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## REPRODUCTIVE FACTORS AND BREAST CANCER



# Reproductive Factors and Breast Cancer

## *Parity, Breastfeeding and Genetic Predisposition in Relation to Risk and Prognosis*

Salma Butt M.D.

### Akademisk avhandling

Som med vederbörligt tillstånd av Medicinska Fakulteten vid Lunds Universitet  
för avläggande av doktorsexamen i medicinsk vetenskap kommer att offentlig  
försvaras i Kvinnoklinikens aula, Ing 74, plan 3, SUS Malmö,  
fredagen den 11/2 2011 kl 09.00

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Title and subtitle REPRODUCTIVE FACTORS AND BREAST CANCER - PARITY, BREASTFEEDING AND GENETIC PREDISPOSITION IN RELATION TO RISK AND PROGNOSIS			
Abstract  The aim of this thesis was to study reproductive factors and genetic polymorphisms in relation to breast cancer risk and survival. An important component of this was to investigate the risk of specific breast cancer subgroups. The association between parity and breast cancer-specific survival was studied among 4,453 women diagnosed with breast cancer in Malmö, Sweden. It was found that: <ul style="list-style-type: none"> <li>• Nulliparity and multiparity (<math>\geq 4</math> children) were associated with a worse survival after breast cancer than that of women with one child.</li> </ul> Parity, age at first childbirth and breastfeeding were examined in relation to the risk of specific breast cancer subgroups among 17,035 women in The Malmö Diet and Cancer Study. It was found that: <ul style="list-style-type: none"> <li>• Nulliparous women had a higher risk of more aggressive breast cancer subgroups than women with one child.</li> <li>• Women with a late first childbirth (<math>&gt;30</math> years) had a higher risk of more aggressive breast cancer subgroups than women with an early first childbirth (<math>\leq 20</math> years).</li> <li>• Long duration of breastfeeding was associated with relatively aggressive breast cancer subgroups.</li> </ul> The potential interaction between parity/age at first childbirth and single nucleotide polymorphisms (SNPs) was studied in The Malmö Diet and Cancer Study. It was found that: <ul style="list-style-type: none"> <li>• Seven out of 14 investigated SNPs showed a statistically significant association with breast cancer risk. Certain combinations of parity/age at first childbirth and SNPs might alter the susceptibility to breast cancer.</li> </ul> We conclude that parity, breastfeeding and genetic predisposition are related to breast cancer risk and/or prognosis.			
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To my beloved family

“Although nature commences with reason and ends in experience  
it is necessary for us to do the opposite, that is to commence with  
experience and from this to proceed to investigate the reason.”

*Leonardo da Vinci*

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# Contents

List of Papers .....	9
Abbreviations .....	10
1 Introduction .....	13
2 The breast .....	14
2.1 <i>Breast development</i> .....	14
2.2 <i>Sex hormones: physiology and effect on breast tissue</i> .....	14
3 Breast cancer .....	15
3.1 <i>Breast cancer pathogenesis</i> .....	15
3.1.1 Breast tumour characteristics .....	16
4 Epidemiology of breast cancer .....	16
4.1 <i>Time trends</i> .....	16
4.2 <i>Global perspective/geographical differences</i> .....	17
4.3 <i>Determinants/markers</i> .....	17
5 Factors related to risk and prognosis .....	19
5.1 <i>Genetic factors</i> .....	19
5.2 <i>Endogenous hormones</i> .....	20
5.3 <i>Exogenous hormones</i> .....	20
5.4 <i>Reproductive factors</i> .....	20
5.4.1 Parity .....	20
5.4.2 Age at first childbirth .....	21



5.4.3	Breastfeeding .....	21
5.5	<i>Other reproductive factors</i> .....	22
5.6	<i>Lifestyle factors</i> .....	22
5.7	<i>Vitamin D, parathyroid conditions and thyroid hormones</i> .....	22
6	Study aims .....	23
7	Material and methods .....	23
7.1	<i>Healthcare in Malmö</i> .....	23
7.1.1	Breast cancer management in Malmö .....	23
7.2	<i>The Malmö Breast Cancer Database</i> .....	24
7.3	<i>The Malmö Diet and Cancer Study</i> .....	24
7.4	<i>Studied factors</i> .....	25
7.4.1	Parity .....	25
7.4.2	Age at first childbirth .....	26
7.4.3	Breastfeeding .....	26
7.5	<i>Genetic polymorphisms</i> .....	26
7.6	<i>Study populations and cancer endpoints</i> .....	27
7.7	<i>Tumour characteristics</i> .....	28
7.8	<i>Follow-up and cause of death</i> .....	28
7.9	<i>Statistical methods</i> .....	28
8	Main results .....	30
8.1	<i>Paper I</i> .....	30
8.1.1	Parity and mortality distributions .....	30
8.1.2	Parity in relation to breast cancer survival .....	31
8.2	<i>Paper II</i> .....	32
8.2.1	Parity .....	32
8.2.1.1	<i>Distribution of risk factors</i> .....	32
8.2.1.2	<i>Risk of different breast cancer subgroups</i> .....	32
8.2.2	Age at first childbirth .....	32
8.2.2.1	<i>Distribution of risk factors</i> .....	32
8.2.2.2	<i>Risk of different breast cancer subgroups</i> .....	32
8.3	<i>Paper III</i> .....	33
8.3.1	Total duration of breastfeeding .....	33
8.3.1.1	<i>Distribution of risk factors</i> .....	33
8.3.1.2	<i>Risk of different breast cancer subgroups</i> .....	33
8.3.2	Average duration of breastfeeding .....	33
8.3.3	Breastfeeding duration of first child .....	34

<b>8.4 Paper IV</b> .....	34
8.4.1 Distribution of potential risk factors .....	34
8.4.2 Breast cancer risk .....	34
8.4.3 Parity and risk of breast cancer .....	34
8.4.4 Age at first childbirth and risk of breast cancer .....	35
8.4.5 Sensitivity analysis including subjects with 80% complete data on SNPs .....	35
<b>9 General discussion</b> .....	36
<b>9.1 Findings</b> .....	36
9.1.1 Parity and breast cancer survival .....	36
9.1.2 Parity and age at first childbirth in relation to risk for different breast cancer subgroups .....	37
9.1.3 Breastfeeding in relation to risk for different breast cancer subgroups .....	38
9.1.4 Genetic predisposition, parity, age at first childbirth and risk for breast cancer .....	38
<b>9.2 Methodological issues</b> .....	39
9.2.1 Ethical considerations .....	39
9.2.2 Representativity .....	40
9.2.2.1 Mammography screening and detection of breast cancer .....	40
9.2.3 Definition and validity of parity .....	40
9.2.4 Validity of age at first childbirth .....	41
9.2.5 Assessment of breastfeeding and comparability .....	41
9.2.6 Validity of SNP analyses .....	42
9.2.7 Validity of tumour endpoints and cause of death .....	42
9.2.8 Confounding .....	42
9.2.9 Chance findings and statistical power .....	43
9.2.10 Implications and future research .....	44
<b>10 Conclusions</b> .....	45
<b>Acknowledgements</b> .....	46
<b>Populärvetenskaplig sammanfattning på svenska</b> .....	49
<b>References</b> .....	52
<b>Papers</b>	
<i>Paper I</i> .....	61
<i>Paper II</i> .....	71
<i>Paper III</i> .....	91
<i>Paper IV</i> .....	125



## List of Papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

- I Parity in relation to survival following breast cancer. Butt S, Borgquist S, Garne JP, Landberg G, Tengrup I, Olsson A, Manjer J. *Eur J Surg Oncol* 2009;35:702-8.
- II Parity and age at first childbirth in relation to the risk of different breast cancer subgroups. Butt S, Borgquist S, Anagnostaki L, Landberg G, Manjer J. *Int J Cancer* 2009;125:1926-34.
- III Breastfeeding in relation to risk of different breast cancer subgroups. Butt S, Borgquist S, Anagnostaki L, Landberg G, Manjer J. (Submitted).
- IV Genetic predisposition, parity, age at first birth and risk for breast cancer. Butt S, Borgquist S, Anagnostaki L, Landberg G, Manjer J. (Submitted).

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## Abbreviations

ALNI	Axillary lymph node status	IHC	Immunohistochemical
BMI	Body mass index	IGF	Insulin-like growth factors
BRCA1	Breast cancer gene 1	LH	Luteinising hormone
BRCA2	Breast cancer gene 2	MAF	Minor allele frequency
CHRT	Combined hormone replacement therapy	MBCD	Malmö Breast Cancer Database
CI	Confidence interval	MDCS	Malmö Diet and Cancer Study
CIS	Cancer in Situ	NHG	Nottingham grade
EGF	Epidermal growth factors	OC	Oral contraceptives
ER $\alpha$	Oestrogen receptor alpha	OR	Odds ratio
ER $\beta$	Oestrogen receptor beta	PgR	Progesterone receptor
FGF	Fibroblast growth factors	PTH	Parathyroid hormone
FSH	Follicle-stimulating hormone	RR	Relative risk
GnRH	Gonadotropin-releasing hormone	SD	Standard deviation
GWAS	Genome-wide association study	SNP	Single nucleotide polymorphism
hCG	Human chronic gonadotropin	T3	Thyroid hormone 3
HER2	Human epidermal growth factor 2 receptor	TMA	Tissue microarray
hGH	Human growth hormone	TNM	Tumour, node, metastases
HRT	Hormone replacement therapy	TRH	Thyroid-releasing hormone
		TSH	Thyroid-stimulating hormone
		WHO	World Health Organisation

# 1 Introduction

Breast cancer is the most common cancer in females worldwide <sup>[1]</sup> and accounts for about 30% of all cancers in females in Sweden <sup>[2]</sup>. Every year, about 7,000 women in Sweden are diagnosed with breast cancer and one in every ten women will develop breast cancer during their lifetime <sup>[2]</sup>. Five years after diagnosis, almost 90% of the women are still alive and the ten-year survival rate is about 80%. In 2006, the total number of deaths due to breast cancer was 1,506 in Sweden. The mean age of developing breast cancer is about 60 years, and only 5% of breast cancer cases are diagnosed among women younger than 40 years of age <sup>[2]</sup>. However, international data suggest that, among women 40–45 years of age, approximately 20% of all deaths are attributable to breast cancer <sup>[3]</sup>. Fig. 1.

There are many well-established risk factors for breast cancer, most of them related to reproductive life. Important risk factors are parity, age at first childbirth and breastfeeding <sup>[3]</sup>.

There have been a large number of epi-

demiological studies on parity in relation to breast cancer risk, but very few on parity and survival, i.e., prognosis following a diagnosis of breast cancer.

Even though it is well recognised that breast cancer is a heterogeneous disease, relatively few epidemiological studies on breast cancer risk have focused on selected risk factors in relation to the risk of different breast cancer subgroups.

It is evident from a large number of studies that environmental factors, i.e., reproductive, hormonal and lifestyle factors, have an important impact on breast cancer risk <sup>[3]</sup>. There is also a well-established association between hereditary factors and breast cancer risk, i.e., a family history of breast cancer or carrier-ship of BRCA genes <sup>[4]</sup>. Recently, several genome-wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) associated with breast cancer risk <sup>[5–8]</sup>. There are, however, very few studies that have investigated breast cancer risk with regard to the potential interaction between environmental factors and SNPs.

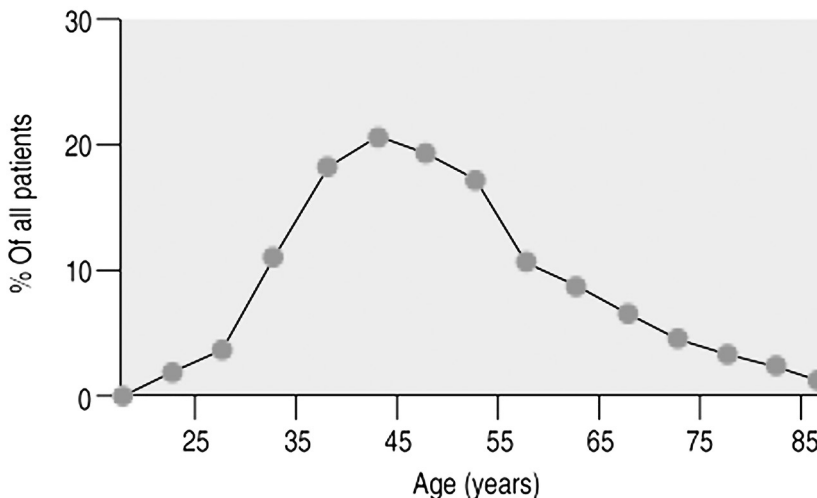


Fig. 1 Percentage of all deaths in women attributable to breast cancer, according to age.

From: *BMJ*. 2000 September 9; 321(7261): 624–628. Copyright © 2000, British Medical Journal. Printed with permission.

## 2 The breast

### 2.1 Breast development

Breast tissue is recognisable from about the sixth gestational week and develops further in the subsequent weeks to form an epidermal layer, mesenchyme and mammary ducts with lumens<sup>[9]</sup>. In newborns, the breast buds are responsive to maternal hormones. In females, the breast tissue develops further during puberty under the influence of the cyclic hormones oestrogen and progesterone. The epithelium reaches into the nipple and creates a double-layered epithelium layering the ducts. This is then successively branched into large ducts and later to terminal duct lobular units<sup>[10]</sup>. These terminal duct lobular units consist of epithelium that differentiates into intralobular collecting ducts and intralobular stroma, and in pregnant and lactating women, into secretory acini<sup>[9]</sup>. During the first pregnancy, the lobules increase both in size and in number and, by the end of the pregnancy, most of the breast is composed of lobules. After birth, the luminal cells start to produce milk. Hence, the ducts and lobules are lined by two different types of cell: milk-producing luminal cells and contractile myoepithelial cells that assist in milk ejection during lactation<sup>[10]</sup>. After cessation of lactation, the breast undergoes further remodelling. The lobules regress and the breast size is diminished, although the increases in size and number of lobules are permanent<sup>[10]</sup> and thus the breast tissue is thought to fully evolve after the first pregnancy<sup>[11]</sup>.

The breast tissue undergoes remodelling under hormonal influence and varies during the female lifespan from more dense fibrous tissue in young women to more adipose tissue in postmenopausal women<sup>[9]</sup>. Russo *et al.* have proposed a remodelling chart where puberty initiates the first remodelling from lobular 1 tissue into lobular 2 tissue. During the first pregnancy, the breast tissue is further differentiated into lobular 3 tissue. When the breast

tissue is preparing to lactate, it undergoes further remodelling and becomes lobular 4 tissue. After breastfeeding cessation, the tissue is remodelled back to lobular 2 tissue and to lobular 1 tissue in postmenopausal females<sup>[12]</sup>. Russo *et al.* have proposed that lobular 3 and 4 tissues are more resistant to carcinogenic influence; therefore, parous women would be expected to be more protected against breast cancer than nulliparous women<sup>[11]</sup>.

### 2.2 Sex hormones: physiology and effect on breast tissue

Women are known to be born with about 250 follicles in each ovary, but it is still not fully understood what initiates menarche<sup>[13]</sup>. Moreover, the interplay between hormones in the female body is complex and there are most likely several hormonal pathways that have yet to be identified. It is thought that the hypothalamus starts to secrete hormones leading to the production of pituitary human growth hormone (hGH), which is responsible for the growth spurt observed in menarche<sup>[14]</sup>. Later, hGH is suppressed by ovarian oestrogen leading to the production of gonadotropin-releasing hormone (GnRH) from the hypothalamus to the pituitary gland<sup>[14]</sup>. The pituitary gland then produces follicle-stimulating hormone (FSH) and luteinising hormone (LH), which act on the ovaries making them produce oestrogen and progesterone. The secretions of FSH and LH are regulated by negative and positive feedback from ovarian hormones<sup>[14]</sup>.

The ovarian follicle is stimulated to develop under the influence of FSH. One follicle often develops faster than the rest and produces the hormone oestrogen. The primary three oestrogens produced are oestrone, oestradiol and oestriol<sup>[14]</sup>. Oestrogen levels stop rising on the eighth day of the cycle and, on the 14<sup>th</sup> day, LH levels increase rapidly, leading to rupture of the follicle and hence ovulation occurs. LH and possibly prolactin later stimulate the

formation of the corpus luteum from which progesterone is produced with a peak at day 24. If no fertilisation has occurred by then, the corpus luteum regresses and progesterone and oestrogen levels decline at about the 28<sup>th</sup> day, which causes endometrial bleeding, namely, menstruation <sup>[14]</sup>.

If, however, fertilisation has occurred, FSH level decreases and does not rise until the first ovulation after delivery occurs <sup>[13]</sup>, which is most likely due to high levels of oestrogen and progesterone during pregnancy, which are maintained with the help of human chorionic gonadotropin (hCG) produced by the placenta <sup>[14]</sup>. During pregnancy, the maternal circulating hormones are produced by the ovaries, the placenta and the foetus <sup>[13]</sup>.

The hormone prolactin is present in all females, irrespective of hormonal status, and is increased during pregnancy. However, prolactin cannot exert any effect during pregnancy as oestrogen is bound to the prolactin receptors in the breast <sup>[14]</sup>. After delivery, the levels of oestrogen decrease rapidly and the levels of prolactin increase, which stimulates milk production in the female breast. If the baby is weaned, the levels of prolactin decrease and milk production stops. Prolactin is a hormone that is secreted primarily from the pituitary gland and regulated by thyroid-releasing hormone (TRH) and dopamine. TRH increases prolactin whilst dopamine inhibits its secretion <sup>[14]</sup>. Studies have also shown that the breast itself can produce prolactin, which shows that the interplay between these hormones is highly complex <sup>[15]</sup>.

During delivery and later during breastfeeding, another important hormone is secreted, namely, oxytocin. This hormone is also produced by the pituitary gland and acts as a neurotransmitter. Oxytocin is important for initiation of the delivery but also during breastfeeding when the hormone is responsible for letting the milk down. The production of oxytocin is stimulated by the breastfeeding of the child <sup>[14]</sup>.

## 3 Breast cancer

### 3.1 Breast cancer pathogenesis

Most breast cancers develop from the epithelium lining the terminal ducts and lobes <sup>[16]</sup> and are thought to be the result of a multifactorial and multistep process, often described as a triad of malignant growth, invasion and metastatic capability <sup>[17]</sup>. Sequential DNA mutations and a series of DNA repair and immune system errors are required for this to occur <sup>[17]</sup>. Thus, carcinogenesis is regarded as the net result of genetic and epigenetic alterations leading to genomic instability. Still, it is unclear why these errors occur; however, both inherited and acquired mutations seem to be responsible. Among acquired or environmental agents that damage DNA, chemicals, radiation and DNA viruses are potential causative factors <sup>[16]</sup>. Important contributors to carcinogenesis are regulatory genes necessary for cell division, cell differentiation, angiogenesis and invasion, among others. These genes are often classified as oncogenes or suppressor genes. In cancer, oncogenes are up-regulated and responsible for the initiation and progression of the cancer whereas suppressor genes are inactivated and unable to function as important inhibitors of cancer development <sup>[16]</sup>. In summary, Hanahan *et al.* have proposed causal features of cancer cells: the ability to react to or produce growth signals, to ignore suppressor signals, to exhibit genomic instability and anomalous programmed cell-death, the ability to divide endlessly and to initiate angiogenesis along with the ability to metastasise <sup>[18]</sup>.

Hormones and certain growth factors play a critical role in breast cancer progression <sup>[16]</sup>. As described above, breast tissue is under hormonal influence by systemic sex hormones as well as growth factors like epidermal growth factors (EGF), fibroblast growth factors (FGF) and insulin-like growth factors (IGF). Moreo-



ver, tumour cells are probably more sensitive to these signals than normal cells or might even have acquired the ability to produce stimulatory signals <sup>[17]</sup>.

### 3.1.1 Breast tumour characteristics

Breast cancer has long been defined by different prognostic and predictive measurements <sup>[17]</sup>. Staging of tumours started as early as the 1940s on the basis of the clinical size of the tumour (T), palpable axillary lymph nodes (N) and the presence or absence of distant metastases (M), named the TNM classification <sup>[17]</sup>. Today, breast cancer characterisation is expanded and includes additional features such as invasiveness, vascular invasion, histological type and grade <sup>[17]</sup>.

Histological type according to the World Health Organisation (WHO) classification comprises six different types: ductal and lobular, which are the two most frequently occurring types, and phyllodes, medullary, mucinous and tubular types <sup>[17]</sup>. Breast cancer is further graded according to the Nottingham grade (NHG), which is a scoring system for tumour aggressiveness based on mitotic count, tubular formation and degree of nuclear atypia <sup>[19]</sup>. This grade is an independent determinant of survival with almost 100% five-year survival among grade I patients and 60% survival in grade III patients <sup>[16]</sup>.

Breast cancers are also classified according to the expression of different receptors as this is of prognostic value and, more importantly, of predictive value, as targeted therapy is available for certain tumours <sup>[3]</sup>. Frequently studied receptors are oestrogen receptor  $\alpha$  (ER $\alpha$ ), oestrogen receptor  $\beta$  (ER $\beta$ ), progesterone receptor (PgR) and human epidermal growth factor 2 receptor (HER2). ERs are important in activating transcription and ER $\alpha$  is often up-regulated in tumour cells <sup>[16]</sup>, whereas the function of ER $\beta$  remains less clear <sup>[3]</sup>. The progesterone receptor is also often up-regulated in tumour cells and is regarded as an ER-regulated gene

<sup>[16]</sup>. HER2 is a growth factor receptor that is important in cell growth and differentiation. Moreover, targeted therapy is available for tumours that express HER2 <sup>[16]</sup>.

Tumour aggressiveness can further be studied by the assessment of levels of important cell cycle regulating proteins such as the oncogene cyclin D1 and suppressor genes <sup>[16]</sup>, for instance, p27 <sup>[20]</sup>. Cyclin D1 is a transcriptional factor activated by mitotic signals and amplification of this gene or high protein expression is often observed in breast cancer cells <sup>[16]</sup>. Finally, the consequence of cell cycling in terms of tumour proliferation can be estimated by the expression of the antigen Ki67 <sup>[16]</sup>. Currently, cell cycle markers are frequently studied in research settings, but are less frequently utilised in clinical settings <sup>[16]</sup>.

## 4 Epidemiology of breast cancer

### 4.1 Time trends

Breast cancer incidence has increased markedly over time <sup>[21]</sup>, and in the last two decades, an increased incidence has been observed in almost all countries <sup>[22]</sup>. In Europe, the average increase was about 25% between 1990 and 2002 <sup>[22]</sup>, and the incidence in Sweden approximately doubled between 1960 and 2008 <sup>[23]</sup>. The annual increase in Sweden during the last 20 years has been 1.2%, but slightly lower during the last 10 years: 0.8% per year <sup>[23]</sup>. The most pronounced increase in incidence has been seen in developing countries, where the incidence used to be low; this has often been attributed to improved healthcare facilities, allowing the diagnosis and identification of breast cancer cases <sup>[22]</sup>. Another group for which there has been a specifically high increase is among Western women older than 50 years of age. Moreover, it has been observed that women with a comparatively

high socioeconomic status have experienced a pronounced increase in incidence [24, 25]. It has been suggested that these latter two observations are effects of changed circumstances for diagnosis, that is, the introduction of, and relatively high participation in, mammography screening [22]. However, the introduction of mammography screening cannot fully explain the change over time concerning breast cancer incidence [21]. Other factors that may have contributed are changes in risk factors, for example, lower parity, higher age at first childbirth, changed breastfeeding patterns and increased use of exogenous hormones [3]. This is compatible with more pronounced changes in developing countries, where reproductive patterns are changing, and with the observation of a larger increase in women from high socioeconomic groups who are characterised by a late first childbirth and frequent use of hormone replacement therapy (HRT) [26, 27].

Concerning trends over time for breast cancer mortality, the pattern is more complex than that for incidence [28, 29]. Globally, an increase was mainly observed from the 1950s until about 1980. After that, mortality rates have decreased in most countries [21]. Specifically, the decrease has been most pronounced among young women, that is, aged 35–49 years [30]. In Sweden, the patterns have been slightly different and mortality rates for breast cancer, as expressed per 100,000, have been almost unchanged since the early 1960s [2]. It is difficult to fully explain the mechanisms behind the breast cancer mortality pattern as several factors may affect mortality over time; increased mortality may be an effect of an increased incidence whereas other factors may decrease mortality, for example, mammography screening and early detection together with improved adjuvant treatment [3, 21].

## 4.2 Global perspective/ geographical differences

Breast cancer incidence is high in all developed countries, apart from Japan, with the highest incidence in the USA and the Netherlands [31]. In most developing countries, the incidence is still low compared with those of Europe and the USA [31], and the lowest incidence is seen in African women [3]. Fig. 2.

In parallel to the change over time, an important likely explanation for incidence differences is the availability of mammography and healthcare facilities, which increase the ability to find breast tumours. This cannot, however, explain differences in mortality [21]. In addition, differences with regard to surgery and adjuvant treatment cannot explain mortality differences as some of the countries with the most developed healthcare systems have the highest mortality, for example, Denmark, Canada and The Netherlands [3]. Instead, mortality differences seem to be mainly determined by underlying, true, incidence differences.

In parallel to “true” incidence changes over time, incidence and mortality differences between countries may be the result of different prevalence of risk factors such as reproductive history, use of exogenous hormones [27], and possibly other lifestyle factors such as alcohol, obesity and diet [26]. Hereditary factors differ between ethnic groups and countries, but the importance of environmental factors is shown by classical studies of immigrants from Japan to Hawaii and the USA who themselves, and their daughters, soon experience an increased risk of breast cancer [3]. Fig. 3.

## 4.3 Determinants/markers

Age is the most important determinant of breast cancer incidence [3]. Incidence is low in women before 40 years of age, but then increases steeply [3]. The mean age of developing breast cancer in Sweden is about 60 years and only 5% of the breast cancer cases are diag-

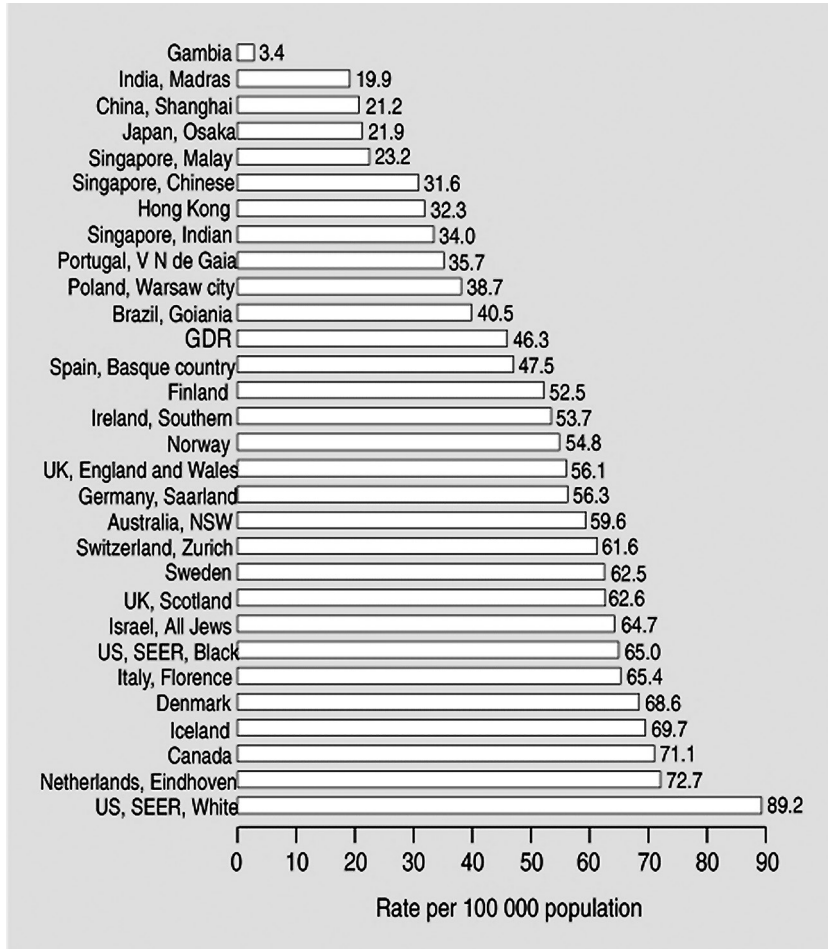


Fig. 2 Standardised mortality for breast cancer in different countries. From: BMJ. 2000 September 9; 321(7261): 624–628. Copyright © 2000, British Medical Journal. Printed with permission.

nosed among women younger than 40 years of age [2]. It has been suggested that the very young [32] and the very old [33] may have a worse prognosis. In young women, this has been explained by the tendency for these women to present with more aggressive tumours [34]. In old women, poor survival may be related to co-morbidity, which may weaken the patient and affect the probability of surviving the disease. Another possibility is that old women are “under-treated”, that is, they receive adjuvant therapy less often than would be appropriate

given their tumour characteristics [33].

High socioeconomic status and/or high educational level are positively associated with the incidence of breast cancer [3]. A potential explanation is that attendance to mammography screening is higher among women with a high socioeconomic status [35, 36]. Another explanation may be that these women have a truly increased risk due to certain risk factor patterns, for example, late first childbirth and use of exogenous hormones [37]. In contrast, high socioeconomic status and/or high edu-

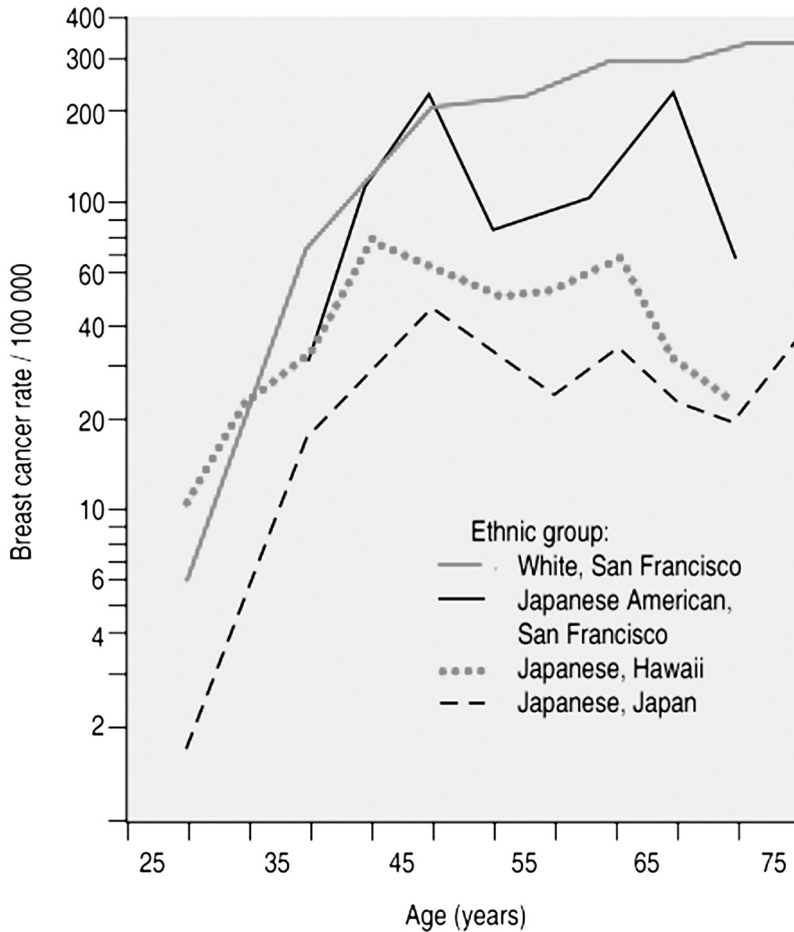


Fig. 3 Annual incidence of breast cancer in Japanese women in Japan, Hawaii, and San Francisco and in white women from San Francisco. From: *BMJ*. 2000 September 9; 321(7261): 624–628. Copyright © 2000, British Medical Journal. Printed with permission.

cational level are associated with a relatively good prognosis and low breast cancer mortality<sup>[38]</sup>. Potential explanations for this include early detection resulting in tumours with less advanced stage, more frequent screening that detects tumours, and more aggressive surgical and adjuvant therapies<sup>[39]</sup>. However, when adjusting for these factors, the relationship between socioeconomic status and survival/mortality remains, making socioeconomic status an independent marker for breast cancer mortality<sup>[38, 39]</sup>.

## 5 Factors related to risk and prognosis

### 5.1 Genetic factors

It has long been evident that family history of breast cancer is an important risk factor<sup>[3]</sup> and about 10% of all breast cancers are thought to be caused by genetic factors<sup>[4]</sup>. In the 1980s and 1990s, breast cancer genes 1 and 2 (BRCA1 and BRCA2) were identified<sup>[40]</sup>.

They are both tumour suppressor genes and have been shown to be involved in breast and ovarian cancer initiation [4]. Women carrying either of these genes are at 10 to 30 times higher risk of developing breast cancer than the general population, and these genes have also been associated with relatively aggressive breast cancer subgroups [40]. BRCA1 and BRCA2 mutations are found in 30–40% of patients with hereditary breast cancer, and they account for about 3–4% of all breast cancer cases [41]. Other high-risk genes have been identified in certain families and moderate-risk alleles have also been found [40]. However, there is still a large group of breast cancer patients, with a family history of the disease, for whom no specific gene can be identified.

The human genome is inherited from parents to children and is comprised of 3 billion base pairs composed of four bases: adenine (A), tyrosine (T), cytosine (C) and guanine (G). The Human Genome Project was initiated with the aim of improving the detection, treatment and prevention of diseases like cancer. In 2003, the work was completed and it was concluded that 99.9% of the genome is the same in all humans [42]. The 0.1% variation in the genome is due to changes in the base pairs. Much of this variation is due to single nucleotide polymorphisms (SNPs), and recently, genome-wide association studies (GWAS) have identified several SNPs associated with breast cancer [5–8]. Certain combinations of these polymorphisms and reproductive factors might affect susceptibility to breast cancer.

## 5.2 Endogenous hormones

Normal breast development and tumour initiation, promotion and progression in breast cancer are strongly dependent on hormonal stimulation by sex steroid hormones and there is an established association between risk of breast cancer and endogenous levels of both female sex hormones (e.g. oestradiol and oestrone) and male sex hormones (e.g. testoster-

one and androstenedione) [43, 44].

Prolactin is also important for the function of the breast (see above). The results of studies on the potential association between prolactin and breast cancer risk have, however, been less clear than those of studies concerning sex steroid hormones [44, 45].

## 5.3 Exogenous hormones

Exogenous hormones, oral contraceptives (OC) and hormone replacement therapy (HRT) have also been associated with breast cancer [3]. Current users of OC have a slightly increased risk [3, 46], but the association between HRT and breast cancer risk is more evident and a high risk has been seen for women using HRT for 15 years or longer [47]. The risk associated with HRT seems to be mainly limited to combined hormonal therapy, that is, medication including both oestrogens and progesterone [48]. Concerning HRT, there also seems to be an association mainly with tumours that exhibit more favourable characteristics [47, 48].

## 5.4 Reproductive factors

### 5.4.1 Parity

An association between parity and breast cancer was already proposed in the 18<sup>th</sup> century when it was observed that breast cancer among nuns was more common than that among other women [49]. Later epidemiological studies have consistently shown parity to be inversely associated with breast cancer risk [50–58]. There are many aspects related to parity, such as age at first birth, time since last birth and breastfeeding [27], but it has been shown that parity is an independent determinant of breast cancer risk and that each additional childbirth confers an approximately 7% risk reduction for breast cancer [59]. Whether this effect is consistent throughout life irrespective of hormonal status, or is conferred to pre- or postmenopausal women, is still controversial [53, 60].

It has been shown that the first pregnancy induces a genomic signature in the human breast that makes it less susceptible to tumour-initiating factors [61]. It has been shown in many studies that parity keeps conferring risk reductions for each additional child, and at least two studies that examined “grand multiparity”, namely, five or more children, found a risk reduction for every additional childbirth [52, 62]. Furthermore, it has been proposed that during every pregnancy there is a shift of more stem cells to a stage where they become resistant, or less sensitive, to carcinogenic stimuli [11].

A dual effect of parity has been observed with a short-term increased risk after childbirth but a long-term decreased risk [63–67]. It has also been shown that breast cancer diagnosed during or shortly after pregnancy is associated with poor prognosis [68, 69] and higher mortality [70–73]. Potentially, tumours in these women are a result of the changed hormonal milieu causing tumour growth in already malignant cells [74]. Studies on breast cancer survival in relation to parity are, however, rare and their results have been contradictory. A few studies could not find any prognostic effect of parity [69, 71, 75, 76], while some found parity to be associated with better prognosis, but only in older women [77].

There are few studies in relation to different breast cancer subgroups, but nulliparity has been associated with the risk of more unfavourable breast tumour characteristics like grade III tumours [63] and tumours with high expression of Ki67 [78]. Hormone receptor status in relation to parity has been reviewed by Ma *et al.* who found a risk reduction for ER-positive but not for ER-negative tumours [79]. No association between WHO tumour types and parity has been described [80].

#### 5.4.2 Age at first childbirth

Age at first childbirth has been shown to be an independent determinant of breast cancer

risk [59]. Hinkula *et al.* found a doubled risk for women postponing their first childbirth from 20 years to 30 years of age [51]. Other studies have, however, indicated differences in risk among women stratified in terms of menopausal status. In premenopausal women, every postponed year for the first birth gave a 5% risk increase, while in postmenopausal women, the risk increase was 3% [81]. Moreover, age at first birth may modify the risk reduction achieved from parity. In one study, high parity (five or more children) was associated with a breast cancer risk of 0.46 compared with that of uniparous woman. This association was stronger among women giving birth for the first time before the age of 20, and less apparent in women giving birth for the first time after the age of 30 [62].

One study by Kroman *et al.* showed poor survival among women with an early first childbirth [75]; however, a more recent study by the same research group reported that an early first childbirth was associated with better survival [69]. This later study also showed that time since last birth was of importance, and that women with a recent pregnancy had poorer survival [69].

In studies of the risk of specific breast tumour subgroups among women with a late first childbirth, one study showed an increased risk of ductal carcinoma and a low risk for lobular carcinoma [82]. A more recent review by Althuis *et al.* found an association between late first childbirth and an increased risk of ER-positive but not ER-negative tumours [83].

#### 5.4.3 Breastfeeding

It has long been discussed whether or not breastfeeding confers any protective effect on the development of breast cancer. The Collaborative Group on Hormonal Factors in Breast Cancer analysed 47 epidemiological studies performed in 30 different countries in order to assess whether breastfeeding had any association with breast cancer risk [59]. They

found that the risk of breast cancer decreased by 4.3% for each additional 12 months of breastfeeding. Furthermore, a short total duration of breastfeeding in Western countries may contribute to the increasing incidence of breast cancer over time [59]. A subsequent review by Yang *et al.*, including the Collaborative Group's study, could however not find any obvious protective effect of breastfeeding [84].

At least one study has examined the relationship between breastfeeding and breast cancer survival, but no clear association was found [71]. Another two studies have examined the association between breastfeeding and the risk of certain breast cancer subgroups [80, 85]. One found that long total duration of breastfeeding reduced the risk for ductal tumour types and both ER/PR-positive and ER/PR-negative tumours [80], while the other study found breastfeeding to be associated with a decreased risk for triple-negative tumours, that is, ER/PR/HER2-negative tumours [85].

## 5.5 Other reproductive factors

Both early menarche and late menopause have been associated with an increased risk of breast cancer [3]. It has been shown that, for each year that menarche is postponed, there is a 9% lower breast cancer risk in premenopausal women and a 4% lower risk in postmenopausal women [59]. For every year that menopause is postponed, a 3% risk increase has been reported [59].

## 5.6 Lifestyle factors

Lifestyle factors have been associated with both breast cancer risk and breast cancer mortality [3]. One review found that a healthy lifestyle, defined as high metabolic equivalent task hours, was associated with lower breast cancer mortality [86], while excess body weight or high BMI has been associated with higher breast cancer risk in postmenopausal women [87] and

high mortality [86]. A high level of physical activity has been attributed a small protective effect in several studies [88], and high alcohol consumption has been associated with a modest, but increased, risk of breast cancer [89]. There have been a large number of studies investigating the potential association between dietary factors and breast cancer risk, and between smoking and breast cancer risk, but no strong associations have been established [3, 89].

## 5.7 Vitamin D, parathyroid conditions and thyroid hormones

Given the role of sunlight exposure in the synthesis of vitamin D, ecological studies suggest that vitamin D protects against breast cancer [90]. However, studies on the relationship between dietary intake of vitamin D, or dairy products, and risk of breast cancer have not provided consistent evidence for an association [91]. There have been several prospective studies on vitamin D levels in the blood and breast cancer risk, and they generally show a weak (not statistically significant) association between high vitamin D levels and a decreased risk of breast cancer [92]. A factor strongly related to vitamin D is parathyroid hormone (PTH). Several studies have linked hyperparathyroidism with an increased subsequent risk of breast cancer, but the only prospective study to date on PTH levels and subsequent breast cancer risk found no association [92–94].

Patients with thyroid conditions have sometimes been reported to have an increased occurrence of breast cancer compared with that of healthy women, and a large number of studies have compared levels of thyroid hormones and thyroid-stimulating hormone (TSH) between breast cancer cases and controls. However, taken together, these studies have not been conclusive regarding the potential association between thyroid conditions and breast cancer risk [95–97]. With few exceptions, previous studies of thyroid hor-

monal levels have been cross-sectional, but a recent prospective study from Malmö, Sweden, showed a strong dose-response relationship between thyroid hormonal levels (T3) and subsequent risk of breast cancer [98].

## 6 Study aims

The main objective of this thesis is to study reproductive factors in relation to breast cancer. Specifically, this thesis will investigate:

1. If there is an association between parity and survival following a diagnosis of breast cancer.
2. If parity and age at first childbirth are associated with the incidence of specific breast cancer subgroups.
3. If breastfeeding is associated with the incidence of specific breast cancer subgroups.
4. If there is an interaction, with regard to breast cancer risk, between parity/age at first childbirth and a number of recently identified genetic polymorphisms (SNPs).

## 7 Material and methods

### 7.1 Healthcare in Malmö

Malmö is the third largest city in Sweden and has 293,909 inhabitants (January 2010) [99]. In Malmö, there is only one hospital: The University Hospital of Malmö. It opened in 1896, almost a century after Lund University Hospital, and for a long period these two hospitals worked side by side. In 2010, the hospitals in Malmö and Lund were fused and renamed Skåne University Hospital, at which 1.7 million people in Southern Sweden are now able to obtain healthcare [100].

Mammography screening was introduced in Malmö in 1977 as part of a randomised trial to which 50% of all women aged 45 to

69 years of age were invited [35]. Following the results of this screening study, a general service of screening was introduced in 1991 [35].

### 7.1.1 Breast cancer management in Malmö

Breast cancer patients living in Malmö have been treated at Malmö University Hospital and there have been no referrals to or from the hospital regarding breast cancer patients. Since 1977, each patient with breast cancer has been reviewed at a weekly multidisciplinary conference [101].

Triple assessment for palpable tumours was introduced to the scientific world in 1975 [17] and was introduced in clinical settings in Malmö in 1977. The triple assessment is based on three different examinations of the “lump”: physical examination, imaging (mammography and sometimes ultrasound) and fine-needle aspiration [17]. After these three investigations, a multidisciplinary conference takes place. The radiologist, cytologist, surgeon, pathologist and oncologist are all present at these conferences. Following a malignant diagnosis, the patient is usually called back to the Department of Surgery for the results and further investigation, planning and information.

After surgery, a new multidisciplinary conference takes place. This conference is held about two weeks after surgery, when the pathologists have evaluated the tumour and different markers have been investigated. The pathologist report will be the basis for decisions on future treatment and follow-up. The patient is called back to the surgeon for information and later to the oncologist for potential adjuvant treatment.

Breast cancer patients are followed by the surgeon or, if referred to the Department of Oncology, by the oncologist. If there is no recurrence of cancer in five years, the woman is considered to be cured of her breast cancer.

In 2007, a total of 710 women visited the breast cancer facility in Malmö and 538



women with breast tumours were operated on, among whom 88 had benign tumours<sup>[102]</sup>.

## 7.2 The Malmö Breast Cancer Database

The Malmö Breast Cancer Database (MBCD) includes all female invasive breast cancer cases diagnosed in Malmö between 1961 and 1991. Identification of breast cancer cases was performed using The Swedish Cancer Registry, The Southern Cancer Registry (the regional branch of the national registry) and local hospital records. In all, 4,453 women with incident invasive breast cancer were diagnosed and their records form the MBCD. Information was collected by one surgeon, Jens-Peter Garne<sup>[101]</sup>. In order to validate the correctness of the breast cancer diagnosis, medical records, histopathological samples and X-ray examination results were all compared. Information on date of birth, date of diagnosis, age at diagnosis, parity, menopausal status, tumour size, type of surgery, axillary lymph node involvement (ALNI) and distant metastases was also retrieved<sup>[101]</sup>. The histological classification was based on a modification of the WHO classification as proposed by Linell *et al.*<sup>[103]</sup>.

## 7.3 The Malmö Diet and Cancer Study

The Malmö Diet and Cancer Study (MDCS), a prospective cohort, was initiated in 1991 in order to study the association between dietary factors and cancer incidence. Residents in Malmö born between 1923 and 1950 were asked to participate. Out of 74,138 residents, a total of 68,905 eligible individuals were invited. Recruitment was performed by public advertisement and personal invitations<sup>[104]</sup>. Baseline examinations were performed between 1991 and 1996. All participants visited the MDCS centre twice. The first time, baseline examinations took place and instructions were given on how to fill in the questionnaire. The second visit

included the collection of questionnaires and a personal interview regarding dietary habits. At the end of the baseline period, almost 41% of the invited participants, namely, 28,098 individuals, had completed all study parts and, among these, 17,035 were women<sup>[105]</sup>.

The questionnaire included questions on socioeconomic status, reproductive history, lifestyle and medications. Body weight and height were measured by a trained nurse and body mass index (BMI) was calculated as kg/m<sup>2</sup>. Moreover, blood samples were collected from all participating women at baseline and samples have been stored in a biological bank at -80 degrees Celsius.

Information on tumour laterality, size and lymph node metastasis was retrieved from medical records and histopathological reports by one registered nurse<sup>[106]</sup>.

## 7.4 Studied factors

### 7.4.1 Parity

In study I, parity was defined as follows: nullipara, one child, two children, three children and four or more children. Information on parity was based on medical records. There was no information on whether these children were born at full term or if they were twins<sup>[101]</sup>. In studies II, III and IV, parity was assessed from the question: "How many children have you given birth to?" and was defined as: nullipara, one child, two children and three or more children. This different categorisation was made since the patterns of childbirth have changed during recent decades and there were very few women who had given birth to four or more children. In MBCD, there was no reliable information on abortions or miscarriages. In MDCS, a question covered this issue. However, most of the women did not respond to this, meaning that this variable is not reliable. Therefore, information on abortions, spontaneous or induced, could not be included in any of the studies.

### 7.4.2 Age at first childbirth

In studies II, III and IV, age at first childbirth was included either as the main exposure variable or as a potential confounder. Information on this variable was retrieved from the questionnaire in which “year of birth” had been filled in for the first seven children. Subsequently, age at first childbirth was calculated by subtracting the year of birth for the first child from the year of birth for the mother. Age at first childbirth was categorised as follows:  $\leq 20$ ,  $>20 - \leq 25$ ,  $>25 - \leq 30$  and  $>30$  years.

### 7.4.3 Breastfeeding

Duration of breastfeeding was assessed with the help of the baseline questionnaire, and the database included this information for the first seven children.

Mean duration of breastfeeding per child was calculated as the sum of the months of breastfeeding divided by the number of children for which information on breastfeeding was provided.

Total time of breastfeeding was calculated as the mean time of breastfeeding multiplied by parity (an open-ended question that could have any value, see above). This calculation was made since time of breastfeeding had only been given for the first seven children and a small number of women had more than seven children.

Breastfeeding of the first child was investigated in an additional analysis, as it is likely that changes during the first pregnancy and following lactation may be particularly important with regard to the differentiation of the breast tissue. Women that had never breastfed were included in the lowest quartile in all measurements of breastfeeding.

## 7.5 Genetic polymorphisms

SNPs were selected from previous reports on GWAS and candidate gene studies as fol-

lows: eleven from Easton *et al.*: (rs2981582 (FGFR2), rs3803662 (TNRC9), rs12443621 (TNRC9), rs98051542 (TNRC), rs889312 (MAP3K1), rs3817198 (LSP1), rs2107425 (H19), rs13281615 (8q24), rs981782 (5p12), rs30099 (5q), rs4666451 (2p))<sup>[5–8]</sup>; one from Cox *et al.*: (rs1045485 (CASP8))<sup>[5–8]</sup>; one from Stacey *et al.*: (rs13387042 (2q35))<sup>[5–8]</sup>; and one from Harlid *et al.*: (rs7766585 (ESR1))<sup>[107]</sup>.

The SNP analyses were performed using a MALDI-TOF mass spectrometer (SEQUENOM MassArray) using iPLEX reagents and protocol (SEQUENOM) and 10 ng of DNA as the PCR template. Primer sets were from Metabion (Martinsried, Germany). The laboratory methods have previously been described in detail<sup>[107]</sup>. The genotypes for the SNPs were defined as follows: homozygous for the major allele (AA), heterozygous (Aa) and homozygous for the minor allele (aa). In cases with a minor allele frequency (MAF) near 0.5, the same classification as that used in previous studies was used.

## 7.6 Study populations and cancer endpoints

In study I, the MBCD was used. A total of 4,453 women were diagnosed with invasive breast cancer between 1961 and 1991<sup>[108]</sup>. Ten were excluded because of missing information on all variables, and 109 were excluded due to a previous diagnosis of breast cancer, either according to medical records or according to a second record-linkage performed to The Swedish Cancer Registry in 2006. Women registered in the Swedish Cancer Registry with a breast cancer diagnosis more than 90 days prior to the date of diagnosis in the MBCD were considered to have had a previous diagnosis of breast cancer. In addition, 104 cases were excluded due to bilateral cancer (in all 105) as it was difficult to retrospectively interpret information on the tumour characteristics for these cases. A total of 111 women were diagnosed

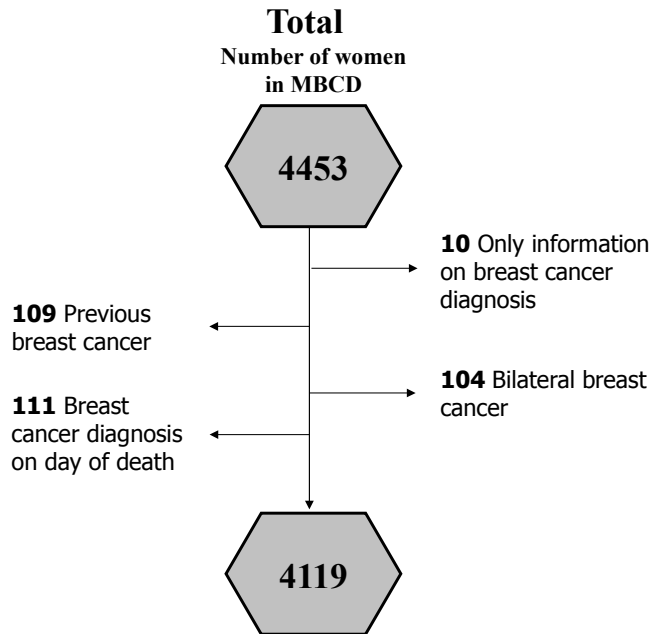


Fig. 4 The Malmö Breast Cancer Database (MBCD). Incident breast cancer cases in boxes.

with breast cancer on the day they died, or even a few days later due to a delay in registration at the tumour registry; they were excluded, as the aim of this study was to investigate survival following diagnosis. Hence, the study population consisted of 4,119 women. Fig 4.

In studies II and III, the MDCS was used. All 17,035 women in the MDCS were followed until 31 December, 2004. Tumour endpoints were retrieved by record linkage with The Swedish Cancer Registry (until 31 December, 2003), and due to a delay in central registration, also with linkage to its regional branch, The Southern Swedish Regional Tumour Registry (for the year 2004). A total of 576 women out of 17,035 had already been diagnosed with breast cancer at baseline and they were excluded from the analyses as prevalent breast cancer cases. Seventy-two out of the 622 incident breast cancer cases involved cancer in situ (CIS) and did not provide any

invasive endpoints in the analysis for all breast cancers. CIS cases did however contribute data on person-years up until the event. Data for a total of 10 women with bilateral breast cancer were not included as endpoints owing to difficulties in determining the relevant side to be used in the analyses of tumour size, axillary lymph nodes and histopathology. A further 39 did not have sufficient tissue for further analyses. Bilateral cases and cases with no tumour material did however provide data on person-years up until the event. Therefore, in study II, the study population consisted of 16,459 women. In study III, a total of 2,089 women (65 cases) were nulliparous and, for 278 (12 cases), no information on parity was available; these were excluded from all analyses. In all, the study population consisted of 14,092 parous women, for whom adequate information was available on breastfeeding and parity, including 424 tumours that were invasive uni-

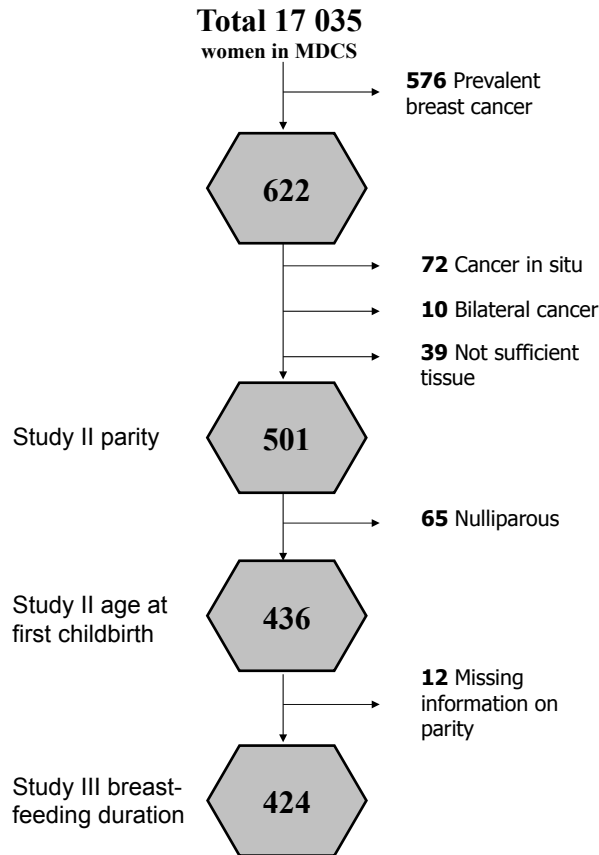


Fig. 5 The Malmö Diet and Cancer Study (MDCS). Incident breast cancer cases included in different analyses, in boxes.

laterally, with sufficient tissue for further histopathological analysis. Fig 5.

The study IV was also based on the MDCS, but the exclusion criteria were different. Women with any prevalent cancer (not including cancer in situ of the uterine cervix) prior to breast cancer diagnosis were excluded. A total of 545 cases with breast cancer diagnosis were identified in a first set with follow-up until 31 December, 2004. One case for which DNA was not available was excluded. The remaining 544 cases were matched to two controls each, a total of 1,088. The matching criteria were age ( $\pm 90$  days) and time of sampling

at baseline ( $\pm 30$  days). A new linkage was performed with follow-up until 31 December, 2007, where an additional 186 cases and 372 controls were identified. A total of 11 controls from the first set were diagnosed with breast cancer during the second follow-up period. They were removed as controls and replaced by other controls matched on the same criteria. For 14 women, there was no DNA available; hence, they were excluded from all analyses (2 cases and 12 controls). The study population consisted of a total of 2,176 women, out of which 728 were cases and 1,448 were controls.

## 7.7 Tumour characteristics

In the MBCD, the histopathological classification was based on the system of Linell *et al.*<sup>[103]</sup>. Invasive ductal carcinomas were divided into comedo- and tubuloductal carcinomas. Furthermore, tubuloductal tumours were subdivided according to the content of tubular structures. All tumours diagnosed between 1961 and 1970 were re-evaluated by one pathologist in order to classify them according to the system of Linell *et al.* From 1981 to 1991, all breast cancers were classified according to this system at diagnosis<sup>[101]</sup>, but for cases diagnosed between 1971 and 1980, there was no information on histological subtype. However, these tumours were reviewed if the status of invasive/in situ was uncertain. The histological type for these tumours was given as “invasive, type not assessed”<sup>[108]</sup>.

In the MDCS, one senior breast pathologist re-evaluated all invasive tumours (Lola Anagnostaki) and tumour type was described according to the WHO classification<sup>[17]</sup>. The tumours were graded according to Elston and Ellis, including tubular formation, nuclear atypia and mitotic index<sup>[19]</sup>. For the construction of tissue microarray (TMA), two cores of 0.6 mm from each tumour were taken and arranged in a recipient block as previously described by Borgquist *et al.*<sup>[48, 106]</sup>. Immunohistochemical (IHC) analyses were performed using specific antibodies, as described previously by Borgquist *et al.*<sup>[48]</sup>, and tumours were evaluated according to the expressions of ER $\alpha$ , ER $\beta$ , PgR, Ki67, cyclin D1 and p27. Tumours were dichotomised with the following groups: 0–10% and 11–100% positive nuclei. HER2 was analysed using IHC as previously described<sup>[48]</sup>. HER2 was classified according to Swedish clinical practice, namely, a breast tumour is considered to be HER2 positive when it scores more than 3+ on IHC staining<sup>[109]</sup>. Tumours that were negative for ER $\alpha$ , HER2 and PR were classified as triple-negative tumours. All arrays were evalu-

ated independently twice by the same person (Signe Borgquist) and, in case of discrepancy, a third evaluation was performed by the same investigator.

Information on tumour laterality, size and lymph node metastasis was retrieved from medical records and histopathological reports by one registered nurse.

## 7.8 Follow-up and cause of death

In study I, all women were followed until 31 December, 2003, using the Swedish Cause-of-Death Registry. They were subsequently divided into the following four groups: (1) women with breast cancer-specific death, that is, breast cancer denoted as the underlying cause of death (1,415), (2) women with breast cancer as a subordinate factor, that is, multiple causes of death (305), (3) women with death unrelated to breast cancer (1,475) and (4) women still alive at the end of follow-up (924). The main outcome in study I was breast cancer as an underlying cause of death, that is, breast cancer-specific death.

In studies II and III, all women were followed until 31 December, 2004. Vital status was obtained from The Swedish Cause-of-Death Registry until 31 December, 2004. In study IV, vital status was obtained from The Swedish Cause-of-Death Registry until 31 December, 2007.

## 7.9 Statistical methods

In study I, different categories of parity were compared regarding the distribution of age, menopausal status and tumour characteristics. Groups defined by vital status and cause of death were also compared with respect to these factors. All women were followed from the date of diagnosis until death or end of follow-up, 31 December, 2003. Breast cancer mortality rate was calculated per 100,000 person-years in different categories of parity. Cor-

responding relative risks (RR) of breast cancer-specific death were analysed, using Cox's proportional hazards analysis with 95% confidence intervals (CI). The time scale for the Cox analysis was time-in-study, that is, date of diagnosis until death or end of follow-up. The proportional hazard was classified with a log-minus-log curve and met the assumption of proportionality. Women with one child were used as a reference in all Cox analyses. All analyses were subsequently adjusted for potential prognostic factors: age at diagnosis, menopausal status, time of diagnosis, size of tumour, histological type, lymph node status and distant metastases. Moreover, all analyses were repeated with all breast cancer deaths (underlying cause of death + multiple causes of death) and with all-cause mortality as the outcome. All analyses were repeatedly stratified for age, menopausal status (pre-/post-menopausal) and different diagnostic periods (1961–1970, 1971–1980 and 1981–1991). Missing information on parity may be related to advanced tumours, and all analyses were repeated excluding women with distant metastases as part of a sensibility analysis.

In study II, different categories of parity and age at first childbirth were compared regarding the distribution of established and potential risk factors for breast cancer. These factors were also compared between breast cancer cases (CIS, invasive with tissue and invasive with no tissue or bilateral) and non-cases. Each subject was followed until the event of breast cancer, death or end of follow-up, 31 December, 2004. The incidence of breast cancer was calculated per 100,000 person-years in different parity classes and in different groups of age at first childbirth. Corresponding RRs of breast cancer risk were analysed using Cox's proportional hazards analysis yielding RR with 95% CI. Women with one child were the reference group in the parity analyses and women with their first childbirth before the age of 20 were the reference group in the age at first childbirth analyses. A log-minus-log curve was

plotted for overall breast cancer risk in relation to parity classes and age at first birth. Both analyses meet the assumption of proportional hazards. All analyses were subsequently adjusted for potential confounders.

The confounders were chosen on the basis of already established and potential risk factors for breast cancer: age at baseline, education, socioeconomic status, marital status, age at menarche, age at first birth, parity, oophorectomy, age at menopause, oral contraceptive use, hormone replacement therapy use, BMI, alcohol consumption, smoking and height. In the analyses, there were several endpoints and adjustment for the same confounders in all analyses made it possible to obtain comparable risk estimates. The confounders were tested one by one in relation to overall breast cancer risk in order to see which confounder resulted in the largest change of risk estimates.

The trend over parity categories was examined from nulliparous women to those with three or more children. When analysing the trend over age at first childbirth, age groups as defined above were used. The "missing data" category was not included in trend analyses. In all trend analyses, a *p*-value less than 0.05 was considered statistically significant.

To examine heterogeneity, comparing risks for different tumour subgroups in relation to specific exposure categories, for example, the risk associated with lobular-type tumour in women with three or more children was compared to the risk of ductal-type tumour in women with three or more children. A case-case analysis using unconditional logistic regression analysis was applied, and *p*-values <0.05 were considered statistically significant.

In study III, the main analyses used total duration of breastfeeding as the main exposure variable. Additional analyses used mean duration of breastfeeding and breastfeeding duration of the first child. Breastfeeding duration was divided into quartiles. Quartile cut-offs for breastfeeding were based on the distribu-

tion for all women in the study cohort. Different quartiles of breastfeeding were compared regarding the distribution of established and potential risk factors for breast cancer. Each subject was followed until the event of breast cancer, death or end of follow-up, 31 December, 2004. The incidence of breast cancer was calculated per 100,000 person-years in different breastfeeding quartiles. Corresponding RRs of breast cancer were analysed using Cox's proportional hazards analysis yielding RR with 95% CI. These analyses were subsequently adjusted for the same potential confounders as in study II.

The trend over breastfeeding categories was examined from the lowest to the highest quartile, excluding the "missing data" category, and *p*-values less than 0.05 were considered statistically significant.

To examine heterogeneity, to test whether effect estimates were similar between for example grade I and grade III tumours in a certain breastfeeding quartile, adjusted case-case models using unconditional logistic regression analysis were used and *p*-values <0.05 were considered statistically significant.

In study IV, cases and controls were compared with regard to established and potential risk factors for breast cancer in order to identify possible confounders. All analyses were subsequently adjusted for matching criteria, age and year of inclusion in study, and for potential confounders. A confounder was defined as a factor with a distribution difference exceeding 5% units between cases and controls. Type of occupation and use of HRT met the criteria of confounders and were hence included in the adjusted analyses.

An unconditional binary logistic regression model was fitted to analyse the association between SNPs and breast cancer. Odds ratios (OR) with 95% CI were calculated using the major allele homozygotes (AA) as a reference group in all analyses. In addition, per allele analysis was performed using a continuous variable with the following values: 0

(AA), 1 (Aa) and 2 (aa). The reported OR for this latter analysis denotes the risk difference when increasing the number of risk alleles by one. Furthermore, analyses were stratified for parity and age at first childbirth in order to study the relationship between selected SNPs and breast cancer risk, reported as OR, in different categories of these reproductive factors. In addition, the material was stratified for single alleles, and the breast cancer risk associated with parity and increasing age at first birth was calculated. These associations were reported using the *p*-values for continuous analysis.

In order to assess any potential interactions between selected SNPs and parity, and between selected SNPs and age at first birth, an interaction term was introduced in the logistic regression model. A *p*-value less than 0.05 was considered statistically significant. In a second step, the *p*-value was corrected for multiple comparisons using the Bonferroni correction, that is, by dividing by the number of comparisons. In the present study, performing 20 interaction analyses, the corrected level for statistical significance was a *p*-value less than 0.0025.

As part of the sensitivity analysis, all analyses were repeated excluding women for whom information on less than 80% of SNPs was available, as this may indicate poor DNA quality. In these analyses, 660 cases and 1,310 controls were included.

## 8 Main results

### 8.1 Paper I

#### 8.1.1 Parity and mortality distributions

Nulliparous women and women with four or more children were relatively old at diagnosis (mean: 65 years, SD: 14) and, subsequently, more often postmenopausal at diagnosis than women with one, two or three children (mean:

62, 60 and 62 years, respectively). Nulliparous women and women with four or more children had a slightly higher proportion of distant metastases at diagnosis than other women. Other tumour characteristics were similar in different parity categories. Women with breast cancer as the underlying cause of death were younger at diagnosis (mean: 62 years, SD: 14) than women who died from other causes (breast cancer as one of multiple causes of death, mean: 72 years, SD: 13; and death unrelated to breast cancer, mean: 69 years, SD: 12). Women still alive at the end of follow-up were the youngest at diagnosis (mean: 54 years, SD: 10). Larger tumours (>20 mm) were more common among women who died from breast cancer (underlying cause of death: 42%; and breast cancer as one of multiple causes of death: 37%) than among women still alive at the end of follow-up (20%). ALNI-positive tumours were more frequent among women who died from breast cancer (underlying cause of death: 56%; and multiple causes of death: 36%) than among women still alive (26%). Distant metastases were more often found in women with breast cancer as the underlying cause of death (16%) than in all other categories (breast cancer as one of multiple causes of death: 7%, death unrelated to breast cancer: 1%, and women still alive: 0%).

### 8.1.2 Parity in relation to breast cancer survival

High parity (four or more children) was associated with a high breast cancer-specific mortality compared with that of women with one child (RR: 1.49; 95% CI: 1.20–1.85). This association was slightly lower when adjusted for potential confounders (1.33; 1.07–1.66). Nulliparous women also had a higher mortality than women with one child (1.27; 1.09–1.47), but this association did not reach statistical significance in the adjusted analyses (1.12; 0.96–1.30). Using all breast cancer death as the outcome, the results were similar

in analyses for nulliparous women (adjusted RR = 1.10; 0.96–1.27) and for women with high parity (1.28; 1.05–1.57). Using all-cause mortality, the adjusted risk ratio for nulliparity was 1.03 (0.94–1.14) and that for high parity was 1.02 (0.88–1.19).

In stratified analyses on age at diagnosis, high parity was statistically significantly associated with high breast cancer-specific mortality among women older than 45 years of age (1.34; 1.07–1.68), but the association was not statistically significant in younger women (1.47; 0.56–3.85). Nulliparity was associated with a higher, although not statistically significant, breast cancer-specific mortality in women younger than 45 years of age (1.28; 0.79–2.09) but not in older women (1.10; 0.93–1.29).

In analyses stratified for menopausal status, the results among premenopausal women were similar to those in women younger than 45 years of age, except for women with three children. In this group, there was no association with breast cancer mortality (0.91; 0.64–1.31) among premenopausal women. Results for postmenopausal women were similar to those found among women older than 45 years of age.

There was a positive association between high parity and mortality from breast cancer in women diagnosed in all diagnostic periods, although confidence intervals were wide and not statistically significant. Nulliparity was not significantly associated with mortality in any of the diagnostic periods. When women with distant metastases were excluded, the adjusted risk estimate related to high parity in premenopausal women was similar, but reached statistical significance (1.41; 1.02–1.96). The RR associated with missing information on parity among postmenopausal women was not similar, and the statistical significance changed (0.98; 0.73–1.33). Moreover, the adjusted RR associated with high parity for the period 1981–91 changed from 1.47 (0.97–2.23) to 1.70 (1.07–2.67). All other results were similar.



## 8.2 Paper II

### 8.2.1 Parity

#### 8.2.1.1 Distribution of risk factors

Nulliparous women were more educated, more often non-manual workers and had to a larger extent not been exposed to oral contraceptives compared with all the other parity categories. Nulliparous women were also more likely not to be married or cohabiting compared with all other groups except for women with missing information on parity. Women with three or more children were younger at first childbirth than all other groups except for women with missing information on parity. All other factors were evenly distributed between different parity categories.

#### 8.2.1.2 Risk of different breast cancer subgroups

Nulliparity was associated with a high risk of breast cancer compared with that of uniparous women, but this association did not reach statistical significance (adjusted RR: 1.39; 0.92–2.08). Nulliparity was however statistically significantly associated with high risks of CIS (3.15; 1.00–9.94), grade III tumours (2.93; 1.29–6.64) and HER2-positive tumours (3.24; 1.02–10.25). Moreover, nulliparity was associated with large tumours (1.89; 0.91–3.91), high Ki67 expression (1.95; 0.93–4.10), high cyclin D1 expression (1.35; 0.83–2.18) and low p27 expression (2.03; 0.99–4.14), but these associations did not reach statistical significance. The breast cancer risks in relation to axillary lymph node status were similar in all parity groups.

Parity was not associated with any specific breast cancer subgroup defined by ER $\alpha$ , ER $\beta$ , PgR and histological type.

Women for whom no information on parity was available were very few in number and missing information was not statistically significantly associated with any of the investigated tumour subgroups. Multivariate analyses

including subjects with missing information on parity were considered unsuitable owing to the very small number of cases in this category.

The analyses did not show any statistically significant trend over parity groups with regard to risk of any breast cancer subgroup. When analysing heterogeneity between different breast cancer subgroups in relation to association with specific exposure categories, there were no statistically significant findings.

An additional analysis was made for triple-negative (ER $\alpha$ -/PR-/HER2-) breast tumours, but this analysis did not show any statistically significant association with parity.

All confounders were tested one by one in the analysis for overall breast cancer risk. Age at first childbirth was the confounder that affected the adjusted risk estimates the most. When only adjusting for socioeconomic status, the relative risks were as follows: 1.12 (0.82–1.54) for nulliparous women, 1.12 (0.89–1.43) for women with two children and 1.03 (0.78–1.35) for women with three or more children.

### 8.2.2 Age at first childbirth

#### 8.2.2.1 Distribution of risk factors

Women older than 30 years of age at first childbirth were more educated and had been less exposed to oral contraceptives than all other age categories. Women younger than 20 years of age at first childbirth were more often current smokers than all other age categories. All other factors were evenly distributed between different categories for age at first childbirth.

#### 8.2.2.2 Risk of different breast cancer subgroups

Old age at first childbirth (>30 years) was associated with a high breast cancer risk compared with that of women who gave birth to their first child before the age of 20, but this association did not reach statistical significance: 1.39 (0.94–2.07). The risk of grade III tumours was 2.10 (1.14–3.89) among women aged 20–

25, and 2.67 (1.19–6.02) among women >30 years of age. Older age at first childbirth was also associated with high expression of cyclin D1 (2.69; 1.18–6.12), and low expression of p27 (2.23; 1.15–4.35). Late first childbirth was associated with lobular breast cancer.

Women who were older at first childbirth were statistically significantly more likely to be lymph node negative than women with their first childbirth before 20 years of age 1.70 (1.05–2.77). There were statistically significant trends related to increasing age at first childbirth with regard to the risk of tumours with negative axillary lymph nodes, overexpression of cyclin D1, low p27 expression and lobular type ( $p$ -value < 0.05). There was no association between age at first childbirth and specific subgroups by invasiveness, Ki67, HER2, ER $\alpha$ , ER $\beta$  or PgR status.

In the heterogeneity test, the risk associated with an old age at first childbirth (>30 years) was statistically significantly higher in cyclin D1-overexpressing tumours compared with the risk of cyclin D1-negative tumours ( $p$ -value 0.046).

Triple-negative breast tumours were evaluated with regard to age at first childbirth, but did not reveal any statistically significant association.

All confounders were tested one by one in the analysis for overall breast cancer risk. HRT was the confounder that affected the adjusted risk estimates the most.

## 8.3 Paper III

### 8.3.1 Total duration of breastfeeding

#### 8.3.1.1 Distribution of risk factors

Women in the highest quartile of breastfeeding duration were more often multiparous and younger at first childbirth than all other groups. Moreover, women in the highest quartile were older at menopause (age > 53 years: 17.4% in comparison to 10.2%, 13.1%

and 15.6% for first, second and third quartile respectively), were less exposed to oral contraceptives (never: 53.5% in comparison to 47.3%, 45.7% and 49.3%) and were less likely to have smoked (49.9% as compared to 38.2%, 40.6% and 45.4%). All other factors were evenly distributed between breastfeeding categories.

#### 8.3.1.2 Risk of different breast cancer subgroups

The overall risks of breast cancer (i.e. the risks of unilateral invasive tumours with biological material) were similar in all breastfeeding quartiles compared to that of the lowest quartile. There was a trend towards a high risk of grade III tumours in women with a longer duration of breastfeeding; however, this association did not reach statistical significance (1.74; 0.89–3.41). The risk of high Ki67-expressing tumours was statistically significantly associated with increased duration of breastfeeding ( $p$ -value < 0.05).

### 8.3.2 Average duration of breastfeeding

Women in the lowest quartile of average duration of breastfeeding were younger at baseline (mean age: 55.7 years, as compared to 56.6, 57.9 and 58.6 years for first, second and third quartile respectively) and were more often pre-/perimenopausal at breast cancer diagnosis than all other groups (40.4% as compared to 35.8%, 30.3% and 30.6%). The RRs for average duration of breastfeeding were similar to the RR of total duration of breastfeeding. The risk of having grade III tumours was statistically significant for women in the highest quartile (1.87; 1.05–3.34) and the trend for having a grade III tumour with increasing time of breastfeeding reached statistical significance ( $p$ -value < 0.05).

### 8.3.3 Breastfeeding duration of first child

The analyses of breastfeeding of first child in relation to risk factors for breast cancer showed that women in the highest quartile were more likely to have had their first child later than the other breastfeeding groups (> 30: 14.8% as compared to 10.0%, 8.0% and 9.8% for first, second and third quartiles respectively). All other risk factors were distributed the same as for total duration of breastfeeding. All RRs for breastfeeding of the first childbirth in relation to the risk of different breast cancer subgroups were similar to those related to total duration of breastfeeding and average duration of breastfeeding. There was a statistically significantly increased risk of having high expression of cyclin D1 with increasing duration of breastfeeding ( $p$ -value < 0.05).

## 8.4 Paper IV

### 8.4.1 Distribution of potential risk factors

Cases were more often non-manual workers than controls (60.0% vs. 52.3%). More cases were users of HRT, particularly combined hormonal replacement therapy (CHRT) than controls (21.2% vs. 12.3%). These factors differed by at least 5% units between cases and controls and were, hence, included in the multivariate analyses. All other factors were similarly distributed between cases and controls.

### 8.4.2 Breast cancer risk

Seven of the 14 tested SNPs were statistically significantly associated with the risk of breast cancer. For rs2981582 (FGFR2), there was a high risk for heterozygous minor allele carriers (1.31; 1.07–1.61) and for women homozygous for the minor allele (1.63; 1.23–2.16). Homozygous minor allele carriers had a high breast cancer risk for rs3803662 (TNRC9)

(1.47; 1.04–2.08), rs12443621 (TNRC9) (1.42; 1.09–1.84) and rs3817198 (LSP1) (1.50; 1.08–2.09).

Rs889312 (MAP3K1) was positively associated with breast cancer for heterozygous minor allele carriers (1.26; 1.03–1.53) but this group was very small. Rs981782 (5p12) was inversely associated with breast cancer risk for both hetero- (0.91; 0.72–1.14) and homozygous carriers of the minor allele (0.74; 0.56–0.98).

### 8.4.3 Parity and risk of breast cancer

Among nulliparous women, homozygote carriers of the minor allele for rs3817198 (LSP1) had a high breast cancer risk (4.38; 1.13–17.0). Among women with one child, heterozygote carriers of minor allele for rs051542 (TNRC9) had a statistically significant risk of breast cancer (1.88; 1.13–3.14).

For women with two children, a high breast cancer risk was seen in both heterozygote (1.55; 1.13–2.13), and homozygote (1.88; 1.23–2.86) carriers of the minor allele for rs2981582 (FGFR2). For rs889312 (MAP3K1), only the heterozygote carriers of the minor allele had a statistically significant positive association (1.37; 1.02–1.85). Moreover, rs13281615 (8q24) was associated with a high breast cancer risk in homozygote minor allele carriers (1.62; 1.05–2.51). There was an inverse association for breast cancer in homozygote minor allele carriers of rs981782 (5p12) (0.57; 0.37–0.87).

For women with three or more children, there was a statistically significant positive association with breast cancer and heterozygote minor allele carriers of rs3803662 (TNRC9) (1.52; 1.01–2.30) and rs12443621 (TNRC9) (1.62; 1.02–2.55). Homozygote minor allele carriers of rs3817198 (LSP1) had a statistically significant high risk of breast cancer (2.00; 1.03–3.89). An inverse relationship was statistically significant for heterozygote minor allele carriers of rs2107425 (H19) in this stratified

group (0.58; 0.38–0.90). There was no statistically significant interaction between parity and the different SNPs.

In women with one child, per allele analyses showed a high breast cancer risk for rs1045485 (CASP8) (2.28; 1.04–4.96). Moreover, low per allele breast cancer risk was seen among nulliparous women carrying rs2107425 (H19) (0.58; 0.36–0.95). For rs981782 (5p12), a high breast cancer risk was seen for nulliparous women (1.64; 1.01–2.67). The per allele analyses further confirmed the results for women with two children regarding rs889312 (MAP3K1) (1.24; 1.00–1.55), rs13281615 (8q24) (1.25; 1.02–1.55) and rs981782 (5p12) (0.76; 0.62–0.94). Results were also confirmed for women with high parity carrying rs3803662 (TNRC9) (1.39; 1.00–1.92) and rs2107425 (H19) (0.69; 0.52–0.94).

There was no statistically significant trend for parity when stratifying for different alleles.

#### 8.4.4 Age at first childbirth and risk of breast cancer

Women that were young at their first childbirth ( $\leq 20$  years) had a high breast cancer risk if they were homozygote carriers of the minor allele of rs3817198 (LSP1) (3.71; 1.55–8.89). Among women with their first childbirth between  $>20$  and  $\leq 25$  years of age, there was a high risk for heterozygote and homozygote minor allele carriers of rs2981582 (FGFR2) (1.57; 1.10–2.23; and 2.25; 1.37–3.68). A high breast cancer risk was also seen for heterozygote minor allele carriers of rs12443621 (TNRC9) (1.48; 1.04–2.13) and for all minor allele carriers of rs889312 (MAP3K1) (1.54; 1.10–2.15; and 1.84; 1.08–3.15).

In women giving birth to their first child between  $>25$ – $\leq 30$  years of age, there was a low risk of breast cancer for homozygote minor allele carriers of rs981782 (0.54; 0.30–0.97).

No statistically significant risks for any SNPs were seen among women  $> 30$  years of age at first birth.

There was a statistically significant interaction ( $p=0.04$ ) between age at first childbirth and rs2107425 (H19). Following the application of the corrected  $p$ -value of 0.0025, there were no statistically significant interactions.

The per allele analyses confirmed the results for women in the youngest group carrying rs3817198 (LSP1) (1.64; 1.13–2.39), while the risk reduction became statistically significant for rs2107425 (H19) (0.64; 0.44–0.93). The results for women with their first birth between  $>20$  –  $\leq 25$  years were confirmed for rs2981582 (FGFR2) (1.51; 1.19–1.91) and rs889312 (MAP3K1) (1.41; 1.11–1.79). Further results were confirmed for women with their first birth between  $>25$  –  $\leq 30$  years of age for rs981782 (5p12) (0.74; 0.56 – 0.99). Women with late first birth ( $>30$  years of age) showed a statistically significant high per allele breast cancer risk for rs1045485 (CASP8); however, the confidence interval was very wide (4.72; 1.00 – 22.14). The risk with rs3817198 (LSP1) reached statistical significance (1.82; 1.09–3.04) for these women.

There was a statistically significant trend for increasing age at first birth when stratified for the minor allele of rs3817198 (LSP1) ( $p$ -value 0.02).

#### 8.4.5 Sensitivity analysis including subjects with 80% complete data on SNPs

When repeating all analyses only including subjects with results for  $\geq 80\%$  of all SNPs, the results were similar in all analyses. In the overall analyses, the adjusted RR for rs3803662 (TNRC9) was, however, no longer statistically significant for homozygote minor allele carriers (1.37; 0.96–1.97). For rs13281615 (8q24), the risk for minor allele carriers became statistically significant (1.35; 1.02–1.79).

In the analyses stratified for parity, rs12443621 (TNRC9) became statistically significant for nulliparous homozygote minor allele carriers (2.27; 1.04–4.97). Rs889312

(MAP3K1) became statistically significant for women with one child and for women with two children that were homozygous for the minor allele (2.33; 1.03–5.28; and 1.44; 1.02–1.89, respectively).

In stratified analyses on age at first childbirth, heterozygote minor allele carriers of rs2107425 (H19) with their first childbirth between >20 – ≤25 years now had a statistically significant lower risk (0.68; 0.48–0.97). To summarise, some analyses with borderline-significance ORs became statistically significant when only individuals with information on ≥ 80% of all SNPs were analysed.

## 9 General discussion

### 9.1 Findings

#### 9.1.1 Parity and breast cancer survival

In accordance with the results of study I, at least three other studies have found high parity to be associated with poor survival following breast cancer diagnosis [70,72,110]. When stratifying the analyses for menopausal status, the present study found similar associations between high parity and poor survival in pre- as well as postmenopausal women, which confirms the findings by Rosenberg *et al.* [68]. Other previous studies found no association between parity and survival [71,111,112], and this discrepancy may to some extent be due to different study designs, such as fewer cases [112] and the use of alternative endpoints (all-cause mortality) [111].

The association between high parity and poor survival may have several explanations. During pregnancy and later during lactation, the breast tissue evolves from immature to fully developed [11]; therefore, there is a fast cell turnover taking place during and after pregnancy. In contrast to nulliparity, high parity might eventually enhance the initiation or progression of malignant tumour cells. Dal-

ing *et al.* have reported that women diagnosed with breast cancer less than two years after parity seem to have a relatively high proportion of p53-positive, PR-negative, lymph node-positive and grade III tumours [78]. This could be the result of initiation and progression of highly malignant cells during and after pregnancy.

Moreover, a delayed diagnosis may be more common in some women if a certain group of patients are overrepresented amongst non-attendees in mammography screening. Lagerlund *et al.* found that women with no children, or with five or more children, had a lower mammography attendance rate than women with two children [113]. Thus, nulliparity and high parity may lead to delayed diagnosis and more advanced breast cancer, resulting in a poor prognosis. However, data on tumour size and ALNI in the current study do not suggest that nullipara or women with high parity had comparatively large tumours. Moreover, in the present study, high parity had similar relationships to survival both before and after the introduction of mammography screening in 1977 [114], and the observed association is not likely to be fully explained by differences with regard to mammography screening or early detection. Another aspect that has to be taken into consideration is the possibility that women from a low socioeconomic class may be characterised by both a specific pattern concerning parity and poor breast cancer survival [38,108,109]. One explanation may be that these women have a delayed diagnosis [35] and more advanced carcinomas at diagnosis [115]. Other explanations of poor survival are that these women may also have unfavourable prognostic factors such as current smoking [116] or obesity [117].

In study I, we also found a high risk of breast cancer-specific death in nulliparous premenopausal women. In contrast, Rosenberg *et al.* reported that, among premenopausal women, nulliparous women had better survival than parous women, whereas in postmenopausal women, the association was

the opposite<sup>[68]</sup>. It is possible that nulliparous women have had a longer exposure to oral contraceptives than parous women. No information on hormonal use was gathered in the MBCD, but the association between oral contraceptives and survival is, however, not clear<sup>[118]</sup>, and the use of oral contraceptives may, indeed, be generally the same in nulliparous and parous women<sup>[73]</sup>.

### 9.1.2 Parity and age at first childbirth in relation to risk for different breast cancer subgroups

In accordance with the results of study II, nulliparity has been associated with increased risk of breast cancer in many studies<sup>[51, 63, 119]</sup>. In study II, nulliparous women were at higher risk of CIS, grade III and HER2-over-expressing tumours. At least one other study has confirmed the findings related to grade III tumours<sup>[63]</sup>, but no other study has confirmed the relationship between nulliparity and HER2 status. The results in study II also suggest that nulliparous women are at higher risk of larger and highly proliferating tumours. Daling *et al.* found similar results regarding nulliparous women and a high level of Ki67<sup>[78]</sup>. To our knowledge, no other study has investigated parity in relation to cyclin D1, p27 or CIS.

High parity, compared with having one child, was associated with a lower risk of breast cancer in the present study; a previous study showed similar results<sup>[62]</sup>. High parity was not related to any specific hormone receptor status as proposed previously, and reviewed by Ma *et al.*<sup>[79]</sup> and Ursin *et al.*<sup>[80]</sup>.

Older age at first childbirth was related to large tumours, grade III, lobular tumours and overexpression of cyclin D1 along with low expression of p27. Previous studies confirm the association with large tumours and high stage at diagnosis<sup>[51, 62, 63, 119–123]</sup>. To our knowledge, no study has investigated associations between age at first childbirth and tumour character-

istics such as histological type, grade, cyclin D1 and p27

The results of this study suggest that nulliparous women develop more aggressive breast tumours. Again, it may be questioned whether these women were less likely to attend mammography screening, as Lagerlund *et al.* have proposed<sup>[113]</sup>. Another possible explanation is that nulliparous women and women with a late first childbirth are more susceptible to promoting factors, leading to more aggressive tumours. Breast tissue undergoes proliferation and maturation during pregnancy<sup>[11]</sup> and it has been shown that time since birth may have an impact on breast cancer prognosis<sup>[124]</sup>.

It is possible that nulliparous women and women with a late age at first childbirth share certain features as women in both of these categories have breast tissue that is not fully evolved during the majority of their fertile life. Therefore, their breast tissue could be more susceptible to initiating and promoting factors<sup>[125]</sup>.

Russo *et al.* have evaluated different genomic changes in breast epithelium and found that the genome in parous women without breast cancer differs from those of both nulliparous and parous women with breast cancer<sup>[61]</sup>. It is possible that such differences contribute to protect against specific phenotypes for breast tumours in these women.

Moreover, at least one *in vivo* study has shown that pregnancy induces alterations in the breast epithelial genes with down-regulation of growth hormone genes (for example, IGF-1) and up-regulation of growth inhibitor genes (for example, TGF- $\beta$ 3)<sup>[126]</sup>. Data on these factors were not available in our study but it is possible that they are involved in the fact that nulliparous women and women with a late first childbirth have more aggressive breast cancer subgroups.

### 9.1.3 Breastfeeding in relation to risk for different breast cancer subgroups

No statistically significant risk association was seen for overall breast cancer risk and duration of breastfeeding in study III. It is known that breastfeeding reduces lifetime ovulatory menstrual cycles<sup>[127]</sup>, namely, by reducing the impact of the levels of hormones present during normal menstrual cycles<sup>[128]</sup> and by specifically reducing the progesterone exposure<sup>[129]</sup>. This may explain the findings in previous studies of a reduced risk of breast cancer in women who had breastfed. It can be hypothesised that an environment with relatively low levels of oestrogen/progesterone may develop certain kinds of tumour sub-groups, namely, hormone-independent tumours that in most cases are prognostically unfavourable. However, in this study, there was no statistically significant association between breastfeeding and ER tumours.

In this study, we found that an increased duration of breastfeeding was associated with breast tumours with a high level of Ki67. To our knowledge, no other study has investigated breastfeeding in relation to tumour proliferation markers such as Ki67. Most previous studies on breastfeeding and breast cancer markers have investigated histological type and hormone receptor status. One previous study found that an increased total time of breastfeeding protected against ductal type of breast cancer<sup>[80]</sup>, which contrasts to the statistically not significant findings in our study. Ursin *et al.* found total duration of breastfeeding to be protective against ER+PR+ but not ER-PR- tumours<sup>[80]</sup>, and yet another study found breastfeeding for more than six months to be protective against triple-negative breast tumours<sup>[85]</sup>. These findings were not confirmed in study III.

Breastfeeding stimulates the production of prolactin, a hormone that has been reported to have tumour-promoting effects<sup>[45]</sup>. The

potential relationship between breastfeeding, prolactin and breast cancer appears, however, to be complex. Even if prolactin levels are high during lactation, it has been reported that, among non-lactating women, prolactin levels in blood are relatively low in those with a previous long duration of breastfeeding<sup>[130]</sup>. Moreover, breast tissue itself may be capable of producing prolactin, which would probably lead to locally increased levels that are not detectable in ordinary blood samples<sup>[15]</sup>. The potential relationship between breast cancer subgroups and prolactin will need to be investigated in experimental studies in order to investigate this further.

Generally, lifestyle factors associated with an increased risk of breast cancer, for example, HRT<sup>[48]</sup> and obesity<sup>[131]</sup>, have been associated with prognostically relatively favourable breast tumours. It is possible that the same biological pattern is seen for breastfeeding, namely, there may be a protective effect against breast cancer (even though no such association was seen in the present study), but a higher risk of more aggressive breast cancer subgroups if these women develop breast cancer. However, the biological mechanism behind this hypothesis has still to be identified.

### 9.1.4 Genetic predisposition, parity, age at first childbirth and risk for breast cancer

In this study, we found that parity/age at first childbirth and certain SNPs may interact with regard to breast cancer risk. To our knowledge, there have been only two previous studies regarding breast cancer risk and the potential interaction between SNPs and parity/age at first childbirth<sup>[132, 133]</sup>.

One recent study examined the potential gene-environment interaction, but did not find any statistically significant result for ten environmental factors (parity and age at first childbirth included)<sup>[133]</sup>. However, in their study, Travis *et al.* defined parity into two cat-

egories: nulliparous and parous. Age at first childbirth was also divided into two groups: younger or older than the age of 25. In our study, both parity and age at first birth were divided into four groups in order to identify threshold effects. In their study, Travis *et al.* studied 12 SNPs (rs2981582 (FGFR2), rs3803662 (TNRC9), rs13387042 (2q35), rs889312 (MAP3K1), rs13281615 (8q24), rs4666451 (2p), rs981782 (5p12), rs1045485 (CASP8), rs3817198 (LSP1), rs30099 (5q), rs1982073 (TGFB1) and rs1800054 (ATM))<sup>[133]</sup>. However, they did not examine four of the SNPs examined in this study (rs8051542 (TNRC9), rs12443621 (TNRC9), rs2107425 (H19) and rs7766585 (ESR1)). A strength of the study by Travis *et al.* was that they were able to include 7,610 cases and 10,196 controls, which made their results less vulnerable to type II errors.

Kawase *et al.* found a statistically significant interaction between parity and rs2981582 (FGR2)<sup>[132]</sup>. They found a high breast cancer risk for nulliparous women and for women that had given birth to one or two children who were homozygous for the T-allele of rs2981582 (FGR2)<sup>[132]</sup>. In this previous study, a total of 456 cases and 912 controls were included, which is comparable to study IV. However, they only studied one SNP<sup>[132]</sup>.

The underlying mechanism for these associations remains to be evaluated and, considering the exploratory nature of our study, our findings need to be replicated.

## 9.2 Methodological issues

### 9.2.1 Ethical considerations

All studies included in this thesis have been approved by the regional ethical committee at Lund University: Dnr 615/2004, LU 51–90, Dnr 652/2005 and Dnr 2009/682.

In study I, the MBCD was used, and this database includes all breast cancer cases from a certain time period in Malmö, Sweden. No

information was given to the patients at the time of diagnosis that their data would be included in future studies. However, at the time of the ethical application, advertisements in local newspapers provided information about the planned studies and the possibility to withdraw. During data analysis, civil registration numbers were removed from the datasets to make sure the patients could not be identified.

In study II, the MDSCS was used. The participants of this study were invited to participate in a research project on cancer. Written informed consent was obtained from the participants. When the dataset was later subjected to additional analyses, advertisements were placed in local newspapers informing former participants that they could withdraw from the study. In relation to the present analyses, no new contact was taken with former participants, thus minimising any additional harm. As in the MBCD, individual researchers only handled information containing sequence numbers, not civil registration numbers. The pathological analyses necessitated the use of civil registration numbers, but following these analyses, the files were returned to the data manager in the MDSCS who replaced these numbers with sequence numbers prior to adding information from the MDSCS database.

For all studies, the findings are primarily applicable to populations, not to individuals. It is not possible to tell if a woman will get breast cancer, or what type of tumour she will get, given information on parity, age at childbirth and breastfeeding status. There are also severe limitations regarding the potential for primary and secondary prevention using alteration of these factors. The main contribution of the present studies is to increase the knowledge about breast cancer epidemiology, which can help us to better understand breast cancer pathogenesis and the mechanisms that affect risk and survival. This is particularly true for study IV, which is of an exploratory nature with the aim of better understanding the interaction between genes and reproductive factors.



Study IV may be subject to more chance findings than the rest of the work, and informing the participants of the results might only put them under psychological stress that is not ethically justifiable.

To summarise, we obtained written informed consent and/or provided the ability for subjects to opt-out from planned studies. There were no additional contacts made with former patients, participants or relatives for these studies. All information was coded with a sequence number and it was not possible for single researchers to link data to an identifiable individual. These circumstances all reduced potential ethical problems.

## 9.2.2 Representativity

In the MBCD, all cases of invasive breast cancer diagnosed in Malmö between 1961 and 1991 were included. That is, there was no selection related to socioeconomic status or reproductive history, and the material represents an unselected breast cancer population.

In the MDCS, 40% of invited women participated in the baseline examination. Participants may have been selected from higher socioeconomic groups, and women in MDCS had a higher incidence of breast cancer than the rest of the female population in Malmö [105]. However, within the cohort, there was a considerable difference in the distribution of examined exposures and tumour characteristics. This allowed internal comparisons, and relative risks are probably less sensitive to a potential selection bias. However, it may be difficult to apply incidence rates, or information on prevalence rates for exposures, to the background population, even if the internal validity is good.

### 9.2.2.1 Mammography screening and detection of breast cancer

In both databases, it can be questioned whether participation in mammography screening may have influenced the incidence, namely, if

there was a potential detection bias. In study II, nulliparous women and women with an older age at first childbirth had a relatively high risk of breast cancer. This may indicate higher attendance of mammography screening among these women. However, women in these parity groups had tumours with more aggressive characteristics, and if anything, patients diagnosed by mammography screening are expected to present with less advanced breast tumours. In study III, there was no clear overall association between breastfeeding and overall risk, but at least, there was no decreased risk, which would have indicated that these women had attended mammography screening less often. This indicates that a low screening attendance was not the main reason for the occurrence of more advanced and more aggressive tumours in these women. As such, true differences in aggressiveness may be even higher than those observed and reported in studies II and III. Considering the results in study I, no screening existed prior to 1977. After 1977, only 50% of all women were invited to mammography screening and this was part of a trial where the randomisation was not related to socioeconomic status or reproductive history [134].

## 9.2.3 Definition and validity of parity

In study I, information on parity was retrieved from medical records and in studies II, III and IV, information on parity was retrieved from a questionnaire provided at the baseline examination. Hence, all parity data were based on self-reported number of children, the validity of which may be questioned. However, other investigators have found self-reported information on parity to be highly accurate, and we consider this information to be valid [135, 136].

Information on parity was missing for some women in both the MBCD and the MDCS. In study I, these women had similar tumour size, histology and laterality to those of other subgroups. However, women with missing in-

formation on parity tended to be slightly older, and to have higher proportions of distant metastasis and unknown nodal status than women for whom such information was available. Still, this group had an intermediate survival compared with other parity groups, and probably contains women representing all parity categories. It is possible that women with advanced tumours were admitted to hospital via different routines than other women, and this may explain why missing information on parity was more common among them. However, when the analyses in study I were repeated excluding women with distant metastases at diagnosis, all results were similar.

In studies II and IV, those with missing parity data formed a separate subgroup and these women did not differ from the rest regarding both risk factors and RRs. Another possible source of misclassification of parity in studies II and IV is that information was retrieved at baseline, and these women could have had subsequent pregnancies. However, all women participating in this database were 44 years or older, thus unlikely to have had additional children following baseline.

One limitation of the information on parity in both databases is that there was no information on multiple pregnancies, only “the number of children”. This means that it was not possible to assess the total number of pregnancies and women with twins will have had fewer pregnancies than indicated by the “parity” variable. This also means that it was not possible to separately study women that had given birth to twins. At least two Swedish studies on twin births and cancer risk<sup>[137, 138]</sup> have been published. In both studies, a risk reduction for breast cancer was seen among women with twin births<sup>[138]</sup>; however, the result was not statistically significant in one of the studies<sup>[137]</sup>. Moreover, the risk reduction was primarily seen in women with twin births before the age of 30<sup>[138]</sup>, indicating age at first childbirth to be an important determinant. Hence, this possible misclassification of parity is not likely

to have affected the results in the present studies to any great extent.

In the MBCD, there was no systematically collected information on total number of pregnancies, including spontaneous and induced abortions. In the MDSCS, there were indeed questions on legal abortions and miscarriages, but answers to these questions were to a large degree incomplete, and this information could not be used. However, previous studies have shown no association between abortions and breast cancer risk<sup>[139, 140]</sup>, nor between abortions and survival<sup>[72, 78]</sup>.

Time since last birth might have influenced the results in all studies<sup>[69]</sup>. In study I, the average age at diagnosis was above 60 in all parity groups, making it unlikely that the time since last birth would have influenced the analyses. The studies II, III and IV, included younger women, and time since last birth may have influenced the risk associated with breast cancer, and potentially also the risk of certain tumour subgroups.

#### 9.2.4 Validity of age at first childbirth

Information on age at first childbirth was available in the MDSCS, while in the MBCD, this information was only available for a small proportion of all women. Hence, this did not allow any separate analyses in study I. There is limited evidence of the validity of self-reported age at first childbirth, but as other self-reported pregnancy-related factors, for example, number of live births, gestational age and birth-weight have been shown to have a very high validity, it is reasonable to assume that there are no large discrepancies between self-reported and true age at first childbirth<sup>[136]</sup>.

#### 9.2.5 Assessment of breastfeeding and comparability

Duration of breastfeeding was retrieved from the questionnaire in the MDSCS. A limitation

of this variable is that the questionnaire did not allow for a distinction between different breastfeeding patterns. That is, some women may have reported the time they were exclusively breastfeeding, while others may have filled in the total duration of breastfeeding. There may indeed be a secular trend in breastfeeding patterns, as the recommendations for exclusive/partial breastfeeding have changed over time in Sweden<sup>[141]</sup>. Today, Sweden is a country with a high rate of women breastfeeding for at least six months<sup>[141]</sup>, yielding a study population where a “short” exposure to breastfeeding may by international standards be relatively long. Moreover, most previous studies have used women who have/have never breastfed as categories related to exposure. In study III, there were 680 parous women (4.8%) who reported that they had never breastfed. When the present data were re-analysed using this categorisation, there was no statistically significant association between overall breast cancer risk and having breastfed (adjusted relative risk = 1.14; 0.70–1.88) compared with women who had never breastfed. Hence, we considered it more valuable to investigate duration of breastfeeding in categories in order to see if there was a threshold effect.

### 9.2.6 Validity of SNP analyses

For study IV, all SNP analyses were performed by two investigators (Sophia Harlid and Malin Ivarsson).<sup>[107]</sup>

It is possible that individuals for whom only some SNP analyses succeeded had damaged blood samples. In order to verify that the results of this study were affected by damaged DNA, the statistical analyses were repeated including only women with results for 80% or more of the total number of SNP analyses. This sensitivity analysis yielded very similar results to the main analysis, indicating that the SNP results were valid.

### 9.2.7 Validity of tumour endpoints and cause of death

Breast cancer endpoints in all studies were retrieved by record linkage to The Swedish Cancer Registry. This is a nationwide registry and all cancer cases in Sweden are to be reported to this registry by both clinicians and pathologists. This registry has previously been validated in Malmö and the completeness was 99% regarding breast cancer<sup>[101]</sup>. In studies II and III, tumour classification with regard to the biomarkers was analysed using the TMA technique, which is a well-documented method for tumour tissue screening and two cores are considered to be sufficient in order to get a representative sample<sup>[142, 143]</sup>. Tumour type and grade was re-evaluated by a single senior pathologist, eliminating inter-individual variation.

In all studies I, II, III and IV, a record linkage was performed to the Swedish Cause-of-Death Registry. The Swedish Cause-of-Death Registry is based on the Swedish Population Registry, which includes 100% of the residents in Sweden. Hence, all deaths are to be reported to the Swedish Cause-of-Death Registry. In study I, breast cancer as the underlying cause of death was used as the primary endpoint. At least three studies have found cause of death amongst breast cancer patients to be highly accurate<sup>[114, 144, 145]</sup>; therefore, we consider this endpoint to be valid.

### 9.2.8 Confounding

In all the present studies, it is important to consider confounding; therefore, all analyses were performed both crudely and adjusted for potential confounders. In study I, women with high parity and nulliparous women had a high risk of dying from breast cancer. The RR was slightly lower in adjusted analyses for women with high parity, and the adjusted risk associated with nulliparity was lower and did not reach statistical significance. Information on

tumour size, type, ALNI and distant metastasis was available in the MBCD, which enabled adjustment for these tumour characteristics. One limitation in study I is that there was no information on tumour grade or hormonal receptors<sup>[16]</sup>. Moreover, it may be questioned whether adjustments for tumour size and ALNI should be carried out as these may be intermediate factors between high parity and poor prognosis. The proportion of women with ALNI was similar in the highest parity category to that of uniparous women. Still, small differences with regard to tumour size and ALNI between parity groups may be important given the strong impact on survival of tumour size<sup>[146]</sup> and ALNI<sup>[16]</sup>. Therefore, we presented the results both crudely and adjusted for tumour size and ALNI. Moreover, all analyses in study I were adjusted for diagnostic period, as diagnostic procedures and breast cancer treatment have changed over time. A limitation of study I is, indeed, that there was no information on adjuvant therapy. To some extent, this problem was solved by adjusting for factors that are used when deciding whether or not to give adjuvant therapy, namely, age, menopausal status, tumour size and ALNI. Another limitation in study I is that there is no information on socioeconomic status, although this has been shown to be related to breast cancer survival<sup>[38, 39]</sup>. Low socioeconomic status has been shown to be related to late diagnosis and relatively advanced tumours; however, even after adjustment for these factors, there was an independent association between low socioeconomic status and breast cancer survival<sup>[38]</sup>. In study I, it was possible to adjust for the effect mediated by tumour size and stage, but there may still be some residual confounding if parity is related to socioeconomic status.

In studies II and III, it is also important to consider confounding by socioeconomic status when studying parity. Moreover, regarding duration of breastfeeding, Thulier *et al.* reviewed variables associated with breastfeed-

ing and concluded that highly educated and married women tend to breastfeed their children for longer periods<sup>[147]</sup>. In the MDCC there was, however, information available on education, type of occupation and marital status/cohabiting, and all multivariate analyses were adjusted for these possible confounders. Hence, socioeconomic status should not have affected the results of the analyses in studies II and III.

In study II, the potential confounders were tested one by one in relation to overall breast cancer risk in order to see which confounder resulted in the largest change of risk estimates. In parity analyses, age at first childbirth conferred the largest difference in risk estimates after adjustment. Adjustment for socioeconomic status did not change risk estimates. In analyses for age at first childbirth, use of HRT affected the analyses the most.

In study IV, covariates included in the multivariate analysis were limited to factors that differed by 5% units or more between cases and controls. This was implemented as some analyses included a small number of cases, which limited the number of covariates that could be included.

### 9.2.9 Chance findings and statistical power

It is important to consider potential type I errors. Since 95% CI were used, there is a 5% risk that the null-hypothesis is correct, that is, that there is no true difference even if the statistical association is significant. Studies II and III include many comparisons, which increases the risk of type I error, namely, the risk of so-called mass-significance. However, the likelihood of type I errors diminishes as the associations seen in studies II and III for different tumour subgroups point in the same direction. In study I, the findings are consistent with previous studies<sup>[70, 72, 110]</sup>, which is another indication that the results were not just caused by chance. In study IV, type I er-

rors are, however, more important to consider since this study is of a more exploratory nature, and hence many comparisons were made without an *a priori* hypothesis. To minimise the risk of a type I error, all *p*-values in the interaction analyses were adjusted by Bonferroni correction. Following this, no statistically significant interactions were seen between SNPs and reproductive factors.

The studies II and III include several comparisons with few cases; this increases the risk of type II error, that is, the statistical power is low and there is a risk that true associations are not detected. However, given the consistent pattern between exposures and different tumour characteristics, even statistically not significant findings are of interest in these studies.

The risk of a type II error is probably important in study IV, as some alleles were rare and included very few cases. In the per allele analyses, some analyses reached statistical significance, which was most likely due to increased power. It is therefore important that the analyses are replicated by others in larger studies in order not to miss any true associations.

### 9.2.10 Implications and future research

In study I, we found nulliparity and multiparity to be associated with poor breast cancer survival. In study II, we found that nulliparous women develop more aggressive breast cancer subgroups, which might explain their poor survival. No such association was seen for multiparous women explaining their poor survival. One important factor that may further explain differences with regard to survival is socioeconomic status. This is of great interest as it might be associated with both breast cancer survival and parity. It would be possible to retrieve population census information for women in the MBCD, and this would allow us to further describe the relationship between parity and breast cancer survival.

Late first childbirth has repeatedly, in accordance with our study, been shown to be associated with the risk of more aggressive breast cancer subgroups<sup>[51, 62, 63, 119–123]</sup>. This is an interesting finding given that childbearing patterns have changed markedly over recent decades. Is an early first childbirth in fact protective against breast cancer or against more aggressive breast cancer subgroups? Should women be advised to have an early first childbirth? The ethical dilemmas included in primary/secondary prevention are indeed complex, but the results are, as previously mentioned, primarily not applicable to individuals nor can a causal relationship be established between the exposure and the outcome. Rather, by increasing our knowledge of breast cancer epidemiology, we can better understand breast cancer pathology and identify important biological mechanisms. One further step will be to retrieve information on age at last childbirth, and time between last childbirth and diagnosis. It will be possible to link our cohorts to the national birth registry and this will allow such studies to be carried out.

Regarding the results in study III, they need to be replicated in order to make sure they are not chance findings. The results indicate that it may be a disadvantage for a woman to breastfeed for a relatively long period. This is a difficult message that may easily get misinterpreted as it contradicts the current WHO recommendations of long breastfeeding duration. If true, the finding about breastfeeding will help us to better understand breast cancer pathogenesis, but it should not be an argument to encourage women not to breastfeed.

Study IV describes an exploratory study on the potential relationship between reproductive history, SNPs and breast cancer risk. It will have to be replicated in other larger studies, but it also serves as a model for future studies on the potential interaction between established risk factors for breast cancer and a number of recently discovered SNPs.

What is most important to remember is

that epidemiological studies can never establish a causal relationship between an exposure and an outcome. They can only report statistical associations and give risk estimates in large samples. If these risk estimates are high, they will motivate further investigations; however, such studies must include clinical and laboratory studies. Ideally, the findings should also be tested in future randomised trials.

## 10 Conclusions

1. Nulliparity and multiparity (four children or more) are associated with worse survival after breast cancer compared with that of women with one child.
2. Nulliparity and a late first childbirth (older than 30 years) are associated with the incidence of relatively aggressive breast cancer subgroups.
3. The total and the average durations of breastfeeding are positively associated with the incidence of relatively aggressive breast cancer subgroups.
4. Certain combinations of parity/age at first childbirth and genetic polymorphisms may be associated with a high risk of breast cancer. These results will need to be replicated in future studies.

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## Reproductive Factors and Breast Cancer

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# Populärvetenskaplig sammanfattning på svenska

## Summary in Swedish

Bröstcancer är den vanligaste cancerformen hos kvinnor världen över och den står för omkring 30% av all cancer hos kvinnor i Sverige. Varje år får ca 7 000 kvinnor i Sverige diagnosen och man räknar att ca en av tio kvinnor insjuknar under sin livstid. Trots att så många får bröstcancer så är det betydligt färre kvinnor som dör av bröstcancer; för närvarande ca 1 500 per år i Sverige. Detta beror på att överlevnaden är relativt god och fem år efter diagnosen så lever fortfarande ca 90%.

Det finns många väl etablerade riskfaktorer för bröstcancer och den starkaste faktorn är ålder där man vet att äldre kvinnor insjuknar mycket oftare. Tidiga menstruationer och om menstruationerna slutar sent ökar också risken. På senare år har det också blivit allt tydligare att hormonmedicinering i samband med klimakteriet ger en tydligt ökad risk. Däremot minskar risken om man föder många barn och om det sker tidigt i livet. Vad gäller genetiska faktorer räknar man med att kanske 10% av all bröstcancer är ärftlig och man har identifierat speciella gener (BRCA1 och 2) som kan förklara 3–4% av alla bröstcancerfall.

Det har under de senaste 50 åren gjorts ett stort antal studier som funnit att många barn, och tidigt barnafödande minskar risken för bröstcancer, dock finns det bara få studier vad gäller överlevnad efter bröstcancer i förhållande till antal barn (paritet).

De flesta studier som rör riskfaktorer för bröstcancer undersöker risken att insjukna i bröstcancer utan att göra skillnad på olika typer av bröstcancer. Detta trots att det är känt att olika former av bröstcancer är mycket olika biologiskt sett och att det är möjligt att olika former, t.ex. mer aggressiva typer, skulle kunna vara kopplade till vissa riskfaktorer.

En annan viktig aspekt är att arvet och miljö kan samverka när det gäller risken för att insjukna i bröstcancer. Tidigare har man mest beskrivit ärftlig risk med hjälp av sjuklighet hos nära släktingar och om man bär på de speciella bröstcancer generna (BRCA1 och 2). Under de senaste åren har dock forskningen gjort stora framsteg och man har hittat flera ”mindre” avvikelser i den mänskliga arvsmassan som ökar risken för bröstcancer. Dessa genetiska förändringar brukar beskrivas som genetiska ”polymorfismer”, dvs. vanligt förekommande förändringar i arvsmassan som är relativt vanliga men bara i vissa fall påverkar risken för bröstcancer. Det engelska uttrycket ”single nucleotide polymorphisms” har gett upphov till förkortningen ”SNP” för denna typ av genetiska faktorer. Det finns dock mycket få studier som har undersökt bröstcancer risk med hänsyn till en eventuell samverkande effekt mellan dessa SNPar och faktorer som paritet och ålder vid första barnets födelse.

Syftet med denna avhandling var att studera:

1. Hur antal barn (paritet) påverkar överlevnaden efter en bröstcancerdiagnos?
2. Hur paritet och ålder vid första barnets födelse påverkar risken för olika bröstcancer typer?
3. Hur amningstidens längd påverkar risken för olika bröstcancer typer?
4. Hur paritet och ålder vid första barnets födelse i kombination med olika SNPar påverkar risken för bröstcancer?

Skånes Universitetssjukhus Malmö, tidigare Universitetssjukhuset MAS, och före detta Malmö Allmänna Sjukhus, har länge varit det enda sjukhuset i Malmö. Alla kvinnor med bröstcancer i Malmö har diagnostiserats och behandlats vid detta sjukhus under den studerade perioden, från 1960-talet fram till nu.

I arbete 1 använde vi oss av ett material som en kirurg, Jens-Peter Garne, samlade in under tidigt 1990-tal. Garne gick igenom alla

journaler för kvinnor med bröstcancer mellan åren 1961 och 1991 i Malmö. Ur journalerna samlades information in om bl.a. paritet och tumörkaraktistika dvs. hur stor tumören var, hur den växte osv. Denna information finns nu samlad i en databas. Denna databas kopplades sedan till det svenska cancerregistret och det svenska dödsorsaksregistret för att säkerställa bröstcancerdiagnosen och för att se vilka kvinnor som hade avlidit och om de hade avlidit av bröstcancer. Totalt ingick 4 453 kvinnor i denna databas. I våra analyser använde vi kvinnor med ett barn som referenskategori, dvs. den grupp som vi jämförde de andra kvinnornas med.

Vi fann att kvinnor med inga barn, och kvinnor som fött fyra eller fler barn, hade en ökad risk för att dö av sin bröstcancer jämfört med kvinnor med ett barn. När man tar hänsyn till faktorer som man vet påverkar överlevnaden, bl.a. tumörstorlek, cancerspridning och menopausstatus, kvarstår bara ett statistiskt säkert samband för kvinnor med fyra eller fler barn. Tidigare studier har visat att många barn skyddar mot bröstcancer och det är mycket intressant att se hur dessa kvinnor möjligen kan ha en sämre överlevnad om de väl insjuknar i bröstcancer. De bakomliggande orsakerna till dessa fynd är oklara men det är möjligt att många graviditeter kan leda till hormonförändringar som ökar risken för bara vissa typer av bröstcancer.

I arbete 2 använde vi oss Malmö Kost Cancer Studien vilket är en stor databas och biobank i Malmö. I början på 1990-talet bjöd man in alla kvinnor i Malmö födda mellan 1923 och 1950 och frågade om de ville delta i en studie där man skulle studera sambandet mellan kost och cancer. Totalt deltog 17035 kvinnor och alla tillfrågades om reproduktiva faktorer; bl.a. "Hur många barn har du fött och vilka år är de födda?". Av de ingående kvinnorna hade en del redan haft bröstcancer och dessa ingick inte i våra analyser eftersom vi ville studera risken att insjukna i bröstcancer. Under tiden som vi följde upp kvinnorna

så insjuknade 622 kvinnor med bröstcancer (fram till och med december 2004). I arbete 2 studerade vi paritet och ålder vid första barnet i förhållande till risk för olika bröstcancertyper. Bröstcancertyper definierades efter olika klassifikationer som idag används vid diagnos och behandling av bröstcancer, samt ett antal nya biologiska markörer. I de statistiska analyserna användes kvinnor med ett barn som jämförelsegrupp. Vid analyserna av ålder vid första barnets födelse valdes den yngsta åldersgruppen som referens (mindre än 20 år).

Vi fann att kvinnor med inga barn hade en ökad risk för mer aggressiva bröstcancertyper när man jämförde med kvinnor med ett barn. Vi fann även att kvinnor som födde sitt första barn sent dvs. efter 30 års ålder, hade en ökad risk för mer aggressiva bröstcancertyper. Tidigare studier har visat att bröstvävnaden mognar först ut helt när man har fött sitt första barn. En möjlig förklaring till våra fynd kan vara att kvinnor med inga barn, och kvinnor som föder sitt första barn sent, har en bröstvävnad som inte är fullt utmognad och vilken därför är mer känslig för faktorer som orsakar bröstcancer.

I arbete 3 användes samma material som i arbete 2, men denna gång studerades amningstidens längd i förhållande till risken för olika bröstcancertyper. Amningstidens längd mättes bl.a. som total amningstid och i dessa analyser använde vi kvinnor med den kortaste amningstiden som referensgrupp.

Vi fann att kvinnor med lång amningstid hade en ökad risk för mer aggressiva bröstcancertyper. Dessa resultat överensstämmer dåligt med tidigare studier där man har funnit att lång amningstid skyddar mot bröstcancer. Tidigare studier har dock studerat all form som bröstcancer som en enda sjukdom, vilket kan vara en möjlig förklaring till dessa skillnader. Därtill kan det vara så att amning skyddar mot bröstcancer men att kvinnor som får bröstcancer trots att de har ammat länge utgör en speciell grupp.

I arbete 4 använde vi samma databas som i arbete 2 och 3, dvs. Malmö Kost Cancer Studien. I arbete 4 studerades olika kombinationer av reproduktiva faktorer (paritet och ålder vid första barnets fördelelse) och genetiska faktorer (olika SNPar) för att se om de påverkade risken för bröstcancer. Denna gång var upplägget på studien annorlunda och nu studerades dessa faktorer hos 728 kvinnor med bröstcancer jämfört med 1 448 kvinnor som inte hade bröstcancer

Vi kunde bekräfta tidigare studier av bröstcancerriksk vad gällde flertalet av de studerade SNParna. Vi fann också att vissa av dessa genetiska variationer påverkade risken för bröstcancer hos kvinnor med en viss paritet eller ålder vid första barnets födelse. En viktig aspekt av den fjärde studien var att det finns en risk att

fynden orsakades av slumpen eftersom många jämförelser gjordes och därför måste analyserna göras om av andra i andra befolkningsmaterial för att bekräfta våra resultat.

Slutsatsen man kan dra av denna avhandling är att paritet, ålder vid första barnets födelse, amningstidens längd samt genetiska faktorer, alla tycks påverka bröstcancerriksk och/eller bröstcanceröverlevnad. Även om epidemiologiska studier av statistiska samband inte kan säga något definitivt om orsak och verkan, och inte heller om risker hos enskilda individer, så är de viktiga pusselbitar i att försöka förstå orsakerna bakom sjukdomar som bröstcancer. Dessa epidemiologiska studier blir då grunden för vidare forskning i laboratorier och i kliniken vilket kan förbättra våra möjligheter att förebygga och behandla bröstcancer.

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