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ORIGINAL ARTICLE

Association of hormone receptor status with grading, age of onset, and tumor size in *BRCA1*-associated breast cancer

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Abstract *BRCA1*-associated breast cancer frequently presents with estrogen-receptor (ERα) and progesterone-receptor (PR) negativity, grade 3, and early onset. In contrast, in *BRCA1*-deficient mice, ERα is highly expressed in early tumorigenesis. In a retrospective cohort study on 587 breast cancer patients with deleterious *BRCA1* mutations, the correlation of ER, PR status, grading, age of onset, and tumor size was investigated. ERα and PR expression decreased from 62% in ductal carcinoma in situ (DCIS) to 20% and 16% in pT3, respectively (p value for ER 0.025 and PR 0.035, Fisher's exact test). The percentage of grade 1/2 tumors decreased from 44% in DCIS to 17% in pT3 (p value 0.074). Moreover, ER/PR positivity increased with increasing age. Our data suggest that early stage *BRCA1*-associated breast cancers are more frequently ERα and PR positive and low grade than advanced stages.

Keywords *BRCA1* · Breast cancer · Estrogen receptor · Progesterone receptor

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Introduction

Women with deleterious mutations in the breast cancer susceptibility gene *BRCA1* are predisposed to breast and ovarian cancer with an estimated lifetime risk of about 80% [1–3].

As *BRCA1* acts as a tumor-suppressor gene, the inactivation of the wild-type allele is thought to be mandatory for cancer development. Although the potential for disruption of function of the second *BRCA1* allele exists in all somatic cells with inherited germ-line mutations, the increased risk of cancer in mutation carriers is most evident in hormone-sensitive tissues, such as breast and ovarian tissue in women and prostate tissue in men. The BRCA1 protein has been implicated in DNA damage repair, cell cycle checkpoint control, and transcriptional regulation [4]. The specific suppression of breast and ovarian carcinogenesis by the *BRCA1* gene has been attributed to its regulation of estrogen receptor alpha (ERα) and progesterone receptor (PR) [5, 6], which play important roles in breast development [7, 8]. BRCA1 directly interacts with ER and down regulates ligand-dependent and -independent transcription activities [9, 10]. In line with these findings, it could be shown that ERα is highly expressed in the premalignant mammary gland and initiation stages of tumorigenesis in a mouse model lacking the full-length form of *BRCA1* [11]. In contrast, normal breast tissue of human *BRCA1* mutation carriers stains positive for ER to the same extent as in women with wild-type *BRCA1*. The majority of BRCA related breast cancers are ERα and PR negative and of high grade [12, 13]. Clinical observations, however, indicate that reduced exposure to steroidal hormones after removal of the ovaries leads to a reduction in breast cancer risk around 50% [14]. Additionally, tamoxifen reduces contralateral breast cancer risk in *BRCA* mutation carriers [15].

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King et al. presented data indicating that PR is highly and aberrantly expressed in normal breast epithelium of *BRCA1* mutation carriers [16]. They could show that this is due to the diminution of PR ubiquitination and degradation in the absence of functional *BRCA1*. Furthermore, the treatment of the *BRCA1*-deficient mice with the progesterone antagonist mifepristone (RU 486) prevented mammary tumorigenesis [17]. These observations indicate that ovarian hormones contribute to breast cancer development in *BRCA1* mutation carriers. Therefore, the purpose of this study was to analyze ERα and PR expression during breast cancer development in *BRCA1* mutation carriers.

Study population and methods

The German Consortium for Hereditary Breast and Ovarian Cancer (GCHBOC) comprises 12 university centers. Using uniform inclusion criteria and standard operating procedures, families with clustering or early onset of breast or ovarian cancer are registered and tested for the presence of deleterious germ line mutations in *BRCA1* and *BRCA2*. Comprehensive data on familial cancer history including a detailed pedigree, pathology reports, and results of molecular testing are documented in a central database using standardized electronic case report forms. Inclusion criteria and methods for genetic testing are described elsewhere [18]. All patients gave their written informed consent to be enrolled in the registry. The registry has been approved by the institutional review boards of each participating center.

Between 1997 and 2008, 8,622 women underwent *BRCA* genetic testing. Overall, 2,655 women were identified with a polymorphism, 340 women with an unclassified variant (UV), and 1,853 with a deleterious mutation in the *BRCA1* gene. Of the *BRCA1* mutation carriers, 1,042 had developed breast cancer between 1976 and 2008. Of the latter, medical reports were available from 587 women.

In order to exclude a potential recruitment bias, we compared the median age of onset of the 587 women included in our study with the median age of onset of 455 women who could not be included in the study because of missing data. The median age of onset for the study group is 39 years (range 23–80 years) and for the 455 excluded women, 40 years (range 17–81 years), i.e., there was no difference in menopausal status which may have exerted a major effect on hormonal receptor status.

In the case of metachronous tumors, only the first breast cancer was considered. Patients with pT4 tumors were excluded because these tumors represent a small and inhomogeneous group of malignancies with different tumor extent and partially inflammatory component. A total of 587 patients with complete clinical and pathology reports including ERα, PR status, pT status, and age at diagnosis were analyzed. In 541 patients, information on tumor grading was available.

We stratified the patients by tumor size, histological grade, and age and calculated the percentage of ER/PR-positive tumors for each stratum. The median age of onset our study participants was 39 years (range 23 to 80 years), 38 years (25 to 66 years) for patients with ductal carcinoma in situ (DCIS), 39 years (23 to 80 years) for patients with T1, 38 years (23 to 79 years) for patients with T2, and 39 years (24 to 54 years) for patients with T3. There was no significant difference in the age pattern between tumor sizes. Sixty-five patients were younger than 30 years at the time of the first breast cancer diagnosis; 251 were between 30 and 39 years, 193 between 40 and 49 years, 51 between 50 and 59 years, and 27 above 60 years of age.

Tumor pathology

Information regarding the histological type of breast cancer, ER and PR status, and grading were obtained from institutional pathology reports and were reviewed by reference pathologists. All carcinoma in situ and invasive breast cancer specimens were routinely evaluated for ER and PR status using immunohistochemistry. Monoclonal antibodies were used to stain for ERα and PR. Three classification systems, i.e., percentage of positive-stained nuclei, Remmele score, and Allred score, have been applied according to the current S3 guideline [19]. Tumors were considered hormone-responsive in the case of >10% positive cell nuclei, Allred score >=4, or Remmele score >=2. As the revised St. Gallen guideline 2005 considers tumors exhibiting 1% to 10% positive nuclei of uncertain endocrine responsiveness, there remained some impreciseness in the categorization of tumors with very low ERα and PR expression.

Statistical analysis

Two-sided Fisher's exact test was used to asses the association between hormone receptor positivity, tumor size, grading, and age at diagnosis. A p value equal to or less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS 15.0.1.1 (SPSS Inc., Chicago, IL, USA) for Windows.

Results

Thirteen women were diagnosed with DCIS, 321 women with pT1-size tumors, 228 women with pT2 tumor, and 25 women with pT3 tumor. Detailed characteristics are given in Table 1. The ERα and PR expression gradually diminished (Fig. 1) from 62% in pTis to 20.0% in pT3 for ERα and from 61.5% to 16.0% for PR, respectively (p=0.025 and p=0.035). In

Table 1 Clinicopathological characteristics of *BRCA1*-associated breast cancer cases

Characteristics	*BRCA1* mutation carriers (n=587)
Tumor size, no. (%)	
pTis	13 (2.3)
T1	321 (54.7)
T2	228 (38.7)
T3	25 (4.3)
Grading, no. (%)	
G1	7 (1.2)
G2	147 (25.1)
G3	387 (65.9)
Not specified	46 (7.8)
Estrogen receptor status, no. (%)	
Positive	158 (26.9)
Negative	429 (73.1)
Progesterone receptor status, no. (%)	
Positive	159 (27.0)
Negative	428 (73.0)
Age (years) at first breast cancer, no. (%)	
<29	65 (11.0)
30–39	251 (42.9)
40–49	193 (32.8)
50–59	51 (8.7)
>60	27 (4.6)

541 patients, we had information on histological grade, ERα, and PR status. The proportion of cancers that were ERα-positive decreased considerably from grade 1 to 3 (grade 1, 57.1% ERα positive; grade 2, 45.6%; and grade 3, 17.8% ERα positive; p value <0.001). A similar relationship was found for PR status and grading (grade 1, 42.9% PR positive; grade 2, 39.5%; and grade 3, 20.7% PR positive; p value <0.001; Fig. 2). It is well known that ERα and PR are expressed in an age-dependent manner in sporadic breast cancer which was seen in our cohort of *BRCA1* mutation carriers as well. We observed an increase in ERα and PR positivity for tumors diagnosed under 30 years (24.6% ERα and 20.0% PR) to tumors diagnosed above 60 years (55.6% ER and 40.7% PR; Fig. 3; p_{trend}=0.009 and p_{trend}=0.004, respectively).

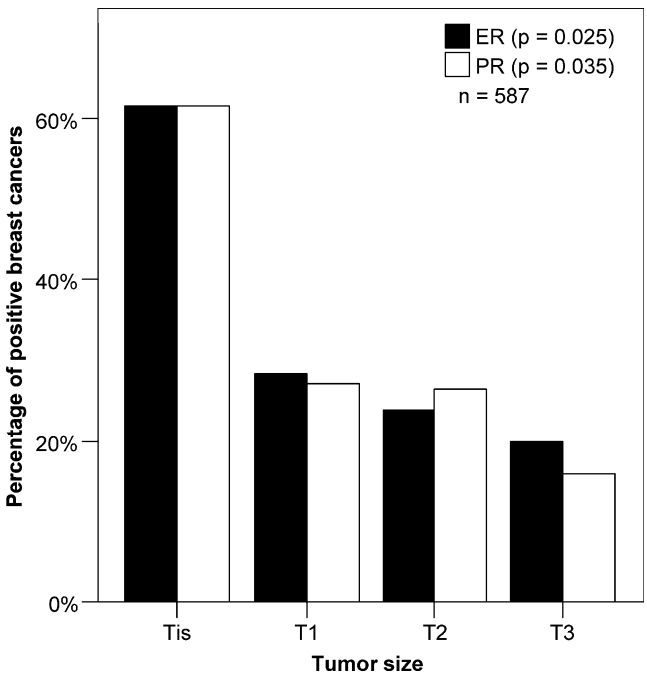

Fig. 1 Percentage of ER and PR positive breast cancer by tumor size in 587 *BRCA1* mutation carriers

Additionally, we found a trend for an association between histological grading and tumor size. Grade 1/2 tumors decreased from 44.4% in DCIS to 16.7% in pT3 tumors (p value 0.074; Fig. 4).

Discussion

Numerous studies pointed out that 70–90% of invasive *BRCA1*-associated breast cancers do not express ERα which is in line with our observation [20, 21]. The overall frequency of high-grade tumors (G3) in our study population was 71.5% which is slightly lower than that reported by Atchley et al. (85.4%) but consistent with the results by Foulkes et al. (73.6%) [20, 21]. However, most studies did not stratify the patients according to tumor size. For

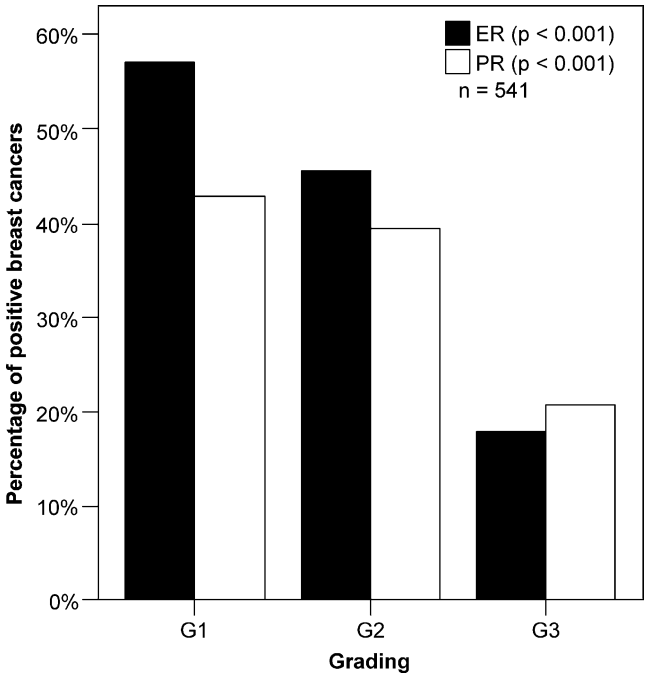

Fig. 2 Percentage of ER and PR positive breast cancer by grading in 541 *BRCA1* mutation carriers

instance, among 56 *BRCA1* mutation carriers described by Atchley et al., 71.5% presented with advanced tumor stage (pT2–3) compared to only 43.0% (pT2–3) in our study population [20]. Foulkes et al. even excluded patients with preinvasive stages (DCIS) from their evaluation [21].

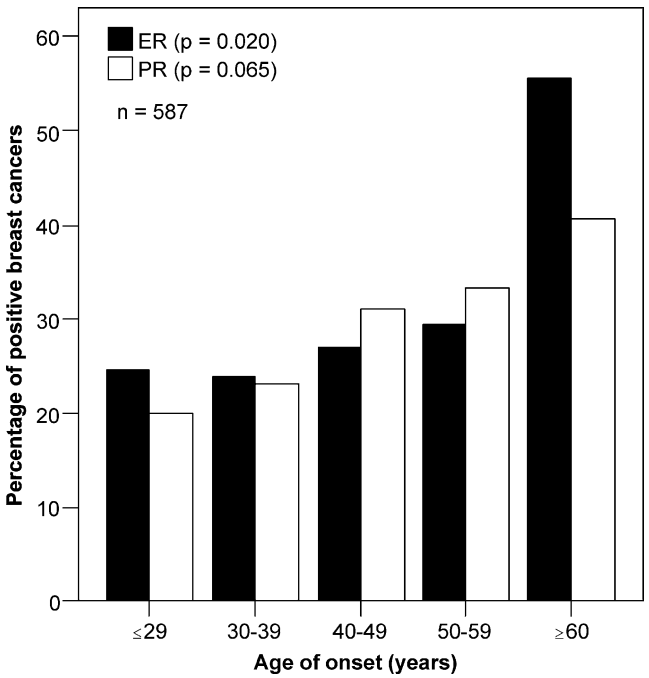

Fig. 3 Percentage of ER and PR positive breast cancer by age of onset in 587 *BRCA1* mutation carriers

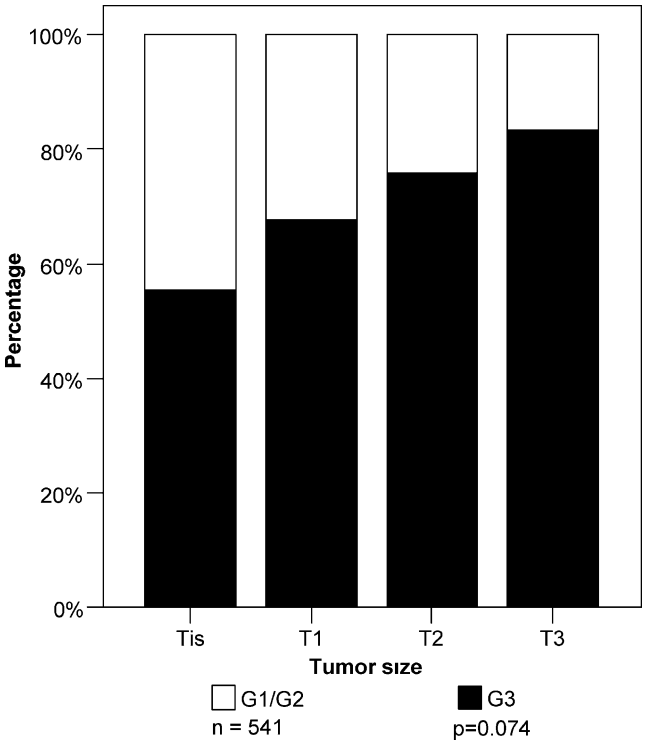

Fig. 4 Association of grading and tumor size in 587 *BRCA1* mutation carriers

In our study, we found an inverse correlation between hormone receptor status and tumor size. In agreement with our data, Li et al. found that ERα is highly expressed in initiation stages of tumorigenesis in *BRCA1*-deficient mice [11]. The majority of cells in hyperplasia (95.8%), carcinoma in situ (94.7%), and small tumors <0.5 cm in diameter (82.4%) were ERα positive and gradually decreased to less than 1% positivity in larger tumors of approximately 2 cm in size.

We detected an inverse correlation between ERα and PR expression and grading which is in agreement with Atchley et al. who found that the proportion of tumors that were ERα-positive decreased as the histological grade increased [20].

Finally, we observed an increase in the percentage of tumors that were ERα/PR positive with increasing age. Accordingly, Vaziri et al. found that ERα and PR were less frequently expressed in tumors of *BRCA1* mutation carriers (25% ERα/PR) than in controls (59.5% ERα and 57.1% PR) if breast cancer appeared before age 50 [22]. A similar trend was observed by Foulkes et al. who found that 19% of *BRCA1*-related breast cancers were ERα-positive cancers occurring in women in their premenopausal years compared to 38.0% in postmenopausal years after age 55 [21].

A potential limitation of our study is the impreciseness in the classification of tumors with very low hormone receptor

levels, i.e., tumors with 1–10% positively stained nuclei have been considered non-endocrine responsive in the past while they are considered of uncertain responsiveness since 2005 (St. Gallen, S3 GL). Therefore, the percentage of hormone responsive *BRCA1*-associated tumors might have been slightly higher which should not have a major influence on the observed association between hormone receptor levels and tumor progression.

Our observations point to a role of hormones in early BRCA1-associated carcinogenesis rather than in tumor progression. This is further supported by the observation that oophorectomy in human *BRCA1* mutation carriers and in mouse mutants significantly reduced the frequency of breast cancer formation [23, 24]. Taken together, these data suggest that *BRCA1*-related breast carcinogenesis is sensitive to anti-hormonal prevention.

In this context, Narod et al. and Metcalfe et al. demonstrated that prophylactic tamoxifen intake significantly reduced the risk for contralateral breast cancer in *BRCA1* mutation carriers [15, 23]. However, these observations conflict with results by Jones et al. [25]. They described a proliferative effect of tamoxifen on mammary cancer development in *BRCA1*-depleted mice while oophorectomy was protective. This was explained by an agonistic activity of tamoxifen in the absence of functional *BRCA1*.

An alternative strategy may therefore be the targeting of PR which showed a concomitant expression with ERα in our cohort. PR expression is even elevated in benign tissue adjacent to BRCA1-associated breast cancers [16]. This is further supported by Poole et al. who pointed out that *BRCA1* deficiency in mice correlates with PR accumulation [17]. They were able to show that functional *BRCA1* leads to degradation of PR by ubiquitination. Consequently, administration of the progesterone antagonist mifepristone (RU 486) substantially reduced branching and tumor development in these mice. Our finding of predominantly PR positive staining in early tumor formation in *BRCA1* mutation carriers supports the assumption that progesterone is involved in breast cancer development. Therefore, PR is a promising new target for the prevention of *BRCA1*-associated carcinogenesis that deserves further investigation.

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Conflict of interest None.

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