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A prospective study on predictive factors linked to the presence of *BRCA1* and *BRCA2* mutations in breast cancer patients

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Abstract

We prospectively screened a hospital-based population of 1000 successive breast cancer patients receiving adjuvant radiotherapy for predictive factors associated with the presence of *BRCA1* and *BRCA2* mutations. We offered genetic counseling and DNA analysis to selected patients. About 52% of patients showed at least one presumed predictive factor. Hundred and thirty-seven patients underwent DNA analysis. We identified 14 deleterious mutations (10.2%, 95% CI: 5.2–15.3%): 8 *BRCA1* mutations and 6 *BRCA2* mutations and 14 variants of uncertain clinical significance. Ovarian cancer in the family history was the only factor significantly associated with the presence of a disease-causing mutation ($P < 0.01$). Eight of the 14 (57%) mutation carriers had no affected first-degree relatives and in 4 of these there was no family history of breast or ovarian cancer. Clinicians should offer genetic counseling and DNA testing to breast cancer patients from families with breast and ovarian cancer, and to patients who are younger than 45 years when they are diagnosed with breast cancer.

Keywords: Breast cancer; Ovarian cancer; *BRCA1* gene; *BRCA2* gene; Hereditary breast cancer; Hospital population

1. Introduction

Physicians involved in the treatment of breast cancer patients are often confronted with patients asking about the possible hereditary nature of their disease. Referral for genetic counseling and diagnostic genetic testing is increasingly offered by clinicians, enabling patients to make more informed decisions on risk-reduction options, with the ultimate goal of reducing morbidity and mortality [1]. Surveillance may also be recommended for family members at risk. However, it remains a challenge to identify those breast cancer patients who have a higher probability of carrying a *BRCA1* or *BRCA2* mutation. Simple and reliable criteria for selection are lacking.

Germ-line mutations in either of two tumour suppressor genes, *BRCA1* and *BRCA2*, predispose women to breast and ovarian cancer. Mutations have been found in families with multiple cases of both breast and ovarian cancer and with cases of early-onset breast cancer [2]. The prevalence of mutations in young patients unselected for family history has been determined in several population-based series, with percentages ranging from 3.3% to 13% [3–9]. In breast cancer patients, not selected for family history or age at diagnosis, the prevalence of mutations was 1.6% in the Netherlands [10], 1.8% in Finland [11] and 10.3% in a cohort

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of Ashkenazi Jewish breast cancer patients [12]. Apart from family history and age at diagnosis, several authors consider patients with bilateral breast cancer to have an increased prevalence of *BRCA* mutations [8,13,14]. One could hypothesise that this also holds true for multifocality and multicentricity of breast cancer, as well as for lobular carcinoma *in situ* (LCIS).

We performed a prospective hospital-based study to determine which factors, in this context, are associated with a mutation in the *BRCA1* or *BRCA2* gene.

2. Patients and methods

2.1. Study population

All of 1000 successive breast cancer patients treated with radiation therapy as part of curative treatment at the Department of Radiotherapy, University Medical Center Utrecht, between March 1997 and June 1999 were included in our study. The cohort constituted approximately 60% of all the patients diagnosed with breast cancer in the region during that period.

2.2. Procedures

A checklist with eight factors presumed to be predictive for hereditary breast cancer was used at the patients' first visit to the Department of Radiotherapy. Predictive factors included prevalence of breast and ovarian cancer in relatives, and the following patient characteristics: age at diagnosis if under 45 years, bi-laterality, multifocality and multicentricity of infiltrating carcinoma, accompanying LCIS and a personal history of ovarian cancer. Patients demonstrating one or more predictive factors were offered the option of having an extensive family pedigree compiled, focusing on breast cancer (age at diagnosis, bi-laterality), ovarian cancer, and other malignancies. After compilation of the pedigree, patients who fulfilled the criteria for referral to the family cancer clinic (FCC) (Table 1) were offered genetic counseling and DNA analysis. Malignancies in relatives of patients visiting the FCC were verified through medical records. Written informed consent was given by all patients who agreed for DNA mutation analysis.

Table 1
Four criteria for referral to the family cancer clinic

A	Breast cancer in patient or affected relative[a] <45 years of age
B	Patient has 2 or more relatives[b] with breast cancer
C	Bilateral, multifocal or multicentric breast cancer in patient or affected relative[a]
D	Ovarian cancer in patient or relative[a]

[a] First- or second-degree relative.
[b] First-degree, or one first-degree relative plus one second-degree relative on the paternal side.

If a *BRCA1* or *BRCA2* mutation was detected, presymptomatic DNA analysis was offered to first- and second-degree relatives. All patients visiting the FCC were informed about their estimated risk of developing breast and ovarian cancer and the risk for their relatives. A breast and ovarian cancer prevention and screening program, according to Dutch national guidelines was offered to all patients. This study was approved by the hospital's Medical Ethics Committee.

2.3. Mutation analysis

High-molecular weight genomic DNA was isolated from peripheral blood lymphocytes. We used a combination of different detection methods to investigate the *BRCA1* and *BRCA2* genes for the presence of mutations. Exons 2 and 20 of the *BRCA1* gene were scanned for mutations by denaturing gradient gel electrophoresis (DGGE) [15]. Exon 11 of the *BRCA1* gene as well as exons 10 and 11 of *BRCA2*, were screened for chain-terminating mutations by protein truncation test (PTT) [14,16]. To detect genomic deletions of exons 13 and 22 of the *BRCA1* gene, we employed a specific PCR-deletion assay [17]. In addition, the entire coding region of both genes was analysed by DGGE [15], followed by direct automated DNA sequencing.

2.4. Statistical methods

All eight predictive factors were tested by univariate analysis for the association with the prevalence of *BRCA1* or *BRCA2* mutations and where appropriate 95% confidence intervals were calculated.

3. Results

3.1. Study population

Of 1000 patients receiving curative radiation therapy, the great majority (717 patients, 71.7%) had radiotherapy applied to the breast as an integral part of breast-conserving therapy. Adjuvant locoregional radiotherapy following radical mastectomy or breast-conserving therapy was performed in 198 patients (19.8%), indicated by unfavorable histological findings. A minority of 85 patients (8.5%) was treated with curative intent for a local or regional recurrence, or for locally advanced inoperable breast cancer. Age at diagnosis varied from 26 to 89 years with a mean of 55.9 years.

3.2. Predictive factors for hereditary breast cancer

The prevalence of all the presumed predictive factors is shown in Table 2. Of the 1000 patients studied, 522 (52.2%) had one or more risk factors: 376 patients had

Table 2
Prevalence of predictive factors for hereditary breast cancer in 1000 successive breast cancer patients, DNA-tested patients and mutation carriers

	Risk factors included in checklist	All 1000 patients (predictive factors in 522)		137 DNA tested patients		14 Mutation carriers	
		n	%	*N*	%	*n*	%
1	Age at diagnosis <45 years	184	18.4	76	55.5	8	57.1
2	Bilateral breast cancer	40	4.0	14	10.2	2	14.3
3	Ovarian cancer in personal history	3	0.3	2	1.5	1	7.1
4	Multicentricity/multifocality	58	5.8	26	19.0	3	21.4
5	Lob. C.I.S.	54	5.4	14	10.2	–	–
6	Breast cancer in family	340	34.0	73	53.3	9	64.3
7	Ovarian cancer in family	20	2.0	8	5.8	3	21.4

one predictive factor, 117 had two, 27 had three, and 2 had four factors. Breast cancer was diagnosed before the age of 45 years in 184 patients (18.4%); in 104 of these (10.4%) young age was the only predictive factor. Relatives with breast cancer were reported by 340 (34.0%) of the patients, and relatives with ovarian cancer by 20 patients (2.0%).

3.3. Pedigree composition and referral to the family cancer clinic

The flow scheme for the study and the numbers of patients are shown in Fig. 1. Of 522 patients who had predictive factors for hereditary breast cancer, 374 (72%) agreed to having a pedigree compiled. 371 patients had a pedigree drawn up, and 240 (65%) met criteria for further counseling. Of them 189 opted for referral to the FCC, and 179 actually visited the FCC. After having received more detailed information at the FCC, 35 patients had no further interest in genetic counseling. Seven patients died before blood samples had been taken. DNA analysis was performed in 137 patients.

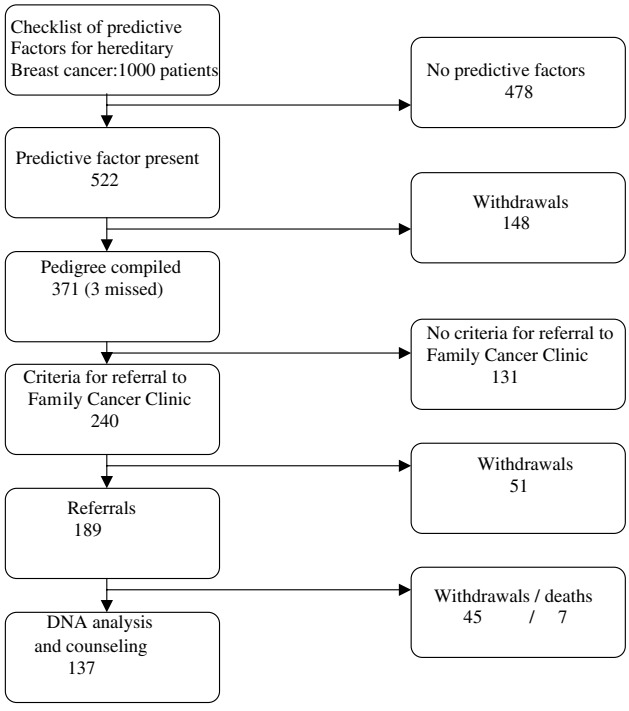

Fig. 1. Flow scheme for a study of 1000 successive breast cancer patients and the patient numbers involved.

3.4. Definitive pedigree analysis at the family cancer clinic

The pedigrees of all 189 patients who had been referred to the FCC disclosed that 167 (89%) belonged to 'breast cancer only' families (in 86 of these families, the index patient was the only member to have breast cancer). Twenty two (11%) of the index patients belonged to 'breast and ovarian cancer' families (3 index patients and 1 relative had both breast and ovarian cancers).

3.5. Outcome of DNA analysis

DNA analysis was performed for 137 patients, and revealed 14 deleterious mutations (10.2%; 95% CI: 5.2–15.3%): 8 *BRCA1* mutations (5.8%; 95% CI: 2.6–11.2%) and 6 *BRCA2* mutations (4.4%; 95% CI: 1.6–9.3%). One of the mutations disclosed in our series was a *de novo* germline mutation of *BRCA2* [18]. Table 3 shows details of the mutations detected, as well as several characteristics of the individual mutation carriers and their families. We detected 14 variants of uncertain clinical significance in 12 patients (1 of whom had a deleterious *BRCA2* mutation) (see Table 4).

3.6. Factors associated with the presence of a BRCA1 or BRCA2 mutation

We analysed the predictive value of the factors for hereditary breast cancer used in our check-list (Table 2) for a *BRCA1* or *BRCA2* mutation. Univariate analysis identified a significant difference between mutation carriers and those patients receiving a non-conclusive test outcome, but only for ovarian cancer in the proband and/or relatives. From the 22 'breast and ovarian cancer families', we tested 17 patients and found that 5 carried a mutation (29.4%) (2 of *BRCA1* and 3 of *BRCA2*), in

Table 3
Characteristics of carriers of breast cancer gene mutations

Age of proband at diagnosis (years)	Gene		Mutation	Family category	Total *n*[a]	Number of affected first-degree relatives	Predictive factors on check list (Table 2)	Criteria for referral to FCC (Table 1)
30	*BRCA1*		IVS20 + 1 g > a	BC only	3	0	1 + 6	A
35	*BRCA1*		Gln1395Stop	BC only	7	2	1 +4 + 6	A + B + C
38	*BRCA1*		1411insT	BC only	4	1	1 + 6	A
39		*BRCA2*	3034delAAAC	BC only	1	0	1 + 4	A + C
40, 44	*BRCA1*		Ser510Stop	BC and OC	2	1	1 + 2 + 7	A + C + D
40	*BRCA1*		2804delAA	BC and OC	3	0	1 + 7	A + D
42	*BRCA1*		3604delA	BC only	1	0	1	A
42	*BRCA1*		185delAG	BC only	2	1	1	A
46, 51		*BRCA2*	Cys2689Stop	BC and OC	1	0	2 + 4 + 6	C
47	*BRCA1*		4184delITCAA	BC only	6	1	6	C
50		*BRCA2*	Cys2689Stop	BC only	3	0	6	A
50		*BRCA2*[b]	7647delTG	BC and OC	2	0	6 + 7	D
51, 49 (OC)		*BRCA2*	7647delTG	BC and OC	1	0	3	D
51		*BRCA2*	6503delTT	BC only	3	1	6	B

BC = breast cancer; OC = ovarian cancer; FCC = family cancer clinic.
[a] Total number of affected patients, including the index patient.
[b] This patient also has an uncertain variant in *BRCA2*: I3412V.

contrast to 120 tested patients from 'breast cancer only families', who carried 9 mutations (7.5%) (6 of *BRCA1* and 3 of *BRCA2*) ($P < 0.01$). Eight of the 14 (57.1%) mutation carriers had no affected first-degree relative and in 4 of these there was no family history of breast or ovarian cancer (Table 3).

3.7. Role of age at diagnosis

Median age at diagnosis of mutation carriers was 42 years (range 30–51 years), and of carriers of uncertain variants of *BRCA1* or *BRCA2* genes 43 years (range 33–71 years). In all patients who received a non-conclusive test outcome, the median age at diagnosis was 43 years (a non-significant difference with the mutation carriers). Comparing age at diagnosis between *BRCA1* and *BRCA2* mutation carriers, we found a median age of 40 years for *BRCA1* carriers (range 30–47 years) and of 50 years for *BRCA2* carriers (range 39–51 years). The prevalence of *BRCA1* and *BRCA2* mutations in three different age groups is shown in Table 5.

4. Discussion

We report here the first hospital-based prospective study on the systematic screening of breast cancer patients for possible predictive factors associated with the presence of a *BRCA1* or *BRCA2* mutation. All 1000 breast cancer patients who received curative radiotherapy in one department during a 28-month period were included. Hospital-based populations consist of

Table 4
Characteristics of carriers of uncertain variants of *BRCA1* and *BRCA2* genes

Age of proband at diagnosis	*BRCA-UV1*		Designation-*UV1*	*BRCA-UV2*	Designation-*UV2*	Family category	Total *n*[a]
33		*BRCA2*	K2339N	*BRCA2*	H2440R	BC only	1
34	*BRCA1*		R841W			BC only	1
35	*BRCA1*		V271L			BC only	1
38		*BRCA2*	H179R			BC only	1
39		*BRCA2*	I505T			BC and OC	2
43		*BRCA2*	1623A > C			BC only	2
43, 46	*BRCA1*		K1254R			BC only	2
50		*BRCA2*[b]	IVS20 + 21C > A			BC only	2
50		*BRCA2*	IVS4 + 33A > G			BC only	3
53		*BRCA2*	R174H	*BRCA2*	I2828M	BC only	2
57		*BRCA2*	D2913E			BC only	3
71	*BRCA1*		1184G > A			BC only	3

BC = breast cancer; OC = ovarian cancer.
[a] Total number of affected women, including the index patient.
[b] This patient also has disease causing mutation.

Table 5
Prevalence of *BRCA1* and *BRCA2* mutations in breast cancer cases by age

Age at diagnosis (years)	Number in study population	Number of DNA analyses	Number of mutations	
<45[a]	184	76	8	(7 *BRCA1*/1 *BRCA2*)
45–59[b]	452	41	6	(1 *BRCA1*/5 *BRCA2*)
>59[b]	364	20	0	

[a] All of them were given the option of having a pedigree compiled and DNA analysis instigated.
[b] These groups were given the option of having DNA analysis only if criteria for referral to the FCC were present.

patients who have no or few relatives affected with breast cancer and very few relatives affected with carcinoma of the ovary, and whose age at diagnosis is over 50 years in the vast majority (our data, [12,19]). Current knowledge of predictive factors for detecting *BRCA1* or *BRCA2* mutations is mainly based on data gathered from research families and from other selected families seeking advice at family cancer clinics [20,21]. They, in contrast to our population, have a high prevalence of breast and/or ovarian cancer in relatives and a young mean age at diagnosis.

Of all eight factors studied (Table 2), ovarian cancer in the (individual's or family) history, was the only variable to show a statistically significant association with detecting a mutation. Large population-based series of early-onset breast cancer patients showed that *BRCA* mutations were more frequent among those cases with relatives with breast or ovarian cancer, [8,9,12]. In a very large population-based study in unselected Finnish breast cancer patients, Syrjäkoski and colleagues [11] showed that 7 of 19 mutations were detected in patients who had relatives affected with ovarian cancer, compared to 5 of 14 in our study. In their study, a family history of ovarian cancer was a strong predictor of mutations, in contrast to a family history of breast cancer. In our study we could not show breast cancer in relatives to be a statistically significant predictive factor. Bi-laterality of breast cancer and the histological factors of multifocality, multicentricity and LCIS were not seen very often in our study and we were not able to discover any predictive value.

Factors predictive for hereditary breast cancer used in the current study, such as breast and ovary cancer in relatives, ovary cancer in the proband, and young age at diagnosis, have all been studied before [3,5,6,10,11,20,21]. The other possible factors that we explored in our study have not been uniformly documented e.g. bi-laterality of invasive breast cancer proved to be a risk factor in some studies [8,13,14] but not in others [6,10]. Multifocality and multicentricity as predictive factors for hereditary breast cancer have not been studied in this context as far as we know. LCIS is associated with an increased risk of developing infiltrating cancer [22,23], but the Breast Cancer Linkage Consortium did not find an increased incidence of LCIS in carriers of a *BRCA1* or *BRCA2* mutation [24].

Importantly, almost 30% of our mutation carriers had no family history of breast or ovarian cancer (Table 3). In addition, over 50% of the mutation carriers had no affected first-degree relatives with breast cancer. Two other population-based studies recently showed that 25–50% of mutation carriers had a negative family history [9,12] suggesting that *BRCA* screening policies based on family histories alone would miss a considerable proportion of mutation carriers.

The frequency of *BRCA* mutations in patients diagnosed before the age of 45 years and tested was 10.5%, indicating that young age at diagnosis is a useful selection criterion for DNA testing. With respect to an association between young age at diagnosis and the probability of carrying a *BRCA1* mutation, another Dutch population-based study by Papelard and colleagues [10] showed comparable results. They studied only the *BRCA1* gene, in a hospital-based series of surgically treated breast cancer patients, not selected for family history or age. DNA analysis in 210 patients diagnosed at age less than 50 years revealed 10 mutations (4.8%) compared to no mutations in 432 patients diagnosed at age 50 or over. The age of their mutation carriers ranged from 30 to 48 years with a median age of 43 years. Our results almost mirror those of Papelard: age of *BRCA1* mutation carriers ranged from 30 to 47 years with a median age of 40.

In our study, in agreement with Meijers-Heijboer and colleagues [25], age at diagnosis of breast cancer in *BRCA2* mutation carriers was generally higher than in *BRCA1* carriers (*BRCA2* carriers ranged from 39 to 51 years with a median of 50 years). Only one *BRCA2* mutation carrier was younger than 45 years when diagnosed with breast cancer, while the four eldest mutation carriers in our whole series (50 and 51 years) harbored *BRCA2* mutations.

The following features of this study must be considered when interpreting our results:

1. This study was performed at a radiotherapy department and this might have led to a biased ascertainment of probands, as our study population might not be representative of the general breast cancer population. Cancer registry information from the Comprehensive Cancer Center of the central Netherlands revealed that curable breast cancer patients not

receiving radiotherapy did indeed differ from the study population. The mean age at diagnosis was higher (63.7 *versus* 55.9 years), surgical treatment was less extensive, and systemic treatment was applied more frequently (data not shown).
2. We identified variants of uncertain clinical significance in 12 patients (of whom one also had a deleterious *BRCA2* mutation). In the future some of these variants might be classified as deleterious mutations.
3. Eighteen patients withdrew from genetic testing because of relatives already undergoing DNA analysis. A selection bias leading to a lower detection rate of mutations in our cohort was unlikely however, because no deleterious mutations were discovered in these families.
4. We ended up detecting a relatively small number of mutations. This could have been partly due to selection and withdrawal (Fig. 1). In the study, 609 patients (60.9%) were excluded from DNA analysis. Of the remaining 391 patients at risk, 244 withdrew from genetic counseling. We missed three patients for pedigree composition and seven patients died before starting DNA analysis. In the end, 137 patients underwent DNA analysis and 14 deleterious mutations were detected. The small number of mutations hampered the statistical power of the analysis when evaluating predictive factors for mutation carriership.
5. In the current study an age at diagnosis <45 years in itself allowed for genetic testing, while older patients had to be selected by other means (Table 1). We may have missed some older breast cancer patients who might carry *BRCA1* or *BRCA2* mutations with lower penetrance.

In conclusion, our results and those from other population-based studies [3,4,6,8,9,11] still have not fully answered the question of how clinicians can best select breast cancer patients for genetic counseling. Selection of patients for referral is necessary because of the costs involved and the limits on capacity for DNA analysis and genetic counseling. It is clear that a breast cancer patient who has had ovarian cancer or whose relatives have a history of ovarian cancer should be offered a referral to the family cancer clinic (our results, [8,9,11,12]). Age at diagnosis less than 45 years is also a useful criterion for referral. Choosing specifically to counsel younger patients is more justifiable when we realise that it is especially the young mutation carriers who are likely to benefit from genetic counseling. They are at higher risk of developing contra-lateral breast cancer [26,27], and they more often opt for preventive surgery [25]. Younger mutation carriers may have a higher chance of increasing their health and life expectation with preventive measures [28].

In view of the growing demand from breast cancer patients for more information, including genetic counseling, we need to gain better insight into how to select breast cancer patients for referral to the family cancer clinic. Prospective population-based studies with DNA analysis of all the breast cancer patients involved might become feasible in the near future, pending progress in DNA analysis techniques leading to less expensive testing.

Conflict of interest statement

None declared.

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