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ARTICLE

BRCA1/2 testing in newly diagnosed breast and ovarian cancer patients without prior genetic counselling: the DNA-BONus study

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Germline *BRCA1/2* testing of breast and ovarian cancer patients is growing rapidly as the result affects both treatment and cancer prevention in patients and relatives. Through the DNA-BONus study we offered *BRCA1/2* testing and familial risk assessment to all new patients with breast ($N = 893$) or ovarian ($N = 122$) cancer diagnosed between September 2012 and April 2015, irrespective of family history or age, and without prior face-to-face genetic counselling. *BRCA1/2* testing was accepted by 405 (45.4%) and 83 (68.0%) of the patients with breast or ovarian cancer, respectively. A pathogenic *BRCA1/2* variant was found in 7 (1.7%) of the breast cancer patients and 19 (22.3%) of the ovarian cancer patients. In retrospect, all *BRCA1/2* mutation carriers appeared to fulfill current criteria for *BRCA1/2* testing. Hospital Anxiety and Depression Scale (HADS) scores showed that the mean levels of anxiety and depression were comparable to those reported for breast and gynecological cancer patients in general, with a significant drop in anxiety symptoms during a 6-month follow-up period, during which the test result was forwarded to the patients. These results show that *BRCA1/2* testing is well accepted in newly diagnosed breast and ovarian cancer patients. Current test criteria based on age and family history are sufficient to identify most *BRCA1/2* mutation carriers among breast cancer patients. We recommend germline *BRCA1/2* testing in all patients with epithelial ovarian cancer because of the high prevalence of pathogenic *BRCA1/2* variants.
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INTRODUCTION

Breast cancer is by far the most common cancer in women worldwide, with more than 1.6 million new cases diagnosed each year. Ovarian cancer is substantially less common, with ~240 000 new cases each year, but with higher mortality.[1] Most cases of breast and ovarian cancer are sporadic, but a minor fraction (2–8% and 8–15%, respectively) is caused by inheritance of pathogenic germline variants in *BRCA1* or *BRCA2*, with variation in prevalence and relative contribution of *BRCA1* and *BRCA2* in different populations.[2–8] It is important to identify these patients because the presence of such germline variants affects treatment, follow-up and further cancer prevention in patients with breast or ovarian cancer.[9,10] In addition, it may strongly influence upon their close relatives, as *BRCA1/2* testing can identify healthy *BRCA1/2* mutation carriers at high risk and thereby prevent cancer and cancer-related deaths through increased surveillance and prophylactic surgery.[10–16]

The most common current practice of *BRCA1/2* testing is based on referral of suspected high-risk patients to clinical genetics services for specialized face-to-face genetic counselling. This procedure traditionally includes collection and confirmation of family history, risk assessment and eventually *BRCA1/2* testing followed by a post-test counselling with dissemination of test results and advice concerning surveillance and follow-up.[17–19] Based on family history, *BRCA1/2*-negative families with increased risk of familial breast cancer can also be identified.[18,20]

However, this traditional approach is time consuming and resource demanding for both the patient and the health-care system, with an inherent risk of focusing too much on healthy relatives and not reaching all the cancer patients in question. Moreover, the discovery that *BRCA1/2* status can inform treatment decisions in breast and ovarian cancer patients has led to an increased demand for *BRCA1/2* testing at the time of cancer diagnosis.[9,21] New approaches to *BRCA1/2* testing and genetic counselling may be needed to meet this situation. The aim of this project was therefore to assess the feasibility and impact of offering *BRCA1/2* testing to all newly diagnosed patients with breast or ovarian cancer without prior face-to-face genetic counselling. We here report the uptake of *BRCA1/2* testing, the incidence of pathogenic *BRCA1/2* variants and the individual risk profiles among these unselected breast and ovarian cancer patients. As the psychosocial impact of such *BRCA1/2* testing in newly

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diagnosed cancer patients without prior genetic counselling is scarcely described,[22] we also examined the symptoms of anxiety and depression at inclusion and during the follow-up period of 6 months.

PATIENTS AND METHODS

Recruitment of patients

The patients were recruited from four hospitals in Western Norway (Haukeland University Hospital, Stavanger University Hospital, Haugesund Hospital and Førde Central Hospital), including three surgical departments and two gynecological departments, from September 2012 to April 2015. All patients with newly diagnosed breast or ovarian cancer were invited to participate in the study (for overview, see Figure 1). The patients received written information on the project and general information on hereditary breast and ovarian cancer, including the mode of inheritance and the potential consequences of a positive test results; such as the elevated cancer risk, recommended follow-up and risk-reducing strategies for the patient and healthy relatives. They were also informed that a positive test result could affect the surgical treatment of breast cancer patients, whereas specific information on novel therapies, like PARP-inhibitors, was not given. In addition, the patients had the opportunity to contact a genetic counselor on telephone for any further questions. All participants signed informed consent and filled in a structured questionnaire on personal and family medical history. The patients could choose *BRCA1/2* testing with or without participating in an associated study of psychosocial aspects (see below). A blood sample was then collected at the local hospital and sent to a central laboratory for *BRCA1/2* analysis. The study protocol was approved by the Regional Committee for Medical and Health Research Ethics (reference number REK Vest 2012-60).

DNA isolation, BRCA mutation analysis and clinical assessments

Genomic DNA was purified from EDTA-anticoagulated blood using the QiaSymphony instrument (Qiagen, Hilden, Germany). Genotyping of a panel of 20 pathogenic *BRCA1* and 10 pathogenic *BRCA2* variants that are recurrent in the Norwegian population was carried out using TaqMan Low-Density Arrays on the ABI 9700 instrument (Applied Biosystems, Foster City, CA, USA) as recommended by the manufacturer. An overview over the variants and sequences for the corresponding primers and probes is given in the Supplementary Table 1. In addition, the *BRCA1* and *BRCA2* genes were analyzed for deletions and insertions by Multiplex Ligation-dependent Probe Amplification (MLPA) technology (P002 BRCA1 and P045 BRCA2 MLPA probe mixes; MRC-Holland, Amsterdam, The Netherlands).

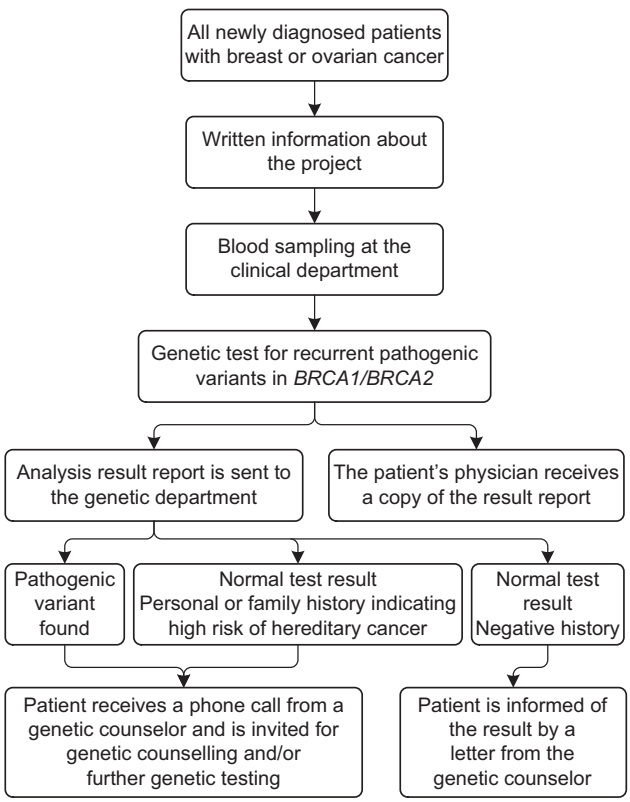

Figure 1 Flowchart showing the inclusion of patients and reporting of results in the DNA-BONus study.

The result of the *BRCA1/2* testing was given to the patient by a genetic counselor within 3 weeks after blood sample collection (Figure 1). In addition, the result was reported to the clinician who was responsible for treating the patient, to be filed in the patient's medical record at the hospital. If the test result was negative and there was no increased familial cancer risk, the patient received the result by letter. Patients with a positive test result or with a personal or family history indicative of a high risk of hereditary cancer were contacted over the phone by a genetic counselor and were offered traditional face-to-face genetic counselling and further investigations in one of our outpatient clinics.

Based on collection of family history and confirmation of cancer diagnoses in relatives, selected patients were then offered extended genetic testing, with Sanger sequencing of all exons and flanking intron sequence in both *BRCA1* and *BRCA2*. We used the following reference sequences: *BRCA1*: NG_005905.2 (gene), NM_007294.3 (mRNA), NP_009225.1 (protein); *BRCA2*: NG_012772.3 (gene), NM_000059.3 (mRNA) and NP_000050.2 (protein)).

To classify the sequence variants we followed the recommendations given by the International Agency for Research on Cancer (IARC).[23] Pathogenic (class 5) and likely pathogenic (class 4) variants were regarded as positive genetic test results and have been submitted to the Leiden Open Variation Database (LOVD 3.0 shared installation; www.databases.lovd.nl/shared/genes). In this article we use the term *BRCA1/2* mutation carrier for patients in whom a pathogenic or likely pathogenic variant was found.

All patients were categorized before *BRCA1/2* testing depending on the presence of increased familial cancer risk or not. Increased risk was defined as personal at risk cancer history (eg, patients with young age at diagnosis, bilateral breast cancer or both breast and ovarian cancer) or positive family history (eg, close relative with breast cancer before 50 years of age or ovarian cancer at any age, two or more relatives with breast cancer or both breast and ovarian cancer in relatives) or a combination of personal at risk cancer history and positive family history, according to the current national clinical criteria for *BRCA1/2* testing (see also legend to Table 1). The participants were in addition rated by the Manchester scoring system for *BRCA1/2* testing.[24,25]

Psychological measurements

Participants who gave informed consent for the psychosocial part of the project were asked to fill in questionnaires at baseline when they were offered genetic testing (T1), at 1 week after disclosure of the *BRCA1/2* test result (T2) and 6 months after disclosure of the *BRCA1/2* test result (T3). In the present study, we have used data from the Hospital Anxiety and Depression Scale (HADS).[26] HADS comprises two subscales for symptoms of anxiety and depression, respectively, each with 7 items to be scored on a 4-point (0–3) scale, giving a range of subscores from 0 to 21. The reliability of the HADS subscales in this study, as estimated with Cronbach's α, had a range of 0.83–0.88 for HADS anxiety and 0.80–0.86 for HADS depression at the three assessments. Subscale scores of ≥ 8 were used as cutoff for defining higher, caseness-relevant levels of anxiety and depression.[27]

Statistical methods

Descriptive statistics were used for psychological and clinical variables, reporting the mean values, SD and range. To analyze the changes over time in HADS anxiety and depression scores, we used a paired sample *t*-test and McNemar's exact test. Independent sample *t*-test was used to compare the means of two independent groups and χ^2 test was used to analyze dichotomous variables for independent groups.

Missing values were replaced by the individual's own average score for HADS if 60% or more of the items were filled in by the respondents. All statistical

Table 1 DNA-BONus study population

	Number of patients included	*Mean age (years)*	*Current national criteria for BRCA1/2 testing* [a]					*Manchester scores at inclusion*	
			At risk personal cancer history only (N)	*Positive family history only (N)*	*Fulfilled Both at risk personal history and family history (N)*	*Total number of patients fulfilling criteria, N (%)*	*Not fulfilled Number of patients outside current criteria for BRCA1/2 testing,* [a] *N (%)*	*Number of patients with combined Manchester score ≥ 15, N (%)*	*Number of patients with combined Manchester score < 15, N (%)*
Breast cancer									
Total	405	56.9 Range: 23–89	103	48	51	202 (49.9%)	203 (50.1%)	41 (10.1%)	364 (89.9%)
Pathogenic *BRCA1/2* variant identified, *N* (%)	7 (1.7%)	50.6 Range: 32–76	3	1	2	6 (85.7%)	1[b] (14.3%)	2 (28.6%)	5 (71.4%)
Ovarian cancer									
Total	83	60.5 Range: 24–88	49	4	17	70 (84.3%)	13 (15.7%)	26 (31.3%)	57 (68.7%)
Pathogenic *BRCA1/2* variant identified, *N* (%)	19 (22.3%)	56.5 Range: 44–72	10	0	8	18 (94.7%)	1[b] (5.3%)	11 (57.9%)	8 (42.1%)

Positive family history: first-degree relative with breast cancer before age 50 years or ovarian cancer at any age, two or more breast cancer cases or both breast and ovarian cancer on the same side of the family, male relative with breast cancer or known *BRCA1/2* mutation in the family.
[a]Criteria for clinical *BRCA1/2* founder mutation testing of patients with breast or ovarian cancer, as outlined by the Norwegian Health Authorities: at risk personal history; breast cancer before age 50 years, ovarian cancer before age 70 years, bilateral breast cancer, both breast and ovarian cancer or male breast cancer at any age.
[b]These numbers represent two patients who apparently did not fulfill current national test criteria upon inclusion. They were reclassified after genetic counselling, and in retrospect, both were eligible for diagnostic *BRCA1/2* testing according to the test criteria.

analyses were performed using IBM SPSS Statistics for Windows, Version 22.0 (Armonk, NY, USA).

RESULTS

A total of 1015 patients with either breast cancer ($N=893$) or ovarian cancer ($N=122$) were offered *BRCA1/2* testing at the time of cancer diagnosis, of whom 405 (45.4%) of the breast cancer patients and 83 (68.0%) of the ovarian cancer patients completed the genetic testing. The mean age of the participants was 56.9 years (SD 12.4, range (min–max) 23–89) in the patients with breast cancer and 60.5 years (SD 11.9, range 24–88) in the patients with ovarian cancer (Table 1). Among the participants, 202 (49.9%) of the patients with breast cancer and 70 (84.3%) of the patients with ovarian cancer were eligible for *BRCA1/2* testing according to current national clinical guidelines (Table 1). The median time from diagnosis to blood sampling was 34 days (mean 68, range 0–1402) and the median time from diagnosis to the patient received initial test result was 52 days (mean 87, range 12–1423) (data not shown). For 13 patients, the interval between diagnosis and blood sampling exceeded 1 year.

A pathogenic *BRCA1/2* variant was identified in 7 (1.7%) of the 405 breast cancer patients (mean age of 50.6 (SD 15.8, range 32–76) years; Table 1), of whom 6 carried a *BRCA1* and 1 a *BRCA2* pathogenic variant (Table 2). Three *BRCA1* and one *BRCA2* mutation carriers had a breast cancer that was triple negative (Er-/Pr-/HER2-) and all seven breast cancers were HER2 negative (Table 2). Interestingly, as many as 19 (22.3%) of the 83 ovarian cancer patients (mean age 56.5 (SD 9.1, range 44–72) years) were *BRCA1/2* mutation carriers (Table 1), including 15 with a pathogenic *BRCA1* variant and 4 patients with a pathogenic *BRCA2* variant (Table 2). Most ovarian cancers were serous carcinomas, apart from one poorly differentiated carcinoma and one endometroid adenocarcinoma. The majority of the mutation carriers ($N=21$; 80.8%) were identified by the standard test panel of recurrent mutations (Table 2), where 3 of the most frequent Norwegian pathogenic founder variants in *BRCA1* (c.1556del, c.697_698del and c.3228_3229del) were detected in 15 (57.7%) of the mutation carriers. Four additional pathogenic variants were identified by Sanger sequencing of selected breast cancer ($N=94$) or ovarian cancer ($N=31$) patients with a particularly high risk of carrying a pathogenic *BRCA1/2* variant, based on the personal and family history (see Table 2). During the first (years 2012–2013) and second (years 2014–2015) half of the DNA-BONus study period, 26.1% (55 out of 211) and 25.3% (70 out of 277) of the participants were selected for Sanger sequencing, respectively. Out of the total population of 488 patients, no one had *BRCA1/2* alterations that could be detected by MLPA.

Among the 272 patients fulfilling the current national criteria for diagnostic *BRCA1/2* testing at inclusion, 6 out of 202 breast cancer patients (3.0%) and 18 out of 70 ovarian cancer patients (25.7%) were found to be mutation carriers (Table 1). Among 216 patients not meeting current clinical test criteria at inclusion, the corresponding numbers of *BRCA1/2* mutation carriers were 1 of the 203 breast cancer patients (0.5%) and 1 of the 13 ovarian cancer patients (7.7%). However, it should be noted that the breast cancer patient with a pathogenic *BRCA1* variant and the ovarian cancer patient with a pathogenic *BRCA2* variant, who apparently had negative family histories upon inclusion, were both subsequently reclassified as having familial risk, based on extended pedigrees obtained through the genetic counselling (see below, Discussion section).

The mean combined Manchester score at inclusion was 8.9 (range 2–71) (data not shown), with 67 out of 488 patients (13.7%) having a score of ≥ 15 (Table 1). A pathogenic *BRCA1/2* variant was found in 13 out of 67 patients (19.4%) with a score of ≥ 15 and in 13 out of 421 patients (3.1%) with a score <15 (Table 1; summarized numbers). Among the 26 *BRCA1/2* mutation carriers, the mean combined Manchester score at inclusion was 19.5 (range 4–71) (Table 2; summarized numbers). After genetic counselling and collection of additional clinical information, including pathology reports, the scores could be recalculated for 25 of the 26 mutation carriers (Table 2). The mean combined score increased to 27.7 (range 14–81) (data not shown), with 24 mutation carriers having a score of ≥ 15, whereas the remaining mutation carrier had a score of 14 (Table 2).

All 26 *BRCA1/2* mutation carriers accepted the offer of traditional face-to-face post-test genetic counselling. Among participants with a negative result on the initial *BRCA1/2* panel and MLPA analysis, genetic counselling was offered for 188 patients (40.3% of total) with a personal at risk cancer history indicating further genetic testing (eg, young age at diagnosis or more than one primary cancer) or with a positive family history indicative of either familial breast cancer (eg, two or more breast cancer cases in first-degree relatives) or another hereditary cancer syndrome. The acceptance rate for genetic counselling in this group was 93.6% ($N=176$).

Because of the potential risk of imposing additional psychosocial burden by offering and performing *BRCA1/2* testing in the newly diagnosed cancer patients, we measured the level of anxiety and depression scores before testing and at 1 week and 6 months after disclosure of the test result in a subset of participants (Table 3). Among these 215 patients, the median time from diagnosis to blood sampling was 32 days (mean 56, range 0–436) and median time from diagnosis to received result was 50 days (mean 75, range 12–456) (data not shown). The mean HADS subscale score for anxiety symptoms was 6.84 (SD 4.28) at baseline (ie, time of inclusion), with a significant decrease to 4.88 (SD 3.86) 6 months after disclosure of the *BRCA1/2* test result ($P<0.001$). The percentage of patients with higher levels of anxiety symptoms, defined as scores ≥ 8, decreased significantly from inclusion (39.9%) to 1 week (23.6%, $P<0.001$) and 6 months (19.8%, $P<0.001$) after disclosure of the test result, respectively. During the observation period there was no significant change in depression symptoms, with a mean HADS score of 3.32 (SD 3.07) at baseline and 2.65 (SD 3.04) at 6 months. Approximately 10% of the patients showed higher levels of depression symptoms with a score of ≥ 8, both at baseline and follow-up measurements (Table 3). There were no significant differences in HADS scores between patients with breast ($N=138$) and ovarian ($N=29$) cancer, or between mutation carriers ($N=8$) and noncarriers ($N=159$) (data not shown).

To explore the effect of time after diagnosis on the HADS scores, we divided the sample in two groups, with $N=171$ (83.0%) having less than and $N=35$ (17.0%) having more than 90 days from cancer diagnosis to blood sampling. There were no significant differences in HADS scores between the two groups (data not shown).

Compared with the participants who only agreed to genetic testing (mean age 61.6 years), the patients who also took part in the psychosocial study were significantly younger ($P<0.001$), with a mean age of 56.2 years (data not shown). There were no significant differences between the two groups regarding educational level or type of cancer diagnosis (breast or ovarian).

DISCUSSION

The main findings in this study are that: (1) most patients with newly diagnosed ovarian cancer accept germline *BRCA1/2* testing, with significantly lower uptake among breast cancer patients; (2) there is a high prevalence of *BRCA1/2* mutation carriers in the group of ovarian cancer patients; (3) all patients who were identified with a

Table 2 Clinical and genetic data on the *BRCA1/2* mutation carriers that were identified in the DNA-BONus study

			Clinical information						BRCA1/2 pathogenic variant				
LOVD ID	Cancer	Pathology	Age at diagnosis (5-year interval)	Previous cancer (age in 5-year interval)	Known BRCA1/2 family	Norwegian criteria[a]	Manchester score[b] At inclusion	Manchester score[b] After genetic counselling	Gene	DNA level	Protein level	Clinical classification	Included in panel or not[c]
34523	Breast	Low differentiated carcinoma, Er-/Pr-/HER2-	30–35		No	P	10	18	*BRCA1*	c.3228_3229del	p.(Gly1077AlafsTer8)	BIC: class 5 –– pathogenic	Panel
32380	Breast	Medullary carcinoma, Er-/Pr-/HER2-, grade 3	30–35		No	P	8	21	*BRCA1*	c.697_698del	p.(Val233AsnfsTer4)	BIC: class 5 – pathogenic	Panel
34522	Breast	Ductal carcinoma, Er+/Pr+/HER2-, grade 1	45–50		No	P+F	12	Pending	*BRCA1*	c.5407–25T>A	p.Gly1803GlnfsTer11	Our ranking: class 4[d]	Panel
32381	Breast	Ductal carcinoma, Er+/Pr+/HER2-, grade 1	60–65		No	F	22	19	*BRCA1*	c.5407–25T>A	p.Gly1803GlnfsTer11	Our ranking: class 4[d]	Panel
32382	Breast	Ductal carcinoma, Er+/Pr-/HER2-, grade 3	75–80		No	None[e]	4	19	*BRCA1*	c.5096G>A	p.(Arg1699Gln)	BIC: unknown IARC: class 5 – pathogenic Reduced penetrance[f]	Not in panel
34528	Contralateral breast	Medullary carcinoma, Er-/Pr-/HER2-, grade 3	40–45	Breast cancer age 35–40	No	P	14	26	*BRCA1*	c.1556del	p.(Lys519ArgfsTer13)	BIC: class 5 – pathogenic	Panel
34538	Contralateral breast	Ductal carcinoma, Er-/Pr-/HER2-, grade 2	50–55	Breast cancer age 50–55	Yes	P+F	24	36	*BRCA2*	c.3847_3848del	p.(Val1283LysfsTer2)	BIC: class 5 – pathogenic	Panel
43839	Ovarian	Endometroid adenocarsinoma	40–45		No	P	13	26	*BRCA1*	c.1556del	p.(Lys519ArgfsTer13)	BIC: class 5 – pathogenic	Panel
34529	Ovarian	Serous adenocarcinoma	45–50		Yes	P+F	71	59	*BRCA1*	c.1556del	p.(Lys519ArgfsTer13)	BIC: class 5 – pathogenic	Panel
34530	Ovarian	Serous papillary adenocarcinoma	45–50		No	P	13	15	*BRCA1*	c.1556del	p.(Lys519ArgfsTer13)	BIC: class 5 – pathogenic	Panel
34539	Ovarian	Poorly differentiated serous adenocarcinoma	45–50		No	P	14	24	*BRCA2*	c.7069_7070del	p.(Leu2357ValfsTer2)	BIC: class 5 – pathogenic	Not in panel
34531	Ovarian	Serous adenocarcinoma	50–55		No	P+F	23	31	*BRCA1*	c.1556del	p.(Lys519ArgfsTer13)	BIC: class 5 – pathogenic	Panel
34524	Ovarian	Serous carcinoma	50–55		No	P	13	25	*BRCA1*	c.3228_3229del	p.(Gly1077AlafsTer8)	BIC: class 5 – pathogenic	Panel
43840	Ovarian	Serous adenocarcinoma	50–55		yes	P+F	23	30	*BRCA1*	c.1556del	p.(Lys519ArgfsTer13)	BIC: class 5 – pathogenic	Panel
34532	Ovarian	Serous papillary adenocarcinoma	50–55		No	P+F	27	31	*BRCA1*	c.1556del	p.(Lys519ArgfsTer13)	BIC: class 5 – pathogenic	Panel
34535	Ovarian	Serous carcinoma	50–55		No	P+F	28	26	*BRCA1*	c.4065_4068del	p.(Asn1355LysfsTer10)	BIC: class 5 – pathogenic	Panel
34540	Ovarian	Serous carcinoma	55–60	Colon cancer age 55–60	No	P+F	30	30	*BRCA2*	c.4936_4939del	p.(Glu1646GlnfsTer23)	BIC: class 5 – pathogenic	Not in panel
34526	Ovarian	Serous carcinoma	55–60		No	P+F	33	34	*BRCA1*	c.697_698del	p.(Val233AsnfsTer4)	BIC: class 5 – pathogenic	Panel
34536	Ovarian	Serous adenocarcinoma	55–60		No	P	13	14	*BRCA1*	c.1016dup	p.(Val340GlyfsTer6)	BIC: class 5 – pathogenic	Panel
34533	Ovarian	Serous adenocarcinoma	55–60		No	P	13	15	*BRCA1*	c.1556del	p.(Lys519ArgfsTer13)	BIC: class 5 – pathogenic	Panel
34534	Ovarian	Serous adenocarcinoma	60–65		No	P+F	27	27	*BRCA1*	c.1556del	p.(Lys519ArgfsTer13)	BIC: class 5 – pathogenic	Panel
34537	Ovarian	Serous adenocarcinoma	65–70	Breast cancer age 45–50	No	P	15	17	*BRCA1*	c.1687C>T	p.(Gln563Ter)	BIC: class 5 – pathogenic	Not in panel

Table 2 (Continued)

			Clinical information						*BRCA1/2 pathogenic variant*				
LOVD ID	*Cancer*	*Pathology*	*Age at diagnosis (5-year interval)*	*Previous cancer (age in 5-year interval)*	*Known BRCA1/2 family*	*Norwegian criteria*[a]	*Manchester score*[b] *At inclusion*	*Manchester score*[b] *After genetic counselling*	*Gene*	*DNA level*	*Protein level*	*Clinical classification*	*Included in panel or not*[c]
34527	Ovarian	Poorly differentiated carcinoma	65–70		No	P	10	18	*BRCA1*	c.697_698del	p.(Val233AsnfsTer4)	BIC: class 5 – pathogenic	Panel
34541	Ovarian	Serous papillary adenocarcinoma	65–70		No	P+F	20	20	*BRCA2*	c.7069_7070del	p.(Leu2357ValfsTer2)	BIC: class 5 – pathogenic	Not in panel
34525	Ovarian	Serous adenocarcinoma	70–75		Yes	P+F	16	81	*BRCA2*	c.3228_3229del	p.(Gly1077AlafsTer8)	BIC: class 5 – pathogenic	Panel
43838	Ovarian	Serous carcinoma	70–75		No	None[e]	10	31	*BRCA2*	c.5217_5223del	p.(Tyr1739Ter)	BIC: class 5 – pathogenic	Panel

Abbreviations: BIC, Breast cancer Information Core database, http://research.nhgri.nih.gov/bic/; IARC, International Agency for Research on Cancer, http://www.iarc.fr.
[a]Patients fulfilled Norwegian *BRCA1/2* diagnostic testing criteria because of personal at risk cancer history (P) or positive family history (F) or both of these (P+F).
[b]Combined Manchester score based on (1) patient-reported information at inclusion and (2) pathology adjustments and detailed family history retrieved after genetic counselling.
[c]'Panel' = pathogenic variants included in the panel screening test (Supplementary table 1); 'Not in panel' = pathogenic variants found by Sanger sequencing of the total coding region of *BRCA1* and *BRCA2*.
[d]In our diagnostic lab the BRCA1 c.5407-25T>A variant has been found in six independent families with breast and/ovarian cancer, and mRNA analysis from one mutation carrier has previously indicated partial loss of exon 22, r.5407_5467del.
[e]These patients did not fulfill current test criteria upon inclusion, but additional information obtained at genetic counselling revealed that they had a positive family history and thus in retrospect were eligible for diagnostic *BRCA1/2* testing.
[f]According to Spurdle *et al*,[35] this variant is associated with intermediate risk of breast and ovarian cancer.

pathogenic *BRCA1/2* variant fulfill our current clinical criteria for diagnostic *BRCA1/2* testing; and (4) the level of anxiety and depression symptoms in the participants at inclusion was comparable to what can be found in cancer patients in general.[28,29]

Ovarian and breast cancer patients with pathogenic *BRCA1/2* variants are candidates for targeted drug therapy, such as PARP inhibitors.[21] Recently, the US Food and Drug Administration (FDA) approved a PARP inhibitor for use in ovarian cancer (http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm427554.htm). Our study shows that, even before such treatment options became available, *BRCA1/2* testing was well accepted among newly diagnosed patients, with 68% participation rate among the women with ovarian cancer, whereas 45% of patients with breast cancer chose to undergo *BRCA1/2* testing. There may be a selection bias among the participants because, on average, patients with breast cancer and ovarian cancer in our study were younger (mean age 56.9 and 60.5 years, respectively) as compared with patients with these cancers in the Norwegian population in general. According to national numbers, the mean age of all cases with breast cancer and ovarian cancer diagnosed between 2008 and –2012 was 61.5 and 65.4 years, respectively,[30] thereby indicating that older patients may have declined participation in our study. This could be particularly relevant for breast cancer patients with low *a priori* risk of carrying a pathogenic *BRCA1/2* variant. The assumption of a certain degree of risk-based selection in the uptake is further supported by the fact that among the participants, 50% of the patients with breast cancer and >80% of the patients with ovarian cancer were eligible for diagnostic *BRCA1/2* testing according to the current clinical guidelines. For obvious reasons the uptake will be higher when the result of *BRCA1/2* testing influences treatment options.[21]

In total, we identified 26 patients with a pathogenic *BRCA1/2* variant and by that identified 22 new *BRCA1/2* families. This finding supports a need for increased availability and use of such *BRCA1/2* testing, as a supplement to the existing referral systems and service in cancer genetics. Our study also reports a high prevalence (22.3%) of pathogenic *BRCA1/2* variants in ovarian cancer patients, substantially higher than reported by others.[3–5] This may be caused by a high prevalence of pathogenic founder variants in our population, but surprisingly the prevalence among patients with breast cancer is rather low (1.7%) compared with international data.[2,6] The highest prevalence of *BRCA1/2* mutation carriers has been reported in populations with very strong founder effects, and most studies on the frequency of pathogenic *BRCA1/2* variants in patients with sporadic breast cancer have had some form of selection criteria, for example, young age at onset or triple-negative histology.[2,7,8] In the DNA-BONus study, we offered genetic testing to all patients with newly diagnosed breast cancer that, in combination with a rather low prevalence of pathogenic *BRCA2* variants in the Norwegian population,[6] at least in part may explain the rather low frequency of pathogenic *BRCA1/2* variants among our patients with breast cancer.

At inclusion, all but two of the 26 *BRCA1/2* mutation carriers fulfilled the current clinical recommendations for diagnostic *BRCA1/2* testing in Norway. One patient with breast cancer after the age of 75 years apparently had a negative family history according to the information forwarded at inclusion. However, further examination revealed that her sister died from ovarian cancer before the age of 50 years. The other patient was a woman with ovarian cancer after the age of 70 years. We were informed at inclusion that she had two first-degree relatives with abdominal cancer and cervical cancer, respectively, both after the age of 70 years. During the genetic counselling these diagnoses were both confirmed to be ovarian cancer cases. Thus, all mutation carriers in this study fulfilled current national

Table 3 HADS anxiety and depression subscale scores at various time points for a subset of DNA-BONus participants

	At inclusion (T1)	*One week after disclosure of genetic test result (T2)*	*Six months after disclosure of genetic test result (T3)*
HADS anxiety			
No. of patients	213	191	167
Subscore mean (SD)	6.84 (4.28)	5.29[a] (4.06)	4.88[b] (3.86)
Score ≥8 (%)	39.9	23.6[c]	19.8[d]
HADS depression			
No. of patients	215	190	169
Subscore mean (SD)	3.32 (3.07)	2.90[e] (3.30)	2.65[f] (3.04)
Score ≥8 (%)	10.2	10.0 [g]	10.7[h]

Abbreviation: HADS, Hospital Anxiety and Depression Scale.
[a]T1 *vs* T2: $P<0.001$.
[b]T1 *vs* T3: $P<0.001$; paired sample *t*-test.
[c]T1 *vs* T2: $P<0.001$.
[d]T1 *vs* T3 $P<0.001$; McNemar's exact test.
[e]T1 *vs* T2: $P=0.32$.
[f]T1 *vs* T3: $P=0.11$; paired sample *t*-test.
[g]T1 *vs* T2: $P=1.00$.
[h]T1 *vs* T3: $P=0.42$; McNemar's exact test.

criteria for diagnostic *BRCA1/2* testing when a proper personal and family history had been taken.

The Manchester scoring system is a frequently used tool to identify individuals and families at high risk of having a pathogenic *BRCA1/2* variant.[24] In this study, we found that the Manchester scores obtained at inclusion were markedly lower than the real values (see below). In retrospect, all *BRCA1/2* mutation carriers had combined Manchester scores at ≥14 points, demonstrating that the hereditary breast and ovarian cancer families identified through testing of patients with incidental breast or ovarian cancer do not differ significantly from families identified through the traditional route. These findings indicate that most *BRCA1/2* mutation carriers can be identified through evidence-based clinical criteria, also within a group of incidental patients.

In order to identify patients at risk of non-*BRCA1/2* familial breast cancer and other causes of hereditary cancer, we systematically collected structured family history from the participants before *BRCA1/2* testing and employed a low threshold for our genetic counselors to contact the participants for additional information. Indeed, the importance of family history should not be neglected when the availability of *BRCA1/2* testing increases and more patients with breast cancer are tested in routine clinical practice. Most familial breast cancer risk is not caused by pathogenic *BRCA1/2* variants, and women belonging to *BRCA1/2*-negative breast cancer families are also at increased risk for breast cancer.[20] The importance of obtaining a structured family history was illustrated by the fact that *BRCA1/2* mutation carriers in our study scored significantly higher in the Manchester scoring system when taking into account the information collected during the genetic counselling procedure, as compared with the rating based on the initial self-reported information. In this regard, oncologists and surgeons may need additional support and training to extract a structured and relevant family history.[31,32]

The traditional genetic counselling procedure has obvious benefits with respect to high-quality family history collection, and it has been shown to increase cancer-related knowledge and decrease distress in newly diagnosed cancer patients with an elevated risk of hereditary cancer.[33] However, because this procedure is resource demanding, alternative approaches are needed when treatment-driven genetic testing is offered to larger patient groups with lower probability of carrying a pathogenic *BRCA1/2* variant. Written, telephone-based or digital information provided by a clinical geneticist or genetic counselor, together with adequate information from the oncologist or surgeon, could be considered as an alternative for some patients.[22] Patients at increased risk of psychosocial distress should have easy access to genetic counselling. An open telephone line to a genetic counselor might not be optimal for patients newly diagnosed with cancer, as we experienced that <20 patients actually contacted the genetic counselor for more information before testing throughout the whole DNA-BONus study period of two-and-a-half years. In order to discuss the consequences of the *BRCA1/2* test results for the patient and other family members, as well as to explain complex test results and other hereditary causes of cancer, we also advise genetic counselling in case of a positive *BRCA1/2* test result and in case of a personal or family history suggestive of hereditary cancer.

As the most common current practice of *BRCA1/2* testing is based on referral of selected high-risk subjects to extensive face-to-face procedures of genetic counselling before *BRCA1/2* testing,[17,18] we investigated whether our new simplified approach could lead to increased anxiety or depression in the newly diagnosed patients. Interestingly, the level of anxiety symptoms was comparable to those reported for patients with breast cancer and gynecological cancer in general,[28,29] but higher than normal population values.[34] Approximately 40% of the patients had a HADS subscale score above the defined threshold for symptoms of anxiety[27] at inclusion, and the level of anxiety decreased significantly during the 6-month follow-up period that also included the dissemination of the *BRCA1/2* test result. The drop in the level of anxiety symptoms during the observation period may simply reflect the adjustment to the cancer diagnosis and treatment, and genetic testing in our study did not appear to influence on this expected drop.

There are some limitations to our study. Because of ethical regulations, we had no information about the patients who declined participation in the study. Another limitation is that Sanger sequencing of the *BRCA1/2* genes was only performed on selected high-risk patients, implying that some of the lower-risk patients could be carriers of rare *BRCA1/2* variants that were not covered by the *BRCA1/2* panel test. In this respect, it should be noted that the methods and two-step procedure for *BRCA1/2* testing (ie, multiplex panel test for recurrent variants, plus optional *BRCA1* plus *BRCA2*

Sanger sequencing) remained unchanged during the whole inclusion period, and that the fraction of patients who were sequenced was almost the same in the first and second half of the DNA-BONus study. Another potential weakness is that patients with previously known pathogenic *BRCA1/2* variants, who were diagnosed with cancer during the DNA-BONus study period, might have declined participation because of low relevance, thereby reducing the total count of *BRCA1/2* mutation carriers among the participants. Finally, some of the psychosocial results are limited by a small number of participating *BRCA1/2* mutation carriers and should therefore be interpreted with caution.

In conclusion, we show that *BRCA1/2* mutation testing is well accepted among patients with newly diagnosed breast or ovarian cancer. We further conclude that current clinical guidelines are sufficient to identify the majority of the *BRCA1/2* mutation carriers among patients with breast cancer. Because of the high prevalence of pathogenic *BRCA1/2* variants, we recommend that all patients with epithelial ovarian cancer are offered germline *BRCA1/2* testing, irrespective of age or family history of cancer.

CONFLICT OF INTEREST

N Hoogerbrugge is scientific consultant to AstraZeneca since June 2014. HP Eikesdal has received PARP inhibitors free of charge from AbbVie and AstraZeneca for use in clinical trials in patients with breast cancer. The other authors declare no conflict of interest.

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