

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subjects, each one the focus of numerous PhD-programs), you must know important details, and understand when you are outdated.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 where 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 care givers 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.

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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, p_1 / p_2 or $(a / a + b) / (c / c + d)$

Odds` ratio - Ratio of the odds in two groups, $p_1 / (1 - p_1) / p_2 (1 - p_2)$ or $(a / c) / (b / d)$.

1.3 List of papers/publications

Paper 1: Grindedal EM, Heramb C, Karsrud I, Ariansen SL, Mæhle L, Undlien DE, Norum J, Schlichting E.: “*Current guidelines for BRCA testing of breast cancer are insufficient to detect all mutation carriers*” **BMC Cancer. 2017 Jun 21;17(1):438**

Paper 2: Heramb C et al: “*BRCA1 and BRCA2 mutation spectrum – an update on mutation distribution in a large cancer genetics clinic in Norway*” **Hered Cancer Clin Pract. 2018 Jan 10;16:3.**

Paper 3: Heramb C, Ekstrøm PO, Tharmaratnam K, Hovig E, Møller P, Mæhle L.” *Ten modifiers of BRCA1 penetrance validated in a Norwegian series*
Hered Cancer Clin Pract 2015 May 30;13(1):14

2. GENERAL INTRODUCTION

The breast cancer susceptibility genes, *BRCA1* and *BRCA2*, are well-known for being associated with substantially increased cancer risk in women, mainly breast and ovarian cancer (1). *BRCA1/2* testing is one of the earlier genetic tests performed in our lab, starting up in the nineties. Now, in 2017, about 300 *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, *BRCA1/2* mutation carrier number 4000 was identified in Section for Hereditary Cancer (SHC), and 1163 *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 *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 *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 *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 *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 *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; *BRCA1/2* mutation frequency, breast cancer characteristics and sensitivity of *BRCA1/2* testing criteria in a broader breast cancer population (paper 1).
- 2) Mutation spectrum of *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 *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 *BRCA1/2* - testing and management.

Firstly, the criteria for *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 *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.

<p>BRCA 1/2 test criteria</p>	<p>Are test criteria fulfilled?</p> <p>Diagnostic testing</p> <p>Predictive testing</p> <p>Sensitivity Specificity</p> <p>How many carriers are lost?</p>	<p>Current guidelines for BRCA testing of breast cancer patients are insufficient to detect all mutation carriers</p> <p><i>Abstract</i> Background Identification of BRCA mutations in breast cancer (BC) patients influences treatment and survival and may aid in prognostic for their relatives. Testing is often limited to carriers fulfilling high risk criteria. However, there is limited knowledge of the penetrance of such mutations and of the clinical impact of BC caused by BRCA mutations in this population. The aim of this report was to address these issues by evaluating the results of BRCA testing of BC patients in South-Eastern Norway. Methods 127 newly diagnosed BC patients were tested with sequencing and Multi-Copy-Fusion Amplification (MCA) for mutations in BRCA1 and BRCA2. Characteristics, among others and represents complete penetrance and penetrance of common guidelines for BRCA testing to identify carriers was analyzed. Number of identified breast mutation carriers was assessed. Results A pathogenic BRCA mutation was identified in 31%. Carriers of both full-length and truncated BRCA1 and BRCA2 mutations were included in the analysis. The mean age at diagnosis was 52.5 years. The mutation carrier frequency was 24.4% (95% CI 16.8-32.0). The mean age at diagnosis was 52.5 years. The mutation carrier frequency was 24.4% (95% CI 16.8-32.0). Conclusions Greater penetrance of BRCA mutations in breast cancer patients is observed compared to the general population. Current guidelines for BRCA testing are insufficient to detect all mutation carriers. Testing of breast cancer patients for BRCA mutations is recommended to identify carriers of BRCA mutations in this population. Keywords Breast cancer, BRCA mutations, Genetic testing, Norway</p>
<p>BRCA 1/2 mutation test</p>	<p>Mutation spectrum</p> <p>Founder mutations</p> <p>Rare variants</p> <p>How many carriers are lost?</p>	<p>BRCA1 and BRCA2 mutation spectrum – an update on mutation distribution in a large cancer genetics clinic in Norway</p> <p><i>Abstract</i> Background Founder mutations in the breast cancer genes BRCA1 and BRCA2 have been identified in various populations, among them in the Ashkenazi Jewish, Finnish, Hungarian and Italian. Founder mutations among the population in South-Eastern Norway have been identified. The aim of this study was to update the mutation spectrum of BRCA1 and BRCA2 mutations in this population. The aim of this study was to update the mutation spectrum of BRCA1 and BRCA2 mutations in this population. Methods A total of 2015 BRCA1 and BRCA2 mutation carriers were included in the study. The mutation spectrum of BRCA1 and BRCA2 mutations was analyzed. The mutation spectrum of BRCA1 and BRCA2 mutations was analyzed. Results The mutation spectrum of BRCA1 and BRCA2 mutations was analyzed. The mutation spectrum of BRCA1 and BRCA2 mutations was analyzed. Conclusions The mutation spectrum of BRCA1 and BRCA2 mutations was analyzed. The mutation spectrum of BRCA1 and BRCA2 mutations was analyzed. Keywords BRCA1, BRCA2, Founder mutations, Genetic testing</p>
<p>BRCA 1/2 risk management</p>	<p>Risk prediction</p> <p>Genetic modification of risk</p> <p>Individualization of risk</p> <p>How to choose?</p>	<p>Ten modifiers of BRCA1 penetrance validated in a Norwegian series</p> <p><i>Abstract</i> Background Carrier status of BRCA1 mutations is associated with an increased risk of breast and ovarian cancer. The aim of this study was to validate ten modifiers of BRCA1 penetrance in a large Norwegian series. Methods The ten modifiers of BRCA1 penetrance were validated in a large Norwegian series. The ten modifiers of BRCA1 penetrance were validated in a large Norwegian series. Results The ten modifiers of BRCA1 penetrance were validated in a large Norwegian series. The ten modifiers of BRCA1 penetrance were validated in a large Norwegian series. Conclusions The ten modifiers of BRCA1 penetrance were validated in a large Norwegian series. The ten modifiers of BRCA1 penetrance were validated in a large Norwegian series. Keywords BRCA1, Modifiers, Cancer risk</p>

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 *BRCA2* carriers, these average cumulative risks were 66% and 12%, respectively. After a first breast cancer, *BRCA1* and *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 *BRCA1* carriers to be 60% for breast cancer, 59% for ovarian cancer, and 83% for contralateral breast cancer. For *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 *BRCA1/2* breast cancers

The pathology of *BRCA1/2* breast cancers shows the variable expression of *BRCA1/2* mutations. Tumors arising in *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 *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 *BRCA1* germline mutations are significantly more often “triple negative”, i.e. negative for estrogen receptor, progesterone receptor, and HER-2 (57). *HER2*-status has not been found to be a reliable predictor of *BRCA*-status (58, 59), and hence it has not been suggested as selection criteria for testing.

The pathology of *BRCA1* breast cancers has been somewhat better studied than pathology of *BRCA2*. Mavvadat et al. (2011) performed a large study on comparing pathology of breast cancer in *BRCA1* and *BRCA2* mutation carriers. The study included pathology data on 4325 *BRCA1* and 2568 *BRCA2* mutation carriers (60) and was able to study smaller subsets of disease characteristics and give age-specific proportions of tumor subtypes in *BRCA1* and

BRCA2. 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 *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 *BRCA1* mutations in 2001 were haplotyped and found to originate from different parts of the country.

The Icelandic *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 *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 *BRCA1*-carriers and 33% and 76% for *BRCA2*-carriers in this study.

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 *BRCA1* or *BRCA2* gene (65). The Breast Cancer Linkage Consortium suggested early on, that variants in a region known as the Ovarian Cancer Cluster Region in *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 *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 *BRCA1* and *BRCA2* mutation carriers (65). This study also identified multiple breast cancer cluster regions (BCCRs) in *BRCA1* and *BRCA2* and two OCCRs in *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 *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 *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.

Environmental modifiers of *BRCA1/2* have been the subject of many studies. In general, environmental risk factors for cancer are better studied for *BRCA1* than for *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 *BRCA1/2* mutation carriers were evaluated (69). Only high age at first life birth received the characteristic “probable association” for *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 *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 *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 *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 *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 *BRCA2* mutation carriers, but not for *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 *BRCA2*, and a decreased for *BRCA1* (76).

Milne and Antoniou (2016) summarizes that to date, a total of 26 and 16 SNPs are associated with breast cancer risk for *BRCA1* and *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 *BRCA1* and *BRCA2* mutation carriers. The joint effects of all SNPs and family history have not been estimated for *BRCA1* and *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 *ESR1*-locus, 19p13.1, (77, 78) and *TERT* (27). Associations with ER-negative cancer have been found for *TERT*-loci.

Genetic and environmental modifiers of *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 mammae 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.

$$\text{HWE: } p^2 + 2pq + q^2 = 1$$

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 where genetic counselling is offered, (www.lovdatabank.no). 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 rationale 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 in 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 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 *BRCA* testing of “treatment consequence” was added. Breast cancer patients not fulfilling the traditional criteria could then be offered *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 carriers, 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 were registered at the time of inclusion. Most patients included in the registry for breast cancer were 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-Dye Terminator 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 \geq 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 were 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:

Genotype	Young	Old	total	RR
QQ	14	20	34	1.00
qq	9 (a)	10	19	1.15
Qq	19	14	33	1.40
Qq or qq	28	24	52	1.3

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. (Odds ratio was calculated as the relation between “odds of risk allele in young group”/ vs “odds of risk allele in old group”).

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 represents an effort to get updated information on important aspects regarding *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 focus 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 *BRCA1/2* genetic testing practice in the era of personalized medicine.

6.1 Diagnostic testing

Paper 1 shows the results after offering *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, *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 *BRCA1/2*-status. But in doing so, specificity decreases. This could be illustrated with one of the highly specific breast cancer characteristics in *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 *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 *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 *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 *BRCA1/2*-carrier from the highest risk measure.

Penetrance studies in *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 spectrum 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 *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 *BRCA1/2* mutation carriers (72). An EMBRACE-led prospective study on cancer risk in *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 *BRCA2* carriers. Mavaddat et al (2012) argue that the apparent difference in SNP associations between *BRCA1* and *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 *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 *BRCA1*, the five percent of *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 *BRCA1*-carriers. We found that five of the ten *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 *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 *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 *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 *trying* to identify "knowledge gaps", and then not to give up *trying* to fill them.

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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.

9.2 Supplementary table 2: Guidelines for testing

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:

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2c: Norwegian Breast Cancer Group (NBCG)

NBCG guidelines

- Breast cancer <50 years
- Two close relatives with breast cancer, mean age <55 years
- Three close relatives with breast cancer at any age
- Male breast cancer
- Bilateral breast cancer <60 years
- Female breast cancer and close relative with ovarian cancer
- Female breast cancer and close relative with prostate cancer <55 years
- Ovarian cancer at any age

ERRATA

Page	Line	Original text	Type of correction	Corrected text
19	1	... «BRCA carriers»..	Cor	... «BRCA2 carriers»...
22	12	...”environmental risk factors for are better studied for BRCA1 are better studied than for BRCA2”	Incomplete sentence	.. .”environmental risk factors for cancer are better studied for BRCA1 than BRCA2”
30	19	..”<10%”..	Cor	..”>10%”..
42	26	(ref) x 2 in text	Cor	(109) for both.
43	9	..”paper 3 and 4”..	Cor	..”paper 3”.. (not 4)
43	18	..”family history”..	Cor	..”family history (as defined by criteria).”...
45	27	“ The nine mutation carriers with the”	Incomplete sentence	Should be deleted.
52	22	..”as an evaluation”	Incomplete sentence	..”as an evaluation of the framework of a screening program.

PAPER I

RESEARCH ARTICLE

Open Access



Current guidelines for *BRCA* testing of breast cancer patients are insufficient to detect all mutation carriers

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Abstract

Background: Identification of *BRCA* mutations in breast cancer (BC) patients influences treatment and survival and may be of importance for their relatives. Testing is often restricted to women fulfilling high-risk criteria. However, there is limited knowledge of the sensitivity of such a strategy, and of the clinical aspects of BC caused by *BRCA* mutations in less selected BC cohorts. The aim of this report was to address these issues by evaluating the results of *BRCA* testing of BC patients in South-Eastern Norway.

Methods: 1371 newly diagnosed BC patients were tested with sequencing and Multi Ligation Probe Amplification (MLPA). Prevalence of mutations was calculated, and BC characteristics among carriers and non-carriers compared. Sensitivity and specificity of common guidelines for *BRCA* testing to identify carriers was analyzed. Number of identified female mutation positive relatives was evaluated.

Results: A pathogenic *BRCA* mutation was identified in 3.1%. Carriers differed from non-carriers in terms of age at diagnosis, family history, grade, ER/PR-status, triple negativity (TNBC) and Ki67, but not in HER2 and TNM status. One mutation positive female relative was identified per mutation positive BC patient. Using age of onset below 40 or TNBC as criteria for testing identified 32-34% of carriers. Common guidelines for testing identified 45-90%, and testing all below 60 years identified 90%. Thirty-seven percent of carriers had a family history of cancer that would have qualified for predictive *BRCA* testing. A Variant of Uncertain Significance (VUS) was identified in 4.9%.

Conclusions: Mutation positive BC patients differed as a group from mutation negative. However, the commonly used guidelines for testing were insufficient to detect all mutation carriers in the BC cohort. Thirty-seven percent had a family history of cancer that would have qualified for predictive testing before they were diagnosed with BC. Based on our combined observations, we suggest it is time to discuss whether all BC patients should be offered *BRCA* testing, both to optimize treatment and improve survival for these women, but also to enable identification of healthy mutation carriers within their families. Health services need to be aware of referral possibility for healthy women with cancer in their family.

Keywords: Breast cancer, *BRCA* mutation, Genetic testing, Norway

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Background

Germline mutations in the *BRCA1* and *BRCA2* genes are associated with a high lifetime risk of breast and ovarian cancer [1, 2]. Knowledge of one's *BRCA* status is of importance for healthy women as cancer may be prevented through risk-reducing mastectomy and salpingo-oophorectomy [3–5]. Identification of a pathogenic *BRCA* mutation in a woman diagnosed with breast cancer (BC) may influence treatment and prognosis of her current cancer but also enable prevention of future cancers [6–12]. Consequently, surgeons and oncologists more and more frequently want to offer genetic testing at time of diagnosis.

Because of the high costs associated with genetic analyses, *BRCA1/2*-testing has traditionally been restricted to BC patients having an a priori high risk of being a carrier. These factors include young age at diagnosis (below 45 years), triple-negative breast cancer (TNBC) or a family history of breast- and/or ovarian cancer [13–22]. The American Society of Clinical Oncology (ASCO), The National Comprehensive Cancer Network (NCCN) in the US and the Norwegian Breast Cancer Group (NBCG) all have guidelines for *BRCA* testing of BC patients based on these risk factors (Additional file 1: Figure S1), and according to The National Institute for Health and Care Excellence (NICE) in the UK, *BRCA* testing should be offered to BC patients with a probability of having a mutation is 10% or more [23–26]. There are also corresponding guidelines for predictive testing of healthy women.

During the recent years, the cost of genetic testing has decreased due to the advent of new and more efficient DNA-sequencing technologies. Consequently, BC patients are now often offered multi gene panel testing. These panels include *BRCA1/2* and the other high risk breast cancer genes *TP53* and *PTEN*, but also genes with more moderate cancer risk and genes whose clinical significance is still not resolved [27, 28]. Testing is nevertheless still mostly restricted to patients fulfilling certain high risk criteria for being mutation carriers, and few studies have described *BRCA* testing of unselected groups of BC patients [29–35]. To our knowledge, only two studies have performed testing with sequencing and Multi-Ligation Probe Amplification (MLPA) of all patients included [30, 35]. Knowledge of the clinical characteristics of BC caused by *BRCA* mutations in unselected BC cohorts is therefore limited. Moreover, there is also limited information about the sensitivity and specificity of current guidelines for *BRCA* testing to identify carriers in cohorts not selected for high risk factors. With the ongoing changes in opportunities for genetic testing we believe it is necessary to assess whether the current strategies for *BRCA* testing are sufficient to enable mutation positive women to benefit from the potential of both cancer cure and prevention that lies

within such testing. Observations from *BRCA* testing of less selected groups of BC patients are necessary for this evaluation.

The NBCG guidelines used in Norway are regularly revised. Because it became clear that identification of a *BRCA* mutation could have implications for treatment, a subjective criteria was introduced a few years ago. If the treating physician considered the test result to be of importance for treatment decisions, testing could be offered even in the absence of other high risk factors such as young age or family clustering. As a consequence, testing could be offered also to BC patients with an a priori low risk of being carriers. Due to this change in practice we have been able to compare the sensitivity of previous and present national and international guidelines for *BRCA* testing in BC patients without the selection bias described.

This report summarizes the results of *BRCA* testing in South-Eastern Norway according to these revised Norwegian guidelines from 1st of January 2014 to 31st of August 2015. The study had three specific aims: Firstly, it was to calculate the prevalence of *BRCA* mutations in this cohort of BC patients that as a whole had an a priori low risk of being mutation carriers, describe the spectrum of mutations, and the number of mutation positive female relatives identified. Secondly, we wanted to describe and compare clinicopathological features of BC among carriers and non-carriers. The third aim was to calculate the sensitivity and specificity of different guidelines used for diagnostic testing [23–26], and also to evaluate how many mutation carriers that had a family history of cancer that qualified for predictive testing before they were diagnosed with BC [26].

Methods

Patients

During the study period, a total of 1371 BC patients were tested. Two cohorts of patients are described in this report: Cohort 1: Patients tested at The Breast Cancer Surgery Unit, Department of Oncology, Oslo University Hospital, Ullevål (OUH-U), and Cohort 2: Patients tested at the other hospitals in the health administrative area of South Eastern Norway called South-Eastern Norway Regional Health Authority trust. This cohort is referred to as SERHA.

OUH-U (cohort 1)

This is the largest unit treating BC patients in Norway. Six hundred and seven patients underwent BC surgery, and 440 (72.5%) of them were tested. Two of these were men. A quality of care database was established at the unit to evaluate the practice of *BRCA* testing among this group of patients. Information on age of onset, receptor status, grade, stage, nodal involvement, Ki67 and family

history was accessed from the Electronic Patient Record (EPR) system (DIPS®) and registered in the quality database. Family history was taken by the doctor admitting the patient to the hospital according to ordinary routines. No standardized or quality assured methods were used. The information on family history recorded in the patient record of both carriers and non-carriers was evaluated and scored according to the old diagnostic and predictive test criteria of NBCG [26]. No information on size (number of family members) of the families was recorded. One hundred and sixty-seven patients were not tested. Of these, 96 either directly declined testing or wanted to think about it. For the remaining 71, there was no record in the hospital's EPR system on whether testing was offered or not.

SERHA (cohort 2)

We do not have the exact number of all BC patients undergoing treatment at these hospitals the other hospitals in the health region during the study period, but based on numbers from the Norwegian Breast Cancer Registry (NBCR) at the Cancer Registry of Norway (CRN) we estimated that the number was around 2400 [36]. Nine hundred and thirty-one (39.0%) were tested. Information on age of onset, receptor status and family history was registered on all carriers in the EPR at the Department of Medical Genetics (DMG) OUH. No information was collected on mutation negatives in this cohort.

Genetic testing

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.

Results 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 BC, and patients with a Variant of Uncertain Significance (VUS) 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 confirmed. A quality of care database was established at DMG OUH and all BC patients with a pathogenic *BRCA* mutation and their relatives who were tested for the mutation were registered here. Both male and female relatives of the mutation positive BC patients were offered testing for the mutation in question. Testing was offered not only to first degree relatives, but to all blood relatives who were referred to DMG OUH.

Statistics

Mutation carriers from both cohorts were scored according to the ASCO, NCCN, NICE and NBCG guidelines [23–26]. Carriers were scored according to the NBCG criteria as they were before the revision that opened for testing based on implication for treatment decisions. In the remainder of the article these will be referred to as the “old NBCG criteria”. To score patients according to the NICE guidelines, the BOADICEA Web Application (BWA v3) [38] was used to calculate risk of carrying a *BRCA* mutation. Sensitivities of criteria to identify carriers were calculated excluding the patients with a known family mutation.

Tests for trends were performed to compare the differences in BC 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 \geq 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.

Results

Identified mutation carriers, spectrum and frequency of mutations

A pathogenic mutation in *BRCA1/2* was identified in 42 of the 1371 (3.1%) BC patients. Thirteen mutation carriers were identified in Cohort 1 (13/400 = 3.0%), and 29 in Cohort 2 (29/931 = 3.1%). All mutation carriers were women. Twenty-eight (2.0%) had a mutation in *BRCA1* and 14 (1.0%) in *BRCA2*. Median and mean age at diagnosis was 45 years (range 26–77 years) and 46.1 years (46.3 years for *BRCA1* and 45.6 years for *BRCA2*) respectively. Four of the 42 women belonged to families where a *BRCA* mutation already had been detected, but had not sought

predictive genetic testing. Four of the mutation carriers were detected through MLPA (dup exon 3-16, dup exon 13 and del exon 22 in *BRCA1* and dup exon 20 in *BRCA2*), and the remaining carriers with sequencing. A VUS was identified in 67 (4.9%) patients.

When considering only those with Norwegian ancestry, we revealed that 13/29 (44.8%) had one of the known Norwegian founder mutations [39]. 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. One of these was *BRCA2* c.614delG. Two patients carried this mutation and were related. Of the 13 mutation carriers that were not of Norwegian ancestry, three were from Poland and two from Morocco. The following nationalities were represented with one carrier each: Canadian, Swedish, Iraqi, Latvian, Indian, Turkish, and Greek. Three different *BRCA2* mutations were identified in the three BC patients from Poland. None of them were among the mutations known to be frequent in the Polish population [40–42], and only one of them had been reported previously (c.9403delC) [42]. The other two (c.4797_4797delCAAT and c.7024C > T) were not found to be reported previously in the Polish population. Mutation, age of onset, nationality, fulfilling criteria for predictive testing or not and clinicopathological aspects of tumors among mutation carriers is presented in Table 1. Age at diagnosis is given in age ranges to prevent disclosing patient information.

As of August 2016, 67 female and 19 male relatives of the 42 mutation positive BC patients have been tested for the mutation identified in their family. Forty female relatives have tested positive for the mutation identified in their family. Five of the 42 BC patients had no adult female relatives living in Norway. Excluding these 5, 40/37 = 1.1 female mutation positive female relative has so far been identified per mutation positive BC patient. This number is likely to increase as more relatives are informed and tested. The mean age in this group of carriers was 46.7 years (range 20–84). All were offered annual MRI and mammography from the age of 25, and they were given the opportunity of choosing risk-reducing surgery. Seven of the relatives had already had cancer before the mutation was identified in their relative. Five of these had had BC and two OC. In addition, after being tested for the mutation in their family, one woman has been diagnosed with BC at first MRI and one has been diagnosed with OC with FIGO stage 1B when undergoing prophylactic salpingo-oophorectomy. In addition to those who have been tested, 37 female relatives (first degree or second degree through a man) aged above 18 years and 17 below 18 years have been identified, but they have not yet been referred for testing.

Comparison of clinicopathological characteristics of tumors in mutation positive and mutation negative from the OUH-U cohort

No information was collected about the mutation negative BC patients in Cohort 2 from SERHA. A detailed comparison of the clinicopathological characteristics of tumors in mutation positive and mutation negative was therefore only possible to perform in cohort 1 from OUH-U. The results are presented in Table 2. Compared to the mutation negative, mutation positive women were younger ($p < 0.001$), had tumors of higher grade ($p = 0.001$), higher Ki67 ($p < 0.001$ (comparing mean) and $p = 0.004$ (comparing number with <30% activity) and more of them had TNBC ($p < 0.001$). In addition, more mutation carriers had family histories of breast and/or ovarian cancer compared to BC patients without mutation ($p = 0.035$). No significant difference was observed in TNM- status ($p = 0.396$) and HER2-profile ($p = 0.84$). In cohort 1 from OUH-U, 167 patients were not tested. They had a higher age at diagnosis ($p < 0.001$), a lower Ki67 score ($p < 0.05$) and a lower proportion fulfilled the old NBCG criteria ($p < 0.05$) compared to the patients tested (Additional file 2: Table S1).

To indirectly assess whether the two cohorts were similar in terms of risk distribution, we compared mutation positive patients in the two groups in terms of age at diagnosis, receptor status and family history of cancer. There was a tendency towards a higher mean age of onset in Cohort 2 compared to Cohort 1 (48 vs 42, $p = 0.09$). There was no significant difference in terms of TNBC and whether or not they fulfilled the diagnostic NBCG criteria (Additional file 3: Table S2).

Sensitivity and specificity of criteria for genetic testing

The old NBCG, the NCCN, and ASCO guidelines had a sensitivity ranging from 84.2% to 89.5%. The NICE guidelines had the lowest sensitivity, and would have identified only 44.7% of the mutation positive women. Testing only women below 40 years or only those with TNBC would have identified 31.6% and 34.2% of the mutation carriers. Testing all BC patients below 60 years would have identified 89.5%. Almost 40% of the BC patients found to carry a *BRCA* mutation had a family history of cancer that fulfilled the NBCG criteria for predictive *BRCA* testing, before they were diagnosed with BC themselves. See Table 3 for details.

The specificity of the different criteria for testing was calculated for Cohort 1 from OUH, and is presented in Table 4. The highest specificity was found for the high-risk criteria separately of each other. Breast cancer <40 years of age and TNBC both had a specificity of 94%. The specificity of fulfilling the NBCG criteria was 70%, while having breast cancer below 60 years of age

Table 1 Identified *BRCA1/2* carriers

Gene	Cohort	Mutation	Effect	Age at diagnosis	Norwegian ancestry	Qualifying for predictive testing	Triple negative disease	
<i>BRCA1</i>	1	c.2019delA	Frameshift	20-29	No	No	Yes	
	1	c.1016dupA ^a	Frameshift	30-39	Yes	Yes	Yes	
	1	del exon 3-16	Deletion	30-39	No	No	No	
	1	c.3228_3229delAG ^{a*}	Frameshift	30-39	Yes	No	No	
	1	c.3178G > T ^a	Nonsense	40-49	Yes	No	No	
	1	c.3084_3094delTAATAACATTA ^a	Frameshift	40-49	Yes	No	Yes	
	1	c.3228_3229delAG ^a	Frameshift	40-49	Yes	No	Yes	
	1	c.5047G > T ^b	Nonsense	40-49	Yes	Yes	Yes	
	1	c.3607C > T ^b	Nonsense	50-59	Yes	Yes	No	
	1	c.4484G > A ^c	Missense. Leads to skipping of exon 14	50-59	Yes	No	No	
	2	c.5407-2A > G ^c	Frameshift, skipping of exon 23	60-69	Yes	No	No	
	2	c.1072delC ^b	Frameshift	60-69	Yes	No	No	
	2	c.1556delA ^a	Frameshift	40-49	Yes	Yes	No	
	2	c.5153G > C ^b	Missense	40-49	Yes	Yes	No	
	2	c.3756_3759delGTCT	Frameshift	40-49	No	No	No	
	2	del exon 22	Frameshift	40-49	No	No	Yes	
	2	c.5309G > T	Missense	30-39	No	Yes	Yes	
	2	c.697delGT ^a	Frameshift	70-	Yes	Yes	Yes	
	2	c.3228_3229delAG ^a	Frameshift	50-59	Yes	Yes	Yes	
	2	c.445G > T	Nonsense	40-49	No	Yes	Yes	
	2	c.1016dupA ^a	Frameshift	50-59	Yes	No	Yes	
	2	c.5266dupC	Frameshift	30-39	No	Yes	No	
	2	c.2989_29x0dup ^b	Frameshift	50-59	Yes	No	No	
	2	c.1016dupA ^a	Frameshift	30-39	Yes	No	No	
	2	c.1556delA ^a	Frameshift	50-59	Yes	Yes	No	
	2	dup exon 13 ^b	Frameshift	50-59	Yes	No	No	
	2	c.5309G > T	Missense	30-39	No	No	Yes	
	2	c.5503C > T	Nonsense	50-59	No	Yes	Yes	
	<i>BRCA2</i>	1	c.4710delA	Frameshift	30-39	No	No	No
		1	c.3847_3848delGT ^a	Frameshift	40-49	Yes	No	No
1		c.614delG ^c	Frameshift	50-59	Yes	Yes	No	
2		c.4936_4939delGAAA ^b	Frameshift	40-49	Yes	No	No	
2		c.3847delGT ^a	Frameshift	40-49	Yes	No	No	
2		c.9403delC	Frameshift	40-49	No	No	No	
2		c.5722delCT ^b	Frameshift	40-49	Yes	No	No	
2		c.6059_6062delAACA ^b	Frameshift	30-39	Yes	No	No	
2		c.5722delCT ^b	Frameshift	50-59	Yes	Yes	No	
2		c.4794_4797delCAAT	Frameshift	40-49	No	Yes	No	
2		c.614delG ^c	Frameshift	30-39	Yes	Yes	No	

Table 1 Identified *BRCA1/2* carriers (Continued)

2	c.7024C > T	Nonsense	30-39	No	Yes	No
2	c.9699_9702delTATG ^c	Frameshift	70-	Yes	No	No
2	dup exon 20 ^b	Frameshift	40-49	Yes	Yes	Yes

1: Tested at Oslo University Hospital Ullevål (OUH-U)

2: Tested at other hospitals in South-Eastern Norway Regional Health Authority trust's coverage area (SERHA)

^aCommon Norwegian founder mutation³⁸

^bIdentified in 1-9 families at Department of Medical Genetics (DMG), OUH (unpublished data)

^cNot identified previously at DMG, OUH

BC Breast cancer

OC Ovarian cancer

had a specificity of 48%. Mutation frequency and number needed to test (NNT) to identify one mutation carrier depending on different test criteria are shown in Table 5. Testing all BC patients below 60 years would give mutation frequency of 5.5% and by using this criteria, 18 BC patients had to be tested to identify one carrier.

Discussion

We have reported the results of diagnostic *BRCA* testing of women diagnosed with BC in the South-Eastern part of Norway according to the NBCG guidelines. These guidelines opened up for testing independently of the common high risk factors i.e. also when the treating physician considered the test result to be of importance for treatment decisions. To our knowledge, this is therefore the largest and least selected series reported where BC patients were tested with both sequencing and MLPA of both genes, and it does not have the selection bias arising when only high-risk patients are tested.

We identified a mutation in 3.1% of BC patients. In a recent study from the Western region of Norway, 405 BC patients were tested for 30 specific *BRCA1/2* mutations and with MLPA [32]. Sequencing was performed on 94 of these. A mutation was found in only 1.7% of participants. Both studies are small and consequently they do have limitations. However, the observed difference may at least partly be explained by the fact that all patients in our study were tested with sequencing and MLPA and not for selected mutations only. In our study, 16 out of 29 (55%) women with Norwegian ancestry did not have any of the 10 most common Norwegian founder mutations [39], and five (17%) had a mutation that had not been previously observed in our population. In comparison, in 2007 the 10 founder mutations accounted for about two-thirds of all detected mutation carriers at our department [39]. This reflects that in 2007 most patients were tested for a limited number of mutations, whereas today sequencing and MLPA is offered to all who qualify for testing in our health region. Our findings also illustrate that there are mutations within our population that are and may remain rare. By testing only for frequently observed

mutations in the Norwegian population, a substantial number of mutation positive women with a pathogenic *BRCA* mutation will not be found.

A VUS was identified in 4.9% of the tested patients. Our numbers are comparable to what others have revealed [43]. Studies have reported that physicians, with limited formal training in genetics, may misinterpret VUS results [44–46]. This was dealt with in the current study as all patients with a VUS were referred to genetic counseling. There is a worry that information about a VUS may have a negative psychological impact on the patient [47]. However, studies have also demonstrated that it is interpreted as more similar to a test result where no pathogenic variant has been detected than to a result with an identified pathogenic variant [46]. Addressing the issue of patients' interpretation of risk and possible psychological impact was beyond the scope of this study, but should be closer evaluated in future studies. By offering testing only for a set of already known and described mutations one would avoid the challenges associated with identifying VUS. We have however described that a substantial number of mutation carriers will be missed by testing only for known mutations. It is our opinion that the benefits associated with identifying all carriers (and the corresponding risk associated with not identifying a mutation carrier) outweigh the current challenges associated with identifying VUS. One may also hypothesize that the frequency of VUS may decrease in the future as more people are undergoing testing.

By comparing carriers and non-carriers tested at OUH-U we observed that even though testing was offered broadly, mutation positive women still differed from mutation negative in terms of the known high risk aspects for being carriers: age of onset, triple negativity and family history. We found no difference in HER2-status between the two groups, and these findings are in accordance with a recent study where HER2-status was not found to be a reliable predictor of *BRCA*-status [48]. Mutation carriers had a higher score for Ki67 than mutation negatives, and this has also been described in a few studies [49, 50]. The observed differences between the two groups are also illustrated by the fact that each of the test criteria has a high specificity (see Table 4).

Table 2 Comparison of clinical and pathological characteristics of carriers and non-carriers tested at Oslo University Hospital Ullevål (OUH-U)

	BRCA 1/2 carriers (n = 13)	Non-carriers (n = 427)	p-values
Below 40 years	5 (38.5%)	24 (5.6%)	
Below 50 years	10 (76.9%)	106 (24.8%)	
Below 60 years	13 (100%)	222 (52.0%)	
Age at diagnosis			
Mean (95% CI)	42 (36.1-47.9)	57.9 (56.8-59.1)	<0.001
Median (range)	43 (26-58)	58 (23-93)	
Predictive test criteria fulfilled	4 (30.8%)	49 (11.5%)	0.035
Stringent NBCG criteria fulfilled	12 (92.3%)	130 (30.4%)	<0.001
TNM	(n = 13)	(n = 422)	
T1	5 (38.5%)	261 (61.8%)	
T2	5 (38.5%)	121 (28.7%)	0.396
T3	2 (15.3%)	26 (6.2%)	
T4	1 (7.7%)	13 (3.1%)	
N0	8 (61.5%)	295 (72.0%)	
N1	2(15.4%)	82 (19.4%)	0.078
N2	3(23.1%)	24 (5.7%)	
N3	0	12 (2.8%)	
Distant metastasis	0	2 (0.5%)	0.76
Grade	(n = 13)	(n = 419)	
1	0	101 (24.1%)	0.001
2	4 (30.8%)	211 (50.4%)	
3	9 (69.2%)	107 (25.5%)	
Estrogen receptor status	(n = 13)	(n = 423)	
Positive	7 (53.8%)	371 (87.7%)	<0.001
Negative	6 (46.2%)	52 (12.3%)	
Progesterone receptor status	(n = 13)	(n = 422)	0.045
Positive	5 (38.5%)	276 (65.4%)	
Negative	8 (61.5%)	146 (34.6%)	
HER2 status	(n = 13)	(n = 423)	
Positive	2 (15.4%)	57 (13.5%)	0.84
Negative	11 (84.6%)	366 (86.5%)	
Triple negative breast cancer	(n = 13)	(n = 422)	
	5 (38.5%)	30 (7.1%)	<0.001
Ki67	(n = 13)	(n = 412)	
Mean (95% CI)	59 (45.3-72.8)	31.3 (29-33)	<0.001
< 30% activity	11 (84.6%)	182 (44.1%)	0.004

NBCG Norwegian Breast Cancer Group

TNM Scoring of tumors according to the TNM Classification of Malignant Tumors

T Size of original tumor

N Involvement of regional lymph nodes

M Distant metastasis

Whereas the mutation positive differed as a group from mutation negative, selecting patients for testing based on the known high risk factors will identify

carriers with varying sensitivity (see Table 3). Testing only those with BC below 40 years or TNBC identified 31.6% and 34.2% of carriers respectively, and less than

Table 3 Sensitivity of criteria for testing to identify *BRCA1/2* carriers

Test criteria	<i>BRCA1/2</i> mutation carriers (<i>n</i> = 38 ^a)
BC <40 years	12 (31.6%)
BC <50 years	28 (73.7%)
BC <60 years	34 (89.5%)
TNBC	13 (34.2%)
Fulfilling stringent NBCG criteria for testing	32 (84.2%)
Fulfilling ASCO guidelines for testing	34 (89.5%)
Fulfilling NICE guidelines for testing	17 (44.7%)
Fulfilling NCCN criteria for testing	32 (88.9%)
Family history fulfilling NBCG criteria for predictive testing before index person contracted BC	14 (36.8%)

^aThe four women belonging to families where a mutation had already been identified were excluded from this analysis

BC Breast cancer

TNBC Triple Negative Breast Cancer

NBCG Norwegian Breast Cancer Group

ASCO American Society of Clinical Oncology

NICE National Institute for Health and Care Excellence

NCCN The National Comprehensive Cancer Network

50% of carriers qualified for testing according to the NICE guidelines. By use of the comprehensive ASCO, NCCN and old NBCG criteria, where the different single characteristics are combined in order to increase sensitivity, between 84.2 and 89.5% would be identified. NBCG has recently suggested that testing should be offered to women with TNBC under the age of 60 [26]. By adding this aspect to the original stringent criteria, 34/38 (89.5%) would have been identified. In a recent study where 488 women with BC were tested for mutations in 25 cancer susceptibility genes, Tung et al. found that all *BRCA*-mutation carriers fulfilled the NCCN guidelines [35]. We do not know whether the difference in observed sensitivity is due to chance or systematic differences between the two cohorts.

The ASCO, NCCN and NBCG criteria include an assessment of the patient's family history of cancer. The family histories of the mutation positive BC patients

Table 4 Specificity of criteria for *BRCA1/2* testing

Test criteria	Specificity ^a
BC < 40 years	(403/427) 94.4%
BC < 50 years	(321/427) 75.2%
BC < 60 years	(205/427) 48%
TNBC	(397/422) 94.1%
Fulfilling NBCG criteria for diagnostic testing	(297/427) 69.5%
Fulfilling NBCG criteria for predictive testing	(378/427) 89%

^aSpecificity is calculated only for Cohort 1, OUH-U

BC Breast cancer

TNBC Triple negative breast cancer

NBCG Norwegian breast cancer group

Table 5 Number needed to test to identify one mutation carrier according to test criteria

Test criteria	Mutation frequency	Number needed to test (NNT) to identify one mutation carrier
BC < 50 years	10/116 = 8.6%	12
BC < 60 years	13/235 = 5.5%	18
TNBC	5/35 = 14%	7
NBCG criteria	12/147 = 8.2%	12

identified in our study were thoroughly investigated by genetic counselors and medical geneticists following the identification of the mutation, resulting in the sensitivity estimates presented. The observed estimates may therefore be higher than what is realistic in the clinical setting when family history is taken by the admitting physician at time of diagnosis. It may be difficult for the patient to know or recall detailed information about their family history of cancer when asked in a possibly stressful diagnostic setting. In line with this, Høberg-Vetti et al. found in their study from the Western part of Norway that 2 out of 26 (7.7%) mutation carriers reported a negative family history of cancer at time of diagnosis and testing, but closer evaluation revealed that they did have a family history of breast and/or ovarian cancer [32]. We also worry that the complexity of the NCCN, ASCO and NBCG criteria make them difficult to use and implement systematically in a busy clinical setting. Both these aspects could lead to fewer patients being offered testing, even those fulfilling the criteria. This is illustrated in several studies. Febraro and colleagues observed that only 34% of breast cancer patients fulfilling NCCN guidelines were referred to genetic counseling and testing [51]. In a recent Swedish study where all BC patients were tested retrospectively, it was found that 65% of the mutation carriers fulfilled Swedish criteria for testing, but only 18% had been identified in regular clinical routine [52]. Moreover, even though all mutation carriers fulfilled the NCCN criteria in the study by Tung et al., 13.3% of the carriers identified through this research project had not been tested clinically [35].

The fact that 37% of the women had a family history of cancer that according to the Norwegian guidelines qualified for referral to predictive genetic testing before their own disease, may be another illustration of the challenges with using assessment of family history as a criteria for genetic testing or referral to genetic counseling. The low number leads us to conclude that the current system of referring healthy women to genetic testing based on their family history is suffering from lack of compliance. These women contracted cancers that could have been prevented had they known about their risk and undergone prophylactic surgery. The

reasons for this lack of referral and how it can be improved need to be further explored, but this was not the scope of the current study.

Using age of onset as a criteria for testing will likely lead to increased adherence by surgeons and oncologists compared to guidelines requiring a detailed and complicated assessment of the patient's family history of cancer. Testing all BC patients below 60 years identified as many or more carriers than all guidelines assessed (see Table 3). Due to the lowered cost of testing and the clinical impact of detecting a *BRCA* mutation, Finch et al. [53] have recently argued that the threshold for testing should be lowered from a 10% prior probability of being a carrier to 5%. Testing all under 60 in the OUH-U cohort gave a mutation detection rate of 5.5% (see Table 5), i.e. within this threshold. By using this criteria one would have to test 18 BC patients to identify one carrier. As of August 2016, testing these 18 patients had also led to the identification of one female relative per index patient. In Cohort 1 from OUH-U, 235 out of the 440 tested (53.4%) were younger than 60 and 132/440 (30%) fulfilled the old NBCG criteria (see Table 2). In 2014, 3324 Norwegian women contracted BC [54]. Using the calculations from the OUH-U cohort indicate that testing all below 60 years will involve 800 more analyses annually compared to testing only those fulfilling the old NBCG criteria.

One year after the last BC patient in our cohort was tested, 1.1 female relative per identified carrier had tested positive for the mutation and were given the opportunity of cancer prevention. It is likely that this number will increase as more relatives are informed and tested. According to Finch et al., "the value of a cancer genetic testing program comes from the number of cancers prevented" [53]. Even though testing all below 60 years may be feasible and effective, we observed that 10% will still be missed by this strategy. Two mutation carriers were older than 70 years. One may argue that the identification of a mutation in a woman who is 70 years or older may not influence treatment decisions, life expectancy or lead to a significant gain in quality adjusted life years (QALY) for this woman. However, it is likely that women over 70 have adult female relatives that may be at high risk of cancer due to the mutation.

We observed that more than half of the mutation carriers did *not* have a family history of breast and/or ovarian cancer before they were diagnosed with breast cancer themselves. These findings are in line with other studies reporting that family history has limited value in predicting carrier status [33, 38, 55], and our findings illustrate the difficulties with finding these women prior to disease development. Today, these women cannot obtain genetic testing while still healthy, as a population-based screening protocol is not accessible. Mary Claire

King and colleagues consider that the identification of "a woman as a carrier only after she develops cancer is a failure of cancer prevention" [56] and based on their finding that *BRCA* mutation carriers have a high risk of cancer regardless of their family history [57], argue for population based screening to all women aged 30 years [56].

In a cost analysis of the cancer genetic services in the UK, Slade et al. have demonstrated that the most cost efficient genetic service model is to identify unaffected mutation carriers through an affected mutation positive index person [58], and argue for more comprehensive testing of all cancer patients fulfilling the NICE criteria. Patients fulfilling these criteria have an a priori 10% risk of being carriers. We identified a mutation in 3.1% of carriers, and one may argue that this is too low to warrant testing of all BC patients. We have however, recently shown that the practice of *BRCA* testing at OUH-U is cost-effective within the frequently used thresholds in Norway [59]. The cost-effectiveness was mainly due to the prevented breast and ovarian cancers in their female relatives who tested positive for the mutation. Possible life years gained (LYG) due to prophylactic surgery among the BC patients was not included in the calculations in this study. The calculations may therefore be considered a conservative estimate. In addition, the cost of testing is constantly dropping, making the cost-effectiveness of a broad application of *BRCA* testing to BC patients even larger in the coming years.

Our results indicate that by testing only for founder mutations in the BC population of the South-Eastern part of Norway, and by testing only those with a family history of cancer, a significant number of mutation carriers will be missed. One may ask whether these results are relevant for screening strategies in other populations. The prevalence of *BRCA* mutations vary between populations [34, 60, 61], and the indication for genetic screening of all breast and ovarian cancer patients may be stronger in populations with a higher frequency of mutation carriers than in Norway. In populations where there is a stronger founder effect, the number of mutation carriers missed by offering testing for only founder mutations will be lower than what we have observed. However, recent studies have demonstrated that 13% of *BRCA1* mutations and 7.2% of *BRCA2* mutations in Ashkenazi Jews were non-founders [62]. Similarly, a Polish study found that in families with a family history of breast and/or ovarian cancer having tested negative for Polish *BRCA* founder mutations, sequencing revealed 31 other *BRCA* mutations. The detection rate of these mutations was 10% [63]. Sequencing and MLPA may therefore be warranted also in populations with a stronger founder effect than in Norway. We observed that only 40% of mutation carriers had a family history of

breast and/or ovarian cancer. There are various reasons for this: Small family size, mutations may be inherited through several generations of men and incomplete penetrance. Family history as a selection tool for testing may have a higher sensitivity in populations with higher birth rates than in Norway. However, most western countries have had a declining birth rate since the 1960s and now have a birth rate between 1.5 and 2 [64]. One may therefore hypothesize that the value of using family history as a selection tool for testing will be even lower in the future.

BC patients are now often offered multi gene panel tests, and this is the direction in which the field of genetic testing is moving rapidly. There are several advantages with this strategy compared to testing only for the *BRCA* genes. More carriers of pathogenic mutations in other known BC risk genes such *TP53*, *PTEN* or *PALB2* will be identified. In addition, carriers of mutations in genes that likely would not have been investigated when testing only for one gene at a time will be identified. By testing a sequential series of breast cancer patients for 25 cancer predisposition genes, Tung and colleagues identified carriers of mutations in the *MSH6* and *PMS2* genes [35]. Conversely, by testing families suspected to have Lynch Syndrome for 112 known or candidate colorectal cancer genes, Hansen and colleagues identified one *BRCA1* carrier and two *BRCA2* carriers [65]. In sum, through multi gene panel testing more mutation carriers and their mutation positive relatives will be identified and given the opportunity of appropriate cancer surveillance and/or prevention. In the coming years this technology will also likely become more cost effective than traditional Sanger sequencing of one gene at a time. The aim of this study was not to argue against the value of multi gene panel testing, but rather to investigate whether the current strategies for *BRCA* testing, regardless of technology used, are sufficient to identify all carriers of mutations in these well-known and defined genes.

One limitation to our study is that we have not tested all BC patients. In the OUH-U cohort (Cohort 1) 167/607 = 27.5% of all women diagnosed with BC were not tested. These women were older and fewer filled the NBCG criteria than those who were tested. Unfortunately, we do not have access to the exact number of untested patients in the SERHA series or clinical information about these. If 2400 were treated in SERHA in the study period, about 39% of these (931/2400) were tested. The reason for the lower number of tested in Cohort 2 may be that there was a lower awareness of the possibility of genetic testing at these hospitals, but we cannot exclude that this cohort may be more selected. To assess this, we

compared the two cohorts indirectly by comparing the mutation positive BC patients. There was a tendency towards a higher age of onset in Cohort 2 from SERHA, but this difference was not statistically significant. No significant differences were found between mutation carriers in the two cohorts in terms of TNBC and family history (Additional file 3: Table S2). We also observed the same frequency of mutation carriers in the two series. The two cohorts may therefore be similar, and it is likely that the untested in Cohort 2 were older and that fewer filled the NBCG criteria than the tested. If there are mutation carriers among the untested in both series, the total frequency of carriers might have been lower, but it is likely that even fewer would have fulfilled the different high-risk criteria.

Conclusions

By offering *BRCA* testing to a broad group of BC patients we found that 3.1% carried a deleterious mutation, and so far this has led to the identification of 1.1 female mutation positive relative per mutation positive BC patient. Even though mutation carriers differed as a group from mutation negative, criteria for testing based on the high-risk aspects did not detect all *BRCA* carriers in this BC population. Testing all BC patients below 60 years had a sensitivity matching the commonly used guidelines, and will likely be easier to apply, but 10% of mutation carriers would still be missed. Thirty-seven percent of the women had a family history of cancer prior to their own BC that qualified for predictive genetic testing. They contracted cancers that could have been prevented if the health care system had identified their increased genetic risk. Based on our combined observations, we conclude that the current strategies for *BRCA* testing are insufficient to detect all carriers. We suggest that it is time to discuss whether *BRCA* testing should be offered also to BC patients not belonging to a high risk group. If all BC patients are offered *BRCA* testing, the potential for cancer cure and prevention associated with such testing can be improved even further than what today's strategies for testing allows. In case of lack of economic resources to fulfill this strategy, at least those aged 60 years or less at time of BC diagnosis should be tested. Our observations also indicate that health services need to be aware of referral possibilities for healthy women with cancer in the family, and the reasons for the low compliance should be explored. Improved strategies both for diagnostic and predictive *BRCA* testing will identify more mutation positive women prior to cancer development than the current practice.

Additional files

Additional file 1: Figure S1. Guidelines for testing. (DOCX 26 kb)

Additional file 2: Table S1 Clinical and pathological characteristics of breast cancer in mutation carriers, non-carriers and not tested at OUH-U. (DOCX 28 kb)

Additional file 3: Table S2. Comparison of mutation positive breast cancer patients in Cohort 1 (OUH-U) and Cohort 2 (SERHA). (DOCX 14 kb)

Abbreviations

ASCO: The American Society of Clinical Oncology; BC: Breast Cancer; CRN: Cancer Registry of Norway; DMG: Department of Medical Genetics; EPR: Electronic patient record; LYG: Life years gained; M: Distant metastasis; MLPA: Multi Ligation Probe Amplification; N: Involvement of regional lymph nodes; NBCG: The Norwegian Breast Cancer Group; NBCR: Norwegian Breast Cancer Registry; NCCN: The National Comprehensive Cancer Network; NICE: The National Institute for Health and Care Excellence; NNT: Numbers needed to test; NSD: Norwegian Social Science Data Services; OC: Ovarian cancer; OUH-U: Oslo University Hospital, Ullevål; REK: Regional Committees for Medical and Health Research Ethics; SERHA: South-Eastern Norway Regional Health Authority; T: Size of original tumor; TNBC: Triple negative breast cancer; TNM: Scoring of tumors according to the TNM Classification of Malignant Tumors; VUS: Variant of Uncertain Significance

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

EMG: Conception and study design. Data collection and assembling. Data analysis, interpretation, manuscript writing. Final approval. CH: Conception and study design. Data collection and assembling. Data analysis, interpretation, manuscript writing. Final approval. IK: Conception and study design. Data collection and assembling. Data analysis, interpretation, manuscript writing. Final approval. SLA: Conception and study design. Data collection and assembling. Data analysis, interpretation, manuscript writing. Final approval. LOM: Conception and study design. Data analysis, interpretation, manuscript writing. Final approval. DEU: Conception and study design. Data analysis, interpretation, manuscript writing. Final approval. JN: Conception and study design. Data analysis, interpretation, manuscript writing. Final approval. ES: Conception and study design. Data analysis, interpretation, manuscript writing. Final approval.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Both predictive and diagnostic genetic testing was performed according to the guidelines for the Norwegian health care system and the Norwegian Biotechnology Act. All tested BC patients in both cohorts gave a written informed consent to genetic testing. All relatives undergoing predictive genetic testing were given genetic counseling and gave a written informed consent to testing. All patients in both cohorts, whose clinical information has been registered and included in the analyses, have been registered in

the quality register at the Department of Medical Genetics at OUH or the quality register at the Breast Cancer Surgery Unit at OUH. All clinical information was registered in the EPR system at OUH. The study was carried out as a quality of care analysis approved by the Data Protection Officer at the hospital, and consequently no approval from the Regional Committees for Medical and Health Research Ethics (REK) or from the Norwegian Social Science Data Services (NSD) was necessary.

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PAPER II

RESEARCH

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BRCA1 and *BRCA2* mutation spectrum – an update on mutation distribution in a large cancer genetics clinic in Norway

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Abstract

Background: Founder mutations in the two breast cancer genes, *BRCA1* and *BRCA2*, have been described in many populations, among these are Ashkenazi-Jewish, Polish, Norwegian and Icelandic. Founder mutation testing in patients with relevant ancestry has been a cost-efficient approach in such populations. Four Norwegian *BRCA1* founder mutations were defined by haplotyping in 2001, and accounted for 68% of *BRCA1* mutation carriers at the time. After 15 more years of genetic testing, updated knowledge on the mutation spectrum of both *BRCA1* and *BRCA2* in Norway is needed. In this study, we aim at describing the mutation spectrum and frequencies in the *BRCA1/2* carrier population of the largest clinic of hereditary cancer in Norway.

Methods: A total of 2430 *BRCA1* carriers from 669 different families, and 1092 *BRCA2* carriers from 312 different families were included in a quality of care study. All variants were evaluated regarding pathogenicity following ACMG/ENIGMA criteria. The variants were assessed in AlaMut and supplementary databases to determine whether they were known to be founder mutations in other populations.

Results: There were 120 different *BRCA1* and 87 different *BRCA2* variants among the mutation carriers. Forty-six per cent of the registered *BRCA1/2* families (454/981) had a previously reported Norwegian founder mutation. The majority of *BRCA1/2* mutations (71%) were rare, each found in only one or two families. Fifteen per cent of *BRCA1* families and 25% of *BRCA2* families had one of these rare variants. The four well-known Norwegian *BRCA1* founder mutations previously confirmed through haplotyping were still the four most frequent mutations in *BRCA1* carriers, but the proportion of *BRCA1* mutation carriers accounted for by these mutations had fallen from 68 to 52%, and hence the founder effect was weaker than previously described.

Conclusions: The spectrum of *BRCA1* and *BRCA2* mutations in the carrier population at Norway's largest cancer genetics clinic is diverse, and with a weaker founder effect than previously described. As a consequence, retesting the families that previously have been tested with specific tests/founder mutation tests should be a prioritised strategy to find more mutation positive families and possibly prevent cancer in healthy relatives.

Keywords: *BRCA1*, *BRCA2*, Founder mutations, Genetic testing

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Background

Breast cancer genes 1 and 2, *BRCA1/2* have been very well studied since their discovery in 1994 and 1995. Disease-causing mutations in these genes give a high lifetime risk of both breast and ovarian cancer [1–3]. An increased risk of aggressive prostate cancer for male *BRCA2* mutation carriers has been described [4], as well as elevated risk of pancreatic cancer [5]. Risk for other cancers is less evident [5, 6]. Preventive measures such as prophylactic mastectomy and oophorectomy or surveillance with breast MRI seem to improve survival for *BRCA* mutation carriers without significantly reducing quality of life [7–10]. More recently also cancer treatment choices are influenced by *BRCA*-status, especially for ovarian cancer [11].

Founder mutations in *BRCA1* and 2 have been described in many populations, i.e. the Ashkenazi-Jewish, Polish, Norwegian, and Icelandic [12–14]. Therefore, founder mutation testing in patients with relevant ancestry and family history has been a cost-efficient approach during the years with limited access to sequence analysis. A founder mutation may be defined as “a genetic alteration observed with high frequency in a group that is or was geographically or culturally isolated, in which one or more of the ancestors was a carrier of the altered gene”. Founder effect is frequently defined as “the loss of genetic variation that occurs when a new population is established by a very small number of individuals from a larger population” [15]. Different historical, societal and geographic factors may influence development of a founder effect including bottle neck phenomenon, genetic drift, selective mating /inbreeding, and high reproduction.

One of the first studies carried out on *BRCA* epidemiology in Norway by Moller et al. in 2001, showed that 68% of the mutation carriers had one of the four most frequent Norwegian founder mutations in *BRCA1* [16], c.1016dup, c.1556del, c.3328_3229del, c.697_698del, all demonstrated to be true founder mutations through haplotyping [13]. The variant c.1016dup was shown to originate in the south-eastern part of the country, while the other three originated from the south-western part of the country, before the Bubonic plague. Later, in 2007, four more *BRCA1* variants and two *BRCA2* variants c.3847_3848del and c.2808_2811del were published as frequent mutations in the Norwegian population, but no haplotype study has been carried out to establish a true founder origin in these [12, 17].

Founder mutation testing and MLPA (multiplex ligation-dependent probe amplification) have a lower sensitivity compared to sequencing of the entire genes and MLPA especially when used on a population with mixed genetic background. This has become increasingly obvious in our clinical practice over the years. Due to the multicultural population served by Oslo University

Hospital (OUH), as well as the falling costs of testing, sequencing and MLPA as initial test has been chosen over founder mutation testing when *BRCA*-testing is indicated. Following this, sequencing and MLPA have become the standard test since January 2014. This practice is in line with the fact that genetic variation in any gene is abundant, and rare, pathogenic variants in any gene are expected to exist [18].

The knowledge on frequencies and spectrum of disease-causing variation in *BRCA1/2* both nationally and locally is however incomplete. The aim of this study has been to describe the results from the *BRCA* testing during the last 15 to 20 years. This will give necessary overview of mutation frequencies in our region, and the results may give directions for both future research and serve as an evaluation of the current testing practice.

Methods

Study design

The study was carried out in the Section of Hereditary Cancer, Department of Medical Genetics, Oslo University Hospital, OUH, and was approved by the Data Protection Officer at OUH as a quality of care study. The study group was the full mutation carrier population registered in the clinic. Data collection was done in May 2016, and mutation carriers registered in the clinic before 5th of May 2016 were included. The study subjects were both men and women tested over the years, affected with cancer or not. Families registered with a positive mutation test were included. The lowest number of mutation carriers in a family was set to one. In this study, “family” was defined by the practice of giving an index patient a separate family number if he/she did not already have family members registered in the clinic. A thorough job looking up relatives have been done in each case and if relatives were found, the person have been included in the already registered family. Genetic testing was performed both diagnostically and predictively. All activities fulfilled the requirements of genetic counselling, information and consent stated by the Norwegian Act on Biotechnology, www.lovdata.no. All clinical information was registered in the electronic patient journals at OUH. Close to all positive mutation tests were confirmed in a separate blood sample. On the basis of the selection criteria, 2430 *BRCA1* mutation carriers from 669 different families, and 1092 *BRCA2* carriers from 312 different families were included in the study.

Genetic testing and testing strategies

Our cancer genetics clinic has offered both diagnostic and predictive testing to individuals fulfilling criteria for *BRCA1/2* testing given by the health authorities. Initially our clinic served the whole country with genetic counselling and *BRCA* testing. Since the late 90s, the

department has mainly served the South-Eastern part of Norway. The south-eastern part of Norway contains 2.9 million people, which is a little more than half of the Norwegian population of 5.2 mill.

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.1556del, c.3328_3229del, c.697_698del and c.1016dup) [13]. 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 *BRCA1* and 2 genes has increasingly been offered to our 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 until January 2014, when Sanger or high throughput sequencing (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 is suspected. This applied to fourteen families where two mutations in *BRCA1* /*BRCA2* were identified.

Founder mutation search method

To establish whether the variants found in our cohort were described as founder mutations elsewhere, we used the following strategy: All variants were described with HGVS standard nomenclature (*BRCA1* NM_007294.3 and *BRCA2* NM_000059.3). A search was performed in Alamut Visual per variant, first with default settings, then adding “founder” to the variant search terms. Alamut searches automatically after all known notations of the variant, mainly in Google. Depending on the search results, the variants were termed either F = Founder, when documentation of this was retrieved, NF = Not founder, when the variant was previously reported but not shown to be a founder anywhere, or NPR = Not previously reported if there were no documents retrievable on the variant. A double check on all variants initially classified as NPR was performed in databases ClinVar, HGMD, UMD, LOVD and *BRCA* Exchange.

Classification

The original laboratory reports were from different time periods with different routines for variant interpretation and reporting. In order to ensure up to date quality of the variant classification, we reevaluated all mutations reported in the *BRCA1/2* carrier population as part of

this quality of care study. Variants were interpreted according to the recommendations of the American College of Medical Genetics [19], and ENIGMA (Evidence-based Network for the Interpretation of Germline Mutant Alleles) using the five-class system: pathogenic (class 5), likely pathogenic (class 4), variant of uncertain significance (class 3), likely benign (class 2), or benign (class 1). A disease-causing mutation was defined as a class 4 or 5 variant according to ACMG/ ENIGMA criteria. The types of mutations for both genes are listed in Table 1, all classified as 4 or 5. The majority of variants were straight forward to classify as they introduce stop or frameshift, or constitute rearrangements or alter splicing. The splicing mutations were either in canonical +/−1 or 2 splice sites or analyzed in functional test by us or others. The missense mutations were identified in the well-known domains, RING and BRCT in *BRCA1* and DNA binding domain in *BRCA2*. Published multifactorial likelihood scores and/ or functional studies were part of the evidence in these cases. Disease-causing missense mutations were found to constitute 9% of *BRCA1* and 5% of *BRCA2* mutations in this study. Others have suggested that approximately 7% of the load of pathogenic sequence variants in *BRCA1* is attributable to missense substitutions [20, 21]. Variants of unknown significance (VUS) were not included in this study.

For the purpose of this study, a *founder mutation* was defined as a variant previously reported as such, and this may include common ancestry proven by haplotype studies. A *recurrent* mutation was defined as a variant to occur in one mutational hot spot as separate events, this term is however used synonymously with frequent variant in many publications. In this study, a *frequent mutation* was defined as a mutation found in three or more families and subdivided into three categories for systematic purposes. Mutations found in >30 different families each were termed *highly frequent*, mutations found in 10–30 families were termed *moderately frequent*. Mutations found in 3–9 families each were termed *less frequent*. A *rare mutation* was defined as a mutation found in one or two families. A frequent mutation from any of

Table 1 Types of mutations

Type of mutation	<i>BRCA1</i>	<i>BRCA2</i>
Frame shift	49	45
Stop	38	25
Rearrangement	12	4
Missense	11	4
Splice variant	9	7
Start loss	1	1
In frame deletion	0	1
	120	87

the three groups may in some cases be considered a founder candidate, depending on e.g. the geographical origin of the families. Any mutation, both frequent and rare in our study, may be known as founder mutations in a specific population.

Mutation frequencies

Throughout this study we have calculated mutation frequencies both as number of mutation carriers per variant, and number of different families per variant. The fraction of mutation carriers carrying the four well-known *BRCA1* founder mutations are directly comparable to the numbers obtained in the previous studies done on the subject. The number of families per variant would be indicative of possible new founder mutations, which again may be of separate interest for future studies. A calculation of number of mutation carriers per family was included in the work-up for each variant. Establishing a carrier frequency for the population on the whole was beyond the scope of this study.

Results

The *BRCA1* results are shown in Fig. 1 and Table 2, the *BRCA2* results are shown in Fig. 2 and Table 3. There were 120 different *BRCA1* variants and 87 different *BRCA2* variants found among the mutation carriers, 669/981 families had a *BRCA1* mutation (68%), and 312/981 had a *BRCA2* mutation (32%). Forty-six per cent of the registered *BRCA1/2* families (454/981) had a previously known Norwegian founder mutations, identified through the founder search in Alamut. There were five *BRCA1* variants and one *BRCA2* variant among the six most frequent *BRCA1/2* variants (Table 4). These six variants accounted for 47% (1643/3522) of the mutation carriers. In total, 70 % of *BRCA1/2* mutation carriers

(2466/3522) had a moderately or highly frequent variant (found in more than 10 families). Sixteen per cent (577/3522) had a less frequent variant found in 3–9 families. Fourteen per cent of *BRCA1/2* carriers (479/3522) had a rare mutation.

BRCA1

Each of the four well-known founder mutations in *BRCA1*, c.1556del, c.3328_3229del, c.697_698del and c.1016dup, were found in more than 30 different families each and classified as highly frequent (Table 2, Fig. 1). These four mutations accounted for 52% (1266/2430) of *BRCA1* carriers in this study, or 44% of *BRCA1* families (295/669). The variant c.1016dup was the most frequent mutation with 471 mutation carriers from 111 families. Together with the fifth highly frequent mutation, c.3178 G > T, also found in more than 30 families, the top five *BRCA1* mutations accounted for 58% (1413/2430) of the mutation carriers, or 51% of the families (341/669).

Twenty-seven per cent (33/120) of the variants were classified as moderately frequent (10 variants) and less frequent (23 variants). These accounted for 32% of mutation carriers (775/2430) or 34% of *BRCA1* mutation families (229/669).

Sixty-eight per cent (82/120) of the *BRCA1* variants were rare. Ten per cent of the *BRCA1* mutation carriers (242/2430), 15% of the *BRCA1* families (99/669), had one of these mutations.

BRCA2

The single most frequent *BRCA2* variant, c.5217_5223del, was found in 230 individuals from 61 different families. This variant accounted for 21% (230 /1092) of *BRCA2* carriers, or 19% of families (61/312). It was also the third

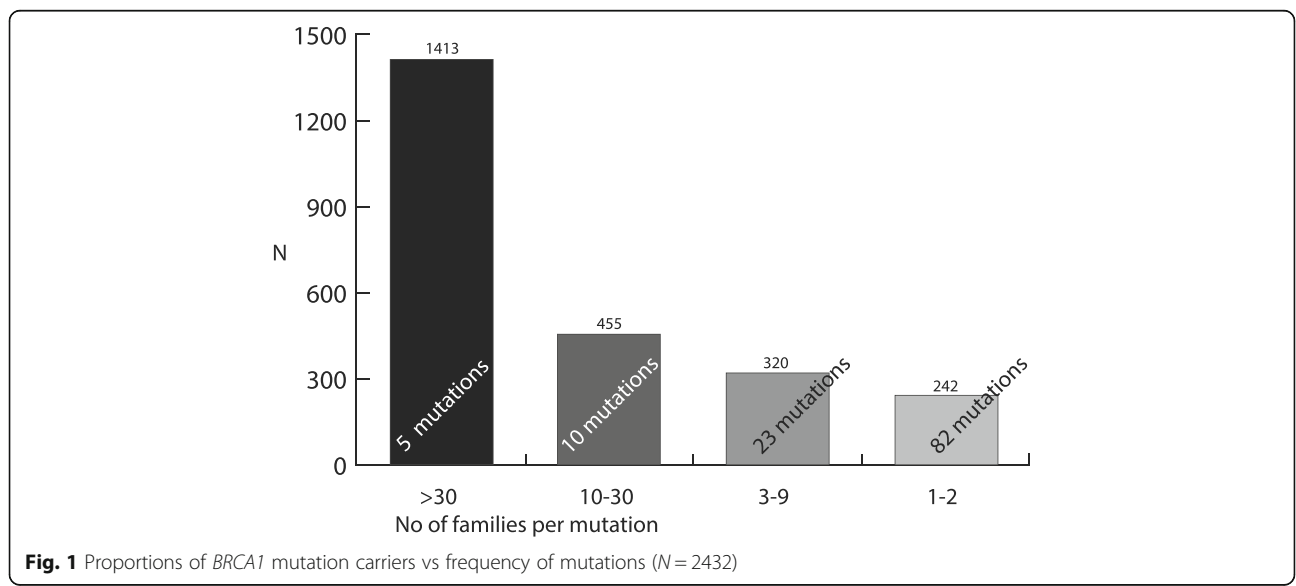


Table 2 BRCA1 variants

No of families	HGVS		Type of mutation	No.of ind.	No. of fam.	Average no of carr./fam	Percentage of carriers/families	Previous reports*
>30	c.1016dup	p.Val340Glyfs*6	fs	471	111	4.2	58%	Norwegian, Danish and Swedish
	c.1556del	p.Lys519Argfs*13	fs	399	95		1413/2430	Norwegian
	c.3228_3229del	p.Gly1077Alafs*8	fs	214	45			Italian, Norwegian
	c.697_698del	p.Val233Asnfs*4	fs	182	44		51%	Norwegian
10–30	c.3178G > T	p.Glu1060*	stop	147	46		341/669	Norwegian
	c.1A > G	p.Met1Val	start codon	69	21	3.5	19%	Norwegian
	c.3048_3052dup	p.Asn1018Metfs	fs	44	16		455/2430	Swedish founder
	c.5266dup	p.Gln1756Profs*74	fs	30	16			European, Russian
	c.3084_3094del	p.Asn1029Argfs*5	fs	43	13			Norwegian
	dup exon 13/c.(4185 + 1_41861)_(4357 + 1_4358-1)dup	p.?	rearr	41	11		19%	Norwegian and British
	dup exon 22 /c.(5332 + 1_5333-1)_(5406 + 1_5407-1)del	p.?	rearr	29	11		128/669	Dutch
	c.4745del	p.Asp1582Alafs*19	fs	78	10			Norwegian
	c.2351_2357del	p.Ser784Trpfs*6	fs	54	10			Norwegian
	del exon 8–13 / c.(441 + 1_442-1)_(4357 + 1_4358-1)del	p.?	rearr	37	10			British, European founder
	c.3607C > T	p.Arg1203*	stop	30	10			Greek founder, Belgian, Korean recurrent
	3–9	c.1072del	p.Leu358Cysfs*16	fs	22	8	3.2	13%
c.68_69del		p.Glu23Valfs*17	fs	13	7		320/2430	Ashkenazi, Polish, Italian, Spanish
c.4065_4068del		p.Asn1355Lysfs*10	fs	12	7			British and German
c.5047G > T		p.Glu1683*	stop	39	6			NF
c.3319G > T		p.Glu1107*	stop	16	8		15%	Danish
c.5075-2A > C		p.?	splice var	39	5		101/669	Norwegian
c.2475delC		p.Asp825Glufs*21	fs	15	5			Swedish and Danish
c.3700_3704del		p.Val1234Glnfs*8	fs	7	5			Rec. Greek, Czech
c.3331_3334del		p.Gln1111Asnfs*5	fs	21	4			Hispanic, Portuguese founder
c.2591C > G		p.Ser864*	stop	16	4			NF
c.3966delA		p.Lys1322Asnfs*3	fs	15	4			NF
del exon 3–16/ c.(80 + 1_81-1)_(4986 + 1_4987-1)del		p.?	rearr	14	4			NF
c.130 T > A		p.Cys44Ser	missense	8	4			NF
c.1450G > T		p.Gly484*	stop	18	3			NF
c.5513 T > G		p.Val1838Gly	missense	13	3			NF
c.3756_3759del		p.Ser1253Argfs*10	fs	10	3			Recurrent Polish
c.5251C > T		p.Arg1751*	stop	10	3			Finnish
c.1687C > T		p.Gln563*	stop	6	3			European, Austrian, Slovenian founder
c.3710del		p.Ile1237Asnfs*27	fs	6	3			Danish, Swedish, rec. Polish

Table 2 BRCA1 variants (Continued)

No of families	HGVS		Type of mutation	No.of ind.	No. of fam.	Average no of carr./fam	Percentage of carriers/families	Previous reports*
	c.4035del	p.Glu1346Lysfs*20	fs	6	3			Slovenian, Polish, Latvian, Lithuanian
	c.5309G > T	p.Gly1770Val	missense	6	3			Moroccan founder
	c.181 T > G	p.Cys61Gly	missense	5	3			Central and eastern European founder
1-2	c.843_846del	p.Ser282Tyrfs*15	fs	3	3			NF
	c.5511G > A	p.Trp1837*	stop	18	2	2.4	10%	NF
	c.2869C > T	p.Gln957*	stop	14	2		242/2430	NF (?)
	c.66dupA	p.Glu23Argfs*18	fs	12	2			NF
	c.1058G > A	p.Trp353*	stop	10	2		15%	NF
	c.5407-25 T > A	p.?	splice var	9	2		99/669	NF
	c.1292dup	p.Leu431Phefs*5	fs	8	2			NF
	c.794_795del	p.Ser265Cysfs*21	fs	8	2			NF
	c.2558ins356	p.?	stop	7	2			NPR
	c.5503C > T	p.Arg1835*	stop	6	2			Pakistani founder
	c.2681_2682del	p.Lys894Thrfs*8	fs	5	2			Scottish
	c.2989_2990dup	p.Asn997Lysfs*4	fs	5	2			NF
	c.4689C > G	p.Tyr1563*	stop	5	2			NF
	c.5153G > C	p.Trp1718Ser	missense	5	2			NF
	del exon 1-3 / c.(?_1-1)_(134 + 1_135-1)del	p.?	rearr	4	2			Norwegian founder
	c.1287del	p.Ile429Metfs*12	fs	2	2			NPR
	c.3937C > T	p.Gln1313*	stop	1	1			NF
	c.5095C > T	p.Arg1699Trp	missense	1	2			NF
	c.3874del	p.Ser1292Leufs*15	fs	7	1			Danish founder
	del exon 1-13/ c.(?_1-1)_(4357 + 1_4358-1)del	p.?	rearr	5	1			Norwegian founder
	c.5534del	p.Glu1346Lysfs*20	fs	5	1			NPR
	c.1793 T > G	p.Leu598*	stop	4	1			NF
	c.115 T > G	p.Cys39Gly	missense	2	1			Greenlandic/Danish founder
	del exon 5-7/c.(134 + 1_135-1)_(441 + 1_442-1)del	p.?	rearr	3	1			NF
	del exon 8 c.(441 + 1_442-1)_(547 + 1_548-1)del	p.?	rearr	3	1			European founder
	c.848 T > A	p.Leu283*	stop	3	1		NF	
	c.929del	p.Gln310Argfs*4	fs	3	1			NF
	c.1434_1435del	p.Glu479Lysfs*10	fs	3	1			NF
	c.2257dup	p.Ser753Lysfs*9	fs	3	1			NPR
	c.3770_3771del	p.Glu1257Glyfs*9	fs	3	1			Spanish founder
	c.4612C > T	p.Gln1538*	stop	3	1			NF
	c.457_458ins21	p.?	stop	6	2			NPR

Table 2 BRCA1 variants (Continued)

No of families	HGVS		Type of mutation	No.of ind.	No. of fam.	Average no of carr./fam	Percentage of carriers/families	Previous reports*
	del exon 18–24/c. (5074 + 1_5075–1)_ (5592 + 1_?-1)del	p.?	rearr	2	1			NPR
	c.1059G > A	p.Trp353*	stop	2	1			NF
	c.1360_1361del	p.Ser454*	stop	2	1			Italian
	c.1695dup	p.Lys566Glufs*4	fs	1	1			NF
	c.65 T > C	p.Leu22Ser	missense	2	1			NF
	c.2138C > G	p.Ser713*	stop	1	1			NF
	c.2389G > T	p.Glu797*	stop	2	1			NF
	c.2438dup	p.Leu814Thrfs*9	fs	2	1			NF
	c.3477_3479delinsC	p.Lys1160Glyfs*4	fs	2	1			NF
	c.3689 T > G	p.Leu1230*	stop	2	1			NF
	c.3835del	p.Ala1279Hisfs*28	stop	2	1			NF
	c.4186C > T	p.Gln1396*	stop	2	1			NF
	c.4484G > A	p.Ala1453Glyfs*10	splice var.	2	1			NF
	c.386del	p.Gly129Alafs*34	fs	2	1			NPR
	c.4932_4933dup	p.Arg1645Lysfs*14	fs	2	1			NF
	c.4972delA	p.Thr1658Profs*19	fs	2	1			NPR
	c.4986 + 1G > T	p.?	splice var	2	1			NF
	c.5407-2A > G	p.?	splice var	2	1			NF
	c.445G > T	p.Glu149*	stop	2	1			NPR
	c.510del	p.Ile171Tyrfs*63	fs	2	1			NPR
	del exon 11/c.(670 + 1_671–1)_(4096 + 1_ 4097–1)del	p.?	rearr	1	1			NPR
	del exon 16/c.(4675 + 1_4676–1)_(4986 + 1_4987–1)del	p.?	rearr	1	1			NF
	del exon 20–24/c. (5193 + 1_5194–1)_ (5592 + 1_?-1)del	p.?	rearr	1	1			NF
	c.1175_1214del	p.Leu392Glnfs*5	fs	1	1			NF
	c.1674dup	p.Gly559Argfs*2	fs	1	1			NPR
	c.1823_1826del	p.Lys608Ilefs*3	fs	1	1			NF
	c.1961dup	p.Tyr655Valfs*18	fs	1	1			NF
	c.2019del	p.Glu673Aspfs*28	fs	1	1			NF
	c.2185G > T	p.Glu729*	stop	1	1			NF
	c.2293G > T	p.Glu765*	stop	1	1			NF
	c.140G > T	p.Cys47Phe	missense	1	1			NF
	c.2727_2730del	p.Asn909Lysfs*90	fs	1	1			NF
	c.2864C > A	p.Ser955*	stop	1	1			Hispanic, Californian
	c.188 T > A	p.Leu63*	stop	1	1			Japanese
	c.2981_2982del	p.Cys994*	stop	1	1			NF
	c.3005del	p.Asn1002Thrfs*22	fs	1	1			NF
	c.213-5 T > A	p.?	splice var	1	1			NF
	c.3400G > T	p.Glu1134*	stop	1	1			NF

Table 2 BRCA1 variants (Continued)

No of families	HGVS		Type of mutation	No.of ind.	No. of fam.	Average no of carr./fam	Percentage of carriers/families	Previous reports*
	c.241C > T	p.Gln81*	stop	1	1			NF
	c.3544C > T	p.Gln1182*	stop	1	1			NF
	c.3644_3648del	p.(Asn1215Ilefs*2)	fs	1	1			NPR
	c.3813dupT	p.(Asn1272*)	stop	1	1			NF
	c.3817C > T	p.Gln1273*	stop	1	1			NF
	c.4146_4155dup	p.Ser1386Leufs*8	fs	1	1			NF
	c.5074 + 2 T > C	p.?	splice var.	1	1			NF
	c.5030_5033del	p.Thr1677Ilefs*2	fs	1	1			French
	c.5212G > A	p.Gly1738Arg	missense	1	1			Greek
	c.5193 + 2del	p.?	splice var.	1	1			NF
	c.5434C > G	p.Gly1803Glnfs*11	splice var.	1	1			NF
	c.514C > T	p.Gln172*	stop	1	1			NF
	c.5213G > A	p.Gly1738Glu	missense	1	1			Danish, Iranian

*Founders are reported with indication of origin, NF not founder through search, NPR not previously reported

most prevalent variant when both *BRCA1/2* were taken together (Table 4), but it was not found to be reported as a founder from the Alamut search.

Four moderately frequent mutations were found, (c.4821_4823delTGAinsC, c.2808_2811del, c.8331 + 2 T > C and c.3847_3848del), and they accounted for 34% (368/1092) of the *BRCA2* mutation carriers, or 30% (94/312) of the families.

Twenty per cent (17/87) of the *BRCA2* variants were classified as less frequent, accounting for 23% (257/1092) of the mutation carriers, or 26% (80/312) of the families.

Seventy-five per cent (65/87) of the *BRCA2* variants were considered rare, found in 1–2 families each. Twenty-two per cent (237/1092) of the mutation carriers, (25%

(77/312) of the families) had one of these rare *BRCA2* mutations.

Founder mutation search

Among the variants found in more than ten families each, ten out of fifteen *BRCA1* variants and two out of five *BRCA2* variants were previously reported as founder mutations in Norway, including the four demonstrated by haplotyping. Another three *BRCA1* variants were reported as Norwegian founders, and these were found in the less frequent or rare category. The remaining highly frequent variants were either described as founder mutations in neighbouring/European countries, or previously reported in other countries, but not as founders, which was the case with the two most frequent *BRCA2* variants

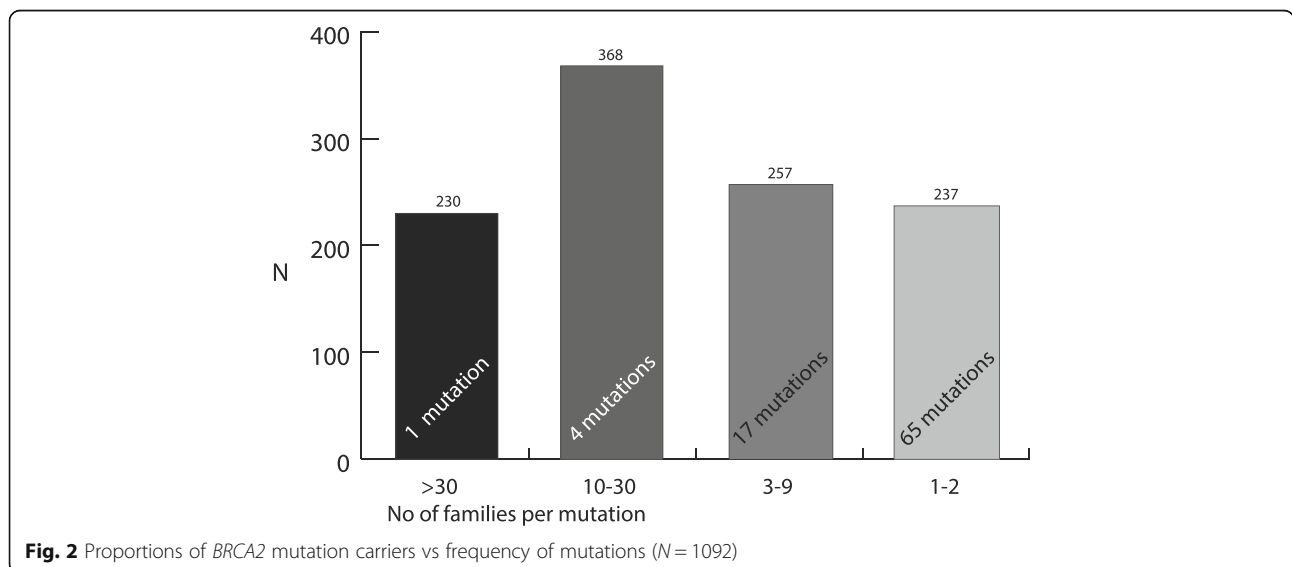


Fig. 2 Proportions of *BRCA2* mutation carriers vs frequency of mutations (N = 1092)

Table 3 BRCA2 variants

No of families	HGVS		Type of mutation	No.of ind.	No. of fam.	Average no of carrier/ fam	Percentage of carriers/families	Previous reports
>30	c.5217_5223del	p.Tyr1739*	stop	230	61	3,8	21% 230/1092 19% 61/312	NF
10–30	c.4821_4823delTGainsC	p.Glu1608Aspfs*6	fs	126	25	3,9	34%	NF
	c.2808_2811del	p.Ala938Profs*21	fs	89	26		368/1092	Norwegian founder
	c.8331 + 2 T > C	p.?	splice variant	71	29			Danish founder
	c.3847_3848del	p.Val1283Lysfs*2	fs	82	14		30% 94/312	Norwegian, Iranian founder
3–9	dup exon 20/ c.(8487 + 1_8488–1)_(8632 + 1_8633–1)dup	p.?	rearr	34	9	3,2	23%	NF
	c.9118-2A > G	p.?	splice variant	44	8		257/1092	Finnish founder, recurrent Polish
	c.5723_5722del	p.Leu1908Argfs*2	fs	23	8			NF
	c.2047_2050del	p.Ser683Argfs*46	fs	28	6		26% 80/312	NPR
	c.8229_8243del	p.Arg2744_Gly2748del	in frame del	18	5			NF
	c.5946del	p.Ser1982Argfs*22	fs	11	5			Ashkenazi, Hungarian
	c.771_775del	p.Asn257Lysfs*17	fs	6	5			Finnish, Icelandic
	c.1905_1909del	Asp635Gluufs*15	fs	22	4			NF
	c.5576_5573del	p.Ile1859Lysfs*5	fs	11	4			NF
	c.6059_6062del	p.Glu2020Valfs*19	fs	9	4			NF
	c.9117G > A	p.Val2985Glyfs*4	splice variant	5	4			NF
	c.171C > G	p.Tyr57Ter*	stop	15	3			NF
	c.8177A > G	p.Tyr2726Cys	missense	9	3			NF
	c.2830A > T	p.Lys944Ter*	stop	8	3			NF
	c.3847_3848del	p.Val1283Lysfs*2	fs	8	3			NF
	c.7069_7070del	p.Leu2357Valfs*2	fs	3	3			NF
	c.7480C > T	p.Arg2494*	stop	3	3			Finnish founder
1–2	del exon 3	p.?	rearr	8	2	3,1	22%	NF
	c.3G > A	p.Met1?	start loss	6	2		237/1092	NF
	c.5157_5161del	p.Asn1719Lysfs*6	fs	2	2		25%	NF
	c.6373dup	p.Thr2125Asnfs*4	fs	5	2		77/312	NF
	c.6486_6489del	p.Lys2162Asnfs*5	fs	2	2			Danish founder
	c.7558C > T	p.Arg2520*	stop	14	2			NF
	c.7617 + 1G > A	p.?	splice	2	2			Danish founder
	c.8130del	p.Ser2710Argfs*23	fs	9	2			NF
	c.8323dup	p.Met2775Asnfs*7	fs	10	2			NF
	c.631 + 4A > G	p.?	splice	11	2			NF
	c.9154C > T	p.Arg3052Trp	missense	9	2			NF
	c.9699_9702del	p.Cys3233Trpfs*15	fs	12	2			NF

Table 3 BRCA2 variants (Continued)

No of families	HGVS		Type of mutation	No.of ind.	No. of fam.	Average no of carrier/ fam	Percentage of carriers/families	Previous reports
	del exon 19–21/c. (8331 + 1_8332–1)_ (8754 + 1_8755–1)del	p.?	rearr	1	1			NPR
	del exon 20/c.(8487 + 1_8488–1)_(8632 + 1_8633–1)del	p.?	rearr	1	1			NF
	c.1296_1297del	p.Asn433Glnfs*18	fs	1	1			NF
	c.1429del	p.His477Ilefs*8	fs	10	1			NPR
	c.1456C > T	p.Gln486*	stop	5	1			NF
	c.1642C > T	p.Gln548*	stop	3	1			NF
	c.1658 T > G	p.Leu553*	stop	4	1			NF
	c.1945C > T	p.Gln649*	stop	4	1			NF
	c.2636_2637del	p.Ser879*	stop	2	1			NF
	c.3158 T > G	p.Leu1053*	stop	1	1			NF
	c.3307_3308dup	p-Leu1103Phefs*2	fs	1	1			NPR
	c.3545_3546del	p.Phe1182*	stop	1	1			NF
	c.3596_3599del	p.Asp1199Valfs*9	fs	8	1			NF
	c.3720_3721del	p.Phe1241*	stop	2	1			NPR
	c.3751dup	p.Thr1251Asnfs*14	fs	2	1			NF
	c.171del	p.Tyr57*	stop	4	1			NPR
	c.3860del	p.Asn1287Ilefs*6	fs	1	1			Austrian founder
	c.196C > T	p.Gln66*	stop	2	1			NF
	c.4095 T > A	p.Cys1365*	stop	3	1			NF
	c.4258del	p.Asp1420Ilefs*28	fs	2	1			Swedish founder
	c.4710del	p.Glu1571Argfs*8	fs	1	1			NF
	c.4794_4797del	p.Asn1599Metfs*17	fs	1	1			NF
	c.5073dup	p.Trp1692Metfs*3	fs	1	1			NF
	c.316 + 1G > T	p.?	splice variant	5	1			NF
	c.5577del	p.Val1862*	stop	1	1			NF
	c.5645C > A	p.Ser1882*	stop	7	1			French founder
	c.5682C > G	p.Tyr1894*	stop	4	1			NF
	c.6034dup	p.Ser2012Phefs*6	fs	3	1			NPR
	c.6084_6088del	p.Glu2029Tyrfs*18	fs	5	1			NPR
	c.407del	p.Asn136Ilefs*16	fs	1	1			NF
	c.6611dup	p.Val2205Cysfs*20	fs	2	1			NF
	c.6591_6592del	p.Glu2198Asnfs*4	fs	3	1			NF
	c.469_470del	p.Lys157Valfs*25	fs	3	1			NF
	c.7024C > T	p.Gln2342*	stop	1	1			NF
	c.517-2A > G	p.?	splice variant	2	1			NF
	c.7234del	p.Thr2412Leufs*57	fs	4	1			NF
	c.7673_7674del	p.Glu2558Valfs*7	fs	3	1			NF
	c.7680dup	p.Gln2561Serfs*5	fs	1	1			NF
	c.7753G > A	p.Gly2585Arg	missense	1	1			NF
	c.7829dupT	p.Asp2611Glyfs*7	fs	1	1			NF

Table 3 BRCA2 variants (Continued)

No of families	HGVS	Type of mutation	No.of ind.	No. of fam.	Average no of carrier/ fam	Percentage of carriers/families	Previous reports
	c.7878G > C	p.Trp2626Cys	missense	3	1		NF
	c.7913_7917del	p.Phe2638*	stop	1	1		Czech founder
	c.8090_8105del	p.Ser2697Lysfs*31	fs	2	1		NPR
	c.8396dup	p.Pro2800Thyfs*12	fs	9	1		NPR
	c.658_659del	p.Val220Ilefs*4	fs	1	1		Lithuanian founder
	c.8878C > T	p.Gln2960*	stop	1	1		Korean
	c.614delG	p.Ser205Ilefs*6	fs	4	1		NPR
	c.9127G > T	p.Glu3043*	stop	1	1		NF
	c.9227del	p.Gly3076Aspfs*7	fs	2	1		NF
	c.9253dup	p.Thr3085Asnfs*26	fs	10	1		NF
	c.9382C > T	p.Arg3128*	stop	1	1		Jewish founder
	c.9403del	p.Leu3135Phefs*28	fs	1	1		NF
	c.9523G > T	p.Glu3175*	stop	3	1		NF

*Founders are reported with indication of origin, NF not founder through search, NPR not previously reported

(c.5217_5223del and c.4821_4823delTGAinsC). There were 14 founder mutations among the 23 less frequent mutations in *BRCA1* (61%), mainly Central-European, one Norwegian and three Swedish/Danish. There were four founder mutations among the 17 less frequent *BRCA2* variants (23.5%), none of them were Norwegian. 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 Tables 2 and 3.

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 *BRCA2* variant c.2047_2050del, found in six families, was the only frequent variant not previously described.

Discussion

We aimed at describing the *BRCA1/2* mutation distribution in the largest genetic clinic in Norway after many years of *BRCA* testing. Over the last 10 years, the total

number of mutation carriers ($N = 3522$) is almost 2.5-fold and the total number of mutations has almost tripled ($N = 207$), compared to 2007 when 1300 carriers and 75 distinct mutations were identified [17].

There are three main findings. Firstly, the distribution of both *BRCA1* and *BRCA2* mutations is quite extreme: A few mutations are very frequent and many mutations are very rare. The four proven *BRCA1* founder mutations by Møller et al. in 2001 are still the most common variants among *BRCA1* mutation carriers, but these variants account for a smaller proportion of carriers than previously described. Secondly, there is an increasing amount of moderately and less frequent variants in both genes, among which many are considered to be founders. This is especially true for the *BRCA2* variant c.5217_522del which is not previously described as a founder, but is shown to be the third most frequent mutation in the *BRCA1/2* carrier population as a whole. Thirdly, 71% (148/208) of the *BRCA1/2* variants are rare and found in only one or two individuals/families.

Even though the four most common *BRCA1* mutations are the exact same in 2016 as in 2001 [13], the

Table 4 BRCA1/2 variants found in more than 30 families

Gene	Variant		No. of individuals	No. of families
<i>BRCA1</i>	c.1016dup	p.Val340Glyfs*6	471	111
<i>BRCA1</i>	c.1556del	p.Lys519Argfs*13	399	95
<i>BRCA2</i>	c.5217_5223del	p.Tyr1739*	230	61
<i>BRCA1</i>	c.3178G > T	p.Glu1060*	147	46
<i>BRCA1</i>	c.3228_3229del	p.Gly1077Alafs*8	214	45
<i>BRCA1</i>	c.697_698del	p.Val233Asnfs*4	182	44
Total			1643	403

proportion of *BRCA1* mutation carriers accounted for by the four founders has fallen from 68% in 2001 to 52% in the present study. In the 2001- study, 82 patients who contracted breast cancer prospectively after being recommended breast cancer screening based on their family history, were *BRCA1/2* tested. No *BRCA2*- mutation were found. The patients were included for breast cancer screening based on selection criteria similar to traditional testing criteria. The present study has a retrospective method, and a much higher number of patients compared to the previous study, and any rare mutation will be easier to detect.

As expected when testing more patients, some of the rare/less frequent variants described in 2001 are shown to be frequent, as is the case with the *BRCA1* variant c.3178G > T [16]. On the other hand, the *BRCA1* variants c.794_795del, c.2558ins356, c.2869C > T and c.5511G > A were all identified in 2001, and have not turned out to be frequent in the patient population over time. It remains to be seen from future testing which of the rare variants in 2016 that remains rare.

The second main finding is that a substantial number of carriers have moderately or less frequent mutations, many of which are founder candidates. The laboratories have, as specified in Method section, offered specific testing for the frequent mutations that have been detected over time. Finding a mutation in more than three families is a liberal but well recognized threshold of suspecting a founder candidate [12, 22]. Both the well-known Icelandic/Finnish founder, *BRCA2* c.771_775del, some of the European recurrent mutations, i.e. *BRCA1* c.5266dup and, c.181 T > G as well the Ashkenazi Jewish founders are all present in the groups of low or moderately frequent mutations in our study. A recently identified Moroccan *BRCA1* variant has been demonstrated in three families at OUH and have been shown to share the same haplotype as in a series of Moroccan patients [23]. Systematic collection of information on geographic or ethnic origin per individual/family was beyond the scope and permits of this study, but was performed in 2007, where it was found to be an apparent geographical connection for some of the frequent mutations in *BRCA1* and *BRCA2* [17].

The third important finding is that 71% of the *BRCA1/2* variants are classified as rare, and that 14% of the mutation carriers in total have one of these rare variants, 10% of *BRCA1* carriers and 22% of *BRCA2* carriers. Some of these variants are actually reported as founders, mainly from other populations (15 and 17% of variants for *BRCA1* and *BRCA2* respectively), and some are not previously reported in the available databases (8.2% (*BRCA1*) and 11.5% (*BRCA2*)). These NPR variants may possibly represent unique variants in our population, or they are simply not reported to international databases from other laboratories yet.

The amount of rare mutations found in our study may be similar in other countries assumed to have a strong founder effect. In a recent study from Bulgaria, 200 individuals from breast/ovarian cancer families were genotyped with sequencing, and comparable results were found [24]. Two new, and five previously known mutations were identified in *BRCA2*, while two new and six previously known mutations were identified in *BRCA1*. In a Danish study on *BRCA1/2* founder mutations, a majority of the mutations identified were found one individual or family, which is similar to our study [25]. A rate of 7–13% rare, non-founder mutations has been described in Ashkenazi-Jewish *BRCA1/2* carriers [26]. In the Polish population, 10 % of breast/ovarian cancer patients that previously tested negatively for the Polish founder mutations were found to carry other recurrent or founder candidate variants [27]. The questions following from this are what should the indication for *BRCA1/2* testing be, and which method for testing will have sufficient sensitivity. Founder mutation testing alone will, according to this, even in founder populations have lower sensitivity than favourable.

To establish the frequencies of rare pathogenic *BRCA1/2* mutations is very important due to their significance in cancer prevention [28], also when a broader testing approach for other breast cancer genes with lower penetrance is applied through gene panels [26, 29]. Founder mutations and their effect will dilute in a multi-cultural society as described in this study. If presymptomatic population screening should be discussed in Norway, as it has been piloted for Ashkenazi Jews in the United States, such knowledge is nevertheless crucial [30]. When discussing screening for any disease, rare or common, establishing test sensitivity and specificity is central [31]. If a similar offer of voluntary founder testing in subgroups of the Norwegian society should be planned for, these data can be used to establish the expected false negative rate. On the other hand, if sequencing/MLPA is considered a better choice because of higher detection rate of pathogenic variants, the rate of detecting VUS and the practice of reporting these variants and reevaluation over time must be considered. To establish the frequency of VUS in the patient database was outside the scope of this study, but after the conversion by the laboratory to full gene tests by sequencing combined with MLPA in January 2014, the rate of VUS in diagnostic testing of *BRCA1/2* has been 4.9% [32].

Knowing the local mutation spectrum also makes it possible to plan for future epidemiological studies in the larger population, haplotype studies and possibly genotype/phenotype studies. Norwegian founder mutations have previously been considered to have somewhat lower penetrance and lower cancer risk per year than the rarer mutations in the population regarding both breast and ovarian cancer [33]. The issue of possible

genotype-phenotype effects in *BRCA1/2* mutation carriers have been explored in several studies [22, 34, 35]. Rebbeck et al. presented in 2015 one of the largest studies performed on the subject, confirming the existence of areas with relative variation in breast and ovarian cancer risk. The results await appropriate validation before findings may be transferred to clinical counselling practice.

There are some selection biases due to the changing practice of both patient inclusion and testing over the years. During the first 10 years of *BRCA* testing in OUH, patients were mainly tested for the known founder mutations. Family members of identified mutation carriers were informed by their relatives of the possibility of predictive testing, and over time, quite large families with founder mutations were identified. An overall larger percentage of carriers than of families for the most frequent mutations illustrate this, as well as the rate of mutation carriers per family (stated in Tables 2 and 3). The rarer mutations in *BRCA1* have a larger fraction of families compared to the fraction of mutation carriers, and therefore a lower number of mutation carriers per identified family. The high number of different families per founder mutation may however indicate that this family testing strategy is not the sole reason for the high variant frequency, but rather confirm what is known about these mutations already. The variants are old, present before the historical event of the Bubonic plague in fourteenth Century. The carriers have been the object of high selection i.e. through bottle neck phenomenon, non-random mating/inbreeding, as well as other historical factors favouring establishing large families [13, 16]. Over time the families have grown so large that the descendants loose contact, and hence the number of seemingly unrelated families increase. For *BRCA2*, the average number of mutation carriers per family is quite similar between the frequent and the rare mutations. This may be due to a true, but weaker founder effect for the most frequent *BRCA2* variant, c.5217_5223del, i.e. a younger age of this variant than the *BRCA1* founder variants, and hence a true, lower frequency in the population. This may in turn be caused by less selection favouring the mutation, e.g. lower degree of inbreeding, smaller families and other historical factors. However, the numbers may also simply reflect a shorter time span both since the most frequent mutation, c.5217_5223del, was identified in our clinic.

Defining a mutation as rare when identified in two families, and as “less frequent” when found in three families or individuals may seem a bit arbitrary, and even misleading. As shown in Tables 2 and 3, variants found in i.e. three small families consisting of 1–2 persons each may really also represent a rare mutation, and if counted as such it would lead to a higher number of rare mutation carriers. The material represents more than half of the Norwegian population, but are not representative for

the nation as such. There are well-known local, founder effects present in both the Western and Northern part of the country that will influence on the national frequencies of founder mutations. Lower inclusion rate of patients especially from Western Norway in the later years may also bias the result presented here towards a lower proportion of these mutations in our patient cohort.

In sum, we find that while the well-known founder effect in Norway is still present, it is weaker than previously described. Several frequent mutations detected over the last 15 years are considered founder candidates, and previously described founders from other populations are also found among rare variants in our population. Due to the significant presence of rare mutations we suggest that in order to identify as many *BRCA1/2* mutation positive families as possible one should consider to systematically offer retesting with sequencing and MLPA to individuals and families that have previously only been tested with a limited, founder mutation test. The study also supports the continuation of the introduced testing practice of using sequencing and MLPA as initial test in individuals fulfilling testing criteria. Such a testing practice will over time allow detection of variants, both rare and frequent, that otherwise would be missed. Cost-efficiency of such a test approach will vary among health care systems. However, a similar practice has been shown to be cost-efficient in a recent UK study, especially when allowing healthy mutation positive relatives to be identified before they contract cancer [36].

Conclusions

The mutation spectrum of *BRCA1* and *BRCA2* mutations in the largest hereditary cancer clinic in Norway is diverse. The four *BRCA1* founder mutations identified in 2001, are still the most frequent *BRCA1* mutations, but account now for 52% of *BRCA1* mutation carriers, compared to 68% in 2001. In total, 46 % of the registered *BRCA1/2* families (454/981) had one of the previously known Norwegian founder mutations, identified through the founder search in Alamut. Moreover, several frequent mutations have been identified during the last 15 years, many of which are considered founders in the Norwegian population. Lastly, a majority of mutations are rare, but as a group these rare mutations account for 15% of *BRCA1* and 25% of *BRCA2* mutation families. The results presented therefore support the current practice of using sequencing and MLPA over limited testing for only founder mutation in our patient population. Only through this strategy will new *BRCA1/2* mutations, both rare and frequent be identified. Families and individuals who previously have tested negative for founder mutations should systematically be offered retesting with sequencing and MLPA in order to identify healthy *BRCA1/2* carriers and enable them to prevent cancer.

Abbreviations

ACMG: American College of Medical Genetics; *BRCA1/2*: Breast cancer gene 1 / 2; BRCT-domain: *BRCA1* - C terminus domain; ClinVar: Public Archive of variants, hosted by National Center of Biotechnology Information; ENIGMA: Evidence-based Network for the Interpretation of Germline Mutant Alleles; F: Founder mutation; HGMD: Human gene mutation database; HGVS: Human genome variation society; HTS: High throughput sequencing; LOVD: Leiden open (Source) variation database; MLPA: Multiplex ligation-dependent probe amplification; MRI: Magnetic resonance imaging; NF: Not founder mutation; NPR: Not previously reported; OUH: Oslo University Hospital; RING-finger domain: Really interesting new gene - finger domain; UMD/BRCAShare: Universal mutation database - BRCA; VUS: Variants of unknown significance

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Availability of data and materials

May be made available upon request, but such release of data must also be approved by Data Protection Office.

Authors' contributions

CH and LOM designed the study, CH performed the data management work. TW, SL, SLA and CH did the variant searches, TW, SL, SLA classified the variants. All authors contributed to the writing of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was carried out in the Section of Hereditary Cancer, Department of Medical Genetics, Oslo University Hospital, OUH, and was approved by the Data Protection Officer at OUH as a quality of care study. All activities fulfilled the requirements of genetic counselling, information and consent stated by the Norwegian Act on Biotechnology, as part of the health service.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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PAPER III

RESEARCH

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Ten modifiers of *BRCA1* penetrance validated in a Norwegian series

Cecilie Heramb¹, Per Olaf Ekstrøm², Kukatharmini Tharmaratnam³, Eivind Hovig², Pål Møller^{1*} and Lovise Mæhle¹

Abstract

Background: Common genetic variants have been shown to modify *BRCA1* penetrance. The aim of this study was to validate these reports in a special cohort of Norwegian *BRCA1* mutation carriers that were selected for their extreme age of onset of disease.

Methods: The ten variants rs13387042, rs3803662, rs8170, rs9397435, rs700518, rs10046, rs3834129, rs1045485, rs2363956 and rs16942 were selected to be tested on samples from our biobank. We selected female *BRCA1* mutation carriers having had a diagnosis of breast or ovarian cancer below 40 years of age (young cancer group, $N = 40$), and mutation carriers having had neither breast nor ovarian cancer above 60 years of age (i.e., old no cancer group, $N = 38$). Relative risks and odd ratios of belonging to the young cancer versus old no cancer groups were calculated as a function of having or not having the SNPs in question.

Results: Five of the ten variants were found to be significantly associated with early onset cancer. Some of the variation between our results and those previously reported may be ascribed to stochastic effects in our limited number of patient studies, and/or genetic drift in linkage disequilibrium in the genetically isolated Norwegian population. This is in accordance with the understanding that the SNPs are markers in linkage disequilibrium with their respective disease-causing genetic variants, and that this may vary between different populations.

Conclusions: The results confirmed associations previously reported, with the notion that the degree of association may differ between other populations, which must be considered when discussing the clinical use of the associations described.

Keywords: *BRCA1*, Modifiers, Genetic drift

Introduction

Mutations in the *BRCA1* gene constitute a high life-time risk of breast and ovarian cancer. Risk reducing salpingo-oophorectomy over the age of 35 years is advocated to reduce the risk of cancer and early death [1]. Breast cancer may be prevented by prophylactic mastectomy and patient prognosis improved when breast cancer is detected early with mammography and MRI [2]. Because *BRCA1*-associated breast cancer has an early onset, prophylactic mastectomy must be undertaken at younger age to provide a maximum protective effect [3].

BRCA1-associated cancer is age-dependent, and whether or not this is stochastic or influenced by other factors (modifiers of penetrance) is a question that has not been

fully explored: Both stochastic elements and modifying factors may be instrumental in diseases causation. Modifying factors may be genetic, environmental, or both. This study was designed to validate previous reports of normal genetic variants that contribute to modifying *BRCA1* penetrance.

A number of normal single nucleotide polymorphisms (SNPs) associated with breast cancer in the general population have been demonstrated to modify the penetrance of *BRCA1* [4–13]. We decided not to participate in the initial studies of these modifiers of breast cancer penetrance, and we now have one of the few sufficiently large series of well-described *BRCA1* mutation carriers to validate the findings reported by others.

The aim of this study was to determine whether SNPs reported to be associated with cancer risk in *BRCA1* mutation carriers in other populations had the same association in the Norwegian population.

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Materials and methods

Selection of patients

All study subjects were demonstrated *BRCA1* mutation carriers at the outpatient Cancer Genetics Clinic, Oslo University Hospital and the respective mutations were as previously reported [14]. Two groups were selected for analysis: Mutation carriers having had breast and/or ovarian cancer under the age of 40 years, hereafter described as the young cancer group, and mutation carriers who were completely disease free until their 60th birthday, or older (the old no cancer group). If there were to be significant associations between age at onset of cancer and the SNPs considered in our material, they should be identified by comparing the extremes by this approach. The study was approved by both the Ethical review board (ref S02030) and the Norwegian Data Inspectorate (ref 2001/2988-2). All genetic counseling and testing was performed according to Norwegian law, and all patients gave written informed consent. The present report is one in a series to meet a request from the Norwegian Parliament to report the results of our studies into inherited breast and or ovarian cancer risk. We did not discriminate between breast or ovarian cancer, as we have previously shown that there is no sib pair concordance for breast and ovarian cancer in *BRCA1* mutation carriers in our population [15]. Patients having had prophylactic mastectomies under the age of 60 years were excluded from the study, patients having had prophylactic salpingo-oophorectomy, but not mastectomy, were included in the old group. Power calculations indicated that, if reasonable prevalence of the variant alleles for each of the modifiers tested, we would reach significance if the OR > 2 or < 0.5, and with 50 participants in both the young cancer and old no cancer groups. Preliminary analyses on the number of women having consented to participate, indicated that we would reach significance by selecting affected women aged less than 40 years and women unaffected at over 60 years as mentioned previously.

Test panel/selection of SNPs

Not knowing the prevalence of the genetic variants in question in Norway, nor their association with disease, we selected the ten genetic variants reported to have the highest association with early/late onset of breast cancer among *BRCA1* mutation carriers in 2011 when the study was designed. The test panel consisted of nine single nucleotide polymorphisms (SNPs) and one deletion shown to be associated with cancer risk in *BRCA1* mutation carriers as shown in Table 1. For simplicity, we will in this report refer to the deletion as a SNP. Seven of the SNPs were reported to increase cancer risk [4–10] and three were reported to decrease risk for breast cancer in *BRCA1*-mutation carriers [11–13].

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

Genotyping

Samples

Blood samples were obtained after informed consent and stored at -20°C (or -70°C). DNA was extracted from 200 μl of whole blood by using a Qiagen BioRobot M48 Robotic Workstation, following the protocol of the MagAttract DNA Blood Mini M48 kit (Qiagen, Hilden, Germany).

Fragment design

Default Primer3 (<http://bioinfo.ut.ee/primer3/>, last accession date 03062014) parameters were applied when designing primers used to amplify fragments around each DNA variant, identified by the NCBI SNP reference numbers (rs) [16]. A 42-bp artificial high melting domain, labeled with 6-FAM, was incorporated at one end of the amplified target using a set of three primers in the PCR setup [17].

PCR

The PCR reaction mixtures were as described by the manufacturer (Life technologies Carlsbad CA) without modification. Annealing temperatures are given in Additional file 1.

Electrophoresis

We used cycling temperature capillary electrophoresis (CTCE) to detect allelic variants as described previously [16, 17].

Statistics

We confirmed that the prevalence of the SNPs in the young cancer and old no cancer groups assessed together were all in Hardy–Weinberg equilibrium.

Since this was a one-sided study, we used Fishers' exact to identify any significant association.

Results

The selection criteria applied to our data set revealed 40 patients in the young cancer group and 38 participants in the old no cancer group, which was considered sufficient to reveal any difference in the frequency of SNPs between the two groups. Forty-seven (60 %) patients had eight different founder mutations previously reported [18], of whom 25 belonged to the young onset cancer

Table 1 Distribution of genotypes in the ten SNPs determined in *BRCA1* mutation carriers with breast or ovarian cancer before 40 years of age (young cancer) and in carriers not having had breast or ovarian cancer before 60 years of age (old no cancer), and with calculated RR and OR, and HR/OR from previous reports [according to references given in left column]

SNP	Genotype	Young cancer (number of cases)	Old no cancer (number of cases)	Reported risk	ObservedRR (95 % CI)	ObservedOR (95 % CI)	Fishers' exact p – one sided
rs13387042 2q35 [4]	GG	13	18		1	-	
	AA	9	9	HR 1.05	1.19 (0.64–2.22)	1.38 (0.43–4.45)	0.40
	GA	17	11	HR 1.14	1.45 (0.87–2.41)	2.14 (0.76–6.06)	0.12
	AA or GA	26	20		1.35 (0.83–2.19)	1.80 (0.72–4.52)	0.15
rs3803662 16q12 TOX3, LOC643714 [5]	CC	18	26		1	-	
	TT	1	2	HR 1.24	0.81 (0.16–4.20)	0.72 (0.06–8.58)	0.65
	CT	20	10	HR 1.11	1.63 (1.05–2.52)	2.89 (1.10–7.61)	0.03*
	TT or CT	21	12		1.56 (1.00–2.41)	2.53 (1.00–6.40)	0.04*
rs8170 19p13 [6]	GG	29	27		1	-	
	AA	0	1	HR 1.35	-	-	-
	GA	11	10	HR 1.22	1.01 (0.63–1.63)	1.02 (0.38–2.80)	0.58
	AA or GA	11	11		0.97 (0.59–1.57)	0.93 (0.35–2.50)	0.65
rs9397435 6q25 ESR1 [7]	AA	32	34		1	-	
	GG	1	0	HR 1.37	2.06 (1.61–2.64)	-	-
	AG	7	4	HR 1.31	1.31 (0.79–2.19)	1.86 (0.50–6.96)	0.27
	GG or AG	8	4		1.38 (0.86–2.20)	2.13 (0.58–7.75)	0.20
rs700518 CYP19 [8]	AA	12	14		1	-	
	GG	16	6	OR 2.81	1.58 (0.97–2.57)	3.11 (0.92–10.48)	0.06
	AG	11	17	OR 1.41	0.85 (0.46–1.58)	0.75 (0.26–2.23)	0.41
	GG or AG	27	23		1.17 (0.72–1.91)	1.37 (0.53–3.54)	0.34
rs10046 CYP19 [9]	CC	11	15		1	-	
	TT	17	7	OR 1.37	1.67 (1.00–2.81)	3.31 (1.02–10.72)	0.04*
	TC	12	15	OR 1.26	1.05 (0.57–1.94)	1.09 (0.37–3.23)	0.55
	TT or TC	29	22	OR 1.29	1.34 (0.81–2.23)	1.80 (0.69–4.67)	0.17
rs3834129 CASP 8 [10]	nor/nor	8	9		1	-	
	del/del	12	6	HR 1.60	1.42 (0.78–2.58)	2.25 (0.57–8.82)	0.20
	nor/del	19	23	HR 1.83	0.96 (0.53–1.76)	0.93 (0.30–2.88)	0.56
	del/del or nor/del	31	29		1.10 (0.63–1.92)	1.20 (0.41–3.54)	0.48
rs1045485 CASP 8 [11]	GG	33	25		1	-	
	CC	1	6	HR 0.86	0.25 (0.04–1.56)	0.13 (0.01–1.12)	0.04*
	GC	5	5	HR 0.83	0.88 (0.45–1.70)	0.76 (0.20–2.91)	0.47
	CC or GC	6	11		0.62 (0.31–1.23)	0.41 (0.13–1.27)	0.10
rs2363956 9p13 ABHD8, ANKLE1, C19orf62 [12]	AA	15	5		1	-	
	CC	7	13	HR 0.7	0.47 (0.24–0.89)	0.18 (0.05–0.70)	0.01*
	AC	18	19	HR 0.89	0.65 (0.43–0.98)	0.32 (0.10–1.05)	0.05*
	CC or AC	25	32		0.58 (0.40–0.86)	0.26 (0.08–0.81)	0.02*
rs16942 BRCA1 [13]	TT	20	23		1	-	
	CC	5	9	HR 0.85	0.77 (0.35–1.66)	0.64 (0.18–2.22)	0.35
	TC	15	6		1.54 (1.01–2.34)	2.88 (0.94–8.82)	0.05*
	CC or TC	20	15		1.23 (0.80–1.89)	1.53 (0.62–3.77)	0.24

*: $p < 0.05$

group. Thirty-one (40 %) had altogether 19 different mutations, of which 15 had young onset cancer. The observed results and the calculated RRs, ORs and significance levels are shown in Table 1, together with the previously reported HRs and ORs [4–13]. All ten SNPs tested showed point estimates of being positively or negatively associated with having early onset breast cancer similar to previous reports. Because the references had used different ways of ascertaining patients, including different methods by which to calculate HRs and ORs, we had no exact notion of what RRs and ORs would be calculated for our study, and could not compute theoretical significance levels against those expected.

We found that rs3803662 was significantly associated with early onset breast cancer ($p = 0.026$ for heterozygous cases and $p = 0.040$ for homo- or heterozygous cases). The SNP rs10046 was positively associated with early onset disease in the homozygous state ($p = 0.040$), and rs104585 was negatively associated with early onset breast cancer ($p = 0.039$ for homozygous). The SNP rs2363956 was negatively associated with early cancer ($p = 0.012$, $p = 0.049$ and $p = 0.015$ for homo-, hetero- or homo- or heterozygous, respectively). Finally, rs16942 was significantly associated with early onset breast cancer ($p = 0.05$ for heterozygous). The distributions for homozygous versus heterozygous for rs16942 were conflicting and remain to be precisely defined. The rs16942 SNP is within linkage distance from the BRCA1 gene, and haplotyping of the patients/families in question may be necessary to consider this further.

Discussion

In principle, we have confirmed the reported association between the presence of variant SNPs and early onset of breast cancer in *BRCA1* mutation carriers. Five of the SNPs tested revealed significant associations with early ages of onset cancer, whereas five did not. The lack of association may be due to different associations in the Norwegian population compared to other populations, which may be a result of genetic drift [18]. Also, stochastic variation in our restricted number of patients may have obscured the associations examined.

Sixty-three percent of the mutation carriers included had one of the frequent Norwegian founder mutations, in which we have determined the penetrance to be similar in retrospective series of extended pedigrees, which we later confirmed through prospective studies of new cases in the same families [18, 19]. There were no associations with the presence of founder mutations or less frequent mutations with early onset cancer. For this reason, we do not have the confounder of putative different penetrance of the causative mutations as discussed in other reports [4–13]. This may be a third possible cause for a stronger association in the Norwegian population.

Yet another possible cause for the stronger associations in our population is that we scored both breast and ovarian cancer as affected phenotypes, while most reports considered only breast cancer.

Also, most previous reports calculated HR from continuous distribution by other methods. The described differences between the young cancer cases and the old no cancer group might have been expected to show stronger associations than those previously reported [4–13], due to the methods applied, and not because of differences in the populations studied.

We find that the limited number of cases in our study, and some discrepancies between the previously reported distribution between homozygous and heterozygous carriers in comparison to our findings, is likely to result in insufficient power to evaluate the underlying mechanisms of the associations observed. Our results may, however, be considered to contribute towards a future combined effort to precisely define the contribution of risk provided by these polymorphisms. We would like to add, however, that some of the discrepancies found may not be methodological artifacts, but rather related to differences in linkage disequilibrium between the SNPs studied and disease in different populations. If this is verified, the search for an actual risk value for the association between breast cancer and the presence or absence of a given SNP that is population specific may be a useful approach in risk stratification. Some of the slight variations in associations reported in the different populations may have been caused by such mechanisms, commonly referred to as genetic drift.

In conclusion, our validation gave similar, but not identical results compared to those published by others.

Also, it is not currently established whether or not the association to these SNPs are of clinical interest. We have previously shown that BRCA1 carriers in our population have on average 25 % risk of developing breast cancer at 40 years of age [19]. The associations reported here may give a ten percent higher or lower cancer risk estimate at the time. Calculating the combined modifying effects will apply to a very few cases, and the majority will be close to 25 %. The clinical utility of the findings is a question we leave open for discussion. Through this report, we make the findings available for *BRCA1* mutation carriers in our population and for international meta-analyses.

Additional file

Additional file 1: Annealing temperatures PCR.

Competing interests

The authors declared that they have no competing interests.

Authors' contributions

CH, EH, PM and LM designed the study, POE performed the genotyping experiments, KT and PM undertook the statistical analyses, CH, KT, LM, POE, EH and PM wrote the manuscript. All authors approved the final manuscript.

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