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Treatment of *BRCA1/2*-associated breast cancer and identification of mutation carriers among breast cancer patients

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Title: Treatment of *BRCA1/2*-associated breast cancer and identification of mutation carriers among breast cancer patients

Abstract:

The general aim of the research presented in this thesis was to contribute to the understanding of how breast cancer patients with germline *BRCA1/2* mutations should be treated medically and surgically, and furthermore, to characterize the limitations and strengths of different procedures for BRCA testing.

In Paper I, the long-term prognosis was assessed in a cohort of early-onset breast cancer patients (n = 221). *BRCA1/2* mutation carriers (n = 20) had an inferior overall survival compared to non-carriers (HR 1.8; 95% CI 1.0-3.3), but not when the analysis was restricted to patients who received adjuvant or neoadjuvant chemotherapy (HR 1.1; CI 0.5-2.5). The results lend support to the notion that all, or almost all, patients with BRCA-associated breast cancer should be offered chemotherapy.

In Paper II, breast-conserving therapy (BCT) was compared to mastectomy in a cohort of *BRCA1/2* mutation carriers (n = 162). Patients treated with BCT had a high risk of local recurrence (15-year risk: 32%), some of which were probably new primary breast tumors rather than true recurrences.

In Paper III, the "real world" performance (effectiveness) of the Swedish BRCA testing criteria was compared with the sensitivity (efficacy) of those criteria in a group of germline *BRCA1/2* mutation carriers (n = 20), identified within a biobank research study of unselected breast cancer patients. The effectiveness was much lower than the efficacy (18% vs 65%), suggesting that currently used clinical BRCA testing routines need to be critically revised.

In Paper IV, the results of a prospective, non-randomized study (BRCAsearch; ClinicalTrials.gov Identifier: NCT02557776) were reported. Patients with newly diagnosed breast cancer were offered germline BRCA testing regardless of age at diagnosis or family history of cancer. Pre-test information was provided by a standardized invitation letter instead of in-person genetic counseling. Out of 818 patients who received the invitation letter, 542 (66.2%) consented to analysis of *BRCA1* and *BRCA2*. Eleven (2%) pathogenic mutations were found (*BRCA1*, n = 2; *BRCA2*, n = 9). Very few patients contacted us for telephone genetic counseling, suggesting that a majority felt that the written pre-test information was sufficient for them to make a decision on testing.

In conclusion, the results of the work presented in this thesis indicate that germline BRCA status could contribute to personalized treatment decisions for breast cancer patients, and consequently, the results lend support to the idea that breast cancer patients should be offered BRCA testing at the time of diagnosis. The procedure for BRCA testing used in the BRCAsearch study offers an example of how genetic testing could be undertaken on a large scale in a feasible way.

Key words: BRCA1, BRCA2, breast cancer, prognosis, chemotherapy, breast-conserving therapy, testing criteria, prevalence, genetic counseling

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Treatment of *BRCA1/2*-associated breast cancer and identification of mutation carriers among breast cancer patients

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To Eme-Lie, Kerstin, Stina, and Karla

Contents

List of papers

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

I. **Nilsson MP**, Hartman L, Idvall I, Kristoffersson U, Johannsson OT, Loman N. Long-term prognosis of early-onset breast cancer in a population-based cohort with a known BRCA1/2 mutation status. *Breast Cancer Res Treat*. 2014, 144(1):133-42.

II. **Nilsson MP**, Hartman L, Kristoffersson U, Johannsson OT, Borg Å, Henriksson K, Lanke E, Olsson H, Loman N. High risk of in-breast tumor recurrence after BRCA1/2-associated breast cancer. *Breast Cancer Res Treat*. 2014, 147(3):571-8.

III. **Nilsson MP**, Winter C, Kristoffersson U, Rehn M, Larsson C, Saal LH, Loman N. Efficacy versus effectiveness of clinical genetic testing criteria for BRCA1 and BRCA2 hereditary mutations in incident breast cancer. *Fam Cancer*. 2017, 16(2):187-93.

IV. **Nilsson MP**, Törngren T, Henriksson K, Kristoffersson U, Kvist A, Silfverberg B, Borg Å, Loman N. BRCAsearch: Written pre-test information and BRCA1/2 germline mutation testing in a large prospective cohort of unselected patients with newly diagnosed breast cancer. *Manuscript*.

Related publications not included in this thesis:

- Winter C*, **Nilsson MP***, Olsson E, George AM, Chen Y, Kvist A, Törngren T, Vallon-Christersson J, Hegardt C, Häkkinen J, Jönsson G, Grabau D, Malmberg M, Kristoffersson U, Rehn M, Gruvberger-Saal SK, Larsson C, Borg Å, Loman N, Saal LH. Targeted sequencing of BRCA1 and BRCA2 across a large unselected breast cancer cohort suggests that one-third of mutations are somatic. *Ann Oncol*. 2016, 27(8):1532-8.
  *Both authors contributed equally to this article

- **Nilsson MP**, Loman N. Is Breast-conserving Therapy Really a Good Option for BRCA1/2 Mutation Carriers? *Clin Oncol (R Coll Radiol)*. 2016, 28(3):223-4.

Abbreviations

BCT	breast-conserving therapy
BPM	bilateral prophylactic mastectomy
BRCA1	breast cancer 1 (gene)
BRCA2	breast cancer 2 (gene)
BRCA-BC	BRCA1/2-associated breast cancer
CBC	contralateral breast cancer
CGH	comparative genomic hybridization
CI	95% confidence interval
CK	cytokeratin
CMF	cyclophosphamide, methotrexate, 5-fluorouracil
CPM	contralateral prophylactic mastectomy
DCIS	ductal carcinoma in situ
DNA	deoxyribonucleic acid
EGFR	epidermal growth factor receptor
EMT	epithelial-mesenchymal transition
EOC	epithelial ovarian cancer
ER	estrogen receptor
GWAS	genome-wide association study
HBOC	hereditary breast and ovarian cancer
HER2	human epidermal growth factor receptor 2
HR	hazard ratio
HRR	homologous recombination repair
HRT	hormone replacement therapy

IBTR	in-breast tumor recurrence
LOH	loss of heterozygosity
LR	local recurrence
MRI	magnetic resonance imaging
NCCN	National Comprehensive Cancer Network
NHEJ	non-homologous end joining
ORR	objective response rate
OS	overall survival
PARP	Poly (ADP ribose) polymerase
pCR	pathological complete response
PD-1	programmed cell death protein 1
PgR	progesterone receptor
PM	prophylactic mastectomy
RCT	randomized controlled trial
RNA	ribonucleic acid
RRSO	risk-reducing salpingo-oophorectomy
SNP	single nucleotide polymorphism
TFGT	treatment-focused genetic testing
TNBC	triple-negative breast cancer (negative for ER, PgR, and HER2)
TP53	tumor protein 53
VUS	variant of uncertain significance

Background

Hereditary factors in breast cancer

By comparing concordance rates of monozygotic and dizygotic twins, the heritability of breast cancer has been estimated to be 27-31% [1, 2]. In other words, the contribution of hereditary genetic factors to the causation of breast cancer is around 30%. "Heritability of breast cancer" is however not synonymous to "Hereditary breast cancer". Hereditary breast cancer usually refers to cancers that follow an autosomal dominant pattern of transmission within families, and account for approximately 10% of all breast cancer cases [3]. Hereditary breast cancer often has a younger age at onset compared to sporadic cases. In 20-30% of the families with hereditary breast cancer, a pathogenic germline mutation in the *BRCA1* gene or in the *BRCA2* gene can be identified. In families with multiple cases of early-onset breast cancer, and specifically if ovarian cancer is also present in the family, the proportion of BRCA mutations is higher. Furthermore, in some populations with strong founder mutations and in certain breast cancer subtypes, the proportion of BRCA mutations is also higher.

Genes other than *BRCA1* and *BRCA2* have also been associated with hereditary breast cancer. Generally, the phenotypes and risks conferred by mutations in these genes are much less studied than the consequences of pathogenic mutations in *BRCA1* and *BRCA2*. Concerns have therefore been raised regarding clinical validity and clinical utility, as these other genes are already part of multigene testing panels offered to a broader and broader range of cancer patients [4]. All of them, except for *TP53*, seem to confer lower average cumulative risks of breast cancer than mutations in *BRCA1* and *BRCA2*. Some genes are considered high-penetrant ($> 40\%$ lifetime risk), others are moderate-penetrant (20-40% lifetime risk).

"The missing heritability", accounting for the difference between the heritability of breast cancer and the currently known breast cancer genes, is mainly consisting of low-penetrant common alleles acting multiplicatively; most of these common alleles have not been identified yet [5].

BRCA1 and *BRCA2*

Historical background

In 1866, the French neurologist Paul Broca was the first to describe a family with hereditary breast cancer. His wife, as well as women in four generations of her family, had suffered from breast cancer [6]. In 1971, Henry Lynch and Anne Krush observed an increased risk of ovarian cancer in some families with hereditary breast cancer, which was later termed the hereditary breast and ovarian cancer (HBOC) syndrome [7, 8]. By linkage analysis in families with multiple cases of early-onset breast cancer, the *BRCA1* locus was mapped to chromosome 17 in 1990 [9]. A few years later, in 1994 and 1995, respectively, *BRCA1* and *BRCA2* were identified and sequenced [10-13]. The consequence of a germline mutation in *BRCA1* or *BRCA2* is the HBOC syndrome.

Mutations

More than 4000 different pathogenic mutations have been described in *BRCA1* and *BRCA2*. A majority of these mutations cause a non-functional truncated protein, and are thus termed "truncating" mutations. Some non-truncating missense mutations are also pathogenic. In *BRCA1*, pathogenic mutations are predominantly located in the RING finger and BRCT domains. In *BRCA2*, pathogenic mutations are located mainly in the DNA binding domain. Large genomic rearrangements account for 2-14% of the pathogenic mutations, and are more prevalent in *BRCA1* than in *BRCA2* [14]. Bi-allelic germline mutations in *BRCA1* or in *BRCA2* are extremely rare but have been described in individuals with Fanconi anemia-like syndromes [15, 16].

Normal function

Homologous recombination repair (HRR) is the main mechanism by which DNA double strand breaks and DNA lesions that stall the DNA replication fork are repaired in human cells. Both *BRCA1* and *BRCA2* are crucial for functional HRR [17]. In the absence of HRR, DNA double strand breaks are repaired by more error-prone pathways, such as non-homologous end joining (NHEJ), which could result in acquired mutations and facilitate cancer development. Furthermore, *BRCA1* may also have other roles in genomic maintenance and cell cycle control [18].

Inheritance and haploinsufficiency

Germline mutations in *BRCA1* and *BRCA2* are inherited in an autosomal dominant way, meaning that a first-degree relative of a mutation carrier is at 50% risk of having inherited the mutation. At a cellular level, the function of the genes is not dominant, but recessive, classifying *BRCA1* and *BRCA2* as tumor suppressor genes

rather than proto-oncogenes. However, due to the concept of haploinsufficiency, the DNA repair in a cell with a heterozygous BRCA mutation is not entirely normal. In experimental studies, heterozygous *BRCA1* inactivation results in genomic instability in breast cells, and heterozygous *BRCA1* mutations confer hypersensitivity to genotoxic stress [19].

If the function of the normal allele is lost in a cell with a heterozygous germline BRCA mutation, HRR is no longer present, resulting in a high risk of tumor development. The normal allele could be inactivated by several different mechanisms, including loss of heterozygosity (LOH) and somatic point mutations.

Prevalence of BRCA mutations

The prevalence of BRCA mutations refers to the proportion of individuals within a defined population that are carriers of a germline mutation in *BRCA1* or *BRCA2*. The prevalence of BRCA mutations has been studied in various types of populations, for example:

- Unselected individuals within a country, a region, or an ethnic group, who were not actively recruited for testing. An example of such a study design is anonymized testing in biobanks. This prevalence is also referred to as "population frequency of BRCA mutations". With all other study cohorts listed below, selection bias has to be accounted for when trying to estimate the true population frequency.
- Unselected individuals that were actively recruited (convenience cohorts). For instance, recruitment through advertisement in newspapers.
- Unselected breast cancer patients.
- Breast cancer patients selected based on age, e.g. < 40 years, < 50 years, or < 65 years.
- Breast cancer patients selected based on tumor phenotype, e.g. TNBC.
- Unselected ovarian cancer patients.
- Unselected patients with other types of cancer, e.g. pancreatic cancer, prostate cancer, and colorectal cancer.
- Individuals and families that fulfilled certain criteria to merit testing.

Population frequencies of BRCA mutations

Without any exceptions, mutations in *BRCA1* and *BRCA2* have been found in every population studied throughout the world, at varying frequencies. If the following

variables are known, the population frequency of BRCA mutations could be extrapolated: (i) the prevalence of mutations in breast cancer patients; (ii) the cumulative incidence of breast cancer in mutation carriers; (iii) the cumulative incidence of breast cancer in the general population. Another way of estimating the population frequency of BRCA mutations – which is less sensitive to assumptions – is to carry out mutation analysis on a cross section of the population, either from biobanks or from convenience cohorts. More research is needed before the true population frequency of BRCA mutations in populations without strong founder mutations (such as Sweden) could be definitely determined. A reasonable guess at the moment is that the combined frequency of pathogenic *BRCA1* and *BRCA2* mutations is somewhere between 1/200 and 1/400 in populations without strong founder mutations. In most populations, *BRCA2* mutations seem to be more frequent than *BRCA1* mutations [20].

Prevalence of BRCA mutations in breast cancer patients

In the following, a breast cancer in a patient with a germline BRCA mutation is denoted "BRCA-associated breast cancer" (BRCA-BC). Since BRCA-BC differs from sporadic breast cancer in a number of ways, the prevalence of BRCA mutations is highly dependent on the characteristics of the cohort studied. For instance, the prevalence of BRCA mutations is increased in young patients, in patients with triple-negative breast cancer (TNBC), and in populations with strong founder mutations. For practical and financial reasons, previous studies have mostly been carried out in populations enriched for BRCA mutations, or have used targeted analyses of founder mutations. Only in the recent years, comprehensive analyses of the entire coding regions of *BRCA1* and *BRCA2* have become feasible in studies of unselected breast cancer patients. Since the prevalence of mutations in these cohorts is rather low, sample size is still a problem.

Another important aspect when interpreting data on mutation prevalence, is to assess background factors of the country studied. In countries with a high incidence of breast cancer, the median age of diagnosis is higher than in countries with a lower incidence of breast cancer. Since the median age of BRCA-BC is lower than sporadic breast cancer, and the phenotype of breast cancer among older women is less "BRCA-like", the prevalence of BRCA mutations among unselected breast cancer patients in high-incidence countries is probably lower than in low-incidence countries.

Very few studies, if any, have been carried out with comprehensive analysis of *BRCA1* and *BRCA2* in truly unselected breast cancer patients in high-incidence countries without strong founder mutations. Therefore, data has to be extrapolated from studies on selected cohorts, and from studies that have used panel testing of previously identified mutations instead of full-length sequencing of both genes.

Based on that, the prevalence of BRCA mutations in unselected breast cancer patients from such countries is in the range of 1-5% [21-25]. In these countries, the prevalence of BRCA mutations in breast cancer patients diagnosed ≤ 40 years is around 10% [26], and in patients with TNBC around 11% [27].

Due to founder mutations, the prevalence of BRCA mutations in unselected breast cancer patients in some countries in Eastern Europe is 3-8% [28-31], in Iceland 5-10% [32, 33], and among Ashkenazi Jews 11% [34].

The highest prevalence of BRCA mutations in unselected breast cancer patients (27%) has been found in Bahamas, probably due to a combination of founder mutations and a low incidence of breast cancer at older ages [35].

Prevalence of BRCA mutations in ovarian cancer patients

The prevalence of BRCA mutations in unselected patients with epithelial ovarian cancer (EOC) is 10-15% [36, 37]. In high-grade serous ovarian cancer and in young patients the prevalence is even higher (15-20%). Family history of ovarian cancer or breast cancer increases the prevalence even further. BRCA mutations are very rarely found in mucinous ovarian cancer, but are found in frequencies of approximately 3-10% in endometroid, clear cell, and low-grade serous ovarian cancer [36].

Increased risk of cancer

BRCA mutation carriers have a markedly increased risk of breast cancer and ovarian cancer compared to non-carriers. There is also ample evidence for increased rates of pancreatic cancer, prostate cancer, and male breast cancer, and some evidence for an increased risk of early-onset colorectal cancer. EOC, fallopian cancer, and high-grade serous primary peritoneal carcinoma are thought to arise by similar mechanisms in the same cells of origin, and in the following, "ovarian cancer" refers to either one of them.

Breast cancer and ovarian cancer

The penetrance of BRCA mutations refers to the cumulative risk of BRCA-associated cancer up to a certain age, e.g. 70 years or 80 years, and has been the subject of a large number of studies [38, 39]. Most of them have used retrospective study designs, where various types of bias correction methods are needed. The large variation in penetrance estimates reported in these studies is partly due to what bias correction method the authors used, and partly due to true differences between populations and cohorts [40]. In a pooled analysis of 22 population-based studies [38], the average cumulative risks by age 70 years were:

- *BRCA1*, breast cancer: 65%

- *BRCA2*, breast cancer: 45%

- *BRCA1*, ovarian cancer: 39%

- *BRCA2*, ovarian cancer: 11%

The penetrance is however not the same for all mutation carriers; it is affected by other factors modifying the risk [41]. Consequently, the penetrance estimates have generally been higher in clinically ascertained cohorts, enriched for carriers with a strong family history, than in population-based cohorts. In counseling of mutation carriers regarding their risk of cancer, this is very important to keep in mind.

Over the last years, a few prospective studies have been published, all with a relatively short follow-up time [42-44]. Prospective study designs overcome some of the bias inherent to retrospective studies. However, one should note that the carriers that have been followed prospectively for incident cases of cancer in these studies are mostly derived from high-risk families, and therefore, ascertainment needs to be considered also in prospective studies.

For counseling purposes at our department in Lund, Sweden, we have often estimated the lifetime risk of breast cancer in *BRCA1/2* carriers to be in the range of 50-80%; the higher end of the range is probably more applicable to a woman with a strong family history. Known modifying factors are not yet used for individual risk assessment at our department, but these could probably widen the penetrance interval for breast cancer to 30-90%, and might be used for a more precise individual risk prediction in the future. For ovarian cancer, the lifetime risk is 30-60% for a *BRCA1* mutation carrier, and 10-20% for a *BRCA2* mutation carrier.

Not only invasive breast cancer, but also ductal cancer in situ (DCIS), is a part of the *BRCA1/2* tumor spectrum. The prevalence of germline BRCA mutations is similar among patients with DCIS and patients with invasive breast cancer [45].

Prostate cancer

BRCA-associated prostate cancer makes up a distinct subgroup of prostate cancer, with a young age at onset, an aggressive tumor phenotype, a propensity for metastatic spread, and an inferior prognosis compared to sporadic cases [46, 47]. Accordingly, cohorts enriched for young patients and patients with metastatic disease are enriched for mutation carriers. The prevalence of germline *BRCA2* mutations has been reported to be 1.2% in prostate cancer patients < 65 years, and 5.3% in patients with metastatic disease [48, 49]. The corresponding prevalence estimates for germline *BRCA1* mutations were 0.4% and 0.9%, respectively [49, 50]. Thus, the risk of prostate cancer is more pronounced in *BRCA2* than in *BRCA1* mutation carriers. In a study from England, the cumulative risk of prostate cancer by age 70 years was estimated at 15% for *BRCA2* mutation carriers and 8.5% for *BRCA1* mutation carriers [48, 50].

Pancreatic cancer

Previously, only *BRCA2* mutations were thought to increase the risk of pancreatic cancer, but recent studies show that also *BRCA1* mutations are associated with an increased risk of pancreatic cancer [51, 52]. In a consecutive series of 306 unselected pancreatic cancer patients from a hospital in Toronto, 11 germline *BRCA2* and 3 germline *BRCA1* mutations were found (*BRCA1* + *BRCA2* = 4.6%). The age at diagnosis was not different between mutation carriers and non-carriers. Among patients with Ashkenazi descent, 12% were mutation carriers [53]. The prevalence of BRCA mutations among unselected pancreatic cancer patients thus seems to be similar to the prevalence among unselected breast cancer patients, but since pancreatic cancer is a rather rare disease, the corresponding cumulative lifetime risk of pancreatic cancer is much lower.

Colorectal cancer

The results of studies regarding the risk of colorectal cancer in BRCA mutation carriers have been inconsistent. There is some evidence for an increased risk of early-onset (< 50 years) colorectal cancer in *BRCA1* mutation carriers, but not in *BRCA2* mutation carriers or in older carriers [54]. In absolute numbers, the risk for early-onset colorectal cancer is low, but colonoscopy screening for mutation carriers between ages 40 and 50 years has been proposed [55].

Male breast cancer

The lifetime risk of male breast cancer in the general population is very low (0.1%). The cumulative risk of male breast cancer is around 7% in *BRCA2* mutation carriers and 1% in *BRCA1* mutation carriers [56, 57].

Other types of cancer

No consistent findings have been reported regarding increased risks for other cancers than the ones summarized above. At this point, it could not be ruled out that BRCA mutation carriers are at increased risks also for other types of cancer, for instance malignant melanoma, salivary gland cancer, endometrial cancer, and stomach cancer [58-61].

Excess mortality beyond cancers?

By using mortality data from first-degree relatives of participants in the Washington Ashkenazi Study, Mai et al found an excess non-cancer mortality in BRCA mutation carriers [62]. Could there be intrinsic or extrinsic factors in mutation carriers that are associated with non-cancer mortality and morbidity at older ages? In mouse models, BRCA proteins have been found to have an important role in endothelial repair and cardioprotection [63], and BRCA-deficient mice have an increased mortality in response to ischemic stress [64]. Barac et al therefore hypothesized that mutation carriers could be more sensitive to the cardiotoxic effects of anthracyclines. However, in contrast to animal data, they found no difference in left ventricular ejection fraction (LVEF) between mutation carriers and non-carriers treated with adjuvant anthracyclines [65]. Postoperative radiotherapy to the breast and chest wall increases the risk of cardiac disease [66], but whether that risk differs between mutation carriers and non-carriers is unknown, as no studies have been carried out in mutation carriers. Premenopausal oophorectomy has been associated with increased morbidity and mortality in the general population, and is discussed in the "Risk-reducing strategies" section of this Background.

Modifying factors

The prevalence of BRCA mutations is higher in Iceland than in the other Nordic countries due to a founder mutation in *BRCA2* [32]. Thanks to a combination of widespread population genetic testing within biobank studies, and comprehensive nation-wide pedigree information, Iceland is well suited for registry-based studies on hereditary breast cancer. In an Icelandic study of 847 unselected breast cancer cases, diagnosed in the years 1921-1985, eighty-eight carriers of the *BRCA2* founder mutation were found [67]. Breast cancer incidence in first-degree relatives was estimated by cross-referencing of different nationwide registries. In the year of 1920, the cumulative incidence of breast cancer by age 70 years was 18.6% for mutation carriers. In the year of 2002, this cumulative incidence had increased 4-fold, to 71.9%. Among non-carriers, the cumulative incidence had also increased, from 2.6% to 10.7% [67]. An increasing incidence of breast cancer in subsequent generations is termed "the cohort effect". The cohort effect is an indirect evidence of the existence of factors that modify the risk of breast cancer. As nicely shown in the Icelandic study, the cohort effect is a strong effect. Furthermore, the study shows that modifying factors are affecting the risk both in mutation carriers and in non-carriers. The relative increase is similar in the two groups, suggesting that the factors that affect the risk of breast cancer in the general population are the same factors that affect the risk in mutation carriers.

Other examples of indirect evidence for the existence of modifying factors in BRCA mutation carriers include:

- A strong family history is a risk factor for cancer not only in the general population, but also within cohorts of BRCA mutation carriers [44]. Ovarian cancer in the family increases the risk of ovarian cancer to a larger extent than breast cancer, whereas breast cancer in the family increases the risk of breast cancer to a larger extent than ovarian cancer [68].

- The cumulative risk of breast cancer by age 70 years is higher for mutation carriers in North America than for carriers of the same mutations in Poland (72 vs 49%) [69].

- A previous breast cancer in a BRCA mutation carrier is a risk factor for incident breast cancer during prospective follow-up [70].

Modifying factors can broadly be divided into genetic factors and environmental factors. Direct evidence of modifying factors has since long existed in the general population [71], and has emerged in the BRCA mutation carrier population over the last decade [41].

Genetic factors

Genetic factors could be divided into:

A. Mutation-specific factors, meaning that not all pathogenic mutations in *BRCA1* and *BRCA2* confer the same risk.

B. Genetic factors in other parts of the genome modifying the risks conferred by mutations in *BRCA1* or *BRCA2*.

Some evidence of mutation-specific risks in BRCA mutation carriers has existed for more than a decade [38]. In 2015, a study from the CIMBA consortium convincingly showed that this is actually the case. Breast cancer risks, as well as ovarian cancer risks, were associated with both type of mutation and location of the mutation within the *BRCA1* and *BRCA2* gene [72].

Using hypothesis-free genome wide association studies (GWAS) has been a successful way of identifying genetic factors that modify breast cancer risk, both in the general population and in BRCA mutation carriers. GWAS can identify single nucleotide polymorphisms (SNPs) throughout the genome that are markers of elevated risks, but do not establish what functional variants that are mediating the increase. More than 100 SNPs have been associated with breast cancer risk in the general population [41]. Each of them is associated only with a small effect (relative risks per copy of the minor allele < 1.3), but they seem to act multiplicatively rather than additive, so the summary relative risk of multiple SNPs can be substantial. Despite including data from > 35.000 BRCA mutation carriers, GWAS conducted in mutation carriers have suffered from power problems. However, in general, it seems like SNPs associated with ER-negative breast cancer in the general population are also associated with ER-negative breast cancer in mutation carriers, and SNPs associated with ER-positive breast cancer in the general population are also associated with ER-positive breast cancer in mutation carriers [41]. In a study from the EMBRACE consortium, the association between 7 SNPs (combined multiplicatively into a risk score) and breast cancer incidence in *BRCA2* mutation carriers was investigated. During prospective follow-up, *BRCA2* mutation carriers in the highest tertile of risk defined by the combined SNP risk score had a significantly higher risk of incident breast cancer compared to *BRCA2* mutation carriers in the lowest tertile, confirming the results from previous retrospective studies [43].

Environmental factors

A large number of reproductive, lifestyle, and other factors have been associated with breast cancer risk in the general population [71]. An important question is whether these factors also are risk factors for BRCA mutation carriers. Multiple

studies have been carried out trying to answer this question, elegantly summarized by Milne and Antoniou in a recent review article [41]. Various types of bias in retrospective studies and limited sample sizes in prospective studies have made robust interpretations of the results difficult, and many of the findings have been inconsistent. However, with a few exceptions, such as age at first full-term pregnancy in *BRCA1* mutation carriers, available data indicates that the factors that modify breast cancer risk in the general population also modify breast cancer risk in BRCA mutation carriers, to a similar relative extent [41]. Although not entirely consistent, most studies have found that the following factors are associated with the risk of breast cancer in *BRCA1* and *BRCA2* mutation carriers:

- Mammographic density [73-75].

- Exposure to diagnostic radiation other than mammography at early ages (< 30 years) [76-79].

- Number of full-term pregnancies [41].

Breastfeeding > 1 year, later age at menarche, and later age at first pregnancy has been associated with a decreased risk of breast cancer in *BRCA1* mutation carriers [80-82]. Oral contraceptive use has consistently been associated with a decreased risk of ovarian cancer in mutation carriers (RR 0.50-0.58) [83, 84]. Two meta-analyses of oral contraceptive use and breast cancer risk in mutation carriers have not shown any statistically significant associations; Moorman et al, OR 1.21 (95% confidence interval, CI 0.93-1.58) [84], and Iodice et al, RR 1.13 (CI 0.88-1.45) [83]. A moderately increased breast cancer risk conferred by oral contraceptives can however at this point not be excluded, especially in some subgroups of carriers, such as *BRCA2* mutation carriers and women who start use before age 25 years [41, 85]. In small exploratory studies, various trace elements measured in plasma or toenails (folate, iron, antimony, selenium) have been associated with breast cancer risk in mutation carriers [86-88].

The evidence for a protective effect of prophylactic oophorectomy and tamoxifen use on breast cancer incidence will be described in the "Risk-reducing strategies" section of this Background.

Breast tumor phenotypes

For reasons that are poorly understood, the breast cancer phenotypes differ between *BRCA1* and *BRCA2* mutation carriers.

The luminal ER-negative mammary epithelial progenitor is thought to be the cell of origin of *BRCA1*-associated breast cancer [89]. In mouse models, disruption of

BRCA1 activates epithelial-mesenchymal transition (EMT) and induces dedifferentiation of luminal stem cells [90]. Compared to sporadic breast tumors, medullary features, high grade, pushing margins, lymphocytic infiltration, and areas of necrosis are more prevalent in *BRCA1*-associated breast tumors [91]. By immunohistochemical staining, a majority are ER-negative, PgR-negative, and HER2-negative (triple-negative breast cancer, TNBC). In a large cohort, Mavaddat et al reported that 69% of *BRCA1*-associated breast tumors were triple-negative, and 78% were ER-negative [92]. These proportions are markedly different from sporadic breast tumors, where 8-15% are triple-negative and 15-20% are ER-negative [93]. Furthermore, *BRCA1*-associated breast tumors frequently express basal markers such as CK5/6, CK14 and EGFR [91]. In terms of intrinsic molecular subtypes based on gene expression, most are "basal-like" [94].

Apart from higher grade and higher proliferation, *BRCA2*-associated breast tumors are rather similar to sporadic breast tumors in terms of histology and immunohistochemistry [91]. A majority of the *BRCA2*-associated tumors cluster in the "luminal B" molecular subtype [94].

The frequency of HER2-postivity is lower in both *BRCA1*- ($< 5\%$) and *BRCA2*-associated breast tumors ($< 10\%$) compared to sporadic breast tumors (10-20%) [95]. *TP53*-mutations are very frequently found in both *BRCA1*- and *BRCA2*-associated breast tumors [96]. Results from preclinical studies have suggested that secondary mutations in *TP53* are needed to rescue homozygous BRCA-deficient cells from apoptosis [97].

The phenotypic differences between BRCA-associated breast tumors and sporadic breast tumors could potentially be used in a number of ways [94], including:

- Improvement of algorithms that assess the likelihood of finding a mutation in a proband.

- Triaging of patients to BRCA testing on the basis of tumor phenotype instead of family history or age at diagnosis.

- Assessment of whether a variant of uncertain significance (VUS) is pathogenic or not.

- Defining tumors among sporadic breast cancer patients that present phenotypic similarities with BRCA-associated tumors, due to a defect in homologous recombination repair. This phenomenon has been termed "BRCAness" [17], and might be used for treatment prediction.

Treatment of BRCA-associated breast cancer

Surgery

For a mutation carrier diagnosed with breast cancer, there are 3 different options regarding local surgical treatment:

- Mastectomy

- Breast-conserving therapy (BCT)

- Bilateral mastectomy, i.e. ipsilateral mastectomy + contralateral prophylactic mastectomy (CPM).

In the general breast cancer population, sufficiently powered randomized controlled trials (RCTs) carried out in the United States and in Italy in the 1970s and 1980s convincingly showed that BCT followed by postoperative radiotherapy to the ipsilateral breast is non-inferior to mastectomy. In the NSABP-trial the 20-year risk of local recurrence following BCT + radiotherapy was 14.3%, and in the Italian trial it was 8.8% [98, 99].

For mutation carriers, no randomized trials comparing BCT to mastectomy have been carried out. Whether or not the results from the RCTs should be generalized to the mutation carrier population has been a matter of debate [100-102]. As mutation carriers are at a high risk of breast cancer, there might be an increased risk of second primary breast cancers in the ipsilateral breast following BCT, since breast tissue is left for tumor development. It is not always possible to separate a true local recurrence from a new primary breast tumor, and in the following "in-breast tumor recurrence" (IBTR) refers to either of them. The proportion of IBTRs that are in fact new primary breast tumors will increase with observation time following the primary breast cancer diagnosis.

A majority of the studies that have compared the outcomes following BCT reported hazard ratios > 1 regarding IBTR in mutation carriers vs non-carriers, but in most of the studies the difference did not reach statistical significance [103, 104]. Key studies comparing the risk of IBTR in BRCA mutation carriers vs non-carriers treated with BCT:

- Haffty el al reported an increased risk of IBTR in 22 carriers vs 105 non-carriers; 12-year risk IBTR 49% vs 21% (p = 0.007). None of the carriers had received adjuvant antihormonal treatment, and none had undergone oophorectomy [105].

- With a median follow-up of 7.1 years, Pierce et al found no significant difference between 160 carriers and 445 matched non-carries (15-year risk

IBTR 24% vs 17%, HR 1.37; p = 0.19). Oophorectomy decreased the risk of IBTR and when patients that had done an oophorectomy were excluded from the analysis, the difference in IBTR between carriers and non-carriers reached statistical significance (HR 1.99; p = 0.04) [106].

- Both Robson et al [107] and Kirova et al [108] found that young age, but not mutation status, was a predictor of an increased risk of IBTR.

In a recent meta-analysis, including 6 retrospective cohort studies and 4 case-control studies, no significant difference was found regarding IBTR in BRCA mutation carries vs non-carriers (RR 1.45; CI 0.98-2.14) [104]. However, when restricted to studies with a median follow-up ≥ 7 years, a higher risk of IBTR was observed for mutation carriers (RR 1.51; CI 1.15-1.98), supporting the notion that mutation carriers have an increased risk for new primary breast tumors in the treated breast, but not for true recurrences. Only two studies in the meta-analysis reported separate risks for new primary breast tumors and true recurrences, and in these two studies the risk of new primary breast tumors (RR 2.07; CI 0.99-4.36), but not the risk of true recurrences (RR 1.37; CI 0.44-4.21), was increased in mutation carriers [104]. One should note that, related to the paucity of data, the confidence intervals in this meta-analysis are wide, and the included studies are heterogeneous regarding many potentially important factors such as follow-up time, year of diagnosis, ascertainment, and adjuvant treatment.

In a relatively large retrospective cohort of BRCA mutation carriers treated with BCT (n = 396), without any group for comparison, Metcalfe et al analyzed predictive factors of IBTR [109]. Among the patients in this cohort, 70% were treated with chemotherapy, 87% with radiation therapy, 38% with tamoxifen, and 64% had undergone an oophorectomy. Mean age at diagnosis was 42.4 years. The 15-year risk of IBTR was 15.8%. In a multivariable model, radiation therapy, oophorectomy, and chemotherapy decreased the risk of IBTR [109].

At this point, it should be noted that it is unknown whether the protective effect mediated by chemotherapy and hormonal interventions is sustained beyond 15-20 years after diagnosis. Mutation carriers are often diagnosed with breast cancer at a young age, and should hopefully live another 40-50 years. Accordingly, very long follow-up is needed to estimate lifetime risks of IBTR.

Previous to our study (Paper II in this thesis), only one other study directly compared BCT to mastectomy in mutation carriers [110]. In that study, no difference in survival was seen between BCT and mastectomy, but patients treated with BCT had a higher risk of local recurrence: the 15-year risk of local recurrence was 23.5 vs 5.5%. Chemotherapy decreased the risk, so in the subgroup of BCT patients treated with chemotherapy the cumulative risk of local recurrence after 15 years was only 11.9% [110]. An interpretation of why the increased risk of local recurrence did not

translate into an inferior survival could be that many IBTRs were new primary cancers, which are more often curable than true recurrences. However, multiple known and unknown factors affect surgical decision making, and survival analyses from retrospective studies should always be interpreted with caution.

A survival benefit associated with CPM is not expected to be distinguishable during the first 10 years of follow-up [111], but has now begun to emerge in mutation carriers [112-114]. In cohorts with a pronounced difference in ipsilateral events between BCT and mastectomy, a difference in survival could possibly be emerging with longer follow-up time, given that sample sizes are large enough.

The survival benefit conferred by CPM in BRCA mutation carriers is a consequence of their markedly increased risk of CBC; whereas the annual risk of CBC in sporadic breast cancer patients is 0.5%, it can be estimated at 2-3% in mutation carriers from high-risk families [115, 116]. Adjuvant tamoxifen reduces the risk of CBC, both in *BRCA1* mutation carriers and in *BRCA2* mutation carriers [117, 118]. Premenopausal oophorectomy also reduces the risk of CBC, which has more convincingly been shown for *BRCA1* than for *BRCA2* mutation carriers [119-121]. In some studies, but far from all, adjuvant chemotherapy decreased the risk of CBC [122]. Importantly, even within cohorts of mutation carriers, young age at diagnosis of the first breast cancer and other cases of early-onset breast cancer in the family increase the risk of CBC [120, 123]. This is an example of how modifying factors affect the risk of cancer in mutation carriers. In line with this, estimates of CBC risk have generally been higher in cohorts derived from Hereditary cancer units [43, 123, 124], enriched for carriers with a strong family history and a young age at breast cancer diagnosis, than in population-based breast cancer cohorts [125, 126].

Chemotherapy

Many different types of chemotherapy are used for the treatment of cancer. Some, such as alkylating agents and platinum agents, are DNA interacting and cause DNA double strand breaks. Others, such as taxanes and vinca alkaloids, are not DNA interacting but have their mode of action through other mechanisms, thereby inhibiting mitosis. Traditionally, the choice of which chemotherapeutic agents to choose for which patient has been based on the site of the primary tumor. Breast cancer patients have mainly been treated with some agents, while ovarian cancer patients have been treated with other agents. The goal of personalized medicine is to change this outdated paradigm, and to improve the treatment outcomes by using predictive factors to guide individual treatment decisions, giving the right agent to the right patient.

Standard chemotherapy

In the 1970s, chemotherapy was introduced as a treatment for breast cancer, both in the metastatic setting and in the adjuvant setting. The first generation of chemotherapy regimens were CMF or CMF-like. In the second generation of chemotherapy regimens, introduced mainly in the 1990s, an anthracycline was added in the adjuvant setting. The third generation of chemotherapy regimens, that are considered standard today, also include a taxane, either together with an anthracycline, or instead of an anthracycline ("taxane-based").

A number of studies, mainly non-randomized cohort studies, have investigated the efficacy of standard chemotherapy in BRCA-BC. Most of them have shown that BRCA-BC is equally, or more, sensitive to standard chemotherapy compared to sporadic breast cancer. Here, some key studies are summarized:

- In a retrospective cohort study of patients who had received neoadjuvant chemotherapy at MD Anderson, mostly anthracycline-based, Arun et al found that *BRCA1* mutation carriers had a higher rate of pathological complete response (pCR) compared to non-carriers (pCR for *BRCA1*: 46%; *BRCA2*: 13%; non-carriers: 22%) [127].

- In a more recent study from China, Wang et al investigated pCR rates among 652 patients with TNBC that had been treated with neoadjuvant chemotherapy. *BRCA1* mutation carriers had a higher rate of pCR compared to non-carriers (54 vs 30%), especially in the subgroup who had received regimens that included an anthracycline (57 vs 29%). *BRCA2* mutation carriers also had a higher rate of pCR (54%), although the difference compared to non-carriers did not reach statistical significance [128].

- In the metastatic setting, Kriege et al studied the objective response rates to first line palliative chemotherapy. The most commonly used regimens were CMF and anthracycline-based. Mutation carriers had higher objective response rates (ORR) than sporadic cases (ORR for *BRCA1*: 66%; *BRCA2*: 89%; sporadic: 50%) [129].

- In studies investigating the prognosis of BRCA-BC compared to sporadic breast cancer, both Rennert et al [130] and Huzarski et al [131] found an interaction between *BRCA1* mutation status and effect of chemotherapy, meaning that the benefit of chemotherapy was greater for *BRCA1* mutation carriers than for non-carriers.

As outlined above, standard chemotherapy is not a poor option for BRCA mutation carriers with breast cancer. An exception might be taxane-based regimens without an anthracycline, since the effect of taxanes is not dependent on DNA repair [132]. It is also likely that BRCA-BC is less sensitive to vinca alkaloids, which are sometimes used for breast cancer patients in the metastatic setting. No studies on

vinca alkaloids in BRCA-BC have been reported, but there is some indirect evidence from other types of cancer (e.g. mesothelioma) that BRCA deficiency might be a negative predictive factor for response to vinca alkaloids [133].

Platinum agents

Platinum agents cause DNA crosslinks that stall the replication fork [17]. Mounting evidence suggests that BRCA-deficient cells are less capable of repairing the DNA damage caused by platinum agents than BRCA-proficient cells. In contrast to ovarian cancer, where platinum agents are the backbone of first line treatment, platinum agents have not been widely used in breast cancer, and are therefore not considered standard chemotherapy. Key studies of platinum agents in BRCA-BC:

- The randomized phase III TNT trial compared carboplatin with docetaxel in metastatic TNBC, and found that patients with germline BRCA mutations had a higher response rate with carboplatin (ORR: 68% with carboplatin vs 33% with docetaxel), which was not the case for patients without BRCA mutations (ORR: 28% vs 37%) [134].

- In a small study of single-agent cisplatin for metastatic *BRCA1*-BC, Byrski et al reported an impressive objective response rate of 80% [135].

- The same group has also published papers on their experience of treating *BRCA1*-BC with 4 cycles of neoadjuvant cisplatin, followed by surgery and adjuvant standard chemotherapy. In the most recent update of this cohort, the pCR rate was 61%, which is higher than in almost any other study of any type of chemotherapy in the general breast cancer population or among BRCA mutation carriers [136].

At the molecular level, basal-like breast cancers are remarkably similar to high-grade serous ovarian carcinomas [137]. For patients with high-grade serous ovarian cancer, BRCA mutations have consistently been associated with increased response rates to platinum agents, adding some evidence for the use of platinum agents also for breast cancer patients with BRCA-deficient tumors [138].

Addition of platinum agents to standard chemotherapy

At least two randomized phase II trials have evaluated the benefit of adding carboplatin to standard chemotherapy in the neoadjuvant setting of TNBC [139, 140]. Both trials showed improved rates of pCR with the addition of carboplatin (53% vs 37%, and 54% vs 41%, respectively), but whereas the German GeparSixto trial also demonstrated an improved event-free survival, the CALGB/Alliance 40603 trial failed to so, possibly due to lack of power for survival endpoints [141, 142]. Patients from the GeparSixto trial were analyzed for a panel of recurrent germline BRCA mutations, and mutation carriers derived more benefit from the addition of carboplatin than non-carriers [143].

Side effects of chemotherapy

As outlined in the sections above, BRCA mutation carriers seem to derive more benefit from some chemotherapeutic agents compared to non-carriers, likely reflecting the impaired DNA repair capacity of BRCA-deficient tumor cells. Due to the concept of haploinsufficiency, also cells with a heterozygous BRCA mutation – i.e. all somatic cells in an individual with a germline BRCA mutation – might be more sensitive to chemotherapeutic effects compared to cells without a BRCA mutation. This leads to the question: do BRCA mutation carriers experience more side effects to chemotherapy than non-carriers? In BRCA-deficient mouse models, a higher susceptibility of anthracycline-induced cardiotoxicity has indeed been reported [144]. However, most clinical studies have not found increased rates of early toxicity in mutation carriers compared to sporadic cases [145-147]. In the long term, the risk of treatment-related leukemia might be increased in mutation carriers, but the absolute numbers are so small that it should not influence treatment decisions [148-150].

Radiation therapy

Ionizing radiation causes DNA double strand breaks. BRCA mutation carriers might therefore be at an increased risk of radiation damage, such as acute radiation toxicity or radiation-induced contralateral breast cancers and sarcomas. Indeed, there is ample evidence from preclinical studies that cells with a heterozygous BRCA mutation are more radiosensitive than normal cells [151, 152]. However, clinical studies have not convincingly shown any clinically significant differences in early or late radiation toxicity between mutation carriers and non-carriers [146, 153].

It is well-known that ionizing radiation could cause secondary cancers. For instance, Hodgkin´s lymphoma patients treated with mantle irradiation have a 2.7-fold increased risk of breast cancer; the median latency period is 18 years and the risk is more pronounced in younger patients [154, 155]. Studies in the general breast cancer population have shown that postoperative radiotherapy could increase the risk of both radiation-induced sarcomas and contralateral breast cancer (CBC). However, the absolute risks are small, and the results from different studies are inconsistent [155]. Importantly, any increased risk of CBC is evident first after more than 10 years of follow-up. Most studies of BRCA mutation carriers have not shown an increased risk of CBC from scatter radiation to the contralateral breast [110, 120, 156], at least not any multiplicative interaction between CBC risk and BRCA mutation status [157]. Given that these studies were retrospective and relatively small, an increased risk of scatter radiation-induced CBC for mutation carriers could however at this point not be excluded, especially not for young patients [155]. In a cohort of 2885 breast cancer patients from Israel, including 470 BRCA mutation carriers, seven developed sarcomas in the field of irradiation [158]. Out of these

seven patients, three were BRCA mutation carries. The relative risk of radiation-induced sarcomas was doubled in BRCA mutation carriers, but since the absolute numbers were so small, the authors felt that BRCA mutation status should not be considered in the decision regarding radiotherapy [158].

For patients with node-negative T1-T2 tumors, mastectomy offers a non-radiation alternative to BCT, which should be discussed with mutation carriers (as well as with non-carriers) at the time of breast cancer diagnosis. For patients with node-positive or locally advanced tumors, multiple RCTs have shown a survival benefit conferred by postoperative radiotherapy, and in that setting, there is no evidence to suggest that mutation status should influence treatment decisions regarding radiotherapy [159].

PARP inhibitors

Poly (ADP ribose) polymerase (PARP) has an important role in the repair of single strand breaks in DNA. Inhibition of PARP function by PARP inhibitors results in the accumulation of single strand DNA breaks, which are subsequently converted to double strand breaks [160]. As opposed to cells with intact HRR, BRCA-deficient cells are not able to repair these double-strand breaks, and PARP inhibitors can thereby specifically target BRCA-deficient cells – a concept known as "synthetic lethality" [161].

Proof-of-principle trials showing that a proportion of BRCA mutation carriers with metastatic breast cancer or ovarian cancer can indeed achieve an objective response to the PARP inhibitor olaparib were published in 2010 [162, 163]. In 2014, on the basis of the results from a randomized phase II trial, olaparib was approved for use in the maintenance setting of platinum-sensitive high grade BRCA-mutated ovarian cancer, regardless of the mutation being germline or somatically acquired [164].

Outside of clinical trials, PARP inhibitors are currently only used for BRCA-mutated ovarian cancer, but a number of phase III trials are ongoing for different types of cancers, including BRCA-BC. As responses to PARP inhibitors have been observed in various types of BRCA-associated tumors, as well as in sporadic tumors displaying "BRCAness", the use of PARP inhibitors is expected to increase within a near future.

Immunotherapy

Anti-cancer drugs targeting the immune system have already improved the prognosis for patients with some types of cancer – most notably metastatic melanoma – but it is not a daring guess to say that we have only seen the beginning of this revolution in cancer therapy. Currently, PD1-inhibitors are the most effective immuno-modulating agents. PD1-inhibitors have only been in use outside of clinical trials for about two years, so predictive markers of response have not been fully elucidated yet. In some studies, high mutational load, expression of PD-L1, and an immunogenic tumor profile with lymphocytic infiltration have been associated with an increased efficacy of PD1-inhibitors. Of note, these are also characteristics of BRCA-associated tumors. Although intriguing and a reason for optimism, it remains to be proven if BRCA-carriers derive benefit from immunotherapy.

Prognosis of BRCA-associated breast cancer

The prognosis of BRCA-BC has been a matter of debate over the last 20 years, and the debate is still ongoing. It has been argued that the prognosis is better, the same, or worse compared to sporadic breast cancer [165]. Before the conducted studies could be merited on their strengths and weaknesses, one has to realize that the prognosis of BRCA-BC is a very complex issue with multiple confounding factors of importance. Furthermore, most studies are retrospective and prone to various other types of bias. The unadjusted hazard ratios or other unadjusted effect measures are relevant for some aspects, and the adjusted hazard ratios or stratified analyses are important for other aspects – both are needed.

Results from univariable analyses are useful for counseling of mutation carriers who have not yet been diagnosed with breast cancer. In other words, a mutation carrier would want to know not only her risk of breast cancer, but also the overall prognosis of a BRCA-BC. The introduction of MRI-based breast cancer screening in mutation carriers could possibly affect such estimates, illustrating a problem that clinical breast cancer researchers are always facing: many years of follow-up is needed for survival endpoints, so the evidence of today is partly reflecting yesterday´s treatment and surveillance protocols.

Results from multivariable analyses are needed for the treating physician at the time of breast cancer diagnosis. Ideally, all factors that are currently used for prognostication of breast cancer should be included in the models. Important questions are if BRCA status add prognostic information beyond standard prognostic factors, and if BRCA status is predictive of response to standard chemotherapy or to other types of treatments. If so, treatment decisions could be

affected or guided by BRCA status. By including univariable hazard ratios from some studies and multivariable hazard ratios from other studies into a single random-effects model, such as Baretta and colleagues have done in a recent meta-analysis [166], the important distinction between unadjusted and adjusted risks is lost. A few other meta-analyses have been published [167-169], but given the large heterogeneity between different studies in terms of ascertainment, treatment, statistical adjustment etc., at this point it is more useful to assess the results of the most informative studies separately:

- At some centers in Poland, consecutive breast cancer patients have been offered mutation analysis of founder mutations in *BRCA1* since many years. Huzarski et al studied the overall survival (OS) of 233 *BRCA1* mutation carriers within a cohort of 3345 patients with stage I-III breast cancer diagnosed ≤ 50 years [131]. Patients who underwent genetic testing > 2 years after breast cancer diagnosis were excluded, minimizing survival bias. A very high proportion of the mutation carriers (91%) were treated with chemotherapy and 50% of them had done an oophorectomy. 10-year OS was similar for carriers vs non-carriers (80.9 vs 82.2%). In a multivariable model of OS that included most prognostic factors except for tumor grade, and also included both oophorectomy and chemotherapy, the hazard ratio for a *BRCA1* mutation was 1.81 (CI 1.26-2.61). Importantly, an interaction was found between *BRCA1* status and chemotherapy, indicating that mutation carriers derived more benefit from chemotherapy than non-carriers. The authors chose not to sensor patients at time of CBC or ovarian cancer. Thereby, a potential impact of various treatments is taken into account, but on the other hand the survival reported might not be a true surrogate of first breast cancer specific survival due to a higher risk of second primary cancers in mutation carriers.

- In a population-based cohort study conducted in Canada, The United States, and Australia, breast cancer patients were retrospectively ascertained using sampling criteria to enrich for BRCA mutations, and then followed prospectively. 94 *BRCA1* mutation carriers and 72 *BRCA2* mutation carriers were compared with 1550 sporadic cases. Compared to sporadic cases, mutation carriers were younger at diagnosis, were more likely to have high-grade tumors, and more often received adjuvant chemotherapy (*BRCA1*: 85%; *BRCA2*: 79%; sporadic: 60%). As expected, most *BRCA1*-BC were ER-negative. *BRCA1* mutation carriers and sporadic patients had similar risks of distant recurrence and death in both univariable and multivariable analysis. Distant recurrence and OS was worse for *BRCA2* mutation carriers compared to sporadic patients in univariable analysis (OS HR 1.81; CI 1.15-2.86). After adjustment for age, tumor stage and grade, nodal status, hormone receptors, and year of diagnosis, an inferior prognosis for *BRCA2*

mutation carriers was no longer seen (OS HR 1.12: CI 0.70-1.79). The authors concluded that the inferior prognosis for *BRCA2* mutation carriers in unadjusted analyses seems to reflect the presence of more adverse tumor characteristics, rather than the *BRCA2* mutation per se [170].

- In a study from Iceland, 285 breast cancer patients carrying the *BRCA2* 999del5 founder mutation were matched with 570 non-carriers. *BRCA2*-associated tumors were larger and more often node positive. A positive *BRCA2* mutation status was associated with a worse OS in both unadjusted and adjusted analyses. However, when the analyses were stratified for use of chemotherapy, the inferior prognosis for *BRCA2* mutation carriers was only seen in the subgroup of patients not treated with chemotherapy (adjusted HR: 2.38; CI 1.31-4.34), not among patients treated with chemotherapy (adjusted HR: 1.21; CI 0.74-2.00). The authors concluded that the disparity in survival between *BRCA2* mutation carriers and non-carriers might be eliminated through the use of chemotherapy, and that chemotherapy should not be withheld from *BRCA2* mutation carriers on the basis of ER status. However, they also noted that further studies are needed to support this clinical recommendation [171].

- At least two studies have used a retrospective anonymized study design with analysis of Ashkenazi founder mutation in archived paraffin-embedded tumor blocks, thereby avoiding survival bias and minimizing inclusion bias. In the first study, Robson et al found that *BRCA1* mutations were an independent predictor of breast cancer mortality. When stratified for use of chemotherapy, the inferior prognosis for *BRCA1* mutation carriers was only seen in the subgroup that did not receive chemotherapy [172]. In the second study, Rennert et al found no difference in unadjusted or adjusted breast cancer mortality between mutation carriers and non-carriers. Just like in the study by Huzarski et al, they found an interaction between *BRCA1* mutation status and chemotherapy [130].

- In a large study from Holland, published in March 2017, breast cancer patients diagnosed < 50 years during the years 1970-2003 (n = 6478) were retrospectively and anonymously analyzed for a panel of 92 recurrent *BRCA1* and *BRCA2* mutations; 3.2% were *BRCA1* and 1.2% were *BRCA2* mutation carriers. Among mutation carriers, 59% were treated with chemotherapy, mostly CMF-like. The absolute 10-year OS was worse for mutation carriers compared to non-carriers (*BRCA1*: 61.4%; *BRCA2*: 60.9%; non-carriers: 70.4%). For *BRCA1* mutation carriers, the worse overall survival was partly explained by an increased risk of ovarian cancer. Furthermore, the difference between *BRCA1* mutation carriers and non-carriers was attenuated after adjustment for tumor and treatment

characteristics (adjusted HR: 1.20; CI 0.97-1.47), and disappeared when the analysis was restricted to patients who received chemotherapy (adjusted HR: 1.05; CI 0.79-1.41). For *BRCA2* mutation carriers, the results are more difficult to interpret, partly due to small numbers, and partly due to outdated treatments. Only 21% of the *BRCA2* mutations carriers received endocrine treatment, despite 77% of their tumors were ER-positive. *BRCA2* mutation carriers had a worse overall survival compared to non-carriers (unadjusted HR: 1.26; CI 0.91-1.73), which was more pronounced after more than 5 years of follow-up (unadjusted HR: 1.56; CI 1.06-2.28). The difference was attenuated, but did not disappear, after adjustment for tumor and treatment characteristics. In contrast to *BRCA1* mutation status, *BRCA2* mutation status did not seem to be a predictor of response to the chemotherapy regimens used, mostly CMF-like. The authors concluded that *BRCA2*-BC might be less chemotherapy-responsive, but that prospective cohorts treated according to more modern protocols are needed to resolve this question [173].

- All of the studies summarized above have been carried out in population-based cohorts. In breast cancer patients ascertained through Hereditary cancer units, Brekelmans et al found no difference in survival between *BRCA1/2* mutation carriers and sporadic controls [174, 175].

In total, more than 60 studies have been carried out on the prognosis of BRCA-BC [168]. The prevalent occurrence of negative prognostic factors in BRCA-BC has implicated an inferior unadjusted survival for mutation carriers compared to sporadic controls, especially in older cohorts. In more modern cohorts, where a larger proportion of patients have been treated with adjuvant chemotherapy, as well as in models adjusted for prognostic factors, any observed difference in survival is small or absent. In some subgroups, for instance TNBC patients, the prognosis might in fact be superior for mutation carriers [166, 176, 177], probably explained by a larger benefit of DNA-damaging chemotherapy. It should be noted that currently available evidence is based mostly on *BRCA1* mutation carriers, and only to a lesser extent on *BRCA2* mutation carriers.

Prognosis of BRCA-associated ovarian cancer

Up until 3-4 years ago, it was generally accepted that patients with BRCA-associated ovarian cancer had a superior survival compared to patients with sporadic ovarian cancer. For instance, in a pooled analysis of 26 observational studies, Bolton et al reported a 5-year OS of 44% in *BRCA1* mutation carriers, 52% in *BRCA2* mutation carriers, and 36% in non-carriers [178]. However, as cohorts were

followed for a longer period of time ($\geq$ 10 years), it turned out that the long-term prognosis was not superior for mutation carriers [179]. In fact, the difference might eventually be reversed [180, 181]. These findings could be explained by the longer progression free survival and higher ORR to platinum-based chemotherapy in BRCA-mutated ovarian cancer. However, postoperative chemotherapy in ovarian cancer very seldom has a curative potential, and even patients with a favorable initial response will often recur and eventually succumb to the disease.

Risk-reducing strategies

For a woman with a BRCA mutation, three specific strategies could decrease her risk of cancer and cancer-related death beyond population screening with mammography:

- Risk-reducing surgeries: risk-reducing salpingo-oophorectomy (RRSO), and prophylactic mastectomy (PM)
- MRI-based breast screening
- Chemoprevention

No randomized trials have been carried out to evaluate the potential benefits of risk-reducing surgery or MRI-based breast screening in mutation carriers. Therefore, one has to rely on results from retrospective and prospective cohort studies and case-control studies, prone to confounding and other types of bias, when assessing and comparing the efficacy of various interventions. Kurian et al developed a Monte Carlo simulation model to simulate risk-reducing strategies. In their model, the probability of being alive at age 70 years was 53% for a woman with a *BRCA1* mutation, but increased to 79% if she underwent PM at age 25 years and RRSO at age 40 years. Delaying PM to 40 years decreased the survival by 1-2%, and opting for MRI-based breast screening instead of PM decreased the survival by 2-3%. RRSO was thus the most effective single intervention. The corresponding numbers for a woman with a *BRCA2* mutation in their simulation model was 71 and 83%, respectively, and for a woman in the general population 84% [182].

Risk-reducing salpingo-oophorectomy

The cumulative incidence of ovarian cancer in *BRCA1* mutation carriers is 2-3% at age 40 years, and 11-21% at age 50 years [34, 38, 183-186]. In *BRCA2* mutation carriers, ovarian cancers are rare < 50 years and very rare < 40 years. Ovarian cancer surveillance with clinical examination, ultrasound, and Ca-125 has not been effective in reducing advanced-stage ovarian cancer or ovarian cancer deaths, and is therefore not considered a good option for mutation carriers [187]. Current

practice in Sweden is to recommend women with a *BRCA1* mutation to undergo RRSO at age 35-40 years when childbearing is completed, and to recommend women with a *BRCA2* mutation RRSO at age 40-45 years. The uptake of RRSO has increased steadily, and is now > 90% in some countries [188].

BRCA-associated ovarian cancer is thought to arise in the distal fallopian tube, so the fallopian tubes need to be removed together with the ovaries for adequate risk reduction. Despite optimal surgery, there is a remaining lifetime risk of primary peritoneal carcinoma – a tumor considered identical to serous ovarian cancer – of approximately 2-4% [189]. Risk-reducing salpingectomy with delayed oophorectomy has been proposed as an alternative to RRSO; a non-randomized prospective study (TUBA study) is ongoing [190], but evidence regarding efficacy is still lacking.

RRSO decreases the risk of ovarian cancer by approximately 80%. In a study from the PROSE consortium, undergoing RRSO was associated with lower all-cause mortality (HR 0.40; CI 0.26-0.61), ovarian cancer specific mortality (HR 0.21; CI 0.06-0.80), and breast cancer specific mortality (HR 0.44; 0.26-0.76) [191]. This study and at least four other studies found that RRSO in BRCA mutation carriers with no previous breast cancer decreased the risk of subsequent breast cancer [186]. However, over the last two years the findings regarding breast cancer risk reduction have been questioned. Using a study design that minimizes bias, Heemskerk-Gerritsen et al and Kotsopoulos et al found no protective effect of RRSO on breast cancer incidence in mutation carriers without a history of breast cancer [192, 193]. Older studies might have overestimated the effect of RRSO in this setting. Still, a protective effect regarding breast cancer incidence is likely to be found with longer follow-up of patients who have undergone premenopausal RRSO. Importantly, the main reason for RRSO in cancer-free mutation carriers is not breast cancer risk reduction, but ovarian cancer risk reduction [194].

Following a diagnosis of breast cancer, mutation carriers do not only have a risk of breast cancer recurrence, but also a high risk of ovarian cancer [195]. In this setting, RRSO improves survival both due to a decreased risk of ovarian cancer, and a decreased risk of breast cancer death. RRSO should therefore be recommended to mutation carriers soon after a breast cancer diagnosis [119].

For a mutation carrier with breast cancer, one could conclude that RRSO confers a protective effect against IBTR after BCT [104], against CBC [104], and improves survival [119, 121], with a relative risk reduction of about 50% for all of these endpoints. First, these findings could be used as an argument for expanding the BRCA testing criteria to include all patients with newly diagnosed breast cancer [119]. Second, these findings underscore that the uptake of oophorectomy needs to be considered when interpreting the results of studies on breast cancer outcomes in mutation carriers.

Compared to mutation carriers who have not undergone RRSO, women who have opted for RRSO are more satisfied with their risk management decisions [196], and are less worried about ovarian cancer [197]. Although there is no difference in general quality of life, RRSO is associated with menopausal symptoms like vaginal dryness and dyspareunia. Furthermore, in the general population, premenopausal oophorectomy has been associated with increased long-term risks of cardiovascular disease, osteoporosis, and possibly with a cognitive decline [189]. The magnitude of these risks, and ways to reduce them, are areas where more research is needed. In Sweden, hormone-replacement therapy (HRT) is recommended for mutation carriers from the time of RRSO up to the age of natural menopause, to alleviate menopausal symptoms and to mitigate the long-term non-cancer risks.

Prophylactic mastectomy

In population-bases cohorts, the cumulative incidence of breast cancer in *BRCA1* mutation carriers is approximately 13% at age 40 years, and 31% at age 50 years. *BRCA2* mutation carriers have a later median age at onset of breast cancer, and the cumulative risks in population-based cohorts are approximately 7% and 20% at age 40 years and 50 years, respectively [38, 183, 186]. As pointed out previously, these risk estimates could be underestimations in families with multiple cases of early-onset breast cancer.

Following unilateral mastectomy for breast cancer, mutation carriers could opt for CPM, either concurrent with the initial mastectomy at the time of breast cancer diagnosis, or at a later point of time. Breast cancer-free mutation carriers and carriers that have been treated with BCT could opt for bilateral prophylactic mastectomy (BPM). PM is often accompanied by breast reconstruction. PM reduces the risk of breast cancer by at least 90-95%. Small invasive or in situ breast tumors are found en passant in 3-4% of PM surgical specimens [198].

There has been a steady increase in the uptake of PM over the last decade [199]. An initiative (SWE-BRCA) is underway to survey the uptake rates of PM in Swedish BRCA mutation carriers, and preliminary results show that the uptake is at least 50% (N Loman, personal communication). Predictors of uptake of PM among mutation carriers include younger age, previous unilateral mastectomy and RRSO, country of residence, and family history of breast cancer [200-202].

Since breast cancer is a disease that is often curable, and patients with metastatic disease can live for a considerable time with palliative treatments, any significant survival benefit associated with PM is not expected to be seen during the first decade following surgery, but only with longer follow-up [111]. However, evidence of a survival benefit is now beginning to emerge [112-114, 203].

PM has not been found to have any major negative impact on anxiety, depression, or general quality of life, but a negative impact on sexuality and body image has been reported [204].

Counseling regarding the management of breast cancer risks in mutation carriers needs to be undertaken on a case-by-case basis. In Sweden, as well as in other countries, both PM and MRI-based breast screening are considered acceptable options. For mutation carriers with ovarian cancer, the subsequent risk of breast cancer during the first 10 years of follow-up is low, supporting nonsurgical management of breast cancer risk in that setting [205, 206].

MRI-based breast screening

All Swedish women are offered screening mammography every 18-24 months, starting at age 40 years. Subject to much international debate over the last three decades, screening mammography in the general population probably offers a small breast cancer specific survival benefit. The incidence and age at onset of breast cancer is markedly different in mutation carriers compared to non-carriers. Therefore, separate and more intense screening programmes are offered to mutation carriers. By starting earlier, adding MRI, and decreasing the screening intervals to 12 months, tumors could be detected at an earlier stage.

The sensitivity to detect tumors is doubled if MRI is added to mammography. The sensitivity of the two modalities combined is 90% or more [207]. Mammography offers an added value primarily for the detection of *BRCA2*-BC and DCIS [208]. In non-randomized comparisons with patients who were not screened with MRI, MRI-based screening is associated with a decreased incidence of stage II-IV breast cancer, and a corresponding increased incidence of stage 0-I breast cancer [209]. Despite this, results regarding survival following an MRI-detected breast cancer have been inconsistent [210-212]. Small, node-negative tumors in mutation carriers might follow a more aggressive course than in non-carriers, especially if not treated with chemotherapy [213].

In Sweden, mutation carriers are screened with MRI of the breasts yearly starting at age 25, combined with yearly mammography from age 30.

Chemoprevention

Chemoprevention refers to various medical interventions aimed at decreasing the risk of subsequent breast cancer in healthy women. In the general population, there is convincing evidence from randomized trials that primary prevention with tamoxifen taken for 5 years decreases the risk of ER-positive breast cancer by approximately 50% [214]. From a subgroup analysis of the NSABP-P1 breast

cancer prevention trial, there is some evidence to suggest that the results might be applicable to *BRCA2* mutation carriers as well [215]. Data on secondary prevention indicates that tamoxifen decreases breast cancer incidence not only in *BRCA2*, but also in *BRCA1* mutation carriers, despite the fact that most *BRCA1*-associated tumors are ER-negative [118]. A randomized trial of primary prevention with an aromatase inhibitor in mutation carriers is ongoing in France (LIBER trial) [216].

In Sweden and in most other countries, both patients and physicians have been minimally interested in chemoprevention. Currently, it is almost nonexistent among Swedish mutation carriers.

Identification of mutation carriers

BRCA testing criteria

Since the discovery of the *BRCA1* and *BRCA2* gene more than 20 years ago, various criteria have been used to select patients for genetic testing ("BRCA testing criteria"). A cut-off that has commonly been used is a 10% a priori probability of carrying a mutation to merit testing. Currently used Swedish BRCA testing criteria are listed in Figure 1 in "Summary of materials and methods". These criteria are mainly based on age at diagnosis and family history of cancer. They have only marginally been changed over the last decade, and are more strict than in many other countries [217, 218]. A new updated, less strict, version of the Swedish BRCA testing criteria will be launched by the end of 2017. In the first 5-10 years after the cloning of the genes, the frequency of pathogenic BRCA mutations among index persons tested in Sweden was approximately 20%. Now, 15-20 years later, the frequency has dropped to close to 10% (Å Borg, personal communication).

Different statistical models are used to assess the likelihood of finding a mutation [219]. The predictive value can be improved by incorporating tumor phenotype into these models [220]. In Sweden, the BOADICEA model is mostly used [183].

Many studies have investigated the efficacy of various BRCA testing criteria, assessing the sensitivity of the criteria for accurate identification of mutation carriers [21, 23, 25, 221]. However, a mutation carrier can evade detection for other reasons than not fulfilling the selection criteria; i.e. incomplete family history reported or recorded, was not referred, did not pursue testing etc. In recent years, studies on the effectiveness of BRCA testing criteria have also been published [25, 222-234]. Whereas efficacy can be defined as the performance of an intervention under ideal and controlled circumstances, effectiveness refers to the performance under "real world" circumstances [235]. The distinction between efficacy and effectiveness is

important but often poorly understood [236]. Of note, regarding most clinical aspects, effectiveness is a more important measure of diagnostic success than efficacy.

The effectiveness of BRCA testing criteria is by definition always lower than the efficacy. It should be pointed out that a person's decision to pursue genetic testing is preference-sensitive. For a preference-sensitive decision, the goal is to make a "quality" decision rather than a "right" decision [237]. Still, in some studies, the effectiveness of BRCA testing criteria was less than half of the efficacy, which is very unlikely to reflect the true preferences of the patients.

Ways to improve the effectiveness of BRCA testing criteria are needed, and a few recent studies have reported encouraging results with training of on-site nurses to triage patients eligible for genetic testing [238, 239], inclusion of a genetic counselor in weekly multidisciplinary team meetings [240, 241], and implementation of easy-to-use screening tools for identifying patients at risk for hereditary cancer [242, 243].

A number of studies have found that both referral rates and uptake of genetic testing has increased sharply over the last decade in patients diagnosed with breast cancer under an age that merits testing (i.e. < 45 years according to NCCN guidelines) [226, 227, 244]. This could be due to an increased awareness of hereditary cancer in the general population and among health care providers. For reasons related to study design, family history criteria have been harder to evaluate than age criteria. Of note, family history criteria (identifying older carriers) are more often overlooked than age criteria [229]. It is also important to note that most previous studies on the effectiveness of BRCA testing criteria have used study designs prone to bias, i.e. sampling through questionnaires sent home to patients, and ascertainment from academic centers, quality-focused practices, and medical insurance companies.

Among unselected breast cancer patients, up to half of the mutation carriers are missed due to not fulfilling the testing criteria [23]. Because the identification of a BRCA mutation carrier is associated with potential benefits, new methods for triaging patients to testing are needed.

New approaches

An approach different from family history based ascertainment, is the use of tumor characteristics suggestive of BRCA mutations to select patients for genetic testing. Some examples of tumor features that could be used for prediction of BRCA status include:

- Morphologic features: trabecular growth pattern, high mitotic index [245]

- Immunohistochemistry: TNBC [25], basal cytokeratins (e.g. CK5/6) and EGFR [246]

- Gene expression: basal-like and luminal B [94]

- Array CGH classifier [247]

- miRNA classifier [248]

Despite multiple retrospective studies suggesting that the method could be feasible, with the exception of TNBC very little has translated into any prospective evaluations of feasibility and clinical validity [249]. Instead, other means of triaging patients for BRCA testing, based on an expansion of the criteria to include a much wider range of individuals, have been successfully implemented, both in research studies and in routine health care. For example, testing for a panel of common BRCA mutation have been offered to unselected incident breast cancer cases in Mid-Norway from 1999 and onwards [21, 23]. By testing of all breast cancer cases, the efficacy of the testing criteria increases to 100%, but only among carriers that have been diagnosed with breast cancer. By testing of *all* individuals in a population for BRCA mutations – not only cancer patients – index mutation carriers could be identified even *before* a diagnosis of cancer. Studies of population-based screening for BRCA founder mutation in the Ashkenazi Jewish population have been carried out in the United States, Canada, England, and Israel [250-253]. Based on a high population frequency of BRCA mutations in these studies (1.1 – 2.4%), and a high risk of cancer in the relatives of BRCA mutation carriers ascertained in this way [250], population-wide screening of BRCA mutations has been advocated [254].

Genetic counseling

Genetic counseling before and after testing for hereditary mutations in high-penetrant genes is considered mandatory since many years and has many positive effects regarding decision-making conflict, knowledge, and cancer management strategies [255, 256]. The procedure used in conjunction with germline BRCA testing has traditionally been based on guidelines originally developed for Huntington´s disease. In Sweden, as well as in most other countries, pre-test and post-test counseling is undertaken at 30-60 minutes in-person sessions at a Hereditary cancer unit. Although proven to be effective, this traditional approach is costly and time-consuming, which has limited testing to a rather small number of individuals. Furthermore, carrying a BRCA mutation is different from carrying a mutation predisposing to Huntington´s disease, not least due to the fact that preventive options could be offered to BRCA mutation carriers.

As outlined in the previous sections, randomized trials are very few in hereditary breast and ovarian cancer. For issues regarding genetic counseling, there are however a number of randomized trials. Most of them have been carried out in the United States, in Holland, or in Australia. Superiority trials have focused on ways to improve standard genetic counseling. The addition of decision aids [257-259], peer-support programmes [260], and psychosocial telephone counseling [261] have been shown to increase knowledge, and decrease decisional conflict and cancer-related distress, at least in the short term.

The availability of genetic counselors is already today a problem [262] and if genetic testing should be expanded to a larger number of individuals, the counseling process needs to be simplified. Non-inferiority trials have therefore focused on simplified ways of conducting genetic counseling. In an early study, Calzone et al showed that group education followed by brief individual counseling was non-inferior to standard counseling in terms of knowledge and distress [263]. The results from two large randomized trials on telephone genetic counseling for women at a high risk of BRCA mutations have recently been published [264, 265]. Both of them were carried out in the United States. In these trials, pre-test and post-test telephone genetic counseling was cost-effective and fulfilled the criteria for non-inferiority to standard in-person counseling for all psychosocial and decision making outcomes. The uptake of genetic testing was lower among patients randomized to telephone counseling: 28% vs 37% in the study by Kinney et al [264], and 84% vs 90% in the study by Schwartz et al [265]. In one of these trials, ethnicity moderated the association between randomization group and uptake of testing, meaning that telephone counseling resulted in lower uptake rates of testing especially among minority women [266].

A further simplification of the testing procedure is to offer written pre-test information instead of in-person or telephone counseling. The first randomized trial of written pre-test information in hereditary breast cancer was published in 2016. In this study from Australia, 135 women with newly diagnosed breast cancer, all of whom fulfilled clinical criterial for BRCA testing (> 10% likelihood of carrying a mutation), were offered treatment-focused genetic testing (TFGT; testing prior to surgery, that could inform surgical decisions). Instead of standard pre-test genetic counseling, the intervention group received a brief educational pamphlet. Following testing, all patients received the test result during a face-to-face appointment at a Hereditary cancer unit. The results of the study indicated that the intervention arm was cost-effective and non-inferior to the standard arm on the primary outcome decisional conflict [267].

No randomized trials on written pre-test information have been carried out in cohorts of unselected breast cancer patients. In a prospective, single-arm study from Norway (DNA-BONus study), written pre-test genetic information and BRCA

testing was offered to all patients with newly diagnosed breast cancer [21]. The uptake of genetic testing was 45.4%. Due to ethical regulations, no predictors of uptake could be analyzed. A psychosocial part of the study evaluated symptoms of anxiety and depression by self-reported questionnaires, and showed results comparable to what has previously been reported in breast cancer patients from the general population.

If given a choice between standard pre-test genetic counseling and a simplified procedure, the results of a non-randomized study from Holland suggest that a majority of the patients would opt for the simplified procedure. In that study, 233 breast cancer patients referred for genetic counseling could choose between either standard in-person pre-test genetic counseling or an intervention called "DNA-direct". Patients who opted for DNA-direct received telephone, written, and digital information instead of pre-test genetic counseling. Important to note, patients with psychological problems or difficulty with reading Dutch text were excluded. 161 patients (59%) opted for DNA-direct. Six out of eight BRCA mutation carriers who opted for DNA-direct were satisfied with the procedure and would choose DNA-direct again [268].

Taken together, the results of previous studies indicate that the standard procedure for pre-test genetic counseling could be modified in a cost-effective way without any negative impact on psychosocial outcomes or decisional conflict. Written pre-test information or telephone counseling might result in slightly lower uptake rates of genetic testing. However, simplified procedures enable genetic testing to be expanded to a much larger number of patients.

Psychosocial aspects

In a meta-analysis from 2009, Hamilton et al found that BRCA mutation carriers experienced a transient increase in distress shortly after the genetic test results were delivered, but the levels of distress returned to pre-test levels within a year [269]. In 2012, Graves et al published the results from the first prospective study on long-term psychosocial impacts of BRCA testing. Compared to the global distress measures used in most other studies, the measure of genetic testing distress used in their study (MICRA) might be more sensitive to capture distress specifically connected to genetic testing. With a median follow-up of 5 years, BRCA carriers who had been affected with cancer reported modestly increased levels of genetic testing distress and uncertainty compared with women who received negative/uninformative BRCA testing results. Importantly, no difference was observed in measures of global psychologic dysfunction [270].

Despite no evidence for any long-term clinically significant dysfunction, undergoing genetic testing might still be detrimental to psychological well-being in a small minority of the patients. The most important factor for high levels of distress following genetic testing seems to be high pre-test levels of distress [271, 272].

Aims

The overall aim of the research presented in this thesis was to contribute to the understanding of how breast cancer patients with germline *BRCA1/2* mutations should be treated medically and surgically, and furthermore, to characterize the limitations and strengths of different procedures for BRCA testing.

The specific aims were:

- To study the long-term prognostic impact of germline *BRCA1/2* mutations in young patients with early breast cancer (Paper I).

- To compare the risk of local recurrence and survival between *BRCA1/2* mutation carriers treated with breast-conserving therapy and carriers treated with mastectomy (Paper II).

- To determine the difference between efficacy and effectiveness of the Swedish BRCA testing criteria in a population-based cohort of breast cancer patients (Paper III).

- To evaluate a simplified BRCA testing procedure in patients with newly diagnosed breast cancer (Paper IV).

Summary of materials and methods

Paper I

As previously reported in a paper not included in this thesis [26], all women in the Southern Health Care Region in Sweden with an invasive breast cancer diagnosed before the age of 41 years between 1990 and 1995 (n = 262) were contacted in 1996 and offered mutation analysis of the *BRCA1* and *BRCA2* genes. Women who had died could be included if samples of their blood or other tissues were available. In total, mutation analysis was carried out in 89% of the patients and twenty-three (10%) pathogenic mutations were found: 18 in *BRCA1* and 5 in *BRCA2*. Patients with a previous cancer in the contralateral breast or metastatic disease at the time of diagnosis were excluded, leaving 221 patients for the present study. Due to small numbers, *BRCA1* and *BRCA2* mutation carriers were grouped together for analyses.

Study endpoints for Paper I were overall survival (OS) and incidence of contralateral breast cancer (CBC; invasive or DCIS) for mutation carriers compared to non-carriers. Clinical data was abstracted from medical records and pathology reports. Vital status was controlled in the Swedish Census Register. Selection of variables for exploratory subgroup analyses and for multivariable analyses was based on results of previous work from other researchers. Tumor grade was not selected for inclusion in the multivariable models because of many missing values. Age at diagnosis was stratified into 3 age groups to account for non-linear associations. Differences in tumor, patient and treatment characteristics between mutation carriers and non-carriers were tested using Fisher's exact test for all covariates except for age, where the difference in median age was tested using the Wilcoxon rank sum test. For univariable analysis, OS and CBC were estimated using the Kaplan-Meier method and compared using the log-rank test. For calculation of hazard ratios and for multivariable analysis, the impact of different prognostic factors on OS and CBC were assessed by the Cox proportional hazards model.

Paper II

All patients that have undergone BRCA mutation analysis in Lund, Sweden, are registered in an institutional database. From this database, all *BRCA1/2* mutation carriers with an invasive breast cancer stage I-III diagnosed between 1975 and 2011 were identified (n = 204). Patients with no consent to follow-up, lost to follow-up, or a diagnosis of ovarian cancer within 10 years preceding the breast cancer diagnosis were excluded. Out of 173 remaining patients, 11 were treated with breast-conserving therapy (BCT) without postoperative radiotherapy; they were also excluded. For the present study, 162 patients thus constituted the study population (*BRCA1*, n = 114; *BRCA2*, n = 48).

Study endpoints were local recurrence as first recurrence (LR), OS, breast cancer death and distant recurrence, for the pre-specified subgroups of patients treated with BCT and mastectomy, respectively. Clinical data was abstracted from medical records and pathology reports and supplemented by information from self-reported questionnaires. Vital status was controlled in the Swedish Census Register. Cumulative incidence curves were calculated for LR in presence of other recurrences or death as competing risks, and for breast cancer death and distant recurrence in presence of death of other cause than breast cancer as competing risk. All LR were invasive. Kaplan-Meier curves were used to illustrate OS. Cause-specific log-rank tests and Cox regression analyses were used to compare event rates between the treatment groups.

Paper III

Patients diagnosed with invasive breast cancer who were scheduled for surgery during the years 2007-2009 in Malmö, Sweden, were asked to participate in the population-based ABiM study by agreeing to donate a blood sample for research purposes and to consent the use of blood and tumor tissues for molecular analyses. Approximately 80% of the patients were included in the ABiM study; the remaining 20% were not asked or declined to participate. In 2014, analyses of germline and tumor DNA from 273 out of 538 patients in the ABiM study were conducted within a research project. The patients included in this research project in 2014 had on average larger tumors, of higher grade, and with higher Ki-67 compared to ABiM patients not included in this research project, but the median age at diagnosis did not differ between the two groups [273]. As previously reported in a paper not included in this thesis, pathogenic germline mutations were detected in 20 patients (*BRCA1*, n = 10; *BRCA2*, n = 10) [273]. The ABiM study was aimed to be a biobank research study, and the patients did not expect to be recontacted at a later point of

time. Accordingly, patients were not biased to participate because of a wish to get individual information about their breast cancer, such as whether it was of a hereditary type or not.

After thorough discussions within a group of experts, we decided to recontact the mutation carriers and give them the individual information about their mutation carrier status. For deceased patients, we recontacted their next of kins. The recontacting was undertaken in 2015, five to seven years after the breast cancer diagnosis. Following confirmatory genetic testing, a detailed family history of cancer at the time of breast cancer diagnosis was retrospectively obtained. Clinical data, documented personal and family history of cancer, and any comments about genetic counseling or genetic testing was abstracted from the surgical and oncological medical records.

The aim of the study was to compare the efficacy and the effectiveness of the Swedish BRCA testing criteria, listed in Table 1. Efficacy was defined as the proportion of mutation carriers among the breast cancer patients in the study population that fulfilled the Swedish BRCA testing criteria at the time of breast cancer diagnosis. In other words, efficacy referred to the performance of the Swedish BRCA testing criteria under ideal and controlled circumstances. Effectiveness was defined as the proportion of mutation carriers among the breast cancer patients in the study population that were referred for genetic counseling as probands, without the occurrence of new incident cases of breast cancer or ovarian cancer in the family, and subsequently actually underwent genetic testing. Patients, in whom a mutation was already known in the patient or in the family at the time of breast cancer diagnosis, were excluded from the analysis of effectiveness. In other words, effectiveness referred to the performance of the Swedish BRCA testing criteria under "real world" circumstances.

Table 1. Swedish Breast Cancer Group criteria for screening of mutations in *BRCA1* and *BRCA2*

Any of the following:
■ Three cases of breast cancer in first degree relatives, or second degree relatives through a male, with at least one diagnosed ≤ age 50 years, and/or ovarian cancer (regardless of age).
■ Two cases of breast cancer or ovarian cancer in first degree relatives, or second degree relatives through a male, with at least one case of breast cancer diagnosed ≤ age 40 years, or two cases of ovarian cancer (regardless of age).
■ One case of breast cancer ≤ age 35 years.
■ One case of triple-negative breast cancer ≤ age 40 years*.
■ One case of male breast cancer.
■ Breast cancer and ovarian cancer in one individual.

Cases of bilateral breast cancer, prostate cancer, and pancreatic cancer may strengthen the indication for screening of mutations in *BRCA1* and *BRCA2*, but are not defined in any specific criterion

* This criterion was not fully applied during the study period; however, this does not affect the conclusions of the study

Paper IV

In Paper IV, the results of the prospective, single-arm study BRCAsearch (ClinicalTrials.gov Identifier: NCT02557776) are reported. For reasons related to ethical permits and funding, only patients included in another study called SCAN-B (ClinicalTrials.gov Identifier: NCT02306096) [274] were eligible for inclusion in BRCAsearch. During the study period of BRCAsearch, 86% of all invasive breast cancer cases at the participating hospitals were included in SCAN-B. SCAN-B was a biobank research study and the results from that study had no implications for individual participants. Main exclusion criteria for SCAN-B were inability to understand written Swedish, and severe psychological problems.

Inclusion criteria for BRCAsearch were (all): (i) The patient is included in the SCAN-B study; (ii) The patient is recently diagnosed with an invasive breast cancer; (iii) The patient has signed an informed consent form for BRCAsearch. Exclusion criteria for BRCAsearch included the following: (i) The patient is unable to understand the written information in Swedish. (ii) The patient is in a psychological state, due to chronic or temporary reasons, where one could suspect that information about the study substantially could be detrimental to the psychological well-being.

Here is a summary of the study procedure for BRCAsearch:

1. An invitation letter (see appendix I in Paper IV) was given to the patient by the nurse at the visit to the surgeon a week after primary surgery, or by the oncologist at the time of information about neoadjuvant chemotherapy. The invitation letter contained information about the study as well as possible implications of genetic testing, an informed consent form, psychosocial questionnaires, and contact information to a genetic counselor. The patient was invited to contact a genetic counselor for pre-test telephone counseling if she felt a need for more information.

2. If consent was given and a separate blood sample was sent in, *BRCA1* and *BRCA2* were analyzed by full sequencing (see appendix II in Paper IV for information about mutation analysis and variant calling).

3. For patients who had not returned the consent form, the study protocol dictated that a reminder should be sent by mail 2-4 months after the invitation letter to the study. However, due to the workload of individual researchers, the median time from the invitation to the study to the reminder was in fact 4.5 months.

4. Non-carriers were informed about the test result through a letter. Mutation carriers were telephoned and given a time for an appointment within one week.

5. Psychosocial self-reported questionnaires were delivered at 3 times: at invitation to the study, one month after information about test results, and one year after information about test results.

According to the study protocol, patients that fulfilled the Swedish BRCA testing criteria should receive the invitation letter for BRCAsearch, but should also be referred for an assessment at the Department of Clinical Genetics, in order to enable testing of genes other than *BRCA1* and *BRCA2*.

BRCAsearch enrolled patients from three hospitals in south Sweden during the time period March 2, 2015 – August 26, 2016. The study flowchart is presented in Figure 1.

In Paper IV, the following outcomes from BRCAsearch are reported: the uptake of genetic testing, the prevalence of *BRCA1/2* mutations, the proportion of the mutation carriers that did not fulfil current criteria for BRCA testing, how many of the patients that contacted us for questions, as well as some biological characteristics of the BRCA-associated breast tumors.
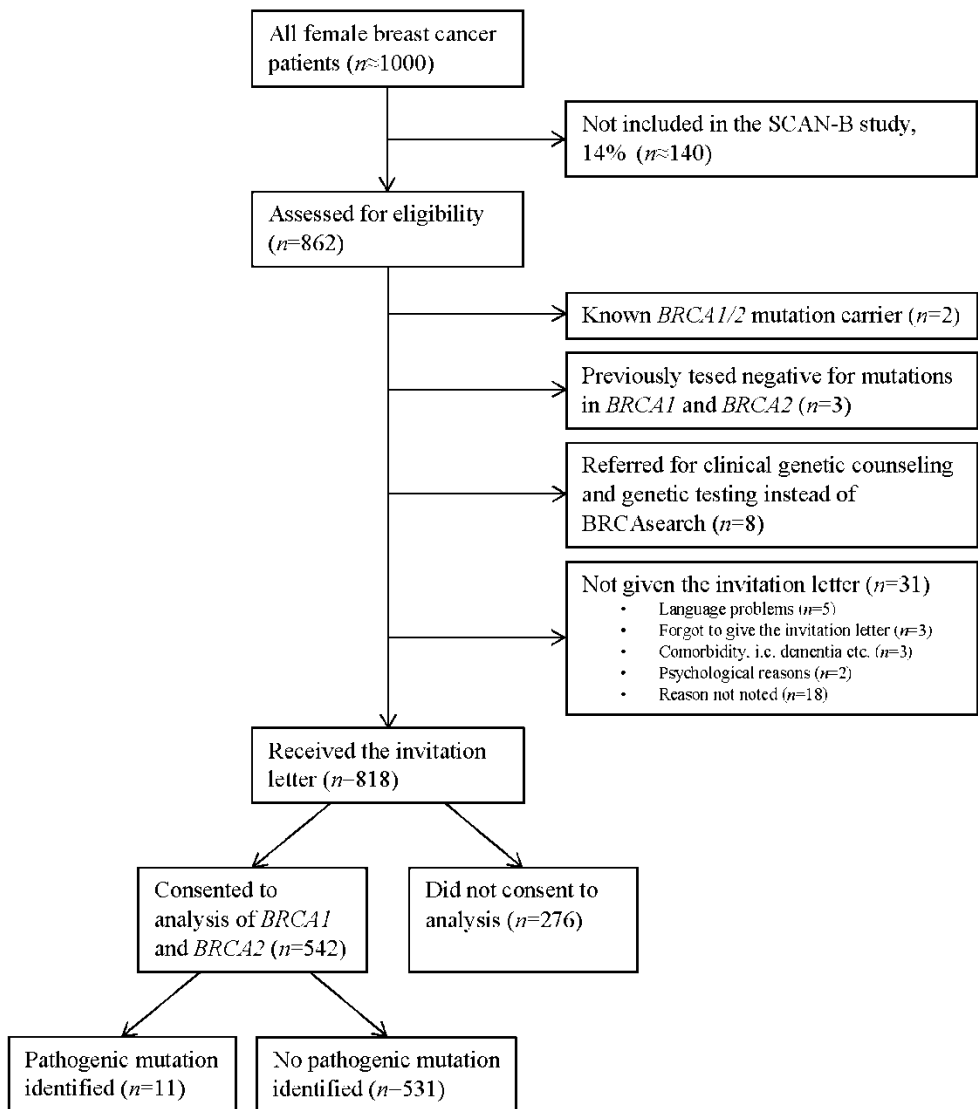
All female breast cancer patients ($n \approx 1000$)

Not included in the SCAN-B study, 14% ($n \approx 140$)

Assessed for eligibility ($n=862$)

Known *BRCA1/2* mutation carrier ($n=2$)

Previously tesed negative for mutations in *BRCA1* and *BRCA2* ($n=3$)

Referred for clinical genetic counseling and genetic testing instead of BRCAsearch ($n=8$)

Not given the invitation letter ($n=31$)
- Language problems ($n=5$)
- Forgot to give the invitation letter ($n=3$)
- Comorbidity, i.e. dementia etc. ($n=3$)
- Psychological reasons ($n=2$)
- Reason not noted ($n=18$)

Received the invitation letter ($n-818$)

Consented to analysis of *BRCA1* and *BRCA2* ($n=542$)

Did not consent to analysis ($n=276$)

Pathogenic mutation identified ($n=11$)

No pathogenic mutation identified ($n-531$)

Figure 1. Flowchart of patient inclusion for BRCAsearch and genetic analyses

Summary of results and discussions

Paper I

The median follow-up was 19 years. As expected, mutation carriers were younger at diagnosis (median 34.5 vs 37.0 years) and were more likely to have ER-negative (87% vs 46%), PgR-negative (80% vs 38%) and grade III (83% vs 40%) tumors, compared to non-carriers. Mutation carriers more often received adjuvant or neoadjuvant chemotherapy (65% vs 44%), and less often received anti-hormonal treatment (0% vs 17%). Both among patients who did and among patients who did not receive chemotherapy, BRCA-associated tumors were more often grade III and more often ER-negative. The 5-, 10-, 15-, and 20-year OS was 60%, 45%, 39%, and 39% for mutation carriers and 82%, 70%, 59%, and 53% for non-carriers, respectively (5-year log-rank p = 0.013; 10-year p = 0.008; 15-year p = 0.020; 20-year p = 0.046) (Figure 2). In unadjusted Cox regression analysis, there was a trend for an inferior OS for mutation carriers (HR 1.8; 95% CI 1.0-3.3), which was not due to second primary tumors, e.g. ovarian cancers or other competing deaths. When the analysis was restricted to patients who did not receive chemotherapy, mutation carriers had an inferior prognosis (HR 3.0; CI 1.2-7.7) (Figure 3). In the subgroup of patients who received chemotherapy, mutation carriers had a prognosis comparable to non-carriers (HR 1.1; CI 0.5-2.5) (Figure 4). We used stratified analyses to investigate the impact of chemotherapy on survival. A different approach is to use interaction terms in multivariable models. Others have found an interaction between chemotherapy and mutation status, indicating that mutation carriers in those studies derived a greater benefit of chemotherapy compared to non-carriers, in line with the results of our study [130, 131].
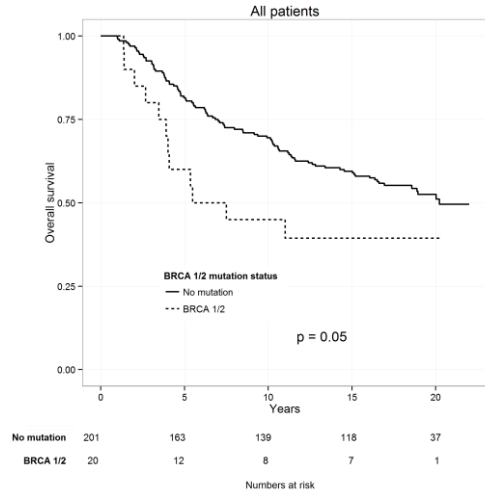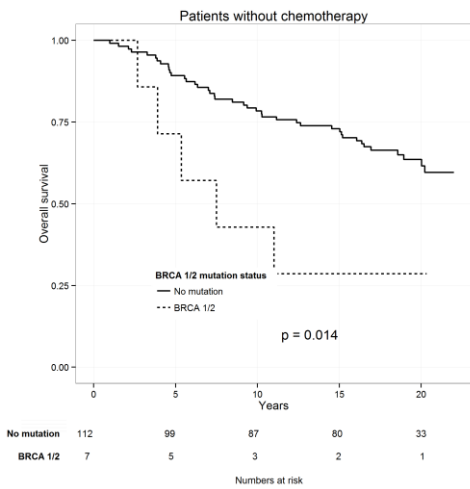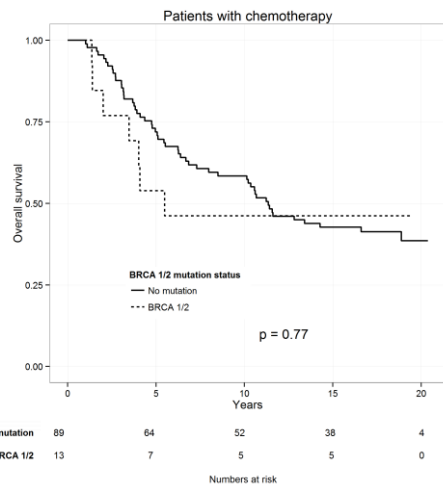
Figure 2

Figure 3

Figure 4

Figure 2. Overall survival for all patients

Figure 3. Overall survival for patients not treated with chemotherapy

Figure 4. Overall survival for patients treated with chemotherapy

The multivariable analyses in our study should be interpreted with caution due to small numbers and missing values, making proper adjustments for all prognostic factors impossible. The inferior OS for mutation carriers remained significant when adjusting for tumor stage, age, and chemotherapy, but not when ER status was also included in the model.

Our study confirmed the results of multiple previous studies regarding a high risk of CBC in mutation carriers with early-onset breast cancer; the 15-year risk of CBC was 53% for mutation carriers and 10% for non-carriers. Of note, the analysis regarding CBC in mutation carriers was based on only 4 events, adding much uncertainty to the point estimate. Among non-carriers, the family history of cancer modified the risk of CBC; the 15-year risk was 5%, 22%, and 30% for patients without close relatives with breast cancer, for patients with second-degree relatives with breast cancer, and for patients with first-degree relatives with breast cancer, respectively. A relatively large impact of family history on CBC risk suggests that this group of patients with early-onset breast cancer without an identified BRCA mutation is likely to include some individuals with mutations in other breast cancer genes.

Cancers where late recurrences are not unusual - such as breast cancer – require long follow-up in order to properly assess survival. However, cancer treatments are rapidly improving and evolving, so therefore, to some extent, observational studies of today are only offering evidence of yesterday´s treatments. Of note, the indications for adjuvant chemotherapy are nowadays mainly based on tumor characteristics rather than tumor stage. Because BRCA-associated tumors are usually luminal B or triple-negative, most young breast cancer patients with germline BRCA mutations will today receive chemotherapy, regardless of whether their mutation status is known or not.

Due to small numbers, *BRCA1* (n = 16) and *BRCA2* (n = 4) mutation carriers were grouped together in our study cohort. If the prognostic and predictive value of mutations is not the same for *BRCA1* and *BRCA2*, the results of our study are probably most applicable to *BRCA1* mutation carriers.

Paper II

The mean follow-up for OS was 12.9 years for patients alive at the end of follow-up. Some time period trends were seen: BCT was common in 1990-1999, whereas mastectomy was more common both before and after that. Tumors treated with mastectomy were more often stage III and less often stage I compared to tumors treated with BCT. Patients treated with mastectomy more often received adjuvant

or neoadjuvant chemotherapy (59% vs 42%) and adjuvant endocrine therapy (37% vs 13%). In both groups, 67% underwent a bilateral oophorectomy.

Following mastectomy, all LR were seen in the first 5 years. As opposed to this, following BCT the rate of LR continued to be high also after the first 5 years. The cumulative risk of LR in the BCT group was 15%, 25% and 32% after 5, 10 and 15 years, respectively (Figure 5). In our study, we were not able to differentiate a true local recurrence from a new primary breast cancer in the treated breast. Based on the results of other studies, it is likely that a majority of the ipsilateral events in the BCT group were in fact second primary tumors rather than true recurrences [110]. Second primary tumors might influence the survival to a lesser extent than true recurrences from aggressive tumors. Compared to mastectomy, BCT was associated with an increased risk of LR in univariable analysis (HR 4.0; 95% CI 1.6-9.8) and in multivariable analysis adjusting for tumor stage, age, and use of chemotherapy (HR 2.9; CI 1.1-7.8). In this multivariable model, younger age was associated with a higher risk of LR (< 43 years vs ≥ 43 years: HR 2.7; CI 1.0-7.6) and use of chemotherapy resulted in a point estimate below 1 but a wide confidence interval (HR 0.6; CI 0.2-1.7).

Since ipsilateral events where compared between BCT and mastectomy in our study, we denoted them "local recurrences". Some other studies have denoted ipsilateral events following BCT "in-breast tumor recurrence" (IBTR). Most of the studies to date have not differentiated between true recurrences and new primary breast tumors, and IBTR usually refers to either of them. Supported to some extent by the results of our study, but even more so by the results of other studies (summarized in the Background of this thesis), it is important to note that not all mutation carriers are at the same risk of an IBTR. Factors that have been found to decrease the risk of IBTR in previous studies include oophorectomy, chemotherapy, and a higher age at breast cancer diagnosis. These factors might decrease both the risk of true recurrences and the risk of new primary tumors in the ipsilateral breast, and need to be taken into account when the results regarding IBTR from different studies are compared. Also, the ascertainment of the cohort needs to be considered. We point out that the cohort in our study consisted of mutation carriers ascertained through a Hereditary cancer unit. Consequently, a majority of them belonged to families with multiple cases of cancer. Under a model where the risk of new primary breast tumors is influenced by modifying factors, the risk of IBTR is higher for these mutation carriers than for mutation carriers ascertained through population-based programmes.

No significant differences in survival endpoints were seen for patients treated with BCT compared to patients treated with mastectomy, neither in univariable nor in multivariable analyses (Figure 6). However, we emphasize that the survival analyses in this study should be interpreted with caution. Important baseline

characteristics were not balanced between the groups and the cohort is not large enough to properly adjust for all possible confounders. Furthermore, in observational studies on surgical decisions, bias can never be fully accounted for.
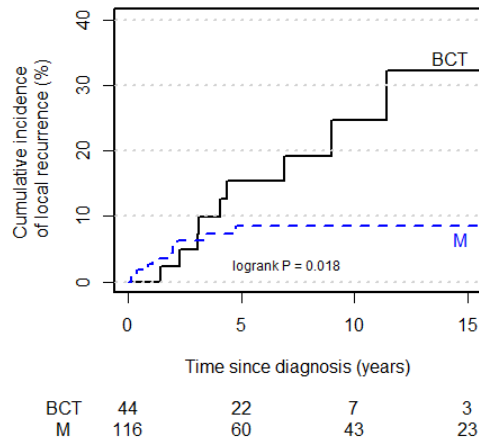

Figure 5. Cumulative incidence of local recurrence as first recurrence by type of surgery (*BCT*, breast-conserving therapy; *M*, mastectomy)
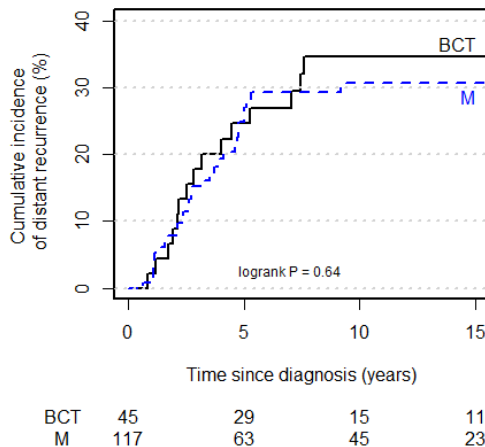

Figure 6. Cumulative incidence of distant recurrence by type of surgery (*BCT*, breast-conserving therapy; *M*, mastectomy)

Paper III

According to the family history as it was documented by the treating physicians at time of diagnosis, 11 out of 20 BRCA mutation carriers fulfilled the Swedish BRCA testing criteria. For 3 of these patients, a BRCA mutation was already known in the patient or in the family at the time of breast cancer diagnosis. Of the remaining 8 patients, 6 were referred for genetic counseling by the treating physicians. Two of those 6 patients did not attend genetic counseling. Out of 4 patients attending genetic counseling, one was not tested and 3 were tested (all three were *BRCA1* mutation carriers).

Upon retrospectively thoroughly assessing the family history of cancer at the time of breast cancer diagnosis, it was revealed that another 2 patients actually fulfilled the Swedish BRCA testing criteria, although this had not been documented in the medical records by the treating physicians at the time of breast cancer diagnosis. Accordingly, 13 out of 20 mutation carriers (65%) actually fulfilled the Swedish BRCA testing criteria at the time of breast cancer diagnosis. Excluding the three patients in whom a mutation was already known at the time of diagnosis, 17 mutation carriers remained that could potentially have been identified through routine health care. As detailed above, only 3 of them were identified as probands and subsequently underwent testing, corresponding to an effectiveness of the testing criteria of only 18%. Table 2 lists the reasons for mutation carriers in our cohort not being tested as probands through routine health care. As the table clearly shows, the currently used procedure of BRCA testing involves multiple steps, all of whom have to be passed in order for a breast cancer patient to be tested for BRCA mutations. In order to be able to identify more mutation carriers among breast cancer patients, the clinical routines for BRCA testing – at least the routines used in Sweden, but very likely also the routines used in other countries – need to be critically revised.

Table 2. Reasons for BRCA mutation carriers not tested as probands following breast cancer diagnosis.

n	Reason for not being tested as proband
7	Did not fulfil the Swedish BRCA testing criteria
2	Family history not reported/assessed/documented correctly in the medical records, thus appeared as not fulfilling the Swedish BRCA testing criteria
3	BRCA mutation already known in the patient/family
2	Not referred for GC
2	Referred for GC, but did not attend GC
1	Attended GC, but not tested

GC, Genetic counseling

The Swedish BRCA testing criteria are more strict than in many other countries. As an example, 18 out of 20 mutation carriers in this cohort fulfilled the NCCN BRCA testing criteria [217].

The small sample size is a limitation of our study, adding uncertainty to the point estimates. It should also be noted that the patients in our cohort were diagnosed with breast cancer almost a decade ago. As summarized in the Background of this thesis, others have found that the uptake of genetic testing has increased over the last decade. Our results can therefore not automatically be generalized to present circumstances.

Paper IV

The invitation letter was given to 818 patients with newly diagnosed breast cancer. The mean age was 63.6 years (median age: 65.4 years), which is close to the mean age of all breast cancer patients in Sweden. Through Jan 31, 2017, five-hundred and forty-two (66.2%) consented to analysis of *BRCA1* and *BRCA2*. Among consenting patients, 459 (84.7%) consented without a reminder, and 83 (15.3%) consented following a reminder. Following the reminder, the uptake of genetic testing in our study thus increased from 56.1 to 66.2%. In a previous study from Norway, that was similar to our study in design, the uptake of genetic testing was only 45.4%. The lower uptake compared to our study could partly be due to no reminder in the Norwegian study, but also other factors related to study design and awareness of hereditary breast cancer might account for the difference in uptake rates.

Eleven pathogenic mutations were found (*BRCA1*, n = 2; *BRCA2*, n = 9) in 542 tested patients (Table 3). In addition, there were two patients who were assessed for eligibility that were already known BRCA mutation carriers. The prevalence of BRCA mutations among unselected breast cancer patients can be assessed in different ways within a single cohort, yielding slightly different results. Among the 542 patients tested within our study, the prevalence was 2.0% (CI 1.1-3.6%). Including the two patients who were already known mutation carriers at the time of breast cancer diagnosis, the prevalence was 2.4% (CI 1.4-4.1%). Factors increasing the likelihood of finding a mutation – such as younger age, family history of cancer, etc. – are probably more common among tested patients than among patients not consenting to analysis. Consequently, the prevalence of BRCA mutations among all breast cancer patients might be somewhat lower than these estimates.

Table 3. Characteristics of mutation carriers (n = 11)

Mutation (HGVS)	Age at diagnosis (years)	St.Gallen subtype	TNM stage (AJCC 7th Edition)
BRCA2 c.9580_9581delCC	70	LumB HER2-	T1N0M0
BRCA2 c.8575_8575delC	49	LumB HER2-	T1N1miM0
BRCA1 c.1687C>T	46	Basal	cT2N0M0; ypTisN0**
BRCA2 c.5946_5946delT	68	LumB HER2-	T1N0M0
BRCA2 c.6267_6269delGCAinsC	63*	LumB HER2-	cT2N1M0; ypT0N0**
BRCA2 c.8953+1G>T	65	LumB HER2-	T1N0M0
BRCA2 c.4258_4258delG	47*	LumB HER2-	T1N2M0
BRCA2 c.4258_4258delG	72	LumB HER2-	T1N0M0
BRCA1 c.1687C>T	57*	LumB HER2-	T1N0M0
BRCA2 c.4258_4258delG	68*	LumB HER2-	cT2N1M0; ypT0N0**
BRCA2 c.5219_5219delT	40	LumB HER2+	T1N0M0

* Previous diagnosis of breast cancer in the contralateral breaast; age refers to age at diagnosis of the second primary breast cancer

** Neoadjuvant chemotherapy

Multiple factors influence the prevalence of BRCA mutations among breast cancer patients, and previous studies have yielded diverse results. The prevalence is increased in populations with strong founder mutations. Sweden is a country without strong founder mutations; families carrying the five most recurrent mutations account for ~23% of the total number of families with a BRCA mutation in Sweden (Å Borg, personal communication). The prevalence is also increased in breast cancer cohorts selected for younger age and certain tumor characteristics. In contrast to most previous studies of comprehensive analysis of both the *BRCA1* and *BRCA2* gene (not only panel testing for recurrent mutations), our study cohort is close to population-based. Another factor that needs to be taken into account is the uptake of prophylactic mastectomy among known mutation carriers in the population. In the catchment area of the participating hospitals in our study, 102 known living female mutation carriers had undergone prophylactic mastectomy by Feb 2, 2015, when the study started (*BRCA1*, n = 67; *BRCA2*, n = 35), and were thus very unlikely to get breast cancer. The ratio of *BRCA1* to *BRCA2* mutations (ratio ~ 2:1) among these known mutation carriers, of whom a majority belong to families that have fulfilled the clinical BRCA testing criteria to merit testing, is in contrast to the ratios presented in Paper III and Paper IV of this thesis. Among unselected breast cancer patients in Sweden, *BRCA2* mutations seem to be at least as common as *BRCA1* mutations, but the low number of mutation carriers in our cohorts precludes any firm conclusions.

The mean age of the eleven mutation carriers previously not identified was 59.2 years, which is only marginally lower than the mean age of all tested patients in the study (mean age: 61.8 years). However, four of these mutation carriers had

previously been diagnosed with a breast cancer in the contralateral breast. Six out of 11 fulfilled the Swedish BRCA testing criteria and 9 out of 11 fulfilled the NCCN BRCA testing criteria. Combining the cohorts in Paper III and Paper IV, the efficacy of the Swedish BRCA testing criteria was 61%, and the efficacy of the NCCN BRCA testing criteria was 87%.

Out of 542 tested patients, only eleven (2.0%) contacted us for questions related to genetic counseling, and nineteen (3.5%) contacted us for practical questions. In other words, a great majority went through the whole process of pre-test written information, genetic testing, and receipt of the test results without contacting us at all. The results presented in Paper IV do not offer any information on whether these study participants did not contact us because they felt that they had already received sufficient information to make an informed decision on testing, or whether they did not fully comprehend what kind of analysis they were consenting to. In a follow-up part of BRCAsearch, we are currently sending out questionnaires to study participants in order to evaluate their attitudes towards the procedure used for genetic testing within the study.

The median time from the breast cancer diagnosis to the delivery of the test result was approximately 3 months in BRCAsearch. Treatment decisions on neoadjuvant/adjuvant chemotherapy were therefore not affected by mutation status. Despite that, nine out of eleven mutation carriers received chemotherapy, reflecting that none of the mutation carriers had a luminal A tumor. Decisions regarding treatments other than chemotherapy were changed in four cases following information about a positive mutation status; two patients opted for bilateral prophylactic mastectomy (BPM) instead of postoperative radiotherapy following BCT, one patient was included in an adjuvant PARP inhibitor trial, and one patient with a small (T1b) luminal B tumor was prescribed an aromatase inhibitor. Through Feb 15, 2017, ten mutation carriers have opted for risk-reducing salpingo-oophorectomy (RRSO), and nineteen relatives have undergone predictive testing; eleven of whom have turned out to be mutation carriers. Given the short follow-up, the number of identified mutation carriers in these families is expected to increase.

Conclusions

- The long-term survival is inferior for BRCA mutation carriers compared to non-carriers diagnosed with early-onset breast cancer in the 1990s.

- The inferior prognosis for mutation carriers compared to non-carriers is abrogated by the use of chemotherapy, supporting the notion that all – or almost all – mutation carriers with early breast cancer should be offered chemotherapy.

- Mutation carriers diagnosed with breast cancer < 41 years of age are at a high risk of metachronous contralateral breast cancer, supporting that contralateral prophylactic mastectomy should be discussed with them.

- Mutation carriers treated with breast-conserving therapy, and who resemble our cohort regarding ascertainment, age, adjuvant treatment and uptake of oophorectomy, have a high risk of in-breast tumor recurrence, many of which are probably in fact new primary breast tumors.

- The real world performance (effectiveness) of the Swedish BRCA testing criteria is far lower than the sensitivity (efficacy) of those criteria. Therefore, currently used clinical BRCA testing routines need to be critically revised.

- Written pre-test information without in-person pre-test genetic counseling is associated with an uptake of genetic testing in approximately 2/3 of the patients.

- The prevalence of *BRCA1/2* mutations among unselected breast cancer patients in southern Sweden is in the range of 1-4%.

- Among unselected breast cancer patients in southern Sweden, *BRCA2* mutations are at least as common as *BRCA1* mutations.

To summarize, the results of the work presented in this thesis indicate that germline BRCA status could contribute to personalized treatment decisions for breast cancer patients, and consequently, the results lend some support to the idea that breast cancer patients should be offered BRCA testing at the time of diagnosis. The procedure for BRCA testing used in the BRCAsearch study offers an example of how genetic testing could be undertaken on a large scale in a feasible way.

Future perspectives

In the next decade, the prognostic and treatment-predictive value of *BRCA1* and *BRCA2* mutations in breast cancer patients treated according to modern protocols will be further elucidated, mainly through subgroup analyses of randomized trials and long-term follow up of prospective cohort studies. Currently available evidence suggests that a great majority of the mutation carriers diagnosed with breast cancer should be offered adjuvant or neoadjuvant chemotherapy. Some questions regarding chemotherapy remain to be solved, though. For instance, should breast cancer patients with small tumors (< 5 mm), luminal B tumors 5-10 mm, or low-stage luminal A tumors – i.e. patients that are usually not treated with chemotherapy today – be offered chemotherapy if they turn out to be mutation carriers? Is standard anthracycline-containing chemotherapy good enough for mutation carriers in the adjuvant setting, or could the prognosis be improved even further by the addition of platinum agents? Do *BRCA2* mutations have the same prognostic and predictive impact as *BRCA1* mutations?

The paradigm of personalized medicine as the basis for cancer treatment has sparked much optimism during the past 10-20 years, but also disappointments, as quite a few approaches that have been promising in the preclinical setting subsequently have failed to improve survival outcomes in clinical trials. PARP inhibitors for the treatment of BRCA-deficient breast cancer is certainly very promising and randomized phase III trials are ongoing. While the results of these trials have to be awaited before any definitive conclusions are made, I consider it likely that PARP inhibitors will be approved for use in BRCA-associated breast cancer within the next 1-3 years in the metastatic setting, and within 7-15 years even in the adjuvant setting. If that will actually be the case, it will have a huge impact on genetic testing and genetic counseling of breast cancer patients. Testing patients on a larger scale will require a streamlining of the testing procedure. As the testing procedure changes, it is important to evaluate any psychosocial impacts conferred by the new methods. Furthermore, as more and more mutation carriers are identified, the health care system must allocate sufficient resources for genetic counseling, MRI-based screening programmes, and prophylactic surgeries.

Most of the studies to date on the treatment predictive value of BRCA mutations have been carried out in breast cancer or ovarian cancer patients. However, the impact on treatment efficacy is not limited to these types of cancer. Recent small

studies and case series have shown impressive response rates to PARP inhibitors and platinum agents in metastatic prostate cancer and pancreatic cancer. In contrast to most other types of cancer, adjuvant chemotherapy has not proven to be effective for prostate cancer patients in the general population. BRCA-associated prostate cancer makes up a distinct subgroup with an aggressive "BRCA-like" phenotype. A randomized trial evaluating adjuvant cisplatin to BRCA mutation carriers with early prostate cancer would be of great interest, but might be very hard to carry out due to power problems. As an alternative to randomized trials in this specific subgroup, evidence might be extrapolated from patients with "BRCA-like" tumors of other primary locations, or from studies in the metastatic setting.

Germline BRCA mutations differ from other treatment predictive factors in a very important way: information is not only obtained about the current cancer, but also about the risk of other cancers later in life, and the risk of cancer in relatives. Traditionally, testing has been offered to probands with early-onset cancer or with multiple cases of cancer in the family. Finding out a hereditary cause of their cancer has often not come as a total surprise to them. If all breast cancer patients are to be offered mutation testing, a proportion of the mutation carriers identified will be old and without any family history of cancer. The information about a germline BRCA mutation could come as a surprise or even a chock to them and their family members. The psychosocial impact of proactive BRCA testing in unselected cohorts is an area where more research is needed. Also, the penetrance of BRCA mutations identified in older patients without a family history of cancer needs more study. Due to genetic modifiers, the penetrance is probably lower in such families. More precise penetrance estimates are needed for proper genetic counseling. However, not only accurate penetrance estimates, but also research about the impact of individualized penetrance estimates on patient-related outcomes such as risk management and psychosocial adjustment is needed. In other words, does it matter for a person if she hears that her risk of breast cancer is 30-40% compared to if she hears that her risk is 80-90%?

Presymptomatic testing in family members, increased uptake of prophylactic surgeries, and modern cancer treatments have improved the survival for women carrying germline BRCA mutations, which is very satisfying. However, as more and more mutation carriers reach older ages, other questions arise. For example, what are the long-term effects of premenopausal oophorectomy and how could these long-term effects be mitigated? Should BRCA status influence the treatment or prevention of cardiovascular disease or other non-cancer diseases? With international collaboration, inclusion of patients into randomized trials, and close follow-up within prospective cohorts, these and other important questions could be answered in the future. The long-term goal is that a person carrying a germline mutation in *BRCA1* or *BRCA2* should have the same life expectancy and quality of life as a person in the general population.

Populärvetenskaplig sammanfattning

Bröstcancer är den vanligaste cancerformen för kvinnor i Sverige. Risken att drabbas någon gång under livet är drygt 10%. I de flesta fallen vet man inte varför en kvinna drabbas av bröstcancer, men i en minoritet av fallen finns en förklaring i form av en medfödd förändring i någon gen – man talar då om ärftlig bröstcancer. Hos vissa personer med ärftlig bröstcancer kan man identifiera exakt vilken gen och vilken mutation i den genen som är orsak till cancern. En mutation i en gen är att jämföra med ett stavfel. Stavfelet gör att genen inte kan läsas av korrekt och genens funktion faller då i de flesta fall bort. De viktigaste generna för ärftlig bröstcancer är *BRCA1* och *BRCA2*. Alla människor bär på två kopior av *BRCA1*, en som man ärvt från sin mor och en som man ärvt från sin far. På samma sätt bär alla människor på två kopior av *BRCA2*. Mutationsbärare har en normal kopia av genen och en muterad kopia av genen i varje cell i kroppen (förutom i könscellerna som antingen bär på den muterade eller den normala kopian). Den normala kopian räcker till för att funktionen av genen ska vara normal eller nästan normal. Om den andra kopian i en cell inaktiveras någon gång under livet, exempelvis genom en förvärvad mutation, kommer genfunktionen emellertid att slås ut i den cellen. Både *BRCA1* och *BRCA2* behövs för att laga skador i arvsmassan. Celler som helt saknar *BRCA1* eller *BRCA2* kommer att ha kraftigt försämrad förmåga att laga de skador i arvsmassan som kontinuerligt uppstår i levande celler. Cellerna löper då hög risk att utvecklas till cancerceller.

Mutationer i *BRCA1* eller *BRCA2* ärvs genom så kallad dominant nedärvning. Det betyder att ett barn till en mutationsbärare har 50% risk att ärva mutationen. En kvinnlig mutationsbärare har en kraftigt förhöjd risk att drabbas av vissa typer av cancer. Risken för bröstcancer är 50-80% och risken för äggstockscancer är 30-60% (*BRCA1*) respektive 10-20% (*BRCA2*). Manliga mutationsbärare har också en ökad risk för vissa typer av cancer, men inte alls i samma utsträckning som sina kvinnliga släktingar. Med anledning av de höga riskerna för cancer erbjuds mutationsbärare speciella kontrollprogram vars syfte är att diagnosticera cancer i ett så tidigt stadium som möjligt, för att därigenom öka chanserna till bot. Ett alternativ till kontrollprogram är att genomgå förebyggande operationer, d.v.s. att i förebyggande syfte operera bort äggstockarna och brösten. Om en mutationsbärare låter operera bort sina äggstockar i förebyggande syfte då familjebildningen är avslutad blir hennes förväntade livslängd ungefär som för vilken kvinna som helst. Det finns

uppenbara fördelar med förebyggande operationer, men naturligtvis också potentiella nackdelar, varför ett sådant beslut måste föregås av både betänketid och stöd från sjukvården. För personer som själva tidigare haft cancer, eller som på nära håll sett cancersjukdomens fasor, kan beslutet dock ofta vara mindre dramatiskt.

En förutsättning för att botas från bröstcancer är att kirurgen opererar bort cancern. Det kan göras på två sätt: antingen opereras hela bröstet bort (mastektomi), eller så opereras en bit av bröstet bort (bröstbevarande kirurgi). För att maximera chanserna till bot, så följs operationen nästan alltid av en eller flera onkologiska tilläggsbehandlingar. Dessa kallas vanligen för adjuvanta, eller förebyggande, behandlingar. De vanligaste adjuvanta behandlingarna är cellgiftsbehandling, antihormonell behandling, och strålbehandling. Tack vare förbättrad kirurgi och modern adjuvant behandling så botas numera ca 80% av dem som drabbas av bröstcancer.

Den aktuella avhandlingens övergripande mål var dels att studera hur bröstcancer hos mutationsbärare ska behandlas på ett så bra sätt som möjligt, och dels att undersöka nya metoder för att identifiera fler mutationsbärare bland bröstcancerpatienter. Som det är idag så testas nämligen inte alla bröstcancerpatienter för BRCA-mutationer. Bara de som uppfyller vissa kriterier erbjuds testning. Kriterierna baseras på patientens ålder (mutationer är vanligare hos yngre patienter) samt om det finns flera fall av cancer i släkten.

I den första artikeln utgick vi ifrån en grupp av patienter som drabbades av bröstcancer i ovanligt ung ålder (före 41 år) under åren 1990-1995, varav samtliga hade testats för BRCA-mutationer. Var tionde av dem bar på en mutation i *BRCA1* eller *BRCA2*. Med nästan 20 års uppföljning kunde vi jämföra överlevnaden mellan mutationsbärare och icke-bärare. Det visade sig att mutationsbärare hade sämre överlevnad, vilket sannolikt beror på att själva tumörerna hos mutationsbärare ofta är av en extra aggressiv typ. Emellertid så var det endast mutationsbärare som inte behandlats med cellgift adjuvant som hade sämre överlevnad; de mutationsbärare som fått cellgift adjuvant hade samma överlevnad som icke-bärarna. Det verkar alltså som att cellgift kan balansera upp den försämrade överlevnaden. Konklusionen blir att det är extra viktigt för mutationsbärare att få cellgift adjuvant.

I den andra artikeln utgick vi ifrån en grupp av mutationsbärare med bröstcancer. Vissa av dem hade opererats med mastektomi, medan andra hade opererats med bröstbevarande kirurgi. Det främsta syftet med studien var att jämföra risken för återfall av bröstcancer på platsen för den första cancern, antingen på bröstkorgsväggen efter mastektomi eller i samma bröst efter bröstbevarande kirurgi. De patienter som opererats med bröstbevarande kirurgi hade en hög risk för återfall av cancer i det opererade bröstet; risken var 32% efter 15 år. Sannolikt är minst hälften av dessa "återfall" inte verkliga återfall av den första cancern, utan helt nya bröstcancrar. Konklusionen blir att bröstbevarande kirurgi kan vara ett mindre bra

alternativ just för mutationsbärare, eftersom de har så hög risk för helt nya cancrar, även i samma bröst.

Som nämnts ovan så finns det i dagsläget speciella kriterier för vilka som ska erbjudas testning avseende BRCA-mutationer. Syftet med den tredje artikeln var att studera hur pass bra dessa kriterier är på att identifiera mutationsbärare bland bröstcancerpatienter. Vi utgick ifrån en grupp av patienter som frivilligt gått med i en forskningsstudie i samband med att de behandlades för bröstcancer i Malmö under åren 2007-2009. Som en del av den forskningsstudien så gjordes år 2014 analyser avseende mutationer i *BRCA1* och *BRCA2*. Det visade sig att 20 av studiedeltagarna var mutationsbärare. I efterhand så tog vi kontakt med dem som inte redan kände till att de var mutationsbärare, för att delge dem denna viktiga information. Trots att 13 av mutationsbärarna (65%) uppfyllde kriterierna för testning så hade färre än hälften av de personerna blivit remitterade för testning och de facto blivit testade i samband med sin bröstcancer. Konklusionen blir att kriterierna för testning missar att upptäcka vissa mutationsbärare, men att ännu fler mutationsbärare undgår upptäckt trots att de egentligen uppfyller kriterierna för testning. Om man vill identifiera fler mutationsbärare måste sålunda inte bara kriterierna ändras, utan även implementeringen av själva proceduren för testning måste förbättras. Ett sätt att vidga kriterierna och förenkla proceduren som omger testningen är att helt enkelt erbjuda samtliga bröstcancerpatienter mutationsanalys.

Tidigare har genetisk testning alltid föregåtts av så kallad genetisk vägledning. Vid genetisk vägledning får patienten och hennes släktingar träffa en läkare eller en specialutbildad sjuksköterska under ca 1 timme för att tala om testningens fördelar och nackdelar, möjligheter och implikationer. Genetisk vägledning är välbeprövat och välfungerande. Eftersom bröstcancer är en så pass vanlig sjukdom så finns det emellertid inga praktiska möjligheter att erbjuda alla bröstcancerpatienter dylik genetisk vägledning. Vidare kan man ifrågasätta om det är nödvändigt, eller ens lämpligt, att tala om ärftlighet under 1 timme med en person som just opererats för cancer. För de allra flesta kommer ju samtalet att vara i onödan, eftersom det bara är en liten minoritet som visar sig vara mutationsbärare. I den fjärde artikeln presenteras resultaten av en studie som vi själva initierat och genomfört. Studien kallas BRCAsearch. Inom ramen för BRCAsearch så erbjöds under perioden februari 2015 – augusti 2016 samtliga bröstcancerpatienter i Helsingborg, Kristianstad och Malmö BRCA-testning. Istället för vanlig genetisk vägledning före testning så fick de skriftlig information via ett informationsbrev som delades ut av sjuksköterskan på bröstkirurgen ungefär en vecka efter operationen. Patienterna fick även ett telefonnummer att ringa om de hade ytterligare frågor, d.v.s. behov av vanlig genetisk vägledning. Informationsbrevet delades ut till 817 patienter. Av dessa så tackade 542 (66%) ja till genetisk analys. 11 av dem visade sig vara bärare av en mutation i *BRCA1* eller *BRCA2*. Endast en liten andel av patienterna kontaktade oss per telefon för frågor. Konklusionen blir att det är genomförbart att

tillhandahålla information om genetisk testning på ett förenklat vis, för att därigenom kunna erbjuda ett mycket större antal patienter testning. Vidare visar studien att ca 2-3% av bröstcancerpatienterna i Skåne bär på en medfödd mutation i *BRCA1* eller *BRCA2*.

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