BRCA2 Mutations and Androgen Receptor Expression as Independent Predictors of Outcome of Male Breast Cancer Patients

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ABSTRACT

Purpose: **Germline mutations of the *BRCA2* gene are involved in the development of a considerable number of male breast cancer cases. Although phenotypic differences have been observed between sporadic and *BRCA*-related breast carcinomas, conflicting data exist on the differences in prognosis of women with hereditary and sporadic breast cancer. The purpose of the study was to investigate the prognostic value of *BRCA2* status in male breast carcinoma (MBC).**

***Experimental Design*: We studied 43 male breast cancer patients, including 12 with *BRCA2* mutations. Tumor samples were characterized immunohistochemically using antibodies to estrogen receptor, progesterone receptor, and androgen receptor (AR).**

***Results*: *BRCA2*-related tumors presented at the earlier age compared with sporadic tumors ($P = 0.005$). Patients positive and negative for *BRCA2* mutations did not differ with respect to tumor size, lymph node involvement, histological grade, and sex hormone receptor status. Five-year disease-free survival (DFS) and overall survival (OS) were significantly decreased in *BRCA2*-positive patients (67% *versus* 28% for *BRCA2*-negative *versus* positive patients, respectively, $P = 0.017$ for DFS; 86% *versus* 25%, $P = 0.006$ for OS). Shorter survival was also correlated with expression of AR in tumor tissue (74% *versus* 33% for patients with tumors staining negatively and positively for AR, $P = 0.029$ for DFS; 71% *versus* 57%, $P = 0.05$ for OS).**

***Conclusions*: The *BRCA2* mutations and AR expression in tumor tissue are independent adverse factors for MBC prognosis. *BRCA2*-related MBC presents at the earlier age compared with non-*BRCA2*-related cancer, but do not differ with respect to other clinicopathological features.**

INTRODUCTION

Breast cancer is an uncommon disease in men. It represents ~1% of all breast cancer cases and <1% of cancers in men (1). Several factors have been reported to influence the risk for breast carcinoma in men. These include clinical conditions causing hypoandrogenism (Klinefelter's syndrome, testicular trauma, infertility), liver cirrhosis causing hyperestrogenism, the use of exogenous estrogens, obesity, gynecomastia, environmental factors such as exposure to electromagnetic field and ionizing radiation, or family history of breast cancer (1–9).

The overall survival for male breast cancer patients has ranged between 49 and 87% at 5 years (10–14). The male breast cancer is usually more advanced at diagnosis than female breast cancer. In men, skin infiltration and ulceration with involvement of axillary lymph nodes are more common (15). More advanced stage and higher incidence of lymph node metastases have been linked to a poorer prognosis (1, 11, 16). However, the survival when corrected for age and stage is similar in men and women. The histological grade tends to be lower in men, whereas estrogen receptor and progesterone receptor is higher (17). It is postulated that aggressive behavior of male breast cancer may be a result of close proximity to skin and nipple which facilitates early invasion of dermal lymphatics and spread to axillary lymph nodes (15).

Beside classical prognostic factors such as tumor size, lymph node involvement, histological grade, prognostic value of a number of new molecular markers including c-myc, c-erbB-2, p53, MIB-1, cyclin D expression, DNA ploidy, and microvascular density have been investigated (10, 12, 18–20). However, for the majority of markers analyzed, the results are inconsistent.

One of the most important risk factors for breast cancer in both women and men seems to be inherited predisposition. Two genes, *BRCA1* and *BRCA2*, account for the disease in large majority of breast cancer families (21). Unlike *BRCA1* mutations, germline mutations of *BRCA2* are involved in development of a considerable number of male breast cancer (22–26). In women, the pathological features of hereditary breast cancers, especially tumors that occur in *BRCA1* mutation carriers, *i.e.*, high grade and proliferation rate, aneuploidy, lack of estrogen receptor, are associated with poor prognosis (27, 28). *BRCA2*-related breast cancers are also higher grade tumors, are more frequently lobular type, and show less tubule formation than do sporadic cases (29). Several studies have investigated the outcome of *BRCA1*- and *BRCA2*-associated breast cancer (29–34). However, there are conflicting data on the differences in prognosis of hereditary and sporadic cases.

To our knowledge, there are no data on prognostic value of

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BRCA2 status in male breast cancer. In our previous studies analyzing 43 MBC[2] patients, we have identified 12 patients positive for *BRCA2* mutation (26, 35, 36). The aims of the present study were to: (*a*) characterize the clinicopathological features of male breast cancer patients; (*b*) investigate the expression of sex hormone receptors (ER, PR, and AR) in tumor tissue; (*c*) compare *BRCA2* status with clinicopathological features and hormone receptor expression; and (*d*) evaluate prognostic significance of *BRCA2*, ER, PR, and AR status.

MATERIALS AND METHODS

Patients and tumors. Forty-three MBC patients diagnosed in Great Poland Cancer Center between 1986 and 2000 were included in the study. The selection criteria were: known *BRCA2* status; available clinicopathological data; and archival samples for immunohistochemistry. *BRCA2* mutation analysis identified eight cases with *BRCA2* frameshift mutations, seven germline and one somatic, and four carriers of *BRCA2* missense variants, three germline and one somatic (Table 1). The remaining 31 patients (noncarriers) were considered as control group for comparison with mutation carriers. There was no selection bias toward *BRCA2* mutation-positive tumors. Written consent from all patients to participate in the study was obtained, and an ethical committee approved the study. Histological diagnosis was obtained in all cases. Carcinomas were pathologically staged according to the tumor-node-metastasis classification system (37). Histological grade was assessed according to the system of Elston and Ellis (38). The average age of 43 patients at the time of diagnosis was 60.8 years (median 65; range 29–85). The median age at diagnosis was chosen as the cutoff age for statistical analysis. Two of 43 patients had a positive history (affected first-degree relative) of breast cancer. Thirty patients (70%) were diagnosed with invasive ductal carcinoma, 2 patients had only ductal carcinoma *in situ* (5%), and 1 had lobular cancer. Other cases were: two papillary carcinomas (5%), one mucinous carcinoma and seven patients had variant histology (16%), including three cases of invasive ductal carcinoma and ductal carcinoma *in situ*, and four cases of invasive ductal p. lobular carcinoma. Two patients (5%) presented with stage 0 disease, 10 patients presented with stage I (23%), 11 (26%) presented with stage IIA, 7 (16%) presented with stage IIB, and 13 (30%) presented with stage III. Eighteen carcinomas were pT1, 12 were pT2, and 13 were pT3. Eighteen cases were lymph node negative, and 25 were node positive. Histological grade was assessed in 40 cases: 7 (17.5%) tumors were grade 1; 18 (45%) were grade 2; and 15 (37.5%) were grade 3.

Mutation Detection. Genomic DNA was extracted by standard procedure from peripheral lymphocytes of MBC patients for germline mutation detection. For somatic mutation analysis DNA was isolated from paraffin-embedded tumor tissues using Wizard Genomic DNA Purification Kit (Promega, Madison, WI). The entire coding region of the *BRCA2* gene and exon/intron splice junctions were amplified from genomic DNA with 63 primer pairs, and the length of amplified fragments varied from 136 to 300 bp. The mutation analysis of *BRCA2* gene was performed using single-strand conformation polymorphism-heteroduplex analysis. PCR products from variant conformers detected in single-strand conformation polymorphism-heteroduplex analyses were purified and subsequently sequenced in both directions using fmol DNA Sequencing System (Promega).

Immunohistochemistry. Immunohistochemical detection of ER, PR, and AR in paraffin sections was performed using the immunoperoxidase staining procedure with mono-

Table 1 MBC patients included in the study and their *BRCA2* mutation status

Patient	*BRCA2* status with exact location of alteration	Patient identification: Y series (26)	Patient identification: T series (35)
1	Exon 11, 3764G>A, *S1179N*, germline	9Y	3T
2	Exon 17, 8138del5, stop2638, germline	10Y	4T
3		11Y	39T[a]
4	Exon 18, 8457insA, stop2763, germline	12Y	1T
5		13Y	40T[a]
6		14Y	15T
7		15Y	41T[a]
8		16Y	18T
9		17Y	42T[a]
10	Exon 17, 8138del5, stop2638, somatic	18Y	16T
11		19Y	43T[a]
12		20Y	19T
13	Exon 25, 9599A>T, *N3124I*, germline	21Y	9T
14	Exon 11, 6495del3insC, stop2090, germline	22Y	8T
15		23Y	44T[a]
16		24Y	13T
17		25Y	32T
18		26Y	45T[a]
19		27Y	31T
20		28Y	17T
21		29Y	11T
22	Exon 26, 9814A>G, *K3196E*, germline	30Y	12T
23		31Y	14T
24		32Y	28T
25		33Y	29T
26		34Y	46T[a]
27		35Y	27T
28		36Y	25T
29		37Y	47T[a]
30		38Y	22T
31		40Y	20T
32		41Y	21T
33		42Y	24T
34		43Y	26T
35	Exon 11, 4967G/A, *C1580Y*, somatic	44Y	23T
36	Exon 10, 2045insA, stop615, germline	45Y	37T
37	Exon 11, 6621del4, stop2136, germline	46Y (36)	38T
38			2T[a]
39			5T[a]
40			6T[a]
41	Exon 11, 6495del3insC, stop2090, germline		10T[a]
42			34T[a]
43	Exon 11, 6495del3insC, stop2090, germline		35T[a]

[a] Unpublished data.

[2] The abbreviations used are: MBC, male breast carcinoma; ER, estrogen receptor; PR, progesterone receptor; AR, androgen receptor; OS, overall survival; DFS, disease-free survival; CI, confidence interval.

clonal mouse antihuman PR antibodies (DAKO A/S, Glostrup, Denmark), monoclonal mouse antihuman ER antibodies, and monoclonal mouse antihuman AR antibodies (Novacastra Laboratories, Ltd., Newcastle upon Tyne, United Kingdom). Carcinomas with no or weak staining (<10% positive cells) were considered as receptor negative. Sections from formalin-fixed, paraffin-embedded specimens were cut at 4–5 μm, mounted on 3-aminopropyltriethoxysilane-coated glass slides, and incubated for 20 min at 60°C. Sections were dewaxed and rehydrated according to routine procedure, and were incubated in citrate buffer (pH 6.0) in a microwave oven for 10 min at 750 W. Slides were rinsed in Tris-buffered saline, pH 7.4, for 15 min. Endogenous peroxidase activity was blocked using 3% H_2O_2 for 10 min. After rinsings in water for 10 min and in Tris-buffered saline for 15 min, sections were incubated at 4°C overnight with primary antibodies. After a washing, sections were incubated with En Vision/HRP$^+$ for 30 min. Reaction products of all markers under investigation were visualized using 3,3′-diaminobenzidine as chromogen. Sections were counterstained with Mayer's hematoxylin, dehydrated through graded ethanol, cleared in xylene, and mounted.

Statistical Methods. The relationship among clinical and pathological tumor features, sex hormone receptor status, and *BRCA2* mutation status was analyzed with a Yates-corrected χ^2 test. Survival curves were estimated according to the Kaplan-Meier method. OS was calculated from the date of surgery until death or the date patients were last known to be alive. DFS was calculated from the date of surgery until relapse or the date patients were last known to be alive. Univariate survival analyses were based on Kaplan-Meier product-limit estimates of survival distribution, and differences between survival curves were tested using the *F* Cox test. The relative importance of multiple prognostic factors on survival was estimated using the Cox proportional hazard regression model. $P < 0.05$ was considered to be significant.

RESULTS

The *BRCA2* mutation status was analyzed in relation to clinical, pathological, and biochemical features such as age, stage of disease, tumor size, lymph node involvement, tumor grade, and sex hormone receptor expression (Table 2). There was a significant difference between carriers of *BRCA2* germline mutations (frameshift and missense) and the control group ($P = 0.05$). Carriers tended to be younger at presentation than the control group (mean 54.4 *versus* 62.3, median 51 *versus* 66 years, respectively). The difference was more significant when carriers of frameshift *BRCA2* mutations were compared with the control group ($P = 0.002$, mean 48.1 *versus* 62.3, median 46.5 *versus* 66). There was no significant difference with respect to tumor size, lymph node status, or histological grade between mutation carriers and the control group (Table 2). Sex hormone receptor status (ER, PR, and AR) was studied in 39 patients. Twenty-four tumors were positive for ER (61.5%), 28 for PR (71.8%), and 15 for AR (38.5%; Table 2). No significant difference was observed between *BRCA2*-positive/negative patients and receptor status.

The median follow-up was 48 months (range 3–130). Thirty-three patients were alive at the time of last follow-up, 7 *BRCA2* mutation-positive patients and 26 controls. The duration of follow-up for these patients ranged from 12 to 130 months, and the median length of follow-up was 60 months. Twenty-six patients were alive and had not progressed (6 *BRCA2* positive, 20 controls), and seven patients had progressed and were still alive (1 *BRCA2* positive, 6 controls). Ten patients were known to have died, all because of breast carcinoma with documented recurrence, 5 *BRCA2*-positive patients and 5 controls. OS and DFS were analyzed according to *BRCA2* status, clinical, pathological, and biochemical parameters. OS for all 43 patients was 70% at 5 years, 86% *versus* 25% for controls and *BRCA2*-positive patients, respectively ($P = 0.006$; Fig. 1*A*). The 5-year DFS for the entire group was 60%. DFS, similarly to OS, was found to differ with respect to *BRCA2* mutation status and was significantly decreased in *BRCA2* mutation-positive patients (68% *versus* 28%, $P = 0.017$; Fig. 1*B*).The 5-year OS and DSF rates were also significantly decreased for men in more advanced stages. OS was 75% for stage I group, 70% for stage II, and 52% for stage III ($P = 0.034$). DFS was 60% for stage I, 51% for stage II, and 44% for stage III ($P = 0.053$). Shorter survival was correlated with increasing tumor size ($P = 0.03$ for OS) and lymph node metastases ($P = 0.037$ for OS; $P = 0.01$ for DFS; Fig. 2). The 5-year OS and DFS were significantly decreased for men with tumors staining positively for AR ($P = 0.05$ for OS; $P = 0.029$ for DFS; Fig. 3). There was no difference in either OS or DFS rates with respect to ER and PR status. The results are presented in Table 3. Multivariate survival analysis was performed by testing adverse factors identi-

Table 2 *BRCA2* mutation status in MBC patients in relation to clinical and pathological features and ER/PR/AR expression

Variable	No.	No. of *BRCA2*-positive cases	No. of *BRCA2*-negative cases	*P*
Age at diagnosis[a]				
<65 yr	20	7 (70)[b]	13 (42)	
>65 yr	23	3 (30)	18 (58)	.24
Mean (yr)	60.8	54.4	62.3	.05
Median (yr)	65	51	66	
Histological grade				
G1	7	1 (8)	6 (21)	
G2	18	4 (33)	14 (50)	.30
G3	15	7 (58)	8 (29)	
T stage				
pT1	18	3 (25)	15 (48)	
pT2	12	4 (33)	8 (26)	.58
pT4	13	5 (42)	8 (26)	
Lymph node status				
Negative	18	4 (33)	14 (45)	
Positive	25	8 (67)	17 (55)	.71
ER				
Negative	15	5 (45)	10 (36)	
Positive	24	6 (55)	18 (64)	.84
PR				
Negative	11	4 (36)	7 (25)	
Positive	28	7 (64)	21 (75)	.75
AR				
Negative	24	8 (73)	16 (57)	
Positive	15	3 (27)	12 (43)	.59

[a] For carriers of *BRCA2* germline mutations (frameshift and missense) *versus* noncarriers.
[b] Numbers in parentheses, percentage.

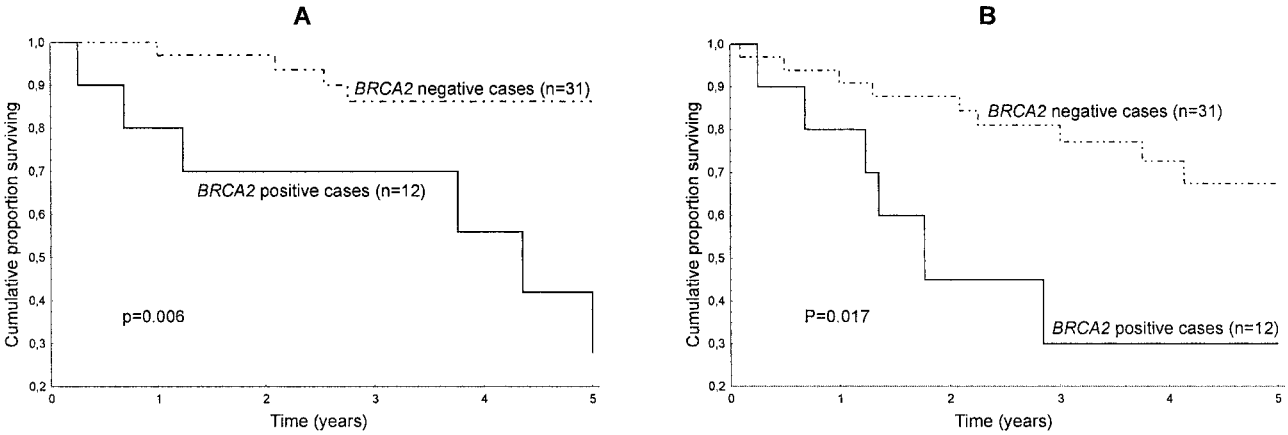

Fig. 1 OS (*A*) and DFS (*B*) of MBC patients by *BRCA2* status.

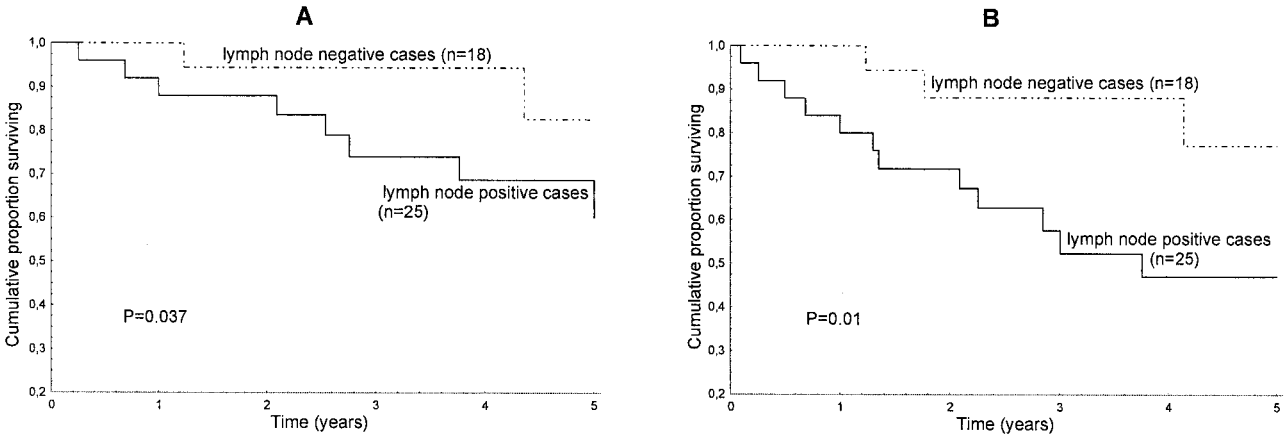

Fig. 2 OS (*A*) and DS (*B*) of MBC patients by N stage.

fied in univariate analysis in the Cox model. Only *BRCA2* status (χ^2 = 7.61, P = 0.006, hazard ratio 5.96, 95% CI 1.84–21.11 for DFS; χ^2 = 8.77, P = 0.003, hazard ratio 23.33, 95% CI 2.9–187.17 for OS) and AR status (χ^2 = 9.74, P = 0.002, hazard ratio 7.83, 95% CI 1.26–11.37 for DFS; χ^2 = 7.64, P = 0.006, hazard ratio 26.71, 95% CI 2.6–273.96 for OS) retained independent prognostic significance for both DFS and OS. The similar result was obtained when all factors tested in univariate analysis were included in the Cox model, and only *BRCA2* status (P = 0.04 for DFS; P = 0.007 for OS) and AR status (P = 0.003 for DFS; P = 0.007 for OS) had independent prognostic significance.

DISCUSSION

There are three major findings of this study: (*a*) the presence of *BRCA2* mutations and AR expression in tumor tissue were associated with shorter survival of male breast cancer patients; (*b*) *BRCA2* and AR status were independent factors for MBC prognosis; (*c*) *BRCA2*-related breast cancer presented at the earlier age compared with non-*BRCA2*-related cancer in men but did not differ with respect to other clinicopathological features.

Tumor-node-metastasis stage, tumor size, and lymph node metastases have prognostic importance in men (11, 13, 16, 39, 40). In our study in univariate analyses, shorter survival was correlated with increasing tumor size and lymph node metastases. However, none of these factors was found to be an independent predictor for poor prognosis by multivariate analysis. Approximately 64–85% and >70% of all MBC cases express ER and PR, respectively (11–14, 17). In our study, 61.5% of tumors were ER positive, and 71.8% were PR positive. Conflicting data exist on prognostic significance of ER status in MBC. Donegan *et al.* (11) showed that both ER positivity and PR positivity were prognostically favorable. Our data and results reported by Pich *et al.* (18) indicate lack of prognostic value of ER and PR status.

Susceptibility to breast carcinoma in approximately 5–10% of all cases is a result of inheritance of mutation in *BRCA1* and *BRCA2* breast cancer genes. Tumors from *BRCA1* and *BRCA2* carriers are characterized by a significantly higher number of chromosomal aberrations than are found in sporadic cancers (41, 42). Clinical and histopathological analyses of BRCA-related tumors showed phenotypic differences between sporadic breast carcinomas and tumors occurring in individuals carrying germ-

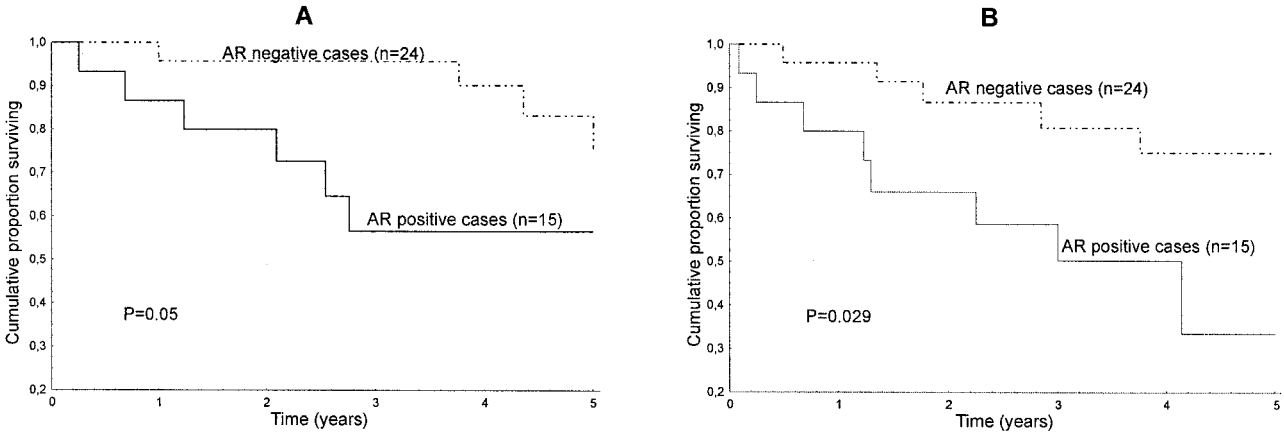

Fig. 3 OS (*A*) and DFS (*B*) of MBC patients by AR status.

Table 3 Associations of *BRCA2* status, clinical and pathological features, and sex hormone receptor status with survival of MBC patients

Variable	No.	Median survival (mos)		5-yr survival rate (%)		*P*	
		OS	DFS	OS	DFS	OS	DFS
Whole series	43	48	39	70	60		
Age at diagnosis							
<65 yr	20	39	32.5	47	46		
>65 yr	23	54	50	82	68	0.16	0.22
Histological grade							
G1	7	45	40	58	71		
G2	18	49	39	70	62		
G3	14	49	33	73	56	0.5	0.57
T stage							
pT1	18	46	38	69	72		
pT2	12	54	44	79	60		
pT4	13	45	34	52	43	0.03	0.18
Lymph node status							
Negative	18	49	44.5	86	76		
Positive	25	48	32	50	46	0.037	0.01
ER							
Negative	15	59	45	76	70		
Positive	24	42.5	35	61	54	0.22	0.22
PR							
Negative	11	55	45	57	52		
Positive	28	43	39	70	64	0.14	0.11
AR							
Negative	24	58	48	71	74		
Positive	15	37	33	57	33	0.05	0.029
BRCA2 status							
Negative	32	48	43	86	68		
Positive	11	45	20	25	28	0.006	0.017

line mutations in *BRCA1* and *BRCA2* genes. In our study, all *BRCA2*-related tumors were invasive ductal carcinomas; however, the lobular type is very rare in men. The *BRCA2*-related tumors tended to be at a slightly higher grade and stage at presentation than non-*BRCA2* tumors, but the difference was not statistically significant. It has been reported that *BRCA1* tumors demonstrate a low frequency of ER and PR expression (43, 44). We and others did not observe significant differences regarding steroid receptor levels between *BRCA2*-related and -nonrelated tumors (44). *BRCA2* tumors in most cases were ER and PR positive. In our study, men with *BRCA2* mutations were significantly younger at presentation than other cases. The similar trend was observed by Loman *et al.* (44) and by Eerola *et al.* (32) in female breast cancer.

Until now, there are only two studies on the survival of *BRCA2*-positive and -negative breast cancer patients with identified *BRCA2* mutations, concerning female breast cancer (30, 34). In our study, for the first time the prognostic significance of *BRCA2* status was investigated in MBC. We found that DFS and OS rates were significantly worse for men with *BRCA2*-associ-

ated than -nonassociated tumors. This difference cannot be explained by pathological factors, given that we did not find significant variation between these two groups with respect to lymph node involvement, tumor stage, or grade. All patients in our study were diagnosed within the last 15 years, and similar treatment was applied to the whole group, with surgery and systemic adjuvant therapy (chemotherapy-cyclophosphamide, methotrexate, and 5-fluorouracil and/or hormone therapy-tamoxifen). Moreover, the *BRCA2* status retained the significant prognostic factor by multivariate analysis.

Breast cancer is an endocrine-related malignancy. Ovarian hormones, estrogen and possibly progesterone, are thought to play an important role in development and progression of breast cancer in women (45). However, the role of androgen in breast cancer etiology is poorly understood. In our study, ARs were detected in 38.5% of MBC patients; this rate is identical with that reported by Munoz de Toro *et al.* (46; 38.5%, 5 of 13) and similar to the rate of 34% observed by Pich *et al.* (47) in a series of 47 primary male breast carcinomas. However, it is much lower than the rate of AR positivity reported by other investigators. Unlike other studies on female breast cancer or MBC, we did not find a correlation between AR and ER status (48–52). There was no association between AR and age, tumor size, or lymph node status, and these results are in accordance with reports on female breast cancer and MBC as well (47, 49, 50). The role of AR as a prognostic factor is controversial. In MBC, Pich *et al.* (47) showed lack of association between AR and survival, whereas Munoz de Toro *et al.* (46) suggested that decreased androgen action (AR−) within the breast might contribute to an earlier development of MBC. In contrast, we found a strong correlation between AR expression and MBC patient OS and DFS. AR positivity was associated with adverse prognosis and AR status had prognostic significance in both univariate and multivariate analysis. In addition, whereas in former studies AR expression has been associated with favorable outcome, we found that AR expression predicted shorter survival. The involvement of AR in MBC development has been also investigated at the DNA level (24, 53–55). Two germline mutations have been associated with predisposition to MBC, which can result in reduced AR function (53, 54). However, we and others found no evidence of germline or somatic AR mutation (24, 26). AR activity can be affected by the highly variable polyglutamine tract (CAG repeat) located in the NH_2-terminal *trans* activation domain of the AR. The length of the tract varies from 12 to 32 residues in normal individuals (56). Expansion of the CAG repeat has been associated with reduced AR expression/*trans* activation, whereas the relatively short CAG repeat sequence increases the level of *trans* activation of the AR (57, 58). The role of CAG repeat sequence in MBC has been investigated in several studies, but there was no statistically significant difference in the number of CAG repeats between MBC patients and controls (24, 55). Divergent responses to androgen have been observed in human breast cancer cell lines. It has been shown that androgen may both stimulate and inhibit the growth of AR-positive breast cancer cell lines *in vitro* (59–61). It has also been suggested that the enhanced transcriptional activity of the AR gene might promote breast cancer progression (62). In an animal model, in both female and male Nobel rats, combination of testosterone and estrogen induced higher incidence of mammary cancer than either hormone treatment alone (63–65). In male Nobel rats, androgen could shorten the latency period, enhance tumor size, and increase the incidence of mammary cancers (66). These results together with our findings may indicate an important role of androgen and AR expression in the MBC progression.

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