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Breast cancer in young women. Aspects of heredity and contralateral disease.

Augustinsson, Annelie

2021

Document Version:

Publisher's PDF, also known as Version of record

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Citation for published version (APA):

Augustinsson, A. (2021). *Breast cancer in young women. Aspects of heredity and contralateral disease*. [Doctoral Thesis (compilation), Department of Clinical Sciences, Lund]. Lund University, Faculty of Medicine.

Total number of authors:

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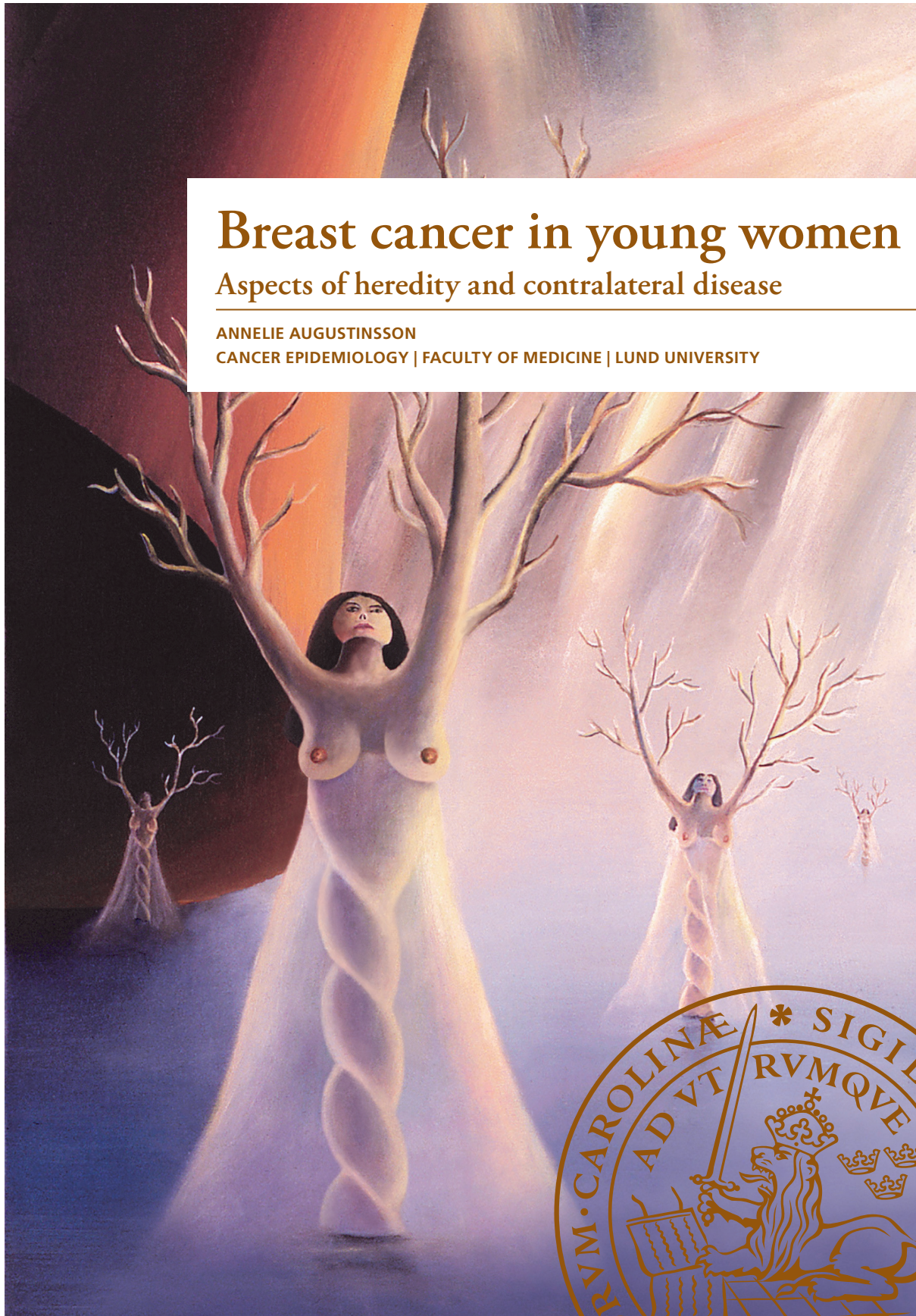
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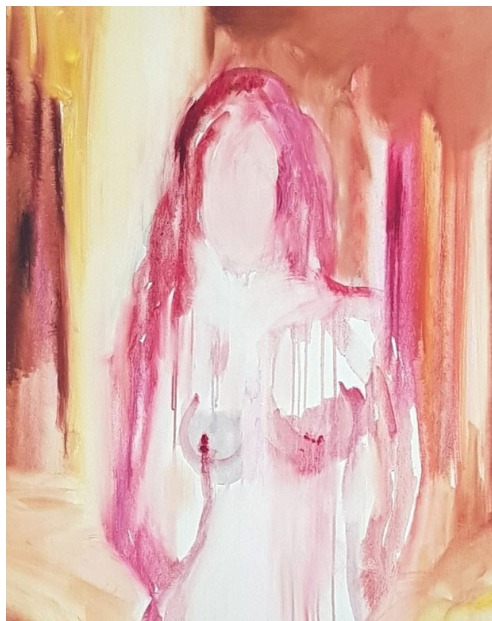
Breast cancer in young women

Aspects of heredity and contralateral disease

ANNELIE AUGUSTINSSON

CANCER EPIDEMIOLOGY | FACULTY OF MEDICINE | LUND UNIVERSITY





I still remember the pains
of poisoned blood running through my veins
many nightmares fears have past
I regained my body at last
and neither water nor wind can kill the newborn flame
because I'm strong again but nevertheless the same



Breast cancer in young women

Aspects of heredity and contralateral disease

Annelie Augustinsson



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DOCTORAL DISSERTATION

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To be defended at Belfragesalen, BMC D15, Klinikgatan 32, Lund,

Friday, May 7, 2021, at 9:00 a.m.

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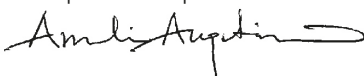
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		Date of issue May 7, 2021
		Sponsoring organization
Title and subtitle: Breast cancer in young women – Aspects of heredity and contralateral disease		
Abstract <p>Breast cancer is the most commonly diagnosed cancer among women in Sweden, as well as worldwide. In Sweden, 8,288 women were diagnosed with invasive breast cancer in 2019, out of whom approximately 1.5% were younger than 35 years of age. Although breast cancer is relatively uncommon in young women, they tend to be diagnosed with more aggressive tumors at a more advanced stage, and have a poorer prognosis compared with older women. Young patients are also more likely to harbor a strong genetic predisposition for breast cancer. In paper I–III, women who were diagnosed with breast cancer at an age of 35 years or younger in the South Swedish Health Care Region were studied. In paper I, the concordance between self- and register-reported information regarding first-degree family history of cancer was evaluated. Almost perfect agreement between reports of family history of breast and ovarian cancers, but lesser agreement for other types of cancer, was observed. In addition, the frequencies of carriers and noncarriers of pathogenic variants and tumor characteristics for each of these group were described. Pathogenic variants were identified in <i>BRCA1</i> (19%), <i>BRCA2</i> (7%), and other genes, i.e., <i>TP53</i>, <i>CHEK2</i>, and <i>PALB2</i> (4.5%). Compared with other groups, women with pathogenic variants in <i>BRCA1</i> were more likely to be diagnosed with high grade, estrogen receptor-, progesterone receptor-, and triple-negative tumors. We also noted that even though all included women fulfilled the criteria for consideration of genetic counseling and testing, many had not been referred to the Oncogenetic Clinic in Lund. In paper II, we subsequently observed that both place of residence at breast cancer diagnosis and treating hospital were associated with the probability for a referral for genetic counseling and testing, and in paper III, most women stated that the main reason for not undergoing genetic testing when they were first diagnosed with breast cancer was that they had not received any information about genetic counseling and testing from their treating physicians. Among women who have previously been diagnosed with breast cancer, both young age and the identification of a pathogenic variant are associated with an increased risk for the development of a new primary breast cancer. The second breast cancer can occur ipsilaterally, i.e., in the same breast, but most occur in the contralateral breast. In paper IV, we evaluated how the incidence of contralateral breast cancer (CBC) has evolved in Sweden since the 1960s. A statistically significant increase in CBC incidence, within ten years from the first breast cancer diagnosis between the 1960s and 1980s, was observed. This increase was seen throughout all age groups, with the steepest increase in women younger than 40 years. However, a subsequent significant decrease in the incidence of invasive CBCs after the 1980s was also seen, in contrast to <i>in situ</i> CBCs, where the incidence stabilized in the years after.</p> <p>In paper III, a Traceback approach, i.e., a retrospective genetic outreach activity, was also evaluated by inviting all the women diagnosed with early-onset breast cancer, who had not previously been referred for genetic counseling, to an analysis of breast cancer predisposing genes. Pathogenic variants were identified in <i>BRCA1</i> (n=2), <i>CHEK2</i> (n=1), and <i>ATM</i> (n=1), i.e., in four (14%) of the participants. The Traceback pilot study procedure, with written pre-test information and genetic testing, followed by in-person counseling for carriers of pathogenic variants only, was well accepted. Based on these results, we will initiate an enlarged Traceback study where all previously untested women diagnosed with breast cancer between the ages of 36 and 40 years will be invited.</p>		
Key words: Breast cancer, early-onset, genetic counseling, genetic testing, pathogenic variants, <i>BRCA1</i> , <i>BRCA2</i> , contralateral breast cancer		
Classification system and/or index terms (if any)		
Supplementary bibliographical information		Language English
ISSN 1652-8220		ISBN 978-91-8021-036-2
Recipient's notes	Number of pages	Price
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Aspects of heredity and contralateral disease

Annelie Augustinsson



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Faculty of Medicine
Department of Clinical Sciences, Lund

Lund University, Faculty of Medicine Doctoral Dissertation Series 2021:30
ISBN 978-91-8021-036-2
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
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MADE IN SWEDEN 

To my parents

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List of papers

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

- I. **Augustinsson A**, Ellberg C, Kristoffersson U, Borg Å, Olsson H. Accuracy of self-reported family history of cancer, mutation status and tumor characteristics in patients with early onset breast cancer. *Acta Oncologica*. 2018;57(5):595-603.
- II. **Augustinsson A**, Ellberg C, Kristoffersson U, Olsson H, Ehrencrona H. Variations in the referral pattern for genetic counseling of patients with early-onset breast cancer – a population based study in southern Sweden. *Public Health Genomics*. 2020;23(3-4):100-109.
- III. **Augustinsson A**, Nilsson MP, Ellberg C, Kristoffersson U, Olsson H, Ehrencrona H. Genetic testing in women with early-onset breast cancer – a Traceback pilot study. *Manuscript submitted*.
- IV. **Augustinsson A**, Fritz I, Ellberg C, Kristoffersson U, Ehrencrona H, Olsson H. Incidence trends of contralateral breast cancer over five decades: a nationwide population-based study. *Manuscript*.

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Related publications not included in this thesis:

1. Dörk T, Peterlongo P, Mannermaa A, Bolla MK, ... **Augustinsson A**, ... Easton DF. Two truncating variants in *FANCC* and breast cancer risk. *Scientific Reports*. 2019;9(1):12524.
2. Kapoor PM, Lindström S, Behrens S, Wang X, ... **Augustinsson A**, ... The Breast Cancer Association Consortium. Assessment of interactions between 205 breast cancer susceptibility loci and 13 established risk factors in relation to breast cancer risk, in the Breast Cancer Association Consortium. *International Journal of Epidemiology*. 2020;49(1):216-232.
3. Liu J, Prager-van der Smissen WJC, Collée JM, Bolla MK, ... **Augustinsson A**, ... Hollestelle A. Germline *HOXB13* mutations p. G84E and p. R217C do not confer an increased breast cancer risk. *Scientific Reports*. 2020;10(1):9688.
4. Kramer I, Hooning MJ, Mavaddat N, Hauptmann M, ... **Augustinsson A**, ... Schmidt MK. Breast cancer polygenic risk score and contralateral breast cancer risk. *American Journal of Human Genetics*. 2020;107(5):837-848.
5. Coignard J, Lush M, Beesley J, O'Mara T, ... **Augustinsson A**, ... Antoniou A. A case-only study to identify genetic modifiers of breast cancer risk for *BRCA1/BRCA2* mutation carriers. *Nature Communications*. 2021;12(1):1078.
6. Johnson N, Maguire S, Morra A, Kapoor PM, ... **Augustinsson A**, ... Fletcher O. *CYP3A7*1C* allele: linking premenopausal oestrone and progesterone levels with risk of hormone receptor-positive breast cancers. *British Journal of Cancer*. 2021;124(4):842-854.
7. Morra A, Jung AY, Behrens S, Keeman R, ... **Augustinsson A**, ... Chang-Claude J. Breast cancer risk factors and survival by tumor subtype: pooled analyses from the Breast Cancer Association Consortium. *Cancer Epidemiology, Biomarkers & Prevention*. 2021;cebp.0924.2020.

Abbreviations

AI	Aromatase inhibitor
BBC	Bilateral breast cancer
BC	Breast cancer
CBC	Contralateral breast cancer
CI	Confidence interval
CIS	Cancer in situ
DNA	Deoxyribonucleic acid
ER	Estrogen receptor
GDPR	General data protection regulation
GnRH	Gonadotropin-releasing hormone
GWAS	Genome-wide association study
HBOC	Hereditary breast and ovarian cancer
HER2	Human epidermal growth factor receptor 2
IHC	Immunohistochemistry
IR	Incidence rate
IRR	Incidence rate ratio
ISH	In situ hybridization
MBBC	Metachronous bilateral breast cancer
MRI	Magnetic resonance imaging
NHG	Nottingham histological grade
NKBC	National Quality Registry for Breast Cancer
OR	Odds ratio
OvC	Ovarian cancer

PARP	Poly (ADP-ribose) polymerase
PR	Progesterone receptor
SBBC	Synchronous bilateral breast cancer
SCTAT	Sex cord tumor with annular tubules
SERM	Selective estrogen receptor modulator
SNP	Single-nucleotide polymorphism
TAM	Tamoxifen
TDLU	Terminal duct lobular unit
TNBC	Triple-negative breast cancer
TNM	Tumor node metastasis
VUS	Variant of uncertain (or unknown) significance

Genes

<i>ATM</i>	ATM serine/threonine kinase
<i>BRCA1</i>	BRCA1 DNA repair associated
<i>BRCA2</i>	BRCA2 DNA repair associated
<i>CDH1</i>	Cadherin 1
<i>CHEK2</i>	Checkpoint kinase 2
<i>PTEN</i>	Phosphatase and tensin homolog
<i>PALB2</i>	Partner and localizer of BRCA2
<i>STK11</i>	Serine/threonine kinase 11
<i>TP53</i>	Tumor protein p53

Abstract

Breast cancer is the most commonly diagnosed cancer among women in Sweden, as well as worldwide. In Sweden, 8,288 women were diagnosed with invasive breast cancer in 2019, out of whom approximately 1.5% were younger than 35 years of age. Although breast cancer is relatively uncommon in young women, they tend to be diagnosed with more aggressive tumors at a more advanced stage, and have a poorer prognosis compared with older women. Young patients are also more likely to harbor a strong genetic predisposition for breast cancer.

In paper I–III, women who were diagnosed with breast cancer at an age of 35 years or younger in the South Swedish Health Care Region were studied. In paper I, the concordance between self- and register-reported information regarding first-degree family history of cancer was evaluated. Almost perfect agreement between reports of family history of breast and ovarian cancers, but lesser agreement for other types of cancer, was observed. In addition, the frequencies of carriers and noncarriers of pathogenic variants and tumor characteristics for each of these groups were described. Pathogenic variants were identified in *BRCA1* (19%), *BRCA2* (7%), and other genes, i.e., *TP53*, *CHEK2*, and *PALB2* (4.5%). Compared with other groups, women with pathogenic variants in *BRCA1* were more likely to be diagnosed with high grade, estrogen receptor-, progesterone receptor-, and triple-negative tumors. We also noted that even though all included women fulfilled the criteria for consideration of genetic counseling and testing, many had not been referred to the Oncogenetic Clinic in Lund. In paper II, we subsequently observed that both place of residence at breast cancer diagnosis and treating hospital were associated with the probability for a referral for genetic counseling and testing, and in paper III, most women stated that the main reason for not undergoing genetic testing when they were first diagnosed with breast cancer was that they had not received any information about genetic counseling and testing from their treating physicians.

Among women who have previously been diagnosed with breast cancer, both young age and the identification of a pathogenic variant are associated with an increased risk for the development of a new primary breast cancer. The second breast cancer can occur ipsilaterally, i.e., in the same breast, but most occur in the contralateral breast. In paper IV, we evaluated how the incidence of contralateral breast cancer (CBC) has evolved in Sweden since the 1960s. A statistically significant increase in CBC incidence, within ten years from the first breast cancer diagnosis between the

1960s and 1980s, was observed. This increase was seen throughout all age groups, with the steepest increase in women younger than 40 years. However, a subsequent significant decrease in the incidence of invasive CBCs after the 1980s was also seen, in contrast to in situ CBCs, where the incidence stabilized in the years after.

In paper III, a Traceback approach, i.e., a retrospective genetic outreach activity, was also evaluated by inviting all the women diagnosed with early-onset breast cancer, who had not previously been referred for genetic counseling, to an analysis of breast cancer predisposing genes. Pathogenic variants were identified in *BRCA1* ($n=2$), *CHEK2* ($n=1$), and *ATM* ($n=1$), i.e., in four (14%) of the participants. The Traceback pilot study procedure, with written pre-test information and genetic testing, followed by in-person counseling for carriers of pathogenic variants only, was well accepted. Based on these results, we will initiate an enlarged Traceback study where all previously untested women diagnosed with breast cancer between the ages of 36 and 40 years will be invited.

Thesis at a glance

Papers	Study questions	Participants	Results	Conclusions
I	Is there a concordance between self-reported and registry-reported information regarding family history of BC, OvC, and other types of cancer in first-degree relatives of women diagnosed with early-onset BC?	All women ($n=231$) diagnosed with BC at ≤ 35 years between 1970 and 2013 in the South Swedish Health Care Region who were registered at the Oncogenetic Clinic in Lund.	Almost perfect agreement between self-reported and registry-reported information regarding first-degree family history of BC and OvC, but lesser agreement of other types of cancer, was observed.	Physicians and genetic counselors can rely on self-reported family history of BC and OvC, but family history of other types of cancer is not communicated as efficiently.
II	Is there an association between 1) place of residence at BC diagnosis and 2) treating hospital and the fact that not all women diagnosed with early-onset BC have attended genetic counseling and testing?	All women ($n=279$) diagnosed with BC at ≤ 35 years between 2000 and 2013 in the South Swedish Health Care Region.	Women with early-onset BC from two regions, rural settings ($<10,000$ inhabitants), and two hospitals were significantly less likely to be registered at the Oncogenetic Clinic in Lund.	Variations in the referral pattern for genetic counseling and testing indicates a need for an extended oncogenetic service and educational outreach in regional hospitals to improve care.
III	What is the main reason for not having attended genetic counseling and testing when first being diagnosed with early-onset BC? What are the experiences of the Traceback approach, with written information and genetic testing, and in-person counseling for women with pathogenic variants only?	All women ($n=63$) diagnosed with BC at ≤ 35 years between 2000 and 2017 in the South Swedish Health Care Region who were not registered at the Oncogenetic Clinic in Lund.	The main reason for not previously having attended genetic counseling and testing was a lack of information and referrals from treating physicians. The Traceback approach was well accepted by the 27 women (four carriers and 23 noncarriers of pathogenic variants) who answered the questionnaire.	Improvement regarding information and referrals for genetic counseling and testing for women who are diagnosed with early-onset BC is warranted. After minor adjustments of the study protocol, an enlarged Traceback study will be initiated by inviting all women diagnosed with BC at an age of 36–40 years.
IV	How has the CBC incidence among all women in Sweden evolved since the 1960s?	All women ($n=210,746$) who were diagnosed with a first BC between 1960 and 2006 in Sweden, and all women ($n=11,533$) who subsequently were diagnosed with CBC between 1960 and 2016.	CBC incidence significantly increased between the 1960s and 1980s, with the steepest increase observed in young women. However, CBC incidence also significantly decreased after the 1980s.	Despite the positive result of a decrease in CBC incidence during the last decades, efforts are still needed to prevent the development of new primary breast cancers.

Abbreviations: BC, breast cancer; CBC, contralateral breast cancer; OvC, ovarian cancer

Introduction

Breast cancer in history

Because of visible signs and symptoms, and palpability of lumps at later stages, breast cancer has been recognized for a long time. The earliest mention of breast cancer has been identified in a text produced in the 17th century BC; The Edwin Smith Surgical Papyrus. This document was discovered in Egypt in 1862 and is regarded to be one of the most important known medical documents because of its descriptions of multiple cases of trauma and surgery, as well as eight cases of tumors or ulcers of the breast [1-4]. In approximately 400 BC, Hippocrates, the father of western medicine, described breast cancer as a disease caused by imbalances of bodily humors (fluids), especially black bile. Because of their crab-like appearances, he named the tumors *karkinos*, a Greek word for crab [3, 4].

Nevertheless, there are not many works of art from antiquity that provide clear representations of breast pathologies. At the beginning of the Renaissance, however, they became more frequent. For instance, two paintings dated to the 16th century, *The Night* painted by Michele di Rodolfo del Ghirlandaio (1503–77) and *The Allegory of Fortitude* painted by Maso da San Friano (1531–71), have been proposed to be the earliest pictorial representations of breast cancer [5].



Figure 1. *The Night* by Michele di Rodolfo del Ghirlandaio (close-up image).

The painting displays a bulge over the nipple, an almost complete nipple retraction, as well as a distortion and consistent size reduction of the entire left breast. © www.galleriacolonna.it. Printed with permission.

Historically, the incidence of breast cancer has not been as high as it is today. Because most breast cancers develop amongst older women, most women before the 19th century had died too young to have developed breast cancer. In addition, women had more children, at a younger age, and breastfed for a longer time than today, which are all factors that are associated with decreased risk for breast cancer.

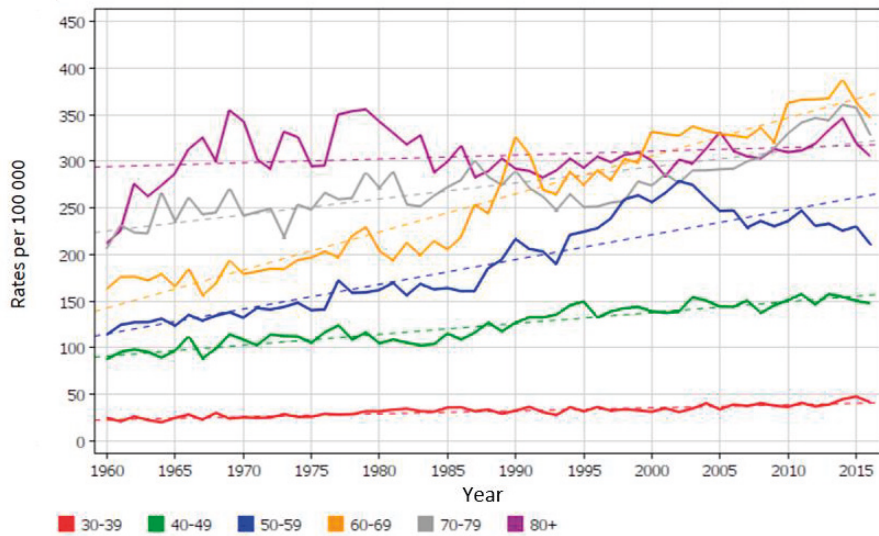
Hereditary breast cancer was first described by the French physician Pierre Paul Broca, who is best known for his research on Broca's area (the region in the frontal lobe that is named after him), in the 19th century. Broca's wife was diagnosed with breast cancer at a young age, and the pedigree of her family displayed four generations of women diagnosed with breast cancer [6].

Breast cancer today

Breast cancer is the most commonly diagnosed cancer among women, both worldwide and in Sweden. Globally, the breast cancer incidence in women was estimated to be 2.1 million cases in 2018 [7]. In Sweden, the annual incidence of breast cancer has increased from 3,392 cases (84 cases per 100,000 women) in 1970 to 10,829 cases (212 cases per 100,000 women) in 2019, whereas breast cancer mortality has decreased from 1,494 women in 1997 to 1,353 in 2019 [8]. However, the number of incident cases is not equivalent to the number of women being diagnosed with breast cancer. Each diagnosed tumor is reported as a case, and the reporting of multiple tumors per individual has increased in Sweden since 2003. Nevertheless, the number of women being diagnosed with breast cancer has also increased during the years. This incidence trend is most likely a combination of a true increase and a detection effect due to mammographic screening [9], since the highest increase in breast cancer incidence is observed in the age groups that are covered by screening [10]. The early detection through mammographic screening may also be one of the reasons for the decrease in mortality [11], in combination with better tumor profiling and adjuvant treatments [12]. Globally, breast cancer is the leading cause of cancer-related deaths in women [7]. In Sweden, however, lung cancer has taken over, during the last decade, as the leading cause of cancer-related deaths in women [8].

The reason why breast cancer develops is multifaceted, and many different risk factors for breast cancer have been established. These risk factors can be divided into nonmodifiable and modifiable factors. The nonmodifiable risk factors include sex, age, height, genetic constitution, and exposure to endogenous hormones. The modifiable factors include pregnancy, breastfeeding, weight, lifestyle factors, and exposure to exogenous hormones [13, 14].

Incidence, women
Sweden
Breast cancer



Mortality, women
Sweden
Breast cancer

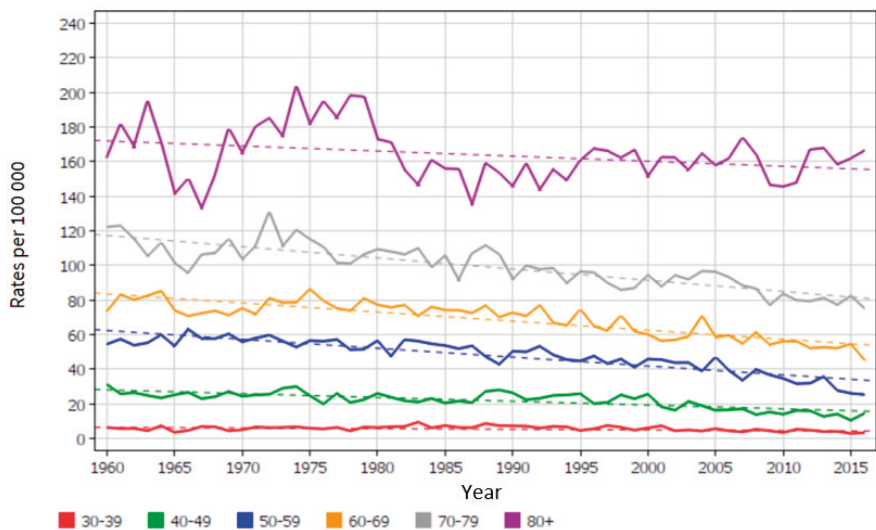


Figure 2. Breast cancer incidence and mortality in Swedish women, stratified by age groups, over time. Between 1960 and 2016, breast cancer incidence has increased across most age groups in Swedish women, while mortality has decreased. Graph from NORDCAN [10].

The most important risk factor for the development of breast cancer is being a woman. In Sweden, only 64 cases of breast cancer in men were reported in 2019 [8]. Another important risk factor is age. The median age at breast cancer diagnosis among women in Sweden is 66 years [15], and only 1.5% of all cases are younger than 35 years [8]. Although breast cancer is relatively uncommon in young women, early-onset breast cancer tends to be diagnosed at a more advanced stage and be more aggressive compared with breast cancer in older women. In addition, they also tend to have a poorer prognosis [16].

Most breast cancers are sporadic and not coupled to strong heredity. In certain families, however, you can find germline pathogenic alterations. A breast cancer diagnosis at a young age increases the probability of a hereditary cause for the diagnosis [16], and the Swedish national breast cancer guidelines therefore recommend that all women diagnosed with breast cancer at an age of 40 years or younger should be offered a referral for genetic counseling at their regional oncogenetic clinic, and subsequently be given the option of an analysis of genes linked to suspected hereditary breast cancer [12].

This thesis focuses on breast cancer in young women, in relation to both heredity and contralateral disease. In addition, it addresses family history of different types of cancer, as well as genetic counseling and testing.

The normal breast

The mammary glands, which are located in the breasts, are organs whose primary function is lactation, i.e., production, secretion, and ejection of milk. Externally, each breast has a raised nipple, which is surrounded by a pigmented area called the areola. Internally, each breast is composed of 15–20 separate sections, or glandular lobes, which each contains several secretory lobules [17]. In addition, each of the lobes consists of a duct system between the lobules and the nipple, where small ducts that leave the lobules converge into one single lactiferous duct [18]. Near the nipple, each lactiferous duct enlarges and forms a lactiferous sinus. Normally, 15–20 of these sinuses open onto the surface of each nipple [19].

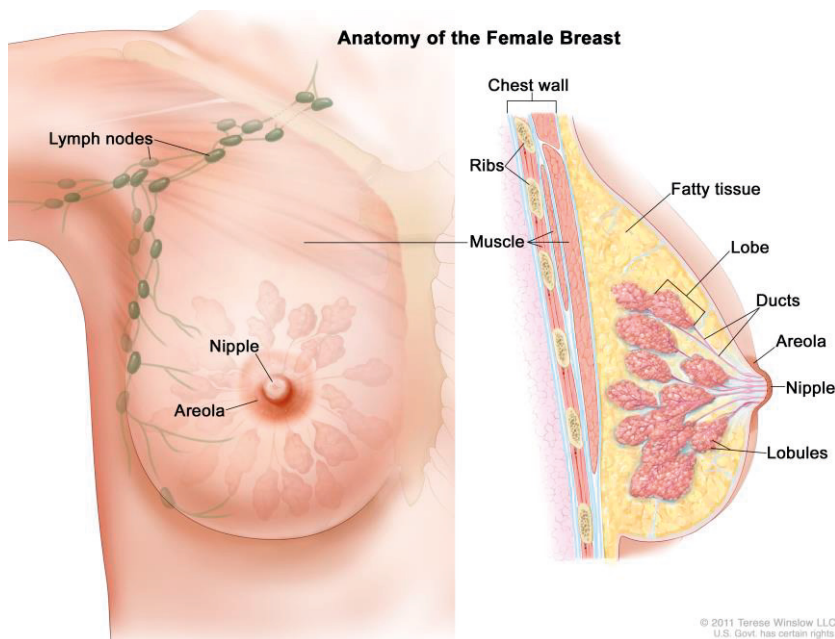


Figure 3. Anatomy of the female breast.

Externally visible are the nipple and the areola. Internally, the lactating breast has a well-developed duct system, which includes the lobes, lobules, and ducts. In addition, the adipose tissue that surrounds each mammary gland, the pectoralis major muscle, ribs, and lymph nodes are visible internally. © 2011 Terese Winslow LLC, U.S. Govt. has certain rights. Printed with permission.

Dense connective tissue surrounds the duct system in each breast and forms partitions between the lobes and the lobules. As support, these bands of connective tissue (suspensory ligaments) extend from the fascia over the pectoralis major muscle to the inner side of the overlaying skin [19].

In children, the breast structures of girls and boys are very similar. However, as girls reach puberty, ovarian hormones, i.e., estrogen and progesterone, stimulate the development of the mammary glands [20]. Terminal duct lobular units (TDLUs) develop, which branch and grow, forming multiple bulbous ends [21]. Fat is also deposited, so that each mammary gland becomes surrounded by adipose tissue, except for the area of the nipple and the areola.

An inactive mammary gland is dominated by the duct system, and the branches of the lactiferous ducts end as small tube-like structures. Hence, the size of the breasts in a nonpregnant woman is predominantly reflected by the amount of adipose tissue rather than the amount of glandular tissue. Normally, the secretory parts of the breasts do not complete their development unless pregnancy occurs [19].

During pregnancy, the breasts proliferate and differentiate in preparation for lactation, resulting in lengthened ducts and profuse branching of the breast parenchyma. The ends of these branches subsequently expand, forming secretory sacs called alveoli. Surrounding these alveoli are myoepithelial cells, which contract to eject the milk during breastfeeding [20]. Throughout lactation, the breasts are fully differentiated [19, 22], however, after pregnancy, and at cessation of lactation, the secretory units of the breasts regress through involution [23].

Breast cancer development

Breast cancer is a disease in which breast cells become abnormal and multiply to form a malignant tumor [22]. Most breast cancers arise from the epithelial cells lining the mammary ducts and lobules (the TDLUs) [21]. Breast cancer exist in two forms; invasive and cancer in situ (CIS). CIS respects the basal membranes and do not invade the surrounding tissue.

There are many mechanisms and signaling pathways that are identical for normal breast development, tumor development, and the transition from CIS to invasive cancer, including recruitment of fibroblasts, leucocytes, and other stromal components [21, 24]. However, breast cancer is more disorganized compared to the constitution of a normal breast, and has escaped the control mechanisms.

Cancer is associated with acquired (somatic) genetic alterations over time. This type of alterations occurs at some time during a person's life and are present only in certain cells. However, the transition from a normal cell into a cancer cell is a multistep process, resulting in an accumulation of such genetic alterations, as well as from epigenetic factors that may silence genes that should be active, or switch on genes that should be silent [25, 26], which usually takes many years. The mechanisms behind the transition from a normal cell into a cancer cell are today known as the hallmarks of cancer.

In 2000, Hanahan and Weinberg published a review article where they suggested six biological capabilities necessary for most forms of cancer to develop, which they called the hallmarks of cancer. These capabilities were: sustained chronic proliferation, evasion of growth suppressors, resistance to cell death, enabling of replicative immortality, induction of tumor angiogenesis, and activation of invasion and metastasis [27]. In 2011, the authors published an update containing four new hallmarks. Two of these, the development of genome instability and mutation, and the induction of tumor-promoting inflammation, were described as enablers of the six previously suggested biological capabilities. The other two were: deregulation of cellular energetics and avoidance of immune destruction [28].

In breast cancer, carcinogenesis is strongly affected by the balance between oncogenes and tumor suppressor genes, i.e., genes that are activated and inactivated in tumors, respectively. The cancer progression and growth are subsequently stimulated by hormones and different growth factors. Female sex hormones have a

significant effect on the mammary glands, and the effect is highest when both estrogen and progesterone levels are high [29].

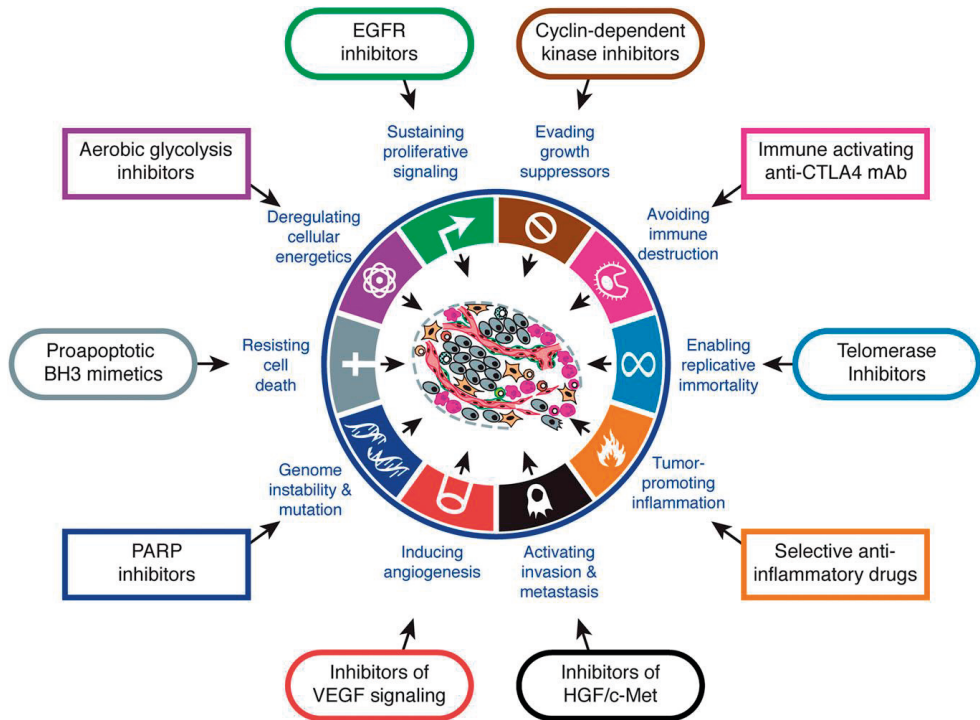


Figure 4. The hallmarks of cancer.

Illustrative examples of treatments that interfere with each of the acquired capabilities for tumor growth and progression.
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Risk factors for breast cancer

The assessment of an individual's risk for breast cancer is complex and based on a combination of several personal, lifestyle, environmental, and reproductive factors [30-32]. There are many different established risk factors associated with breast cancer, of which the two nonmodifiable risk factors of female sex and age are the most important [13]. The incidence of breast cancer is extremely low before the age of 30 years, however, subsequently increases with age. In Sweden, the highest incidence is observed in women between 60 and 69 years of age [10]. Other important risk factors for breast cancer are a previous personal and/or familial history of breast cancer, and a genetic predisposition.

Previous history of breast cancer

Personal history of breast cancer

Studies have reported an estimated 2–6-fold increased risk for the development of a second primary breast cancer among women who have a personal history of breast cancer, compared with the risk of developing a first primary cancer among women in the general population [33, 34], and the increased risk is highest in women who were diagnosed with their first primary breast cancer at a young age [33-36]. Some of the new primary cancers occur ipsilaterally, i.e., in the same breast, but most occur in the contralateral (the other) breast [35]. Among women diagnosed with breast cancer, the incidence of bilateral breast cancer (BBC), i.e., cancer in both breasts, is estimated to range between 1.4% to 11.8% [33].

Family history of breast cancer

Most breast cancers are sporadic. However, it has been proposed that around 15% of all breast cancers are associated with a family history of breast cancer [37, 38], i.e., that one or more close blood relatives have been diagnosed with breast cancer. Having one first-degree relative (such as mother, sister, or daughter) with breast cancer approximately doubles a woman's risk of developing breast cancer compared

with women in the general population, and the risk increases with increasing number of first-degree relatives diagnosed with breast cancer [39, 40]. In addition, the risk is even higher if the relative was diagnosed at a young age or had BBC [40-42].

Other familial risk factors are if one or more second-degree relatives (such as grandmother, aunt, or niece) from either the mother's or the father's side of the family had breast cancer, a relative had BBC before menopause, two or more relatives had breast or ovarian cancer, a relative had both breast and ovarian cancer, or a male relative had breast cancer [38].

In a study from 1971, Lynch and Krush reported an increased risk for ovarian cancer in certain families with familial breast cancer [43]. This finding was later termed the hereditary breast and ovarian cancer (HBOC) syndrome.

Hereditary breast cancer

Germline pathogenic variants in cancer-predisposing genes are associated with increased risk for breast cancer. However, the breast cancer risk is not identical for all women harboring such pathogenic variants. Some variants are highly penetrant, while others have less penetrance. In addition, the penetrance for each of the different pathogenic variants is affected by other factors that modify the risk, e.g., family history of cancer, and therefore, the risk for each carrier of a specific pathogenic variant is not equal either [44, 45].

High penetrance genes

Pathogenic variants in highly penetrant genes are associated with the highest lifetime risks (>30%) for breast cancer [46]. However, pathogenic variants in these genes are rare. Out of all breast cancer cases, approximately 5% have been estimated to have a strong hereditary background, and the prevalence of pathogenic genetic variants in the specific genes *BRCA1* and *BRCA2* in unselected breast cancer patients has been estimated to be 2–2,5% [46, 47]. The prevalence of pathogenic variants in *BRCA1* and *BRCA2*, however, varies between populations.

BRCA1 and *BRCA2*

The *BRCA1* and *BRCA2* genes, both identified in the mid-1990s [48-52], provide instructions for the synthesis of the proteins BRCA1 and BRCA2, respectively. These proteins are tumor suppressors, normally expressed in the cells of the breasts, as well as in other tissues, where they, e.g., are part of a complex involved in the repairing of double-strand breaks in damaged DNA through homologous recombination [53]. High-penetrance alterations in these genes cause a loss of tumor

suppressive function, which is associated with an increased risk for breast cancer. However, according to the Knudson hypothesis, two “hits” to the deoxyribonucleic acid (DNA) is necessary to cause a phenotypic change, i.e., that most tumor suppressor genes require both alleles to be inactivated to cause cancer [54]. Hence, if one *BRCA1* or *BRCA2* allele is inactivated through a germline pathogenic alteration, an inactivation of the other allele, through e.g., a somatic alteration, would be required for homologous recombination deficiency to occur.

In a prospective cohort study of 6,036 women with pathogenic variants in *BRCA1* and 3,820 women with pathogenic variants in *BRCA2*, the cumulative breast cancer risk to the age of 80 years was estimated to be 72% (95% confidence interval (CI), 65–79%) for women with pathogenic variants in *BRCA1* and 69% (95% CI, 61–77%) for women with pathogenic variants in *BRCA2* [55]. Pathogenic variants in *BRCA1* and *BRCA2* also substantially increase the risk for contralateral breast cancer (CBC). In the same study, the cumulative risk for CBC, 20 years after the first breast cancer diagnosis, was estimated to be 40% (95% CI, 35–45%) and 26% (95% CI, 20–33%) for women with pathogenic variants in *BRCA1* and *BRCA2*, respectively, and the risk was highest in women who were diagnosed with their first breast cancer at a young age [55].

In addition, pathogenic variants in *BRCA1* and *BRCA2* are also associated with an increased risk for other types of cancer, especially ovarian cancer [56, 57]. In the prospective cohort study, referred to above, the cumulative risk for ovarian cancer to the age of 80 years was estimated to be 44% (95% CI, 36–53%) and 17% (95% CI, 11–25%) for women with pathogenic variants in *BRCA1* and *BRCA2*, respectively [55]. No strong evidence of an increased risk for any other types of cancer than breast and ovarian cancer among individuals with pathogenic variants in *BRCA1* have been indicated. However, pathogenic variants in *BRCA2* are also associated with an increased risk for pancreatic cancer, as well as prostate cancer and male breast cancer [58, 59].

A breast cancer diagnosis at a young age increases the probability of a hereditary cause, and out of all patients who are diagnosed with breast cancer before age 35 years, 10–15% are estimated to harbor a pathogenic variant in *BRCA1* or *BRCA2* [16, 60]. At the age of 40 years or younger, the relative risk of breast cancer among women with pathogenic variants in *BRCA1* has been estimated to be more than 30-fold, and among women with pathogenic variants in *BRCA2* more than 15-fold, compared with the relative risk among women in the general population [56].

Women with pathogenic variants in *BRCA1* are more often diagnosed with estrogen receptor (ER)-negative breast cancer and triple-negative breast cancer (TNBC), i.e., breast cancer that is ER-negative, progesterone receptor (PR)-negative, and human epidermal growth factor receptor 2 (HER2)-negative, compared with both women with *BRCA2* pathogenic variants and women without pathogenic variants [61, 62].

PALB2

PALB2 is a tumor suppressor gene that encodes a protein that interacts with the BRCA2 protein during homologous recombination and double-strand break repair [63]. A truncating variant in *PALB2* increases the risk for breast cancer, especially in families with previous cases of early-onset breast cancer. In an international study of 524 families with pathogenic variants in *PALB2*, the relative risk for breast cancer, constant with age, was estimated to be 7.18 (95% CI, 5.82–8.85). The absolute risk for the development of breast cancer was estimated to be 17% (95% CI, 13–21%) to the age of 50 years and 53% (95% CI, 44–63%) to the age of 80 years. Pathogenic variants in *PALB2* are also associated with other types of cancer, and the estimated risks to the age of 80 years were 5% (95% CI, 2–10%) for ovarian cancer, 2–3% (95% CI, women, 1–4%; 95% CI, men, 2–5%) for pancreatic cancer, and 1% (95% CI, 0.2–5%) for male breast cancer [64].

Moderate penetrance genes

For carriers of moderate-penetrant genes, the estimated average absolute risk for breast cancer by the age of 80 years lies within the range of 17 to 30% [46].

CHEK2

The *CHEK2* gene encodes a serine/threonine kinase, a protein that acts as a tumor suppressor while being involved in the repair of double-strand breaks, cell cycle arrest, and apoptosis in response to DNA damage. The loss of normal *CHEK2* function leads to unregulated cell division, accumulated damage to DNA, and a potential tumor development. Certain pathogenic variants in *CHEK2* have been associated with breast cancer [65].

The *CHEK2**1100delC, where the deletion of a single cytosine at position 1100 in exon 10 results in a stop codon, is a common protein-truncating variant found in individuals of European descent [59]. In a previously published analysis of patients and controls from 33 studies, the proportion of *CHEK2**1100delC carriers was estimated to be 0.5% among controls, 1.3% among women with breast cancer from population- or hospital-based studies, and 3.0% among women from familial or genetics center-based studies. The estimated odds ratio (OR) for invasive breast cancer among all *CHEK2**1100delC carriers, compared with noncarriers, was 2.26 (95% CI, 1.90–2.69), and among women diagnosed before age 35 years, the estimated OR was 2.59 (95% CI, 1.23–5.47). The cumulative risk for breast cancer development was estimated to be 23% to the age of 80 years, and it was also proposed that carriers of the *CHEK2**1100delC have a 2–2.5-fold cumulative risk for the development of ER-positive breast cancer to the age of 80 years compared with the general population [66].

ATM

The protein encoded by the *ATM* gene is activated by DNA damage and is an important cell cycle checkpoint kinase that regulates many downstream proteins, including the tumor suppressor protein p53 [67]. Pathogenic variants in *ATM* is foremost associated with ataxia telangiectasia, an autosomal recessive disorder that might be inherited by a child if both parents are carriers of a pathogenic variant in *ATM*. In heterozygotic carriers, pathogenic variants in *ATM* are associated with breast cancer, and protein-truncating variants in *ATM* have been proposed to be associated with an absolute lifetime risk for breast cancer of more than 20% to the age of 85 years [46, 47].

Rare syndrome genes

Germline pathogenic variants associated with an increased risk for breast cancer also include rare syndrome genes. However, because of the low prevalence of these pathogenic variants, current estimates of cancer risks for women who carry any of these genes are uncertain.

TP53

The *TP53* gene encodes the protein p53, which acts as a tumor suppressor through several functions, including the regulation of cell division. When the DNA becomes damaged, this protein plays a critical role in determining whether the DNA can be repaired or not. If the DNA cannot be repaired, this protein prevents the cell from dividing and initiates apoptosis. By stopping cells with altered or damaged DNA from dividing, p53 helps to prevent the development of tumors [68].

Inherited pathogenic variants in *TP53* cause the Li-Fraumeni syndrome [69-71]. Families with pathogenic variants in *TP53* tend to have both early-onset and multiple primary cancers, e.g., childhood sarcoma, brain cancer, adrenocortical cancer, and breast cancer [72, 73]. A woman with a pathogenic variant in *TP53* has been estimated to have a 50% lifetime risk for breast cancer by the age of 60 years [74], and an 18–60-fold increased risk for early-onset breast cancer compared with women in the general population [39, 75]. Pathogenic variants in *TP53* is thought to account for 1–4% of breast cancers in women with early-onset breast cancer [76, 77], with a tendency to present at a very young age (<30 years) [74].

In families with familial breast cancer, pathogenic variants in *TP53* are primarily associated with HER2-positive breast cancers, diagnosed at a very early age [12].

PTEN

PTEN, which also acts a tumor suppressor gene, encodes a protein that regulates cell survival and proliferation. Pathogenic variants in *PTEN* result in the inability to

activate cell cycle arrest and apoptosis, which leads to abnormal cell growth and survival [78]. Germline pathogenic variants in *PTEN* are the cause of *PTEN* Hamartoma Tumor Syndrome, which includes Cowden syndrome.

Breast cancer is the most frequent malignancy in women with pathogenic variants in *PTEN*, with an estimated lifetime risk of 85%. However, pathogenic variants in *PTEN* are also associated with thyroid cancer and endometrial cancer, as well as nonmalignant features such as macrocephaly and gastrointestinal polyps. In addition, recent studies have suggested an increased risk for colon cancer and renal cell carcinoma [79].

STK11

The tumor suppressor gene *STK11* encodes a serine/threonine kinase, important for the regulation of cell division [80]. Germline pathogenic variants in the *STK11* gene cause Peutz-Jeghers syndrome, which is characterized by mucocutaneous pigmentation and hamartomatous gastrointestinal polyps. Among women with pathogenic variants in *STK11*, breast cancer risk is estimated to be 8% and 31% to the ages of 40 and 60 years, respectively [81]. In addition to increased risk for breast cancer, women with pathogenic variants in *STK11* have an elevated risk for cancer in other sites, e.g., gastrointestinal cancer and benign sex cord tumors with annular tubules (SCTAT) [82].

CDH1

The *CDH1* gene encodes a protein called cadherin-1. This protein plays an important role in cell–cell adhesion between epithelial cells [83]. Loss of function is thought to contribute to cancer progression by increasing proliferation, invasion, and/or metastasis. Women with pathogenic variants in *CDH1* have a significant lifetime risk of diffuse gastric cancer, as well as breast cancer, particularly lobular breast cancer [79, 84]. Women with pathogenic variants in *CDH1* have been estimated to have an 80% lifetime risk of developing lobular breast cancer to the age of 80 years [85].

Common low risk polymorphisms

Recently, genome-wide association studies (GWAS) have identified multiple low risk polymorphisms [86-88]. These studies have allowed the detection and assessment of small risk loci, which could explain a proportion of all breast cancers, including both early-onset breast cancers and CBCs [89]. The most common genetic alterations are called single-nucleotide polymorphisms (SNPs). SNPs are substitutions of single nucleotides in the DNA sequence that are present in a large proportion of the population, i.e., 1% or more [86]. In the general population, several hundreds of SNPs have been associated with breast cancer risk. Each of these SNPs

is only associated with a small difference in risk, but the combined effect of multiple SNPs can be summarized in a polygenic risk score (PRS), which might be high [44, 90, 91].

Breast cancer associated SNPs, however, do not only increase the risk among women in the general population, but also in carriers of rare high- or moderate-penetrant pathogenic variants. For instance, a woman with a pathogenic variant in *BRCA1* who also carry many of the breast cancer associated SNPs will therefore have a higher risk of developing breast cancer compared with a woman with a pathogenic variant in *BRCA1* who carry less breast cancer associated SNPs.

Reproductive risk factors

Reproductive factors that influence breast cancer risk are linked to the lifetime exposure of female hormones and have foremost been associated with ER-positive breast cancer [92]. The time between menarche and menopause, i.e., the markers of onset and cessation of ovarian activity, respectively, as well as the length of menstrual cycles and the number of pregnancies, all reflect the total number of menstrual cycles a woman undergoes.

Age at menarche

The pubertal transition in girls includes thelarche (the onset of breast development), pubarche (the onset of pubic hair growth), and menarche (the onset of menstrual bleeding). Thelarche, which usually is the first sign of puberty, often occurs two to four years prior to menarche [93]. However, even though a girl's age at thelarche and menarche does not coincide precisely, the two are highly correlated.

In developed countries, menarche usually occurs between the ages of 10 and 16 years in most girls, and an earlier age at menarche is a well-established risk factor for breast cancer [92, 94, 95]. Factors that might influence the age of menarche include genetic factors, socio-economic status, nutritional status, general health and well-being, and certain types of exercise. The average age at menarche has declined during the last 150 years, from an estimated 16.5 years in 1840 to approximately 13 years in the 1990s [96], which might have contributed to the increase in breast cancer incidence during the last century.

Number of menstrual cycles

During a woman's menstrual cycle, more proliferation of the breasts occurs in the luteal phase (when the progesterone exposure is highest) than in the follicular phase [97-99]. The average menstrual cycle length in healthy women of a reproductive age is 28 days, but can range from 21 to 35 days [19]. The variation in length is

observed mainly in the follicular phase, while the luteal phase is rather constant. Hence, women with shorter menstrual cycles undergo more time in the luteal phase, and are therefore exposed to a higher epithelial proliferation compared with women with longer cycles. Subsequently, it has been proposed that many regular (shorter) menstrual cycles either before first full-term pregnancy or during lifetime are associated with a higher risk for breast cancer [100].

In addition, studies have implicated progesterone to be of great importance for the development of breast cancer in relation to both use of oral contraceptives and menopausal hormone therapy.

Use of oral contraceptives

Use of oral contraceptives is an established risk factor for breast cancer [32], especially in women who used high dose oral contraceptives in the 1960s and 1970s [101, 102]. Studies have indicated that younger women may have a higher breast cancer risk due to oral contraceptive use compared with older women [103-106]. Among women between the ages of 20 and 44 years, current use of contemporary oral contraceptives for five years or longer, and long-term use for 15 years or longer, have been associated with increased risk [107]. This increase in breast cancer risk has been observed five years after cessation, but not ten years after [105].

In women with pathogenic variants in *BRCA1* and *BRCA2*, however, the use of oral contraceptives is associated with a decreased risk for ovarian cancer. In a meta-analysis of women at elevated risk for breast cancer, because of pathogenic variants in *BRCA1* or *BRCA2* or a strong family history, the estimated OR for ovarian cancer among oral contraceptive users was 0.58 (95% CI, 0.46–0.73) [108].

Parity and breastfeeding

The epithelium within the breast is considered to be most sensitive to hormonal stimuli between time of menarche and first childbirth. Hence, adding more menstrual cycles prior to the first full-term pregnancy result in the association between high age at first full-term pregnancy, as well as nulliparity, and breast cancer. Delayed childbirth, i.e., having the first child after age 30 years, has been described as an important risk factor for breast cancer [109-111], and postponing childbearing has been estimated to increase the relative risk by 3% for each delayed year [112]. In addition, there is a transient increase in breast cancer risk after giving birth [113], which have been proposed to be strongest after a late first childbirth [110]. A lack of, or a short lifetime duration of, breastfeeding are also proposed to contribute to the high incidence of breast cancer [112].

Menopause and use of menopausal hormone therapy

Even though the focus of this thesis is breast cancer in young women, menopause and use of menopausal hormone therapy as risk factors are addressed briefly in the text below.

When most women are between 45 and 54 years of age, menstrual cycles and ovulation become less regular. Perimenopause is the time from onset of irregular cycles to their complete cessation, and menopause is the marker of cessation of menstrual cycles, i.e., the end of ovarian and endocrine activity associated with reproduction. A late menopause is an established risk factor for breast cancer. Women who have their menopause after the age of 55 years are twice as likely to develop breast cancer compared with women who have their menopause before 45 years of age [32].

Menopausal hormone therapy, also called hormone replacement therapy, is a treatment many physicians may recommend for the relief of common symptoms of menopause. However, menopausal hormone therapy with combined estrogen and progestin is a well-established risk factor for breast cancer [114, 115].

Other lifestyle risk factors

In addition to hereditary and reproductive risk factors for breast cancer, there are some other important lifestyle factors that are associated with an increased risk for breast cancer.

Anthropometric factors

The ovaries produce most of the body's estrogen. However, after menopause, the adipose tissue produces a small amount. Because female sex hormones are involved in breast cancer development, and adipose tissue is the main source of estrogen production in postmenopausal women, weight gain and obesity are established risk factors for postmenopausal breast cancer. In contrast to premenopausal women, where obesity is associated with a decrease in breast cancer risk [116]. However, in premenopausal women, a high birth weight has been proposed to be a risk factor. In both pre- and postmenopausal women, being tall is also considered to increase the risk for breast cancer [117].

In addition, breast size has been proposed to be a risk factor for breast cancer. In a systematic review of breast size and breast cancer risk, the overall results were conflicting, but an increasing breast size appeared to be a risk factor for breast cancer [118]

Dense breasts

Breast density is one of the strongest and most consistent risk factors for breast cancer [119-121]. Dense breasts have more connective tissue, glands, and ducts than adipose tissue, and women with highly dense breast tissue have been estimated to have a 4–5-fold risk of developing breast cancer compared with women with little or no dense breast tissue [122].

Socio-economic status and education

A higher incidence of breast cancer is observed among women with high socio-economic status, which might be explained by mammographic screening attendance, reproductive patterns, use of exogenous hormones, and/or other lifestyle choices [123]. Due to these potential explanations, women with a higher education are also proposed to have a higher risk for breast cancer compared with women with a lower education [124, 125].

Inactivity and sedentary behavior

Lack of physical activity [126] and a sedentary behavior [127] are also factors that are proposed to be associated with an increased risk for breast cancer, both in pre- and postmenopausal women.

Alcohol consumption

Alcohol consumption is also associated with the risk for breast cancer, and the estimated risk increases with increasing intake. One possible reason for the link between alcohol and breast cancer is that alcohol is thought to cause higher levels of endogenous estrogens. Alcohol may also lower levels of some essential nutrients that protect against cell damage, such as folate, vitamin A, and vitamin C. A significantly increased risk with increasing alcohol consumption has been observed, and could therefore be one of the many contributing factors for both pre- and postmenopausal breast cancer [117, 128-130].

Breast cancer prevention

In most cases, the preventive measures are to counteract some of the risk factors for breast cancer. Some of the established risk factors, such as the nonmodifiable risk factors of age and age at menarche, cannot be influenced. However, the modifiable risk factors might.

Lifestyle strategies

Regular physical activity may reduce the levels of endogenous estrogens, and has emerged as a protective factor for both pre- and postmenopausal breast cancer [117, 131]. A meta-analysis reported a significant association between physical activity and a reduced risk for breast cancer, and the authors therefore proposed that physical activity should be advocated for the prevention of breast cancer [126]. In addition, because obesity in postmenopausal women is an established risk factor for breast cancer, keeping the weight within the healthy range and avoiding weight gain are recommended for women after menopause [132]. However, there are no general dietary recommendations for the prevention of breast cancer, except that it would be beneficiary to limit the alcohol consumption [117].

Because of the reduction in the total number of menstrual cycles, a first full-term pregnancy at an early age, as well as multiple childbirths, are considered as being protective against breast cancer [94, 109]. In addition, long-term breastfeeding is considered beneficiary, and in a meta-analysis it was estimated that the risk for breast cancer decreased with 4% for each year of breastfeeding [112]. A recent study has also indicated that breastfeeding might reduce the risk for hormone receptor-negative breast cancer, which could represent a risk-reducing strategy for this more aggressive tumor subtype [133].

Endocrine therapy

For women with a high risk for breast cancer, selective estrogen receptor modulators (SERMs), e.g., tamoxifen (TAM), which have an inhibiting effect on estrogen-mediated cell proliferation, can be used as prevention. In a meta-analysis of women with a normal or increased risk for the development of breast cancer, a statistically significant risk reduction by 38%, with an estimated cumulative incidence of 6.3% in the control group and 4.2% in the SERM group, was observed. However, a

significant increase in the risk of thromboembolic disease (73%) and endometrial cancer (56%) was also observed [134].

Even though TAM reduces the risk of breast cancer by almost 40%, the medication is not used frequently as a prevention strategy for healthy women with an increased risk for breast cancer. Because of severe symptoms, such as hot flashes, night sweats, various gynecological symptoms, and insomnia, many women also fail to adhere to the medication regime. One of the risk factors for breast cancer is dense breast tissue, and TAM has been shown to reduce the mammographic density. In a recent Swedish study, the authors evaluated whether lower TAM doses were inferior in reducing the mammographic breast density compared with the standard TAM dose of 20 mg, and whether the lower doses were associated with fewer symptoms. The results indicated that the minimum dose for a non-inferior mammographic breast density reduction was 2.5 mg. However, this result was confined to premenopausal women. In addition, the severe symptoms were reduced by approximately 50% in the lower dose groups compared with the 20 mg group [135], which would be beneficiary for the adherence.

The hormone estrogen is a key factor in breast cancer carcinogenesis, and a reduction of its synthesis can decrease the risk for breast cancer. Estrogen production is driven by the aromatase enzyme, an enzyme that converts adrenal androgens into estrogens. In a double-blind randomized placebo-controlled trial regarding the use of the aromatase inhibitor (AI) anastrozole for the prevention of breast cancer in postmenopausal women, the predicted cumulative incidence of breast cancer after seven years was 5.6% in the placebo group and 2.8% in the anastrozole group [136].

Risk-reducing surgery

Women with pathogenic variants can be given the option of risk-reducing measures, i.e., prophylactic bilateral mastectomy and salpingo-oophorectomy, to improve both breast cancer specific and overall survival [137, 138]. Retrospective analyses of women with pathogenic variants in *BRCA1* and *BRCA2* have estimated a decrease in breast cancer risk by at least 90% after bilateral risk-reducing mastectomy [139, 140]. However, in Sweden, prophylactic mastectomy due to hereditary indications is only recommended after a consultation at an oncogenetic clinic [12].

In addition, because of the increased risk for ovarian cancer, bilateral salpingo-oophorectomy is recommended for women with pathogenic variants in *BRCA1* and *BRCA2* after the completion of childbearing. Therefore, this risk-reducing strategy should be offered to women with pathogenic variants in *BRCA1* when they are between 35 and 40 years of age, and to women with pathogenic variants in *BRCA2* when they are between 40 and 50 years [12].

Clinical breast cancer

Diagnostics

The Swedish National Board of Health and Welfare recommend that all women between 40 and 74 years of age should be invited to mammographic screening once every two years [141]. Some healthcare regions in Sweden even offer more frequent examinations (once every 18 months) to the youngest women in this age span, because they normally have more dense breast tissue, which makes it more difficult to identify small tumors, and are more likely to be diagnosed with faster growing tumors [12]. Even though a potential breast cancer over-diagnosis among women who attend population-based screening programs has been debated [142], a meta-analysis of randomized studies estimated a reduction in breast cancer mortality by 20% among women who were invited to mammographic screening [143].

For women with high-penetrant pathogenic variants, increased surveillance through annual mammography, as well as magnetic resonance imaging (MRI) screening, is usually recommended [137, 138]. Hence, the Swedish national breast cancer guidelines recommend that women with pathogenic variants in *BRCA1* or *BRCA2* should be offered annual mammographic screening between the ages of 25 and 74 years, in combination with MRI to the age of approximately 55 years [12].

Today, more than 50% of all breast cancer patients are diagnosed after attending mammographic screening in Sweden [12]. However, in 2019, 26% of all diagnosed breast cancers were detected among women who were younger than 40 and older than 74 years of age [8].

Because women who are younger than 40 years of age are not normally invited to mammographic screening, most early-onset breast cancers are detected by the women themselves through signs and symptoms, which usually are palpable lumps or masses [144-146]. Other symptoms might be changes in the shape, size, or appearance of the breast, thickening or swelling of a part of the breast, peau d'orange, scaling, peeling, or flaking of the overlaying skin, changes in the shape of the nipple, and/or discharge from the nipple [12, 146].

Prognostic and predictive markers

Breast cancer, which is a highly heterogeneous disease, is classified according to different tumor characteristics. Clinical guidelines use prognostic and predictive markers to decide whether to recommend adjuvant treatment after breast surgery, and which therapy to choose. Tumor-related prognostic markers predict the risk of recurrence or death from breast cancer, while the predictive markers indicate the likelihood of response to a certain treatment.

Patient characteristics

Age at diagnosis is foremost a risk factor for breast cancer, but is also used in clinical guidelines for the choice of treatment. Although breast cancer is relatively uncommon in young women, they tend to be diagnosed with more aggressive tumors at a more advanced stage, and have a poorer prognosis compared with older women [147-149].

TNM classification

Size of the primary tumor (T), spread to regional lymph nodes (N), and absence or presence of distant metastases (M 0/1) are collectively referred to as the TNM-classification, which is the most important prognostic factor. Tumor size includes stepwise larger tumors: T1=1–20 mm, T2=21–50 mm, T3=>50 mm, and T4=skin and/or chest wall involvement irrespective of tumor size. Axillary lymph node involvement equals the number of involved nodes: N0=node negative, N1=1–3 positive nodes, N2=4–9 positive nodes, and N3= \geq 10 positive nodes. The tumor stage refers to the sum of T, N, and M, and ranges from stage 1 to 4, where the higher stage indicates poorer prognosis [150].

Histological grade

In Sweden, the Nottingham Histological Grade (NHG) system is used when scoring tumor histological parameters which identifies tumor differentiation [151]. The count consists of tubular information, nuclear pleomorphisms, and mitotic count. The sum of these parameters subsequently represent grade 1, 2, or 3. In the NHG system, grade 1 breast cancer is well differentiated and has the best prognosis, while grade 3 is poorly differentiated and has the worst prognosis. Grade 2 is an intermediate group, for which additional assessment of proliferation associated with antigen Ki67, PR status, and gene profiling may facilitate the estimation of the patients' risk for recurrences [12, 152].

Proliferation

Deregulation of cell cycle checkpoints is essential for tumor proliferation. There are several markers for this, including the mitotic count, which is a part of the NHG.

Antigen Ki67 is a nuclear protein that is associated with proliferation. Ki67 is expressed during all the active phases of the cell cycle, but is absent in cell cycle arrest (G0). A high Ki67 score is an independent prognostic marker, and the currently used cut-off regarding a high Ki67 is $\geq 20\%$ [12, 153].

Estrogen receptor (ER) and progesterone receptor (PR) status

Estrogen is a steroid hormone that binds to and activates ERs, which stimulates cell division and therefore also has the potential of activating tumor growth. ERs are expressed in approximately 80% of all invasive breast cancers in Sweden, and are used as a prognostic and predictive marker for the response to endocrine treatment. PRs, i.e., hormone receptors that are closely related to the ERs, but activated by the steroid hormone progesterone, are mainly used as a prognostic marker [12].

In both pre- and postmenopausal women, the effect of obesity on breast cancer risk differ based on ER status. In postmenopausal women, obesity is associated with a higher risk of ER-positive breast cancer, particularly in women who have never taken menopausal hormone therapy, but only a modest or no association with ER-negative breast cancer. In premenopausal women, however, obesity is associated with a lower risk of ER-positive breast cancer, but a higher risk of TNBC [154]. Among all breast cancer patients in Sweden, TNBC is diagnosed in approximately 10% [12].

Human epidermal growth factor receptor 2 (HER2) status

HER2, which is a transmembrane receptor tyrosine kinase within the epidermal growth factor receptor family, is amplified in approximately 15% of all breast cancers in Sweden. Women with HER2-positive tumors have a poorer prognosis compared with women with HER2-negative, and a high risk for metastases. The assessment of HER2 is based on immunohistochemistry (IHC) analysis, where a score between 0 and 3+ will be obtained. With a score of 3+, the tumor will be regarded as HER2-positive. In ambiguous cases (2+), HER2-positivity can be confirmed with in situ hybridization (ISH) analysis [155]. HER2 is both a prognostic and predictive marker for response to targeted treatment, such as the monoclonal antibodies trastuzumab and pertuzumab [12].

Histopathology

Invasive breast cancer can be divided into different histopathological subtypes. Approximately 30% of all breast cancers are classified as *special types*. The most common special type of breast cancer is invasive lobular carcinoma, which counts for approximately 20% of all breast cancers. Other special types are, e.g., mucinous, tubular, medullary, and metaplastic breast cancer, which each count for 1–2% of all breast cancers, respectively. Seventy percent of breast cancers do not fulfill the criteria for any of the special types. These breast cancers have historically been

called invasive ductal carcinomas. However, since the WHO classification of tumors in 2012, this histopathological subtype is called *no special type* [12].

Molecular subtypes

During the last decades, five intrinsic molecular subtypes of breast cancer have been characterized; luminal A, luminal B, HER2-enriched, basal-like, and normal breast-like. ER-positive tumors resemble normal glandular cells, i.e., luminal epithelial cells, while ER-negative tumors resemble myoepithelial cells, i.e., basal-like [156].

Table 1. Molecular subtypes

Luminal A-like	Luminal B-like	HER2-positive/ Luminal	HER2-positive/ Non-luminal	Triple-negative
ER-positive (>10%)	ER-positive (>10%)	ER-positive (>10%)	ER-negative (≤10%)	ER-negative (≤10%)
HER2-negative	HER2-negative	HER2-positive	HER2-positive	HER2-negative
NHG 1 or NHG 2 and low Ki-67 or NHG 2, intermediate Ki-67, and PR ≥20%	NHG 2, intermediate Ki-67, and PR <20%, or NHG 2 and high Ki-67 or NHG 3	Regardless of NHG, Ki-67, and PR	PR-negative (≤10%)	PR-negative (≤10%)

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor 2; PR, progesterone receptor; NHG, Nottingham Histological Grade

These subtypes have shown significant differences in incidence, risk factors, prognosis, and treatment sensitivity. Both luminal A and luminal B breast cancers predict response to endocrine treatment, and have a better outcome than the rest of the subtypes among breast cancer patients who receive various adjuvant systemic treatments [157].

Breast cancer treatment

The treatment for each breast cancer patient is discussed and decided on at multidisciplinary conferences, before and after primary surgery, and is based on the available prognostic and predictive markers according to the clinical guidelines, such as invasiveness, ER, PR, HER2, and Ki67 [12].

In Sweden, only a few percent of patients with primary breast cancer are diagnosed with locally advanced disease. Neoadjuvant chemotherapy and/or endocrine therapy is recommended for all patients with locally advanced and primarily inoperable tumors. These therapies are used prior to a local treatment, such as surgery, and is designed to shrink the tumor, so it can be removed with less extensive surgery. They can also be used by women with a high risk for micrometastatic disease. For instance, women with HER2-positive tumors with an increased risk are recommended neoadjuvant chemotherapy therapy in combination with dual HER2 blockade with trastuzumab and pertuzumab. In addition, neoadjuvant chemotherapy of an operable tumor indicates a survival rate equivalent to adjuvant chemotherapy, but increases the possibility for breast-conserving surgery [12].

Locoregional treatments

Surgery

Surgery is, and has historically been, the most important part of breast cancer treatment. In the adjuvant setting, the primary choice of surgery, if the tumor can be radically removed with a good cosmetic result, is breast-conserving surgery [12]. Prospective, randomized trials have estimated that the survival rates after breast-conserving surgery, in combination with radiotherapy, are equivalent to the survival rates after mastectomy [158]. Hence, the proportion of breast-conserving surgery in Sweden has successively increased. In recent years, oncoplastic surgery has also emerged to improve the cosmetic result. Due to smaller tumors at breast cancer diagnosis, as well as an increased use of oncoplastic surgery and neoadjuvant treatment, the proportion of mastectomies has decreased in Sweden. Nevertheless, mastectomies still have indications, e.g., for large tumors that have progressed during neoadjuvant treatment, multifocal tumors where a good esthetic result cannot be accomplished with breast-conserving surgery, and local recurrence after breast-

conserving surgery [12]. It has previously been reported that young women have a higher risk for local recurrence after both breast-conserving surgery and mastectomy [159]. However, a decrease in the recurrence risk among women diagnosed with early-onset breast cancer has been observed over time [160] and is now less than 1% per year [12].

Along with surgery of the breast, a sentinel node biopsy is also standard procedure to detect eventual metastases in the axillary lymph nodes. Tissue samples from both breast and first axillary lymph node are used in biological analyses, which will be the basis for prognosis and the decision regarding adjuvant treatment [12].

Radiotherapy

Adjuvant treatment with radiotherapy is used to reduce the risk for local recurrence and increase breast cancer specific survival after both breast-conserving surgery and mastectomy [12]. In a meta-analysis, where the risk for recurrence after different types of surgery in combination with radiotherapy was evaluated, breast-conserving surgery followed by radiotherapy was estimated to decrease the risk for local recurrence by two-thirds in women with node-negative breast cancers and by more than two-thirds in women with node-positive breast cancers, as well as breast cancer specific mortality with 5–6%, after 15 years [161]. In Sweden, adjuvant local radiotherapy after breast-conserving surgery is the standard procedure for patients with node-negative tumors [12].

In the same meta-analysis, mastectomy followed by radiotherapy was estimated to decrease the risk of local recurrence by approximately 50% in women with advanced tumors and node-negative breast cancers, and by more than two-thirds in women with advanced tumors and node-positive breast cancers, as well as breast cancer mortality by 3–5%, after 15 years [161]. In Sweden, patients with node-negative breast cancers are not recommended adjuvant radiotherapy after mastectomy unless the tumor is larger than 5 cm. However, most patients with axillary lymph node involvement receive adjuvant radiotherapy [12].

Systemic treatments

Chemotherapy

Because the risk of breast cancer-related deaths is strongly associated with the development of distant metastases, the main goal of both neoadjuvant and adjuvant treatment with chemotherapy is to eliminate micrometastases. Adjuvant chemotherapy (primarily with anthracyclines and taxanes) is given after the local treatment (surgery and/or radiotherapy). Chemotherapy can be used when there is little evidence of cancer being present, but a risk for recurrence. It can also be used

to kill any cancerous cells that might have spread to other parts of the body. These micrometastases can be treated with adjuvant chemotherapy and thereby reduce recurrence. In Sweden, adjuvant chemotherapy is recommended to breast cancer patients with ER-positive/HER2-negative tumors larger than 10 mm with risk factors (luminal B), diagnosed at a young age (<35 years), or lymph node involvement. Chemotherapy is also recommended to patients with triple-negative tumors larger than 5 mm or lymph node involvement [12].

Endocrine therapy

Basically, all women with ER-positive tumors are recommended adjuvant endocrine treatment, and the two main therapies are TAM and AIs. Endocrine therapy should be initiated after chemotherapy, and before or after radiotherapy [12].

TAM is a SERM, an oral anti-estrogen medication that is effective for both pre- and postmenopausal women [162]. For pre- and perimenopausal women with a low risk of recurrence, TAM is recommended for five years, and for pre- and perimenopausal women with a high risk of recurrence, an additional five years of endocrine treatment should be offered. In postmenopausal women, however, treatment with AIs have become more and more frequent during the last decade [12].

AIs do not have an inhibiting effect on the estrogen receptors. Instead they reduce the production of estrogen [162]. In postmenopausal women, the ovaries have ceased to produce estrogen, but estrogen synthesis can still occur (primarily in the adipose tissue). By inhibiting aromatase, AIs can effectively reduce the synthesis of estrogen at the site of the cancer, i.e., the adipose tissue of the breast. AIs, however, are generally ineffective as a treatment for pre- and perimenopausal women [163]. On the other hand, AIs can be used as an extended endocrine treatment for five years for women who were premenopausal when they first started their five-year treatment with TAM, but have become postmenopausal five years later [12].

Among younger premenopausal women (<40 years) with ER-positive tumors, who have received a prior treatment with chemotherapy, ovarian suppression with a gonadotropin releasing hormone (GnRH) agonist, in addition to TAM, could be considered. However, the effect of ovarian suppression is modest, and due to side effects such as hot flashes, various gynecological symptoms, loss of sexual interest, and insomnia [164], this treatment is not recommended for all premenopausal women diagnosed with ER-positive breast cancer, but only for young women diagnosed with tumors with unfavorable prognostic factors [12].

Targeted therapy

Patients with HER2-positive breast cancer with remaining cancer cells after neoadjuvant chemotherapy combined with dual HER2 blockade are recommended adjuvant treatment with trastuzumab for one year. One year of adjuvant treatment

with trastuzumab is estimated to decrease the relative risk of recurrence by 19% and overall mortality by 22% compared with shorter treatments (nine weeks or six months). However, adjuvant treatment with trastuzumab is almost always recommended in combination with chemotherapy, i.e., taxanes (preferably) and/or anthracyclines, which is estimated to give a relative decrease in mortality by 34% for patients with a primary HER2-positive breast cancer [12].

For patients with metastatic triple-negative *BRCA1* and *BRCA2* associated breast cancer, platinum based chemotherapy should be offered as an early treatment alternative in addition to anthracyclines or taxanes [12]. Recently, poly (ADP ribose) polymerase (PARP) inhibitors have become a treatment option for patients with pathogenic variants in *BRCA1* and *BRCA2* [165]. PARP activity is essential for single-strand break repair in DNA, and in both normal cells and cancer cells without germline pathogenic variants in *BRCA1* or *BRCA2*, these breaks can be repaired through homologous recombination. In cells with pathogenic variants in *BRCA1* or *BRCA2*, however, homologous recombination cannot function, which makes them sensitive to PARP inhibitors [166]. For patients with metastatic *BRCA1* and *BRCA2* associated breast cancer, who have been prescribed at least one prior systemic treatment, PARP inhibitors significantly increase progression free survival compared with the standard chemotherapy treatment [165].

Bisphosphonates

Bisphosphonates inhibit osteoclast function and thereby decrease bone resorption. In a meta-analysis of benefits and risks of adjuvant bisphosphonate treatment in breast cancer, a reduction in skeletal recurrence and improved survival was observed in postmenopausal women. However, in premenopausal women, no significant reduction was indicated [167]. In addition, because bisphosphonates are accumulated in the bone tissue and could remain there for years after cessation, young women who intend to start a family in the near future should not be treated with bisphosphonates, as they are classified as having a potential harmful effect on the reproductive process. Hence, adjuvant treatment with bisphosphonates, in addition to current postoperative treatments, is recommended to postmenopausal women with primary lymph node involvement, regardless of ER status, but not for pre- or perimenopausal women [12].

Genetic counseling and testing

A breast cancer diagnosis at a young age increases the probability of a hereditary cause, and therefore it is recommended in the Swedish national breast cancer guidelines that all women who have been diagnosed at an age of 40 years or younger (previously, 35 years or younger) should be offered a referral to their regional oncogenetic clinic for genetic counseling, and subsequently be given the option of analysis of genes linked to suspected hereditary breast cancer.

Table 2. Swedish recommendations for oncogenetic testing

These criteria have been revised during the follow-up period of this thesis [12, 168, 169].

Any of the following
BC ≤40 years of age.
BC ≤50 years of age, if there is at least one additional case of BC in first- or second-degree relatives in the same family branch. BBC counts as two cases. The second case can also be OvC, early-onset prostate cancer (≤65 years of age), or pancreatic cancer.
BC ≤60 years of age, if there are at least two additional cases of BC in first- or second-degree relatives in the same family branch. BBC counts as two cases. The other cases can also be OvC, early-onset prostate cancer (≤65 years of age), or pancreatic cancer.
TNBC ≤60 years of age.
Male BC regardless of age.
OvC including tubal cancer and primary peritoneal carcinomatosis (non-mucinous, non-borderline) regardless of age.
In cases where a pathogenic variant is present in <i>tumor tissue</i> , a complementary analysis of normal tissue (blood) is required to determine or exclude heredity.
In cases where a positive result from a genetic analysis would have an immediate significance for the treatment of a patient diagnosed with cancer, regardless of family history.
Criteria fulfilled for other inherited syndromes, where BC and OvC are included.

Abbreviations: BBC, bilateral breast cancer; BC, breast cancer; OvC, ovarian cancer; TNBC, triple-negative breast cancer

In Sweden, genetic counseling both before and after an analysis of germline pathogenic variants has been considered mandatory, since genetic counseling is a process that guarantees a discussion regarding both benefits and limitations of the genetic testing. It also provides risk estimates for cancer development, recommendations for early detection and preventive measures, information of reproductive options, and support for psychological well-being [138].

However, this traditional approach with pre- and post-test counseling is both costly and time-consuming. Hence, the counseling process needs to be simplified. Two large randomized trials of pre- and post-test telephone genetic counseling for women at high risk of pathogenic variants in *BRCA1/2*, conducted in the United

States, reported strong evidence that telephone counseling was not inferior to in-person counseling for decision making and psychosocial outcomes [170, 171].

Further simplification of the procedure of genetic testing has been trialed by offering written pre-test information instead of in-person (or telephone) counseling. The first randomized trial regarding this simplified genetic counseling approach, with regards to hereditary breast cancer, was conducted in Australia and published in 2016. Among women newly diagnosed with breast cancer, the intervention group received an educational pamphlet instead of the pre-test genetic counseling, a strategy that was deemed as cost-effective and non-inferior to the standard procedure [172].

In a non-randomized trial conducted in the Netherlands, breast cancer patients were given a choice between standard pre-test genetic counseling and a simplified approach called DNA-direct. More than half of the patients opted for DNA-direct, and subsequently received telephone, written, and digital information instead of the standard pre-test genetic counseling. Six out of eight carriers of pathogenic variants in *BRCA1/2* were satisfied with the DNA-direct procedure [173].

In a prospective study from Norway (the DNA-BONus study), written pre-test genetic information and analysis of the *BRCA1/2* genes were offered to all newly diagnosed breast cancer patients. The results from this study showed that symptoms of anxiety and depression were comparable to previously reported symptoms in breast cancer patients in general [174, 175].

In a previously published study regarding simplification of the procedure in our region, with written pre-test information and testing of the *BRCA1/2* genes, it was reported that very few newly diagnosed breast cancer patients contacted the study management with practical questions or for genetic counseling over the telephone, suggesting that most of the women felt that the written information was sufficient [176-178].

These results indicate that the standard procedure for pre-test counseling could be simplified (in a cost-effective way) without a negative impact on decision making and psychosocial well-being.

In 2016, a framework for retrospective identification of germline pathogenic variants in *BRCA1/2* in previous ovarian cancer patients, who have not been referred for genetic testing, and their families was discussed and designated 'Traceback' at a workshop at the US National Cancer Institute [179]. In Sweden, this type of retrospective genetic outreach for individuals who have previously been diagnosed with cancer is not normally used in clinical practice. However, the identification of pathogenic variants in previous breast cancer patients, who otherwise would not have knowledge of their carrier status, is crucial for the prevention of new primary cancers through increased surveillance and risk-reducing measures.

Aims

Paper I

To evaluate the concordance between self-reported and register-reported information regarding family history of breast cancer, ovarian cancer, and other types of cancer in first-degree relatives of women diagnosed with early-onset breast cancer, and if there was a difference in agreement whether the self-reported information was reported by the young woman or by a relative.

To determine the frequencies of carriers and noncarriers of germline pathogenic variants, and to describe tumor characteristics for each of these groups.

Paper II

To evaluate whether place of residence at breast cancer diagnosis and/or treating hospital were associated with the fact that not all women diagnosed with breast cancer at the age of 35 years or younger in the South Swedish Health Care Region had attended genetic counseling and testing.

Paper III

To gain a deeper understanding why not all women with early-onset breast cancer attended genetic counseling and testing when they were first diagnosed.

To evaluate a Traceback counseling strategy, with possible adaptations for broader Traceback studies and future clinical implementation.

Paper IV

To evaluate how the incidence of CBC among women in Sweden has evolved over five consecutive decades.

Materials

Data sources

Sweden, as well as all other Nordic countries, are unique for keeping comprehensive registers with information about their citizens. The Swedish registers can be linked through the compulsory civic registration numbers that are assigned to all residents. The system for these civic registration numbers was implemented in 1947. Since then, all Swedish citizens are provided with a number containing the date of birth (six digits; year, month, day), a birth number (three digits), and one control digit. This tenth digit can be calculated using the other nine digits [180]. To collect data regarding the women who were diagnosed with breast cancer for the studies in paper I–IV, several national and regional registers were utilized.

The Population Register

In Sweden, population registration was originally administered by the Church of Sweden, and the oldest preserved registers dates all the way back to the early 17th century. However, due to the separation of the church and state, the administration of the Population Register was transferred to the Swedish Tax Agency in 1991. The register contains information regarding all Swedish citizens, e.g., civic registration numbers, names, addresses, places of birth, citizenship, spouses, children, parents, legal guardians, adoptions, migration in and out of the country, deaths, and burial sites [180].

The Multi-Generation Register

The Multi-Generation Register, which is a part of the Total Population Register, is a register of all individuals who have been registered in Sweden at any time since 1961, and who were born in 1932 or later. The register contains links between individuals and their biological and/or adoptive parents, which, in addition, result in links to siblings, grandparents, cousins, and other family members. The Multi-Generation Register, which has good coverage and quality, can be used for scientific research and statistical purposes [181].

The Swedish Cancer Register

The National Board of Health and Welfare has since 1958 maintained a register of all malignant (and certain types of benign) cases of tumor disease called the Swedish Cancer Register. It is compulsory for all healthcare providers under public or private administration in Sweden to report cancer cases to this register, and the report should include clinical information as well as information from pathologists and cytologists on surgical removed tissues and biopsies [182]. The overall completeness of the Swedish Cancer Register is high [183]. However, in early cancer statistics, data on if a tumor was a first primary tumor, a recurrence, or a new primary tumor are missing. In addition, it is only during the last years that the number of individuals diagnosed with breast cancer has been reported, and not only incident cases [182].

The Southern Swedish Regional Tumor Registry

In the mid-1980s, The Swedish Cancer Register was subdivided into six regional registers [182]. The Southern Swedish Regional Tumor Registry, affiliated at the Regional Cancer Centre South [184] and managed by Region Skåne, was responsible for the regional cancer registration in the South Swedish Health Care Region until 2008.

The National Quality Registry for Breast Cancer

The National Quality Registry for Breast Cancer (NKBC) is affiliated at the Regional Cancer Centre Stockholm-Gotland and managed by Region Stockholm. This register has been responsible for breast cancer registration since 2008, and facilitates research and developments in breast cancer by providing data regarding preoperative diagnostics, tumor characteristics, type of surgery, waiting periods, complications, recurrence rates, and patient satisfaction [185].

The OnkGen Register

The OnkGen Register at Region Skåne in Lund contains data on all the individuals who have attended genetic counseling at the Oncogenetic Clinic in Lund since it was opened in 1993. Included in this register is information regarding pedigrees in relation to probands, questionnaire data, diagnosis verifications, and results from genetic analyses.

Table 3. Different registers used in paper I–IV

Registers	Administered by	Papers
The Population Register	The Swedish Tax Agency	I–III
The Multi-Generation Register	Statistics Sweden	I
The Swedish Cancer Register	The National Board of Health and Welfare	I, IV
The Southern Swedish Regional Tumor Registry	Region Skåne	I–III
The National Quality Registry for Breast Cancer	Region Stockholm	III
The OnkGen Register	Region Skåne	I–III

Study inclusion

Paper I–III included overlapping populations based on all women diagnosed with breast cancer at the age of 35 years or younger in the South Swedish Health Care Region. This region is one out of six health care regions in Sweden, and encompasses approximately 20% of the total Swedish population [186]. The South Swedish Health Care Region consists of the four counties Skåne, Blekinge, Kronoberg, and Southern Halland.

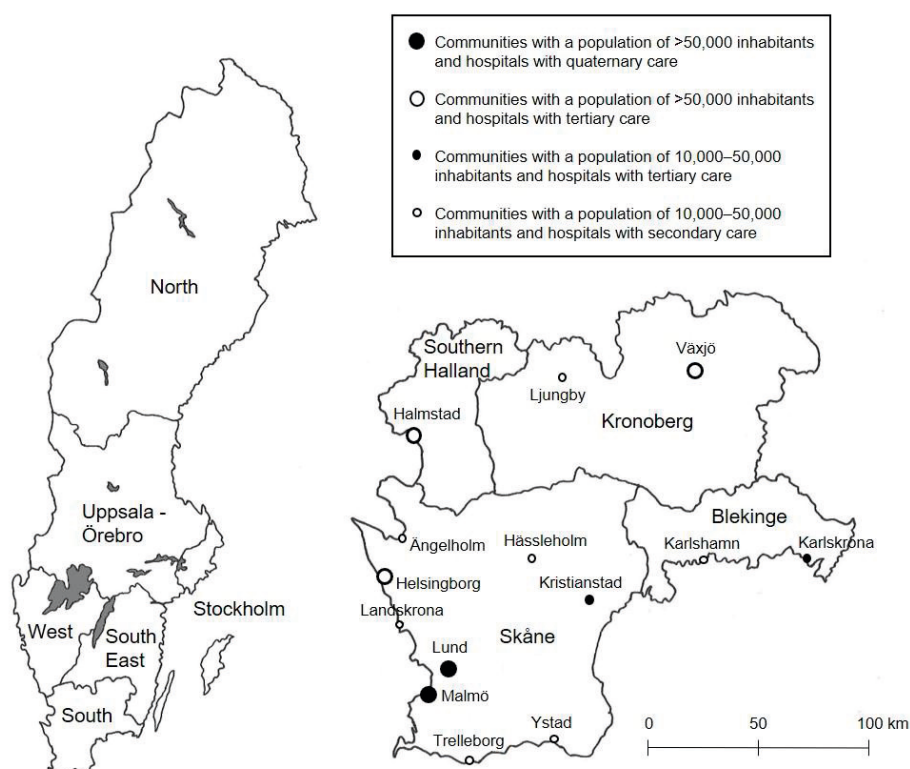


Figure 5. The six national healthcare regions in Sweden (left) and the South Swedish Health Care Region (right). The inhabitants of the South Swedish Health Care Region have access to one hospital in each of the included communities.

Paper I

All women who were diagnosed with early-onset breast cancer between 1970 and 2013, and registered at the Oncogenetic Clinic in Lund, were included in the study. Out of the 231 women who were registered at the clinic, 161 had attended the genetic counseling sessions themselves, and for 70 of the registered women, a relative had attended the session.

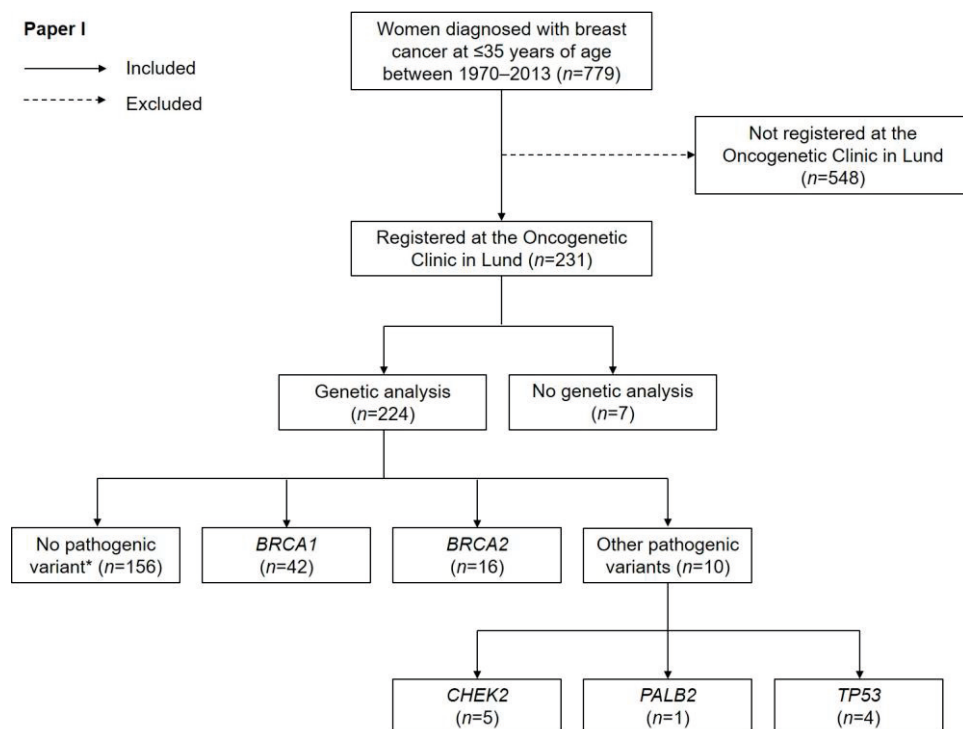


Figure 6. Flow chart of study inclusion and carrier status of the 224 women who underwent genetic testing.

*Including variants of uncertain significance (VUS) in *BRCA1* (n=6), *CHEK2* (n=1), *TP53* (n=2), *CDH1* (n=1), and *PTEN* (n=1).

Paper II

All the 279 women who were diagnosed with early-onset breast cancer between 2000 and 2013 were included in the study. Out of these women, 179 were registered at the Oncogenetic Clinic in Lund and 100 were not.

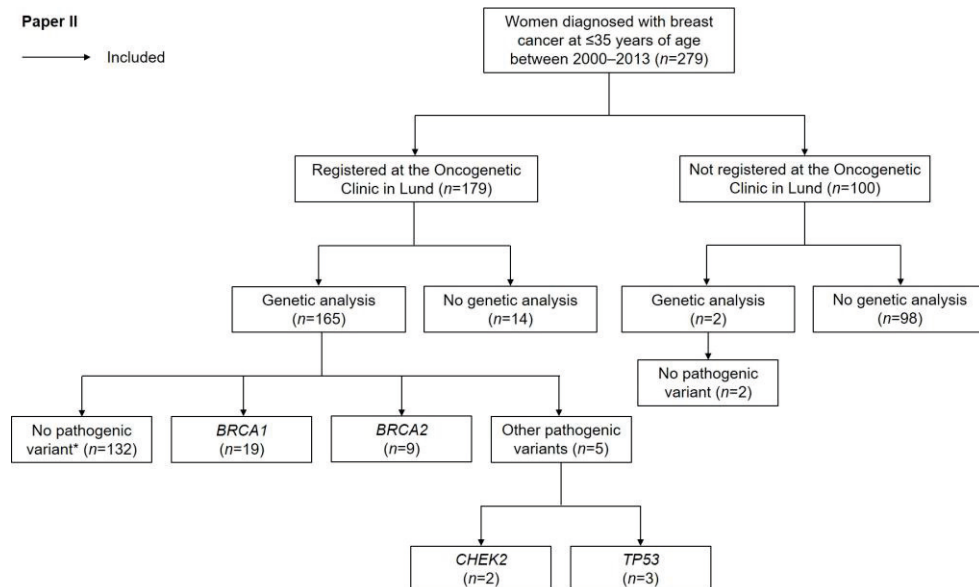


Figure 7. Flow chart of study inclusion and carrier status of the 167 women who underwent genetic testing.

*Including VUS in *BRCA1* (n=2), *BRCA2* (n=1), *CHEK2* (n=1), *TP53* (n=1), and *CDH1* (n=1).

Paper III

All women who were diagnosed with early-onset breast cancer between 2000 and 2017, who had not previously been registered at the Oncogenetic Clinic in Lund and were not deceased, emigrated, or had moved to another healthcare region in Sweden, were invited to participate in the study. The invitation letter, which contained an offer of analysis of the genes *BRCA1*, *BRCA2*, *PALB2*, *CHEK2*, and *ATM*, was sent to 63 women. For the Traceback pilot study procedure, see paper III.

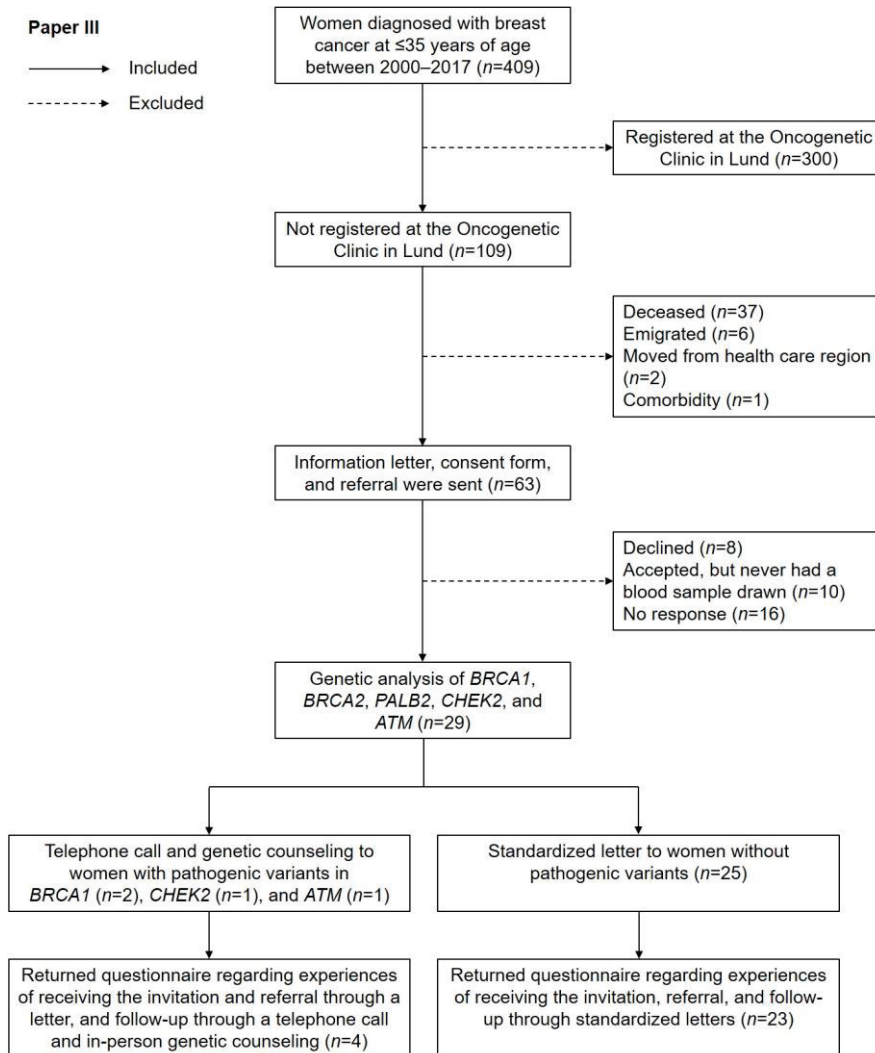


Figure 8. Flow chart of study inclusion, genetic analyses, and return of questionnaires.

Paper IV

After excluding 258 women due to clerical errors or inconclusive data, 210,746 women registered with a first primary breast cancer (both invasive and in situ) at the Swedish Cancer Register between 1960 and 2006 were included in the study. Each woman was counted for once, and all women were stratified within groups based on in which decade they were diagnosed with their first primary breast cancer. These women were then followed, with a follow-up period that was limited to a maximum of ten years. However, if they were diagnosed with CBC, died, or emigrated within ten years from the first breast cancer diagnosis, the follow-up period was shorter. The total time of follow-up, for all included women, added up to 1,456,346 person-years.

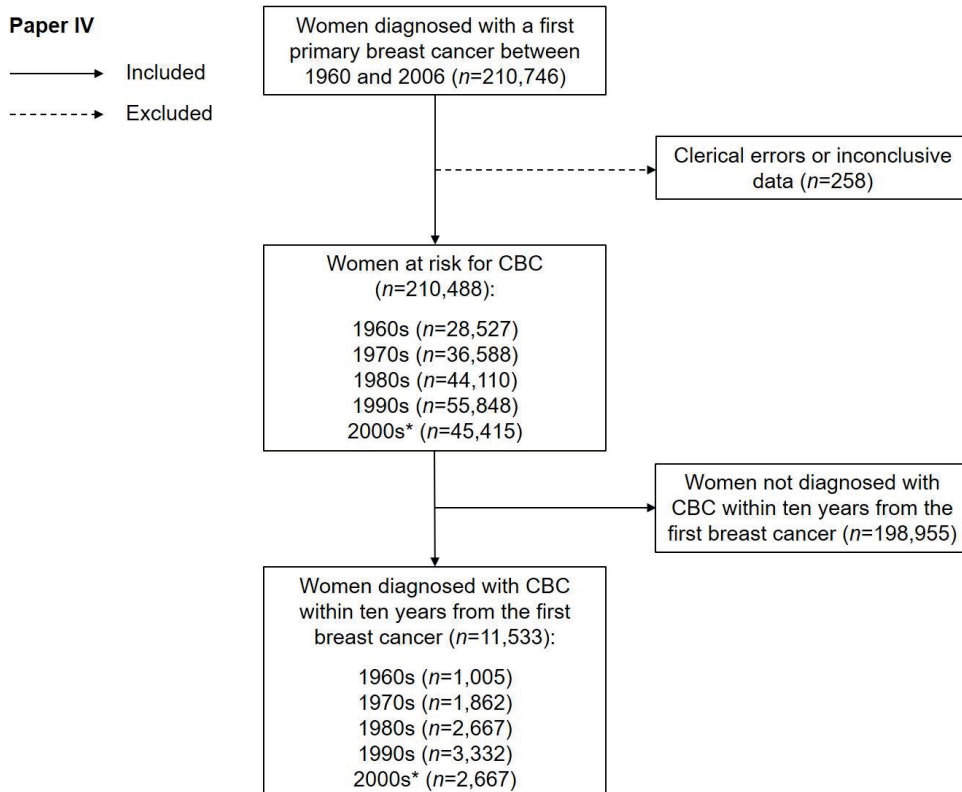


Figure 9. Flow chart of study inclusion and CBC diagnoses within ten years from the first primary breast cancer.

*Includes the years 2000–2006.

Methods and methodological considerations

Statistical analyses

The statistical software used to perform analyses within this thesis were SPSS (IBM Corp. IBM SPSS Statistics for Windows. Armonk, NY: IBM Corp), version 22.0 in paper I and version 25.0 in paper II–IV, and Stata 16 (StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC) in paper IV.

In paper I, Cohen's kappa (κ) was used to determine the agreement between self- and register-reported information regarding family history of breast cancer, ovarian cancer, and other types of cancer. κ is a non-parametric test that can be used to measure inter-rater reliability for categorical data. When interpreting the results from the κ -analysis, we used the criteria proposed by Landis and Koch in 1977, where κ -values of <0 show no agreement, 0–0.20 show slight, 0.21–0.40 show fair, 0.41–0.60 show moderate, 0.61–0.80 show substantial, and 0.81–1 show almost perfect agreement [187].

In addition to the κ -analysis, sensitivity and specificity were calculated in paper I. Sensitivity measures the proportion of positives that are correctly identified, and specificity measures the proportion of negatives that are correctly identified. The terms *true positive*, *false positive*, *true negative*, and *false negative* refer to the correctness of the classification of the results. For example, if the 'condition' is self-reported breast cancer in a first-degree relative, *true positive* means 'correctly reported as diagnosed with breast cancer', *false positive* means 'incorrectly reported as diagnosed with breast cancer', *true negative* means 'correctly reported as not diagnosed with breast cancer', and *false negative* means 'incorrectly reported as not diagnosed with breast cancer'.

Fisher's exact test is a statistical test for categorical variables that needs to be used when evaluating the association between variables when sample sizes are small. Because of the relatively small number of participants in paper I and III, Fisher's exact test was therefore used. In paper II, logistic regression analysis was used to examine differences in 1) place of residence at breast cancer diagnosis and 2) treating hospital between the women who were registered at the Oncogenetic Clinic

in Lund and those who were not, as well as the registration trend over time. The results from these analyses were given as ORs and 95% CIs.

Non-parametric tests are used to compare the distribution between groups when data are not normally distributed. For continuous data, the Mann-Whitney *U* test is used, and for categorical data, the chi-squared test is used. Because of the small and skewed sample sizes in paper III, the Mann-Whitney *U* test was therefore used, and in paper IV, Pearson's chi-squared test was used. When data are normally distributed, parametric tests, such as the *t*-test for continuous data, are instead used. Student's *t*-test was used in paper IV.

Table 4. Statistical methods used in paper I–IV

Statistical methods	Paper I	Paper II	Paper III	Paper IV
Cohen's kappa (κ)	X			
Fisher's exact test	X		X	
Logistic regression analysis		X		
Mann-Whitney <i>U</i> test			X	
Pearson's chi-squared test				X
Student's <i>t</i> -test				X

Additional analyses

Descriptive statistics were used to recap questionnaire answers from one closed-ended question and six scaled-response questions with Likert rating scales, ranging from 1 (strongly disagree) to 5 (strongly agree), in paper III. Likert scales may vary, using either an odd or an even number of points. A scale with an even number of points makes the participant choose side, either *for* or *against*. However, we chose to use an odd number of points, because then the scale has a midpoint that provides the participant with a neutral answer. In addition, content analysis was used to group spontaneous answers from three open-ended questions into categories. This method is used to systematically label the content of, for instance, a set of texts, either quantitatively or qualitatively, for the analysis of patterns and/or meanings [188].

Person-years at risk were calculated as the time from the first primary breast cancer until either a CBC diagnosis, date of death, or emigration, and with a limit of ten years of follow-up for each woman in paper IV. Incidence rates (IRs) and 95% CIs for CBCs were calculated per 10,000 person-years, and incidence rate ratios (IRRs) were calculated as the ratios between two IRs. Crude IRs accurately represent the incidence of breast cancer in each decade. However, when comparing the incidence between two different time periods, an alternative approach is to use age-standardized IRs, which also consider the differences in the age structure of the populations. Hence, age-standardized IRs of both first breast cancers and CBCs per 100,000 person-years in the Swedish population were also calculated, using the Swedish census population in the year 2000 as standard.

Study design

This thesis is mostly based on observational register-based studies. In this type of studies individuals are observed, and outcomes are measured, but no attempts are made to affect the outcome. However, the study in paper III was a Traceback pilot study, where we evaluated a retrospective approach to genetic testing in women who had previously been diagnosed with early-onset breast cancer, but not tested.

When designing a study or interpreting results, two alternative explanations to the statistical associations, in addition to the true associations, must be considered; chance and bias, i.e., random errors and systematic errors, respectively. Chance reflects the random variability in the data that cannot be explained by selection or confounding, and the impact of such random errors can be reduced by increasing the sample size. This will, however, not reduce the effect of systematic errors. Hence, the accuracy of a study depends on total error, which includes both precision and validity [189].

Precision

Statistical significance is based on rejecting or retaining the null hypothesis, an indicator of no association between the investigated variables. The null hypothesis is compared with the alternative hypothesis; the indicator of an association. To be able to make a statement regarding an association between variables, the null hypothesis must be rejected. To measure the strength of the evidence against the null hypothesis, a probability value (P -value) can be calculated. A significance level of $P < 0.05$ is generally considered as statistically significant [189]. In this thesis, significance was considered with a P -value of < 0.05 in all statistical analyses, even though a value close to 0.05 usually only is considered as moderate evidence against the null hypothesis. In paper I, II, and IV, most results indicated a P -value of either 0.001 or < 0.001 , which might be more reasonable when considering the provision of strong evidence [189, 190].

CIs express the precision with which the outcome is measured, i.e., the statistical variation (or random error) that underlies the estimate. A CI is the mean of the estimate plus/minus the variation in that estimate, i.e., the range of values that the estimate is expected to fall within if you repeat the test within a certain level of confidence. The desired CI level is usually one minus the α -value. When using the most common α -value, $P < 0.05$, for statistical significance, the CI will be $1 - 0.05 = 0.95$, or 95%. Because of less precision, CIs in a small study will be wider than in a large study [189], which was seen in the results regarding differences between tumor characteristics in the subgroups in paper I. The sample sizes in paper I–III were all

numerically relatively small, and results from small cohorts should always be interpreted with caution.

Validity

Validity is used to describe to which extent the study really measures what it is supposed to measure. This can be assessed by analyzing how well the results correspond to established theories and other measures of the same concept. Validity is divided into internal and external validity. Internal validity is used to describe the accuracy of the study, i.e., to which extent the study represents the underlying population. Through the measurement of internal validity, alternative explanations for the results can be addressed or eliminated. External validity is used to describe whether the results in the study can be generalized or not, i.e., to which extent the research is valid for other populations than the studied. For external validity, internal validity is crucial. However, internally valid results might not always be generalizable beyond the underlying population [189].

Internal validity

For the results to be internally valid, the study must have enough statistical precision, and it cannot have considerable systematic errors. Systematic errors stem from systematically incorrect measurements or non-random inclusion, and can be classified within three major categories: selection bias, information bias, and confounding [189].

Selection bias arises when study participants differ from nonparticipants in such a way that it affects the association between exposure and outcome. In paper I, a potential sample selection bias was discussed because the prevalence of pathogenic variants in *BRCA1* and *BRCA2* among the women with early-onset breast cancer in our study was much higher than had previously been reported. In paper III, a potential nonresponse bias was discussed, because more than half of the invited women chose not to participate in the study. Even though none of the women who participated expressed any serious concerns regarding their experiences with being contacted retrospectively, we could not exclude the possibility that some of the women who did not respond might have reacted in an alternative way.

Information bias is used to describe when the collected information is either misclassified or incorrect. One example of information bias is recall bias. It is established that individuals that have been diagnosed with a disease are more likely to recall exposures more accurately than healthy individuals. This type of bias is less likely to have occurred in this thesis. In paper III, information was collected exclusively from women who were diagnosed with breast cancer, which decreased the risk for recall bias. However, when answering the question regarding the main

reason to why they had not undergone genetic testing when they were first diagnosed with breast cancer, most women stated that they had not received any information about genetic counseling and testing from their physicians. These statements are probably true, but the conversation might also have been forgotten.

A confounder is a factor that might be related to both exposure and outcome. In observational studies, such confounders are usually not equally distributed among participants in various subgroups, which can cause systematic errors. To adjust for potential confounders, established factors, such as risk factors, can be included in multivariable analyses. In paper II, information regarding many potential risk factors for not being referred for genetic counseling and testing were missing. Hence, when analyzing whether place of residence and/or treating hospital were associated with the fact that not all women with early-onset breast cancer were registered at the Oncogenetic Clinic in Lund, confounders such as socio-economic status and ethnicity were addressed in the discussion section, but could not be adjusted for.

External validity

The study populations in paper I–III included all women who were diagnosed with breast cancer at 35 years or younger in the South Swedish Health Care Region. Since all young breast cancer patients in this region, which encompasses approximately 20% of the total Swedish population, would be referred to the Oncogenetic Clinic in Lund, the results in these papers are generalizable to the underlying population. In paper II, the results regarding differences in the possibility of being offered genetic counseling and testing at an oncogenetic clinic may therefore also be valid for the entire country, and perhaps also internationally. The study population in paper IV included all women diagnosed with a first primary breast cancer in Sweden between 1960 and 2006, and with CBC between 1960 and 2016. Hence, study and target populations overlap to the highest possible degree. Regarding the generalizability to other parts of the world, caution is, however, needed when attempting to transfer research findings between different cultures. Breast cancer is a highly heterogeneous disease with regards to incidence, stage at diagnosis, treatment, and survival, and estimates of global external validity is therefore difficult to make [191].

Table 5. Overview of strengths and limitations in paper I–IV

Papers	Strengths	Limitations
I–III	<p>Study populations representing all women diagnosed with BC at an age of ≤ 35 years and all known carriers of pathogenic variants in this age group in the South Swedish Health Care Region.</p> <p>Retrieval of information from the Population Register, the Multi-Generation Register, the Swedish Cancer Register, the Southern Swedish Regional Tumor Registry, NKBC, and the OnkGen Register contributes to good quality and control of BC diagnoses and carriers of pathogenic variants.</p>	<p>A limited number of women with early-onset BC.</p> <p>Substantial amounts of data regarding tumor characteristics were missing in paper I, mainly because many women were diagnosed before clinical use of ER, PR, and HER2.</p> <p>The Oncogenetic Clinic in Lund was opened in 1993. Prior to 2000, the clinic was mostly research-oriented and the referred BC patients probably had a more pronounced family history of cancer.</p> <p>Information regarding potential confounders, e.g., socio-economic status (marital status, education levels, and income) and ethnicity, was missing in paper II.</p> <p>Less than half of the invited women diagnosed with early-onset BC completed the full study procedure in paper III.</p>
IV	<p>Study population representing all Swedish women diagnosed with BC between 1960 and 2006, and all Swedish women diagnosed with CBC between 1960 and 2016.</p> <p>Retrieval of information from the Swedish Cancer Register contributes to good quality and control of BC diagnoses.</p>	<p>In the 1960s, information regarding which breast the tumor was located in was not registered at the Swedish Cancer Register.</p> <p>Information regarding factors of possible importance, e.g., genetic information and BC pathological subtypes, was not available.</p>

Abbreviation: BC, breast cancer; CBC, contralateral breast cancer; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; MBBC, metachronous bilateral breast cancer; NKBC, National Quality Registry for Breast Cancer; PR, progesterone receptor; SBBC, synchronous bilateral breast cancer

Ethical considerations

All studies within this thesis included human participants, and were conducted in accordance with the 1964 Declaration of Helsinki, and its later amendments or comparable ethical standards, and national legislation. Ethical approvals were obtained from the Ethical Review Board in Lund and the Swedish Ethical Review Authority.

In paper I, all women with early-onset breast cancer and/or their relatives had signed consent forms for scientific follow-up within their families. In paper I, as well as in paper II and IV, already collected data were used and were therefore not a burden to the participants. However, the participants had no option of deciding whether they wanted to be included in the studies or not. Hence, all results from the analyses were presented without the possibility for identification.

In paper III, the Traceback pilot study, signed consent forms were obtained from all participants prior to study entry. The potential participants received an information letter regarding the purpose of the study through regular mail, in which we invited them to a genetic analysis of breast cancer predisposing genes and explained the voluntary nature of their subsequent participation. There were no medical risks identified for the women who chose to participate. However, information regarding possible transient feelings of anxiety or depression, caused by the knowledge of being a carrier of a pathogenic variant, and the subsequent risk that one's children might have inherited the alteration, was given.

In the Traceback pilot study, the only invasive procedure for each participant was one blood sample, which was drawn at a hospital or a local health center of the woman's own choice. The blood samples and the results from the genetic analyses are stored at a biobank (BD41) at the Department of Clinical Genetics and Pathology, Region Skåne, until further notice. Region Skåne is responsible for handling all personal data according to the General Data Protection Regulation (GDPR), and the participants may request that the handling of their personal data should be limited, that information about them should be removed, or that their blood samples must not be used in the future.

No economic compensation was given to the women who chose to participate in the Traceback pilot study. All parts of the study, however, were free of charge for the participants.

Results and discussion

Paper I

Concordance between self- and register-reported information of family history

The accuracy, sensitivity, and specificity of self-reported information regarding first-degree family history of cancers are outlined in Table 6, which is a duplicate of Table 2a in paper I.

Overall, almost perfect agreement between self- and register-reported information regarding first-degree family history of breast cancer and ovarian cancer, however, lesser agreement regarding other types of cancer, was observed.

In addition, both sensitivity and specificity of self-reported family history of breast cancer and ovarian cancer were high. The specificity of self-reported family history of other types of cancer was also high, but sensitivity was lower.

Table 6. The accuracy, sensitivity, and specificity of self-reported information regarding first-degree family history of cancer for all women, as well as noncarriers and carriers of pathogenic variants in *BRCA1* and *BRCA2*

	κ -value	Sensitivity		Specificity		<i>P</i> -value
		<i>n/N</i>	%	<i>n/N</i>	%	
No family history						
All	0.70	98/121	81.0	73/80	91.3	<0.001
No pathogenic variant*	0.70	53/70	75.7	63/67	94.0	<0.001
<i>BRCA1</i>	0.60	27/30	90.0	4/5	80.0	<0.001
<i>BRCA2</i>	0.44	10/12	83.3	2/3	66.7	0.08
Family history of breast cancer						
All	0.92	61/64	95.3	133/137	97.1	<0.001
No pathogenic variant*	0.92	31/32	96.9	102/105	97.1	<0.001
<i>BRCA1</i>	0.83	18/20	90.0	14/15	93.3	<0.001
<i>BRCA2</i>	1.00	6/6	100.0	9/9	100.0	<0.001
Family history of ovarian cancer						
All	0.86	10/10	100.0	188/191	98.4	<0.001
No pathogenic variant*	0.80	2/2	100.0	14/135	99.3	<0.001
<i>BRCA1</i>	0.92	8/8	100.0	26/27	96.3	<0.001
<i>BRCA2</i>	N/A	N/A	N/A	15/15	100.0	N/A
Family history of other cancer						
All	0.51	41/77	53.2	117/124	94.4	<0.001
No pathogenic variant*	0.55	29/52	55.8	81/85	95.3	<0.001
<i>BRCA1</i>	0.51	6/12	50.0	22/23	95.7	0.001
<i>BRCA2</i>	0.60	5/7	71.4	7/8	87.5	0.02

*Analyzed without findings of pathogenic variants, including VUS in *BRCA1*, *CHEK2*, *TP53*, *CDH1*, and *PTEN*. κ -values of almost perfect agreement are highlighted in bold.

In relation to information provider, the agreement between reports regarding first-degree family history of breast cancer was almost perfect in both reports from women diagnosed with early-onset breast cancer ($\kappa=0.93$) and their relatives ($\kappa=0.90$). However, regarding no family history of cancer and family history of ovarian cancer, lesser agreement was observed in the reports from relatives ($\kappa=0.54$ and $\kappa=0.78$, respectively) compared with reports from the women previously diagnosed with early-onset breast cancer ($\kappa=0.73$ and $\kappa=0.92$, respectively).

The results in our study were both concordant and contradictory to previous studies, where agreement between self-reported and register-reported first-degree family history of breast cancer has been reported as high, but agreement between reports of ovarian cancer has been lower [192, 193]. Regarding reports of family history of other types of cancer, the result in our study was also concordant with previous findings [192], exhibiting both lesser agreement and an underreporting. Our study comprised a relatively small number of women, which makes it difficult to draw strong conclusions. However, the results indicate that information of other types of cancer in first-degree relatives is not communicated as successfully as information regarding breast cancer and ovarian cancer. The reasons for this inconsistency might be that certain types of cancer may be less openly discussed within families, e.g., cervical cancer, uterine cancer, endometrial cancer, or prostate cancer, or if cancer history is discussed, the information may not always be entirely accurate or might also be forgotten. Another reason could be that physicians may focus on retrieving information regarding family history of breast cancer and ovarian cancer, with possible pathogenic variants in *BRCA1* and *BRCA2* in mind. However, other types of cancers should not be disregarded, since some pathogenic variants lead to an increased risk for many different types [42, 194].

Frequencies of carriers and noncarriers of pathogenic variants

Out of the 224 women who underwent genetic testing, 68 (30%) tested positive for a pathogenic variant; 42 (19%) in *BRCA1*, 16 (7%) in *BRCA2*, and 10 (4%) in other genes (Figure 6). Between 1993 and 1999, genetic testing at the Oncogenetic Clinic in Lund was mostly angled towards research, and out of the 37 women who underwent genetic testing during these years, 22 (59%) tested positive for a pathogenic variant in *BRCA1* or *BRCA2*; 15 (40%) in *BRCA1* and seven (19%) in *BRCA2*. However, from 2000 and onwards, a more even recruitment was sanctioned by funds that enabled genetic testing free of charge for the referring clinic. Out of the 187 women who underwent genetic testing between 2000 and 2013, 36 (19%) tested positive for a pathogenic variant in *BRCA1* or *BRCA2*; 27 (14%) in *BRCA1* and nine (5%) in *BRCA2*.

The prevalence of carriers of pathogenic variants in women diagnosed with breast cancer at the age of 35 years or younger in our study was higher than the previously reported 10–15% [16]. Two explanations for this discrepancy could be 1) that the

women who were tested in our study might have had a more pronounced family history of breast cancer and/or ovarian cancer, and thereby increased their probability for a referral, and 2) that family members at risk might have referred themselves more often because of a higher understanding of the hereditary aspects of breast cancer [195].

Tumor characteristics

Medullary carcinoma and high grade, ER-, PR-, and triple-negative tumors were more common among women with pathogenic variants in *BRCA1* compared with both women who were analyzed without pathogenic variants and women with pathogenic variants in *BRCA2* (see Table 3 in paper I).

Even though data regarding tumor characteristics were missing to a large extent, which was mostly due to the number of women who had been diagnosed with breast cancer before clinical use of ER, PR, and HER2, our observations were concordant with previously described tumor characteristics associated with pathogenic variants in *BRCA1*, such as high grade [196] and triple-negative tumors, as well as medullary carcinoma [61, 197].

Even though all women diagnosed with early-onset breast cancer in Sweden should be offered a referral to an oncogenetic clinic for genetic counseling, and have the possibility of genetic testing, we observed that a large proportion of the women in our study had not received genetic counseling and testing. Even after the year 2000, when genetic counseling and testing was implemented into clinical care in our region, more than half of the women with early-onset breast cancer were not referred to the Oncogenetic Clinic in Lund. One of the conclusions in paper I, i.e., that the reason behind this must be further elucidated, was the background for the studies in paper II and III.

Paper II

Even though all women ($n=279$) included in this study fulfilled the Swedish national breast cancer guidelines for consideration of a referral for genetic counseling and testing, we identified 100 (36%) women who had not received genetic counseling at the Oncogenetic Clinic in Lund.

The registration at the Oncogenetic Clinic in Lund and the trend of registration over time in relation to the young women's place of residence at breast cancer diagnosis and treating hospitals are outlined in Table 7, which is a duplicate of Table 4 in paper II.

Table 7. Registration at the Oncogenetic Clinic in Lund and trend of registration over time in relation to the women's place of residence at breast cancer diagnosis and treating hospitals

	Registered at the Oncogenetic Clinic			Trend over time	
	<i>n/N (%)</i>	OR (95% CI)	<i>P</i> -value	OR (95% CI)	<i>P</i> -value
Regions					
Western Skåne	132/184 (71.7)	1.00 (ref.)		1.16 (1.06–1.26)	0.001
Eastern Skåne	20/30 (66.7)	0.79 (0.35–1.80)	0.57	1.06 (0.87–1.30)	0.55
Blekinge	6/26 (23.1)	0.12 (0.05–0.31)	<0.001	0.99 (0.78–1.25)	0.94
Kronoberg	13/21 (61.9)	0.64 (0.25–1.63)	0.35	1.64 (1.07–2.53)	0.03
Southern Halland	8/18 (44.4)	0.32 (0.12–0.84)	0.02	1.25 (0.94–1.67)	0.12
Population					
>50,000	85/117 (72.6)	1.00 (ref.)		1.09 (0.98–1.21)	0.12
10,000–50,000	45/67 (67.2)	0.79 (0.41–1.51)	0.47	1.18 (1.02–1.37)	0.02
<10,000	49/95 (51.6)	0.39 (0.22–0.70)	0.001	1.21 (1.08–1.36)	0.001
Hospitals					
Lund	43/65 (66.2)	1.00 (ref.)		1.17 (1.01–1.36)	0.04
Malmö	50/62 (80.6)	2.13 (0.95–4.81)	0.07	1.10 (0.92–1.31)	0.30
H-borg/Ä-holm/L-krona/Trelleborg	27/43 (62.8)	0.86 (0.39–1.93)	0.72	1.25 (1.04–1.50)	0.02
Kristianstad/Hässleholm/Ystad	24/33 (72.7)	1.36 (0.54–3.43)	0.51	1.04 (0.86–1.27)	0.67
Karlskrona/Karlshamn	6/21 (28.6)	0.21 (0.07–0.60)	0.004	1.02 (0.81–1.29)	0.85
Växjö/Ljungby	13/21 (61.9)	0.83 (0.30–2.31)	0.72	1.64 (1.07–2.53)	0.03
Halmstad	8/18 (44.4)	0.41 (0.14–1.18)	0.10	1.25 (0.94–1.67)	0.12
Missing	8/16 (50.0)				

Statistically significant associations are highlighted in bold.

Place of residence

Women from two regions, Blekinge and Southern Halland, were significantly less likely to be registered at the clinic compared with women from Western Skåne. The trend of registration over time, from 2000 to 2013, was significantly improved in Western Skåne and Kronoberg, in contrast to Blekinge, where no improvement was indicated. In addition, women from rural settings with a population of less than 10,000 inhabitants were significantly less likely to be registered at the clinic compared with women from urban settings with a population of more than 50,000 inhabitants. However, the trend of registration over time indicated significant improvement for women from both communities with less than 10,000 inhabitants and communities with between 10,000 and 50,000 inhabitants.

Consistent with previously published studies [198, 199], our study indicated an improvement regarding trend of registration at the clinic over time, which was true for all regions except for Blekinge. One explanation for this discrepancy might be that the distance between this region and the Oncogenetic Clinic in Lund was considered too far, and that the women did not see the benefit of a visit to the clinic [200]. However, since the results in this study reflected the probability of registration at the clinic, the distance between place of residence at breast cancer diagnosis and the Oncogenetic Clinic in Lund should not have been an important factor. Because of their young ages, all women should have been referred, and subsequently have been given the option of genetic counseling and testing. In addition, significant improvement over time was observed in other regions with a

similar distance to the clinic as Blekinge. Another explanation might be that the treating physicians in rural areas might be less likely to be adherent to the clinical recommendations compared with physicians in urban areas [201].

Treating hospital

Women who were treated at the hospitals in Karlskrona and Karlshamn were significantly less likely to be registered at the Oncogenetic Clinic in Lund compared with women treated in Lund. In contrast, women who were treated at the hospital in Lund were less likely to be registered at the clinic compared with women treated in Malmö. The trend of registration at the clinic over time was significantly improved for women treated at the hospitals in Lund, Helsingborg/Ängelholm/Landskrona/Trelleborg, and Växjö/Ljungby.

Among high-risk breast cancer patients, previously published studies have reported that the clinical setting can be associated with the probability of a referral for cancer genetic counseling [199, 202]. Structural differences at regional hospitals and/or the physicians' awareness of referral criteria could therefore be plausible explanations for the low referral rates in our study.

Paper III

Out of the 63 women who were offered genetic testing through a standardized letter, 29 (46%) chose to have their blood sample drawn for DNA extraction and genetic analysis. Four women were identified as carriers of pathogenic variants; two in *BRCA1*, one in *CHEK2*, and one in *ATM*. These four women, as well as 23 (92%) of the 25 women without pathogenic variants, completed all parts of the study by subsequently answering the follow-up questionnaire.

Main reason for not having been tested previously

Out of the 27 women who answered the questionnaire, 20 (74%) stated that the main reason for not undergoing genetic testing when they were first diagnosed with breast cancer was that they had not received any information about genetic counseling and testing from their treating physicians.

In a previously published study, in which the authors evaluated why not all women receive genetic counseling despite clinical recommendations, it was reported that the physicians' attitudes toward genetic counseling and testing, a lack of knowledge regarding hereditary aspects, and a lack of discussions with the patients were possible explanations [202]. Considering the results from this previous study, these might also be some of the reasons for the regional differences that we observed in paper II.

Satisfaction with the Traceback approach

Most of the women, both with and without pathogenic variants, stated that they were satisfied with the written pre-test information, the possibility for further contacts, and the genetic testing (see Table 1 in paper III). The main reasons why the women chose to participate in the study, and have their genes analyzed, were that they saw benefits for themselves, their daughters, and/or other family members, as well as a need for increased knowledge. Most women without pathogenic variants were satisfied with being informed of the result through a standardized letter, as well as the women with pathogenic variants, who were satisfied with being informed of the result through a telephone call and the subsequent in-person genetic counseling (see Table 2 and Supplementary Table 1 in paper III).

In a previous study, where the authors evaluated *BRCA1/2* testing after a written pre-test information, it was reported that very few of the newly diagnosed breast cancer patients contacted them with questions, or for genetic counseling, over the telephone. According to the authors, this suggested that most of the patients felt that the written information was sufficient [178], which seems to be concordant with the findings in our study. The main reasons why the women chose to participate in the study, and undergo genetic testing, were consistent with reasons reported in another previously published study, e.g., that they needed to see a benefit for themselves or their families, especially daughters, to undergo genetic counseling and testing [203]. Most of the women who were tested without pathogenic variants in our study reported that they were ‘totally OK’ with being informed of the result from the genetic analysis through a standardized letter. Two out of the four women with pathogenic variants were clearly positive to being informed of the test result through a telephone call and the subsequent in-person genetic counseling. One woman’s answer was that she ‘was a bit sad’, which was not a reflection of the question. The other woman’s answer related to the time between the telephone call and the subsequent genetic counseling session. Considering these results, this indicates that the women felt that the written pre- and post-test information was enough.

Paper IV

Incidence of contralateral breast cancer

A significant increase in the incidence of CBC, from 3.5% (IR 6.0) to 6.0% (IR 9.0), within ten years from the first breast cancer diagnosis, from the 1960s to the 1980s was observed. The increase was seen in all age groups, with the steepest increase, from 4.4% (IR 6.3) in the 1960s to 8.7% (IR 11.8) in the 1980s, among women who were younger than 40 years when they were diagnosed with their first breast cancer. In addition, a subsequent significant decrease of women diagnosed with invasive

CBC within ten years from the first breast cancer diagnosis after the 1980s was observed, in contrast to CBC *in situ*, where the incidence stabilized in the years after (see Table 2 in paper IV).

This observed incidence increase has been considered to be caused by the lifestyle changes women have undergone in the recent decades [95, 101, 102, 114, 126, 127, 204, 205] and national mammography service screening [206]. The subsequent decrease in CBC incidence is considered to be caused by the introduction of adjuvant therapy with TAM [207] and AIs [163]. One factor that is an established risk factor for CBC is an early age at first breast cancer diagnosis. It has previously been reported that the cumulative incidence of CBC was highest among women who were younger than 45 years when they were diagnosed with their first breast cancer [208], which is concordant with the results in our study, where the steepest increase in CBC incidence, within ten years after the first breast cancer diagnosis, was observed among young women between the 1960s and the 1980s.

Age-standardized incidence of contralateral breast cancer

A steady increase in the age-standardized incidence of CBC per 100,000 person-years in the Swedish population was observed among all women diagnosed with CBC, women with invasive CBC, and peri- and postmenopausal women, within ten years after their first breast cancer diagnoses. However, among young women, the age-standardized CBC incidence increased in women who were diagnosed with their first primary breast cancer between 1960 and 1989, but then stabilized in the following years. Among women diagnosed with *in situ* CBCs, a steeper increase was seen in women who were diagnosed with their first primary breast cancer between 1975 and 1984, compared with the other groups (see Figure 2 in paper IV).

Since early-onset breast cancer is also associated with a hereditary predisposition, a proportion of the women diagnosed with CBC might also be carriers of germline pathogenic variants. The risk for CBC among women carrying pathogenic variants is increased compared with noncarriers, and for women with pathogenic variants in *BRCA1* and *BRCA2*, the risk for CBC has been estimated to be 4.5-fold and 3.4-fold, respectively, compared with noncarriers [209], and the risk is even higher when diagnosed before the age of 40 years [55]. In the early 1990s, genetic counseling for women with early-onset breast cancer was initiated in Sweden, which may have given them the option of contralateral risk-reducing mastectomy. This might have contributed to the decrease, and the following stabilization, in CBC incidence in the 1990s and onwards.

Synchronous and metachronous bilateral breast cancer

Among all women diagnosed with CBC, 23–35% were diagnosed with synchronous bilateral breast cancer (SBBC), i.e., within three months of their first breast cancer diagnosis. There was a significant increase observed in SBBC among women who

were diagnosed with their first breast cancer during the 1960s up until the 1980s, and a subsequent decrease in SBBC among women diagnosed between the 1980s and 1990s. The portion of women diagnosed with metachronous bilateral breast cancer (MBBC), i.e., after three months of their first breast cancer diagnosis, were analyzed in two settings; in 1-year periods and 5-year periods. In the 5-year setting, there was a significant incidence increase in MBBC within five years after the first breast cancer diagnosis between the 1960s and the 1970s, and a subsequent decrease after the 1980s. In the 1-year setting, a similar increase and decrease during the first five years after the first breast cancer diagnosis was observed, however, throughout the decades, the incidence of MBBC seems to have been relatively stable (see Table 4 in paper IV).

The most plausible explanation to why the incidence of MBBC in the first 5-year period decreased in women who were diagnosed with their first breast cancer after the 1980s, is the introduction of adjuvant treatment with TAM in the late 1970s [207] and AIs in the 2000s [163]. In addition, during all decades, the SBBC incidence was significantly higher than the MBBC incidence in each following year. This is probably related to the fact that the women who are diagnosed with breast cancer undergo comprehensive screening for metastases.

Conclusions

The results from the papers included in this thesis suggest that:

- Physicians and genetic counselors can trust self-reported information regarding family history of breast cancer and ovarian cancer in first-degree relatives. However, self-reported information regarding other types of cancer is not communicated as successfully.
- Self-reported information regarding family history of cancers from relatives is almost as accurate as self-reported information from women diagnosed with early-onset breast cancer, which indicates that relatives are a good substitute for information when women diagnosed with breast cancer are themselves not able to.
- The differences in the referral pattern for genetic counseling between regions and treating hospitals, as well as reports of not discussing genetic counseling and testing with their treating physicians from women who were diagnosed with breast cancer at an age of 35 years or younger, indicates that there is a need for improvement regarding discussions about, and referrals for, genetic counseling and testing for women who are diagnosed with breast cancer at a young age.
- The incidence of CBC in Sweden increased between the 1960s and the 1980s, with the steepest increase observed in young women. However, the CBC incidence also decreased during the following decades. In the age-standardized incidence of CBC, using the Swedish census population in the year 2000 as standard, a continuous increase over five decades was observed, except among women younger than 40 years, where there was a subsequent decrease after the 1980s, with a subsequent stabilization in the years after.
- The Traceback approach, i.e., the retrospective genetic outreach, with written pre-test information and genetic testing, followed by in-person counseling for women with pathogenic variants only, was well accepted by the women who chose to participate.

Clinical implications and future perspectives

Ideally, all carriers of pathogenic variants within families with hereditary breast cancer should be identified within the healthcare system. When assessing the need for genetic counseling and testing it is worth considering that hereditary breast cancer is more substantial than pathogenic variants in the *BRCA1* and *BRCA2* genes. Other types of cancer should not be neglected, as some pathogenic variants lead to an increased risk of many cancers.

In the Traceback pilot study, we sought to identify pathogenic variants in women previously diagnosed with early-onset breast cancer, and who, according to the Swedish national breast cancer guidelines, should have been recommended genetic counseling and testing when they were first diagnosed. Pathogenic variants were identified in four (14%) women who would otherwise not be aware of their carrier status. For the prevention of new primary cancers, a knowledge of carrier status is crucial. Among women with high-penetrant pathogenic variants, prevention strategies for the development of new primary breast cancers and breast cancer related deaths are recommended. These prevention strategies include both increased surveillance through annual mammography and MRI screening, as well as the option of risk-reducing bilateral mastectomy and salpingo-oophorectomy. The identification of pathogenic variants is also associated with potential benefits for these women's families. Among healthy relatives harboring the same pathogenic variants, future cancers and cancer-related deaths could be prevented through increased surveillance and prophylactic surgery.

In Sweden, there was a peak in CBC incidence at the end of the 1980s, which most likely was caused by the introduction of the Swedish national mammographic screening program, in combination with hormonal risk factors in diverse time periods and age groups. In the last decades, however, a decrease in CBC incidence was observed. Despite this positive result, efforts to prevent the development of second primary breast cancers are still warranted.

The Traceback pilot study procedure, with written pre-test information and genetic testing, followed by in-person counseling for carriers of pathogenic variants only, was well accepted. Based on these results, our next step will be to initiate an

enlarged Traceback study were all previously untested women diagnosed with breast cancer between the ages of 36 and 40 years will be invited, which will be in concordance with the current Swedish national breast cancer guidelines, to further evaluate the Traceback strategy for possible future clinical implementation.

The long-term goal must be that young women, with or without germline pathogenic variants, should have the same quality of life and life expectancy as women in the general population.

The results presented in this thesis indicate a need for extended oncogenetic service in regional hospitals, as well as in other healthcare units that treat women with breast cancer, which implies a need for more genetically trained nurses and/or genetic counselors. Further educational and outreach activities may also be needed to ensure the integration of the Swedish national breast cancer guidelines regarding referrals for genetic counseling and testing into clinical practice, so that all women diagnosed with early-onset breast cancer will receive proper care.

Populärvetenskaplig sammanfattning

Varje år drabbas ungefär 8 000 kvinnor i Sverige av bröstcancer. Av dessa är det endast ungefär 120 (1,5 %) kvinnor som är yngre än 35 år när de får sin diagnos. Även om det är ovanligt att drabbas av bröstcancer tidigt i livet, så är risken att drabbas av en lokalt framskriden och mer aggressiv bröstcancer, med sämre prognos, större hos yngre kvinnor.

Flera olika faktorer är associerade med en ökad risk för bröstcancer hos kvinnor, och en av de viktigaste är en tidigare familjehistoria av bröstcancer. Bröstcancer är i de flesta fall inte starkt ärftligt, men i vissa av de familjer där det finns flera fall av bröstcancer kan man hitta förändringar i gener som kan förklara varför en individ insjuknar i bröstcancer. Om man har en sådan genetisk förändring kan det påverka hur bröstcancer bör behandlas och följas upp.

Sannolikheten att det skulle kunna finnas en ärftlig orsak till diagnosen ökar om man drabbas av bröstcancer tidigt i livet, och därför rekommenderas det i Nationellt vårdprogram för bröstcancer att alla kvinnor som drabbas vid en ålder av 40 år eller yngre (tidigare 35 år eller yngre) ska erbjudas en remiss till en cancergenetisk mottagning för genetisk vägledning och ställningstagande till analys av gener som är kopplade till misstänkt ärftlig bröstcancer.

I tre av fyra studier som ligger till grund för denna avhandling har de kvinnor i Södra sjukvårdsregionen (det vill säga i Skåne, Blekinge, Kronoberg och södra Halland) som drabbats av bröstcancer vid en ålder av 35 år eller yngre studerats. I studie I ingick de kvinnor som hade varit i kontakt med Onkogenetiska mottagningen i Lund och därmed även lämnat information om familjehistoria av cancer. I denna studie undersökte vi hur väl deras självrapporterade familjehistoria av bröstcancer, äggstockscancer och andra typer av cancer hos förstagrads släktingar överensstämde med de cancerfall som har rapporterats till Cancerregistret. Det vi såg var att självrapporterad information om bröstcancer och äggstockscancer hos nära släktingar överensstämde väldigt bra, men att andra typer av cancer var mindre väl överensstämmande. Det vi också uppmärksammade i denna studie var att inte alla kvinnor som drabbats av bröstcancer vid denna unga ålder i Södra sjukvårdsregionen varit i kontakt med Onkogenetiska mottagningen i Lund, vilken är den enda cancergenetiska mottagningen i denna region.

På grund av detta, så valde vi att undersöka om det fanns något samband mellan 1) var de unga kvinnorna med bröstcancer bodde när de fick sin diagnos, och 2) på vilket sjukhus de behandlades, och deras möjligheter att bli remitterade till genetisk vägledning och testning i studie II. I denna studie såg vi att de kvinnor som bodde i två av de län som ingår i Södra sjukvårdsregionen, och i mindre orter, när de fick sina bröstcancerdiagnoser, samt de kvinnor som behandlats på två av de sjukhus som ingår i Södra sjukvårdsregionen, i mindre utsträckning hade blivit remitterade till Onkogenetiska mottagningen i Lund.

I studie III undersökte vi därför vad som var den viktigaste orsaken till att alla kvinnor som drabbats av bröstcancer när de var 35 år eller yngre inte varit i kontakt med Onkogenetiska mottagningen i Lund via en enkät. Det svar vi fick från de flesta var att de inte hade fått någon information om genetisk vägledning och testning från sina läkare. I denna studie erbjöd vi även dessa kvinnor att få gener som är kopplade till ärftligt ökad risk för bröstcancer analyserade via ett blodprov. I den genetiska analysen ingick generna *BRCA1*, *BRCA2*, *PALB2*, *CHEK2* och *ATM*, eftersom tidigare forskning har visat att ungefär en av fem kvinnor (20 %) som drabbas av bröstcancer tidigt i livet bär på en ärftlig förändring i någon av dessa gener. En sådan förändring är dock vanligast i någon av de båda generna *BRCA1* eller *BRCA2*.

Kvinnor som har en ärftlig förändring i *BRCA1* eller *BRCA2*, och som redan har insjuknat i bröstcancer en gång under livet, har inte någon högre risk att få återfall av sjukdomen jämfört med kvinnor utan ärftliga förändringar. Däremot har kvinnor med ärftliga förändringar en betydligt högre risk att få en ny bröstcancer, antingen i samma bröst eller i det andra bröstet. På grund av detta så följs kvinnor med ärftliga förändringar upp med fler och extra noga bröstundersökningar, och de har också möjlighet att operera bort det friska bröstet i förebyggande syfte.

De kvinnor som har drabbats av bröstcancer, men som inte har några ärftliga förändringar, har även de en högre risk att drabbas av en ny bröstcancer jämfört med risken att drabbas av en första bröstcancer hos kvinnor generellt. Den risken är dock betydligt lägre än hos de kvinnor som har ärftliga förändringar. På samma sätt som beskrivet ovan, så kan den andra bröstcancern då utvecklas i samma bröst eller i det andra bröstet. Oftast utvecklas en ny bröstcancer i det andra bröstet, vilket kallas kontralateral bröstcancer.

I studie IV undersökte vi hur insjuknandet i kontralateral bröstcancer har förändrats i Sverige under mer än fem decenniers tid; mellan 1960 och 2016. Det vi kunde se var att insjuknandet steg mellan 1960-talet och 1980-talet, med den brantaste stigningen bland unga kvinnor. Efter 1980-talet sjönk insjuknandet därefter igen. Eftersom både antalet kvinnor i Sverige och vilken ålder kvinnorna hade per årtionde har förändrats mellan 1960-talet och 2010-talet, så undersökte vi även hur det åldersstandardiserade insjuknandet har ändrats över tid, och då kunde vi se att där var en konstant ökning av kontralateral bröstcancer i Sverige från 1960 och

framåt i alla åldersgrupper, förutom bland unga kvinnor, där insjuknandet sjönk igen efter 1980-talet, för att sedan hålla sig på ungefär samma nivå ända in på 2000-talet.

Det slutgiltiga målet måste vara att unga kvinnor, både med eller utan ärftliga förändringar, ska ha samma livskvalitet och hälsa som kvinnor i den generella populationen. Resultaten i denna avhandling tyder på att där finns ett behov av utökad cancertgenetisk service på regionala sjukhus och andra vårdinrättningar där kvinnor med bröstcancer behandlas. De antyder också att där finns ett behov av fler genetiskt utbildade sjuksköterskor och/eller genetiska vägledare för att säkerställa att de rekommendationer som finns angående remittering för genetisk vägledning och testning i Nationellt vårdprogram för bröstcancer efterföljs, så att alla kvinnor som drabbats av bröstcancer tidigt i livet får den vård som de har rätt till.

Acknowledgements

Rome wasn't built in a day

Av olika anledningar har denna avhandling tagit lång tid att avsluta. Under vissa dagar har den känts som en "stairway to heaven" och under andra som en "highway to hell". Under arbetets gång har jag dock fått stöd och inspiration från många olika håll. Jag vill särskilt rikta ett tack till följande personer:

Håkan Olsson, tidigare huvudhandledare och nuvarande biträdande handledare. Ett stort tack för att du gjorde det möjligt för mig att få påbörja min forskningsbana. Utan dig skulle denna avhandling inte existera. Din oändliga förmåga att komma på nya idéer och projekt har verkligen varit en stor inspiration. Jag undrar om jag någonsin kommer att kunna säga "Det där gjorde vi redan för 30 år sedan".

Hans Ehrencrona, nuvarande huvudhandledare, som hoppade på tåget när jag redan hade varit halvtidsdoktorand under fem års tid. Ett stort tack för all hjälp med både genomförandet av våra studier och revidering av manuskript. Din snabbhet med att svara på frågor och stora förmåga till problemlösning har absolut gjort mitt arbete som doktorand lite enklare.

Ulf Kristoffersson, biträdande handledare, som är den person som presenterade mig för Håkan för många år sedan, och som sedan dess har stått för ovärderlig support. Jag hoppas verkligen att vi kan fortsätta med våra lunchdejter även i fortsättningen, fastän att min tid som "din doktorand" nu är över.

Carolina Ellberg, biträdande handledare, som var ett enormt stöd under arbetet med det första manuskriptet (och ett par andra studier som tyvärr inte är avslutade ännu). Utan dig vet jag inte om jag någonsin hade blivit klar.

Helena Jernström, som, trots att du inte har varit min handledare, har givit mig massor med tips och råd under min tid som doktorand. Dock inte endast om deadlines, epidemiologi och statistiska metoder, utan även om fördelarna med Marshalls när vi var på konferens i Philadelphia.

Bo Midberg, data manager, för att du alltid har varit så hjälpsam när jag har behövt få fram data till mina studier, och **Philippe Wagner**, statistiker, för diskussioner när jag inte har varit säker på om valda statistiska metoder har varit rätt.

Åke Borg, Martin Nilsson och **Ildikó Fritz**, medförfattare. Tack för gott samarbete och konstruktiv feedback på respektive manuskript.

Susanne André, för strålande administrativ support. Du är verkligen en pärla! Tack också till alla andra kollegor, både nuvarande och före detta, i Kampradhuset och i Wigerthuset.

Anna Johnsson, doktorandkollega, som jag har delat i stort sett hela min doktorandtid med. Jag har verkligen uppskattat våra månatliga lunchträffar på BMC (eller HSC), med efterföljande arbete i våra portfolios och kappor. Tack för alla givande diskussioner och allt stöd.

Rebecca Gagnemo Persson, kollega, som har varit ett stort stöd när arbetet som adjunkt och kursansvarig har känts lite övermäktigt och jag har haft svårt att få tid över till min forskning, och **Charlotta Dykes**, ny kollega, som tog över kursansvaret med bravur när jag var doktorand på heltid under tre månaders tid. Tack också till **Anna Forsberg**, min närmsta chef, som har stöttat mig när jag har behövt det, och till resten av kollegorna på HSC.

Mina bästa vänner, **Thérèse**, för allt från samtal angående vetenskapligt arbete och statistik till alla skratt och tokigheter på våra årliga resor någonstans uti världen, och **Sara** och ”tjajorna” i **STGB**, för att ni lite då och då påminner mig om att det finns annat här i livet än Lunds universitet och akademiskt arbete. Hoppas att vi snart kan ses på riktigt igen, och inte bara via mobiltelefoner eller Zoom.

Daniel, min älskade tvillingsjäl. Tack för att du finns där för mig, både i med- och motgångar. Ett stort tack också för att jag fick lov att använda din tavla och text till omslaget på min avhandling. Du är verkligen det bästa som har hänt mig!

Mamma och **pappa**, som pratade engelska med varandra när ni inte ville att jag och lillebror Magnus skulle förstå när vi var små, och som på så sätt väckte ett aldrig sinande begär hos mig att få lära mig nya saker.

Och, till sist, morfar **Åke**, som började kalla mig för ”professorn” redan 2003, när jag precis hade börjat på mitt första arbete som adjunkt på Högskolan Kristianstad.

Nu morfar!

Nu har jag äntligen kommit en liten, liten bit på vägen!

Jag vill också rikta ett stort tack till de kvinnor som drabbades av bröstcancer vid en ung ålder och som valde att delta i Traceback-studien genom att lämna blodprov och svara på den efterföljande enkäten.

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