ORIGINAL ARTICLE

Distinction between hereditary and sporadic breast cancer on the basis of clinicopathological data

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Background: About 5% of all breast cancer cases are attributable to germline mutations in BRCA1 or BRCA2 genes. BRCA mutations in suspected carriers, however, may be missed, which hampers genetic counselling.

Materials and methods: Different clinicopathological features were compared between 22 breast cancers from carriers of proved BRCA1 mutations and 604 cancers from sporadic controls. In addition, 5 BRCA2-related breast cancers and 66 breast cancers of untested patients at intermediate risk and 19 breast cancers of untested patients at high risk of hereditary disease on the basis of family history were evaluated.

Results: A ''probably sporadic'' class (age $\geqslant$54 years and epidermal growth factor receptor (EGFR) negative; 68% of cases) with a 0% chance of BRCA1-related breast cancer containing 79% of the sporadic cases was yielded by using a decision tree with age, Ki67 and EGFR. A 75% chance of BRCA1-related breast cancer was shown by the ''probably BRCA1-related'' class (age $<$54 years and Ki67 $\geqslant$25%; 8% of cases) with 82% of the BRCA1-related cases but only 1.4% of the sporadic cases. Most cases at intermediate or high risk of hereditary disease on the basis of family history could be classified with high probability as either probably BRCA1 related or probably sporadic.

Conclusion: Breast carcinomas can be classified with a high level of certainty as sporadic or related to BRCA1 germline mutations by using a decision tree with age, Ki67 and EGFR. This can be clinically useful in mutation analysis in families with a borderline risk of hereditary disease.

Family history of breast cancer is an established risk factor for the development of the disease. Among those variables that have been shown to bear a causal relationship with breast cancer, the highest increased risk, after age, is a positive family history of breast cancer.[1] In 5% of the breast cancer cases, the disease occurs as part of a hereditary cancer susceptibility syndrome. In contrast with acquired (somatic) BRCA1 and BRCA2 mutations that do not seem to be an important factor in the development of most sporadic breast cancers, a substantial proportion of hereditary breast cancers can be attributed to germline mutations in either of these genes.

Establishment of a BRCA1 or BRCA2 germline mutation has important consequences. Mutation carriers are at high risk not only of breast cancer but also of cancers of the contralateral breast, ovary and fallopian tube,[2 3] which necessitates preventive strategies in these patients. Furthermore, hereditary breast cancer is associated with a poorer survival and hereditary ovarian cancer with a better survival[4] than their sporadic counterparts, which may have consequences for treatment.

Screening for BRCA1 or BRCA2 mutations is difficult. In a large proportion of patients with breast cancer, or ovarian or tubal cancer, who have a family history strongly pointing to hereditary disease, a BRCA1 or BRCA2 mutation cannot be shown. This may partly be explained by germline mutations in genes other than BRCA1 or BRCA2, such as CHEK2,[5] but mutations in BRCA1 or BRCA2 may be missed in current screening procedures, even with complete sequencing.[6] It is currently possible to detect about 90% of all BRCA1 or BRCA2 mutations by using standard diagnostic procedures, and a mutation is detected in almost 25% of cases presenting with familial cancer. Clinically, a hereditary basis of breast cancer is recognised by early age at onset, family history, bilateral breast cancer, breast cancer in men and cancer of the ovary or of the fallopian tube. Also, family history may be incomplete, even in developed countries. In The Netherlands, incompleteness of medical history is an increasing problem as a law has been introduced that does not permit keeping patient data and material for more than 10 years after the initial diagnosis.[7] Furthermore, families may be small, inheritance may occur through non-affected men and penetrance may be incomplete.

Therefore, in patients at high risk on the basis of family history, additional features pointing to hereditary disease in case of negative mutation screening are useful, for example, to decide on preventive strategies and diagnostic procedures. In patients at an intermediate risk of hereditary disease on the basis of family history, additional features may help to decide on mutation screening. Inversely, such features may rule out the need for mutation screening in case of a suspected family history.

Besides young age, poor tumour differentiation, high proliferation, negative steroid receptor status, p53 and HER-2/*neu* positivity,[8–13] overexpression of epidermal growth factor receptor (EGFR)[14] and cytokeratin 5/6 positivity[15] may point to hereditary breast cancer associated with BRCA1. BRCA2-related tumours show a less conspicuous phenotype.[16]

Few studies, however, have aimed at integrating all these features into a multivariate model to classify breast cancer in patients as hereditary or sporadic, as an aid for genetic counselling.[17–19] We therefore aimed at evaluating a panel of clinicopathological variables to classify breast cancers as hereditary or sporadic using a multivariate approach.

Abbreviations: EGFR, epidermal growth factor receptor, MAI, mitotic activity index

Table 1 Median values (ranges) of different continuous clinicopathological features for sporadic breast cancers, cancers in patients with BRCA1 or BRCA2 mutations and in patients with breast cancer who are at different risks of hereditary disease, on the basis of family history

	Sporadic		Intermediate risk		High risk		BRCA1 mutation		BRCA2 mutation	
Feature	n	Median (range)	n	Median (range)	n	Median (range)	n	Median (range)	n	Median (range)
Age (years)	579	64 (30–87)	66	51 (26–76)	19	47 (31–77)	22	38 (31–57)	5	56 (38–66)
Mitotic activity index	567	10 (0–151)	63	7 (0–130)	19	28 (0–104)	22	30 (0–105)	5	11 (4–56)
Tumour size (cm)	554	2.0 (0.1–10)	63	2.0 (0.2–8.0)	17	3.2 (1–22)	17	2.0 (0.5–4)	5	1.5 (1–3)
Ki67 (%)	421	10 (0–80)	62	10 (1–90)	18	58 (0–100)	22	75 (2–100)	5	20 (10–70)
p53 (%)	419	0 (0–100)	57	1 (0–100)	19	5 (0–100)	22	39 (0–100)	5	0 (0–2)
Oestrogen receptor (%)	423	80 (0–100)	62	78 (0–100)	19	0 (0–90)	22	0 (0–100)	5	0 (0–90)
Progesterone receptor (%)	420	0 (0–100)	65	25 (0–100)	19	0 (0–80)	22	0 (0–90)	5	35 (0–80)
p27 (%)	412	30 (0–100)	0	–	14	10 (0–90)	17	50 (0–100)	2	28 (5–50)
p21 (%)	459	1 (0–65)	56	2 (0–90)	18	2 (0–20)	20	1 (0–80)	4	3 (0–25)
Cyclin E (%)	424	0 (0–20)	59	0 (0–50)	4	0 (0–0)	5	0 (0–1)	3	1 (0–1)
Cyclin A (%)	457	5 (0–50)	48	5 (1–60)	19	25 (2–50)	21	35 (5–90)	5	10 (2–20)
Cyclin D1 (%)	427	1 (0–90)	59	0 (0–60)	18	1 (0–35)	22	0 (0–50)	5	0 (0–80)

SUBJECTS AND METHODS

Patients

The study group comprised 22 stage I or stage II invasive breast cancers from 22 patients with a proved BRCA1 germline mutation and five cancers from five patients with a proved BRCA2 germline mutation. In addition, we studied 19 patients with invasive breast cancer, who were not screened for mutations, but were known to have a proved BRCA1 (n = 17) or BRCA2 (n = 2) mutation in their families, further denoted as being at high risk of hereditary disease. The final group comprised 66 patients with invasive breast cancer ''at intermediate risk'' of hereditary disease on the basis of family history according to Claus's criteria.[20] These patients were not tested for BRCA1 or BRCA2 mutations. All these patients were from the Familial Cancer Clinic of the VU University Medical Center, Amsterdam, The Netherlands. The control group comprised 604 patients with stage I or stage II invasive breast cancer unselected for family history, further denoted as sporadic cases, from the archives of the Department of Pathology of the VU University Medical Center.

Histopathology

Tumour size was measured in the freshly resected specimens, and tumour samples were subsequently fixed in neutral buffered formaldehyde and processed to blocks of paraffin wax according to standard procedures. Four-micrometre-thick sections were cut and stained with haematoxylin and eosin for histopathology. Tumour type was assessed according to the World Health Organization criteria, and tumours were graded according to the criteria by Elston and Ellis. To assess the mitotic activity index (MAI), mitoses were counted as described previously.[21]

Immunohistochemistry

Immunohistochemical analysis was carried out on 4-µm-thick sections. After deparaffination and rehydration, endogenous peroxidase activity was blocked for 30 min in a methanol solution containing 0.3% hydrogen peroxide. After antigen retrieval in citrate buffer (autoclaved, except for oestrogen receptor when the microwave was used, and HER-2/*neu* for which no retrieval was done), a cooling-off period of 30 min preceded the incubation (overnight at 4°C) with the primary antibodies (p53: DO7, Dako, (Glostrup, Denmark) 1:500; p21: Pharmingen, (San Diego, California, USA) 1:500; p27: Transduction Laboratories, 1:1000; cyclin A: Novocastra, (Newcastle-Upon-Tyne, UK) 1:100; cyclin D1: Neomarkers, 1:400; EGFR: Novocastra, 1:10; oestrogen receptor: Dako, 1:50; progesterone receptor: Novocastra, 1:50; HER-2/*neu*: courtesy Dr Marc van den Vijver, Netherlands Cancer Institute, Amsterdam, 1:25; Ki67: Dako, 1:40). The primary antibodies were detected with a biotinylated rabbit anti-mouse antibody (Dako). The signal was amplified by avidin–biotin complex formation and developed with diaminobenzidine, followed by counterstaining with haematoxylin, dehydrated in alcohol and xylene and mounted.

For Ki67, p53, p21, cyclin D1, cyclin A, p27, oestrogen receptor and progesterone receptor, only nuclear staining was considered and diffuse cytoplasmic staining was ignored, leading to an estimated percentage of positively stained nuclei. Stainings of HER-2/*neu* and EGFR were scored positive when a clear membrane staining pattern was seen. Scoring was carried out by a single experienced pathologist (PJvD) who was blinded to BRCA1 or BRCA2 mutation status.

Statistics

Continuous variables were tested for differences between the hereditary and sporadic cases with the Mann–Whitney test, and discrete variables were tested with the χ^2 test using logical classes. Significance level was set at $p<0.05$. Finally, decision tree analysis based on recursive partitioning was carried out with the OMEGA Analytical Engine (KiQ, Amsterdam, The Netherlands) to discern BRCA1 and sporadic cases, by using a maximum of three variables with the highest univariate differences between BRCA1 and

Table 2 Median values (n, %) of different discrete clinicopathological features for sporadic breast cancers, cancers in patients with BRCA1 or BRCA2 mutations and in patients with breast cancer who are at different risks of hereditary disease on the basis of family history

	Type	Sporadic (n, %)	Intermediate risk of hereditary disease (n, %)	High risk of hereditary disease (n, %)	Mutations in BRCA1 (n, %)	Mutations in BRCA2 (n, %)
Grade	1	119 (22)	9 (16)	0 (0)	0 (0)	0 (0)
	2	181 (34)	25 (46)	5 (26)	4 (18)	1 (20)
	3	232 (44)	21 (38)	14 (74)	18 (82)	4 (80)
Histological type	Ductal	474 (79)	55 (84)	14 (74)	18 (82)	5 (100)
	Lobular	56 (9)	8 (12)	1 (5)	0 (0)	0 (0)
	Medullary	10 (2)	0 (0)	1 (5)	4 (18)	0 (0)
	Tubular	22 (4)	1 (2)	0 (0)	0 (0)	0 (0)
	Other	42 (7)	1 (2)	3 (16)	0 (0)	0 (0)
EGFR	Neg	360 (84)	24 (77)	3 (20)	7 (33)	0 (0)
	Pos	70 (16)	7 (23)	12 (80)	14 (67)	5 (100)
HER-2/*neu*	Neg	374 (87)	52 (88)	15 (83)	17 (81)	3 (75)
	Pos	55 (13)	7 (12)	3 (17)	4 (19)	1 (25)

EGFR, epidermal growth factor receptor; Neg, negative; Pos, positive.

sporadic cases. Besides the optimal model that was composed of all features in the analysis, two alternate decision trees were designed: one excluding age, to gain insight into the most important primary tumour features pointing to BRCA1-related breast cancer, and the other excluding immunohistochemical variables, which may be useful when tissue blocks are not available for immunohistochemistry. These decision trees had four end points, and for each of these end points the chance for BRCA1-related disease was calculated. This classification model resulted in four classes at an increasing risk of BRCA1-related disease. The class at the lowest risk of BRCA1-related disease was denoted as probably sporadic, and the class at the highest risk as probably BRCA1 related. Next, the cases at intermediate and high risk and the BRCA2-related cancers were classified with these decision trees.

The decision tree approach was chosen as such trees are easy to use in clinical practice. For further statistical clarity, however, we also carried out logistic regression.

RESULTS

Morphologically, tubule formation was virtually absent in cases with BRCA1 and BRCA2 mutations and lymphocytic infiltration was often seen. Mitotic activity was higher and nuclear atypia more outspoken in cases with hereditary cancer. The medullary histological type was relatively frequent in cases with BRCA1 mutations (4/22 *v* 10/604 of sporadic cases, Fisher's exact $p = 0.001$). All remaining cases with BRCA1 mutations were of the ductal type (18/22). All cases with BRCA2 mutations had cancers of the invasive ductal type.

Tables 1 and 2 show the median values (and ranges) of the different continuous variables and the values for the discrete variables for the different risk groups, respectively.

With increasing risk of hereditary disease, age, oestrogen and progesterone receptor expression decreased, whereas MAI, grade and Ki67, p53, and cyclin A expression increased. We found a significant ($p<0.0001$) increase in the frequency of EGFR expression in cancers associated with BRCA1 (14/21, 67%) and BRCA2 mutations (5/5, 100%) than in sporadic cancers (70/430, 16%). EGFR expression in the intermediate-risk group (7/31, 23%) was comparable with that in the group with sporadic cancers, but in the high-risk group EGFR expression (12/15, 80%) was similar to that in the hereditary cases. We detected no relationship between tumour size and HER-2/*neu*, p27, p21 and cyclin D1 expression and no risk of hereditary disease.

For many variables, the five cases associated with BRCA2 mutations showed values between those of cases associated with the BRCA1 mutation and the sporadic cancers. Age was on average 55 years for cases with BRCA2 mutations, compared with 64 years for sporadic cancers and 42 years for cases with BRCA1 mutations. The same intermediate values were seen for MAI, Ki67, oestrogen receptor and progesterone receptor expression. All cases with BRCA2

Table 3 Clinicopathological features that are significantly different ($p<0.05$) between the various groups of sporadic breast cancers, cancers in patients with BRCA1 or BRCA2 germline mutations and in patients with breast cancer who are at different risks of hereditary disease on the basis of family history (Claus's criteria)

	Clinicopathological features differing in groups of patients with breast cancer			
	Intermediate risk of hereditary disease	High risk of hereditary disease	Germline mutations in BRCA1	Germline mutations in BRCA2
Sporadic breast cancer	Age, T size, p53, PR, p21, cyclin E, cyclin D1	Age, MAI, T size, Ki67, p53, ER, cyclin A, EGFR, grade	Age, MAI, Ki67, p53, ER, PR, cyclin A, EGFR, grade	Age, cyclin E, EGFR, grade
Breast cancer in patients at intermediate risk of hereditary disease		MAI, T size, Ki67, ER, PR, cyclin A, EGFR, grade	Age, MAI, Ki67, ER, PR, p21, cyclin A, EGFR, grade	Ki67, cyclin E, EGFR, grade
Breast cancer in patients at high risk of hereditary disease			T size, p27	T size, p53, ER, cyclin A
Breast cancer in patients with germline mutations in BRCA1				Age, Ki67, PR, cyclin A

ER, oestrogen receptor; MAI, mitotic activity index; PR, progesterone receptor; T size, tumour size.
Continuous variables were compared with the Mann–Whitney test and discrete variables with the χ^2 test.

Table 4 Probability of breast cancer cases related to BRCA1 germline mutations for different groups of patients with breast cancer, on the basis of a combination of age at diagnosis, percentage of Ki67-positive cells in the primary tumour and EGFR status of the primary tumour

Classification	Decision tree	Number of sporadic cases	Number of cases with BRCA1 mutations	Probability of BRCA1-related disease (%)	Number of intermediate-risk cases	Number of high-risk cases	Number of cases with BRCA2 mutations
Sporadic	Age ⩾54 years and EGFR = Neg	332	0	0	24	3	0
Intermediate	Age ⩾54 years and EGFR = Pos	62	2	3	2	2	3
Intermediate	Age <54 years and Ki67<25%	21	2	9	26	2	2
BRCA1 related	Age <54 years and Ki67⩾25%	6	18	75	7	12	0

EGFR, epidermal growth factor receptor; Neg, negative; Pos, positive.

mutations showed very low p53 expression, with an average of 0.4% positive nuclei, whereas cases with sporadic cancer and BRCA1 mutations showed an average of 11% and 46% of positive nuclei, respectively. Average cyclin A values of cases with BRCA2 mutations were comparable to those of sporadic cases, but were much lower than those of cases associated with BRCA1 mutations. Cyclin D1 values were remarkably high in cases with BRCA2 mutations. The grade of cases with BRCA2 mutations was similar to those with BRCA1 mutations.

Table 3 shows the markers for major differences when comparing the different groups: cases with sporadic cancer, groups at intermediate risk and high risk, and cases with BRCA1 and BRCA2 mutations.

In decision tree analysis, age was the best univariate predictor, followed by Ki67, oestrogen receptor and EGFR. By using bivariate analysis, the best combinations of variables were found to be age/Ki67, followed by age/EGFR and age/MAI. Logistic regression also yielded age and Ki67 as important predictors. The best combination without including age was Ki67/EGFR. Logistic regression also yielded Ki67 and EGFR as relevant predictors. The optimal decision tree model was composed of age, Ki67 and EGFR. Table 4 shows the probability of hereditary disease for the different groups used in this decision tree.

The probably sporadic and probably BRCA1-related classes contained 68% and 8% of cases, respectively.

In the high-age group (⩾54 years), the chance of hereditary disease was 0% (probably sporadic class) when EGFR was negative and 3% when EGFR was positive. In the low-age group, the chance of hereditary disease was 9% when Ki67 was low and 75% (probably BRCA1-related class) when Ki67 was high. This decision tree classified 79% of the sporadic cases as probably sporadic, and only 1.4% as probably BRCA1 related. Of the BRCA1 cases, none were classified as probably sporadic and 82% as probably BRCA1 related. When classifying the intermediate-risk cases with the decision tree, 41% were classified as probably sporadic and 12% as probably BRCA1 related. Of the high-risk cases, 16% were classified as probably sporadic and 63% as probably BRCA1 related. All cases with BRCA2 mutations were classified into the intermediate categories and none into the probably sporadic or probably BRCA1-related classes. The patients at high risk with proved BRCA2 mutations in their families were also classified into the intermediate-risk category.

By allowing only primary tumour-related features, a decision tree was composed of Ki67, EGFR and the percentage of progesterone receptor-positive cells (table 5).

In the group with low Ki67, the chance of hereditary disease was 0% (probably sporadic class) when progesterone receptor was positive, and 2% when progesterone receptor was completely negative. In the high-Ki67 group, the chance of hereditary disease was 8% when EGFR was negative, but 33% (probably BRCA1-related class) when EGFR was positive. With this decision tree, 44% of the sporadic cases were classified as probably sporadic and only 6% as probably BRCA1 related. Of the cases with BRCA1 mutations, none were classified as probably sporadic class and 59% as probably BRCA1-related class. When classifying the intermediate-risk cases with the decision tree, 56% were classified as probably sporadic and 3% as probably BRCA1 related. Of the high-risk cases, 11% were classified as probably sporadic and 53% as probably BRCA1 related. Three cases with BRCA2 mutations were classified as probably sporadic and two as probably BRCA1 related.

When excluding immunohistochemical features from recursive partitioning, a bivariate model composed of age and MAI was obtained (table 6).

In the high-age group, the chance of hereditary disease was 0% (probably sporadic class) with low MAI and 2% with high

Table 5 Probability of breast cancer cases related to BRCA1 germline mutations for different groups of patients with breast cancer on the basis of a combination of the percentage of Ki67-positive cells, percentage of progesterone receptor-positive cells in the primary tumour and EGFR status of the primary tumour

Classification	Decision tree	Number of sporadic cases	Number of cases with BRCA1 mutations	Probability of BRCA1-related disease (%)	Number of intermediate-risk cases	Number of high-risk cases	Number of cases with BRCA2 mutations
Sporadic	Ki67<25% and PR>0%	184	0	0	33	2	3
Intermediate	Ki67<25% and PR = 0%	128	2	2	15	2	0
Intermediate	Ki67⩾25% and EGFR = Neg	82	7	8	9	5	0
BRCA1 related	Ki67⩾25% and EGFR = Pos	27	13	33	2	10	2

EGFR, epidermal growth factor receptor; Neg, negative; Pos, positive; PR, progesterone receptor.

Table 6 Probability of breast cancer cases related to BRCA1 germline mutations for different groups of patients with breast cancer, on the basis of a combination of classical features: age at diagnosis and MAI

Classification	Decision tree	Number of sporadic cases	Number of cases with BRCA1 mutations	Probability of BRCA1-related disease (%)	Number of intermediate-risk cases	Number of high-risk cases	Number of cases with BRCA2 mutations
Sporadic	Age ⩾54 years and MAI<17	269	0	0	21	4	1
Intermediate	Age ⩾54 years and MAI⩾17	125	2	2	5	1	2
Intermediate	Age <54 years and MAI<17	18	5	22	22	2	2
BRCA1 related	Age <54 years and MAI⩾17	9	15	63	11	12	0

MAI, mitotic activity index.

MAI. In the low-age group, the chance of hereditary disease was 22% with low MAI and 63% (probably BRCA1-related class) with high MAI. With this decision tree, 64% of the sporadic cases were classified as probably sporadic and only 2% as probably BRCA1 related. Of the cases with BRCA1 mutations, none were categorised as probably sporadic and 68% as probably BRCA1 related. When classifying the intermediate-risk cases with the decision tree, 36% were classified as probably sporadic and 19% as probably BRCA1 related. Of the high-risk cases, 21% were classified as probably sporadic and 63% as probably BRCA1 related. One case with BRCA2 mutation was categorised as probably sporadic and none as probably BRCA1 related.

DISCUSSION

In line with previous studies,[8–13 16] the BRCA1-related cancers showed, morphologically, virtually no tubule formation, lymphocytic infiltration remarkably often, more nuclear atypicality and higher mitotic activity. The medullary histological type was relatively frequent in cases with BRCA1 mutations with the remaining BRCA1 cases being of the ductal type. All cases with BRCA2 mutations had cancers of the invasive ductal type.

We found a clear inverse relationship between age and risk for hereditary disease, as expected.[11 22–24] The mean age of patients with sporadic breast cancer was 64 years, whereas the mean age of patients with a proved BRCA1 mutation was 42 years. As age is a powerful discriminator between patients with BRCA1-related and sporadic breast cancer, we have deliberately not matched for age in this study. Primary tumour features that were associated with BRCA1-related cancers were low expression of oestrogen and progesterone receptors, overexpression of EGFR, high MAI and grade, and high expression of Ki67, p53 and cyclin A. Although some studies showed no differences between BRCA-related breast cancers and sporadic cancers,[25] low expression of oestrogen and progesterone receptors has been described previously in BRCA1-related cancers.[8 9 12 16 24 25] We recently described for the first time the high expression of EGFR in breast cancers related to BRCA1 or BRCA2 mutations.[14]

With regard to the proliferation markers, we found high expression levels of Ki67 and high MAI in BRCA-associated tumours as in previous studies,[12 23–25] but we describe here, for the first time, high cyclin A expression as another useful marker of proliferation.[26] Accumulation of p53 in BRCA-related tumours is also in agreement with other studies,[8 24 27] indicating that p53 inactivation is, next to BRCA1 inactivation, an important event in BRCA1-associated carcinogenesis or progression. We found no relevant differences for p21, cyclin D1, p27 and HER-2/*neu* between BRCA1-related cases and sporadic controls. For p21[27] and cyclin D1,[11] these results are in agreement with previous studies. Chappuis *et al*[9] found in general a lower percentage of HER-2/*neu* positivity in BRCA1-related cancers. Therefore, p21, cyclin D1 and HER-2/*neu* do not seem to have a differential role in sporadic and BRCA1-related breast cancers.

Although we studied only five cases of BRCA2-related breast cancers, it appeared nevertheless that these cancers show a phenotype between sporadic and BRCA1-associated breast cancers. Age at presentation, MAI, Ki67,[9 24] oestrogen and progesterone receptor values[8 9 12 16 24] of the cases with BRCA2 mutations were between those of sporadic and BRCA1-mutated cases. All cases with BRCA2 mutations showed very low p53 expression, which has also been shown previously,[24] although some disagree.[9] As low p53 expression usually indicates the presence of the wild-type gene, p53 inactivation seems to be much less relevant as a next hit for cases with BRCA2 mutations compared with those with BRCA1 mutations. Further, cyclin A values were comparable to values in sporadic cases, and cyclin D1 expression was remarkably high. The grade and frequency of EGFR expression were similar to those of BRCA1-related cases, and for cyclin D1, BRCA2-related cases showed remarkably high values unlike those in BRCA1-related cancers and in contrast with a previous study.[28] HER-2/*neu* overexpression seems to be rare in BRCA2-related cancers.[9 28]

In decision tree analysis, the best classifier was composed of age, Ki67 and EGFR. In the age group ⩾54 years, the chance of BRCA1-related cancer was as low as 0% when EGFR was negative and 3% when EGFR was positive. In the age group <54 years, the chance of BRCA1-related disease was only 9% when Ki67 was low and as high as 75% when Ki67 was high. Most sporadic cases were classified as probably sporadic, and only a few as probably BRCA1 related, which can be expected as our group of sporadic cases will also inherently contain some BRCA1-related cancers because the controls were ''unselected for family history'' owing to non-availability of data on family history. None of the BRCA1-related cases were categorised as probably sporadic and as many as 82% were classified as probably BRCA1 related, which is not unexpected, as patients with BRCA1 mutations also have the baseline breast cancer risk of the population without mutations, which is about 10% in The Netherlands. Therefore, some breast cancers in the population with BRCA1 germline mutations, especially in elderly patients, will not be attributable to BRCA1, but will arise according to sporadic carcinogenetic pathways.

Obviously, the 75% chance of BRCA1-related disease in the probably BRCA1-related group is relative, because this chance is influenced by the ratio between BRCA1-related cancers and controls in this study. A ratio of 22 cases of BRCA1-related cancers to 604 controls, however, is in the order of 3–4% of BRCA1-related cases we would expect in a random Western population of patients with breast cancer. Therefore, we believe that the 75% chance may reflect the

Take-home messages

- In many familial breast cancer patients, a BRCA1 or BRCA2 mutation cannot be shown.
- Several tumour pathological features point to hereditary breast cancer and can help to decide on mutation screening.
- Low age, negativity to oestrogen and progesterone receptors, high proliferation and high expression of epidermal growth factor receptor (EGFR) characterise BRCA1-related breast cancer.
- Most breast cancer cases can be classified with high probability as either probably BRCA1 related or probably sporadic on the basis of age and expression of Ki67 and EGFR.

actual chance of BRCA1-related disease for patients with the described profile.

When the decision tree was used to classify cases at intermediate risk of hereditary disease, 41% were classified as probably sporadic and only 12% as probably BRCA1 related. Although we have no gold standard for this group, this distribution makes sense and shows that at least many of these patients can be classified by using the decision tree with high probability. Of the cases at high risk of hereditary disease (with family members having a proved BRCA1 mutation), 16% were classified as probably sporadic and 63% as probably BRCA1 related. The patients with a BRCA2 mutation in the family could not be classified as either probably BRCA1 related or probably sporadic. This distribution makes sense also because most breast cancers in this group (especially those arising at a young age) will be due to BRCA1, as many of these women will have the BRCA1 mutation that has been established in their family. All cases with BRCA2 mutations fell into the intermediate categories, underlining their intermediate nature with regard to age at presentation and biological characteristics of the tumour.

Therefore, this decision tree seems to be quite useful. It may help to decide on the need for DNA testing in families at intermediate risk. Further, it may be of great value to decide on preventive strategies for women in families that are most likely carrying a germline mutation in one of the genes associated with hereditary breast cancer syndromes, but have not shown a mutation on DNA testing.

As the differences between BRCA1-related and sporadic cancers with regard to some of the clinicopathological variables may be related to age, we also designed a decision tree excluding age. Allowing only primary tumour-related features, a decision tree emerged with Ki67, EGFR and the percentage of progesterone receptor-positive cells. Also, here, grade did not emerge. This indicates that these are the features most strikingly different between hereditary and sporadic cancers. By using this decision tree, a group at 0% chance of hereditary disease can be identified, but the chance of BRCA1-related disease in the probably BRCA1-related class was much lower than when age was included. This underlines the significance of young age as a feature of BRCA1-related breast cancer. The significance of young age is further strengthened as only 2 of the 396 patients aged $\geqslant$54 years were BRCA1 carriers, in contrast with 20 of 47 carriers in the younger group. As Eerola *et al*[29] recently pointed out, breast cancers occurring at a higher age in BRCA1 carriers do not have the typical BRCA1 phenotype and are therefore probably due to the baseline sporadic risk and are not related to the BRCA1 germline mutations.

A further improvement in classification functions may be expected from molecular techniques. Wessels *et al*[30] reliably (84% accuracy) classified sporadic and hereditary breast cancers by using chromosomal gains and losses assessed with comparative genomic hybridisation. Differences in gene expression between sporadic and hereditary breast cancers are also shown in two studies.[31 32] These techniques, however, are much more complicated, quite expensive or require fresh tissue, owing to which simple immunohistochemistry on blocks of paraffin wax will remain a practical approach for many laboratories.

In conclusion, most invasive breast carcinomas can be classified as sporadic or BRCA1-related with a high degree of certainty by using a decision tree based on age, Ki67 and EGFR. This could be clinically useful to decide on mutation testing in families at a borderline risk of hereditary disease.

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