Hereditary breast cancer in South-Eastern Norway

BRCA1/2- testing of breast cancer patients.
Mutation spectrum and potential modifiers in Norwegian BRCA1/2 carriers

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1. PREFACE

Before I started the PhD-program leading to this thesis, I had an idealistic or naïve idea that embarking on a scientific carrier could be a way to do good. To qualify as a scientist, develop an ability to pinpoint problems, develop research projects to better understand those problems, or even solve them! It all seemed to be a way of working towards better care and better medicine, even a better and more just world! I pictured the scientific community to be a place where ideals of honesty, truthfulness and humility would be highly valued. I had worked some years as a medical doctor at the time, in differing fields like psychiatry, general medicine, youth medicine, gynecology, obstetrics, as well as clinical and cancer genetics. In the work as a medical doctor, and in trying to be a good one, which is important and difficult, I thought it was necessary to have a thorough academic understanding of all the different subjects studied in medical school. But as much as to have a sound grasp of “the overall picture” (in 20-30 different subjects, each one the focus of numerous PhD-programs), you must know important details, and understand when you are outdated. You also need to be practical and present. In communicating with the patients, you need to act thoughtfully, considerate and caring, listen, and not necessarily tell everything you know or think. You need to build the patient’s trust to be able to help them. You also need to be alert, a systematic data-collector like the lead detective solving a serial murder mystery. “Find the real diagnosis, or it may strike again, and more devastating next time!” Most of the clues are here… you “only” need to think about them, detect them, know what they mean and so forth.

Being a medical doctor already, which was to me both meaningful and rewarding, why study even more? The world can probably do very well without every doctor becoming a researcher? The circumstances, however, gave me an opportunity to look deeper into hereditary breast cancer, and as I had just started working with this group of patients, I felt this was an area were the modern, theoretical medicine really could make a difference in preventing early death from cancer, in young women, young mothers, or even grandmothers. Families suffering from a lot of cancers are often looking for answers that can help other family members live their lives without being too scared about getting cancer themselves. All this is very easy to understand. If some of this familiar cancer is hereditary and preventable through controls or even prophylactic surgery, many of family members will seek such opportunities. Meeting the women and families carrying these mutations made me realize how essential it is to have trustworthy information showing that the measures are really
needed. Also, being able to reassure a family that they do not have an elevated high risk for getting cancer is equally valuable as both situations may give both better management and life quality.

As the work started, I soon realized it is not easier to do good in the scientific world than other places. As with many things it all depend on “the eyes of the beholder “, the perspective you are able to see or are given the opportunity to see. It also depends on hard work and some kind of personal sacrifice. Trying to understand all the relevant research done by others in the same field of research but also in related ones (in this case statistics and molecular genetics of course, as well as bioinformatics, epidemiology) is not far from going straight from kindergarten to medical school. Certainly, one will be able to grasp the essentials, “this person has a broken leg”, “this person has a rash in their face”, but the details, the abundance of information, articles, books, and all different terminology for all the disciplines, the basics AND details in another 20-30 new subjects are overwhelming. Is it even possible to do any good at all...?

Medical research needs to be translated into practical medicine, and the main aim behind research in cancer genetics is to serve the families with hereditary cancer better. To give advice in these situations, I think doctors, like me, working in this field have an obligation to understand the research on behalf of the patients, individually, but also collectively. Some patients will be just as suited to understand the complex research in the field as us, but the majority won’t. This is a problem for autonomy, and in modern medicine, which in many ways are thought to be less patriarchal than the previous eras of medical tradition, the public still need to trust it’s caregivers’ scientific understanding and knowledge. Therefore, the caregivers must REALLY do an effort to be worthy of that trust. Maybe, the skepticism towards medical “truth” as it is shown in e.g. anti-vaccine campaigns has its roots in the fact that to many members of the public, the scientific world is hard to understand, and its messengers may be unclear or say seemingly contradicting things. The field of medical genetics is aiming at giving evidence-based information, to patients or healthy family members about their disposition and risks for disease. The goal is to enable them to prevent complications and sickness, but the message needs to get through.

My distinct impression as a medical doctor, is that patients facing serious illness as well as people wanting to make right decisions for their health are very well able to pinpoint their
interests and ask very good questions, often with a very well-functioning bullshit detector. As a medical doctor in cancer genetics, a practical approach is necessary, and I must be able to communicate my understanding of the research in a way that is understood by the patients and public, and seem useful to them.

As a Philosophical Doctor in the field of Medical Cancer Genetics I must also be able to translate these practical interests of the patients having hereditary, increased risk for cancer into new, good research projects, and read other researchers projects with humility but also expecting them to make it possible for me to understand their research. After all, we are all part of a scientific community, exploring “the truth” on behalf of the public, and our patients. Research in medical genetics requires cooperation between many different specialists, all talking and understanding their own language/dialect/sociolect the best. It is not as easy “to do good”, as I hoped it to be, maybe I was wrong believing that research somehow is inherently good. But it is still the goal for me, personally; at least never give up trying, whether as MD or PhD or “just me”. And that is the personal background for this thesis. It has indeed a long and winding road, not at all stream-lined, a marathon, but now I’ve hopefully reached the stadium. I hope you will enjoy it.
1.1 Acknowledgements

I want to place a great THANK YOU to everyone having had a role in developing this project, and keeping spirits up through the less stream-lined phases of this PhD-period, never giving up on me. I could not have accomplished this without you.

Firstly, I want to thank Lovise for being my main supervisor, as well as an extremely hard-working, knowledgeable colleague and leader. I also want to thank her for your patient and caring personality. I also want to thank everyone at Section of hereditary cancer, especially Teresia, Eli Marie, Kjersti for your supportive words and presence. I want to thank the research group on inherited cancer and Pål Møller, for developing the modifier project, AMG, avdeling for medisinsk genetikk, Dag, Ketil, Sarah and everybody contributing to the quality studies. I want to thank Seksjon for brystkreftbehandling OUS, represented by Ellen og Inga for making the study on diagnostic testing possible, as well as Jan Norum at UNN, University of Northern Norway for his contribution.

I want to thank everyone at Nasjonal kompetansetjeneste for kvinnehelse, Siri, Pernille, Ritsa, and the ever-changing group of PhD-students for educative presentations and seminars, Kathrine for marvelous teaching skills and inspired statistics lessons. I want to thank the University of Oslo for accepting the project and for practical support.

I want to thank my sponsors, Sanitetskvinnetene, represented by Elisabeth Swärd, and Extrastiftelsen for funding the project, as well as all co-authors; Eivind Hovig, Per Olaf Ekstrøm, Kukatharmini Tharmaratnam, Pål Møller, Sarah Louise Ariansen, Eli Marie Grindedal, Dag Erik Undlien, Ellen Schlichting, Inga Karsrud, Jan Norum, Ketil Riddervold Heimdal, Sheba Lothe, Teresia Wangensteen and Lovise Olaug Mæhle for your work, time and effort.

I want to thank the patients for participating, all my colleagues in AMG for taking an interest and showing support during the various phases of the project, and co-supervisors Eirik Frengen and Sophie D. Fosså.

One last, very special “thank you” goes to Matilde, my kind daughter, for your good humor, presence and warm personality. I am also very grateful for the support from the rest of my family and like to thank all my kind, supportive friends for being present and interested.
1.2 Abbreviations/ terms explained

**BRCA1/2** - Breast Cancer genes 1 and 2
DMG - Department of Medical Genetics
OUH - Oslo University Hospital
SHC - Section of Hereditary Cancer
CSGs - Cancer Susceptibility Genes
ER - Estrogen Receptor
PR - Progesterone Receptor
HER2 - Human Epidermal growth factor receptor 2
HBC - Hereditary Breast Cancer
FBC - Familial Breast Cancer
HBOC - Hereditary Breast and Ovarian Cancer
SNP - Single Nucleotide Polymorphism
CIMBA - The Consortium of Investigators on Modifiers of *BRCA1/2*
ACMG - American College of Medical Genetics
ENIGMA - Evidence-based network for the interpretation of germline mutant alleles
EMBRACE - Epidemiological Study of Familial Breast Cancer
NBCG - Norwegian Breast Cancer Group
NICE - The National Institute for Health and Care Excellence
ASCO - American Society of Clinical Oncology
NCCN - National Comprehensive Cancer Network
NICE - The National Institute for Health and Care Excellence
SERHA - South-Eastern Norway Regional Health Authority Trust

Variant - This term is used for any change in DNA, for variants of all classes.
Mutation - This term is used for class 4 and 5 variants
VUS - Variant of unknown significance.
Relative risk - Ratio of two probabilities, p1 / p2 or (a /a +b) / (c /c + d)
Odd’s ratio - Ratio of the odds in two groups, p1 / (1-p1) / p2 (1- p2) or (a / c) / (b / d).
1.3 List of papers/publications


2. GENERAL INTRODUCTION

The breast cancer susceptibility genes, \textit{BRCA1} and \textit{BRCA2}, are well-known for being associated with substantially increased cancer risk in women, mainly breast and ovarian cancer (1). \textit{BRCA1/2} testing is one of the earlier genetic tests performed in our lab, starting up in the nineties. Now, in 2017, about 300 \textit{BRCA1/2}-tests are done each year/month in the laboratory of Department of medical genetics (DMG), Oslo University Hospital (OUH), and this testing constitute almost 50% of the activity in the laboratory unit of Cancer Genetics. During the fall of 2017, when this thesis was written, \textit{BRCA1/2} mutation carrier number 4000 was identified in Section for Hereditary Cancer (SHC), and 1163 \textit{BRCA1/BRCA2} families had been registered in total (personal communication).

The benefits of identifying mutation carriers and preventing cancer have been established through many different cost-efficiency analyses. The exact savings vary between different models of economic analysis, test strategies as well as between health systems (2-8). In general - the efficiency is linked to the number of \textit{healthy carriers} identified by the testing strategy, i.e. carriers that have not developed cancer yet (2). Identifying mutation carriers prior to cancer development is crucial to benefit from the established prophylactic procedures, while testing breast cancer patients diagnostically is important to tailor new treatment options related to \textit{BRCA}-status (9). The effect of the prophylactic measures is considered to have high impact on increasing life-years (10).

The field of medical cancer genetics is young and rapidly developing, potentiated by more and more efficient genetic testing techniques. Because of this, panel testing for Cancer Susceptibility Genes (CSGs) may in many cases be just as feasible as testing for a few, selected genes, such as \textit{BRCA1/2}. Panel testing may seem to be an efficient alternative for identifying more carriers of different breast cancer disposition genes and rarer variants in the chosen genes, if a founder mutation testing approach is considered too narrow (11). When implementing a broader clinical search for the \textit{different} genetic causes for hereditary breast cancer it nevertheless seems crucial to evaluate to what degree all patients, or all possible carriers, benefit from the evidence-based cancer prevention potential available through \textit{BRCA1/2} testing in today’s clinical practice. It is essential both to have good strategies for an efficient identification of individuals with hereditary cancer risk, as well as making the individual cancer risk prediction for mutation carriers as precise as possible.
This thesis is looking at hereditary breast cancer in South-Eastern Norway, with three major topics in focus:

1) Genetic testing of incident breast cancer patients; \textit{BRCA1/2} mutation frequency, breast cancer characteristics and sensitivity of \textit{BRCA1/2} testing criteria in a broader breast cancer population (paper 1).

2) Mutation spectrum of \textit{BRCA1/2} - an update on mutation distribution in a large cancer genetics clinic in Norway (paper 2).

3) Validation of potential modifiers of penetrance in Norwegian \textit{BRCA1} carriers (paper 3).

To get at grasp of the connection between the papers, let us consider the “thesis at a glance” on p.13. The three papers each represent three important steps in \textit{BRCA1/2} - testing and management.

Firstly, the criteria for \textit{BRCA1/2} testing must be fulfilled. Paper 1 discusses this process, and addresses the concern regarding “how many mutation carriers are lost” through the existing test approach, among other issues. Next, paper 2 is addressing the issue of \textit{BRCA1/2} mutation spectrum, and the same concern “how many mutation carriers are lost” is discussed. Paper 3 is discussing an issue with high relevance for all identified mutation carriers, especially prior to cancer development. May the cancer risk in Norwegian mutation carriers be affected by common genetic factors? If so, in turn, and if validated, may such evidence affect the individual selection process of prophylactic surgery, ideally facilitating a more informed choice?

The following introduction will include background information on different aspects regarding hereditary breast cancer in general, and give an overview on how hereditary and genetic breast cancer risk is studied scientifically as well as how it is determined clinically.
## Thesis at a glance

<table>
<thead>
<tr>
<th><strong>BRCA 1/2 test criteria</strong></th>
<th>Are test criteria fulfilled?</th>
<th>Diagnostic testing</th>
<th>Predictive testing</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>How many carriers are lost?</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BRCA 1/2 mutation test</strong></td>
<td>Mutation spectrum</td>
<td>Founder mutations</td>
<td>Rare variants</td>
<td></td>
<td></td>
<td>How many carriers are lost?</td>
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<tr>
<td><strong>BRCA 1/2 risk management</strong></td>
<td>Risk prediction</td>
<td>Genetic modification of risk</td>
<td>Individualization of risk</td>
<td></td>
<td></td>
<td>How to choose?</td>
</tr>
</tbody>
</table>

13
2.1 Breast cancer in Norway

Breast cancer is the most common cancer among women in Norway (12). The “Cancer in Norway”- report, published annually by Cancer Registry of Norway, states that in 2016, 3371 women and 31 men were diagnosed with breast cancer in Norway. The cumulated risk of developing breast cancer for a woman in the general population (by the age 75) was 8.6 %, (0.1 % for men). The five-year survival estimates have slightly increased from 88.6% (2007-2011) to 89.7% (2012-2016). Median age of developing breast cancer in women was 62 years in 2016. The increasing incidence of breast cancer since the beginning of registration is still largely unexplained, but external factors like hormone use, screening, and better diagnostic opportunities are all thought to be important.

2.1.1 Sporadic breast cancer - causes, treatment and prognosis

Several risk factors for breast cancer have been identified, each contributing to a different degree to cancer development (13). Breast cancer is considered to be a complex and multifactorial disease, and both environmental and genetic factors play important roles in disease development. Overweight, hormonal replacement therapy and alcohol are considered main environmental risk exposures that are modifiable (14). Overweight contributes to increased risk of post-menopausal breast cancer, and hormonal factors are one of several mechanisms (15). Low age at menarche, high age at first live birth, oral contraceptive use and high mammographic density are all factors shown to increase risk for sporadic breast cancer in the general population (13, 16).

Treatment in sporadic breast cancer is tailored according to tumor size, histological grade, location (spread), presence and levels of hormonal receptor status and markers ER/PR/HER2/Ki67 (17). Treatment is both supplementary (anti-hormonal), cytostatic and surgical.

Transcriptomal and genetic profiling of breast tumors are contributing to further classification of breast cancer into molecular subtypes, often divided into the following categories: Luminal-A, luminal-B, HER2-overexpression, basal- like and normal-like (18). The basal-like seem to be the subtype with the worst prognosis (19). A recent study from Danish Breast Cancer Group finds that transcriptome based subtyping of breast tumors is a valuable supplement for traditional immunohistochemistry profiling, that reduces the need for adjuvant
chemotherapy and also improves identification of women with predisposing mutations (20) Multiple breast cancer subtypes are also shown to coexist within one single tumor (21), and research on how such profiling of breast tumor tissue may be best applied in treatment is ongoing.

2.1.2 Breast cancer and inheritance
While the majority of breast cancers are sporadic, without an identifiable hereditary cause (22), ten percent of women with breast cancer have a family history of the disease (23). About 50 % of the familial aggregation is currently explained genetically (24), and up to 25-30 % are accounted for by the highly penetrant Mendelian genes, (BRCA1/2, PTEN, TP53) and these Mendelian genes follow dominant inheritance.

Breast cancer gene 1 and 2, BRCA1 and BRCA2, are the most frequently mutated of the high-risk genes and causes what is often called Hereditary Breast - and Ovarian Cancer syndrome, HBOC. The term hereditary breast cancer (HBC) is used in this thesis for breast cancer in persons or families having a demonstrable disease-causing variant in BRCA1/2. BRCA1/2 mutation carriers account for 20 % of familial aggregation of breast cancer, but less than 5 % of all breast cancers (22, 25). The term familial breast cancer (FBC) is used in this thesis for families with more than one case of breast cancer, but without an identifiable genetic cause, and such families represents 70-80 % of the familial aggregation.

The genes TP53 and PTEN are very rarely mutated, but when they are, they do give raise to Li-Fraumeni Syndrome and Cowden syndrome. These syndromes are characterized by high breast cancer risks, but also risk of other cancers, such as sarcomas and brain tumors (TP53) and endometrial cancer among others (PTEN). ATM, STK11, BRIP1, CHEK2 are considered moderate-penetrant breast cancer genes, but are not routinely tested for in a clinical context. Data are very limited on gene-specific penetrance and the cancer spectrum for these genes and others and are therefore not yet easy to use clinically (26, 27). The search for further highly penetrant breast cancer genes (i.e. BRCA3, BRCA4) has not succeeded (28).

Three well-defined classes of breast cancer susceptibility alleles with different levels of risk and prevalence in the population have become apparent: In addition to the rare high-penetrance alleles and rare moderate-penetrance alleles the common low-penetrance alleles have been identified through Genome-Wide Association Studies, GWAS (29, 30). (More on GWAS, see paragraph 2.4.1). The genetic units studied are single nucleotide polymorphisms
(SNPs), one type of common genetic variation. There is also an increasing understanding that the low-and medium penetrance susceptibility alleles may contribute to a hereditary disposition of breast cancer not caused by BRCA1/2, mainly in a polygenic manner (30-32), but there is some controversy to what degree such risk may translate into aggregation in families, mainly due to its polygenic nature.

With a common disease such as breast cancer, aggregation in families may also result from pure chance. Environmental factors may also contribute to familial aggregation in the general population, but twin studies have shown that genetic factors are more important than environmental factors in explaining cancer clustering in families (33). Some of the “missing heritability” may also be due to mutations in BRCA1/2 not yet possible to detect, causing pathogenicity i.e. by disrupting regulatory areas, deep intron variants or even hypothetically interfering with epigenetic mechanisms not yet accounted for.

On-going research is looking into whether genetic tests for multiple susceptibility loci may be applied clinically on the group of familial breast cancer to differentiate risk, to better inform choices of surveillance through the establishment of a polygenic risk score (31). Evans et al (2016) have studied the risk prediction ability of a 18SNP polygenic risk score, and find that a substantial proportion (18–20%) of women in their familial risk clinic may cross the 25% lifetime risk boundary used in North America to include patients for MRI screening. As a consequence, they suggest that it is likely that use of a polygenic risk score may have more added value than extended gene mutation panel.

There are also clues pointing towards that the group of familiar breast cancer may have a varied cancer risk and prognosis. Møller et al (2014) showed prospectively, that in breast cancer families without a demonstrable BRCA mutation, the risk for breast cancer in female first degree relatives was about twice the risk in the general population (34). Having one relative with early onset breast cancer did not alter risk for contracting early onset breast cancer. However, having more than one affected relative increased risk three-fold compared to population risk.

2.2 BRCA1/2, the genes causing hereditary breast - and ovarian cancer

BRCA1 was identified by Mary-Claire King and co-workers in 1994 through linkage studies in large cohorts of families with early-onset breast cancer (35, 36), and BRCA2 followed in
1995 (37). Clinically, the awareness of families suffering from breast- and possibly bowel cancer was much older, and the first report on such a family was published by the physician Broca in the last decades of 18th century, giving a detailed pedigree of his wife’s family (38). The clinical term of hereditary breast - and ovarian cancer syndrome was presented by dr. Henry Lynch in 1971 (39).

Both BRCA1 and BRCA2 are tumor suppressor genes. Loss of heterozygosity (LOH) in tumor samples from BRCA1-related cancers have shown loss of the wild type (WT) copy of BRCA1 and retention of the inherited mutant copy (40). Venkitaraman (2014) argues that tumor suppression by BRCA1 and BRCA2 may originate from fundamental role in controlling the assembly and activity of macromolecular complexes that monitor chromosome duplication, maintenances and segregation across the cell cycle. With their inactivation many central cellular and chromosomal functions are affected (41). The number and diversity of proteins reported to interact with BRCA1 and BRCA2, as well as how the BRCA1/BRCA2 proteins are localized in many different intracellular compartments and have several roles in cell cycle have confounded attempts to explain exactly how the inactivation of the breast cancer genes promotes carcinogenesis.

BRCA1 and BRCA2 encode large proteins involved in DNA repair by homologous recombination (42). BRCA1 is located at the 17q21 and includes 22 exons transcribed into a 7.2 kB transcript and a 1,863-amino acid protein (36). BRCA1 contains a RING-finger domain, which binds BARD1 protein necessary for the ubiquitin-ligase function of BRCA1. The BRCT-domain interact with p53 – protein during DNA repair, and checkpoint proteins as ATM, CHEK2 and RAD51 interact with BRCA1 through DNA-binding domain and SQ-cluster domain in DNA repair. BRCA2 is located at 13q12-13 and contains 26 exons transcribed into an 11.2kB transcript and a 3,418 amino acid protein (37). BRCA2 contains a transactivation site, which binds to PALB2 and interacts with BRCA1, while RAD51 binds to the BRC-domain of BRCA2. Both BRCA1 and BRCA2 proteins and are relative late-comers in evolution. Both genes work to preserve chromosomal structure and stability, and are expressed in different tissues during certain phases of cell cycle. It was early on speculated that the similarities in phenotypes was due to common cellular pathways. Both genes follow autosomal dominant inheritance pattern, one defective copy of BRCA1 or BRCA2 in the germline is enough to cause cancer predisposition (43). However, biallelic inactivation of
BRCA2 leads to Fanconi anemia (41). Both BRCA2 and BRCA1 have gender- and tissue
specific effects, and the hormone homeostasis seems to be linked with gene function (40).

Variants considered pathogenic are variants that predict truncated proteins or null proteins,
most often by introducing premature termination codons through small frameshift deletions or
insertions, nonsense mutations or splice site alterations, or through large deletions or
duplications (44, 45). Missense variants may be pathogenic if there is evidence that they
compromise function of the protein. Splice mutations may occur at predicted splice sites or
less commonly by altering mRNA expression through another mechanism only recognized
through a direct functional analysis. Several thousand variants have been reported to BIC,
Breast Cancer Information Core. Classification of variants are in general performed following
guidelines established by ACMG (American College of Medical Genetics) and ENIGMA
(Evidence-based network for the interpretation of germline mutant alleles) (45, 46).

2.2.1 Penetrance and expression

Penetrance of any DNA-variant is defined as the probability of a given phenotype to present
itself in mutation carriers and expression is defined as the specific symptoms or signs a
phenotype contains (47). In the setting of BRCA1/2, this translates into the increased cancer
prevalence among BRCA1/2 mutation carriers, and how BRCA1/2 are both highly penetrant
for breast and ovarian cancer (1). Both genes show variable expression, making carriers
prone to different cancers or different subtypes of cancers. Risks for other cancers than breast
and ovarian cancer are thought to be of low magnitude (< 5 % life time risk) but do include
male breast cancer (48) as well as prostate cancer (49). It is shown that male BRCA1/2-
carriers have 1 % lifetime risk of breast cancer as compared to 0.1% in the general population.
Prostate cancer risk is significantly increased especially for BRCA2 mutation carriers before
60 years of age (50), as is the risk for developing more aggressive prostate cancer (49).
The life time risk for pancreatic cancer and colonic cancer are also increased in BRCA1 and
BRCA2 carriers (51).

The high breast and ovarian cancer risks have been confirmed retrospectively and
prospectively in several studies (51-53). The retrospective studies suffer to some extent from
selection biases. It has been possible to carry out prospective studies in mutation carriers not
opting for prophylactic surgery confirming the findings (54). Antoniou et al (2003) found
that among BRCA1 carriers, the average cumulative risk of breast cancer by 80 years of age
was 67% and the average cumulative risk of ovarian cancer was 45%. Among \textit{BRCA2} carriers, these average cumulative risks were 66% and 12%, respectively. After a first breast cancer, \textit{BRCA1} and \textit{BRCA2} carriers also showed a substantial risk of contralateral breast cancer (55).

Mavaddat et al (2013) and the EMBRACE study showed that the average cumulative risks by age 70 years for \textit{BRCA1} carriers to be 60% for breast cancer, 59% for ovarian cancer, and 83% for contralateral breast cancer. For \textit{BRCA2} carriers, the corresponding risks were 55% for breast cancer, 16.5% for ovarian cancer, and 62% for contralateral breast cancer (54). In this study, the risks are also given as the risk of getting cancer per ten years, which may be informative numbers to be included when counseling women on their individual risk of cancer.

2.2.2 Pathology of \textit{BRCA1/2} breast cancers

The pathology of \textit{BRCA1/2} breast cancers shows the variable expression of \textit{BRCA1/2} mutations. Tumors arising in \textit{BRCA1/2} carriers have been shown to differ from sporadic cancers in several studies, and therefore, the clinicopathological characteristics have been a way of selecting patients for genetic testing. Evidence regarding pathological characteristics may also prove valuable in risk prediction and have impact on management.

Armes et al (1998) showed that breast carcinomas in \textit{BRCA1} mutation carriers were associated with a distinct histologic appearance; a higher mitotic count and a higher histological grade (56). Data from the International Breast Cancer Linkage Consortium, among others, have shown that breast cancers in patients with \textit{BRCA1} germline mutations are significantly more often “triple negative”, i.e. negative for estrogen receptor, progesterone receptor, and HER-2 (57). \textit{HER2}-status has not been found to be a reliable predictor of \textit{BRCA}-status (58, 59), and hence it has not been suggested as selection criteria for testing.

The pathology of \textit{BRCA1} breast cancers has been somewhat better studied than pathology of \textit{BRCA2}. Mavvadat et al. (2011) performed a large study on comparing pathology of breast cancer in \textit{BRCA1} and \textit{BRCA2} mutation carriers. The study included pathology data on 4325 \textit{BRCA1} and 2568 \textit{BRCA2} mutation carriers (60) and was able to study smaller subsets of disease characteristics and give age-specific proportions of tumor subtypes in \textit{BRCA1} and
Triple negative breast cancers were present in both BRCA1 and BRCA2 carriers, but while the relative frequency of ER-negative and triple negative tumors decreased with age at diagnosis for BRCA1-mutation carriers, it increased with age for BRCA2 mutation carriers, and this was also shown by Atchley et al (59). In Mavaddat’s study, ER-negative tumors were of a higher histological grade than ER-positive tumors, both in BRCA1 and BRCA2 carriers. HER2-status did not vary with age at diagnosis. Increasing age at diagnosis was associated with decreasing number of high grade tumors in BRCA1-carriers, this tendency was not found in BRCA2 carriers. However, a relative increase in the ratio of low-grade tumors vs high-grade tumors were registered with increasing age for BRCA2-carriers. ER-positive tumors were more likely to arise in BRCA2-carriers, and this was true for all morphological categories. ER-negativity in first breast cancer was predictive of ER-negativity in contralateral breast cancer. The evidence regarding age-specific effects may be considered valuable in individual counselling on risk management. Knowledge on differences in pathology between BRCA1 and BRCA2 tumors are important because criteria for testing are usually not differentiating between the two genes.

2.2.3 Founder mutations BRCA1/2

Being a BRCA1/2 mutation carrier is a rare event. The frequency of BRCA1/2 mutations in the general population is estimated to be about 0.2% - 0.02% (28). However, local frequencies vary a lot, and the phenomenon of founder mutations give raise to much higher frequencies in some populations and in some geographic locations. Founder mutations in BRCA1/2 are found in several countries, both in Europe and northern America, and are described in Denmark, Sweden, Iceland, Poland, Norway and Canada. The Ashkenazi Jewish founder mutations have been found to have a frequency of 2.17% (1.14% BRCA1 and 1.03% BRCA2) in a recent study of a US male population of Ashkenazim background (61). Other studies offering population-based screening for women of Ashkenazim decent have found mutation frequencies between 1.1-4.5%. A similar study of Polish founder mutations showed a frequency of 3.9% carriers of three founder mutations, this study did however use family history of breast cancer as inclusion criteria. Norwegian studies on this subject have mainly been performed in breast cancer populations, and the prevalence of the ten most frequent BRCA1/2 mutations recognized in 2007 were found to be 2.5% among breast cancer patients tested regardless of family history.
The identification of founder mutations gave a cost-efficient test approach in times when genetic analysis was a limited and expensive resource, but knowing their distribution may also serve as a model for studying possible differences in penetrance and expression, so-called genotype/phenotype correlation. Previous reports on Norwegian founder mutation carriers have suggested that the founder mutations may have a lower annual cancer risk compared to the rare mutations (53). The general population frequency of \textit{BRCA1}/2 mutations in Norway is not known, but is estimated to be less than 0.5% on the whole. There are however well-known geographical differences in mutation frequencies, illustrated in several studies (62, 63). The four most common \textit{BRCA1} mutations in 2001 were haplotyped and found to originate from different parts of the country.

The Icelandic \textit{BRCA2} founder c.771_775del is expected to have a general population frequency of 0.6%, but is found among 6-8% of Icelandic women with breast cancer, and this may be suggestive of a higher penetrance than other \textit{BRCA2} mutations (64). The high penetrance of Ashkenazi founder mutations was confirmed in a recent study without the selection bias of recruiting persons with a high family cancer load. In this study Ashkenazi-Jewish men were tested regardless of cancer history, and retrospectively the cancer prevalence in mutation families was noted and found to be as high as in previous studies (61). The cumulative risk of developing either breast or ovarian cancer by age 60 and 80, respectively, was 60% and 83% for \textit{BRCA1}-carriers and 33% and 76% for \textit{BRCA2}-carriers in this study.

\subsection*{2.2.4 Genotype/phenotype correlation}

Many studies have explored the possibility of genotype/phenotype effects due to a certain variant’s position in the \textit{BRCA1} or \textit{BRCA2} gene (65). The Breast Cancer Linkage Consortium suggested early on, that variants in a region known as the Ovarian Cancer Cluster Region in \textit{BRCA2} may give relative higher ratio for ovarian cancer versus breast cancer than variants outside this region. It was also reported that the ratio of ovarian to breast cancers were significantly higher for variants in a central region of \textit{BRCA1} than that for mutations outside this region (66, 67). Using the largest dataset analyzed to date, CIMBA (see 2.2.5) found results consistent with previous findings from the Breast Cancer Linkage Consortium for both \textit{BRCA1} and \textit{BRCA2} mutation carriers (65). This study also identified multiple breast cancer cluster regions (BCCRs) in \textit{BRCA1} and \textit{BRCA2} and two OCCRs in \textit{BRCA2}. The findings need appropriate validation, but may have implications for risk management. A recent
publication from ENIGMA- consortium was able to identify an intermediate penetrant variant in \textit{BRCA1}, finding a cumulative risk of breast cancer and ovarian cancer by age 70 years was 20\% and 6\%, respectively (68).

2.2.5 Modifiers of penetrance and expression

Modifying factors are suggested to be one of the main external causes of incomplete penetrance and variable expression in \textit{BRCA1/2}. Such external modifiers could be either other genetic variation, other constitutional factors or environmental factors. The modifier approach is explored to understand how cancer risk may be affected in a given individual. Consequently, harmful environmental factors may be avoided, or if genetic modifiers exist and are measurable, testing for such factors may influence risk prediction.

\textbf{Environmental modifiers} of \textit{BRCA1/2} have been the subject of many studies. In general, environmental risk factors for cancer are better studied for \textit{BRCA1} than for \textit{BRCA2}.

It was early on shown that recent birth-cohorts (born after 1940) showed higher penetrance of breast cancer than older and this was interpreted as an indicator that important environmental factors have changed over time (1). This effect has also been confirmed in more recent studies (61). In a systematic review and meta-analysis by Friebel et al (2014) many exposures and their consequent associations with breast or ovarian cancer risk in \textit{BRCA1/2} mutation carriers were evaluated (69). Only high age at first life birth received the characteristic “probable association” for \textit{BRCA1}, the highest level of assessment, as the included papers in the meta-analysis were scored according to a) amount of evidence, b) replication and c) protection from bias. Tamoxifen was considered to have a “possible protective association” against breast cancer, while oral contraceptives were considered to have a “possible association” in increasing breast cancer risk for both \textit{BRCA1/BRCA2} mutation carriers, as well as possible protective effect against ovarian cancer. However, the results from separate meta-analysis of case-control and prospective cohort studies differed for oral contraceptives especially, but not for cigarette smoking. Regarding oral contraceptive use Kotsopoulos et al (2014) performed a case-control study on 2,492 matched pairs of women with a deleterious \textit{BRCA1} mutations, and found that oral contraceptive use before age 20 and even before 25 significantly increased the risk of early-onset breast cancer at < 40 years of age with 40 \% (OR 1.4) among women with a \textit{BRCA1}-mutation, and moreover, the risk increased with duration of use (70).
Hormonal replacement therapy (HRT) may be another important modifier of cancer risk. Friebel et al found the evidence on HRT insufficient. Eisen et al (2008) performed a case-control study on 821 postmenopausal BRCA1-mutation carriers concluding HRT for three years may protect against breast cancer (71), however increasing risk for endometrial cancer and cardiovascular disease remains.

High mammographic density has been shown to be an independent risk factor for sporadic breast cancer, but the evidence for BRCA1/2 mutation carrier have been conflicting, somewhat in favor of increasing breast cancer risk (72).

The Consortium of Investigators on Modifiers of BRCA1/2 (CIMBA) is a major contributor to research in genetic modifiers through Genome-wide-association-studies on BRCA1/2-carriers. CIMBA’s main aim has been to provide sufficient sample sizes to allow large scale studies to evaluate reliably the effects of genetic modifiers in BRCA1/2 carriers. Their findings across populations of mutation carriers suggest that many susceptibility alleles associated with breast cancer in the general population may act as modifiers of BRCA1/2 penetrance, especially in combinations. The susceptibility alleles, Single Nucleotide Polymorphisms (SNPs), typically show low relative risks per copy of the minor allele. Acting together, they may statistically offer a way to evaluate differentiation in individual risk (72-74). By defining a risk score based on this assumption, CIMBA estimated empirically that mutation carriers being among the highest 5 % of the risk distribution had a statistically significant increased HR of 2.64 of breast cancer risk compared to the lowest 5 % (73). It is reasonable to assume that the relative risks associated with several common genetic variants and/or lifestyle/hormonal factors in combination are larger than the associations per variable. Further, because women with BRCA1 and BRCA2 mutations are already at high risk of developing breast or ovarian cancer, the combined effects of SNPs and lifestyle/hormonal risk factors may translate into large differences in the absolute risks of developing the diseases (73-75). Also, statistically, the utility of any risk factor information critically depends on whether this added information show an ability to stratify risk between the groups studied.

It has also been shown that the common breast cancer genetic susceptibility loci interact pairwise in a way that do not deviate from a multiplicative model of interaction on the risk of developing breast cancer (30). This multiplicative model is however theoretical and needs confirmation in terms of biological mechanisms (75). Polygenic risk scores (PRS) based on
large numbers of SNPs are expected to result in even larger differences in the absolute cancer risks estimated for mutation carriers at the extremes of the combined SNP distributions, compared with the limited SNP profiles investigated so far (72).

Ingham et al (2012) calculated an overall breast cancer risk SNP score (OBRS) for \textit{BRCA1/2} mutation carriers from a large genetics clinic in UK based on 19 SNPs from 18 loci, to predict breast cancer risk. They found that OBRS and age of onset for breast cancer to be associated for \textit{BRCA2} mutation carriers, but not for \textit{BRCA1} mutation carriers (74). Prosperi et al (2014) studied the ability of different statistical models to predict cancer risk, depending on General predisposition score (GPS) of risk alleles and different clinical characteristics included in a risk prediction model, concluding with an ability to predict increased cancer risk for \textit{BRCA2}, and a decreased for \textit{BRCA1} (76).

Milne and Antoniou (2016) summarizes that to date, a total of 26 and 16 SNPs are associated with breast cancer risk for \textit{BRCA1} and \textit{BRCA2} mutation carriers, respectively (72). The corresponding numbers for ovarian cancer risk are 11 and 13. These genetic modifiers are estimated to account for a relatively small proportion (<10%) of the modifying genetic variance for \textit{BRCA1} and \textit{BRCA2} mutation carriers. The joint effects of all SNPs and family history have not been estimated for \textit{BRCA1} and \textit{BRCA2} mutation carriers. These are required before the genetic susceptibility findings can be implemented in the genetic counselling process, and such data are underway from CIMBA.

Fine-mapping for some loci has been performed following GWAS-studies, i.e. for the \textit{ESR1}-locus, 19p13.1, (77, 78) and \textit{TERT} (27). Associations with ER-negative cancer have been found for \textit{TERT}-loci.

Genetic and environmental modifiers of \textit{BRCA1/2} penetrance and expression are likely to act together. The general approach to work around this problem has been to assume that these factors act independently in modifying risk, unless evidence to the contrary is observed (72).
2.3 BRCA1/2 management and treatment options

2.3.1 Survival

A recent Danish publication by Soenderstrup et al (2017) show that ten-year overall survival (OS) and disease-free survival (DFS) for BRCA1 breast cancer patients were 78% and 74%, while for BRCA2 mutations carriers, OS and DFS were 88% and 84%, respectively (79).

If a woman survives her first cancer she will benefit from preventing second cancers. This is supported by the increased 20-year survival rate after contralateral mastectomy showed in different studies (80, 81). There was a significant reduction of death rate after ten years in the study by Soenderstrup et al. for both BRCA1 and BRCA2 mutation carriers performing contralateral mastectomy.

In a recent Chinese study on genetic testing of unselected breast cancer patients, BRCA1 mutation carriers had a significant worse disease-free survival than did non-carriers, whereas there was no significant difference in survival found between BRCA2 mutation carriers and non-carriers (82).

2.3.2 Prophylactic options: Screening versus surgery

When preventing cancer, life-years are saved. Life expectancy for mutation carriers opting for risk-reducing surgery is considered normalized (10). Mutation carriers in Norway are offered MRI surveillance of breasts from 25 years of age aiming at early detection and cure. Intensive combined breast cancer screening with annual MRI and mammography appear to improve survival from breast cancer in BRCA2 mutation carriers (83), while BRCA1-mutation carriers have been found to have 5-year breast cancer specific survival rate of 75 % and 10-year of 69% when choosing surveillance (84). Even stage 1 tumors in this study had a 5-year survival of 82% as compared to 98% in the general population.

Prophylactic/ risk-reducing surgery reduces cancer incidence and this have been shown in several studies, summed up in a metaanalyses and systematic review by Li et al in 2016 (85). Risk-reducing bilateral mastectomy (RRBM) offers high protection against breast cancer, > 90 % reduction of prevalence of breast cancer has been found in several studies (48).
Risk-reducing bilateral salpingo-oophorectomy (RRBSOE) offers at least 80% protection against ovarian, fallopian tube or peritoneal cancer (86). The procedure may offer secondary protection against breast cancer as well, although this last issue has been discussed as less significant due to possible selection and statistical biases (48, 87, 88).

The risk-reducing mastectomy is in generally well tolerated, with dissatisfaction mainly associated with surgical complications (48). Risk-reducing salpingo-oophorectomy has shown some effect on sexual pleasure, which was not relieved by hormone replacement therapy in all users (89), and could be due to testosterone effect. Favorable effects of salpingo-oophorectomy include significantly reduced cancer-related worry in approximately 80% of BRCA1 and BRCA2 carriers and 95% satisfaction with their decision to undergo surgery (90).

2.3.3 Cancer treatment in BRCA1/2 patients

Ablatio mammæ and contralateral mastectomy is the preferred surgical treatment for breast cancer in BRCA1/2 mutation carriers, mainly due to a significant risk of ipsilateral and contralateral breast cancer (80, 81, 91).

Increased survival, as shown in the study by Soenderstrup et al, is linked to the usage of cytostatic treatment in BRCA1/2 - carriers with breast cancer (79). Narod et al (2013) also found that 15-year survival of BRCA1 carriers given cytostatic treatment regardless of tumor size were 89.4% and significant for ER-negative tumors, compared to 73% for those not receiving chemotherapy (92).

It is now recommended nationally that locally advanced breast cancer cases are offered neoadjuvant carboplatin treatment, and ovarian cancer patients with relapse are offered PARP-inhibitor (93). Tamoxifen may have effect on preventing ER-positive breast cancer (48), but is not standard care mainly due to side-effects.
2.4 Methods in genetic epidemiology

2.4.1 Genetic epidemiology
Genetic epidemiology has many definitions, one being “a discipline closely allied to traditional epidemiology that focuses on the familial, and in particular genetic determinants of disease and the joint effects of genes and non-genetic determinants” (47). Genetic epidemiology ranges from local efforts like the one presented in this thesis to large, international collaborations, boosted by the description of the first reference genome. Allele frequencies, phenotype descriptions and its collection in research and/or patient databases is one of the corner stones in population genetics as well as clinical genetics. Such collection of genetic information in databases and even more important, it’s availability, makes the translation between research and clinical work possibly very direct in the field of medical genetics.

2.4.2 Linkage studies and linkage disequilibrium
Linkage and linkage disequilibrium are two key concepts in genetic epidemiology. The earliest genetic achievement was the identification of monogenetic disorders, including the identification of \( BRCA1/2 \). This was done through linkage studies in families affected with presumably hereditary disease. Linkage studies are performed by genotyping such families, where the disease is following a presumably Mendelian pattern and the causative genetic factor has high penetrance. Linkage studies examine how the chosen genetic markers (often microsatellites) segregate with the disease across multiple families (94), identifying genomic areas of interest where candidate genes may be located.

Two genetic loci are **linked** if they are transmitted together more often from parent to offspring than expected under independent inheritance, (i.e. that recombination between them occurs with a probability of less than 50%). Two loci are in **linkage disequilibrium** if they, across the population as a whole are found on the same haplotype more often than expected by chance. Linkage disequilibrium is more likely to persist for loci being located close together, and the concept was developed by population geneticists in an attempt to describe changes in genetic variation within a population over time. Recombination events within a family break apart chromosomal segments, and theoretically eventually linkage equilibrium of all alleles will appear. This will take place over generations, mainly in a population of fixed size, who are undergoing random mating, and these last two conditions are rarely perfectly
met. However, older populations have smaller regions of linkage equilibrium than younger populations, i.e. do African populations have smaller areas of LD than do European populations.

2.4.3 Genome-Wide Association Studies

Genome-Wide Association Studies (GWAS) evolved during the 2000-ies as a method for studying common genetic variation to identify associations with common /complex disease, and are powered to identify small effects per allele. The unit of genetic variation that is studied is typically single nucleotide polymorphisms (SNPs). SNPs serve as markers of a genomic region, and are the most abundant form of genetic variation in the human genome. Genetic association studies aim at detecting association between one or more genetic polymorphisms and a trait/illness. A GWAS is a hypothesis-free search through the genome for variants that are significantly more frequent in cases than controls.

The International HapMap project has cataloged SNP variation in different populations (1.6 million SNPs in 11 populations) thereby enabling the study of linkage disequilibrium in the sense of how an allele of one SNP is inherited or correlated with an allele of another SNP. A marker SNP studied in association studies may have direct/causal association with the disease in question, or have an indirect association (being in linkage disequilibrium with the causal variant) or, the third possibility, the SNP may have a confounded association, due to stochastic factors. Statistically, for genome-wide association studies, different methods are developed with the aim of 1) controlling for population confounding effects, (i.e. a skewed distribution in a study population may falsely establish an association) 2) to correct for the multiple testing involved and corresponding higher risk of false positive results, and 3) to evaluate single - locus effects versus multiple locus effects of the SNPs studied.

Limitations of GWAS to investigate the impact of rare variation on disease are being increasingly recognized. Consequently, attention has shifted recently to investigating the impact of rare variation on disease (24), in genome data.

2.4.4 Variant evaluation

Distinguishing between normal variation and pathogenic/disease-causing variation is central in both genetic epidemiology and clinical genetics. Both the closeness and the distinctions of genetic, epidemiological research versus clinical, medical genetics are very well illustrated by this issue. In paper 2 we aim at updating the knowledge on the BRCA1/2 mutation spectrum
in Southeastern Norway, and in doing so the variants found were classified according to the specifications published by American College of Medical Genetics (46). All new \( BRCA1/2 \) variants found in any lab are encouraged to be included in databases such as ClinVar or ENIGMA. ENIGMA has separate evaluations on especially missense and splice variants, and serves also as an international research database (45).

2.4.5 Hardy–Weinberg equilibrium
In our paper 3 we aim at validating the presence and associations of internationally studied SNPs found to be associated with penetrance of \( BRCA1/2 \) mutations. A central concept for comparing alleles in disease populations (cases) with healthy populations (controls) is Hardy-Weinberg equilibrium (HWE). Observed frequencies of alleles in controls should be consistent with the two alleles being independently sampled from the population as a whole. Hence the distribution in controls is tested, as to whether they are in HWE, \( p \) and \( q \) being the two alleles, minor and major.

HWE: \( p^2 + 2pq + q^2 = 1 \)

2.5 Test requirements/Test quality

2.5.1 Sensitivity and specificity
Any test used for medical purposes must fulfill requirements of test quality. Sensitivity is defined as a test’s ability to correctly identify the test positives, and in this setting: the amount of mutation carriers identified as such, either by a set of criteria or by a genetic test. Specificity is defined as a test’s ability to correctly identify test negatives, i.e. how many non-mutation carriers are correctly identified as such. In a diagnostic test setting both sensitivity and specificity are required to be high, but in a screening test, usually a highly sensitive test is used as a primary test, followed by a highly specific, confirmatory second test (95).

2.5.2 WHO screening criteria
Andermann and colleagues at WHO (2008) suggested revised criteria for screening in the genomic era, based on Wilson and Jungner’s original criteria (96). (Supplementary table 1.) These criteria are considered the gold standard of screening programs, and list different factors to be evaluated before a screening program is to be established. The disease must be a well-recognized health problem, it must be detectable in preclinical/prepathological phase,
acceptable treatment must be available; and potential harm done when testing for the disease or treating it must be considered. Genetic screening, especially for hereditary cancer, differs in some important respects from disease screening. The genetic disposition may be rare, and not a big public health issue on its own, nor is the disposition a disease per se. The acceptance and perceived necessity of such a genetic test by individuals not themselves suffering from cancer or without cancer experiences in the family are yet to be known.

2.5.3 Diagnostic and predictive testing
Norwegian genetic testing is under jurisdiction of the Law on Biotechnology. Diagnostic testing of any genetic illness may be done by any medical doctor in the presence of clinical signs or symptoms of a possibly hereditary or genetic disease. Predictive genetic testing, however, can only be done in special institutions were genetic counselling is offered, (www.lovdata.org). Section of Hereditary Cancer is such an institution in SERHA (South-Eastern Norway Regional Health Authority Trust), and DMG is such a department.

2.5.4 Clinical testing strategies:
There are different criteria sets for BRCA1/2 testing internationally. The reasoning behind clinical testing criteria has been to select individuals for testing with a pre-set probability of having a mutation, (usually > 10%) and this is a common rational behind many medical priorities. The clinical testing criteria were established at a time when BRCA1/2 testing was very expensive and labor-intensive. ASCO, (American Society of Clinical Oncology) in US, NCCN (National Comprehensive Cancer Network) and NICE in UK and NBCG in Norway have all developed criteria for testing that follow this logic (supplementary table 2)

For the same reasons, founder mutation testing was offered as primary test many places, and this was clinical practice also in Norway quite recently, and this is described thoroughly in paper 2. During the later years, sequencing has become much more efficient and less time-consuming, it has also become evident that the sensitivity of founder mutation testing is lower than expected.

The value of a genetic testing program, as stated by Finch et al (2015) among others, comes mainly from the number of cancers prevented (through identifying unaffected carriers)
In a successful population program, the majority of identified \(BRCA1/2\) carriers will be unaffected (97). The same conclusion is reached by Slade et al (2016), who state that the most efficient way of preventing is cancer is through identifying healthy mutation carriers in already identified mutation positive families. This is an argument favoring developing more streamlined service of cancer genetics testing (2). Obviously, the clinical goal is to reach out to as many that may benefit from the testing as possible, within the frame of an evidence-based, proof-read and economically sustainable health program.

2.5.5 Cost-efficiency
Cost-efficiency analysis have shown that preventing cancers, avoiding cancer treatment and sick leave, as well as increasing life-years for mutation carriers are highly cost-efficient (10). The same conclusion is reached in several studies (3, 5, 6)

2.6 Personalized medicine
Genetic testing is one of the main features of personalized medicine. In addition to genetic testing for hereditary hypercholesterolemia and other hyperlipidemias, \(BRCA1/2\) testing is one of the earliest applications of genetics in preventing disease and death, both through prophylaxis, but also recently through tailoring treatment.

2.6.1 Individualized risk prediction
Because of on the knowledge of incomplete penetrance, risk prediction tools have been developed. These tools (BOADICEA (Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm), BRCAPro and Tyler-Cusick model, as well as IBIS, Gail, Claus) aim at using individual information in counseling women on their breast cancer risks, either for women in general, or for \(BRCA1/2\) mutation carriers (98, 99). BOADICEA, and BRCAPro are used to select individuals for \(BRCA1/2\) testing. Their reliability in predicting specific cancer risk estimates have been a matter of discussion. The estimation of cancer risk for any \(BRCA1/2\) mutation carrier in clinical practice is mainly based on group estimates. Individualization of risk prediction should be evidence-based, either through individual assessment (knowledge on genotype – phenotype correlations, exposure to environmental factors), or through a software-based multifactorial risk evaluation. The modifier approach is an attempt to refine such risk prediction (30), as mentioned earlier for both familiar breast cancer and \(BRCA1/2\) mutation carriers.
2.6.2 Population - screening for BRCA1/2 - options and pitfalls.

Offering voluntary *BRCA1/2* testing to healthy persons regardless of family history is suggested to enhance cancer prevention potential. Depending on ancestral background, the a priori probability of having a mutation will vary from 0.5% or lower to 2.5%. Several studies have looked at the benefits from population-screening for *BRCA1/2* mutations. The main strategy has been to test for founder mutations in selected populations, mainly the Ashkenazim in US, Canada and Israel, but also in the Polish populations such studies have been performed as shown in a recent review by Foulkes et al (2016) (100). Mary-Claire King raised the issue of offering *BRCA1/2* mutation screening to all women regardless of ancestry, and received the Lasker award on the subject (101). The critics reviewed by Foulkes et al. include concerns of low cost-efficiency when choosing to screen with sequencing/MLPA due to high pricing and labor-intensive variant assessment. On the other side, the issue of lower sensitivity if choosing to test for too few mutations is also important. A full genetic screening for breast cancer risk genes may be an efficient option also in founder mutation populations, to identify rare *BRCA* mutations in founder mutations populations as well as mutations in other cancer disposition genes (11). A pilot program offering founder mutation screening to US Ashkenazi populations also without significant cancer history was recently performed. Mutation carriers identified through this program were initially somewhat troubled by the findings, but did not regret participating (102). Data are indicating that getting to know this kind of cancer risk induces high information need, and that traditional follow-up from health care professionals may be supplemented with peer-based coaching to increase coping (103).
3. AIMS OF THE STUDY

The main aim of this project has been to study hereditary breast cancer in South-Eastern Norway, focusing on BRCA1/2 epidemiology and clinical implications. We have performed three different studies:

The first study was focusing on diagnostic BRCA1/2-testing in breast cancer patients not selected for high-risk factors. We wanted to study sensitivity and specificity of the traditional criteria applied in a broad breast cancer cohort, the ability of existing guidelines for BRCA-testing to identify mutation carriers. As a descriptive study, we wanted to look at mutation frequency and types of mutations as well as the clinical breast cancer characteristics of the BRCA1/2 mutation carriers in this cohort. We also aimed at studying how many healthy mutation carriers were identified when relatives of the mutation positive breast cancer patients were invited to testing. This study led to paper 1.

The second study was undertaken to get new updated knowledge on the mutation spectrum of BRCA1/2-carriers in South-Eastern Norway. Previously, in 2001, an epidemiological study of BRCA1 mutations was carried out, and at the time 68% of the identified mutation carriers had one of the four Norwegian founder mutations in BRCA1 (62, 63). After fifteen more years of both selective founder mutations testing and sequencing of the entire genes, a different distribution was expected to be found. This study is presented in paper 2.

In the third study, we wanted to validate in Norwegian BRCA1/2 mutation carriers, the international findings of potentially modifying SNPs. The selected SNPs had been shown internationally to modify penetrance in BRCA1/2 mutations carriers from CIMBA-studies, and we wanted to validate these cancer risk associations in Norwegian BRCA1/2 mutation carriers. Our assumption was that if such associations were confirmed across different study populations, this could represent a step in the direction of establishing models of individualized risk prediction. Such an approach could eventually make both the choice and timing of prophylactic surgery and surveillance better informed and more precise. This validation study is presented in papers 3.
4. METHODOLOGICAL CONSIDERATIONS

4.1 Study material

The thesis consists of three patient-based study materials. Study 1 contains patient data obtained from two cohorts: Breast Cancer Surgery Unit at Oslo University Hospital-Ullevål, (OUH-U) and corresponding units in other hospitals in the health region of SERHA, as the only study. Study 1, 2, 3 all contain patient data obtained from Section of Hereditary Cancer, Oslo University Hospital. Study 1 and 2 are approved by Data Protection Officer at OUH as quality of care studies, while study 3 is approved both by the Ethical review board (ref S02030) and the Norwegian Data Inspectorate (ref 2001/2988-2).

4.1.1 Diagnostic testing for BRCA1/2 (study 1/ paper 1): Cohort of breast cancer patients.
A total of 1371 breast cancer patients were included and tested during the study period, running from 1st of January 2014 to 31th of August 2015. The study was made possible due to a revision of NBCG guidelines, where a criterion for \textit{BRCA} testing of “treatment consequence” was added. Breast cancer patients not fulfilling the traditional criteria could then be offered \textit{BRCA}- test, and this represents a less selected approach to testing than previously. The patients were included from different hospitals. Cohort 1 (OUH-U) constituted of 440 patients treated at Breast Cancer Unit at Oslo University Hospital-Ullevål and these patients, both mutation carriers and mutation negatives were included in a quality of care database where details on breast cancer characteristics were registered. Cohort 2 (SERHA) constituted of 931 patients from different hospitals within South Eastern Norway Regional Health Authority Trust. The genetic testing of both cohorts was performed at DMG, Department of Medical Genetics, Oslo University Hospital. Mutation carriers from both cohorts were referred to DMG and included in a quality of care database. In this process, a detailed family history was obtained and relevant diagnosis in relatives confirmed.

After identification of a mutation carrier, family members of the mutation positives, both male and female, were invited to genetic counselling and testing. Testing was offered not only to first degree relatives, but to also more distant relatives.

Mutation carriers from both cohorts were scored according to pre-selected guidelines; ASCO, NCCN, NICE and both old and revised NBCG guidelines. Sensitivity of criteria was calculated for mutation carriers from both cohorts, specificity was calculated for Cohort 1
only where information on mutation negatives was available. Tests for trends were performed to compare the differences in breast cancer characteristics between mutation carriers and non-carriers in Cohort 1. Separate analyses were done to compare tested and non-tested in cohort 1 in order to illustrate potential bias in the group that was not tested. Mutation positives in Cohort 1 and 2 were also compared to investigate similarity of characteristics.

4.1.2 Mutation spectrum of BRCA1/2 (study 2/paper 2): Cohort of mutation carriers
A total of 2430 BRCA1 mutation carriers from 669 families, and 1092 BRCA2 carriers from 312 different families were included in this study. All variants were classified according to ACMG and ENIGMA criteria (45, 46). A search through Alamut was performed on each variant to establish whether the variant was previously described to be a founder mutation. The variant did not have to be proven to be a founder mutation through haplotyping by other researchers.

To measure absolute mutation frequency, we calculated both number of mutation carriers and families carrying each variant. We established three classes of frequency: Highly frequent (>30 families per variant), moderately frequent (10-30 families per variant), less frequent (3-9 families) and rare (1-2 families per variant). The number of families per variant supplied us with information that could point towards not yet recognized founder mutations, if families could be shown to share ancestry. It was however, outside the scope of the study to establish such common ancestry by haplotyping.

4.1.3 Validating modifiers (study 3/paper 3): Extreme groups of mutation carriers
The chosen method for the validation studies was to compare frequencies of the risk alleles between two extreme groups of mutation carries, carriers affected with cancer early in life and carriers affected with cancer late in life or never affected. For the BRCA1 study the “young cancer group” consisted of 40 carriers affected with cancer before or at 40 years of age, and the “old no cancer group” consisted of 38 individuals affected with cancer after 60 years of age or staying healthy throughout life. The same model was applied for BRCA2 mutation carriers. Twenty-nine individuals affected with cancer before or at 48 years of age were included in the young cancer group and 32 individuals not having had breast or ovarian cancer until after 60 years of age were included in the “old no cancer group”.

Power calculations indicated that, if prevalence of the variant alleles for each of the modifiers
tested were > 0.05, we would reach significance if the OR > 2 or < 0.5, and if 50 participants in both the young cancer and old no cancer groups were included. Preliminary analysis indicated that we would reach significance by selecting affected women aged less than 40 years and women unaffected at over 60 years.

Patients showing the features described were made available from the research data base established by the Research group on Inherited Cancer, Radiumhospitalet. This database has included patients since the nineties, and represents data otherwise not easily accessible, on a rare group of patients. All patients included did provide DNA to the research biobank at the point of inclusion or at follow-ups. Personal data, as cancer history and characteristics of present cancer where registered at the time of inclusion. Most patients included in the registry for breast cancer where subject to controls, and prospective cancer development was registered. Patients having had prophylactic salpingo-oophorectomy, but not mastectomy were included in the old group. There was no other systematic collection of background data, and all mutations were considered to be similar in penetrance. Both ovarian and breast cancer was registered as events. Forty-seven (60%) of BRCA1 patients in the validation study had one of eight different mutations considered as founder mutations, and 25 of these founder mutation carriers belonged to the young onset group. The remaining thirty-one (40%) had altogether 19 different mutations, of which 15 had young onset cancer. For BRCA2 the variants demonstrated were all classified as disease causing at the time, and potential genotype-phenotype effects were not known. However, due to low inclusion number of BRCA2 mutation carriers in both groups, it was not possible to finish the BRCA2 part of the study.

4.2 Genotyping, SNP selection, variant classification

4.2.1 BRCA1/2 testing, specific tests, MLPA, sequencing (paper 1 and 2)
For paper 1, Genomic DNA was purified from EDTA-anticoagulated blood using the QiaSymphony instrument (Qiagen, Hilden, Germany). All 23 coding exons of BRCA1 (exons 2 to 24) and 26 coding exons of BRCA2 (exons 2 to 27), were amplified, the primers were designed to cover all coding exons and adjacent 20–base pair introns. The amplified DNA fragments were sequenced using the Big- DyeTerminator Cycle Sequencing kit on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). All sequences were compared with the BRCA1 (NM_007294.3) and BRCA2 (NM_000059.3), reference sequences for
variant detection. In addition, MLPA (P002 BRCA1 and P045 BRCA2 MLPA probe mixes; MRC-Holland, Amsterdam, The Netherlands) was performed to identify deletions and insertions.

For paper 2, summing up the activity over 20 years, the specific test technique has evolved over time. From around 1995 and onwards, the laboratories performing BRCA analysis used various techniques. Initially, by using techniques such as denaturing gradient gel electrophoresis and sequencing methods, four recurrent BRCA1 mutations were identified in Norwegian families (c.1556delA, c.3328delAG, c.697delGT and c.1016dupA). Eventually other cost-efficient/affordable tests, such as multiplex PCR fragment analysis and sequencing of shorter fragments were used to screen larger groups of individuals, as well as to detect mutations already found in the family. When new frequent mutations were identified, these were included in the fragment analysis tests. Sequencing of the whole BRCA1 and 2 genes has increasingly been offered to high-risk cancer families since 2000 and 2002 respectively, and MLPA analysis since 2002. Fragment analysis and sequencing/MLPA were used interchangeably in the work-up of these patients. Since January 2014, only Sanger sequencing and HTS methods have been used combined with MLPA. It should be noted that patients from families with a known genetic mutation have only been tested for this specific mutation except when more than one mutation was suspected.

4.2.2 SNP selection (paper 3)

The SNPs to be validated for BRCA1/2- mutation carriers were selected based on literature search in 2011 when the study was designed. These variants were reported to be the genetic variants with significant association with early/late onset of breast cancer among mutation carriers at the time. For BRCA1, the test panel consisted of nine SNPs and one deletion. Seven of these potential BRCA1- modifiers were reported to increase cancer risk (73, 74, 104-107) and three were reported to decrease risk for breast cancer (108-110). For BRCA2, the initial test panel consisted of eleven single nucleotide polymorphisms (SNPs) that were reported to increase risk (73, 74, 105, 111, 112), and one which was shown to decrease risk of cancer (111).

We demonstrated that the SNPs in the test panel were polymorphic in our population of healthy Norwegian blood donors (N = 3000), and that the rare SNP alleles had a frequency > 5 %. The disease-associated alleles reported in other publications were defined as the minor
or risk allele from which positive or negative associations with cancer were calculated in our study, regardless of whether or not this was the least common allele in our population.

4.2.3 Variant classification (paper 1 & 2)
Results for paper 1 and 2 were interpreted and reported following the recommendations of the American College of Medical Genetics [37], using the five-class system. Patients with a variant class 4 or 5, patients with a normal test, but with a young age of onset and/or a family history of breast cancer, and patients with a Variant of Uncertain Significance (VUS) in study 1 were all referred to genetic counseling at DMG-OUH. Here, they received genetic counseling, a detailed family history was obtained and relevant diagnoses in relatives were confirmed. Throughout this thesis and papers 1 and 2, variant is a general term used for any variant regardless of class, while mutation is used for pathogenic variants only, i.e. class 4 and 5 variants.

4.3 Statistics
In study 1, test for trends were performed to compare the differences in breast cancer characteristics between mutation carriers and non-carriers. Separate analyses were done to compare tested and non-tested in order to illustrate potential bias in the group that was not tested. Mutation positives in Cohort 1 and 2 were compared to investigate how similar the two cohorts were. Pearson’s Chi square and one-way ANOVA were used to compare categorical variables (ER, PR, HER2 status, grade, stage, nodal involvement, family history, Ki67 ≥ 30%) while independent t-tests were used to compare continuous variables (age, mean Ki67). In all analyses, p-values less than 0.05 were considered statistically significant. All statistical analyses were performed using SPSS version 21.0. When missing values were observed, this case was omitted in the analysis of this variable.

For study 2, only descriptive calculations were done and no statistic tests were applied.

For study 3, rates of relative risk and odds ratio were calculated. Being in the “young cancer group” was treated as a function of having the SNP allele in question q or Q, stratified by genotypes qq, Qq and QQ. Hence young cancer/old no cancer was considered the outcome and the SNP in question was considered exposure. We also confirmed that the prevalence of the SNPs in the young cancer and old no cancer groups assessed together where all in Hardy–Weinberg equilibrium. Since this was a one-sided study, we used Fishers’ exact to identify any significant association.
4.3.1 Relative risk (paper 3)
Relative risk (RR) is the probability of an event relative to a chosen type of exposure. We referred to the risk allele as q and the normal allele as Q for any SNP. We used the non-exposed group (QQ) as reference, i.e. we calculated the RR for the three other possible genotypes: Homozygotes qq, heterozygotes Qq or qq/Qq.

As an example:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Young</th>
<th>Old</th>
<th>total</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>QQ</td>
<td>14</td>
<td>20</td>
<td>34</td>
<td>1.00</td>
</tr>
<tr>
<td>qq</td>
<td>9 (a)</td>
<td>10</td>
<td>19</td>
<td>1.15</td>
</tr>
<tr>
<td>Qq</td>
<td>19</td>
<td>14</td>
<td>33</td>
<td>1.40</td>
</tr>
<tr>
<td>Qq or qq</td>
<td>28</td>
<td>24</td>
<td>52</td>
<td>1.3</td>
</tr>
</tbody>
</table>

1. A patient with genotype qq is 1.15 times more likely to contract cancer when young as a patient with genotype QQ.

2. A patient with genotype Qq, is 1.40 times more likely to contract cancer when young as a patient with genotype QQ.

3. A patient with genotype Qq or qq, is 1.3 times more likely to contract cancer when young as a patient with genotype QQ.

The RR for all three groups are of interest: If RR(Qq) = 1, it would indicate that the disease-causing mechanism is absence of Q. If RR(Qq) = RR(qq), it may indicate that presence of q is disease-causing. If RR(Qq) is in-between 1 and RR(qq), it may indicate a dosage effect of q (the exposure). The example above indicates the presence of q (not the absence of Q) to be disease-associated, and there is no dosage effect. The three different RRs will give arguments to consider the biological mechanisms causing them. We also calculated odds ratio as an internal control of the results, and did not get deviations. (Odd’s ratio was calculated as the relation between “odds of risk allele in young group”/ vs “odds of risk allele in old group”.

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5. SUMMARY OF FINDINGS

5.1 Paper 1: Diagnostic testing of breast cancer patients

Offering BRCA1/2 testing to breast cancer patients because of potential treatment consequences resulted in 1371 BRCA-analysis being performed over the study period of 19 months (931 from SERHA and 440 from OUH-U). There were in total 42/1371 mutation carriers (3.1%) among the breast cancer patients. Twenty-eight (2.0%) had a mutation in BRCA1 and 14 (1.0%) in BRCA2. Four of the 42 women belonged to families where a BRCA2 mutation already had been detected and predictive testing had not been performed. When considering only those with Norwegian ancestry, we revealed that 13/29 (44.8%) had one of the known Norwegian frequent mutations. Eleven of 29 (37.9%) had a mutation previously found in 1-9 families at DMG (unpublished data), and 5/29 (17.2%) had a mutation not previously observed in Norway.

The mutation positive breast cancer patients were significantly younger, had tumors of a higher grade, and with a higher fraction of Ki67. Significantly more mutation carriers than mutation negatives had triple negative breast cancer as well as a family history fulfilling predictive testing criteria. This comparison was done in Cohort 1/OUH-U only.

The criteria used for identifying mutation carriers had varying sensitivity from 44.7% (NICE) to 89.5% (ASCO), as shown in table 3 in paper 1. “Fulfilling the NBCG criteria” showed a sensitivity of 84.2%, while “Breast cancer < 60 years of age” reached 89.5%. The amount of carriers fulfilling predictive testing criteria before they contracted cancer themselves was 36.8%, implying that 63.2% of the mutation carriers did not have family history that qualified for referral.

The specificity of the test criteria ranged from 48% at the lowest (breast cancer > 60 years of age) to 98% at the highest for triple negative breast cancer. The criteria for predictive testing had a specificity of 89%, while NBCG criteria for diagnostic testing reached a specificity of 69.5%. Specificity was calculated for cohort 1 only.

The number of breast cancer patients needed to test to identify one mutation carrier varies with sensitivity. Testing all breast cancer patients > 60 years of age gave a mutation frequency of 5.5%, and NNT of 18 compared to a mutation frequency of 8.2%/NNT =12 for
the NBCG-criteria. Within a year after the study was finished we had identified 1.1 healthy female mutation carrier per mutation positive breast cancer patient.

5.2 Paper 2: Mutation spectrum study
There were 120 BRCA1 variants and 87 BRCA2 variants among the 3522 mutation carriers, 669/981 families had a BRCA1 mutation (68%), and 312/981 had a BRCA2 mutation (32%).

There were five BRCA1 variants and one BRCA2 variant among the six most frequent variants. These six variants accounted for 47% (1643/3522) of all the mutation carriers, and each variant were found in more than 30 families. On the other end of the spectrum, 147 rare variants were found in only one or two families each and fourteen per cent (479/3522) of carriers had one of these rare variants.

Fifty-two per cent of BRCA1 carriers (1266/2430) or 44 % of BRCA1 families (295/669), had one of the four BRCA1 founder mutations. These four mutations, c.1445del, c.3328del, c.697del and c.1016dup, were also the four most frequent BRCA1 mutations in 2001. Twenty-one per cent (230/1092) of BRCA2 carriers, or 19% of families (61/312), had the single most frequent BRCA2 variant, c.5217_5223del, which was not found to be reported as founder from the Alamut search.

There were altogether 33 BRCA1 and 21 BRCA2 variants found in 3-30 families each, classified as less frequent (3-9 families each) or medium frequent (10-30 families each). These variants accounted for 32% of BRCA1-carriers (775/2430) (34% of BRCA1-families (229/669)), and 57% of BRCA2-carriers (625/1092) (56% (174/312), of BRCA2-families).

There were 82 rare BRCA1 and 65 rare BRCA2- variants. Ten per cent of BRCA1 carriers (242/2430), 15% of the BRCA1 families (99/669), 22% of BRCA2 carriers (237/1092), and 25% of BRCA2 families (77/312) had one of these rare variants.

5.2.1 Founder mutations and not previously reported variants
Among the variants found in more than ten families, 10 of the 15 BRCA1 variants and two of the five BRCA2 variants were previously reported as founder mutations in Norway. Another three BRCA1 variants were identified as Norwegian founders, in the less frequent or rare categories. The remaining variants were either described as founder mutations in neighboring
or European countries. The two most frequent BRCA2 variants, c.5217_5223del and c.4821_4823delinsC were previously reported in other countries, but not as founders. Sixty-one per cent (14/23) of the less frequent mutations in BRCA1 were previously reported as founders, mainly Central-European, Swedish/Danish, only one was Norwegian. For the less frequent variants in BRCA2, 23.5% (4/17) were reported as founders previously. Seventeen per cent of the rare BRCA1 variants (14/82) and 15.4% of BRCA2 variants (10/65) were previously described as founders. Details on founder mutation origin are listed in table 2 and 3 (paper 2).

Variants not previously reported (NPR) were found mainly among the rare variants for both genes. Thirteen BRCA1 and 10 BRCA2 variants (8.2 and 11.5% of variants, respectively) were not previously described in the Alamut search or in available databases. The frequent BRCA2 variant, c. 2047_2050del was the only frequent variant not previously reported.

5.3 Paper 3: Validation of modifiers
When examining the relative risk for each SNP separately, we found that rs3803626 (TOX3, LOC643714), rs10046 (CYP19), rs104585 (CASP8), rs2363956 (ABHD8, ANKLE1, C19orf62), rs16942 (BRCA1) did confirm previously published associations. These five of the ten possible BRCA1 modifiers considered, showed the expected association with early breast cancer in Norwegian mutation carriers. The associations were statistically significant for rs1045485 and rs10046 (homozygotes), rs16942 and rs3803662 (heterozygotes) as well as for rs2363956 (homo- and heterozygotes). The remaining five SNPs did show point estimates that in varying degree corresponded to reference risk estimates. The significant associations shown were stronger than previously reported, e.g. the results for rs2363956 showed a stronger protective effect for both homo- and heterozygotes than did references: RR (CC) 0.47 (our data) vs 0.7, and RR (AC) 0.65 (our data) vs 0.89 (109).

There was no discrepancy between the directions of the RR and OR estimates. We were not able to compute theoretical significance levels against those expected due to the different statistical methods used in the studies. The low patient number did not allow us to make groups for combinations of SNPs and evaluate the possible interactions and associations between multiple SNPs and disease risk.
6. GENERAL DISCUSSION

The findings presented in this thesis represent an effort to get updated information on important aspects regarding \textit{BRCA1/2} mutation carriers in Southern Norway, focusing on selection for genetic testing, mutation spectrum and genetic modifiers. All the included papers present studies that combine genetic epidemiological research and clinical cancer genetics research performed in patient populations. This general discussion section is considered a supplement to the discussions presented in each paper, and will only briefly discuss identical issues as the papers. Paper 1 and 2 focus on genetic testing for identifying mutation carriers, while paper 3 focuses on testing to refine individual risk estimates. On the whole, the results presented in this thesis may be used in a broader evaluation of the \textit{BRCA1/2} genetic testing practice in the era of personalized medicine.

6.1 Diagnostic testing

Paper 1 shows the results after offering \textit{BRCA1/2} less selectively to breast cancer patients. The findings on the mutation positive breast cancer cohort were in line with findings from other studies: As a group, \textit{BRCA1/2} mutation carriers contracted breast cancer with specific characteristics significantly more often than sporadic cases. The mutation carriers were younger, had tumors of higher grade, they had higher Ki67 and were more often of the triple negative subtype of breast cancer. They also had significantly more often family history (as defined by criteria).

Sensitivity and specificity of the NBCG diagnostic criteria used in Norway today were 84.2% and 69.5% respectively. The highest sensitivity measure of 89.5% was reached by the ASCO-criteria, and hence we conclude that even with the most sensitive diagnostic criteria 10.5% of mutation carriers with breast cancer were not identified. It appears to be a trade-off between sensitivity and specificity.

Sensitivity increases when combining criteria, each highly specific for \textit{BRCA1/2}-status. But in doing so, specificity decreases. This could be illustrated with one of the highly specific breast cancer characteristics in \textit{BRCA1/2} carriers, young age of onset. “Breast cancer $< 40$ years of age” has a specificity of 94.4%, but a sensitivity of 31.6%, it is therefore insufficient on its own for selecting patients efficiently, and many mutation carriers will not be identified.
“Breast cancer < 60 years of age” was found to have a sensitivity of 89.5% and specificity of 48%. This sensitivity measure is identical to that of ASCO-criteria. The predictive criteria had a sensitivity of 36.8% and specificity of 89%. The lower specificity of the diagnostic NBCG criteria (69.5%) over predictive criteria (89%) is probably not surprising given the fact that 70% of hereditary breast cancer is due to other causes than pathogenic mutations in BRCA1/2 as of today. The lower the specificity, the more tests will have to be performed in order to identify one mutation carrier. Testing all breast cancer patients < 60 years of age results in a higher number needed to test (NNT) of 18. With this scenario, six more tests per mutation carrier identified will have to be performed compared to using NBCG-criteria (NNT = 12). Testing breast cancer patients with triple negative breast cancers gives an NNT of 7, as this criterion alone has a specificity of 94%, and a sensitivity of 34.2%. The NBCG/ASCO-criteria as such show the best trade-off between sensitivity and specificity in diagnostic testing, but a high NNT may be considered less problematic if the genetic test is affordable and if the consequences for the non-identified mutation carriers are significant.

When calculating the sensitivity and specificity of predictive testing criteria as of today, 36.8% and 89% correspondingly) paper 1 illustrates the test criteria’s ability to detect mutation carriers before they get cancer, which is considered highly valuable in terms of the prophylactic potential. It is somewhat surprising that 40% of new mutation carriers did have a family history fulfilling criteria for testing before they contracted cancer themselves. This means that our current system of referral for predictive testing is not efficient enough or is even suffering from lack of compliance. Supplementing this, a pilot study on mutation frequency in patients referred for predictive testing in our clinic have described a mutation frequency of 2% which is comparable to the frequency of 3.1% found in paper 1 (113).

In line with these results, a recently published study from Sweden reported a 2% BRCA1/2 mutation carrier rate in a cohort of unselected breast cancer patients (114). In this study, Nilsson et al (2017) found that six out of 11 identified mutation carriers fulfilled the Swedish BRCA testing criteria, and hence 5/11 did not. Nine out of 11 fulfilled the NCCN testing criteria. None of the BRCA-associated tumors in this study were of the luminal A-like subtype. In a recent Chinese study of 8,085 unselected breast cancer patients, 5.3% of patients carried BRCA mutations (1.8% in BRCA1 and 3.5% in BRCA2) (82). In this study, almost three per cent were carriers of mutations in other breast cancer susceptibility genes.
A Swedish study of BRCA1/2 mutations in unselected breast cancer patients showed a 7% germline mutation frequency in BRCA1/2 (115). Another recent, Norwegian study found a BRCA1/2 mutation in 1.7% of the participants (116), (this somewhat low number may partially be explained by a lower fraction of patients offered full test with sequencing and MLPA).

6.2 Mutation frequencies
In paper 2, we have shown that the fraction of mutation carriers carrying one of the founder mutations is lower than previously described. We also found that 10% of BRCA1 and 22% of BRCA2 carried a rare mutation found in only one or two families. The fraction accounted for by the four most prevalent BRCA1 mutations had fallen from 68% in 2001 to 52% in paper 2, due to detection of other mutations, frequent and rare. In paper 1 we reported that 44.8% (13/29) of the identified mutation carriers having Norwegian ancestry, had one of ten BRCA1/2 most frequent Norwegian mutations as published in 2007 (117). Also, the four most prevalent BRCA1-mutations from paper 2 were found in nine of the 29 Norwegian mutation carriers in paper 1, i.e. in about one third of the mutation carriers. Since these four mutations have been subject to family cascade testing for the longest time, one may discuss whether the observed amount of founder mutation carriers detected diagnostically in paper 1 is as expected. If testing by criteria is a sufficient strategy for identifying mutation carriers, these mutations could probably be expected to decrease or even vanish from the “incident” breast cancer group after some time. This could have been an explanation of a lower fraction of BRCA1/2 mutations (1.7%) in the study by Høberg-Vetti et al, as these mutations originated from the West-Norway (116). However, nine of 27 mutation carriers in this study had one of the BRCA1 founder mutations, c.1556del in BRCA1. All but one was identified as they contracted ovarian cancer. It could be argued that it is even more essential to avoid development of ovarian cancer in these carriers, because of its poor prognosis. Our paper 1 has only included breast cancer cases, and the spectrum of mutations in an incident ovarian cancer cohort is not studied in our material.

Paper 1 and 2 are closely linked in several ways, showing the mutation frequencies and spectrum over a chosen period of time and with a certain approach to which individuals are tested, and as such they complement each other. In Paper 1, full testing is given broadly to breast cancer patients over a period of 18 months, while in paper 2, families are initially
offered specific founder testing to families fulfilling criteria, resulting in cascade testing in the carrier families. The test criteria used in the nineties were more restrictive than the ones presented in paper 1 (118), and over time broader testing on a more liberal indication has been practiced.

Both papers 1 and 2 are troubled with selection biases, as they are patient-based, observational studies. In Paper 1, the selection bias mainly arises from the fact that within Cohort 1/OUH-U, almost 30% of the breast cancer patients were not BRCA1/2-tested. We do not have the exact numbers for uptake to testing in SERHA. An estimation based on number of BRCA1/2 tests divided by number of breast cancer cases (931/2400) in SERHA was performed, giving an uptake of 39%, and this is even lower than for OUH-U. As a group, the non-tested in Cohort I were older, and fewer fulfilled the NBCG criteria than those who were tested. If there are mutation positives among the untested, the total mutation frequency will be lower, but the mutations positives identified will be less likely to fulfill criteria.

In paper 2, the frequencies of founder mutations are probably heavily biased because both diagnostic, predictive testing and family work up have focused on founder mutations from the nineties and up to around 2010. The fraction of families carrying a mutation are therefore more informative than the numbers of carriers, but the percentages of families are not directly comparable to previous studies that did not count mutation families for all of the detected mutations at the time (63, 117). An overall larger percentage of carriers than of families for the most frequent mutations illustrate this selection bias, as well as “rate of mutation carriers per family” which is lower for the rare mutations.

In Paper 1, sensitivity and specificity of test criteria is discussed, showing that with the use of the current criteria, about 10% of mutation carriers will not be detected. The sensitivity and specificity of DNA-testing to identify mutation carriers is also important to consider, but this is not directly studied in paper 2 in the same comparative way. However, some important implications on this issue can be drawn from the results.

Offering specific “founder” testing and MLPA compared to sequencing and MLPA will have a lower sensitivity, especially in outbred, multicultural populations. This is clearly illustrated by how the fractions of carriers previously recognized as founders have fallen from 68% to 52%, but also how 38 BRCA1 and 22 BRCA2 frequent mutations (found in more than three
families) now are accounting for 90% and 78% of BRCA1 and BRCA2 carriers, respectively. When testing for a limited number of these mutations, sensitivity will obviously be directly affected. The founder mutation testing may have a sufficient sensitivity in very homogenous genetic populations with a high founder mutation frequency, i.e. Ashkenazim or Icelandic populations, but a recent study from Ontario by Finch et al showed that even among women with Jewish ancestry who tested negative for founders, and still fulfilling test criteria, 2% of had another mutation in BRCA1/2 (97). Our paper 2 is considering patients and families fulfilling test criteria, hence these are comparable studies. Our number of non-founders will depend on the definition, but we did find that 10% BRCA1 and 22% of BRCA2 carriers had one of the rare mutations.

High sensitivity correlates with low number of false negative test results, high specificity correlates with low number of false positive test results, and while these measures are important they are not readily given for genetic tests. Discovery of new pathogenic BRCA1/2 mutations, for instance in intronic regulatory domains, or more hypothetically, epigenetic mutations, could possibly change the spectrum further. With such new discoveries, the mutation detection rate within the group of hereditary breast cancer will increase, and the sensitivity of the previous test will fall. This is what is demonstrated in paper 2, and this development is likely to continue.

The specificity of a given genetic test refers as to what degree a normal genetic variant is identified as normal. Variants of unknown significance may represent this kind of false positive information if the variant is communicated as risk-associated and later down-graded. The percentage of VUSes would vary with the ethnic background. Finch et al showed that 15.8% had a VUS, high amount of VUS in all categories except for Jewish women. 42% of women with African decent had a VUS and most of them were only tested for founders (97). In our paper 4.9% had a variant of unknown significance. The fact that any variant may, more or less likely, be subject to reclassification as evidence evolve, must be considered, and therefore any variant must be object for repeated reevaluation. Systematic, national and international database cooperation on variation is necessary and important to get new information on variants.

It is very important to establish the frequencies of rare pathogenic BRCA1/2 mutations due to their significance in cancer prevention, but also as background information when a broader
testing approach for other breast cancer genes with lower penetrance are applied through gene panels (101). If pre-symptomatic population screening should be discussed regardless of family history in Norway, as it has been piloted among Ashkenazi Jews in the United States, such knowledge is absolutely crucial (102). When discussing screening for any disease, rare or common, establishing test sensitivity and specificity is central (96). To identify more \textit{BRCA1/2} mutation carriers before they contract cancer a pilot project on screening with Norwegian frequent mutations, i.e. as they have been identified in this study could be considered.

Motivation for testing in the settings studied in paper 1 and 2 are generally quite high both for the individual and the family, and the results of testing is generally well accepted (119). Experiencing cancer in the near family is a motivator for testing. Wiesman et al (2016) described how a pilot program of \textit{BRCA1/2} testing was offered to Ashkenazi Jewish individuals both fulfilling NCCN criteria for testing among Ashkenazi Jewish, and not. The average age of the participants were 50 years of age, and two of mutation carriers were detected that did not fulfill the criteria, but did have cancer in the family, however not \textit{BRCA}-related. It is essential to identify factors that significantly increase coping with hereditary cancer risk that may differ according to age, family history or the lack of it. Such knowledge and how to best to facilitate coping is essential, especially for young women in the age of 18 – 30 when identified as mutation carriers (103).

6.3 Cancer risk estimates, penetrance and modifiers

Paper 3 is focusing on genetic modifiers of penetrance and cancer risk. Penetrance is a major issue facing the mutation carriers, especially when they are aware of their carrier status prior to cancer development. The collection of information done on the mutation carrier’s behalf and communicated in genetic counseling is striving towards a best possible informed choice in medical management. The cancer risk estimates given are often group-based, because the evidence is not solid enough to exclude any \textit{BRCA1/2}-carrier from the highest risk measure.

Penetrance studies in \textit{BRCA1/2} are to different degrees troubled with selection biases, mainly through ascertainment that is a central feature in all patient-based research populations. The risk estimates in an ascertained population may be higher than they would be if obtained from a population-based study, on the other hand such a study would be largely infeasible because
of the low prevalence of mutation carriers in the general population, and the long observation time needed (54). Gabai-Kapara et al recently showed for Ashkenazi-Jewish mutations that penetrance was similar when selected through healthy men regardless of family history (61). If a spectrum of mutations causing different cancer risk is identified, the benefit from testing will vary with the specific cancer risk. This is one of the issues arising when discussing population-screening for more variants than the well-studied founders, especially if the variants selected truly have a specter of penetrance.

In general, studying genetic mechanisms for disease development in humans are not only troubled by selection bias, but also with the existence of unknown confounders. One may hypothesize that genotype/phenotype-correlations and possible interactions with rare modifiers may explain both varying prevalence of \textit{BRCA1/2} mutations in comparable studies of “unselected breast cancer patients” and the varying penetrance shown. One such confounder in genotype/phenotype studies may be unknown genetic (or environmental) modifiers, and vice versa. Unknown interactions between environmental and genetic modifying factors may also confound the result. Large and well-planned studies are therefore essential to control for relevant confounders, also because genetic heterogeneity in mutation effects is up to now largely not taken into account in studies on environmental modifiers (69).

To detect potential effects of rare genetic modifiers another approach is needed, and studies on genomic data are underway, supplementing the evidence from GWAS on susceptibility alleles and their effect on penetrance. Genetic modifiers in the form of common genetic variants, SNPs are found in risk prediction models to be interacting not deviant from a multiplicative model, as studied by CIMBA for many years. A systematic assessment of the pairwise interactions between all SNPs that are known to modify cancer risks is currently ongoing in \textit{BRCA1/2} mutation carriers (72). An EMBRACE-led prospective study on cancer risk in \textit{BRCA1/2} patients included profiling of four SNPs but did not find evidence of an association (54). Nevertheless, in this last study a risk score based on the joint distributions of alleles, and assuming that hazard ratios add up multiplicatively, showed an HR of 4.0 in breast cancer risk between the lower tertile and the higher tertile of combined SNP, statistically significant for \textit{BRCA2} carriers. Mavaddat et al (2012) argue that the apparent difference in SNP associations between \textit{BRCA1} and \textit{BRCA2} and non-carriers observed, may be explained by differences in prevalence of tumor subtypes (60) Many of the common breast susceptibility alleles identified through GWAS are predominantly associated with either ER-
or ER+ cancers, indicating that common mechanisms underlie the phenotype of tumors in both mutation carriers and the general population, and studies controlling for different subtypes of breast cancer are therefore warranted.

The models of risk prediction for multiple SNP on breast cancer risk are statistical prediction models. A new genetic test intended to refine risk must consider the issues of sensitivity and specificity of identifying correct individuals, just as we have done in paper 1 and 2 for test criteria and \textit{BRCA1/2} testing. What precision level for cancer risk estimation mutation carriers accept, will probably depend on the different measures to be undertaken, their safety and price. Couch et al showed that for ten SNPs shown to be associated with cancer risk in \textit{BRCA1}, the five percent of \textit{BRCA1} carriers at lowest risk is predicted to have a 28–50% life time risk of breast cancer, compared to 81–100% for the 5% at highest risk (120). A remaining issue is how to determine the lowest risk estimate that could serve as a cut-off for declining or postponing prophylactic surgery. This may be especially important in a situation when surveillance is considered less safe in terms of survival.

The optimal study design for studying factors that modify cancer risks is a prospective cohort in which unaffected mutation carriers are followed over time to observe prospectively who goes on to develop cancer (72, 121). Many years of follow up is necessary, and information on several background factors must be available to study cancer risk associations and interactions between factors. Any direct intervention (prophylactic mastectomy/oophorectomy) will also lead to the inability to collect prospective data on breast cancer incidence (lost-to-follow-up), further complicating the possibility of studying the feasibility of any test to differentiate in cancer risk. The idea of multiple SNP profiling and its multiplicative model need validation (75), but this kind of evidence may be included in an individual, theoretical assessment of risk, if the limitations is taken in to account and communicated to the patient.

Our paper 3 was aiming at validating the effects of SNPs as modifiers in both \textit{BRCA1}-carriers. We found that five of the ten \textit{BRCA1} modifiers had a significant effect on early cancer in Norwegian mutation carriers. The five SNPs showing significant association with cancer risk were rs38036622, rs8170, rs10046, rs2363956, and rs16942. The lack of significant associations for the remaining five SNPs could be due to different distribution in Norwegian population compared to other populations, also known as genetic drift. The results
were not explained by a difference in distribution of founder versus non-founder mutations that otherwise could have been confounding the results. However, our study is likely to be underpowered, and the results must be interpreted with caution. This lack of power may also explain the discrepancies between heterozygotes and homozygotes. It should be noted, that the results may also be indicative of a difference in population distributions of the SNPs and the potential disease-causing DNA variation in BRCA1-carriers in Norway compared to other populations. If so, the results may be explained by deviating linkage disequilibria on the different haplotypes between Norway and other countries, instead of being a methodological artifact. This interpretation may serve as an illustration on whether a global risk association for any given SNP is expected to be found. The effect of a local modifier effect in carriers from one population may disappear when they are included in a larger group of patients. Combining the populations may therefore lead to an overall weaker association than shown by the separate SNPs in smaller populations.

There is evidence that other factors than genetic modifiers may be included in risk prediction programs. Mavaddat et al (2012) argue that knowing the specific distribution of clinicopathological characteristics for BRCA1/2 breast cancers may give evidence that are relevant for predicting carrier status programs, as well as for the models predicting breast cancer risk. In the light of our current studies, we think such information may be most important for improving individual breast cancer risk predictions and to provide for “state of the art”- informed choices of risks of disease to base a choice of prophylactic surgery vs screening. For instance, the evidence that BRCA2 carriers have increased risk for triple negative breast cancer later in life than BRCA1 carriers may influence medical choices. Milne et al (2016) suggest that health providers should aim to be able to provide such comprehensive counselling based on estimates that consider both the gene mutated, and the position and functional effect of the mutation as well as family history of cancer and all genetic and lifestyle/hormonal factors that modify risk for mutation carriers (72) and this is an ambitious aim, which we support.
7. CONCLUSION

It is well established that the potential of individualized cancer prevention through BRCA1/2 testing is best realized when mutation carriers are identified before they contract cancer. How then can we reach as many as possible of these mutation carriers before they contract cancer? Considering the findings from paper 1, 42 mutation carriers were identified as they contracted breast cancer over the study period, and the main message is that these patients did not get the opportunity of prophylactic surgery. They were on average less than 50 years of age and contracted high grade tumors more often triple negative, nine of ten tested were within the given test criteria after themselves contracting cancer, but 60% of the identified mutation carriers did not have family history that could have alerted them of their risk. This may be due to the variable penetrance, modifiers, small families or stochastic factors. Nine of 29 mutation carriers with Norwegian decent had one of the four most frequent BRCA1 mutations. A 3% rate of mutation carrier identification when testing breast cancer patients regardless of criteria, enables testing of the nearest relatives, and in our study one mutation carrier was detected per breast cancer patient. However, families are smaller today, and fewer have regular contact with more distant relatives, and these facts may explain why new families with the Norwegian mutations that has been subject for testing the longest time still are detected in an “incident breast cancer” cohort. Hence, the prospect of offering testing for disease-causing mutations in voluntary individuals should be evaluated. The main obstacle is the cost and analysis of BRCA1/2 - tests, the issue of reporting variants of uncertain significance to the individuals choosing to get the test, as well as an evaluation of the framework of a screening program.

In conclusion, paper 1 tells us that fewer mutation carriers will be missed if more breast cancer patients are tested regardless of family history. But this does not seem to reach out to all mutation carriers before they contract cancer; however this strategy enables relatives of identified mutation carriers to get the test. Paper 2 shows that if more patients are tested with sequencing, new mutations, rare and frequent are detected and due to this, families previously only given a specific founder mutation test should get an updated genetic test. Both the test criteria and the genetic testing procedure will have a certain false negative rate, which may be possible to calculate when population frequencies and characteristics of carriers in unselected populations are established. Both paper 1 and 2 give indications on such false negative rates,
ten per cent of carriers are missed with the most sensitive test criteria, and fourteen per cent have a rare \textit{BRCA1}/2 mutation, only seen in one or two families. Paper 3 discusses the effect of genetic modifiers in Norwegian mutation carriers in an effort to validate their risk associations. Half of the chosen SNPs, five of the ten modifiers for \textit{BRCA1} penetrance were confirmed.

As mutation carriers are identified the focus must be clinically on what may be considered a threshold of risk that requires medical management, and individualized advice regarding risk could be considered, taking gene, mutation type, life style and age of the patient into account. No separate factor is able to null out the group estimate and this must therefore be considered the outer limit of risk. The uncertainty in risk guidance must be communicated. How the experience of being a risk person is affected by the different contexts of diagnostic, predictive and screening is essential to fully understand in order to facilitate the best coping strategy for mutation carriers regardless of context. A recent study showed an effect of a systematic peer-based coaching program especially in young mutation carriers (103). Such knowledge will also be necessary when planning pilot studies offering relevant \textit{BRCA1}/2 test with reasonable detection rate and will be valuable in the individual counselling setting as well, probably especially for young women detecting their mutation.

7.1 Postface
So, this is my work applying for the title as a PhD, to become a philosophical doctor as well as a medical doctor. I hope you liked it. As I said in the Preface this represents my attempt to become a medical researcher, a doctor that are able, in close cooperation with other health professionals and scientists, to develop research projects that may benefit the patients.

No matter how overwhelming at times, if we don’t try we most certainly don’t succeed. “To do good” is now, to me, not to give up \textit{trying} to identify “knowledge gaps”, and then not to give up \textit{trying} to fill them.
8. REFERENCES


38. Krush AJ. Contributions of Pierre Paul Broca to Cancer Genetics. Transactions of the Nebraska Academy og Sciences. 1979;VII.


9. SUPPLEMENTARY TABLES

9.1 Supplementary table 1: Screening criteria

**Wilson and Jungner classic screening criteria**

1. The condition sought should be an important health problem.
2. There should be an accepted treatment for patients with recognized disease.
3. Facilities for diagnosis and treatment should be available.
4. There should be a recognizable latent or early symptomatic stage.
5. There should be a suitable test or examination.
6. The test should be acceptable to the population.
7. The natural history of the condition, including development from latent to declared disease, should be adequately understood.
8. There should be an agreed policy on whom to treat as patients.
9. The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.
10. Case-finding should be a continuing process and not a “once and for all” project.

**Synthesis of emerging screening criteria proposed over the past 40 years**

The screening program should respond to a recognized need.
The objectives of screening should be defined at the outset.
There should be a defined target population.
There should be scientific evidence of screening program effectiveness.
The program should integrate education, testing, clinical services and program management.
There should be quality assurance, with mechanisms to minimize potential risks of screening.
The program should ensure informed choice, confidentiality and respect for autonomy.
The program should promote equity and access to screening for the entire target population.
Program evaluation should be planned from the outset.
The overall benefits of screening should outweigh the harm.
2a: The National Comprehensive Cancer Network (NCCN)

NCCN Guidelines Version 2.2017

BRCA1/2 testing criteria for breast cancer patients *

- Individual from a family with a known deleterious BRCA1/BRCA2 gene mutation
- Personal history of breast cancer + one or more of the following:
  - Diagnosed ≤45 years
  - Diagnosed ≤50 years with:
    - An additional breast cancer primary
    - ≥1 close blood relative with breast cancer at any age
    - ≥1 close relative with pancreatic cancer
    - ≥1 relative with prostate cancer (Gleason score ≥7)
    - An unknown or limited family history
  - Diagnosed ≤60 years with:
    - Triple negative breast cancer
  - Diagnosed at any age with
    - ≥2 close blood relatives with breast cancer, pancreatic cancer, or prostate cancer (Gleason score ≥7) at any age
    - ≥1 close blood relative with breast cancer diagnosed ≤ 50 years
    - ≥1 close blood relative with ovarian carcinoma
    - A close male blood relative with breast cancer
    - For an individual of ethnicity associated with higher mutation frequency (e.g. Ashkenazi Jewish) no additional family history may be required
  - Personal history of ovarian cancer
  - Personal history of male breast cancer
  - Personal history of prostate cancer with family history**
  - Personal history of pancreatic cancer with family history**
  - BRCA1/2 mutation detected by tumor profiling in the absence of germline mutation analysis**
  - First – or second-degree blood relative (same side of family) meeting any of the above criteria**
  - Third-degree blood relative who has breast/ovarian cancer and ≥2 close blood relatives ≤ 50 years or ovarian cancer**

*Breast cancer includes invasive carcinoma and DCIS

**Criteria not relevant for this study, for details on family history specifications please see the full NCCN guide.
2b: American Society of Clinical Oncology (ASCO)
ASCO Guidelines

Breast Cancer Survivorship Care Guideline
“Those with a family history of breast or ovarian cancer or cancer in a certain age group and/or cancer type should be referred for genetic counseling for consideration of testing for hereditary predisposition to genetic mutations. Specifically, genetic counseling for consideration of testing for hereditary predisposition to gene mutations should be recommended for breast cancer survivors with the following characteristics:

64

2c: Norwegian Breast Cancer Group (NBCG)

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<td>o Breast cancer &lt;50 years</td>
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<td>o Two close relatives with breast cancer, mean age &lt;55 years</td>
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<td>o Three close relatives with breast cancer at any age</td>
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<td>o Male breast cancer</td>
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<td>o Bilateral breast cancer &lt;60 years</td>
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<td>o Female breast cancer and close relative with ovarian cancer</td>
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