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 this 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 \textit{BRCA1} (exons 2 to 24) and 26 coding exons of \textit{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 BigDyeTerminator Cycle Sequencing kit on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). All sequences were compared with the \textit{BRCA1} (NM_007294.3) and \textit{BRCA2} (NM_000059.3) reference sequences for variant detection. In addition, MLPA (P002 \textit{BRCA1} and P045 \textit{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 \textit{BRCA1/2} 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 \textit{BRCA1} 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 ≥ 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 \textit{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 \textit{BRCA1} and 14 (1.0%) in \textit{BRCA2}. Median and mean age at diagnosis was 45 years (range 26–77 years) and 46.1 years (46.3 years for \textit{BRCA1} and 45.6 years for \textit{BRCA2}) respectively. Four of the 42 women belonged to families where a \textit{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 \textit{BRCA1} and dup exon 20 in \textit{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 \cite{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 \textit{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 \textit{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 \cite{40-42}, and only one of them had been reported previously (c.9403delC) \cite{42}. The other two (c.4797_4797del-CAAT 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 relatives 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 \textit{BRCA} mutation had a family history of cancer that fulfilled the NBCG criteria for predictive \textit{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 |
|------|--------|----------|--------|------------------|--------------------|-----------------------------------|------------------------|
| BRCA1 |        | c.2019delA | Frameshift | 20-29 | No | No | Yes |
| 1    |        | c.1016dupA | Frameshift | 30-39 | Yes | Yes | Yes |
| 1    |        | del exon 3-16 | Deletion | 30-39 | No | No | No |
| 1    |        | c.3228_3229delAG | Frameshift | 30-39 | Yes | No | No |
| 1    |        | c.3178G > T | Nonsense | 40-49 | Yes | No | No |
| 1    |        | c.3084_3094delTAATAACATTAA | Frameshift | 40-49 | Yes | No | Yes |
| 1    |        | c.3228_3229delAG | Frameshift | 40-49 | Yes | No | Yes |
| 1    |        | c.5047G > T | Nonsense | 40-49 | Yes | Yes | Yes |
| 1    |        | c.3607C > T | Nonsense | 50-59 | Yes | Yes | No |
| 1    |        | c.4484G > A | Missense. Leads to skipping of exon 14 | 50-59 | Yes | No | No |
| 2    |        | c.5407-2A > G | Frameshift, skipping of exon 23 | 60-69 | Yes | No | No |
| 2    |        | c.1072delC | Frameshift | 60-69 | Yes | No | No |
| 2    |        | c.1556delA | Frameshift | 40-49 | Yes | Yes | No |
| 2    |        | c.5153G > C | 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.5300G > T | Missense | 30-39 | No | Yes | Yes |
| 2    |        | c.697delGT | Frameshift | 70- | Yes | Yes | Yes |
| 2    |        | c.3228_3229delAG | Frameshift | 50-59 | Yes | Yes | Yes |
| 2    |        | c.445G > T | Nonsense | 40-49 | No | Yes | Yes |
| 2    |        | c.1016dupA | Frameshift | 50-59 | Yes | No | Yes |
| 2    |        | c.5266dupC | Frameshift | 30-39 | No | Yes | No |
| 2    |        | c.2989_2990dup | Frameshift | 50-59 | Yes | No | No |
| 2    |        | c.1016dupA | Frameshift | 30-39 | Yes | No | No |
| 2    |        | c.1556delA | Frameshift | 50-59 | Yes | Yes | No |
| 2    |        | dup exon 13 | Frameshift | 50-59 | Yes | No | No |
| 2    |        | c.5300G > T | Missense | 30-39 | No | No | Yes |
| 2    |        | c.5503C > T | Nonsense | 50-59 | No | Yes | Yes |
| BRCA2 |        | c.4710delA | Frameshift | 30-39 | No | No | No |
| 1    |        | c.3847_3848delGT | Frameshift | 40-49 | Yes | No | No |
| 1    |        | c.614delG | Frameshift | 50-59 | Yes | Yes | No |
| 2    |        | c.4936_4939delGAAAt | Frameshift | 40-49 | Yes | No | No |
| 2    |        | c.3847delGT | Frameshift | 40-49 | Yes | No | No |
| 2    |        | c.9403delC | Frameshift | 40-49 | No | No | No |
| 2    |        | c.5722delCT | Frameshift | 40-49 | Yes | No | No |
| 2    |        | c.6059_6062delAACA | Frameshift | 30-39 | Yes | No | No |
| 2    |        | c.5722delCT | Frameshift | 50-59 | Yes | Yes | No |
| 2    |        | c.4794_4797delCAAT | Frameshift | 40-49 | Yes | No | No |
| 2    |        | c.614delG | Frameshift | 30-39 | Yes | Yes | No |
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 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).

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

| # | Mutation | Type        | Score | Identified | Risks | Carriers |
|---|----------|-------------|-------|-----------|-------|----------|
| 2 | c.7024C > T | Nonsense    | 30–39 | No        | Yes   | No       |
| 2 | c.9699_9702delTATG | Frameshift | 70–79 | Yes       | No    | No       |
| 2 | dup exon 20 | 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)
*Common Norwegian founder mutation*
†Identified in 1–9 families at Department of Medical Genetics (DMG), OUH (unpublished data)
*Not identified previously at DMG, OUH
BC Breast cancer
OC Ovarian cancer
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...
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 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. Febbraro 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 NBCG 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.

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

| Test criteria                                      | BRCA1/2 mutation carriers (n = 38) |
|----------------------------------------------------|------------------------------------|
| 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%)                         |

*The four women belonging to families where a mutation had already been identified were excluded from this analysis.

### Table 4: Specificity of criteria for BRCA1/2 testing

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

*Specificity is calculated only for Cohort 1, OUH-U.

### 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                                                            |
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 \textit{BRCA} genes. More carriers of pathogenic mutations in other known BC risk genes such as \textit{TP53}, \textit{PTEN} or \textit{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 \textit{MSH6} and \textit{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 \textit{BRCA1} carrier and two \textit{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 \textit{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.

\section*{Conclusions}

By offering \textit{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 \textit{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 \textit{BRCA} testing are insufficient to detect all carriers. We suggest that it is time to discuss whether \textit{BRCA} testing should be offered also to BC patients not belonging to a high risk group. If all BC patients are offered \textit{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 \textit{BRCA} testing will identify more mutation positive women prior to cancer development than the current practice.
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. DEI: 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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References
1. Antoniou A, Pharoah PD, Narod S, Rich HA, Eiyfjord JE, Hopper JL, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet. 2003;72:1117–30.
2. King MC, Marks JH, Mandell JB, New York Breast Cancer Study Group. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science. 2003;302:643–6.
3. Kauf ND, Domchek SM, Frielieb TM, Robison ME, Lee J, Garber JE, et al. Risk-reducing salpingo-oophorectomy for the prevention of BRCA1- and BRCA2-associated breast and gynecologic cancer: a multicenter, prospective study. J Clin Oncol. 2008; doi:10.1200/JCO.2007.13.9626.
4. Domchek SM, Frielieb TM, Singer CF, Evans DG, Lynch HT, Isaacs C, et al. Association of risk-reducing surgery in BRCA1 or BRCA2 mutation carriers with cancer risk and mortality. JAMA. 2010; doi:10.1001/jama.2010.1237.
5. Evans DG, Baldani AD, Anderson E, Brian A, Shenton A, Vasen HF, et al. Risk reducing mastectomy: outcomes in 10 European centres. J Med Genet. 2009; doi:10.1136/jmg.2008.062232.
6. Metcalfe K, Gershman S, Ghadriar P, Lynch HT, Snyder C, Tung N, et al. Contralateral mastectomy and survival after breast cancer in carriers of BRCA1 and BRCA2 mutations: retrospective analysis. BMJ. 2014; doi:10.1136/ bmj.g226.
7. Isaakoff SJ, Mayer EL, He L, Traina TA, Carey LA, Krag KJ, et al. TBCRC009: a multicenter phase II clinical trial of platinum Monotherapy with biomarker assessment in metastatic triple-negative breast cancer. J Clin Oncol. 2015; doi:10.1200/JCO.2014.57.6660.
8. Stover DG, Winer EP. Tailoring adjuvant chemotherapy regimens for patients with triple negative breast cancer. Breast. 2015; doi:10.1016/j.breast.2015.07.032.
9. Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Merqui-Roelvink M, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med. 2009; doi:10.1056/NEJMoa0900212.
10. Rodler ET, Kurland BF, Griffin M, Gralow JR, Porter P, Yeh RF, et al. Phase I study of Veliparib (ABT-888) combined with Cisplatin and Vinorelbine in advanced triple-negative breast cancer and/or BRCA mutation-associated breast cancer. Clin Cancer Res. 2016; doi:10.1158/1078-0432.CCR-15-2137.
11. Tutt A, Robison M, Garber JE, Domchek SM, Audeh MW, Weitzel JN, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. Lancet. 2010; doi:10.1016/S0140-6736(10)60892-6.
12. Sandhu SK, Schelman WR, Wilding G, Moreno V, Baird RD, Miranda S, et al. The poly(ADP-ribose) polymerase inhibitor niraparib (MK4827) in BRCA mutation carriers and patients with sporadic cancer: a phase 1 dose-escalation trial. Lancet Oncol. 2013; doi:10.1016/S1470-2045(13)70240-7.
13. Robertson L, Hanson H, Seal S, Warren-Perry M, Hughes D, Howell I, et al. BRCA1 testing should be offered to individuals with triple-negative breast cancer patients in Cohort 1 (OUH-U) and Cohort 2 (SERHA). (DOCX 26 kb)

Additional files
1. Figure S1. Guidelines for testing. (DOCX 26 kb)
2. Table S1 Clinical and pathological characteristics of breast cancer in mutation carriers, non-carriers and not tested at OUH-U. (DOCX 28 kb)
3. Table S2. Comparison of mutation positive breast cancer patients in Cohort 1 (OUH-U) and Cohort 2 (SERHA). (DOCX 14 kb)
breast cancer diagnosed below 50 years. Br J Cancer. 2012; doi:10.1038/bjc. 2012.31.

14. Pal T, Bonner D, Crugun D, Johnson S, Akbari M, Servais L, et al. BRCA sequencing and large rearrangement testing in young black women with breast cancer. J Community Genet. 2014; doi:10.1007/s12687-013-0166-9.

15. Young SR, Plunkett RT, Donenberg T, Shapiro C, Hammond LS, Miller J, et al. The prevalence of BRCA1 mutations among young women with triple-negative breast cancer. BMC Cancer. 2009; doi:10.1186/1471-2407-9:86.

16. Fostra F, Tiitladiou M, Papadimitriou C, Pertesi M, Timotheoudou E, Stavropoulou AV, et al. Prevalence of BRCA1 mutations among 403 women with triple-negative breast cancer: implications for genetic screening selection criteria: a Hellenic cooperative Oncology group study. Breast Cancer Res Treat. 2012; doi:10.1007/s10549-012-2021-9.

17. Villarea-Garza C, Weitzel JN, Llacuachaqui M, Sifuentes E, Magallanes-Hoyos MC, Gallardo L, et al. The prevalence of BRCA1 and BRCA2 mutations among young Mexican women with triple-negative breast cancer. Breast Cancer Res Treat. 2015; doi:10.1007/s10549-015-3312-8.

18. Wong-Brown MW, Meldrum CJI, Carpenter JE, Clarke CL, Narod SA, Pal T, Bonner D, Cragun D, Johnson S, Akbari M, Servais L, et al. BRCA identify minority of mutation carriers. Neoplasma. 2010; doi:10.5007/58-015.1966.

19. Easton DF, Pharoah PD, Antoniou AC, Tischkowitz M, Tavtigian SV, Mallo KE, Daling JR, Doody DR, Hsu L, Bernstein L, Coates RJ, et al. Prevalence of BRCA1 and BRCA2 mutations in a population-based series of breast cancer cases. Br J Cancer. 2000; doi:10.1038/jhc.2000.3101.

20. Abubagott J, Llacuachaqui M, Allende YS, Velasquez AA, Velarde R, Cotrina J, et al. Prevalence of BRCA1 and BRCA2 mutations in unselected breast cancer patients from Peru. Clin Genet. 2015; doi:10.1111/cge.12505.

21. Tung N, Lin NU, Kidd J, Allen BA, Singh N, Winstrup RJ, et al. Frequency of Germline mutations in 25 cancer susceptibility genes in a sequential series of patients with breast cancer. J Clin Oncol. 2016; doi:10.1200/JCO.2015.65.347.

22. Annual Cancer Registry of Norway, Norwegian Breast Cancer Registry: Annual Report 2014. https://www.kreftregisteret.no/globalassets/publikasjoner-on- rapporter/arsrapport/publisert-2014/arsrapport_bryskreft_2014.pdf.

23. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015; doi:10.1038/gim.2015.30.

24. BOADICEA Web Application (BWA v3): https://pluto.srl.cam.ac.uk/cgi-bin/bbd3/v3/bbdcgi.

25. Møller P, Hagen AI, Apolod J, Maehle L, Clark N, Fåne B, et al. Genetic epidemiology of BRCA1 mutations family history detects less than 50% of the mutation carriers. Eur J Cancer. 2007; doi:10.1016/j.ejca.2013.1713.

26. Górs G, Jakubowska A, Huzarski T, Byrski T, Gronwald J, Grzybowski E, et al. A high proportion of founder BRCA1 mutations in Polish breast cancer families. Int J Cancer. 2004;110:683–6.

27. Ratajiksa M, Brozek I, Senkus-Konefa E, Jassm J, Steponiusa M, et al. BRCA1 and BRCA2 point mutations and large rearrangements in breast and ovarian cancer families in northern Poland. Oncol Rep. 2008;19:263–8.

28. Wojcik P, Jasiokwa M, Strzyzcz M, Sobol M, Hodorowicz-Zanieviak D, Skotnicki P, et al. Recurrent mutations of BRCA1, BRCA2 and PALB2 in the population of breast and ovarian cancer patients in southern Poland. Hered Cancer Clin Pract. 2016; doi:10.1186/s10353-016-0046-5.

29. Frank TS, Diefenbaum AM, Reid JF, Hulick M, Ward BE, Lingenfelter B, et al. Clinical characteristics of individuals with germline mutations in BRCA1 and BRCA2: analysis of 10,000 individuals. J Clin Oncol. 2002;20:1480–90.

30. Birkenhö RL, Campfield D, Ducaine W, Dohany L, Donenberg T, Shannon K, et al. Errors in delivery of cancer genetics services: implications for practice. Conn Med. 2010;74:413–7.

31. Birxer KL, Bruek L, Gosswe B, Homer JP, Pancinaria D, Stanislaw CL, Matloff ET. Adverse events in cancer genetic testing: medical, ethical, legal, and financial implications. Cancer J. 2012; doi:10.1097/PPO.0b013e318260490.

32. Richter S, Harou I, Graham TC, Eise A, Kiss A, Warner E, et al. Variants of unknown significance in BRCA testing: impact on risk perception, worry, prevention and counseling. An Oncol. 2013; doi:10.1093/onc/mt312.

33. Vos J, Gómez-García E, Oosterwijk JC, Menko FH, Stoel RD, van Asperen CJ, et al. The prevalence of HER2 positivity amongst BRCA1 and BRCA2 mutation carriers. Breast Cancer Res. 2012; doi:10.1038/s10549-016-3697-z.

34. Brink AL, Albarracín CT, Lopez A, Valero V, Amos CL, González-Angulo AM, et al. Clinical and pathologic characteristics of patients with BRCA-positive and BRCA-negative breast cancer. J Clin Oncol. 2008; doi:10.1200/JCO.2008.16.6231.

35. Cencener G, Egel E, Tunca B, Ertuk E, Ak S, Gogoz S, et al. BRCA1/2 germline mutations and their clinical importance in Turkish breast cancer patients. Cancer Investig. 2014; doi:10.1080/07357907.2014.919302.

36. Finkelstein D, Robinson K, Wilbur JS, Laprise J, Bregar A, Lopes V, et al. Adherence patterns to National Comprehensive Cancer Network (NCCN) guidelines for referral to cancer genetic professionals. Gynecol Oncol. 2015; doi:10.1016/j.ygyno.2015.04.029.

37. Nilsson MP, Winter C, Kristoffersson U, Rehn M, Larsson C, Saal LH, et al. Efficacy versus effectiveness of clinical genetic testing criteria for BRCA1 and
53. Finch A, Wang M, Fine A, Atri L, Khalouei S, Pupavac M, et al. Genetic testing for BRCA1 and BRCA2 in the province of Ontario. Clin Genet. 2016; doi:10.1111/cge.12647.

54. Cancer Registry of Norway. Cancer in Norway 2014 – Cancer incidence, mortality, survival and prevalence in Norway. Oslo: Cancer Registry of Norway; 2015.

55. Metcalfe KA, Poll A, Roys R, Llacuachaqui M, Tulman A, Sun P, et al. Screening for founder mutations in BRCA1 and BRCA2 in unselected Jewish women. J Clin Oncol. 2010; doi:10.1200/JCO.2009.25.0712.

56. King MC, Levy-Lahad E, Lahad A. Population-based screening for BRCA1 and BRCA2. 2014 Lasker award. JAMA. 2014; doi:10.1001/jama.2014.12483.

57. Gabai-Kapara E, Lahad A, Kaufman B, Friedman E, Segev S, Renbaum P, et al. Population-based screening for breast and ovarian cancer risk due to BRCA1 and BRCA2. Proc Natl Acad Sci U S A. 2014; doi:10.1073/pnas.141597911.

58. Slade L, Hanson H, George A, Kohut K, Strydom A, Wordsworth S, et al. A cost analysis of a cancer genetic service model in the UK. J Community Genet. 2016; doi:10.1007/s12687-016-0266-4.

59. Norum J, Grindedal EM, Heramb C, Karsrud I, Ariansen SL, Undlien DE, et al. BRCA testing of all breast cancer patients? A cost-effectiveness analysis. Submitted to BMC Cancer September. 2016.

60. Risch HA, McLaughlin JR, Cole DE, Rosen B, Bradley L, Fan L, et al. Population BRCA1 and BRCA2 mutation frequencies and cancer penetrances: a kin-cohort study in Ontario. Canada J Natl Cancer Inst. 2006;98:1694–706.

61. Antoniou AC, Gayther SA, Stratton JF, Ponder BA, Easton DF. Risk models for familial ovarian and breast cancer. Genet Epidemiol. 2000;18:173–90.

62. Rosenthal E, Moyes K, Arnell C, Evans B, Wenstrup RJ. Incidence of BRCA1 and BRCA2 non-founder mutations in patients of Ashkenazi Jewish ancestry. Breast Cancer Res Treat. 2015; doi:10.1007/s10549-014-3218-x.

63. Kluska A, Balabas A, Pawełek A, Kulecka M, Nowakowska D, Mikula M, et al. New recurrent BRCA1/2 mutations in Polish patients with familial breast/ovarian cancer detected by next generation sequencing. BMC Med Genet. 2015; doi:10.1186/s12920-015-0092-2.

64. World Bank Open Data: http://data.worldbank.org/indicator/SP.DYN.TFRT.IN. Accessed 4 March 2017.

65. Hansen NF, Johansen J, Syvander AE, Bjørnsvoll T, Talseth-Palmer BA, Lavik LA, et al. Use of multigene-panel identifies pathogenic variants in several CRC-predisposing genes in patients previously tested for Lynch syndrome. Clin Genet. 2017; doi:10.1111/cge.12994.