%**

Known low-penetrance genes include a group of more than 72 BC susceptibility loci. The increase in the relative risk for low-penetrance genes of BC is seldom above 1.5.\textsuperscript{21} Included in this group are commonly occurring allele variants in genes located in or adjacent to BC susceptibility loci. Some of these increase the risk of BC, while others decrease the risk. It is also very likely that some alleles increase the risk of BC given specific genetic or environmental influences through epigenetic changes. It is very likely that there are several more common low-penetrance alleles to be found.\textsuperscript{21}

**Epigenetics in breast cancer**

The most common epigenetic modification is methylation of CpG islands.\textsuperscript{56} The cytosine (C) residue is followed by a guanine (G) residue with a phosphodiester (p) bond between them, and they frequently occur in clusters, so called CpG islands. The CpG islands are not randomly distributed in the genome and are often in, or in close proximity to promoter regions. CpG islands are found in more than half of all promoter regions in the human genome.\textsuperscript{57} Through methylation of the CpG islands, the histones undergo a conformational change, which causes the promoter to be less accessible or completely inaccessible for transcription. The latter is called epigenetic silencing. In sporadic BC, the expression of BRCA1 is down-regulated both epigenetically and through somatic mutations.\textsuperscript{58} The functioning allele in tumor cells of germline BRCA1/2 mutation carriers is seldom down-regulated epigenetically. However, several other genes, such as CDKN2A and CDH1, are commonly regulated through methylation, sometimes to such a degree that the gene is silenced, which is
called hypermethylation. Hypermethylation occurs in hereditary BC families as well as in sporadic tumors. Epigenetics, which also include other types of regulation of gene expression is a new field within BC research, and much is yet to be understood about how these processes influences both hereditary and as sporadic tumor development.

Endogenous and environmental risk factors

Risk factors that are not genetically or epigenetically inherited from parents are endogenous or environmental risk factors. The influence of endogenous and environmental risk factors starts in utero at the gestational stage via the maternal blood, and their influence continues until death. For several of the factors, the exposure is important and influential in specific time intervals during life associated with different developmental stages of the breast. It should be noted that tumorigenesis in breast carcinoma in most cases is a long and slow process, and exposure throughout life accumulates until the scale is tipped towards cancer development.

Exposures during gestational development

Several studies have reported an increased risk of mammary tumor development in rodents whose mothers were treated with hormone-like substances such as Bisphenol A. The association between prenatal exposure and BC is difficult to assess in humans; however, we are learning a great deal from murine models.

Exposure to radiation

Exposure to ionizing radiation, especially during childhood and early breast development, increases the risk of BC. The increased risk is not observed in women exposed at age 40 and older.

Age at first menarche

An early menarche is an established risk factor for BC; the risk of BC increases with younger age of menarche. The time between menarche and menopause, as well as menstrual cycle length, and number of pregnancies, reflects the number of menstrual
cycles a woman undergoes. A study reported that the number of menstrual cycles was a better risk determinant than the individual risk factors. Later onset of menarche seems to be associated with a decreased risk of mucinous carcinoma.

**Hormonal contraception**

A current use of oral contraceptive pills (OCP) induces a small increase in the risk ratio of a BC diagnosis. The increased risk will last until 10 years after cessation. Initiation of OCP use before age 20 is associated with an increase in BC risk compared with women who are older at the initiation of OCP use, and early initiation of OCP use has been associated with more proliferative tumors. However, tumors of current OCP users at BC diagnosis are more likely to be highly differentiated compared with tumors of never users of OCP at diagnosis. Almost 90% of all women in Sweden are current users of OCP or have a prior history of OCP use. There seems to be a limited or no difference between ILC and invasive ductal carcinoma (IDC) risk and OCP use. The risk induction for OCP use in BRCA1 and BRCA2 mutation carriers is somewhat controversial. Several studies have reported an increased risk of BC among BRCA1/2 mutation carriers with a prior history of OCP use.

**Parity**

Increasing parity is generally considered to be protective against BC, and nulliparity is associated with an increased risk of BC at age 40-45. However, there seems to be a short-term increase in BC risk after each pregnancy followed by long-term protection. With regards to the histological subtypes, patients with IDC, ILC or tubular carcinoma (TC) all have decreased risk of BC compared with nulliparous women. There have also been reports of a decreased risk of BC with increasing number of full-term pregnancies for the different histologic subtypes of BC, especially in mucinous carcinoma. For medullary carcinoma, in contrast, there seems to be an increased risk with increasing parity. In BRCA1/2 mutation carriers, there are observations of both a decreased and increased risk of BC with parity.

**Age at first full-term pregnancy**

Increased age at first full-term pregnancy increases the risk of BC in general. There are observations that women older than 35 years at their first full-term pregnancy
have a similar or even higher risk of BC compared with nulliparous women. With regards to histology, the risk of medullary carcinoma does not seem to increase with increasing age at first full-term pregnancy, but results seem conflicting. For mucinous carcinoma and TC, however, there are several studies that report a lack of effect of age at first full-term pregnancy and risk of BC, whereas patients with ILC had a significantly higher risk with increasing age at first full-term pregnancy compared with other histopathological subtypes, even if the increased BC risk is present in IDC as well. In BRCA1/2 mutation carriers, the present conclusion is that there is no increase in the risk of BC with increasing age at first full-term pregnancy.

**Duration of breastfeeding**

Breastfeeding duration is a debated risk factor for developing BC. However, in a meta-analysis conducted by the ‘Collaborative Group on Hormonal Factors in Breast Cancer’, it was estimated that there is a risk reduction for BC of 4.3% per every twelve months of breastfeeding. There seem to be little or no differences between the histological subtypes with regards to risk reduction of BC. For BRCA1 mutation carriers, a breastfeeding duration of more than 12 months is associated with a substantially reduced risk of BC. There is no observed association between duration of breastfeeding and BC risk in BRCA2 mutation carriers.

**Alcohol consumption and smoking**

A daily intake of alcohol increases the risk of BC. The current consensus regarding smoking and the risk of BC is that smoking is responsible for a modest increase in BC risk. The risk is influenced by timing in the sense that women who start smoking around or immediately after menarche and whom continue to smoke with high consumption until first full-term pregnancy have the highest BC risk.

**Menopause**

Increasing age at natural menopause, increases the risk of BC. However, the increased risk of BC caused by a natural menopause at an older age is generally not observed until after age 65. Surgical menopause through oophorectomy performed prior to natural menopause also reduces the risk.
**Menopausal hormone therapy – MHT**

MHT, including both estrogen and progestins, increase BC risk, with indications of higher risks the closer the start of MHT is to menopause. This increase in BC risk is not limited to hormone receptor positive BC. BC tumors in women who were administered MHT with estrogens and progestins MHT are often of higher grade, and BC mortality is increased compared to BC patients who received placebo. The higher grade is likely caused by the difficulty in mammographic detection because of the increased density of the breast tissue caused by the MHT. There are observations that MHT with estrogen only reduces BC risk. The association between BC and MHT use has been reported to confer both a higher and a lower risk of BC. There is a need to further study this question. A current use of both combination therapy and estrogen only MHT at the time of BC diagnosis seems to increase the risk of tubular carcinoma, even if the risk is increased for ductal and lobular carcinoma as well.

**Anthropometric measures**

Obesity in postmenopausal women is associated with an increased risk of BC. In premenopausal women, however, obesity is associated with a decreased BC risk. The relationship between obesity in premenopausal women and the risk of BC has long been debated. Waist-to-hip (WTH) ratio, weight or body mass index (BMI) has historically often been used as a proxy marker for obesity. A recent meta-analysis concluded that an increase in BMI was associated with a decrease of BC risk in women of Caucasian and African descent, whereas the opposite was observed in women of Asian descent. However, an increased WTH ratio was associated with an increased risk of premenopausal BC across all ethnicities. WTH ratios reflect central obesity, which have been reported to influence hormonal and growth factor production, such as increased levels of free IGF-1, insulin resistance, and so on. Therefore, it might be more appropriate to use WTH for BC risk assessment, especially in premenopausal women. Increased height is also associated with an increased risk of BC, in both premenopausal and postmenopausal women.

**Other factors**

Other endogenous or environmental risk factors implicated in BC risk includes shift work, physical activity, diet, breast size and breast density.
Breast cancer prevention

Prevention against BC development is growing increasingly important as the BC incidence is increasing. An estimation of the decrease in cumulative BC risk for preventative strategies is visualized in figure 6.

Life-style prevention strategies

Several preventative measures can be taken by counteracting some of the risk factors for BC mentioned in the prior chapter. Exercise equal to or more than 30 minutes per day and sustain a healthy weight, avoid excessive alcohol intake, abstain from smoking, keep a healthy diet, and increase duration of breast-feeding. However, there are medical and surgical preventative strategies as well.

Oophorectomy

As described in the menopause section, surgical oophorectomy, both before natural menopause and at natural menopause, decreases the risk of BC. If undertaken before age 40, the risk reduction by oophorectomy could be up to 50% compared to women with natural menopause. However, there have been observations indicating that oophorectomy at the same age as natural menopause also decreases the risk of BC.

Prophylactic surgery in high risk women

For BRCA1 and BRCA2 mutation carriers, prophylactic surgery is an option. In BRCA1/2 mutation carriers, both mastectomy and oophorectomy reduce the risk of BC, and oophorectomy reduces the risk of OvC. A recent study observed a decreased risk of BC in BRCA1/2 mutation carriers associated with oophorectomy performed after menopause.
Chemoprevention

Selective estrogen-receptor modulators (SERMSs)
The SERM tamoxifen reduces the risk of hormone receptor positive BC in high-risk premenopausal women and in postmenopausal women with prior history of hysterectomy. Another SERM used for the prevention of BC is raloxifene, which might be a better option for some postmenopausal women because of the absence of effects on the endometrium and decreased venous thromboembolic events.\textsuperscript{112}

Other preventative therapies
Other preventative therapies include aromatase inhibitors (AI) and NSAIDS. AI could be an option for the prevention in high-risk postmenopausal women, and NSAIDS are an option for the prevention of cancer in general.\textsuperscript{4} The use of tamoxifen or AI decreases the risk of a contralateral BC.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{breast_cancer_risk_reduction.png}
\caption{Breast Cancer risk reduction achievable based on life stage. Risk is illustrated (a) starting in midlife (with 22\% missed because it is diagnosed before age 50) and (b) starting in early life. In figure a (midlife), the factors that are illustrated for nulliparous women (body weight, physical activity, and alcohol intake) also affect the risk in parous women.}
\end{figure}


Breast Cancer

BC is a collective name for different tumor types that all arise within the breast. Most BC tumors arise from the ductal epithelium. The heterogeneity of BC occurs both between tumors and within tumors. To validate the prognosis and to make decisions regarding therapy, several classification systems are used in clinical setting.

**TNM**

The TNM system is one of the prognostic measures used: T is graded from 0 to 4 based on the size of the primary tumor. T0 indicates no sign of the primary tumor, T3 tumors are larger than 50 mm, and T4 is subdivided into four categories depending on certain pathological features, one of which being inflammation. The N indicates nodal status ranging from no involvement to distant nodal metastases. N0 indicates no metastases in local nodes, and N3 indicates more than 10 local lymph node metastases. Finally, the M indicates the occurrence of distant metastases. Added together, they create a score that translates to a stage. Combined with a few other factors, treatment is based upon stage.\(^\text{113}\)

**Nottingham histological grade**

The Nottingham grade is based on tubule formation, degree of nuclear polymorphism and mitotic count, all of which are separately graded and added together to create a score that describes the aggressiveness of the tumor. The scores are then grouped into three categories, 1-3, with 3 being the most aggressive.\(^\text{114}\)

**Age**

Age is a strong prognostic factor, where younger age, particularly diagnosis before age 35, is associated with an unfavorable prognosis.\(^\text{115, 116}\) Similarly, women diagnosed after age 70 have a poor prognosis.\(^\text{115}\)
Estrogen and progesterone receptor status

Complete or partial presence of the estrogen receptor (ER) in the breast tumor denotes a high probability of hormone dependence. Therefore, ER status is a predictive marker for response to endocrine therapy. The progesterone receptor (PR) status is used as well; however, the added benefit is debatable. ER and PR presence in the tumor is determined by immunohistochemistry (IHC). In Sweden, a cut-off of 10% or more positive cells is used to distinguish between hormone receptor negative or positive tumors; this value differs from the international standard of a cut-off of 1% or more.

Human epidermal growth factor receptor 2 (HER2)

HER2 is a proto-onco gene that, when amplified in tumors, is associated with proliferation, increased angiogenesis, increased invasive capability and growth signal independence. The amplification of HER2 or the increased expression of HER2 is a biomarker associated with a poor prognosis. It is also a predictive marker for treatment with targeted monoclonal antibodies, such as trastuzumab. HER2 amplification is determined by fluorescence in situ hybridization (FISH), amplified or not amplified. Overexpression is assessed by IHC, where it is categorized from 0 to 3+, where 0 and 1+ are regarded as no overexpression, and 2+ and 3+ are regarded as overexpression.

Ki-67

The Ki-67 protein is a proliferation marker present in all cell cycle phases of proliferating cells throughout the body and absent in the quiescent (G0) phase. Expression is low in the G1 and S phase, increases and peaks just prior to and during the early M phases, and decreases again during the later phases of mitosis. Its function remains unknown to a large extent; however, blocking Ki-67 prevents proliferation. High proliferation is linked to poor prognosis, especially among grade II, ER-positive BC patients. Currently, there is no international consensus regarding the assessment of Ki-67, but the most commonly used cut-off between high and low proliferation is 10-20% staining of the nuclei in invasive carcinoma.
Histopathology

Invasive ductal carcinoma

The histopathological subtype invasive ductal carcinoma (IDC) is the most common histopathological subtype of breast cancer and comprises approximately 60-90% of all BC tumors. IDC can be further classified into tubular, mucinous, medullary, papillary, micropapillary, metaplastic and IDC–not otherwise specified (NOS) which is alternatively referred to as no special type (NST). IDC-NOS are the tumors that fail to express the different characteristics that distinguish the other subtypes, the special types.9

IDC-NOS is a very heterogeneous group, and there were attempts in Malmö in the 1970s and 1980s to subdivide and categorize these tumors. Linell, Ljungberg and Andersson attempted to subdivide ductal carcinomas into the three different subgroups: tubular, tubuloductal, and comedo carcinoma.130 Tubular carcinomas are described below. Tubuloductal carcinomas are tumors that show a mixed histology, whereas comedo carcinomas are tumors that often have microcalcifications and a necrotic core.130 IDC-NOS tumors are very heterogeneous in their histologic and molecular features. Patients with IDC-NOS have the poorest survival compared with the special histopathological subtypes.9

Classic invasive lobular carcinoma

Classic invasive lobular carcinoma (ILC) is the most commonly occurring special type of BC carcinomas and usually accounts for approximately 5-15% of all BC tumors.9 131 ILCs are often multifocal in the ipsilateral breast and are more often bilateral than other histopathological subtypes of BC. A total of 70% to 80% of ILC tumors co-occur with lobular carcinomas in situ. ILCs are characterized by a loss of E-cadherin expression.9 ILCs are often ER positive and low-grade, and a subset have good prognosis.132 Many ILCs cluster in the luminal A or luminal B molecular groups, and a subset of ILC clusters with the HER2-overexpressive subtype. Many patients with ILC are postmenopausal, and part of the observed increased incidence of ILC is attributed to MHT.133
Tubular carcinoma

Tubular carcinomas (TCs) where so named because of their resemblance to tubules. TCs are rare and usually account for between 0.7% and 10.3% of all BC tumors; patients with TCs are more often postmenopausal than premenopausal. TCs are highly differentiated, and the cell layer is usually one cell-layer thick and is surrounded by an abundant fibrous stroma. The survival of patients diagnosed with TC is similar to the survival of the general population. More than 90% of TCs are ER and PR positive. TCs are HER2 negative. In relation to the molecular subtypes, TCs often cluster with the luminal A subtype.

Mucinous carcinoma

Mucinous carcinoma is characterized by mucin production; mucin accounts for more than 50% of the lesion. Mucinous carcinoma usually account for between 1% and 4% of all BC tumors. Patients with mucinous carcinoma have the highest median age at BC diagnosis. Mucinous carcinomas are often highly differentiated, and patients with mucinous carcinoma have a good prognosis. Similarly to TCs, most mucinous carcinomas are ER and PR positive, HER2 negative and clusters with the luminal subgroups not further specified.

Medullary carcinoma

Medullary carcinomas are characterized by lymphoplasmacytic infiltration, with clear boundaries and a lack of stromal cells and glandular shaped cells. Medullary carcinomas often represent between 1% and 3% of all invasive BC. Many tumors in BRCA1 mutation carriers are of the medullary type. However, medullary carcinoma is not an indication of BRCA1 status. Good prognosis has been observed in patients with medullary carcinomas; however, due to the small numbers of cases, most studies have low power. Tumors of the medullary carcinoma type are typically ER, PR, and HER2 negative. Medullary carcinomas are often of basal-like molecular subtype; however, a subset clusters with the claudin-low subtype.

Molecular subtypes

Using micro-array gene-expression profiling, four intrinsic subtypes were defined by Perou et al. in 2000. Throughout the last decade, these have been further studied and refined, and there are currently six intrinsic subtypes accepted: luminal A, luminal B, HER2 enriched, basal-like, claudin-low and normal-like. Each intrinsic subtype has a surrogate clinico-pathologic definition.
Luminal A - luminal A-like
Luminal A-like is often ER and PR positive and HER2 negative. Luminal A-like tumors are not very proliferative with less than 14 percent staining of the nuclei for Ki-67.149

Luminal B - luminal B-like
Luminal B-like is subdivided into HER2 negative and HER2 positive. Luminal B-like HER2 negative is ER positive and HER2 negative and should be PR negative and/or less than 14 percent staining of the nuclei for Ki-67. Luminal B-like HER2 positive should be ER positive. For luminal B-like HER2 positive, Ki-67 and PR status does not matter.149

Erb-B2 overexpression - HER2 positive non-luminal
The HER2 positive non-luminal tumors should be HER positive or HER2 overamplified together with ER and PR negativity or absence.149

Basal-like – Triple negative ductal NOS histopathology
As indicated by the name, these tumors either have very low presence or absence of ER and PR, and HER2 should not be amplified or overexpressed.149

Claudin-low
Similar to the basal-like, claudin-low tumors are usually triple negative but with a high frequency of metaplastic and medullary differentiation.150
Treatment of breast cancer

To determine the treatment plan for patients, a preoperative and a postoperative multidisciplinary mammary conference is mandatory for all BC patients, as stipulated by the Swedish breast cancer group and according to the Swedish guidelines for BC treatment. If neoadjuvant treatment is an option or is necessary to shrink the tumor, either to make it operable or to make breast-conserving surgery possible, it is based on a pathological report containing histopathological information from a core needle biopsy.

Surgery

Surgery is the major treatment modality used for BC treatment. It is conducted either as modified radical mastectomy, partial mastectomy or breast-conserving surgery. Breast-conserving surgery is the primary choice for patients with early stage BC. This is coupled with removal of lymph nodes from the axilla, which is the primary location of metastasis. Currently, the sentinel node technique is most often used, which is based on the investigation into which lymph node/nodes drainage from the breast occurs. A radioactive isotope is injected prior to the operation, followed by a blue dye injection at the same spot at the time of the operation. The colored assimilation of isotopes indicates the draining node. The lymph node is removed and sent for immediate histopathological analysis, conducted by a pathologist. If metastases are present, axillary dissection is performed; otherwise, the axilla is spared from further dissection.

Radiotherapy

To eradicate possible residual microscopic disease at and around the localization of the tumor, postoperative radiotherapy is administered. Postoperative radiotherapy of the breast and thoracic wall is to be administered when the risk of local recurrence within the next 10 years is greater than 20%. Therefore, radiotherapy is recommended for all BC patients who undergo breast-conserving therapy, for all
mastectomized BC patients, and in certain subtypes of tumors larger than 20 mm.\textsuperscript{119} It has been shown that postoperative radiotherapy administered after breast-conserving therapy reduced the relative risk of recurrence up to 50\%.\textsuperscript{153} Although not proven, there are indications that radiotherapy should perhaps be considered in patients with tumors smaller than 20 mm but with one to three metastatic lymph nodes present.\textsuperscript{154} Loco-regional radiotherapy is recommended for all patients with metastases in four or more lymph nodes.\textsuperscript{119} Radiotherapy induces side effects, both acute, such as erythema of the skin and pneumonitis, and late side effects, including neuropathy of the affected brachial plexus, lymphedema of the upper extremity and increased mortality from cardiac disease. However, it seems that the improved precision of the modern techniques have reduced the risk of developing these side effects.$^{155,156}$

**Systemic treatment**

To systemically remove potential micrometastases adjuvant systemic therapy is recommended. Systemic therapy includes chemotherapy, endocrine therapy and anti-HER2 therapy, either individually or in various combinations. The decision to administer systemic treatment is based on a collection of risk factors and/or the presence or absence of predictive biomarkers.

**Chemotherapy**

Chemotherapy is unselective and targets all dividing cells or proliferating cells. The synergic effect of the simultaneous use of several cytotoxic agents was compared with single-agent use because of the multiple pathways targeted.\textsuperscript{157} Cyclophosphamide, methotrexate and fluorouracil (CMF) was the standard regimen during the late 1970s and the first half of the 1980s. However, there were several reports demonstrating the superiority of treatment with anthracycline-containing (doxorubicin and epirubicin) regimens compared with CMF.\textsuperscript{158-160} Further improvement of breast cancer treatment outcomes was observed after the addition of the taxanes.\textsuperscript{161} Anthracycline-containing treatment alone or with the addition of taxanes has been shown to reduce BC mortality by one third in the adjuvant setting.\textsuperscript{162} The standard chemotherapy regimens used today include different combinations of anthracyclines, taxanes, cyclophosphamide, and fluorouracil. Amplification or overexpression of HER2 warrants the addition of trastuzumab to the chemotherapy regimen. The Swedish guidelines recommend adjuvant chemotherapy for BC patients with one or more of the following factors: ER negative tumor, young age at diagnosis, tumor with high proliferative ability, the presence of micrometastases in the lymph nodes and HER2 overexpression or amplification.\textsuperscript{119}
Endocrine therapy

As previously described, BC can be divided into subtypes based on presence of the ER; 70% to 80% of all BCs are ER positive and depend on estrogen for survival to some extent. That blockage of the ER pathway can be used for treatment of ER-positive tumors has been known for a long time. As far back as 1896, Beatson reported a suggestion for the treatment of inoperable, premenopausal BC patients: oophorectomy. In premenopausal women, the primary source of estrogens is the ovaries, whereas in postmenopausal women estrogens are primarily derived from the aromatization of adrenal and ovarian androgens in the liver, muscles and adipose tissue.

The ER pathway can be targeted in several ways: by blockage of ER (tamoxifen), by degradation of ER (fulvestrant), or by blocking the synthesis of estrogens (aromatase inhibitors (AI)). Removal or blockage of the production of estrogens by oophorectomy, radiation, or treatment with gonadotropin-releasing hormone analogues is also one way of inducing endocrine treatment. Tamoxifen can be utilized for both pre- and postmenopausal BC patients. For pre-menopausal breast cancer patients, oophorectomy, radiation or suppression through gonadotropin-releasing hormone analogues is an option, whereas AI is an option for postmenopausal BC patients. Tamoxifen, AI, and ovarian suppression are all used in the adjuvant setting; fulvestrant, in contrast, is used in the metastatic setting. A combination of chemotherapy followed by tamoxifen for approximately five years decreases survival by half for ER positive, low-risk tumors.

Targeted therapies

One targeted therapy is trastuzumab, which is a monoclonal antibody against HER2. In the adjuvant setting, trastuzumab has been reported to decrease mortality by 30% and recurrence by 50%; it has also been reported to increase progression-free survival in the metastatic setting.

mTOR inhibitors, and Poly (ADP-ribose) polymerase (PARP) inhibitors are other options. The first targets the mTOR pathway, which is indicated to be a key pathway in the development of resistance against therapeutic agents. PARP inhibitors are especially interesting for patients and/or tumors with mutations in BRCA1 and/or BRCA2.
Angiogenesis and breast cancer

Angiogenesis is the development and sprouting of new blood vessels from existing vessels. In cancer development, the induction of angiogenesis is one of the hallmarks of cancer defined by Hanahan and Weinberg. In the adult human tissue, angiogenesis is generally quiescent, with some exceptions, such as wound healing, inflammation, endometrial growth during the menstrual cycle and lactation of the breast.

Figure 7 Physiologic and pathologic angiogenesis pathways.


Hypoxia is a key element in stimulating angiogenesis. When tissue becomes hypoxic, Hypoxia-Inducible Factor 1α (HIF-1α) is stabilized and forms a dimer with HIF-1β. The complex binds to hypoxia regulated/responsive element/enhancer sequences in the 5’ and 3’ regions of the Vascular Endothelial Growth Factors A (VEGF-A) gene. VEGF-A together with several growth factors, for example, Fibroblast Growth Factor (FGF), destabilizes the vessels and initiates sprouting into the tissue. Matrix metalloproteinases (MMPs) facilitate extra cellular matrix (ECM) remodeling so that the sprouting can advance into the hypoxic tissue. Once the hypoxic tissue is normoxic and perfusion exists, HIF-1α is once more destabilized, and VEGF-A levels
drop. Simultaneously, several growth factors, including transforming growth factor 1β (TGF-1β), increase. TGF-1β together with a few other factors recruits vascular smooth muscle cells to stabilize the newly formed vessels. (Figure 7)

In breast tissue and BC cells transcription of VEGF-A has been shown to be regulated by estrogens through ER. This regulation, or at least part of it, likely occurs through a structure with 70% homology to an estrogen responsive element in a promoter of VEGF-A. In tumor angiogenesis, the balance between pro- and anti-angiogenic factors is disrupted. The pro-angiogenic pathway is not down-regulated when the hypoxic tissue is vascularized. VEGF-A, as an example, remains up-regulated: the angiogenic switch is on. Tumor vessels become unorganized and leaky.

A monoclonal antibody against VEGF-A, bevacizumab, was investigate in clinical trials. However, it became clear that bevacizumab did indeed induce tumor shrinkage or tumor stasis, but the BC tumors acquired resistance and transformed into a more aggressive type. There is a need for markers to better identify the selection of patients who would benefit from targeted anti-angiogenic therapy.
Aims of this thesis

Paper I

The aim of paper I was to determine a recessive inheritance pattern by set criteria and relate it to histologic subtypes of breast cancer.

Paper II

The aim of paper II was to investigate the histological subtype of lobular breast cancer in relation to cancer in first-degree relatives to possibly identify new hereditary or familial patterns after exclusion of all known mutation carriers.

Paper III

The primary aim of paper III was to investigate the impact of parental origin of the \textit{BRCA1} and \textit{BRCA2} mutations on age at breast and ovarian cancer diagnosis. The second aim was to elucidate whether the use of oral contraceptives modified the age at diagnosis of breast and ovarian cancer in relation to paternal versus maternal origin of the \textit{BRCA1}/2 mutation.

Paper IV

The aim of paper IV was to investigate the relationship between the manifestation of spider telangiectasias at the time of breast cancer diagnosis, and tumor and patients characteristics, as well as overall survival.
Materials

Paper I-II, IV

In paper I, II, and IV, a clinical consecutive BC series was used. The series consisted of 1676 female BC patients in the two first papers and 1682 female BC patients in paper IV. Part of this material has been used in a previous study, and an updated, larger version of this series was recently published.

At the time of BC diagnosis, all BC patients were interviewed by a clinician using a standardized questionnaire regarding several life-style parameters and family history of cancer (see table below). Information from the pathologic examination of the tumor regarding ER, PR, HER2, TNM, and histologic subtype was also added.

<table>
<thead>
<tr>
<th>Variables collected during the interview on the date of diagnosis</th>
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<tbody>
<tr>
<td>Siblings</td>
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<td>Menstrual cycle</td>
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<td>OCP use</td>
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<td>Occurrence of spiders</td>
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<tr>
<td>Detection of BC</td>
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<td></td>
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<tr>
<td>Family history of cancer</td>
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The Regional Breast Cancer Register, hospital records, and pathological reports were used to confirm the diagnoses and date of diagnosis and to confirm the diagnoses in relatives. The Swedish Population Register was used to confirm date of death. Follow-up was performed until Jan 1st, 2009 for paper I and II and until June 1st, 2009 for paper IV. For emigrants, the date of emigration was used as the last follow-up date.

Information regarding germline mutation status was retrieved from the OnkGen register at the Oncogenetic Clinic, Skåne University Hospital, Lund. Confirmed germline mutation carriers (N=16) were excluded from all analyses in paper I and II with the exception of two survival analyses in paper I, figure 1 and table 3.
Figure 8 The inclusion and exclusion of *BRCA1* and/or *BRCA2* mutation carrying individuals in paper III.

The Regional Breast Cancer Register was used to confirm new incident diagnoses and the date of diagnosis. The Swedish Population Register was used to confirm date of death. Follow-up was performed until Dec 1\(^{st}\), 2011. For emigrants, the date of emigration was used as the last follow-up date.
### Paper I

<table>
<thead>
<tr>
<th>Limitations</th>
<th>Strengths</th>
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<tbody>
<tr>
<td>Limited number of BC patients with tubular carcinoma</td>
<td>Retrieval of information from the Total Population Register and the Regional Cancer Registry contributes to good quality and control of diagnoses and date of death</td>
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<tr>
<td>Unknown histopathology of sisters, concordance unknown</td>
<td>Interviews conducted in a standardized manner</td>
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<tr>
<td>Histological subtypes might be assessed differently over time by different pathologists</td>
<td>Known mutation carriers removed</td>
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<tr>
<td>No information regarding molecular subtype</td>
<td>Long median follow-up</td>
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### Paper II

<table>
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<tbody>
<tr>
<td>Pedigree expansion in families of patients with both lobular breast cancer and a father diagnosed with cancer only</td>
<td>The association between lobular histology and a father diagnosed with cancer remains robust regardless of adjustments for potential confounders</td>
</tr>
<tr>
<td>No information regarding molecular subtypes of BC</td>
<td>Pedigree expansion of the selected families indicated that the association was not driven by BC in grandmothers</td>
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<tr>
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<td>Known mutation carriers removed</td>
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<td>Long median follow-up</td>
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### Paper III

<table>
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<tr>
<td>Changes in the criteria for mutation screening over time, could cause a selection bias favoring younger age in <em>BRCA1</em> mutation carriers with paternal origin of mutation</td>
<td>Several families with multiple generations tested leading to known parental origin instead of probable origin of mutation</td>
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<td>Regarding the history of OCP use, a sampling bias might be present</td>
<td>Cohort representing all known mutation carriers in southern Sweden</td>
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<tr>
<td>Recall bias in cases with BC compared with non-BC cases regarding environmental risk factor information might be present in questionnaire data</td>
<td>Retrieval of information from the Total Population Register and the Regional Cancer Registry, contributes to good quality and control of diagnoses and date of death</td>
</tr>
<tr>
<td>Small sample size of <em>BRCA2</em> mutation carriers</td>
<td>All carriers tested at the same core facility</td>
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### Paper IV

<table>
<thead>
<tr>
<th>Limitations</th>
<th>Strengths</th>
</tr>
</thead>
<tbody>
<tr>
<td>No information regarding VEGF-A and other angiogenic factors</td>
<td>Information on several hormonal risk factors for BC present</td>
</tr>
<tr>
<td>Invasive comedo carcinoma special subtype of ductal, comedo carcinoma has limited international use</td>
<td>Interviews conducted in a standardized manner</td>
</tr>
<tr>
<td>No information regarding molecular subtypes of BC</td>
<td>Long median follow-up</td>
</tr>
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</table>
External and internal validity of materials used

External validity

Cohort used for Paper I, II, and IV

Skåne University Hospital, Lund is responsible for radio- and chemotherapy of BC patients in the Southern Health Care Region. The Southern Health Care Region included 1 459 024 inhabitants in 1980 and 1 705 863 inhabitants in 2010. BC patients only treated with surgery without chemotherapy and radiotherapy are not referred to the Department of Oncology at Skåne University Hospital, Lund. This includes a subset of stage I BC patients and very old patients who, due to comorbidity, will not receive the above mentioned therapies. BC patients referred to the Department of Oncology at Skåne University Hospital, Lund represent 60% of all BC patients diagnosed in the Southern Health Care Region. BC patients are randomly allocated to oncologists. Therefore, this cohort should very well resemble the population of the Southern Health Care region with the exception of some stage I BC patients and very old BC patients.

Germline BRCA1 and BRCA2 mutation carriers used in paper III

The Oncogenetic Clinic at Skåne University Hospital, Lund is responsible for all genetic counseling for potential and confirmed germline mutation carriers in the Southern Health Care Region and was started in 1993. The BRCA-lab at the Department of Clinical Sciences, Lund, Division of Oncology and Pathology conducts mutation screenings for BRCA1 and BRCA2 and the predictive tests. BRCA-lab provides their services to all Oncogenetic clinics and their genetic counseling facilities in Sweden. This cohort is the entire population of BRCA1 and BRCA2 mutation carriers with known mutations in the Southern Health Care Region.
The criteria for mutation screening are as follows:

To qualify for genetic counseling and BRCA1/2 mutation screening in Sweden, the individual must fulfill at least one of the following criteria:

<table>
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<tr>
<th>Criteria</th>
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<tbody>
<tr>
<td>a) Three cases of breast cancer, of which at least one occurred before age 50, and/or ovarian/tubal cancer in a first-degree relative or second-degree relatives if related via a male regardless of age.</td>
</tr>
<tr>
<td>b) Two cases of breast/ovarian/tubal cancer in first-degree relatives or second-degree relatives if related via a male, of which at least one case of breast cancer occurred before age 40. If two cases of ovarian/tubal cancer are present, then a genetic investigation should be considered regardless of age at diagnosis.</td>
</tr>
<tr>
<td>c) One case of breast cancer that occurred before age 35.</td>
</tr>
<tr>
<td>d) Male breast cancer case.</td>
</tr>
<tr>
<td>e) Female with both breast and ovarian/tubal cancer.</td>
</tr>
<tr>
<td>f) Bilateral breast cancer, as well as triple-negative (estrogen, progesterone negative breast cancer without HER-2 amplification) breast cancer increases the implication for mutation screening.</td>
</tr>
</tbody>
</table>
Internal validity

Cohort used for Paper I, II, and IV

Questionnaire data were collected for research purposes and were collected over a time period of approximately 30 years. The questions asked are commonly used in BC research, and the reported information from these questions has been found to be valid and reliable.

A potential recall bias in a case-control study using healthy controls with regards to the propensity of BC patients to better recall family history of cancer when diagnosed themselves compared to controls, if it exists, may be a strength in our setting, where all cases in the cohort are BC patients.

In paper I and paper II, there is the potential for selection bias regarding cancer diagnoses in relatives. Increased age at BC diagnosis of a patient increases the chance that family members have been diagnosed with cancer. In paper I, this potential selection bias was addressed by adjusting for age at diagnosis in the models. In paper II, adjustment for age at diagnosis and an addition of subdividing data based on age at diagnosis (table 6) was performed.

In paper I, II and III, there is a lack of histopathological information regarding the ER, PR, and HER2 status of tumors of BC patients diagnosed before the clinical implementation of ER, PR, and HER2. The clinical standard of how they are scored might also have changed. However, the therapy determining information has been used. Regardless of how they would have been treated currently, they received standard treatment at the time.

Germline BRCA1 and BRCA2 carriers used in paper III

As described in paper III, a potential sampling bias and selection bias might be present.

Sampling bias

The questionnaire data were provided by potential mutation carriers attending a genetic counseling meeting at the Oncogenetic Clinic. The proband is the first person in one family who seeks genetic counseling, while the index case of a mutation family is the person whose diagnosis serves as the basis of a mutation screening and who will
be screened for a mutation. If the family contains several BC cases, if possible, the youngest patient will be selected for mutation screening. The index case in some families died prior to the genetic counseling meeting, which has potentially skewed the information regarding OCP retrieved from questionnaire data. Therefore, information regarding OCP in *BRCA1* and *BRCA2* mutation carriers was not missing completely at random; there was a potential sampling bias present. As described in the manuscript and in the Methods section, MICE was used as an effort to handle this problem.

**Selection bias**

The early criteria for genetic counseling and mutation screening (as described in Óskar Jóhannsson’s thesis, 1997 Lund University, Faculty of Medicine) only included first-degree relatives with BC and/or OvC. However, there was no inclusion of second-degree relatives (especially on the paternal side). This has potentially caused a selection bias, which was present in our study. To minimize the effect of a potential selection bias, we adjusted for year of inclusion in the OnkGen Register.
Methods and methodological considerations

Questionnaire data

Through the use of a structured, standardized questionnaire, information regarding lifestyle factors and family history of cancer was collected. Information collected included known risk factors for BC, and additional hormonal markers were included.

Registers used in this thesis

Swedish Population Registers

The registration of Swedish citizens began in the parishes in the late 1600s. In the mid-1700s, the Swedish Population Register was officially conducted by the Swedish churches. In 1991, the Swedish Tax Agency assumed the responsibility from the churches to register and follow the population of Sweden. In 1947, the system for personal identification numbers was implemented, where all Swedish citizens was provided with a personal identification number containing the date of birth (six digits), a birth number (three digits) and a control digit. The control digit can be calculated using the other nine digits. The register contains information regarding full name, place of residence, place of birth, gender, civil status, citizenship, immigration, family (spouse, children and parents), date of death, and place of burial. In 1968 the Total Population Register was implemented and provided by Statistics Sweden. The Total Population Register is an extract from the Swedish Population Register.

The personal identification numbers for citizens of Sweden have allowed us to follow the patients in different registers until their date of death. The Swedish Population Register also contains information regarding family members, which enables pedigree construction and validation of date of birth and date of death in relatives.
The Southern Swedish Regional Tumor Registry

In 1958, the Swedish Cancer Register was initiated and is estimated to contain approximately 96% of all cancer diagnoses. Subdivided into six regional registers, the Southern Swedish Regional Cancer Registry is responsible for the regional cancer register in our region. It is mandatory both for the physician and the pathologist to report diagnosed malignant tumors and carcinoid tumors, all tumors of the central nervous system (including benign tumors) and in endocrine glands (excluding benign thyroid tumors), and some premalignant lesions. Multiple primary tumors are separately registered. Three classes of information are included in the register:

- Patient data – personal identification number, gender, age, and place of residence
- Medical data – tumor site, histopathological subtype, stage, date of diagnosis, at which hospital and which department the patient was diagnosed, pathological/cytological department, and identification number of tumor specimen
- Follow-up data – date of death, cause of death, and date of migration

The connection between the Swedish Total Population register and the Tumor Registries enables good control and coverage regarding the date and type of diagnosis. Similarly, the date of diagnosis and the type of cancer diagnosis can be validated in relatives, which makes the data more reliable compared with reported data only.

The OnkGen Register

The Southern Swedish regional register, OnkGen, contains all attendees to the Oncogenetic Clinic for genetic counseling from 1993 to present. Included in the register is information regarding mutational screening or predictive testing, pedigree information in relation to the proband, data from questionnaires, and verification status of the diagnoses.
Statistics

The statistical software used in this thesis were SPSS 17.0, IBM SPSS 19.0, SPSS 21.0, and R 3.0.0.

For crude hypothesis testing of the distribution in nominal, dichotomous data, the X²-test (prophylactic surgery, OCP use (IV)), Fishers exact test (Occurrence of diagnosis (IV)) and the binomial test (cancer in grandparents (II)) were used, whereas for non-normally distributed data (age at death, year of birth, and year of inclusion (IV)), the Wilcoxon’s rank-sum test was used.

Binary logistic regression was used to estimate the odds ratios (OR) together with 95% confidence intervals (CI) for associations between a binary outcome, the dependent (e.g., horizontal family history of BC (I), cancer diagnosis in father/mother (II), ILC (III), occurrence of spider telangiectasias (IV)) and one or several independent variables (categorical or linear). The multivariate logistic regression was used to control for possible confounders (age at diagnosis (I-IV), use of MHT (I, II), TNM (I), number of children (I, II)).

Survival analyses or time to event (BC/OvC) was visualized using Kaplan-Meier curves, accompanied by a log-rank test $P$-value to assess the difference between the time to event (death/last-follow-up (I, IV), diagnosis/last follow-up/prophylactic surgery (III)) in the analyzed groups. The risk for the above mentioned death/diagnosis was assessed by estimation of the hazard ratios (HR) and their corresponding 95% CI adjusted for possible confounders (age at diagnosis (I, IV), TNM, etc. (I), year of inclusion in the OnkGen register (III)).

In paper III, Robust Variance Estimation (RVE) was used to adjust for potential influences on age at BC and OvC diagnoses induced by either familial factors or specific mutation. The adjustment by RVE addresses the fact that relatives might not be independent of each other when studying familial patterns. It should be mentioned that this does not take the degree of relation into account. Similarly, mutation carriers with the same exact mutation are hypothetically not independent of each other.

In paper III, Multivariate Imputation by Chained Equations (MICE) was performed to address the selection bias caused by missing information on the history of OCP use
and to increase the power of the study due to the loss of several cases due to the missing information.\textsuperscript{186, 192}
Results and discussion

Paper I

The main finding of this study was the association between a horizontal family history of BC and the histologic type tubular carcinoma (TC). Overall survival of BC patients with a horizontal family history was not significantly different than the overall survival of BC patients with no or vertical family history of BC.

We conducted an array CGH on five TC patients with a horizontal family history of BC. It was conducted on tumor material as a search for large deletions that were similar between cases to identify where a possible recessive mutation could be located. Unfortunately, this investigation was inconclusive. It is difficult to determine whether the study was inconclusive due to a lack of resolution or a genetic alteration that cannot be found using array comparative genomic hybridization (CGH) or whether the potential recessive inheritance is a combination of low-penetrance loci with endogenous or environmental risk factors.

TC has previously been suggested for familial BC. We only included pure histologic subtypes, and mixed subtypes were grouped as other. We were also able to remove previously known mutation carriers, which removes some likely confounders. However, this is a hypothesis generating study, and the results need to be confirmed in other cohorts.

Paper II

The main finding in paper II was the association between the histologic special type invasive lobular carcinoma (ILC) and the occurrence of cancer in the father. The association between ILC BC patients and a paternal cancer diagnosis appears to occur in the BC patients diagnosed at age 59 and below, although the loss of statistical significance among the older BC patients may be caused by the loss of power due to the small sample size. Removing the cancer diagnosis of prostate cancer in fathers
weakened the results slightly, but the association remained significant. Removing all BC patients with a family history of BC did not alter the association between ILC and having a father diagnosed with cancer. We excluded the probability that the association was driven by grandmothers with BC.

ILCS have been reported as a candidate for inherited BC due to the clustering of family history of BC in families of ILC patients.\textsuperscript{80, 86, 194, 195} There was an association between the occurrence of BRCA2 mutations and ILC. The even cancer distribution in grandparents should indicate that this association most likely was not due to \textit{BRCA2} mutations unknown to the potential carriers. However, this is a hypothesis generating study, and the results need to be confirmed in other cohorts.

\textbf{Paper III}

Carriers of \textit{BRCA1} mutations of paternal origin were younger at BC diagnosis compared with carriers of \textit{BRCA1} mutations of maternal origin. In contrast, carriers of \textit{BRCA1} mutations of paternal origin were older at OvC diagnosis compared with carriers of \textit{BRCA1} mutations of maternal origin. These associations were not observed in \textit{BRCA2} carriers. The use of OCP did not influence the age at BC or OvC diagnosis in \textit{BRCA1} mutation carriers.

Our study was the third to report a younger age at diagnosis in carriers of \textit{BRCA1} mutation with a paternal origin. All three studies\textsuperscript{196, 197} have slightly different study designs and different study populations. Whether the younger age at BC or older age at OvC diagnosis in \textit{BRCA1} mutation carriers is caused by paternally specific co-inherited modifiers or whether it is caused by endogenous or environmental factors linked with paternal origin of the mutation is difficult to answer and remains to be determined.

\textbf{Paper IV}

The manifestation of spider telangiectasias at the time of BC diagnosis occurred in older BC patients, in heavier BC patients and in BC patients who had a history of OCP and MHT use. Spider telangiectasias were less likely to occur on BC patients with many children and in BC patients who were diagnosed before age 50. The fact that 9\% of the cohort had comedo carcinoma, and none of them had spider
telangiectasias seems unlikely to be a coincidence. Overall survival was better in postmenopausal BC patients with spider telangiectasias.

Spider telangiectasias, also known as spider angiomas, arterial spiders or spider veins, typically manifest themselves on the upper part of the body\textsuperscript{198}, although they occur on the legs as well. Spider telangiectasias are often present in women and men that have been or are heavily exposed to estrogens,\textsuperscript{199} such as during pregnancy\textsuperscript{200} or in liver cirrhosis.\textsuperscript{201} It is also known that the transcription of VEGF can be increased by estrogens in breast tissue. This regulation, or at least part of it, likely occurs through a structure with 70\% homology to an estrogen responsive element.\textsuperscript{178} Another study reported increased TGF-\(\beta\) levels in patients with spider telangiectasias.\textsuperscript{202} TGF-\(\beta\) is important in physiologic angiogenesis and is responsible for the recruitment of vascular smooth muscle cells to stabilize the newly formed vessels.\textsuperscript{174} TGF-\(\beta\) is regulated by estrogens in some tissues during wound healing.\textsuperscript{203} Whereas vessels generally generated during tumor angiogenesis often have high permeability and are leaky.\textsuperscript{179}

Our speculative hypothesis generated from this finding is that the manifestation of spider telangiectasias, when present is a marker of hormonal exposure and may be eligible as a proxy marker of anti-angiogenic therapy. The presence of spider telangiectasias may also indicate good quality vessels due to a sustained level of TGF-\(\beta\) in the tumor. Should this prove to be true, it could explain the good overall survival observed in BC patients with spider telangiectasias because good vessel quality would be beneficial for delivering patient treatment.
Conclusions

Paper I

We propose that breast cancer patients in the histological subtype tubular carcinoma and a horizontal family history of breast cancer could be a phenotype for recessive inheritance in breast cancer and thus a candidate for genetic studies in the search of recessive inheritance in breast cancer.

Paper II

We identified a possible inheritance pattern present in breast cancer patients who have lobular breast cancer together with a father diagnosed with cancer, independent of previous family history of breast cancer. Prostate cancer and other types of tumors in the father were associated with a lobular carcinoma in the daughter. These findings need to be validated in other materials due to the exploratory nature of the design.

Paper III

We confirmed the previously reported younger age at breast cancer diagnosis in carriers of BRCA1 mutations of a paternal origin compared with those carrying BRCA1 mutations of a maternal origin. We also report an observation of older age at ovarian cancer diagnosis in carriers of BRCA1 mutations of a paternal origin compared with those carrying BRCA1 mutations of a maternal origin. No such associations were found in BRCA2 mutation carriers. The use of OCP did not modify age at breast cancer or ovarian cancer diagnosis in relation to parental origin of the BRCA1 mutation.
Paper IV

We observed an association between the occurrence of spider telangiectasias at the time of breast cancer diagnosis and several hormonal risk factors, such as increased weight, ever-use of OCP and MHT, and parity. The occurrence of spider telangiectasias at the time of breast cancer diagnosis was associated with longer overall survival in older breast cancer patients. We propose that spider telangiectasias might be a reflection of high hormonal levels and that they might indicate good ability to form new vessels.
Future perspectives

It would be very interesting to study the association between horizontal inheritance of BC and the histologic subtype TC, both in a much larger study to increase the power and with the addition of sequencing information on concordant sister-pairs, when they exist. We conducted an array CGH on TC patients with a horizontal family history of BC. Unfortunately, the findings were inconclusive. The next step would be to conduct whole genome sequencing in sister-pairs with horizontal family history in the search for a cause of the recessive phenotype.

With regards to the association between ILC and a father diagnosed with cancer, it would be very interesting to conduct a study in a second cohort with the use of the Swedish Total Population Register, the Swedish Cancer Registries, and a national OnkGen Register equivalent. By comparing the frequency of cancer diagnoses in fathers of lobular BC patients with the frequency of fathers with a cancer diagnosis in IDC BC patients, and by removing known mutation carriers, we could confirm our findings. The appropriate genetic study would be to use trios- mother, father, and daughter (the BC patient) – to determine a possible explanation for the association.

The mechanism behind the younger age at BC diagnosis in carriers of BRCA1 mutations of paternal origin compared with maternal origin, as well as the older age at OvC diagnosis in carriers of BRCA1 mutations of paternal origin compared with maternal origin, is unknown, and studies of epigenetic and genetic factors, such as imprinting, are required.

The occurrence of spider telangiectasias at the time of BC diagnosis potentially reflects hormonal exposure, and whether such exposure could indicate good vessel quality and angiogenesis, and whether they could be used as predictors of response to anti-angiogenic therapy, could be studied in a clinical trial without a significant increase in cost. A nurse or physician at entry or diagnosis can simply examine the BC patients to determine if they exhibit spider telangiectasias.

I det första arbetet studerades förekomsten av ett så kallat horisontellt familjeträd i förhållande till olika typer av bröstcancer. Ett horisontellt familjeträd innebär att det inte finns några släktingar med bröstcancer i tidigare generationer, men att två eller fler syskon diagnostiseras med bröstcancer. De familjer som bär på de gener som tidigare beskrivits och som ger upphov till bröstcancer har ofta familjeträden med bröstcancer i flertalet generationer. Vi fann att patienter med en viss typ av bröstcancer som kallas Tubulär oftare har ett horisontellt familjeträd. Vi föreslår därför patienter med Tubulär bröstcancer som har ett horisontellt familjeträd som kandidater för genetiska studier.

I det andra arbetet studerade vi förekomsten av cancer hos förstgradssläktingar (mamma, pappa, syskon eller barn) hos bröstcancerpatienter med tumörer av lobulär typ. Lobulär bröstcancer är den näst vanligaste typen av bröstcancer. Lobulära tumörer utgör ungefär 5-15 procent av alla fall. Vi fann att de kvinnor som diagnosticerats med lobulär bröstcancer oftare än de som diagnosticerats med andra typer av bröstcancer hade en pappa som diagnosticerats med cancer. Detta samband
är delvis beroende av förekomst av prostatcancer hos papporna, men sambandet fanns även för andra typer av cancer hos papporna.

I det tredje arbetet studerades en tidigare observation: att bärare av \textit{BRCA1} mutationer som ärvts av pappan insjuknar i bröstcancer vid en lägre ålder än de som ärver \textit{BRCA1} mutationen av mamman. Två gener av de som är kända som orsak till bröstcancer är vanligare än andra: \textit{BRCA1} och \textit{BRCA2}. Dessa beräknas vara orsak till mellan 20 och 25 procent av den bröstcancer som räknas som ärfilt, och mellan en och två procent av all bröstcancer. Vi kunde konfirmera det fynd som tidigare påvisats i tre olika studier som pekar på att de som ärver en mutation i \textit{BRCA1} genen från sin pappa insjuknar tidigare i bröstcancer än de som ärver en mutation i \textit{BRCA1} genen från sin mamma. Vidare kunde vi också rapportera en observation där de som ärver en \textit{BRCA1} mutation av sin pappa diagnostiseras med äggstockscancer vid en högre ålder jämfört med de som ärver en \textit{BRCA1} mutation av sin mamma. Vi kunde inte fastställa några sådana fynd för varken bröst- eller äggstockscancer hos bärare av \textit{BRCA2} mutationer.

I det fjärde arbetet studerade vi eventuella kopplingar mellan förekomsten av så kallade telangiectasier (spindel vener) vid tidpunkten för bröstcancerdiagnosen och hormonella faktorer, såsom BMI, p-pillanvändning, övergångshormonanvändning och barnafödande. Vi fann ett samband, t.ex. mellan dessa hormonella faktorer och förekomsten av telangiectasier. Vi studerade även totalöverlevnaden för bröstcancerpatienter med telangiectasier, och vi observerade att äldre kvinnor som hade förekomst av telangiectasier vid diagnos hade en bättre totalöverlevnad jämfört med andra bröstcancerpatienter i samma ålder som inte hade telangiectasier.
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